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Avoidance of biorisks of composting by thermohygienization: influence of the type of system and management on the occurrence of the potentially pathogenic mold *Aspergillus fumigatus* and fecal indicator bacteria

Dissertation presented to the Faculty of Sciences of the University of Neuchâtel, in partial fulfillment of the requirements for the degree of "Docteur ès Sciences"

by

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Neuchâtel, May 2000

IMPRIMATUR POUR LA THÈSE

Avoidance of biorisks of composting by thermohygienization : influence of the type of system and management on the occurence of the potentially pathogenic mold *Aspergillus fumigatus* and fecal indicator bacteria

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Neuchâtel, le 6 septembre 1999

Le doyen:

F. Stoeckli

To my daughter Annette

who was born faster and easier than this thesis

ABBREVIATION LIST

ABPA	allergic broncho-pulmonary aspergillosis
AF	Aspergillus fumigatus
cfu	colony forming unit
DW	dry weight
DWC	dry weight of compost
EAA	exogen allergic alveolitis
FAC	Forschungsanstalt für Agrikulturchemie (Federal Research Institute for Agricultural Chemistry and Environmental Hygiene), Liebefeld, Bern
FWC	fresh weight of compost
IgA	immunoglobulin of the type A
IgE	immunoglobulin of the type E
IgG	immunoglobulin of the type G
LPS	lipopolysaccharides
LTT	lymphocyte transformation test
MPN	most probable number
MSW	municipal solid waste
OEL	occupational exposure limits
ODTS	organic dust toxic syndrome
OM	organic matter
PCR	polymer chain reaction
TTM	Total thermotolerant molds and yeasts

SUMMARY

Composting, a biodegradative process in which putrescible organic wastes are transformed into a stable, humus-like product makes nowadays part of a modern waste management system. If composting is carried out at industrial scale, a number of problems, such as the emanation of bad odors, or the dispersion of potentially pathogenic microorganisms can arise.
In the scope of a research program about biorisks, the occurrence of the thermotolerant, allergenic and potentially infectious mold *Aspergillus fumigatus (AF)* and of coliforms (indicator bacteria of a fecal contamination) in different composting systems was investigated. The approach of our research was to consider the hygienization of compost as the consequence of high temperature formation through optimal composting conditions. It was tried to determine the influence of the main process parameters (composition of the biowaste, size of the heaps, turning frequency, aeration cycles) on the composting action, and thus ultimately on thermohygienization. The composting process was monitored at the sites of our industrial partners, representing the different composting systems used in Switzerland (open-air windrows, aerated and non-aerated boxes, aerated trenches, in-vessel composting), by monitoring physico-chemical (temperature, gas concentrations, pH, water content) and microbiological parameters. The field studies were complemented with laboratory experiments about the physiology, especially the thermoresistance of *AF* (spores and mycelium).

AF was detected in high concentrations in fresh biodegradable waste, at all the composting systems investigated. Although an almost complete thermohygienization was obtained in the core of the compost heaps during the thermogenic phase of the process, potentially pathogenic microorganisms proliferated or persisted in the cooler outer and lower zones, due to the always-existing temperature gradient. Laboratory experiments showed that thermotolerant subpopulations of *AF* spores were generated during heating, possibly explaining the quite high temperatures (>65°C) needed for its destruction.

Recolonization of the compost by *AF*, and to a lesser degree by coliforms, after the thermogenic phase was frequently observed, although concentrations did not reach the initial values. The extent of the recolonization depended on the compost maturity, which itself depended on the organic matter content of the starting material and on the efficiency and homogeneity of the rotting process. Special attention should be given to the curing of the compost after the actual rotting phase. If ever possible, treatment should be continued, although at more infrequent intervals, until the compost is used.

An intensive management of the compost proved to be necessary for good hygienization. The following parameters have to be chosen carefully to assure a correct composting process: composition of the initial substrate (C:N ratio between 30 and 35, good structure), adjustment of humidity between 50 and 60 %, frequent mixing (every 1-2 days during the thermogenic phase) to redistribute microorganisms and substrate and to bring material to the hot center of the heap, adjustment of aeration (frequency, duration), duration of the process long enough to avoid recolonization during maturation, or direct use of fresh compost without storage. In experiments carried out at the open-air windrow site, the importance of frequent turnings was demonstrated: daily turned composts contained significantly less *AF* than such that were turned weekly or monthly.

Frequent turnings also accelerated the degradation process, especially in the experiment with mostly woody material (initial C:N ratio of 40:1).

A homogeneous composting process was difficult to achieve in the box systems, where compost was filled 2-4 m deep. At the point of air inflow, the material was cooled and dried, leading to an inhibition of the microbial activity, and thus to an interruption of the degradation process. Thermohygienization was therefore also uneven: the hot spots showed low *AF* concentrations, while higher numbers were detected in the colder zones. In the unaerated box system, conditions in the rotting boxes were completely anoxic, the actual composting, by definition an aerobic process, started only when the material was piled up for curing. As the treatment was extensive at this moment (turning of the material once a week by a front-end loader), an important proliferation of *AF* at the heap surface was measured. Reduction of *AF* numbers was also slow in the aerated bioreactor, despite of very high temperatures around 80°C during the second half of the process, when airflow was in the sucking mode. The most homogenous composting was obtained in the trench system, demonstrated by little temperature and gas gradients. The quite high, uniform temperatures on the other hand let supposedly to a less intensive degradation of organic matter. The 30 days rotting in the hall were not sufficient to produce a stabilized product.

Bioaerosols containing high numbers of fungal spores were shown to be produced when mechanically treating compost (shredding, turning, and screening). When these activities were carried out in the open, spores were very rapidly diluted, so that health risks for the personnel working on the sites, and for people living in the neighborhood of such sites were judged minimal. Very high concentrations of mold spores were usually detected when composting was carried out in boxes or trenches in closed halls. In spite of a supposed automation of the process, the very frequent presence of maintenance personnel was necessary in the rotting halls.
A good correlation was normally seen between fungal concentrations measured in the compost, and that emitted during turning. The turning system used greatly influenced the importance of bioaerosol generation. More gentle turning lead to less spore emission, but was normally coupled to a slower turning rate, leading to infrequent turnings, and thus to an inhomogeneous composting process.
The efficiency of the biofilters, installed to deodorize vitiated air from closed composting systems, to hold back fungal spores and microbial cells was questioned. Further research would be necessary in this area.

ZUSAMMENFASSUNG

Kompostierung, ein biologischer Prozess, während dessen abbaubare organische Abfälle in ein stabiles, humus-ähnliches Produkt umgewandelt wird, ist heutzutage Bestandteil eines jeden modernen Abfallentsorgungskonzepts. Wenn die Kompostierung im industriellen Massstab ausgeführt wird, können eine Reihe von Problemen, wie Geruchsbelästigung, oder die Verbreitung von potentiell pathogenen Mikroorganismen, auftreten.
Im Rahmen eines Forschungsvorhabens zum Thema „Biorisiken" wurde das Vorkommen des thermotoleranten, allergenen und potentiell infektiösen Schimmelpilzes *Aspergillus fumigatus* (*AF*) und von coliformen Keimen (Indikatorkeime für Fäkalkontaminationen) in verschiedenen Kompostiersystemen untersucht. Unser Forschungsansatz war, die Komposthygienisierung als Folge der hohen Komposttemperaturen zu betrachten, die durch optimale Bewirtschaftung auftreten. Wir versuchten, den Einfluss der wichtigsten Parameter (Zusammensetzung des Bioabfalls, Mietengrösse, Umsetzhäufigkeit, Belüftungszyklen) auf den Kompostierprozess, und letztlich auf die Thermohygienisierung aufzuzeigen. Der Kompostiervorgang wurde auf den verschiedenen Anlagen unserer Industriepartner (offene Mieten, belüftete und unbelüftete Boxen, belüftete Kanäle, Reaktorkompostierung) durch physikalisch-chemische (Temperatur, Gaskonzentrationen, pH, Wassergehalt) und mikrobiologische Messungen verfolgt. Die Feldanalysen wurden mit Laborexperimenten über die Physiologie, insbesondere die Thermoresistenz von *AF* (Sporen und Myzel) ergänzt.

AF wurde in hohen Konzentrationen in frischem Bioabfall in allen Kompostiersystem gefunden. Im Innern der Mieten fand während der heissen Phase der Kompostierung eine weitgehende Thermohygienisierung statt. In den kälteren äusseren oder unteren Zonen hingegen, die durch immer auftretende Temperaturgradienten entstanden, konnten potentiell pathogene Organismen überleben. Laborexperimente zeigten, dass thermotolerante Subpopulationen von *AF*-Sporen während der heissen Phase gebildet wurden, was erklärt, dass Temperaturen über 65°C nötig waren, um diese Sporen ganz abzutöten.

Wiederbesiedlung des Komposts mit *AF*, und in geringerm Mass mit Coliformen, nach der heissen Phase wurde häufig beobachtet; Konzentrationen erreichten hingegen nie die Anfangswerte. Das Ausmass der Wiederbesiedlung war vom Reifegrad des Komposts abhängig, der wiederum vom Anteil der organischen Masse im Ausgangsmaterial, und von der Effizienz und der Homogenität des Rotteprozesses beeinflusst war. Der Reifung des Komposts nach der Heissrottephase sollte spezielle Beachtung geschenkt werden. Wenn möglich sollte die Behandlung fortgesetzt werden, wenn auch in grösseren Intervallen, bis der Kompost ausgebracht wird.

Eine intensive Behandlung des Kompost zeigte sich für eine gute Thermohygiensierung unerlässlich. Die folgenden Parameter müssen sorgfältig gewählt werden, um einen korrekten Kompostierprozess zu erreichen: Zusammensetzung des Ausgansmaterials (C:N-Verhältnis zwischen 30 und 35, gute Struktur), Feuchte zwischen 50 und 60 %, häufiges Umsetzen (alle 1-2 Tage während der Heissrottephase), um Mikroorganismen und Substrat neu zu mischen, und um Material in den heissen Kern zu bringen, angepasste Belüftung (Intervall, Dauer), genügend lange Kompostierdauer, damit die Wiederbesiedlung während der Reife beschränkt bleibt, oder direkte Verwertung des Komposts ohne Lagerung. Versuche, die auf einer offenen Mietenanlage durchgeführt wurden, zeigten deutlich den Einfluss von häufigem Umsetzen: täglich umgesetzte Mieten enthielten deutlich weniger *AF* als solche, die nur wöchentlich oder monatlich umgesetzt wurden. Häufiges

Umsetzen beschleunigte auch den Abbauprozess, vor allem im Material mit viel Holz (C:N-Verhältnis von 40:1 zu Beginn).
Ein homogener Kompostierprozess war in Boxen, die 2-4 m hoch mit Kompost gefüllt waren, schwierig zu erreichen. Am Boden, wo Luft eingeblasen wurde, wurde der Kompost ausgekühlt und ausgetrocknet, mit der Folge, dass die mikrobielle Aktivität eingeschränkt wurde, und der Abbauprozess zu Erliegen kam. Die Thermohygieniserung war ungleichmässig: in den heissen Zonen wurden wenig *AF* gemessen, während die kalten Zonen hohen Pilzkonzentrationen aufwiesen. Die unbelüften Boxen waren völlig anaerob. Die Kompostierung, laut Definition ein aerober Prozess, setzte erst ein, nachdem das Material ausserhalb der Boxen zur Reifung aufgeschüttet wurde. Da die Behandlung zu diesem Zeitpunkt extensiv war (der Kompost wurde 1 x wöchentlich mit einem Trax umgesetzt), konnte an der Oberfläche der Haufen eine starke Vermehrung von *AF* gemessen werden. Im Bioreaktor wurde nur eine langsame Abtötung der Schimmelpilze festgestellt, obwohl sehr hohe Temperaturen um 80°C in der zweiten Hälfte des Kompostierprozesses gemessen wurden, als Luft von oben durch die Kompostmasse gesogen wurde. Der homogenste Kompostierprozess wurde in den Kanälen erreicht, wo nur geringe Temperatur- und Gaskonzentrationsunterschieden gemessen wurden. Die ziemlich hohen, gleichmässigen Temperaturen bewirkten jedoch wahrscheinlich einen geringeren Abbau der organischen Substanz. Die 30 Tage Rotte in der Halle genügten nicht, um ein stabiles Endprodukt herzustellen.

Die mechanische Bearbeitung von Kompost (Schreddern, Umsetzen, Aussieben) erzeugte Bioaerosole, die hohe Konzentrationen an Schimmelpilzsporen enthielten. Wenn diese Arbeiten im Freien ausgeführt wurden, erfolgte eine rasche Verdünnung der Sporen in der Luft. Gesundheitsgefährdung für Leute, die in der Umgebung eines Kompostplatzes wohnen, wurde daher als minimal eingestuft. Sehr hohe Schimmelpilzkonzentrationen wurden in geschlossenen Kompostierhallen gemessen (Boxen- oder Kanalkompostierung). Trotz der angeblichen Automatisierung des Prozesses in solchen Hallen ist die Anwesenheit von Personal sehr häufig nötig.
Eine gute Korrelation wurde zwischen der Schimmelpilzkonzentration, die im Kompost gemessen wurde, und derjenigen in der Luft beim Umsetzen gefunden. Das Umsetzverfahren beeinflusst die Freisetzung von Pilzsporen stark. Weniger intensives Durchmischen erzeugte weniger Pilzemissionen, bewirkte aber auch eine geringere Umsatzrate, und daher einen inhomogenen Kompostierprozess und einen weniger raschen Abbau.

Die Wirksamkeit von Biofiltern, die in erster Linie eingesetzt werden, um die Abluft geruchsarm zu machen, in Bezug auf das Zurückhalten von Pilzsporen wurde in Frage gestellt. Weitere Arbeiten zu diesem Thema sind nötig.

1. INTRODUCTION

1.1 WHAT IS COMPOSTING ?

The last decade has lead to an increasing awareness of the problems associated with the classical methods of waste treatment. It was realized that the elimination of waste materials by burning, or by its dumping in sanitary landfills was not the final solution to all waste problems, but gave sometimes rise to new ones (pollution of air and groundwater, elimination of toxic residues, shortage of suitable sites for landfills). Also, biodegradable wastes are not very well suited for incineration because of their high water content, and create problems when put in landfills (emanation of gases and leachates). Due to the ongoing dehumification of the soils, the necessity to recycle plant derived wastes to return nutritive minerals to the soil, but also to renew the humus fraction, was recognized (ARAGNO, 1994).

That's why today, modern waste treatment programs can not be imagined without source separation and composting, either at individual, local or regional level, to treat part of this organic fraction, namely garden and park waste (green waste), kitchen waste, and to a minor degree also agricultural and biodegradable industrial waste.

However, when composting of large quantities is carried out at industrial level, a number of problems (bad odors, dispersion of potentially pathogenic microorganisms) can arise, which may jeopardize the whole industry. Research at industrial scale should examine the measures to be taken to avoid these problems, and to guarantee the production of compost that poses no risk to the environment or the users.

1.1.1 DEFINITION OF COMPOSTING

Depending on the view of the author (more technical or more economical), different definitions of composting exist. All, though, are justified, and show one or the other aspect of this very complex process.

COMPOSTING

- is the **accelerated natural process** of biodegradation of organic matter by **gathering the material** into heaps to **conserve** part of the **heat** generated by microbial metabolisms so that the temperature of the mass rises and **faster reaction rates** are obtained (BIDDLESTONE & GRAY, 1987a)
- is a **biodegradative** process in which **organic wastes** are transformed and **stabilized** by the metabolic activities of a **succession of mixed microbial populations**, each suited to the environment produced by the previous population (ANDERSON & SMITH, 1987)
- is defined as a **thermophilic** biological process involving the decomposition of **putrescible** organic material into a relatively stable **humus like end product**. When properly managed, the process **decreases the weight, volume,** and **water content** and **kills pathogenic organisms** (HAY & KUCHENRITHER, 1990)
- is the **decomposition of heterogeneous organic matter** by a mixed microbial population in a **moist, warm, aerobic** environment (GRAY & BIDDLESTONE, 1971a)

COMPOSTING (continued)

- is a **controlled** biooxidative process that involves a heterogeneous organic substrate in the **solid state**, it evolves by passing through a thermophilic stage and a **temporary release of phytotoxin** (for example acetic acid) (KEELING *et al.*, 1995)
- leads to production of **carbon dioxide**, **water**, **minerals**, and **stabilized organic matter** (compost). It implies control over **temperature**, **moisture**, **substratum composition**, **oxygenation**, etc. (DE BERTOLDI & ZUCCONI, 1987)

COMPOST

- is the **result of the composting process**. The organic matter must be stabilized or cured and become a humus-like product that can be **stored without further treatment**, and can be **applied to land without damage to crops** (HAY & KUCHENRITHER, 1990)
- is the stabilized and **sanitized** product of composting and is **beneficial to growth** of plants (CHEN & INBAR, 1993)
- has undergone an initial **rapid stage of decomposition**, and is in the process of **humification** (stabilization stage) (DE BERTOLDI & ZUCCONI, 1987)

1.1.2 ADVANTAGES AND DISADVANTAGES OF COMPOSTING

Composting of the organic fraction of the waste leads to numerous **improvements** in the overall waste treatment process: **i)** reduction of the amount of waste that has to be incinerated or put in landfills; and therefore reduction of incinerator ash to be disposed of, and of landfill space; **ii)** in general lower costs than incineration, although treatment costs in very sophisticated, completely enclosed composting systems are now near those for incineration; **iii)** recycling of humus and nutrients into the soil; **iv)** bog conservation, because compost can be used as peat substitute; **v)** beneficial role of compost microorganisms in crop protection, in as much as they compete with plant pathogens.

If composting is not carried out properly, it can also have some **disadvantages**: **i)** the most common complaint about composting installations are odor nuisances; that's why the tendency goes to completely enclosed systems where the outlet air is treated in a biofilter before being emitted. The best way, though, to prevent malodor generation is a composting process with a high degradation rate, in order to remove the putrescible substances as quickly as possible; **ii)** proliferation and dispersion of potentially pathogenic and / or allergenic microorganisms; **iii)** soil pollution if the heavy metal content of the compost is too high. This can be avoided if the starting material is free of these contaminants (source separation of the organic waste; use of sewage sludge only from non-industrial origin); **iv)** groundwater pollution if composting is carried out on a surface that is not made up properly or *where the runoff water is not collected.*

1.2 SITUATION IN SWITZERLAND

On the example of Switzerland, the evolution of composting in the last decade will be shown. Although, due to geographical conditions, the situation might be different than in other European countries, in that the installations are generally smaller.

1.2.1 QUANTITIES

The latest numbers available are from 1996: from the total 4.91 million tons of municipal solid waste (MSW, including household waste and refuse of similar nature produced by local industries and small firms, as well as recycled materials (paper, glass, etc.)) generated by a population of 7.1 million (= 689 kg per habitant and year), 450'700 tons (= 57 kg/habitant and year, 20 % more than 1994) were source separated and composted in installations treating more than 100 t/a[1]; this corresponds to 8.3 % of the MSW (ANONYMOUS, 1998). To this have be added the quantities that are composted in private gardens, community installations and enterprises; they are estimated to another 300'000 to 400'000 t/y (ESTERMANN, 1997). In fact, the ordinance on the treatment of waste (Technische Verordnung über Abfälle vom 10. 12. 1990) states that the cantonal authorities must encourage home composting. Only the compostable waste that cannot be privately composted should be collected separately and treated in centralized installations (FISCHER *et al.*, 1995).
Since 1990, a constant yearly increase of about 10 % of the amount of composted material can be observed. However, in 1996 the MSW collected for incineration or landfilling still contained 23 % compostable matter (ANONYMOUS, 1996). With the extension of curbside collection programs for biodegradable waste, the introduction or augmentation of the waste bag tax (in municipalities that levy a waste bag tax, the amount of compostable matter in the unsorted waste destined for incineration is only half of that of those that levy no waste bag tax), and the interdiction, from the year 2000 on, to put combustible waste in landfills, the amount of MSW recycled by composting is thought to rise further in the near future.

1.2.2 COMPOSTING INSTALLATIONS

At the end of 1993, 149 composting installations treating more than 100 t/a were in operation. More than half were small sites treating between 100 and 500 t/y, and only 1/3 treated more than 1000 t/a. To this number have to be added 8 installation treating sewage sludge (methanization and composting), 2 installations handling the organic fraction of MSW for stabilization prior to landfilling, and 2 installations carrying out methanization of kitchen waste. Also, 22 new installations were planned (ANONYMOUS, 1994a). In 1996, the number of composting installations had risen to 231, the increase was mostly due to middle sized sites, treating between 500-1'000 t/a. The number of installations carrying out methanization had increased to 6.
Concerning the composting systems used, open-air windrows are the most widespread. In 1993, about 2/3 of the installations composted in trapezoid windrows, about ¼ of the installations used slate windrows of 3-4 m height. Only about 10 % of the installations were completely roofed or inside a closed building (ANONYMOUS, 1994a). In 1996 the situation had changed insofar that a new system of composting had developed: the edge-of-the-field composting, which represented ¼ of all installations. Farmers compost their own agricultural waste, together with biowaste from source separated collection, on the edge of the field where it will be applied, once the composting process is completed.

[1] Only these installations are recorded statistically

The revenue of composting installations stems mainly from the tipping fees for biodegradable waste: between CHF 100.-/t and 150.-/t, depending on the type of installation. In the context of waste management economy, this compares to tipping fees for incinerators of around CHF 200.-/t, and of landfills of 150-200.-/t. The sales of finished compost (agricultural grade: given away for free or CHF 2-3.-/m^3; horticulture grade: between CHF 20 and 40.-/m^3) contributes thus only to a minor degree to the profitableness of composting installations.
Data from Germany indicate rising operation costs for high-rate composting systems from DM 100.-/t input material in the year 1991 to DM 180.- to 210.- in 1994, due to technically discriminating systems, more intensive management, and higher maintenance costs of completely closed installations (corrosion) (WIEMER & KERN, 1994).

1.2.3 COMPOST UTILIZATION

The main end user of compost produced in Switzerland is agriculture, including viticulture and vegetable and fruit-growing. About 120'000 tons of compost (62 % of the compost produced in centralized installations) were spread in 1992 on agricultural land. The remaining quantities were used in commercial horticulture (nurseries, landscaping, seedling production), or in private gardens (about 10 % of the total amount of compost produced). A potential market for horticulture grade compost might be the partial substitution of imported peat or peat based potting soils, which amount to 210-260'000 tons annually (ANONYMOUS, 1994a). The farmers get the compost for free, or pay a minimal sum for it (CHF 2-3/m^3). In fact, the good contact between the operator of a composting installation and the farmers that live in its vicinity is of great importance for a regular compost distribution. Quantities that can be utilized in agriculture are restricted to a maximum of 25 t DW per hectare and 3 years (Ordinance about Harmful Substances for the Environment, appendix 4.5 (Verordnung über umweltgefährdende Stoffe vom 9.6.1986, Aenderung vom 16.9.1992), ANONYMOUS, 1995b). This limit was primarily set to restrict the input of heavy metals into the soil. In 1993, compost made up one half of a percent of the total yearly nutrient input into Switzerland's soils (FREI *et al.*, 1994).

1.3 ORDINANCES AND DIRECTIVES

In Switzerland, the production and utilization of compost is regulated in a number of ordinances and directives (ANONYMOUS, 1995b), namely the Ordinance about the treatment of waste (Technische Verordnung über Abfälle vom 10.12 1990), the Ordinance about Harmful Substances for the Environment, appendix 4.5 (Verordnung über umweltgefährdende Stoffe vom 9.6.1986, Aenderung vom 16.9.1992), and the Ordinance about Fertilizers and Equal Products (Verordnung über Dünger und diesen gleichgestellte Produkte vom 10.12.1990). These ordinances are assuring that compost can only be sold or given away if it is such that it does not harm the environment or man if applied correctly. Based on the legal provision mentioned above, guidelines for compost quality were worked out and compiled by the Swiss Federal Station for Agricultural[2] (ANONYMOUS, 1995b):

- Minimal quality requirements concern the heavy metal contents and the content of foreign materials (metal, glass, plastics and stones). In function of the amount of material treated at a composting installation, quality examinations have to be carried out 1-4 times a year by authorized laboratories, according to standardized tests.

[2] Eidgenössische Forschungsanstalt für Agrikulturchemie und Umwelthygiene, 3097 Liebefeld-Berne, Switzerland

- In order to fulfill the request that the amount of human, animal, and plant pathogens and pests as well as parts of plants capable of germinating have to give no cause for concern, the whole composting material has to be exposed to a heat phase that
  - * lasts at least 3 weeks, at > 55°C, or
  - * lasts at least 1 week, at > 65°C, or
  - * gives, on the basis of the temperature reached and its duration, a proven hygienizating effect just as good as the two methods mentioned above.
- To reach the necessary temperature, regular turnings (at least 3 during the heat phase) have to be effected, and the water content of the material adjusted. The operator of a composting site has to measure at least 3 times per week the temperature, and keep a record of the temperature evolution and the turnings. No laboratory test to determine pathogen contents have to be carried out, the hygienic harmlessness being considered to be assured, according to the present state of knowledge, by the heat treatments mentioned above.

The time/temperature relations are very similar to those proposed by several other countries. Table 1 gives an overview of the different temperature/time requirements in several countries.

Table 1: Temperature/time requirements for the hygienic control of biowaste composting plants in various countries (STRAUCH, 1996; BÖHM, 1995; FARRELL, 1993, STALDER, 1993).

country	type of installation	time / temperature relations
Austria	open windrow	> 65°C, for a least 6 days, or 2x3 days[a]
	in-vessel	> 65°C, but < 80°C for at least 3 days, water content 40-60 %, C/N 20-35:1[a]
Belgium		> 60°C for at least 4 days
Denmark		> 55°C for at least 2 weeks
France		> 60°C for 4 days[b]
Germany (draft)	open windrow	> 55°C for at least 2 weeks, or >65°C for at least 1 week
	in-vessel	> 60°C for at least 1 week
Italy		> 65°C for 2-3 consecutive days[c]
Netherlands		> 55°C for at least 4 days
Spain		-[d]
United States of America		
Class B (formerly Process to Significantly Reduce Pathogens (PSRP))		> 40°C for at least 5 days[e]
	windrow	> 40°C for at least 5 days, and > 55°C for 4 hours during these 5 days[e]
Class A (formerly Process to Further Reduce Pathogens (PFRP))	static pile or in-vessel	> 70°C for 30 minutes, or > 55°C for at least 3 days or > 53°C for at least 5 days[f]
	windrow	> 55°C for least 15 days with at least 5 turnings[f]

additional requirements:
[a] no Salmonella or viable ascaride eggs
[b] regular control of pathogens
[c] final material must be biologically stable to prevent pathogen regrowth, no Salmonella and fecal coliforms
[d] no weed seeds
[e] $< 2 \cdot 10^6$ fecal coliforms/g
[f] $< 1 \cdot 10^3$ fecal coliforms/g, < 1 viable helminth ova/4 g, < 3 MPN Salmonellae/4g. If the temperature/time requirements are met, the monitoring of fecal coliforms is sufficient.

- In order to inform the end user about the properties of the compost, each delivery has to be accompanied by a note stating the dry weight, the content of organic matter, total nitrogen, phosphorous, potassium, magnesium calcium and salt, an assessment about heavy metal concentration and hygienic quality (based on temperature measurements) and the amount that can be put on the fields.

Concerning the work-place hygiene in composting installations, no specific guidelines exist, the general Ordinance about the Prevention of Accidents and Occupational Disease (Verordnung über die Verhütung von Unfällen und Berufskrankheiten, 1983) is applied (KULL, 1994).

1.4 COMPOSTING - A MICROBIOLOGICAL PROCESS

Composting is a microbiological process in which a succession of mixed microbial populations is decomposing heterogeneous organic matter. The description of the microorganisms that participate in the composting process is complex, because the populations and communities change continuously as a function of the evolution of temperature, nutrient availability, oxygen concentration, water content and pH in the course of composting. The earliest research by WAKSMAN *et al.* (1939a and b) treated the influence of temperature upon the microbiological populations (bacteria, Actinomycetes and fungi) of manure composts. In the 70's, interest in composting of municipal solid waste and sewage sludge at industrial scale led to further investigations on the nature of the microbial populations participating in the composting process (FINSTEIN & MORRIS, 1975; POINCELOT, 1974). Also, numerous studies where carried out in laboratory composting systems to define process parameters (JÄGER, 1997; ATKINSON *et al.*, 1996a, b and c; BOELENS *et al.*, 1996; HOGAN *et al.*, 1989; NAKASAKI *et al.*, 1996 and 1993; TSENG *et al.*, 1996 and 1995; VON RHEINBABEN, 1995 and 1993; BAGSTAM, 1979; SULER & FINSTEIN, 1977). The preparation of compost for the cultivation of edible mushrooms (*Agaricus bisporus*) has a long tradition, and a rich literature is available treating the influence of the microbial compost flora on the mushroom production. Lately, composting has become a subject of research in the context of bioremediation (FEITKENHAUER *et al.*, 1997; BREITUNG *et al.*, 1996; LAINE & JORGENSEN, 1996; TOMATI *et al.*, 1995). Furthermore, hot composts have been "discovered" as a habitat of thermophilic organisms which are of interest for the production of thermostable enzymes (BAUER *et al.*, 1997; HUANG *et al.*, 1996; STUTZENBERGER *et al.*, 1970). The question of pathogenic microorganisms will be addressed in Chapter 1.8.1.

The typical evolution of temperature and pH, as well as the group of microorganisms present in each phase are shown in Figure 1.

As soon as waste is gathered in a heap, the heat generated by the degrading microorganisms is accumulated due to the insulating properties of the waste, and the temperature starts to increase. First, the compost microorganisms metabolize the soluble organic substances. pH decreases because acids are released as intermediate metabolites. This suits well the yeasts, which prefer a low pH. As temperature continues to increase, yeasts are inhibited, and the remaining flora metabolizes the acids. At about 50°C, the mesophilic bacteria cease their activity, and the thermophiles take over. Temperatures continue to rise until they become limited for the most thermotolerant species. In fact, the highest temperatures measured in compost (82°C) coincide with the maximum growth temperature of *Thermus*, the most heat tolerant species isolated so far from compost (BEFFA *et al.*, 1996). The peak temperatures can be maintained as long as there is enough substrate to support microbial activity, otherwise temperatures fall.

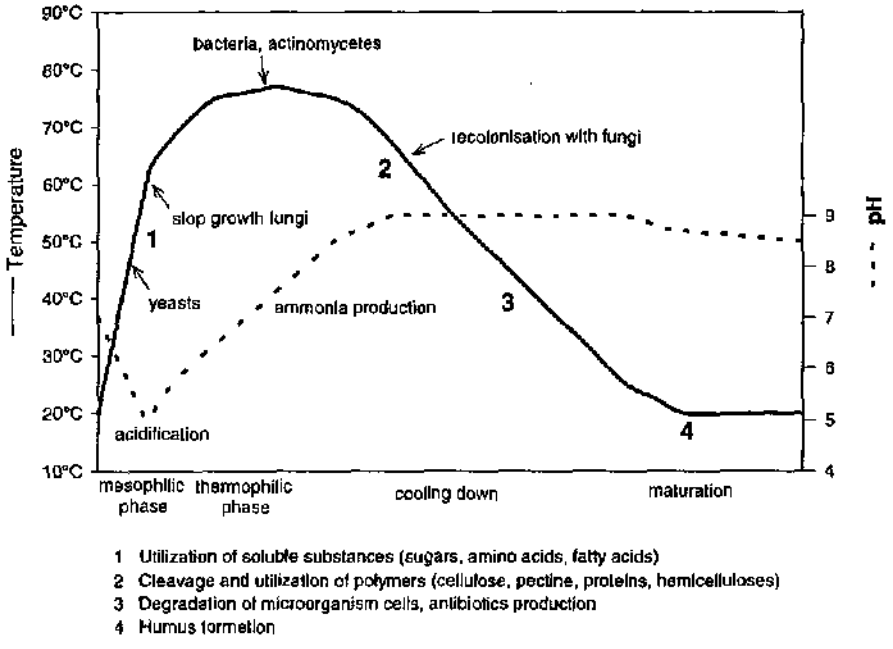

Figure 1: Schematic depiction of the composting process (modified from BIDDLESTONE & GRAY, *1987b)*

The decrease of pH observed in the initial phase of composting (see Figure 1) is due to the accumulation of organic acids that are intermediate metabolites of the aerobic carbon metabolism, or to anaerobic metabolisms which frequently occur due to the high oxygen demand of the microorganisms. Over the whole process, the pH continues to rise, through the liberation of ammonia from the degradation of proteins, up to a value of 8.5-9 (see also Chapter 1.5.4).

A large diversity of microorganisms is involved in the composting process; dead and living microorganisms make up 2-20 % of the composting mass (BEFFA *et al.*, 1995). The different populations vary in their growth temperature range, their substrate utilization, their pH tolerance and their oxygen demand (Table 2).

Table 2: Degradation spectrum of microorganisms that can be found in compost (BEFFA et al., 1995).

Type of micro-organisms	type of metabolism	growth rate	degradation capacity	range of growth
Bacteria except Actinomycetes	aerobic and/ or anaerobic	rapid	• organic and inorganic substances as well as gases (hydrogen, methane, carbon monoxide) • mineralization of nitrogen and sulfur compounds • fixation of atmospheric nitrogen • production of humic substances (exopolysaccharides) from organic monomeres • degradation of lignin not detected	5-82°C
Actinomycetes	aerobic	slow	• organic material and hydrogen • degradation of hemicelluloses, cellulose and lignin	10-65°C
molds	aerobic	rapid	• organic substances • colonization of material with a low water content (wood, dead leaves,..) • important for the degradation cellulose and hemicellulose	5-62°C
yeasts	aerobic / anaerobic	rapid	• utilization of soluble substances (mainly sugars) in the acid phase • formation of alcohol under oxygen shortage	2-45°C
Aphyllophorales Basidiomycetes	aerobic	slow	• degradation of the ligno-cellulose-complex	2-45°C

A succession of mixed populations can be observed as well as temporally as spatially, due to the important temperature and oxygen gradients that build up in a compost heap; each population is suited to the environment produced by the previous one. Generally, heterotrophic organisms (those that utilize organic compounds as energy source) predominate in the whole composting process, but the presence of autotrophs, utilizing inorganic substances either present in the waste or produced by the heterotrophs, has been demonstrated, as well in the thermogenic phase (BEFFA *et al.*, 1996a) as in the maturation phase (MARILLEY, 1994).

A controversy exists whether inoculation of the starting material with thermophilic microorganisms is useful to speed up the composting process. Several authors (FINSTEIN *et al.*, 1986a; DE BERTOLDI *et al.*, 1983; GOLUEKE, 1977; FINSTEIN & MORRIS, 1975) take the view that such inocula have not proved beneficial. Spontaneous self-heating of gathered biodegradable material occurs if the starting conditions (sufficient nutrients, water, oxygen, and insulation) are met. Often, natural seeding with recycled material from the composting process (reject from screening, in the order of 10 %) happens, supplying a large number of microorganisms adapted to the composting process. Also, the fresh material is inoculated by contact with the various machines (shredder, front-end loader, turner) used in the course of the composting process. GRAY & BIDDLESTONE (1971b) however states that in the case of composting of specialized industrial wastes that contain only a low diversity of autochthonous microorganisms, the use of an adapted inoculum might be necessary.

1.5 COMPOSTING - A CHEMICAL PROCESS

Composting can also be regarded as a chemical process, in which the substances present initially in the biodegradable waste are transformed into chemically different ones. Of course, biological and chemical processes are linked, as the enzymes produced by the microflora mainly effect the chemical transformations. Purely chemical reactions (auto-oxidation of lipids, ammonia conjugation reactions, auto-oxidation of some carbohydrates, phenols and quinones, formation of carbon disulfide and dimethyl disulfide (DERIKX *et al.*, 1991) are minor compared to biological activity (MILLER *et al.*, 1989, citing NELL & WIECHERS, 1978).

The input of the process is biodegradable waste, consisting mainly of garden, park and kitchen waste, exceptionally of sewage sludge. Chemically, the main components of these substrates are cellulose, hemicelluloses and lignin, in the case of grass clippings or kitchen waste also free sugars, amino acids and fatty acids, and the polymers fats and proteins. The output under aerobic conditions is new bacterial cells, CO_2, H_2O, NH_3, H_2S and compost; under anaerobic conditions new bacterial cells, CO_2, H_2O, volatile fatty acids, alcohols, NH_3, H_2S, CH_4, H_2, CO and digested residue of material not converted under anaerobic conditions.

1.5.1 LIGNOCELLULOSE

Lignocelluloses, a complex of the polymers lignin, cellulose and hemicellulose, provide the principal carbon and energy substrate for microorganisms in the production of compost. Cellulose is a linear polymer of β(1→4) linked glucose units. Hemicellulose is the collective name of a group of branched heteropolysaccharides containing glucose, mannose, galactose, xylose and arabinose. The major plant hemicellulose is D-xylan, which has a backbone of poly-β(1→4)-xylan linked laterally to arabinose, glucoronic and arabinoglucoronic acid, mannan and galactan.

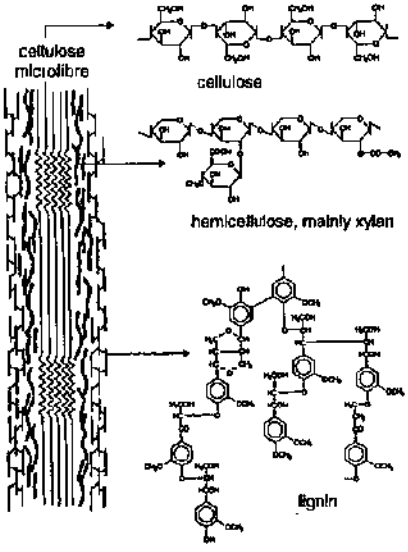

Figure 2: Schematic depiction of the lignin-cellulose complex of plant cell walls (from FRITSCHE, 1998).

Lignin is an aromatic polymer of phenylpropane units, based upon p-coumaryl, conferyl and sinapyl alcohols. Free radical copolymerisation of these alcohols produces a heterogeneous, cross-linked polymer. Because of its structural heterogeneity, lignin is particularly recalcitrant to microbial degradation

Wood is nearly all lignocellulose, straw more than 80 % (LYNCH, 1993). The plant cell wall consists of cellulose microfibres embedded in a matrix of lignin and hemicelluloses (FRITSCHE, 1998; Figure 2).

Lignin and hemicelluloses can be cross-linked . (LYNCH, 1993).

Free cellulose (approximately 50 % of the cellulosic fraction, accordingly to LYNCH, 1993) and hemicelluloses, e.g. the part not bound in the lignocellulose complex, are relatively easily attacked by many microorganisms (see Table 2).

However, the fraction protected by the lignin in the lignocellulose complex (about 40% of the cellulose contained in MSW; STUTZENBERGER *et al.*, 1970) is difficulty accessible to enzymatic attack. Lignin is primarily degraded by fungi (*Basidiomycetes*, especially *Aphyllophorales*), most active in the maturation phase, but also by some actinomycetes and Gram-negative bacteria (PAUL & CLARK, 1989, ZIMMERANN, 1990). The degradation process itself is a chain-reaction, triggered by a lignin-peroxidase, which creates reactive cation-radicals. Lignin degradation is always a co-metabolic process, e.g. the microorganisms can not utilize lignin as carbon and energy source, but need a growth substrate, generally cellulose and/or hemicelluloses. In fact, the degradation of lignin serves mainly to free hemicelluloses and cellulose from the lignocellulose complex, and to make them accessible to the microflora. Lignin degradation compounds (quinones and aromatic radicals) will recompose spontaneously (polycondensation) to form crenic, fulvic or humic acids. These substances, together with exopolysaccharides, peptidic chains and oxidized polyphenols produced by the microorganisms or stemming from the plant material, constitute the lignin-humic complex characteristic of a mature compost (LYNCH, 1993).

All polymeric substances have to be cleaved by enzymes produced by the degrading microflora in mono- or oligomers, in order to be taken up into the microbial cell. Composting is a solid process, e.g. microorganisms can only utilize those substrates they are in direct contact with, by cleaving of polymers with membrane-bound enzymes, and uptake of monomers. Otherwise, excretion of exoenzymes and uptake of monomers takes place in the water film or the mucilage that surrounds compost particles. Turning of compost is important to re-mix microorganisms and/or their enzymes and substrate. Also, by the mechanical action of the turner, clumps of compost and large wood pieces are broken up, and new surfaces are thus available for microbial attack.
The degradation of the monomers then happens like that of simple sugars, via the Krebs-cycle, yielding, under aerobic conditions, CO_2 and H_2O, under anaerobic conditions (fermentation) volatile fatty acids and alcohols, which serve as substrate for methanogenic organisms, and different gases (CO_2, CO, H_2). Intermediate fermentation metabolites are responsible for bad odors, and can be responsible for the phytotoxic action of composts produced under partially anaerobic conditions (MASSIANI & DOMEIZEL, 1996). Methane is a very potent "green-house" gas (HELLMANN *et al.*, 1997).

1.5.2 PROTEINS

Proteins are polymers of amino acids and play an important role in providing nitrogen for the synthesis of microbial cell components and enzymes necessary for the assimilation of the carbon substrate (MILLER, 1992). Microbial cells have a carbon-to-nitrogen (C:N) ratio of about 10:1 (GRAY &v BIDDLESTONE, 1971b). Starting materials for composting should have a C:N ratio between 25 and 35, taking into consideration that a large proportion (60-80 %) of the carbon is used for energy production (GRAY &V BIDDLESTONE, 1971b, citing ALEXANDER, 1961), and that part of the carbon is not available for degradation (KROGMANN, 1994). Higher C/N ratios slow down the degradation process because the microorganisms have to go through many life cycles, oxidizing off the excess carbon, until the C/N ratio is reduced (GRAY & BIDDLESTONE, 1971b). Low C/N ratios will lead to nitrogen losses in from of volatile ammoniac (FRICKE & VOGTMANN, 1994). MILLER (1993) attributed the nitrogen losses observed at higher temperatures to the fact that the thermophilic microorganisms lack the enzymes to degrade part of the carbonaceous substances, lowering thus the available C:N ratio.
Proteolysis, the cleavage of the polymer, and ammonification, the liberation of the amino group from the amino acid, can be carried out by a large number of microorganisms, and starts when the free sugars and amino acids present in the fresh biowaste are used up. Free ammonia (NH_3) is in a

pH and temperature dependent equilibrium with ammonium ions (NH_4^+): the higher both the pH and the temperature rise, the more NH_3 is formed. Free NH_3 is volatile, and can thus lead to N-losses. It is also very reactive, and can form stable products with organic matter (lignin, humic acids) (KROGMANN, 1994; MILLER, 1992). The production of alkaline substances during the protein degradation leads to a pH increase up to a pH of 9. Due to the incorporation of ammonium by the microorganisms and nitrification processes, the pH gets reduced in the maturation phase (FINSTEIN & MORRIS, 1975).
Nitrification (the formation of nitrate from ammonium via nitrite) can either be performed by autotrophic or by heterotrophic microorganisms. Autotrophic nitrification is inhibited by temperatures above 40°C (ALEXANDER, 1977) and high ammonia concentration, and takes thus only place during the maturation phase (KROGMANN, 1994; DE BERTOLDI & ZUCCONI, 1987).
Denitrification denotes the conversion of nitrate to N_2, leading to nitrogen losses from the compost. DE BERTOLDI & ZUCCONI (1987) and CASELLA & RUTILI (1990) mention the presence of denitrifiers in compost, belonging to the genera *Pseudomonas*, *Paracoccus* and *Bacillus*. This process takes only place under reduced oxygen partial pressure, and seems to happen only when enough soluble organic matter and water are available.
DE BERTOLDI & ZUCCONI (1987) also showed the presence of N_2-fixing bacteria (*Azotobacter*, *Enterobacter*, *Klebsiella*, *Bacillus*, *Clostridium*) in compost, especially during the maturation phase, when ammonia and nitrate concentration, low oxygen concentration and temperature were within the bounds permissive for their growth.
At the end of the composting process, most of the nitrogen is either bound in the biomass, or in difficultly degradable matter like lignin or humic substances (KEELING *et al.*, 1994), and only very little is available for plant nutrition in the form of ammonium or nitrate (KROGMANN, 1994).
During the aerobic degradation of sulfur groups containing amino acids, H_2S and other volatile sulfur compounds are liberated, contributing to the bad odors in the initial phase of composting (HAUG & ELLSWORTH, 1990). H_2S can also be formed under anoxic conditions, when sulfate is reduced. H_2S on the other hand can be oxidized by autotrophs (SCHLEGEL, 1985).

1.5.3 DECOMPOSITION RATE / MATURATION

The goal of composting is the production of a stabilized product that can be stored without further treatment, and can be applied to land without damage to crops. Degree of stabilization is synonymous with extent of decomposition, in that putrescible, phytotoxic material is decomposed through aerobic metabolism (MACGREGOR *et al.*, 1981). Composting at industrial level also aims at maximizing the rate of decomposition to reduce the facility space necessary, and to shorten the phase where odor problems could arise (FINSTEIN *et al.*, 1986b). Furthermore, the required maturity depends on the potential utilization: compost that is applied to fields, where it continues the stabilization process, needs to be less mature than compost used in potting mixes (CHEN & INBAR, 1993).
In order to monitor and control the composting process in view of the goals presented above, the extent of decomposition has to be measured. Most often, the organic content of compost samples or extracts thereof is determined. The following classes of organic substances are tested:

Organic matter (OM), often also denoted volatile solids (VS), is the percent of dry solids or dry weight (DW) lost by ignition at 550°C. Biological activity decreases the OM content of the initial substrate by converting organic waste derived C into CO_2. On the other hand, new OM is formed during the composting process (humic substances, microbial cells) (FINSTEIN *et al.*, 1986b). The meaningfulness of the OM content as a maturity indicator is limited, however, because it fails to discriminate among readily metabolizable, putrescible material (sugars, amino acids, fatty acids, etc.), less readily metabolizable material (cellulose, hemicelluloses), and organic material that is only metabolized to a minor degree during any reasonable composting period (lignin).

Total organic carbon (TOC). The test for TOC is basically the same as for OM, only CO_2 production by the combustion of the material is determined, and not ash content. The ratio of OM to TOC for MSW compost was found to be approximately 2.1:1. (FINSTEIN *et al.*, 1986b, citing STEAD & IRWIN, 1970). Therefore, total carbon is often not measured, but calculated by dividing the OM by 2.1. ANONYMOUS (1987b) proposed to use a factor of 2.3 when testing MSW, and 1.9 when testing compost, based of the measured composition of waste in Switzerland. GRAY *et al.* (1973) reported for a divisor of 1.8 an accuracy of 2-10 % compared to more accurate carbon analyses of MSW composts. Regarding the use of TOC as a maturity indicator, the same critics apply as for OM.

Water soluble organic carbon (WEOC). Most microorganisms in composts can only take up substances that are solubilized in water. Soluble substances such as sugars, amino acids, fatty acids, etc., are either present initially in the waste, or are obtained by the hydrolyzation of the polymers contained in the solid substrates by the enzymatic activity of the compost microflora. The solubles are either utilized immediately by the microorganisms for their metabolism and cell growth, or are accumulated in the water phase. As the composting process progresses, the soluble substances decrease. Various authors confirm the utility of WEOC as an indicator of compost maturity (CHEN & INBAR, 1993; GARCIA *et al.*, 1990). IANNOTTI *et al.* (1994) reported a fast decline of soluble organic C in the first 80 days of composting of MSW. The water extract of immature MWS compost consisted of sugars, phenolic substances, organic and amino acids, peptides and other easily biodegradable substances, while in the mature compost (6 months of composting), most of the soluble organic C was present as humic substances which were resistant to further decomposition. CHANYASAK & KUBOTA (1982) showed that with progression of the composting process, the proportion of large molecular weight compounds in the water extract increased, indicating the presence of humic substances.

CHEN & INBAR (1993) proposed to follow the change in soluble organic C spectroscopically at 465 nm (lower end of the visible spectrum) instead of determining organic C by wet oxidation. Because slopes for different kind of composts were different, a calibration curve should be established. LASARIDI & STENTIFORD (1996) found a significant correlation between absorbance at 465 nm and compost age for sludge compost, but not for poultry manure compost.

Biological oxygen demand (BOD). Tests for biological oxygen demand are based on aerobic microbial degradation of the readily bioavailable compounds. The standard 5-day BOD test used for sewage sludge has been adapted for compost suspensions: the oxygen decline was followed at 30°C for about 24 h, with inserted periods of aeration (LASARIDI & STENTIFORD, 1996). Another method consists in measuring the oxygen concentration in the air space over a solid compost sample placed in a sealed container (IANNOTTI *et al.*, 1994). After aeration during 16 h, the disappearance of O_2 is followed during 1 h at 37°C. Comparisons of the two methods showed that the respiration rates were highly correlated, but that the test in the compost suspension exhibited up to 6 times higher values (LASARIDI & STENTIFORD, 1996).

In the methods book for the analysis of compost published by the German Federal Compost Quality Assurance Organization (ANONYMOUS, 1994c), a method measuring the oxygen depletion in a respirometer at 20°C in a 6 h rhythm over 4 days is stipulated.

FORSTER *et al.* (1993) found a very good correlation between organic C and respiration rate in compost suspensions (mg CO_2/gDW·d^{-1}).

One problem encountered with such measurements is the inhomogeneity of the samples, the ideal test temperature and the standardization regarding the water content of the sample. Comparisons between different composts are only possible on the basis of OM, or better WEOC content of the samples.

C/N ratio. The C/N ratio is an important quality parameter when using compost as a soil amendment, because materials with a high C/N ratio can immobilize soil nitrogen by the ongoing decomposition of the carbonaceous substances once the compost has been applied to soil. The ratio decreases as composting progresses because of the conversion of organic C to CO_2 (FINSTEIN *et al.*, 1986b). At the same time, part of the nitrogen can be lost in form of NH_3.
C/N ratio can either be measured in the compost or in an aqueous extract. Normally, a C/N ratio of less than 20 in mature compost is thought to be desirable. However, C/N values measured in sufficiently stabilized composts varied between 5 and 20, depending on the type of raw material. The C/N ratio in the water extract, on the other hand, showed to be a reliable indicator of compost maturity, as it reached, independently of the composition of the starting material, a final value of C/N_{org} of 5-6 (CHANYASAK & KUBOTA, 1981). GARCIA *et al.* (1990) also found a good correlation between water-soluble C/N_{tot} ratio and the degree of lightness of the compost measured with a color analyzer, except for composts with a low (< 15) initial C/N ratio.

Others. The cation exchange capacity (CEC) of composts is related to the amount of humic substances (FORSTER *et al.*, 1993). INBAR *et al.* (1990) showed a quite parallel evolution of CEC and humic material in cattle manure compost. In MSW compost, however, the CEC of the humic substances can vary widely, due to the blocking of their exchange sites by complexing ions (MATHUR *et al.*, 1993).
The German Federal Compost Quality Assurance Organization (ANONYMOUS, 1994c) has developed a test for the determination of the degree of rotting (Rottegrad) based on self-heating of the compost in an open Dewar vessel (1.5 l). Temperature is measured in the lower third of the vessel, for at least 5 days, and the maximum temperature recorded. The rotting degrees are assigned I (T_{max} 60-70°C) to V (T_{max} 20-30°C). Compost with rotting degrees II and III is designated as fresh compost, such with a rotting degree IV and V as finished compost.
The ultimate evaluation of compost has to be based on bioassays, e.g. the germination of seeds or growth of plants (cress, barley, ryegrass, tomato or lettuce seedlings, etc.) in compost/soil mixtures.
Considering maturation of compost, it has always to be taken in mind that the end use dictates the level to which the product must be stabilized. For example if compost is to be used in potting mixes, over 50 % of the organic matter has to be degraded (KEENER *et al.*, 1993), while in composts destined for agriculture, a first stabilization, attained normally after a few weeks of composting, is sufficient.

1.5.4 PH

The pH value of composting material drops in association with organic acid formation at the early stage of composting (NAKASAKI *et al.*, 1993), due to either aerobic or anaerobic processes (GOLUEKE, 1977). In the course of composting, these acids are either further degraded or volatilized. At the same time, ammonium is formed by the hydrolysis and desamination of proteins (ammonification). These two processes combined lead to a rise of the pH (see Figure 1). The pH of fresh biowaste depends on its composition, but also on the duration of storage before delivery to the composting site (KROGMANN, 1994).
Delay of the decomposition of organic matter at the early stages of composting when the pH is low may be caused by an inhibition of microbial protein decomposition in the raw material; optimal protein degradation being observed between pH 7 and 8 (NAKASAKI *et al.*, 1993).

1.6 COMPOSTING - A PHYSICAL PROCESS

Composting is also a physical process, in that factors like temperature, humidity, airflow or porosity affect the microbial community, and therefore the degradation process.

1.6.1 STRUCTURE

The compost matrix is a network of solid particles forming pores of different sizes. Structure is a function of the stiffness of the particles, and their ability to maintain this stiffness also at high water content. It is influenced by the nature of the biodegradable waste. Porosity describes the volume of the free air space, expressed as percentage of the total volume (GOBAT *et al.*, 1998). It is determined by the shape, size and structure of the particles, and the height of the compost pile, as self loading leads to compression of the material at the base (HARPER *et al.*, 1992). The pores are filled with air, water or both (KROGMANN, 1994). For an adequate oxygen supply, the free air pore volume is important. SCHUCHARDT (1990) cited an optimal structure if the free air pore volume is > 50 %, and JERIS & REGAN (1973) suggested that a minimum free air space of 30 % should be maintained. Water, necessary on the one hand for the nutrient uptake by the microorganisms, hinders on the other hand the diffusion of oxygen into the pores by an increase of the aqueous film thickness around individual particles, and by filling the small pores with water by capillary action (see also Chapter 1.6.4). Shredding the material provides an increase of surfaces for microbial attack, but the particles have to be still large enough to maintain a certain porosity (KROGMANN, 1994). Turning of the compost effects a loosening of the material, thereby decreasing bulk density (MICHEL *et al.*, 1996). With ongoing degradation of organic matter, and mechanical size reduction of woody particles, the porosity of the compost decreases.

1.6.2 TEMPERATURE

Temperature is a key factor in the composting process. It determines the growth rate, metabolic activity and type of community structure of the compost organisms. Temperature is also the main factor influencing the survival of pathogens present in compost (ANDERSON & SMITH, 1987). High composting temperatures also increase degradation, considering that for a given enzyme, activity rates double with a 10°C temperature increase, until the inactivation temperature is reached (MILLER, 1992). Usually, enzymes are more thermostable than the organisms that produce them (MADIGAN *et al.*, 1997).

Temperature increases as a function of **metabolic heat evolution** of the degrading microflora and **heat conservation** due to the naturally insulating property of organic waste (MILLER, 1992; DE BERTOLDI & ZUCCONI, 1987). Heat storage is an important factor during the initial stage of rising temperature. It is mostly determined by water because of the high specific heat[3] of H_2O (MILLER, 1992). Heat accumulation leads to high temperatures that severely inhibit further microbial activity (FINSTEIN & MORRIS, 1975). The absolute maximum temperature achievable in composts is 82°C, at which point biological activity and metabolic heat evolution cease (FERMOR *et al.*, 1979; SULER & FINSTEIN, 1977).

[3] The heat capacity (C) of a given mass of a substance is the amount of heat required to raise the temperature of the mass by 1°C. Specific heat is the heat capacity of one gram of a substance. For water: C = 4.18 J/g K^{-1} (MORTIMER, 1977).

The temperature that can be measured at any point in a compost heap is a function of the rates of heat evolution and heat transfer (= distribution of heat within the composting mass, and its removal).

Metabolic heat evolution is affected by the following factors:

- the chemical composition of starting material and thus the nutrient content and its availability for microbial metabolisms (GRAY & BIDDLESTONE, 1971a)
- the moisture content (see Chapter 1.6.4)
- the compost temperature, which effects a feedback control on the activity of different groups of compost microorganisms (growth temperature ranges of the different groups see Table 2)
- the turning frequency: stimulation of the microbial activity by redistribution of nutrients and oxygen
- the oxygen input, because only aerobic metabolisms generate large amounts of heat
- the particle size; size reduction of particles by shredding leads on the one hand to an enhanced substrate availability through increase of the surface (GRAY & BIDDLESTONE, 1971a), on the other hand to a reduction of free air space, and therefore to lower rates of activity because of oxygen transfer limitations (MILLER *et al.*, 1983; see also Chapter 1.6.3)

Heat transfer mechanisms are radiation[4] (a minor factor that can be ignored), conduction[5], convection[6], evaporative cooling[7] and sensible heating.

Conduction in the bulk compost mass is low, between that of wood (0.17 $W \cdot m^{-1} \cdot °C^{-1}$) and that of water (0.56 $W \cdot m^{-1} \cdot °C^{-1}$) (MILLER, 1992). Also, the air-filled pores inhibit conduction. In small composting masses with a large surface area/volume ratio, however, conduction can be a significant factor. Experiments by MICHEL *et al.* (1996) showed lower temperatures in a windrow (4 m wide and 1.2-1.5 m high, with a surface area to volume ratio of 1.6-1.9 m^2/m^3) compared to a pile (radius of 4 m, height of 3 m, area/volume ratio 1 m^2/m^3).

Moving air in a compost heap, either produced by artificial aeration or by convection, or a combination of both, leads to heat removal. About 90 % of the heat is removed by evaporative cooling because of the high heat of evaporation of H_2O; the remaining 10 % by sensible heating of the air passing through the compost (MACGREGOR *et al.*, 1981). Turning of the compost also leads to significant heat removal through evaporative cooling (MILLER, 1992).

Controlled removal of heat can only be achieved by artificial aeration, often executed as temperature feedback-controlled ventilation (FINSTEIN, 1992). The often-recommended action of turning to reduce temperature certainly removes heat, but, at least in the active phase of the composting process, stimulates microbial activity, and leads to more heat production. Measures to control compost temperature by inhibiting heat evolution are not advisable because they would interfere with optimal microbial activity, slowing thus the whole composting process down.

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[4] Radiation: emission of energy (in this case heat) in form of waves or particles (ANONYMOUS, 1987)

[5] Conduction: the transfer of heat between two parts of a stationary system, caused by a temperature difference between the parts (ANONYMOUS, 1987)

[6] Convection denotes a heat-transfer mechanism driven by buoancy differences caused by differential temperatures in a fluid media (air) (MILLER, 1992).

[7] Evaporative cooling: the energy required to evaporate water from compost is transferred from the composting mass, leading to its temperature decrease. The heat of vaporization of water, e.g. the energy necessary to vaporize 1 mol of water, is 40.7 kJ/mol, or 2260 J/g (MORTIMER, 1977).

By the moving air in a compost heap, either due to convection (in the case of open-air windrows) or artificial aeration (in box or trench composting or in a closed bioreactor), a **temperature gradient** builds up (HOGAN *et al.*, 1989; KUTER *et al.*, 1985), according to the physical phenomena explained:

- sensible temperature increase along the airflow pathway
- influx of cold and dry air at the lower part of the windrow due to convection that produces an upwardly curved convective pathway ("chimney effect"), or at the point of air entry of the ventilation system
- heat conduction at the surface of the heap
- less important heat storage, due to dryer material at the surface, or in the case of artificial aeration, at the point of air entry

Figure 3 depicts the possible zones of heat production and heat removal in an open-air compost windrow, as inferred on the basis of extensive temperature measurements in such a heap (see Chapter 3.2.1). The coldest zones are in the middle of the heap, at the base, where little convection occurs, and where free air space is limited, due to the compaction of the material. Also in the lower part of the windrow, but more towards the outer edges, are zones with maximum heat evolution: due to the inflowing fresh air and thus good oxygenation, microbial activity is maximal. At the apex of the heap, hot and humid air leaves the windrow, which can be observed by a small stripe of humid compost material, due to condensation. Heat removal also happens from the sides of the windrow by conduction.

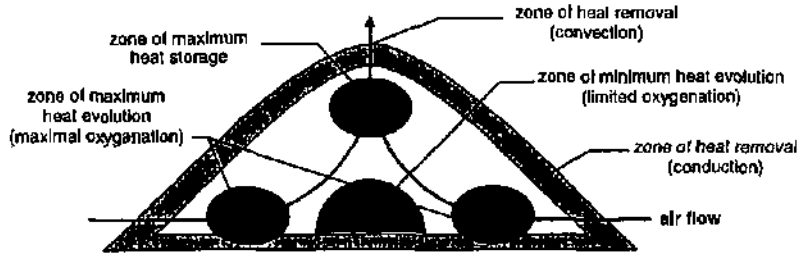


Figure 3: Zones of heat production and heat removal in a compost windrow.

Many authors report this temperature gradient (FERNANDES *et al.*, 1994; INBAR *et al.*, 1990; STENTIFORD *et al.*, 1985; IACOBONI, 1983) and comment on the insufficient pathogen inactivation in the cooler zones. However, no investigations were made about the uneven biological activity, and their effect on final compost quality (MILLER *et al.*, 1991).

Due to the temperature gradient, at any moment all of the three optima mentioned by STENTIFORD (1996) are met in one and the same compost heap: > 55-60°C for maximum sanitization, 45-55°C for maximum biodegradation, and < 40°C for maximum microbial diversity. If a sufficient temperature gradient exists, it is thus not necessary to fix a temperature ceiling of 55-65°C, as often recommended (KUTER *et al.*, 1985; MCKINLEY & VESTAL, 1984; MACGREGOR *et al.*, 1981), based on the works of FINSTEIN & MORRIS (1975). The latter stated that compost temperatures above 65°C lead to "microbial suicide. Their findings were established on the basis of experiments carried out in static bench-scale systems, with homogenous temperatures in the whole composting mass, not representing real situations encountered in even well insulated large-scale systems.

Own experiments at our laboratory with a bench-scale composter (14 liters) showed that when no temperature control was applied, and > 70°C were reached, the composting process came to an end already after 24 hours. Hypothesis is that at these homogeneously high temperatures, no degradation of polymers could happen, and the process stopped as soon as the soluble substances were used up (PIERRE-FRANÇOIS LYON, personal communication). SCHULZE (1962) succeeded to carry out long-term experiments in a bench-scale system by using a continuously fed composting reactor with a constant mixing of the mass. In this way, easily degradable material was constantly supplied, and maximal activity of degradation was seen at approx. 70°C.
The temperature-time function is the major factor influencing the death of pathogens during composting (HAUG, 1993; ANDERSON & SMITH, 1987), even if other mechanisms can lead to the elimination or reduction of pathogens: concurrence by saprophytic microorganisms (antagonism), production of antibiotics (KROGMANN, 1994; HAUG, 1993), or predation by protozoa and microinvertebrates (NESSI, 1994).

Thermohygienization, e.g. the reduction or elimination of potential pathogens by the high temperatures of the thermogenic phase, is very important for industrial scale composting, because the maturation phase, during which antagonism and antibiotic production mainly occur, are normally very short.
The thermoresistance of microorganisms is usually tested in the laboratory, with reference strains, and often in a liquid environment. Under these conditions, most of the mesophilic organisms are destroyed in a short time at temperatures exceeding 55-60°C. However, inactivation under field conditions may be much different from that observed in the laboratory due to clumping of solids, irregular temperature distribution, incomplete mixing and microorganism regrowth (HAY, 1996). FILIP (1978) found that the association of bacteria with compost particles (humic acids, bentonite) in a compost suspension allowed them to tolerate greater heat stress. The release of some metal cations from colloids and the protecting effect of colloidal envelope around the cells may decrease the susceptibility of compost microorganisms to heat.

1.6.3 AERATION / O_2 AND CO_2 CONCENTRATIONS

Composting is by definition an aerobic[8] process: O_2 has to be supplied to the compost in order to compensate for the amount used up by the degrading microorganisms. The O_2 status of a composting mass is therefore determined by its rates of utilization and supply (MILLER, 1992). High or low rates of these two factors can result in similar O_2 levels (MACGREGOR *et al.*, 1981). Air requirements of the microorganisms are dependent on the type of waste (nutrients; structure, which influences the free air space), process temperature, stage of the process and process conditions (moisture content, compaction) (STENTIFORD, 1996). Besides providing oxygen, the provision of air also removes waste gases like CO_2 and NH_3, excess moisture and heat (LETON & STENTIFORD, 1990).

[8] aerobic = needing oxygen for its existence; anaerobic = not needing oxygen for existence (COLLIN, 1992)

Anaerobiosis[9] has to be prevented because it leads to a smaller rate of heat evolution and the production of undesirable intermediate metabolites that are the cause of bad odors (propionic and butyric acid, sulfur compounds and ammonia), phytotoxicity, and the production of greenhouse gases (methane, N_2O). Reference is often made to conditions being oxic[10] or anoxic[11], but a more realistic concept is that of a gradient between highly oxidizing and highly reducing conditions: oxic and anoxic microenvironments can coexist with close proximity (MILLER, 1992). Measurements only allow detecting interstitial gas concentrations, while variations in the microenvironments are most probable. Obviously, oxygen utilization rates strongly affect the apparent interstitial oxygen concentration (MILLER *et al.*, 1989).

In unvented composting systems, air is supplied to the compost through convection-induced mass transfer ("chimney effect", see Figure 3) and gaseous diffusion driven by concentration differences between the interior of the heap and ambient air. Convection will bring fresh, oxygen-rich air into the large pores of the pile, while diffusion from the interstitial atmosphere through the small pores and the water film surrounding the compost particles is responsible for the oxygen supply of the microorganisms. It is stated that oxygen will not limit composting if 10 % interstitial oxygen is present (SULIER & FINSTEIN, 1977). Thick water films in overly hydrated composts (see also Chapter 1.6.4), and small pore sizes will hinder the oxygen diffusion.

Artificially, air can be introduced into the compost by the action of blowers, who either push (positive pressure) or suck (negative pressure) the air through the compost. Experiments carried out by STENTIFORD *et al.* (1985) showed that negative pressure (sucking) aeration normally produced high core temperatures but relatively low peripheral temperatures, whereas the reverse applied to the positive pressure (blowing) system. In the view of maximal pathogen inactivation, the author is of the opinion that a hybrid system with an initial negative pressure would be suited best. In the forced-pressure direction, heat and vapor are removed more efficiently, but vacuum-induced direction is still used widely because of a better odor control (FINSTEIN *et al.*, 1986a).
Air is also introduced into the compost during turning, but the oxygenation is only momentary, because the turning normally leads to an enhanced microbial activity, and thus to a faster O_2 utilization (GRAY & BIDDLESTONE, 1971b). The beneficial effect of windrow turning on aeration is most probably a loosening of the composting mass, bringing about an increase in free air space. The combination of artificial aeration and turning would be ideal (STENTIFORD, 1996), combining the advantages of a maintenance of oxic conditions with the re-mixing of the composting material in the view of homogenous temperature, nutrient and moisture distribution, and the breaking up of preferential air channels which lead to uneven aeration (MILLER, 1992).

Composting windrows without forced aeration tend to vary greatly, both temporally and spatially, in oxygen concentration (MILLER *et al.*, 1991). Different authors (MILLER *et al.*, 1989; RANDLE & FLEGG, 1978) have demonstrated variations between the inner parts of a compost windrow, and the outer layers. But also aerated systems can show oxygen gradients, although to a lesser extent; DE BERTOLDI *et al.* (1985) stated that uniform oxygenation of heaps that exceed a height of 4-5 m can not be obtained: the lower part of the mass gets over-ventilated with excessive cooling and drying, and the upper layers are insufficiently aerated, because the air, while passing through the composing mass, looses oxygen.

[9] aerobiosis = biological activity which occurs in the presence of oxygen; anaerobiosis = biological activity which occurs without the presence of oxygen (COLLIN, 1992)
[10] oxic = oxygen-containing environment, usually highly reducing (MADIGAN *et al.*, 1997
[11] anoxic = oxygen-free environment, frequently highly oxidizing (MADIGAN *et al.*, 1997

In experiments with naturally aerated mushroom composting stacks (2.2 m wide, 1.8 m high), MILLER *et al.* (1989) found the following interesting correlation between temperature and oxygen (Table 3):

Table 3: Relationship between temperature and oxygen concentration in a naturally aerated compost (from MILLER et al., 1989).

temperature	oxygen concentration
at the beginning of the process	
< 65°C	• low (< 2 %) because of high microbial activity • rate of oxygen utilization exceeding the rate of oxygen supply
> 65°C	• medium (2-10 %) because of declining microbial activity due to inhibitively high temperatures • rate of oxygen utilization below the rate of oxygen supply
20-60°C	• high (15-20°%) in zones at the surface, because of gas exchange with the surrounding air
towards the end of the process	
< 65°C	• high (10-15 %) because of low microbial activity due to substrate depletion or drying • rate of oxygen utilization below the rate of oxygen supply

The **control of aeration** in artificially oxygenated systems can be exerted in two ways: by either measuring the **temperature** or the **oxygen content** in the compost or in the outlet air. When temperature is controlled at 60°C, as suggested by the group of Finstein (FINSTEIN *et al.*, 1986b; MACGREGOR *et al.*, 1981) to be in the range of maximal decomposition rate, thorough aeration is believed to be automatically ensured, because about 9 times more air is necessary to remove heat than to supply enough oxygen to maintain aerobic conditions (FINSTEIN & MORRIS, 1975). This was contested by STENTIFORD *et al.* (1985) who showed that in the first phase of the composting process, temperature controlled aeration did not supply enough oxygen, and that a combined system with temperature and oxygen control was required. In any system where the temperature is controlled at a level assuring maximal decomposition (55-60°C), the strong aeration leads to a high water loss of the system, making finally dryness the rate-limiting factor (FINSTEIN *et al.*, 1983).

In any system (windrows or boxes) the point where temperature or oxygen content is measured in the compost heap is of big importance, because of the large temperature and oxygen gradients. A system proposed by STENTIFORD *et al.* (1985) used several thermocouples to measure and control temperature. The evolution of temperature was also monitored, to determine the phase of composting (self heating, temperature plateau under feedback control, cooling due to substrate depletion), and to control temperature accordingly

Air is normally not applied continuously, but in short bursts to allow a better distribution of oxygen and temperature to all portions of the pile (HIGGINS, 1982). There seems to be, however, no "standard aeration cycle", and no literature exists where different modes of aeration are compared. The following aeration regimes were applied:

- LETON & STENTIFORD (1990) reported for sucking aeration of a sludge/woodchips mixture a cycle of 6 minutes operation and 18 minutes pause. He proposed for temperature controlled composting variable aeration times up to 15 minutes in a 20 minute cycle.
- BIDDLESTONE & GRAY (1987a) applied a regime of 7 minutes aeration every hour for the composting of vegetable wastes mixed with straw.
- FINSTEIN *et al.* (1986b) used in his experiments with temperature controlled sludge composting a *baseline blowing aeration of 1.5 minutes every 15 minutes. Only once the set point of the control* temperature was reached did continuous aeration start.

- PEREIRA NETO *et al.* (1985) employed for the composting of MSW mixed with sewage sludge an aeration of 3-10 min. every 15-20 min.
- DE BERTOLDI *et al.* (1982) reported for the composting of MWS mixed with sewage sludge a cycle of 40 seconds suction every 13 minutes.
- HIGGINS (1982) recommended for negative pressure composting on a fixed aeration schedule of 15 minutes aeration and 15 minutes pause.

Comparisons of amount of air used for aeration among different systems from the literature are not easy: compost volume instead of weight is used, and it is often not clear if the total amount of air is indicated, taking into account the moments of the cycle where no aeration occurs, or if the amounts given are that of a single aeration event. HAUG (1986) reported peak demands of 100-150 $m^3/h \cdot t_{fresh\ weight}$ in a temperature controlled (60-65°C) blowing systems. HIGGINS (1982) indicated airflow of 10-15 $m^3/h \cdot t_{fresh\ weight}$ for a fixed schedule sucking operation aiming at maintaining interstitial oxygen content of 5 %.
Only estimations exist about the air supplied by natural aeration. OP DEN CAMP (1987), cited by MILLER *et al.* (1989), reported convective mass flow in naturally aerated mushroom compost stacks of 6-8 $m^3/m^2 \cdot h$, corresponding to 7.5-10 $m^3/h \cdot t_{fresh\ weight}$ (assuming a stack height of 2 m, and a density of 0.4 t/m^3).

The measurements of O_2 consumption or CO_2 production, respectively, either directly in the composting mass after aeration, or in the outlet air in a closed system, can also be used to determine the rate of microbial activity, and therefore the decomposition rate (FINSTEIN *et al.*, 1986b).

1.6.4 HUMIDITY / A_W

Growth of microorganisms is only possible in an aqueous solution, e.g. in the water film that envelops the compost particles. Also, uptake of nutrients takes mainly place if they are dissolved in water (KROGMANN, 1994; MILLER, 1992).
But not only a water deficiency, also a water surplus can impair the composting process, in that the water film surrounding the compost particles increases. The oxygen diffusion through water is 10'000 times slower than through air, explaining why a too high initial moisture content leads to anoxic conditions (MILLER, 1989). The maximum water content a material can hold (up to full capillary saturation) is dependent on the material itself: FERNANDES *et al.* (1994) reported that saturation with water occurred in straw at 77 % water content, in peat at 85 %. KUTZNER & JÄGER (1994) recommended a maximum water content for composting of 74-90 % for wood (sawdust, mulch, bark), 75-85 % for straw, 55-65 % for paper and MSW, and 50-55 % for biowaste (kitchen waste, grass clippings). But in MSW already at a moisture content of 60-65 % the small air pores become water filled (matrix effect due to capillarity), creating water filled zones between particles (MILLER, 1989).
The initial moisture content of mixed garden and kitchen waste ranges from 60-70 % (KUTER *et al.*, 1985).

Water is produced in the course of the composting process by the metabolic breakdown of organic matter, but this is largely compensated by the loss of water through evaporation, caused by the self-heating of the material (FINSTEIN *et al.*, 1986a). Temperature-controlled ventilation causes an intensive drying of the composting material, because heat is removed mostly in the form of the latent heat of vaporization of water (HOGAN *et al.*, 1989). The composting process should operate with moisture contents in the 40-60 % band (STENTIFORD, 1996), the addition of water during the process is therefore often necessary.

This is not possible without re-mixing the material at the same time (KEENER *et al.*, 1994). At the end of the composting process, a water content of 40-50°% should be reached, in order to facilitate screening.

Regarding the minimal water content below which a marked inhibition of microbiological activity occurs different values can be found in the literature: STENTIFORD (1996) stated 30-35 %, KEENER *et al.* (1994) observed reduced activities already at 35-40 % moisture, and BIDLINGMAIER (1994) stipulated a minimal water content during the heat phase of 40 %. In the publication of the U.S. Environmental Protection Agency Office of Solid Waste and Emergency Response (ANONYMOUS, 1993), 40-45 % moisture are indicated as slowing down the process, while below 20 %, no microbial activity is possible any more.
It has to be mentioned, though, that not the water content *per se*, but the "free" water available to the microorganisms, e.g. water in the biofilm surrounding the compost particles and capillary water, but not the osmotically bound water, is of importance. The water activity[12] (a_w), depending on the chemical and physical properties of a substrate and its water content, expresses the amount of water at the disposition of the microflora: most bacteria require an a_w value of more than 0.98, while fungi tolerate generally a lower a_w down to 0.80 (SCHLEGEL, 1985). Xerophilic strains are still growing at an a_w of 0.75 (REISS, 1986). MILLER (1989) proposed not to measure the a_w, which is quite insensitive in moist conditions, but the matric water potential, which describes the association of water with a matrix, based on physical attraction (energy with which water is held within capillaries and on surfaces). He reports a matric potential of -10 kPa at the beginning of the composting process (corresponding to 65 % water content), going down to -90 kPa towards the end, when the water content had been reduced to 25 %.

Table 4: Water content (% of FW) and a_w of source separated biowaste as a function of composting time. Range of 3 samples (NIELSEN et al., 1997b).

week	water content	a_w
0	56.6-59.2 %	0.98-0.99
1	53.6-57.4 %	0.97-0.98
5	55.2-58.5 %	0.98
9	48.5-52.9 %	0.98
11	41.3-49.4 %	0.97-0.98

Table 5: Water content (% of FW) and a_w of different substrates / raw materials for composting.

substrate	water content	a_w	reference
hay or grain	13 %	0.65	LYNCH & HOBBIE, 1988
	25 %	0.93	
	35 %	0.99	
	40 %	1.00	
hay	< 30 %	< 0.96	LACEY, 1994
	30-35 %	0.96-1.00	
	> 35 %	1.00	
shredded newspaper	27 %	0.94	KANEKO & FUJITA, 1988

Only one reference was found about the a_w of compost (Table 4), but several investigations were made about the degree of drying necessary to prevent self-heating of hay or straw (Table 5).

The data from the biowaste compost show that a water content above 40 % provides a water activity largely sufficient for microbial activity for composting (around 0.94; KANEKO & FUJITA, 1988). a_w values at 40 % water content are slightly lower than in hay or grain, most probably due the humic substances produced during the composting process, which can hold a great amount of water. The minimal water content of biowaste providing an a_w sufficient for bacterial growth would thus be higher than with straw or hay, probably in the order of 30

[12] the ratio of the partial water vapor pressure over a substance or a solution compared to that of pure water

Rapid drying to moisture levels less than those mentioned above can produce an apparent end to the initial phase of composting, when in fact the process is stopped prematurely due to desiccation. This can cause difficulties further down the line when the material is rewet: uncontrolled biodegradation takes place, often under anoxic conditions, with the associated odor problems (STENTIFORD, 1996).

YEAGER & WARD (1981) found that potentially pathogenic bacteria in dried sludge were extremely resistant to inactivation, and that regrowth from such material occurred if it was rewetted.

FRICKE (1988) demonstrated in open-air windrows a humidity gradient similar to that of temperature and oxygen. After 2 weeks of composting, the top and the base of the windrow were much wetter (55 % H_2O) than the center or the lateral surfaces (45 % H_2O). The top was more humid because of condensation, the bottom because of the pressure of the material. In ventilated systems, the material tends to dry at the point of the air entry (LETON & STENTIFORD, 1990).

1.6.5 MIXING / TURNING

Turning of compost heap mixes the compost materials, increases porosity, promotes drying through release of water vapor, and exposes the compost mass to high interior temperatures so that adequate pathogen destruction occurs (HAY & KUCHENRITHER, 1990). The widespread belief that turning oxygenates compost is only partially correct. Own measurements showed that the oxygen introduced into the compost by turning was used up in less than one hour in the thermogenic phase of the process (TRELLO BEPPA, personal communication). This can be explained by the activation of the microflora by the mixing of the compost material, redistributing microorganisms, exoenzymes produced by them, nutrients and water, as well as augmenting the surface for microbial attack by breaking apart clumps of material or large wood pieces. Also, the increased porosity and the drying effect have a beneficial effect on aeration, leading to increased microbial activity. Although many authors endorse the opinion that turning accelerates the composting process (ANONYMOUS, 1993; SCHUCHARDT, 1990) astonishingly little scientific information is available about turning machines, their mixing efficiency and the effect of turning frequencies. SCHUCHARDT (1990) gives an overview of the compost turners available at the moment of his publication.

HAY & KUCHENRITHER (1990) proposed that the total number of turnings per cycle should be based on the size of the sub-lethal zone (< 55°C) and the desired degree of disinfection. STENTIFORD (1996) objected that with nutrient rich wastes such as the organic fraction of MSW, cycles based on temperature measurements (55-60°C core temperature) would lead to very frequent turnings in the early stages of the composting process that would not be economically viable. He proposed a modified turning sequence frequency, starting with a frequency of one turning each 3 days and extending later to one turning each 5-7 days. Because in aerated systems, water becomes quickly the rate-limiting factor, KEENER *et al.* (1994) suggested to base the remix frequency on moisture control. The same authors also stressed the importance of mixing for aerated systems, to prevent the formation of preferential air channels witch interfere with a homogenous aeration of the whole composting mass.
Research by IACOBONI (1983), cited by HAY & KUCHENRITHER (1990) had shown that a turning frequency in open-air windrows of 3 times per week provided a satisfactory pathogen kill and ensured adequate drying rates.
HAY & KUCHENRITHER (1990) reported that windrows that were turned 12 times initially did not achieve a higher temperature than windrows turned only 2 times.

Results obtained by MICHEL *et al.* (1996) about the effect of the turning frequency on yard trimming composting showed no big difference between mean temperature and oxygenation in a windrow turned every 4 days with a specialized turning machine, compared to one turned once a month. He found, however, differences in moisture loss, in that the more frequently turned windrow dried faster, and in bulk density (kg compost/m^3), which increased faster under a high turning regime. This influenced reject during screening (10 % by screening at 10 mm for the frequently turned windrow, but 25 % for the monthly turned one), which is an important factor when looking at the throughput of a composting installation. Furthermore, composting time could be reduced by the frequent turning to almost half (60-80 days instead of 120-150).
KUHN *et al.* (1995) compared windrows that were turned twice a week with a special turning machine with such that were only turned every 2-3 months. He found that the former reached a similar maturity (measured as self-heating capacity) in 7.5 weeks than the latter after during 110 weeks of composting.
HELLMANN *et al.* (1997) reported that neither trace gas (N_2O and CH_4) emissions nor microbial diversity were influenced by the turning frequency (twice a week versus once in 3 weeks).
HELM (1995) investigated the influence of the turning frequency (1, 3, 7 or 28 days) on the temperature, dry weight, CO_2 concentration, TOC in the air, pH, OM and bulk density evolution during the composting of mixed garden and kitchen waste. He found for the windrows treated with a high turning frequency less emission of bad odors, a more rapid pH increase, an accelerated degradation (tested by auto-heating in a Dewar container), and a faster increase in bulk density.
SCHUCHARDT (1990) observed an increased drying in an open-air windrow that was turned with a special windrow turner twice a week, compared to one that was turned once a week, as well as a slightly faster reduction of the organic matter content in the former. The determination of temperature zones (> 50°C and > 60°C) lead to the statement that several turnings in the first 2 to 3 weeks of composting with core temperatures > 60°C assured that all material from the cooler outer and lower zones were transferred to the middle of the pile, undergoing there thermohygienization. He did not consider, though, that the hygienized material could be reinfected when it got transported from the core again to the outer layers.
HAUG (1993) analyzed with a mathematical model, based on the fraction of a composting heap having a temperature sufficient to inactivate pathogenic microorganisms, the number of turnings necessary to reduce the number of pathogens from 10^7 cfu to 10^0 cfu. He found that if 80 % of the heap reached high temperatures, 11 turnings were necessary, in the case of only 50 % hot material, 23 turnings. The model will be discussed in more detail in Chapter 3.2.1.3B.

Critics about windrow turning concerned the only periodical oxygenation, the space needed (either beside the windrow when they are displaced by turning, or in general, when the dimensions of turners restrict the pile height), the disturbance of fungal hyphae, the recontamination with pathogens, and the dispersion of *AF* spores (DE BERTOLDI *et al.*, 1985 and 1982; PEREIRA NETO *et al.*, 1985, MILLNER *et al.*, 1980). Also, release of bad odors during turning were evoked (ANONYMOUS, 1993), even if the same publication stated that turnings were necessary to provide aeration. GRAY & BIDDLESTONE (1971b) cautioned against too much agitation to prevent excessive heat and moisture loss, and the shearing of fungal and actinomycetes mycelium. HAY & KUCHENRITHER (1990) also counseled to reduce turning frequency in the later stages of composting, in order not to cause a too rapid temperature decline.

1.6.6 GEOMETRY OF WINDROW / HEAP

Windrow size markedly influences the composting process: the heat liberated by the thermophilic decomposition must exceed the heat lost through the exposed surface. Increasing windrow size greatly decreases the rate of heat loss. HAY & KUCHENRITHER (1990) determined that for each 0.5 m^2 increase in cross-sectional area, there was a 1.2°C increase in temperature. FINSTEIN & MILLER (1985) proposed a maximum height of 2 m for a temperature-controlled aerated static-pile, in order to restrict the temperature to a upper ceiling of 60°C, having determined an upward temperature gradient of 23°C per meter.
DAS & KEENER (1995) examined the compaction of the compost material by its own weight, and the resulting resistance to airflow in compost materials. This concluded that with moist material, e.g. 60 % water content, common in starting material, a height of 2.5 m should not be exceeded, otherwise the free air space gets drastically reduced, leading to a loss of free air space, and therefore to anoxic conditions. Drier material (below 55 % moisture) was much less subject to compaction due to its lower density and better mechanical resistance, meaning that in the later stages of composting, e.g. during curing, higher heaps can be formed. Compost heap size is also a function of the turning machine used (MICHEL & REDDY, 1996).

1.7 TYPE OF SYSTEMS

Composting can be carried out at very different levels and in various degrees of complexity: from the simple «dump» in the backyard to fully automated box composting. In a publication about "state of the art" of composting in Germany, published in 1994, 48 different systems were mentioned (WIEMER & KERN, 1994). Further descriptions of installations can be found with TARDY & BECK (1996); ANONYMOUS (1995a); THOMÉ-KOZMIENSKY (1995); BIDLINGMAIER (1994); ANONYMOUS, (1993); ANDERSON & SMITH (1987).

Independent of the type of installation used, the composting process always proceeds in the same manner, as depicted in Figure 4. The control for contamination with non-biodegradable materials is either carried out visually, or automatically (removing of ferrous materials by a magnet, and possibly of plastic in a wind sifter).

During the actual active composting, often also called the rotting phase, the process is controlled by regular turnings, aeration, and watering. However, the curing or maturation stage, characterized by slow rate of substrate transformations (MILLER, 1992), is often totally uncontrolled. Once the compost is considered ready for use, it should be stable, e.g. storage should not bring about any changes of its properties.

I will only briefly describe the types of installations that were examined in our study. Detailed description of each installation will be given in the Chapter 3 (Results and Discussion).

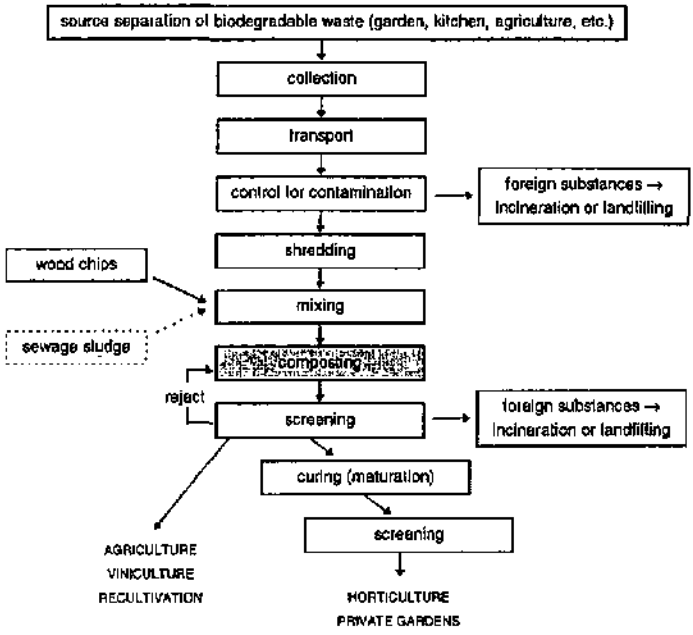


Figure 4: Typical course of a composting process.

Our research focused on composting at industrial level, although the results obtained from the experiments with open-air windrows can possibly also be applied to community or edge-of-field composting, as long as enough biowaste is gathered to allow a sufficient temperature rise in the compost.

1.7.1 COMPOSTING IN OPEN-AIR TRIANGULAR WINDROWS

The composting material is put in long rows (windrows). Depending on the type of turning machine, the windrows have a height of 1-2 m, and a width at the base of 3-4 m. The turning is carried out with a special machine that drives over the windrows. The «old» method of turning the compost with a front-end loader is fortunately not much employed any more, because this time consuming procedure leads to a infrequent turning frequency, an insufficient mixing of the material, and a bad control of compost moisture.

In windrow composting, the control of compost aeration and humidity is only partially feasible: the former through an appropriate structure of the starting material, the geometry of the windrows and the distance between one row and the other, to allow natural aeration to happen; the latter through covering of the heaps during rainy periods with a tarpaulin which allows water vapor to escape, but hinders rain to pass, and the frequent addition of water during dry periods.

Open-air windrows with no artificial aeration depend mainly on convection-induced mass transfer and gaseous diffusion for oxygenation and the loss of metabolic heat. Composting windrows tend to vary greatly, both temporally and spatially, in oxygen concentration, temperature, and other physical factors such as moisture, substrate density, and interstitial concentrations of various gases. Biological activity is thus expressed unevenly throughout a composting windrow. Variations in activity might effect final compost quality (MILLER *et al.*, 1991).

1.7.2 COMPOSTING IN BOXES OR TRENCHES, ROOFED OR IN A CLOSED HALL

Because the composting is carried out in boxes, natural aeration does not occur. It is though necessary to install an artificial aeration system. Air can either be blown into the compost or sucked through it. At the installations investigated, boxes were filled 2-4 m deep with compost, bringing about a reduction of the surface necessary for composting. Turnings in boxes or trenches is normally carried out with an automated system that can either run either on the walls that separate the boxes, or is suspended from the roof covering the boxes. In one installation, compost was taken out from the bottom of the box, mixed and aerated, and refilled at the top. In some of the installations, compost temperature got controlled by aeration; generally it is considered that it should not exceed 65°C, in order to be in the range of optimal degradation (FINSTEIN & HOGAN, 1993). An other way to control temperature is to choose an aeration cycle with preset aeration times that change as a function of degree of maturity of the compost. It seems that there is still little experience in setting the right frequency of aeration (shorter aeration times but more frequent, or longer aeration times but less frequent). Composting in boxes is usually carried out for a short period of time (4-6 weeks), and a curing stage follows outside the boxes.

1.7.3 COMPOSTING IN BIOREACTORS

A bioreactor is a completely closed vessel. In order to assume a homogenous composting process, the material has either to be mixed inside the vessel, or has from time to time to be taken out of the vessel, mixed, and refilled. Compost in bioreactors is always aerated. Composting in a bioreactor has the advantage that input (quantity of air, water) and output (temperature and CO_2 or O_2 content of air, percolate) parameters can be easily recorded, and the process accordingly controlled. On the other hand, visual control or sampling of compost is difficult to execute.

1.7.4 COMBINED METHANIZATION AND COMPOSTING

An interesting concept is the combination of methanization and composting. Very wet and nutrient rich materials such as kitchen waste or sewage sludge can cause problems during the composting process (clogging of the free air space, and therefore creation of anoxic zones and emission of bad odors). These materials are best methanized in a fermentor under anaerobic conditions. The biogas (methane and CO_2) can be utilized to produce energy (electricity and heat) necessary for the total process. The sludge that comes out of the fermentor (20-30 % total solids) is mixed with shredded wooden waste, and composted, either in a bioreactor or in boxes.

1.8 DOES COMPOSTING REPRESENT A HEALTH HAZARD ?

1.8.1 PATHOGENIC MICROORGANISMS IN COMPOST

By its very nature, biodegradable waste can be the vehicle and the breeding ground of a broad spectrum of microorganisms. Most of them are saprophytes, e.g. organisms which live and feed on dead or decaying organic matter (COLLIN, 1992), but the presence of obligate (primary) and facultative or opportunistic (secondary) pathogens[13] is possible. Primary pathogens can get into the waste by spoiled food, contaminated paper handkerchiefs, animal litter, or with animal excrement polluted grass clippings. Another source is sewage sludge, which is sometimes added in small quantities to yard wastes, or water from sewage plants used for humidification.
Since composting is employed on a large scale for the treatment of sewage sludge, many studies were carried about the survival and dispersion of microorganisms pathogenic for man and animal (FARRELL, 1993; HUSSONG *et al.*, 1985; HIGGINS *et al.*, 1982; DUDLEY *et al.*, 1980). When composting plant materials, the elimination of phytopathogenic organisms has to be ensured. Saprophytic microorganisms, which are at the same time opportunistic pathogens (fungi, actinomycetes) have found special attention.

STALDER (1994) cited the following groups of human pathogens as having the most importance in composting environments:

Bacteria:
Enterobacteraceae
E. coli
Pseudomonas
Staphylococci
Streptococci
thermophilic actinomycetes, especially *Saccharopolyspora rectivirgula* and *Saccharomonospora viridis*

Fungi:
Aspergillus fumigatus
Penicillium

Viruses:
Coxsackie-B-Virus
Echo-Virus

As we have limited our investigations to the detection of thermololerant molds, especially *AF*, and Gram⁻ bacteria, these groups will be discussed in the following chapter in more detail.
Adverse health effects may also result from aerosol exposure to metabolic products of microorganisms. Some fungi produce mycotoxins. β,1,3-D-Glucan and galactomannans, both polyglucose structures in fungal cell walls, have been associated with inflammatory responses (SORENSON & LEWIS, 1996). Fever, cough, headache and respiratory impairment can be caused by endotoxins, which are lipopolysaccharides found in the outer membrane of Gram⁻ bacteria (STETZENBACH, 1997). Furthermore, the constant exposition to dust can provoke an unspecific irritation of the mucous membranes of the respiratory tract (Mucous membrane irritation (MMI)), leading to chronic bronchitis (BITTIGHOFER, 1994). It is suspected, as in the Organic Dust Toxic Syndrome (ODTS, see chapter 1.8.1.1E), that often not a single agent, but the combination of fungi, bacteria, and inorganic dust particles, lead to the development of a disease.

[13] Primary pathogens can invade and infect healthy persons, whereas secondary pathogens normally infect debilitated individuals (EPSTEIN, 1996).

1.8.1.1 DISEASES

The following sub-chapters shall give a short overview of the possible diseases caused by microorganisms occurring in compost. Comprehensive treatises on the subject can be found with ANONYMOUS (1994b), LACEY (1995), RICHERSON (1994), CAMPBELL (1994), MILLNER *et al.* (1994), LEWIS *et al.* (1994), YOSHIDA *et al.* (1993), REISS (1986), and GEDEK (1980).

1.8.1.1A Mycoses

Aspergilli, primarily *AF*, but also *A. flavus* and *A. niger* can provoke a disease called aspergillosis, if the spores get to the lung by inhalation. A benign form is the so-called fungus ball, where the fungus grows in preformed cavities, either in the lung or in the paranasal sinus. Complications only arise if blood vessels are perforated.
Invasive aspergillosis, where the fungus penetrates from the lung into the blood stream and attacks other organs, has often a lethal outcome. It requires a considerable immune deficiency through a underlying disease like AIDS or leukemia, but also diabetes or hepatitis can favor the development of a mycosis (STALDER, 1994). Patients with a suppressed immune system after an organ transplantation or in the course of a cancer therapy are especially at risk. Invasive aspergillosis was observed in aged persons presenting no other risk factors (ATHAYDE & SHORE, 1993). Aspergillosis has also been observed in animals: young pigs, lambs, cattle (mycotic placentitis, leading to abortion of the fetus), and chicks (CAMPBELL, 1994).
Molds of the order *Mucorales* cause the so-called mucormycosis, which can manifest itself as a lung, a gastrointestinal or a cutaneous disease.
Cryptococcus neoformans, a fungus found in soil, bird excrement, but also in compost, causes cryptococcosis, which can lead to a meningitis (GROSSE *et al.*, 1997).

1.8.1.1B Mycotoxicoses

Mycotoxicoses are normally provoked by the ingestion of moldy foods, but it has been demonstrated that the spores of various fungi (amongst others *AF* and *A. flavus*) contain mycotoxins. ATHAYDE & SHORE (1993) discussed the significance of inhaled Aflatoxin B1, produced by *A. flavus*, in the pathology of lung tumors. DÉPORTES *et al.* (1997) isolated several potentially mycotoxigenic strains *(A. flavus, A. parasiticus, A. sidowii)* from MSW compost. From 5 strains, three produced mycotoxins after incubation in a liquid medium. No aflatoxins could be extracted from the compost ; a possible interaction between toxins and humic acids was suspected.
The quantities of aflatoxins in the air, and the necessary doses to elicit tumors are not know to date.

1.8.1.1C Allergies

Because fungal spores can only cause infections in the case of reduced host resistance, the main risk of exposure to high concentrations of fungal or actinomycetal spores is the appearance of allergies.
If the hypersensitivity is caused by the inhalation of spores, one speaks of an extrinsic disease, if hyphae are growing in the air passages, it is called intrinsic (HAY, 1988).
Fungal allergies are of type I (immediate type), the reaction is triggered by the immunoglobulin E (IgE), which can be found in large quantities in the serum of affected persons. The clinical manifestations are rhinitis, edema of the nasal mucous membrane, and in later stages asthma bronchiale. About 10-20 % of the population are thought to be atopic[14]. Population studies of the prevalence of IgE antibodies against *AF* gave results of 0-1 %, increasing to 20 % in allergic subsets (SPORIK *et al.*, 1993). In Belgium, about 10 % of allergy sufferers were shown to react to *AF* antigens (LEWIS *et al.*, 1994).

[14] Predisposition to certain allergic responses, due to the inherited presence of IgE (ANONYMOUS, 1987)

Actinomycetes but also fungal antigens can provoke exogen allergic alveolitis (EEA), often also called hypersensitivity pneumonitis (HP), which manifests itself in the form of a pneumonia with severe general reactions such as high fever and shivering attacks which occur 6 to 8 hours after contact with the sensitizing agent. Milder courses of the disease can resemble grippal infections. The disease is known under the name of Farmer's Lung and constitutes an acknowledged occupational illness. PARKER *et al.* (1992), cited by SORENSON & LEWIS (1996), reported an incidence of 0.03 to 0.42 % cases in farming populations. EEA was also reported in other professions with an intensive exposition to bioaerosols (Malsrer's lung, Mushroom worker lung (LEWIS *et al.*, 1994)). EEA is an allergy of type III, mediated by the immunoglobulin G (IgG). In the case of long-time exposition to the allergen, a type IV allergy, mediated by T-lymphocytes (cellular immunity) can follow the humoral[15] immune reaction. Chronic EEA can lead to fibrinization of the lung and to an increasing inhibition of the gaseous exchange (STALDER, 1994). Data from animal experiments showed the provocation of EEA by the inhalation of 10^7-10^8 *AF* spores/m^3 (FOGELMARK *et al.*, 1991, cited by MULLNER *et al.*, 1994).
Allergic Bronchopulmonary Aspergillosis (ABPA) is a characteristic intrinsic condition in atopic, asthmatic patients that can lead to chronic pulmonary damage. It is presumed to be mediated by types I and III hypersensitivity reactions.

1.8.1.1D Exposition to bacterial endotoxine

Gram$^-$ bacteria produce lipopolysaccharides (LPS) as part of the outer layer of their cell wall that have toxic properties. When the cells lyse, the toxins, called endotoxins, are set free (MADIGAN *et al.*, 1997). Inhalation of these can lead to fever and flu-like symptoms (LUNDHOLM & RYLANDER, 1980). NIELSEN *et al.* (1994) measured endotoxin concentrations of 60-260 ng/m^3 during the emptying of biowaste containers, and CLARK *et al.* (1983) between 1 and 42 ng/m^3 during static pile biowaste composting. RYLANDER (1987), cited by KÄMPFER & WEISSENFELS (1997), reported for endotoxin concentrations between 100 and 200 ng/m^3 an impairment of the lung function, and between 500 and 1000 ng/m^3 fever.

1.8.1.1E ODTS

Organic Dust Toxic Syndrome (ODTS) is a non-infectious, flu-like illness, characterized by fever, malaise, muscular pain, and inflammation of the lower respiratory tract, and has been observed in persons exposed to dust containing large amounts of fungi and bacteria (SORENSON & LEWIS, 1996). Because no correlation between the presence of precipitation antibodies and illness could be established, an unspecific immune mechanism is suspected. LACEY & CROOK (1988) proposed an important contribution of mycotoxins.
NOLARD *et al.* (1988) stated that neither clinical nor environmental investigations allowed to clearly distinguish EEA from ODTS, but that these two disorders may represent part of a spectrum of responses to complex organic dusts. While ODTS occurred 30 to 50 times more frequently (0.3 to 0.5 % of Scandinavian farmers) than Farmer's Lung Disease, higher levels of exposure ($2 \cdot 10^9$ spores/day) were necessary (MALMBERG *et al.*, 1993).

[15] pertaining to or proceeding from a fluid of the body (ANONYMOUS, 1987)

1.8.1.2 THERMOTOLERANT FUNGI

Fungi play an important role in the degradation process of composts. While yeasts could be isolated in the first few days of the composting process during the acidification phase, thermophilic or thermotolerant species of the order Zygomycetes, Ascomycetes, Basidiomycetes and Fungi imperfecti predominated during the rotting and *Ceratocystis*, *Doratomyces*, and *Trichoderma* in the maturation phase. In stored compost, the opportunistic human pathogenic species *Paecilomyces varioti* and *Scopulariopsis brevicaulis* were detected (MILLNER *et al.*, 1977).

The fungi found in compost are not necessarily those found in the air of composting sites, too. Normally, only spores can get airborne; the conidiospores from molds (*Aspergillus*, *Penicillium*, etc.) get dispersed very easily, and can be detected in high numbers. Due to their hydrophobicity, they are scattered even in the very humid conditions encountered in compost.

GÖTTLICH et al. (1994) examined the fungi in the air of composting installations, and found the following species, which play a role in human health, in almost all the work places investigated (Table 6):

Table 6: Most frequently detected fungi in the air of composting sites (GÖTTLICH, 1996; GÖTTLICH et al., 1994), and their pathogeneicity (PITT, 1994).

Species	opp.	all.	toxic	Species	opp.	all.	toxic
Aspergillus flavus group	x	x	x	*Penicillium brevicompactum*		x	
Aspergillus fumigatus	x	x	x	*Penicillium crustosum*			x
Aspergillus nidulans	x	x		*Penicillium glabrum*		x	
Aspergillus niger	x	x	x	*Rhizomucor pusillus*	x		
Aspergillus terreus	x			*Rhizopus microsporus var. rhizopodiformis*		x	
Cladosporium ssp.		x		*Scopulariopsis spp.*	(x)		
Doratomyces microsporus		x		*Talaromyces thermophilus*	x		
Penicillium aurantiogriseum var. aurantiogriseum		x					

opp. = opportunistic pathogen; all. = allergen; ( ) molds that cause rarely infection in humans

1.8.1.2A *Aspergillus fumigatus*

The mold the most frequently isolated, and also the most abundant, as well as in compost as in the air of composting plants is *AF* (REINTHALER *et al.*, 1997; ANONYMOUS, 1994b; GÖTTLICH, 1996; MILLNER *et al.*, 1994; STAIB, 1993; KOTHARY & CHASE, 1984; CLARK *et al.*, 1980).

Taxonomy: Taxonomically, *AF* belongs to the class of Deuteromycetes or Fungi imperfecti, meaning that only the asexual form of the fungus is known, and that reproduction happens by conidiospores. The work by GIRARDIN *et al.* (1995), based on genomic studies, showed clearly that *Neosartorya fischeri* (formerly *Aspergillus fischeri*), whose anamorph form can not be distinguished morphologically from *AF*, is a separate species. *AF* can be identified by the characteristics of the Genus *Aspergillus* (Figure 5a and b): terminally expanded (vesiculated) conidiophores arising from thick-walled hyphal cells termed foot cells; crowded phialides on the surface of the vesicle; unicellular conidia produced successively from the tips of the phialides, forming unbranched chains (KWON-CHUNG, 1988). The upper part of the conidiophores as well as the phialides and the conidial spores are green (Figure 5d); the shade of green depending on the culture medium. Phialides are only produced on the upper part of the vesicle, the spore chains forming thus a columnar head (Fig-

ure 5e and 5f). Per conidial head, about 10^4 spores are produced (GIRARDIN, 1993). Maximum sporulation was observed in self-heating of hay after 6 days at 55°C (GREGORY *et al.*, 1963).
Lately, molecular methods, based on random amplified polymorphic DNA analysis (MONDON *et al.*, 1995) or the PCR of primers based on the sequence of an alkaline protease gene (KATZ *et al.*, 1996) have been developed. They allow to differentiate *AF* from other *Aspergillus* spp. and even the identification of individual strains.

Spores: Conidial spores are dry, hydrophobic, and readily airborne (KWON-CHUNG, 1988). The indications about the size of *AF* spores, important for the pathogeneicity of *AF* (the smaller the spores, the further they can penetrate into the lungs when inhaled) as well as for their detection in aerosols (see Chapter 1.8.3A) vary. FELDMANN (1995) indicated 3.8 ± 1.6 µm; ANONYMOUS (1994b) determined an average spore diameter of 100 measured spores of 2.51 µm; MARK, (1992) reported a mean diameter of 2.76 µm; and DERIKX *et al.* (1991) of 2.5-3 µm. The aerodynamic diameter of the spores increases slightly in humid air: at a relative humidity of 100 % from 2.15 to 2.4 µm (REPONEN *et al.*, 1996).

Temperature: *AF* is a so-called thermotolerant mold, e.g. a mold that has a growth maximum above 50°C, and a growth minimum below 20°C. REISS (1986) indicated a minimum growth temperature for *AF* of 10-12°C, an optimum of 37-43°C, and a maximum of 52-55°C. In moist heat (60°C), the mycelium gets inactivated in 5-10 minutes.

Very little information is available about the thermoresistance of *AF* spores. REISS (1986) stated that normally asexual mold spores had a thermoresistance that was 5-10°C above that of the mycelium, but that spores of *AF* in a liquid medium survived 1 hour heating at 80°C, and 10 minutes at 85°C. GEDEK (1980) compared the conidia of *AF* to those of *Paecilomyces fulvus* (the anamorph of *Byssochlamys fulva*), able to survive heating of 15-30 minutes at 80°C. KOTHARY & CHASE (1984) reported the presence of viable *AF* spores in compost of 60-70°C. AMLINGER (1993), however, stated that in laboratory experiments, all spores of *AF* were eliminated at temperatures of 64-72°C in a few minutes. HAINES (1995) asserted that *AF* stopped growing at about 56°C, but that its spores readily withstand higher temperatures, without citing experimental data.

Humidity: Concerning the required a_w of the substrate, REISS (1986) cited for mycelial growth a minimum of 0.85 and an optimum of 0.98, for spore formation a minimum of 0.90 and an optimum of 0.98-0.99. Spore germination happens at lower a_w than growth, but with decreasing a_w, the lag time before germination increases (KOZAKIEWICZ & SMITH, 1994). There exists also a connection between temperature and a_w: mycelial growth and spore germination can occur at lower a_w if the temperature is optimal. SPORIK *et al.* (1993) reported germination of *AF* at a relative air humidity of about 80 %, and an optimum growth at 98 %.

pH: *AF* can grow over a wide range of pH: the minimum is at pH 3, the optimum at pH 6-7, and the maximum at pH 8 (REISS, 1986).

Oxygen: NESSI (1994) carried out experiments about the growth of *AF* isolated from compost under different concentrations of atmospheric oxygen: no inhibition was observed until the O_2 content fell below 2 %. Spores germinated normally, after 1 week at 0.2 % O_2, when they were again incubated in air.

Substrates: *AF* is a very versatile mold when it comes to substrate utilization (VON KLOPOTEK, 1981). Besides utilization of simple substances (sugars, amino acids, etc.), the degradation of polymers such as cellulose (REISS, 1986), starch (DOMINGUES & PERALTA, 1993), pectic substances (BARACAT-PEREIRA *et al.*, 1993), xylan (KADOWAKI *et al.*, 1995; BAILEY & VIIKARI, 1993), and lipids (FOGARTY, 1994) was reported.

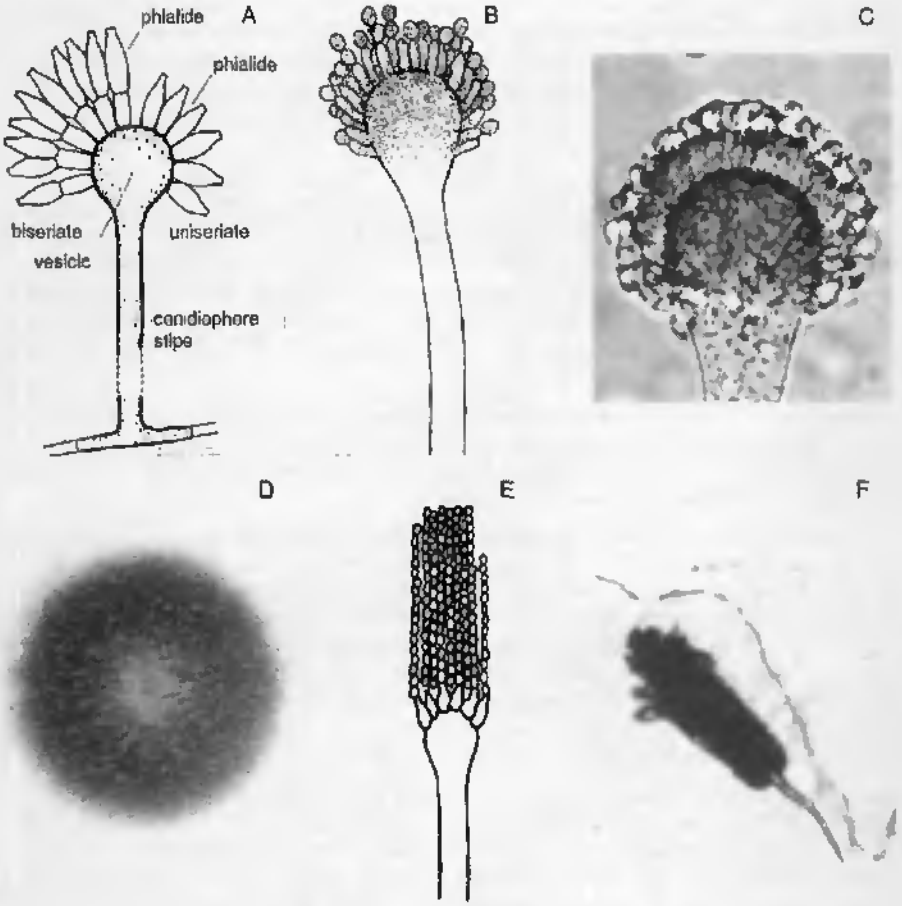


Figure 5: a) morphological structures in Aspergillus (from DE HOOG & GUARRO, 1995); b) conidiophore and conidia of AF (from DE HOOG & GUARRO, 1995); c) microscope photograph of an AF conidial head (x 1600); d) AF colonies on Malt Extract Agar, incubation for 48 h at 40°C; e) columnar shape of the conidial head of AF (from SAMSON & PITT, 1985); f) microscope photograph of an AF conidial head (x 160).

Virulence: Both the small size of the conidia and the ability to grow at 37°C contribute to the pathogenicity (mycosis) of *AF*. However, other virulence factors are presumed to be responsible for the germination of the conidia and the growth of mycelial filaments in the lung tissue. Toxins contained in the conidia are thought to weaken the action of the cilia that cover the surface of the respiratory tract and that are normally mechanically removing all foreign particles. *AF* was also found to produce proteases that inactivate substances which render microorganisms more susceptible to macrophage attack in the alveoli (JATON-OGAY *et al.*, 1994), as well as a toxin, gliotoxin, which reduces the mobility of phagocytes (LATGÉ *et al.*, 1994; PITT, 1994).

However, none of these substances were proven to be alone responsible for the pathogeneicity of *AF*, and no difference was also seen between strains isolated from patients suffering from mycoses and strains isolated from the environment (LATGÉ *et al.*, 1997). Or authors concluded that any strain of *AF* can become pathogenic if the normal defense reactions of the host are suppressed.

Presence and dispersion in composting operations: The two tables below (Table 7 and 8) show that *AF* is present as well in compost as in the air of composting site in often high concentrations.

Already the garden and kitchen waste contained, dependent on the season, up to 10^7 cfu/gDW, the highest concentrations being measured in the spring material. Grass clippings supported higher *AF* levels (10^6) than dead leaves (10^4). Old woodchips were a big source of *AF*, as they contained between 10^6 and 10^7 cfu/gDW. Even undigested sewage sludge had between 10^2 and 10^3 cfu/gDW *AF*. During the composting process, a reduction of *AF* numbers was observed, especially in the active rotting phase, when temperatures were highest. In the investigations that took compost samples in different depths in compost piles, it was shown that *AF* concentrations were always higher on the surface than in the center, where high temperatures and maybe reduced oxygen content limited fungal survival and growth. Screening of the compost often lead to an increase in *AF* numbers, attributed to a break-up of spore chains. In sewage sludge composts, FARRELL (1993) reported the highest *AF* concentrations in composts from static pile facilities.

A direct comparison of the data is difficult, because the starting material (biowaste or biosolids (sewage sludge) with varying addition of wood), composting conditions (static aerated or agitated non-aerated piles), and composting duration were each time different.

Also, for the detection of *AF*, different media were used: Sabourd-2%-Glucose Agar (LAVOIE & ALIE, 1997; MARCHAND *et al.*, 1995); Malt Extract (2-3.1 %) Agar (REINTHALER *et al.*, 1997; NIELSEN *et al.*, 1995; REISS, 1995; KOTHARY & CHASE, 1984); Dichloran Glycerol Agar (NIELSEN *et al.*, 1995), modified Czapek-Dox Agar (KOTHARY & CHASE, 1984; CLARK *et al.*, 1983), Oxgall-Antibiotic Agar[16] (MESSNER & MARK, 1996; STROM, 1991; MILLNER *et al.*, 1977, JONES & COOKSON, 1983), and Rose Bengal Agar (ANONYMOUS, 1994b). While unamended Malt Extract Agar, promoting sporulation, seems to be the most widely used medium (BUTTNER *et al.*, 1997), and is also recommended by the American Conference of Governmental and Industrial Hygienists (BOLEJ *et al.*, 1995), no comparative studies were found in the literature. Formulations containing Rose Bengal, Dichloran or Oxgall inhibit the spread of rapidly growing fungal genera like *Rhizopus* and *Mucor*, and might be advisable to use when enumerating fungi from fresh waste, where these species are numerous. Dichloran Glycerol-18 Agar is a low a_w medium for the detection of xerophilic fungi, and should thus not be used for the detection of *AF*, which does not grow well at low a_w.
Incubation temperature also varied from 30°C to 45°C. To select for thermophilic or thermotolerant species, as is *AF*, incubation should be carried out between 37°C and 45°C (BUTTNER *et al.*, 1997).

[16] per liter: agar 20 g, peptone 10 g, maltose or dextrose 10 g, oxgall 12-15 g, chloramphenicol 2 mg or streptomycin 0.05 mg, penicillin 0.01 mg, aureomycin 0.002 mg

Table 7: Concentration of AF in biowaste and compost.

activity / type and age of compost / sampling depth	*AF* concentration [cfu/gDW]	references
Fresh biowaste	$4 \cdot 10^{5d}$	AMLINGER, 1993
During the rotting phase	$8 \cdot 10^{5d}$	
Mature compost (after 8 months)	$2 \cdot 10^{4d}$	
Garden waste compost, 1 month, 0-10 cm, 33°C	$2 \cdot 10^{7}$	BEFFA *et al.*, 1994
Ditto, 25-35 cm, 50°C	$5 \cdot 10^{4}$	
Ditto, 55-65 cm, 70°C	< 20	
Ditto, 95-10 cm, 53°C	< 20	
Garden waste compost, 4 month, 0-10 cm, 62°C	$2 \cdot 10^{3}$	
Ditto, 25-35 cm, 50°C	$2 \cdot 10^{2}$	
Ditto, 55-65 cm 70°C	< 50	
Ditto, 95-10 cm 53°C	< 50	
Sewage sludge compost, composting phase (3 weeks), surface (25°C)	$4 \cdot 10^{5}$	KOTHARY & CHASE, 1984
Ditto, in 15 cm depth (52°C)	$8 \cdot 10^{4}$	
Ditto, in 30 cm depth (60°C)	$1 \cdot 10^{3}$	
Ditto, in 40 cm depth (65°C)	$< 1 \cdot 10^{3}$	
Sewage sludge compost, curing phase (4 weeks), surface (30°C)	$6 \cdot 10^{3}$	
Ditto, in 15 cm depth (63°C)	$4 \cdot 10^{3}$	
Ditto, in 30 cm depth (73°C)	$< 1 \cdot 10^{3}$	
Sewage sludge compost, screened, surface (25°C)	$1 \cdot 10^{6}$	
Ditto, in 15 cm depth (60°C)	$7 \cdot 10^{3}$	
Ditto, in 30 cm depth (70°C)	$2 \cdot 10^{4}$	
Undigested sewage sludge	$10^{2} - 10^{3}$	MILLNER *et al.*, 1977
Fresh woodchips	$10^{3} - 2 \cdot 10^{5}$	
Old woodchips	$3 \cdot 10^{6} - 6 \cdot 10^{7}$	
Active compost (sewage sludge + woodchips, in zones < 63°C)	$3 \cdot 10^{3} - > 5 \cdot 10^{5}$	
Cured compost	$8 \cdot 10^{5} - 4 \cdot 10^{6}$	
Screened compost	$2 \cdot 10^{4} - 9 \cdot 10^{5}$	
Stored compost (1 month)	$7 \cdot 10^{4} - 1 \cdot 10^{5}$	
Ditto, 4 months	$2.1 \cdot 10^{4}$	
Sewage sludge + wood chips compost, forced aeration, 21 day old	$1.3 \cdot 10^{3}$	MILLNER *et al.*, 1980
Incoming leaves	$3 \cdot 10^{4}$	STROM, 1991
Incoming grass	$2 \cdot 10^{6}$	
Yard waste compost in the thermogenic phase	$1 \cdot 10^{5}$	

[a] first quarter of the composting time
[b] after half of the composting time
[c] at the end of the composting time
[d] mean value of 6 measurements

Table 8: Concentration of AF [cfu/m³ air] in the air at composting sites and other industrial environments.

Activity / type and age of compost / distance	*AF* concentration	Reference
Yard waste composting, on site (30 m from the windrow)	$30 - 3 \cdot 10^4$ (mean $5 \cdot 10^3$)	ANONYMOUS, 1994b
Ditto, 540 m downwind	$2 - 6 \cdot 10^2$ (*mean* $1 \cdot 10^3$)	
Garden waste compost, 4 weeks, turned 1-4x/month, 5 m downwind	$1 \cdot 10^6$	BEFFA *et al.*, 1994
Ditto, 20 m downwind	$7 \cdot 10^4$	
Ditto, 100 m downwind	$6 \cdot 10^2$	
Ditto, 500 m downwind	10	
Garden waste compost, 2 weeks, turned daily, 5 m downwind	$5 \cdot 10^3$	
Ditto, 20 m downwind	$2 \cdot 10^2$	
Delivery of biowaste	$9 \cdot 10^3$	BÖHM, 1995
Sorting	$6 \cdot 10^3$	
Rotting hall	$2 \cdot 10^3$	
On the composting site	$5 \cdot 10^5$	
Ditto, in 50 m distance downwind	$6 \cdot 10^1$	
Ditto, in 100 m distance downwind	$3 \cdot 10^1$	
Sewage sludge composting, air monitoring over 1 year	$0 - 3 \cdot 10^3$	CLARK *et al.*, 1980
Ditto, 150 m from the compost area	0-130	
Sewage sludge composting, collection area (closed hall)	$40 - 2 \cdot 10^6$	CLARK *et al.*, 1983
Ditto, composting area, young compost, open-air	$1 \cdot 10^4 - > 3 \cdot 10^6$	
Ditto, mature compost	$2 \cdot 10^4 - > 6 \cdot 10^6$	
Ditto, screening (closed hall)	$2 \cdot 10^3 - > 4 \cdot 10^6$	
Sewage sludge composting, normal activity (incl. screening), on-site	$2 \cdot 10^7$	CLARK *et al.*, 1984
Ditto, 800-1600 m downwind	5	
Inside the cabin of a front-end loader, turning of biowaste compost	$7 \cdot 10^3$	GÖTTLICH, 1996
Turning of young biowaste compost, inside a hall	$1 \cdot 10^7$	
Close to woodchips pile	$2 \cdot 10^3$	KOTHARY & CHASE,
Close to static piles	$8 \cdot 10^2 - > 5 \cdot 10^3$	1984
Screening	$3 \cdot 10^3$	
Dismantling of a MSW compost pile	$8 \cdot 10^6$	LACEY & CROOK, 1988
Turning, biowaste compost, 0-100 m	$5 \cdot 10^3$	MESSNER & MARK,
Ditto, 100-400 m	$1 \cdot 10^3$	1996
Ditto, 400-1000 m	$7 \cdot 10^2$	
Screening, biowaste compost, 7-8 months, turned 2x/month, 0-400 m	$8 \cdot 10^1$	
Ditto, 400-1000 m	$4 \cdot 10^1$	
Sewage sludge composting	$0 - 6 \cdot 10^4$	MILLNER *et al.*, 1980
MSW collection, personal sampler	$1 \cdot 10^2 - 2 \cdot 10^3$	NIELSEN *et al.*, 1995
Biowaste + garden waste delivery	$4 \cdot 10^4$	REINTHALER *et al.*,
Rotting hall, enclosed table-pile composting, fresh material	$7 \cdot 10^4$	1997
Ditto, 6-8 week old compost	$3 \cdot 10^4$	
Screening	$3 \cdot 10^4$	
Above biodegradable waste containers	$1\text{-}2 \cdot 10^3$	REISS, 1995
Yard waste composting, background level	$37\text{-}6 \cdot 10^2$	STROM, 1991
Ditto, high activity	$5 \cdot 10^3 - > 7 \cdot 10^4$	
Ditto, 100 / 500 m downwind	350 / 86	
Debagging of fresh leaf and yard waste	$8 \cdot 10^2$	VAN DER WERF,
Turning (2-3 times/week, front-end loader) of active windrows	$8 \cdot 10^3$	1996
Processing of curing compost	$7 \cdot 10^3$	
Personal samplers worn by the compost workers	$8 \cdot 10^2 - 8 \cdot 10^3$	

Comparisons are even more difficult when it comes to bioaerosols measured at composting sites (Table 8), where additionally to the different composting systems and detection methods used, results are strongly influenced by the type of the aerosol sampler (see Chapter 1.8.3.2), as well as by the sampling location.
AF concentrations on the sites were most of the time several orders of magnitudes above those measured normally in locations away from composting sites: 0 to 20 cfu/m^3, with maximum concentrations not exceeding 70 cfu/m^3 when there were no self-heating matter in the surrounding area. (GARCIA, 1998; ANONYMOUS, 1994b; MILLNER *et al.*, 1994; MULLINS, 1994 and 1976; RHAME, 1991; DUNGY *et al.*, 1986; JONES & COOKSON, 1983; SOLOMON *et al.*, 1978). While emissions were very low for undisturbed compost piles, any moving of the material during collection, delivery, debagging, sorting, turning, or screening led to elevated *AF* concentrations. During operations carried out in the open, high concentrations were measured in the immediate vicinity (a few meters) of the disturbed compost, but already 20-50 m away, a rapid decline in *AF* numbers occurred. In about 150 m distance, *AF* levels returned to normal, although site activity, specific site design, operations, topography, and prevailing winds can influence the dispersion (FELDMANN, 1995). Manipulations carried out in closed buildings, however, lead to constantly elevated (10^3-10^4 cfu/m^3) *AF* concentrations.
These data show clearly that, if composting presents a certain health risk due to the dispersion of pathogens, this only concerns people who are directly implied in the handling of compost, and not the population who lives in a certain distance (mostly a few 100 m) from a composting site.

The percentage of *AF* on the total fungal flora made out between 20 and 100 %, dependent on the stage of the composting process. In the heating phase, *AF* occurred almost exclusively, while in the maturation phase, due to temperature decrease and thus concurrence by other, mesophilic fungi, as well as the depletion of degradable substances and progressive drying, its percentage got reduced.

No information was found in the literature about the direct connection between *AF* numbers in compost and those emitted in the air, either from non-disturbed or from disturbed material.

When deriving occupational risk assessments from the data presented above (Table 8), it has to be considered that the measurements were most often done with the impaction method (see Chapter 1.8.3.2), detecting only viable organisms. Dead cells, although they will not be able to cause infections, can still provoke allergic reactions (FOGELMARK *et al.*, 1991). NIELSEN *et al.* (1997a) showed a high correlation between total fungal and viable fungal counts in the air during waste collection, the culturable fungi presenting, however, only 0.1-15 %. The difference between total and viable spores was more marked when concentrations were low, meaning that no fungal growth had occurred, and that only "old" spores were collected. In composting, where proliferation of fungi is possible, the viable count may represent more closely the total amount of spores present in the air. NIELSEN *et al.* (1997a) also reported during aerolization of biowaste compost 200 times more total cells than culturable bacteria or actinomycetes.

1.8.1.3 BACTERIA

Among the bacteria, the actinomycetes and the Gram⁻ bacteria are most important in the context of composting, the former as allergens and opportunistic pathogens, and the latter as indicators of an insufficient thermohygienization (fecal coliforms) and as producers of endotoxins. The infection of the personnel or the end users of compost with primary pathogens, although such are regularly isolated from the air of composting sites or from the compost, seems not to constitute a major point of concern if the general rules of hygiene are observed (KÄMPFER & WEISSENFELS, 1997).

We limited our research to the detection of coliforms and *E. coli*, and in some cases to *Enterobacteraceae* and Gram⁻ bacteria in the compost material, and compared their survival to that of *AF*.
A high degree of thermohygienization in the course of the composting process was generally observed with respect to these bacterial groups. Their regrowth at the end of the thermogenic phase was found to be much more influenced by the degree of degradation of the compost than was the case with fungi. This is because they can not metabolize the less bioavailable substrates like lignin or cellulose left at the end of the composting process, in contrast to fungi (SOARES *et al.*, 1995).

1.8.1.3A Gram⁻ bacteria

Gram⁻ bacteria, defined as bacteria with a cell wall consisting of a thin murein layer and an outer membrane of proteins, phospholipids and LPS, being colored red by the Gram differential coloration method, have their importance in composting in the connection with the release of endotoxins (see Chapter 1.8.1.1.D).

1.8.1.3B Enterobacteraceae

The family of *Enterobacteraceae* (formerly *Enterobacteriaceae*) groups 2-3 µm long, Gram⁻, mostly peritrichously flagellated and oxidase-negative bacteria that are rod-shaped. They do not form spores and are facultative anaerobes. Some species have their habitat in the gut of warm-blooded animals and man, whereas others occur normally in water or soil or are plant pathogens. Strains affecting human health are either primary (mostly gastrointestinal disorders) or secondary (*urinary tract disease, pneumonia, septicemia, meningitis, wound infection*) pathogens (BRENNER, 1992).
The most important representatives of this group are members of the genera *Escherichia, Proteus, Enterobacter, Serratia, Erwinia, Klebsiella, Salmonella*, and *Vibrio* (SCHLEGEL, 1985).
The classification of *Enterobacteraceae* is not always easy: The newly named species *Pantoea agglomerans*, most common in organic dusts (DUTKIEWICZ, 1997), is a synonym of *Enterobacter agglomerans* and *Erwinia herbicola*, which by recent research using DNA hybridization, have been shown to be the same species. Also, proposals were made to transfer the species *Enterobacter aerogenes* to the genus *Klebsiella* (BRENNER, 1992).
RÜDEN *et al.* (1994) determined various facultative pathogenic species in different composting installations. The percentage of detection of the different species varied from one installation to the other. A recent publication reported the occurrence of *Salmonella* in 50% of the investigated fresh biowaste samples (KNOP *et al.*, 1996).
The enumeration of *Enterobacteraceae* is recommended by the FAC as indicator for the quality control of pasteurized sewage sludge that is put in the fields, considering that *Enterobacteraceae* have a similar tenacity than *Salmonella* (STADELMANN, 1983). At a concentration of less than 100 cfu's of *Enterobacteraceae*/gDW, it is suggested that the sludge does not contain any *Salmonella* any more.

1.8.1.3C Coliforms

Coliforms are lactose-fermenting *Enterobacteraceae*, which can be of either human, animal or plant origin. Species of the genera *Klebsiella*, *Enterobacter* and *Erwinia* are part of the autochthonous flora of plants (GALLENKEMPER *et al.*, 1995). Instead of total coliforms, fecal coliforms which are supposed to be only of human or animal origin, are determined by incubation at 44°C. GALLENKEMPER *et al.* (1995) found in fresh biowaste, where concentrations up to 10^8 cfu/gDW fecal coliforms were measured, 1-3 times more total coliforms. In a MSW compost, however, where the microorganisms had be exposed to elevated temperatures (maximum 75°C), no difference between total and fecal coliforms was seen (GABY *et al.*, 1972).

Total coliforms are monitored during the composting process because the die-off of coliforms should give a good indication of the completeness of the disinfecting process, and because the population of coliforms can be directly related to the population of human pathogens (CABALLERO, 1985, cited by HAY & KUCHENRITHER, 1990). YANKO, (1988), cited by FARRELL, (1993), showed that in sewage sludge compost, densities of coliforms correlated well with *Salmonella* densities: below 10^3 cfu coliforms/gDW, their was a very low probability to detect *Salmonellae*.

MOTE *et al.* (1988) showed a quite good correlation (correlation coefficient of 0.85) between total coliforms and Gram$^-$ bacteria in dairy waste compost, the former presenting about 80 % of all Gram$^-$'s.

The U.S. Environmental Protection Agency (EPA) regulation demands for sewage sludge compost a final concentration of ≤ 1000 cfu coliforms/gDW (FARRELL, 1993).

1.8.1.3D Escherichia coli

The species *Escherichia coli* (*E. coli*) belongs to the family of *Enterobacteraceae*, and because it is lactose positive, also to the group of coliforms. It is part of the normal intestinal flora of man, where it is present in concentrations of 10^5 to 10^9 cfu/g feces. Some strains are pathogenic. Its presence in water and food is an important indicator for a fecal contamination (SCHMIDT, 1994). Investigations of biowaste has shown its presence in sometimes quite high concentrations (up to 10^7 cfu/gDW), questioning its fecal origin (SCHERER, 1992).

1.8.2 WORKER SAFETY / CASES

1.8.2.1 CASES OF OCCUPATIONAL DISEASES IN THE CONTEXT OF COMPOSTING AND RELATED ACTIVITIES

Below are listed a number of published cases of occupational illnesses that occurred in the context of composting or related activities. It is possible that the incidence of work related health impairments be higher, because the often transient nature of the work force at composting sites results in low reports of illnesses (BEFFA *et al.*, 1998; CLARK *et al.*, 1980). Also, some data is not generally available, as it resides in in-house reports (EPSTEIN, 1996). There is also considerable uncertainty about the immunological diagnosis of the different forms of diseases provoked by *AF*. The used *AF* antigens are heterogeneous and often not well characterized, and can differ considerably due to the use of different strains, culture media, and incubation, extraction and purification methods (PIERRE GUMOWSKI, Allergology, Hôpital de la Tour, Genève, personal communication).

- A compost worker who had to empty rotting boxes and put the compost in windrows developed delayed fever and obstruction of the respiratory passages characteristic for EEA (GRÜNER, 1994).
- 11 out of 26 workers collecting source separated biodegradable waste complained about health problems (headache, fatigue and nausea) during the summer season. The symptoms were thought to derive from the microbiological activity in the waste, and be caused by a complex mixture of endotoxins from the Gram$^-$ bacteria, glucans from the fungi, and possibly enterotoxins from the bacteria (MALMROS, 1994).
- A 52 year old male developed fever, muscle pains, and marked dyspnae 12 hours after shoveling composted wood chips and leaves. Analysis of the dust indicated a predominance of fungal spores and a high concentration of endotoxins (640 to 16'000 units/m^3). Although precipitating antibody tests for the usual antigens were inconclusive, the authors diagnosed EEA or ODTS (WEBER *et al.*, 1993).
- A 57-year of female without any history of asthma, who had cultivated vegetables on straw contaminated with *AF* in a greenhouse without ventilation, developed a case of EEA from *AF* (YOSHIDA *et al.*, 1993).
- A person with a congenital immune defect (chronic granulomatous disease) was occupationally exposed to *AF* spores while shoveling moldy wood chips and subsequently developed a fatal aspergillosis (CONRAD *et al.*, 1992, cited by MILLNER *et al.*, 1994).
- In a newly built MSW sorting plant, the first case of occupational illness occurred only 3 month after its opening, and in the following 5 years, a total of 10 persons out of 15 were diagnosed with asthma bronchiale, chronic bronchitis and allergic alveolitis. Seven of them had to change the job, and only two of these had completely recovered after 2 years away from the plant. High levels of total mesophilic bacteria ($> 2 \cdot 10^5$ cfu/m^3), Gram$^-$ bacteria ($> 6 \cdot 10^3$ cfu/m^3), fungi ($> 1 \cdot 10^5$ cfu/m^3) and endotoxin (480-990 ng/m^3) were measured at the work places (SIGSGAARD *et al.*, 1990).
- A young man living in the immediate vicinity of a leaf composting site developed ABPA related to *AF*. The person was an asthmatic for 16 years and was treated with immunotherapeutic agents (KRAMER *et al.*, 1989).
- A previously healthy 20 year old man developed EEA two months after having started work in a vegetable compost plant, where he turned compost piles with a garden fork. A test for serum precipitins against *AF* was strongly positive, while it remained negative for thermophilic actinomycetes (VINCKEN & ROELS, 1984).

- A man who worked as a gardener for 14 years contracted a fatal locally invasive aspergillosis (ZUK *et al.*, 1989).
- Three asthmatic members of a single family almost simultaneously developed allergic aspergillosis. Their use of potting soil containing *AF* was suggested as the source of infection (VITHAYASAI *et al.*, 1973, cited by MILLNER *et al.*, 1977).

The cases described above show that diseases provoked by the handling of compost were most of the time of allergic nature (EEA or ABPA). Sometimes the symptoms lead to suppose ODTS. Diseases were observed as well in predisposed persons (asthmatics) as in previously healthy ones. Negative health effects were only in one case detected in a person that worked not directly with compost or other biodegradable materials, but lived in the vicinity of a composting installation.

1.8.2.2 EPIDEMIOLOGICAL STUDIES IN COMPOST WORKERS AND WASTE SORTERS

When considering the occupational health problems that can result from composting, one has to assess the risk that such a work might constitute, but this has to be clearly distinguished from a danger.

Danger is defined as 1. a liability or exposure to harm or injury; or 2. an instance or cause of peril, menace. It is the general word for liability to all kinds of injury or evil consequences; either near at hand and certain, or remote and doubtful. **Hazard** suggests a danger that one can foresee but cannot avoid. **Risk** is defined as 1. the exposure to the chance of injury or loss; a hazard or dangerous chance; or 2. the hazard or chance of loss; or 3. the degree of probability of such a loss (ANONYMOUS, 1987a).

In the context of occupational illnesses, BOLEU *et al.* (1995) defined risk as

risk = probability of occurrence x exposure frequency x health effect

While exposure frequency is easily determinable, although dependent of the type of composting systems, and health effects of the legally accepted occupational illnesses are well known, the probability of occurrence can only be determined by broad epidemiological studies. A few of such studies have been undertaken in the last few years:

- A survey was carried out among 209 workers at composting installations in Switzerland treating > 100 t/y. 80 % had been working in the composting industry longer than 2 years. More than 25 % suffered from one or several symptoms of chronic respiratory troubles: chronic sneezing *(25 %), dry cough (14 %), nasal pruritus and rhinitis (11 %), runny nose (11 %), slimy cough* (10 %). Statistically, the prevalence of respiratory symptoms increased significantly with the frequency of exposure to compost, independent if the persons were smokers (40 %) or not. In control groups, respiratory troubles were reported by 4 % (smokers), respectively < 10 % (non-smokers) (GUMOWSKI *et al.*, 1998).
- A total of 117 workers from 5 different waste sorting or composting plants in Austria were examined for subjective health problems, lung function and total IgE blood concentrations. Frequently, mucous membrane irritation of the upper respiratory tract was indicated, but neither the MSW sorting nor the composting plant employees showed impaired lung functions. Total, but *not fungal IgE were significantly higher in the waste workers than in the control group.* However, no correlation with length of employment could be seen (MARTH *et al.*, 1997).

- Work related health troubles were found in workers who manually emptied sacks with garden waste into a waste truck. The control group (workers who collected mixed household waste, sorted household waste and compostable household waste) did not show work related health problems. The troubles reported were airway irritation and asthmatic symptoms (NIELSEN *et al.*, 1997c, citing STENBAEK *et al.*, 1996).
- About 15 workers in a sewage sludge composting facility reported colds, 10 sinus infections, 7 cough and 5 wheezing out of an average of 46 workers tested annually over a period of five years (1987-1991). Reports of asthma, bronchitis, earaches and shortness of breath were very low. Blood tests for *AF* were negative (EPSTEIN, 1996).
- A Danish study initiated at 8 sorting and 4 composting plants showed 15-20 cases of asthma, and 20-30 cases of ODTS. The authors stated that none of the health problems observed in compost workers were infections, but toxic or allergic reactions (MALMROS, 1994).
- No health conditions related to employment were reported for the 20 employees routinely examined every six months from 1982 to 1986 in a sewage sludge composting facility in Columbus, Ohio, USA, nor for 26 workers examined once a year in a facility in Fairfax County, Virginia, USA, nor for the ones employed in Hampton Road, USA (since 1981), or in Montgomery County, USA (since 1983, 40 employees) (EPSTEIN, 1993).
- In a Dutch study, 19 (1.7 %) out of 1122 mushroom workers engaged in closed fermentation rooms were diagnosed with EEA. Serum reactions were positive in all patients against thermophilic actinomycetes (*Excellospora flexuosa*, *Thermomonospora alba*, *T. curvata* and *T. fusca*), in 1 case against *AF* and in 3 cases against *Scytalidium thermophilum*. Titers were significantly higher than in healthy controls, but only slightly higher than in compost workers that showed no symptoms of EEA. In that group, the titers increased with the duration of employment. Respiratory complaints disappeared when protective airstream helmets with a fine dust filter were worn (VAN DEN BOGART *et al.*, 1993).
- STALDER (1994) examined the presence of IgG antibodies against molds (*AF*, *Aspergillus nidulans*, *A. niger*, *A. versicolar*, *A. sidowii*, *Penicillium brevicampactum*, *P. crustosum*) and actinomycetes (*Saccharopolyspora rectivirgula*, *S. hirsuta*, *S. viridis*, *Streptomyces thermovulgaris*) in 30 compost workers from different composting sites in Germany, and compared it to 30 non-exposed persons. There was no statistically significant difference recognizable between exposed and non-exposed persons, neither for molds nor for actinomycetes, although for the latter, 1/3 of the compost workers had anti-actinomycetes-antibodies values that were more than twice the standard deviation above the mean of the non-compost workers. Also, higher titers were observed for workers who had been employed in composting operations for a prolonged time.
- Clinical, microbiological and immunological investigations about the health of the personnel (a total of 65 persons) were carried out at four sludge composting facilities from 1979 to 1981. Although throat and nasal swabs were frequently positive for *AF*, and higher rates of abnormal skin, ear and nose conditions were reported in compost workers, no consistent increase of *AF* antibodies was detected, and pulmonary function was lower on Monday morning compared to Friday. Compost-exposed workers had higher levels of IgG antibodies against LPS than did workers with lower or no exposure to compost. There were also indications of increased white blood cell count, eosinophils (special kind of leukocytes), and hemolytic complement, indicative of a low level inflammatory response (CLARK, 1994, CLARK *et al.*, 1984).
- A Swedish study examining workplace related symptoms among 11 compost workers found complaints about nausea (2), headache (5), fever (1) and diarrhea (4). This compared to only 2 reports of diarrhea in 41 questioned water work employees (LUNDHOLM & RYLANDER, 1980).

Several countries have proposed occupational exposure limits (OEL) for various groups of microorganisms and endotoxin:

- Scandinavia: $5 \cdot 10 \cdot 10^3$ cfu/m^3 total microorganisms, $1 \cdot 10^3$ cfu/m^3 Gram$^-$ bacteria, and $1\text{-}2 \cdot 10^2$ ng/m^3 endotoxins (MALMROS *et al.*, 1992).
- Poland: $1 \cdot 10^5$ cfu/m^3 total microorganisms, $5 \cdot 10^4$ cfu/m^3 fungi, $2 \cdot 10^4$ cfu/m^3 Gram$^-$ bacteria; and half of these values if the respirable fraction exceed 50 % of the total count (DUTKIEWICZ, 1997).
- Netherlands: $1 \cdot 10^4$ cfu/m^3 total bacteria, $5 \cdot 10^2$ cfu/m^3 for one particular species, $1 \cdot 10^3$ cfu/m^3 Gram$^-$ bacteria, 4.5 ng/m^3 endotoxins (8 hour time weighted average) (HEEDERIK & DOUWES, 1997, HEIDA *et al.*, 1995)
- Canada: $1 \cdot 10^4$ cfu/m^3 total bacteria, $1 \cdot 10^3$ cfu/m^3 Gram$^-$ bacteria (LAVOIE & ALIE, 1997)
- Germany (Land Niedersachsen): $1 \cdot 10^4$ cfu/m^3 total bacteria (DANNEBERG *et al.*, 1997)
- Germany: $5 \cdot 10^3$ cfu/m^3 molds (ALBRACHT *et al.*, 1997)

From the studies mentioned above and the OEL proposed, and estimated risk factors, one can try to calculate a risk based on the risk factors presented in Table 9: it would be situated between 6 (for aspergillosis, PO = 0.1, HE = 10), 18 (for ODTS or EEA, PO = 1, HE = 3), and 30 (for mild symptoms like rhinitis, PO = 5, HE = 1), presuming that an exposure to organic dust containing 10^6-10^7 fungal or actinomycetes spores/m^3 would provoke the disease, and that the workers would be exposed daily (EF = 6) to such high concentrations.

Table 9: Factors to calculate a risk (= probability of occurrence x exposure frequency x health effect; BOLEU *et al., 1995).*

Probability of occurrence (PO)	Exposure frequency (EF)	Health effect (HE)
0.1 = almost impossible	0.5 = very seldom (< 1/ year)	1 = minor; damage without absence
0.3 = practically impossible	1 = seldom (yearly)	3 = important; damage and absence
0.5 = possible but unlikely	2 = sometime (monthly)	7 = serious; irreversible effect (disability)
1 = unlikely, but borderline possibly	3 = now and then (weekly)	15 = very serious, one death (acute or future)
3 = unusual	6 = regularly (daily)	40 = disaster; several deaths (acute or future)
6 = most likely possible	10 = constant	
10 = to be expected		

Risk score	Risk class
> 70	important risk; immediate action needed
20-70	possible risk; action needed
< 20	acceptable risk; consider action

However, it has to be repeated that there exists no dose-response for *AF* or actinomycetes, possibly only for endotoxins. Further epidemiological studies are necessary, also to show the effect of a chronic exposure to organic dust in concentrations that are below those known to elicit the acknowledged occupational diseases like Farmer's Lung or ODTS, but well above those encountered in normal air.

1.8.3 WAYS OF POSSIBLE INFECTION

Pathogenic microorganisms are either attached to the compost material, or are emitted in form of bioaerosols often associated with dust particles. Infections or allergic reactions in compost workers can occur by inhalation or swallowing of aerosols, by oral contact with compost due to insufficient personal hygiene, or by entry through wounds (BITTIGHOFER, 1994). While the last two possibilities can be avoided by an adequate instruction of the personnel, and technical measures (protective clothing, etc.), the emergence of bioaerosols can not be completely avoided, unless the composting installation is entirely enclosed and automated, and the vitiated air is filtered before emission to the atmosphere. Although effective personnel protection could be given by the wearing of masks, this is, because of reasons of comfort, only feasible in special situation and for a short period of time, e.g. for control of completely closed installations or biofilters.

1.8.3.1 AEROSOLS

A collection of airborne biological particles is called a bioaerosol. Those generated by agitation of moist compost during the rotting phase are a mixture of microorganisms and plant particles associated with inorganic particles, surrounded by a thin layer of moisture and often consist of aggregates of several organisms (STETZENBACH, 1997).

Human exposure to aerosolized microorganisms and their metabolites happens mainly by inhalation of aerosols: the average amount of air inhaled is approximately 10 m^3/day (STETZENBACH, 1997); one breath accounts to 0.5 (no activity) to 3 (heavy work, sport) liters, which adds, by 16 breaths/min, to an average air volume of 0.8 m^3/ hour by moderate heavy work (MARK, 1992).
Large airborne particles are lodged in the upper respiratory tract (nose and nasopharynx), particles < 6 µm are transported to the lung, and the very small fraction (2-3 µm) gets to the alveoli. There is a difference between mouth breathing and nose breathing: REPONEN *et al.* (1996) reported for *Cladosporium cladosporioides* spores (diameter = 2.3 µm) an alveolar deposition of 15 % when nose breathing, but of 25 % when "mouth" breathing (= 60 % actual mouth breathing and 40 % nose breathing).

Aerosols are, after their release, transported by air. Their settling is affected by the physical properties of the particles (size, density, shape) and the environmental parameters (air currents, relative humidity, temperature) (STETZENBACH, 1997). The dispersion can be calculated by the Gaussian plume dispersion model, based on the idea that the mean concentration of bioaerosol particles are normally distributed about the downwind plume axis from a point source (LIGHTHART, 1994). The model takes into account the following parameters: wind speed; emission strength (cfu/$m^3 \cdot s^{-1}$); initial height of plume rise, which is a function of the height of the source and air temperature at the moment of emission; horizontal and vertical plume spread, which are a function of atmospheric stability (strength of wind, cooling or heating of the air by the ground, roughness of the terrain), and in the vertical direction, particle settling speed as a function of particle diameter; and the death rate of microorganisms. The last point can be neglected in the case of *AF* spores, because they are quite resistant to drying and UV radiation, at least at the short distances at which emissions are measured (LIGHTHART & SHAFFER, 1997). However, little work assessing their survival rates in aerosols has been completed (MOHR, 1997). Settling speed for *AF* spores is, due to their small size, quite low: between 0.02 and 0.05 cm/s (GREGORY, 1973), that means the spores stay airborne quite a long time.

DANNEBERG *et al.*, (1997) used a simplified model, based on the measurements of NO_x-emissions, to determine the dispersal of microorganisms from a biowaste composting plant:

$$s = Q/E^{1.6} \cdot u$$

s = concentration of microorganisms (cfu/m³ air)
Q = emission rate (cfu/s)
u = wind speed (m/s)
E = distance between source and measuring place (m)

Emission rate of total bacteria during sieving of 12 week old compost was calculated as $3 \cdot 10^8$ cfu/s, that for general activity at the site of $1 \cdot 10^7$ cfu/s. Calculated values were one order of magnitude higher than those measured.
MILLNER *et al.* (1980) measured the aerolization of *AF* during the movement of 21 day old sewage sludge compost with a front-end loader. For concentration in the compost of 1.2-3.3 10^3 cfu/gDW, an emission rate of $4.6 \cdot 10^6$ cfu/second was estimated. The authors stated that with a turning machine this rate would most probably be much higher. From this emission, the dispersion was calculated: in 1 km downwind, the residual concentration would amount to $1 \cdot 10^3$ cfu/m³ under stable meteorological conditions (class A), but only to 5 cfu/m³ under very unstable conditions (class F). Besides the meteorological situation (strength of wind, cooling or heating of the air by the ground), the roughness of the terrain will contribute to unstable conditions, leading to a greater dilution of spores.
The geographical location of a site is very important: if a rapid dilution of spores is desired, the site should be as exposed to winds as possible. If, on the contrary, emissions should be minimal, an enclosure of the sites with a wall or trees would be appropriate.

1.8.3.2 PROBLEMS RELATED TO THE OETECTION OF AEROSOLS

There exists a wide variety of bioaerosol samplers, which work on different principles of removing airborne particles from its suspension medium, the air: sedimentation, filtration, impingement (liquid) or impaction (solid surface). The most used is the impaction method on a solid surface (agar or filter). Particles are separated from the airstream by their inertia, forcing their deposition onto a solid medium. The impaction process depends on size, density, and velocity of the particle, and on the physical parameters of the impactor, such as the inlet nozzle dimensions, and the speed at which air is sucked in. The sampled air exits the impactor's inlet nozzle as a laminar air jet directed at the collection surface, and particles with sufficient inertia impact (Figure 6, BUTTNER *et al.*, 1997).

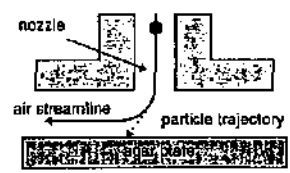


Figure 6: Impaction principle of the collection of bioaerosols (from BUTTNER et al., 1997).

In the studies reported in the literature, different samplers were used to detect airborne microorganisms at composting sites, most often the six-stage Andersen Sampler. The stages have decreasing nozzle diameters so that successive stages collect progressively smaller particles. We used in our study a one-stage sampler, which did not allow a separate detection of particles according to their size. In the literature, a lot of data about the size distribution of fungal aerosols at composting sites exist (JAGER *et al.*, 1994a and b; MILLNER *et al.*, 1980; GREGORY *et al.*, 1963), all showing that a large part of the particles are of respirable size.

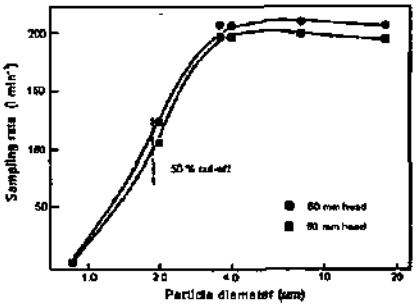


Figure 7: Sampling efficiency for the Surface Air Sampler, tested with artificial aerosols. From LACH *(1985)*

One important feature of aerosol samplers is the collection efficiency, e.g. the ability to remove particles form the airstream and to transfer them to the collection medium. The physical characteristics of the inlet nozzle (diameter, form), and the airflow rate determine the velocity of the laminar air jet at the moment of impaction. This determines the so-called cut-off diameter or d_{50}, indicating the particle diameter at which 50 % of the particles are collected. Because of the sharp cutoff characteristics of impactor samplers, the d_{50} is generally considered to be the particle diameter above which all particles are collected. For efficient collection, the bioaerosol sampler has to have a d_{50} that is below the mean size of the microorganisms being sampled (BUTTNER *et al.*, 1997).

Figure 7 shows the effective sampling rates as a function of the particle diameter for the Surface Air Sampler (50 on 90 mm head). The d_{50} is about 2 µm, just below the reported size of a mean diameter of *AF* spores of about 2.5 µm. However, the cutoff is not very sharp; very small *AF* spores might not be collected.

In principle, all bioaerosol sampler are designed for the detection of small concentrations of airborne microorganisms, as for the control of hygiene in hospitals or food industry. In environmental situations, concentrations are often much higher, leading to plates that are completely overgrown. In order to counter this problem, samplers are on the market where the air gets washed of its particles by a passage through a liquid. From this collection liquid, dilutions can be carried out on different selective media. This method has the disadvantage that the microorganisms are exposed to a great stress when passing from the air to the liquid (BUTTNER *et al.*, 1997, TERZIEVA *et al.*, 1996).

Another problem encountered with many type of air samplers is the imprecision of the amount of air drawn in. MEHTA & PIERSON (1996) found in a study comparing different air samplers that the measured airflow differed sometimes considerably from the manufacturer's stated rate (e.g. Burkhard air sampler: 28.3 l/min, instead of 20 l/min; SAS Super 90 with 55-mm Rodac® contact plates: 85 l/min instead of 90 l/min). LACH (1985) tested the performance of two SAS samplers, equipped with 55 mm (for a 220 hole sieve plate) Rodac® or 90 mm (for a 487 hole sieve plate) Omiko® plates. The nominated airflow for both samplers was 180 l/min, but the measured values were 212 and 200 l/min, respectively. It could be that the filling height of the agar plates were not as recommended by the manufacturer. HECKER & MEIER (1991) had demonstrated that if the plates were overfilled, the air volume was strongly reduced.

MEHTA & PIERSON (1996) found that the Burkhard portable air sampler and the Andersen two-stage impactor recovered more fungal spores (particle size > 3 µm), than the RCS Plus and the SAS Super 90. Comparison between Andersen and SAS Super 90 for the detection of *Penicillium* spores (1.8-3.5 µm diameter) showed a higher sampling efficiency for the Andersen sampler, as well as a higher repeatability (BUTTNER & STETZENBACH, 1993). The latter might be due to the longer sampling time (5 minutes with the Andersen sampler, versus 1 minute for the SAS).

It is generally observed that concentrations of aerosolized microorganisms do vary greatly with time, due to an inhomogeneous distribution in the air, and to irregular emissions (BUTTNER *et al.*, 1997). This is independent of the type of used air sampler, which are by themselves normally quite consistent in their performance. Concentrations of total mesophilic bacteria in a municipal solid waste facility measured simultaneously with two identical samplers differed seldom by more than 20% (LEMBKE *et al.*, 1981).
JAGER *et al.* (1994a) observed variations up to a factor 400 when taking 30 parallel measurements in composting installations,. In this sense, air samplers with a lower sucking speed, collecting over an longer period of time, would show aerosol concentrations that represent better mean concentrations. A technical guideline in Germany specifies the number of samples that have to be taken, as a function of the sampling time: when measuring for one minute, for example, a minimum of 20 single samples should be taken at one position (JAGER & ECKRICH, 1997).

Lately, detection of microbial cells in aerosols with molecular biology methods have been developed (NEEF *et al.*, 1995). This allows the specific determination of certain groups or species by the use of gene probes. If the whole cell hybridization (NEEF *et al.*, 1995) or solid-phase PCR (ALVAREZ *et al.*, 1994) technique is used, the problem of viable, but non-culturable microorganisms due to stress of aerosolization and sampling, can be overcome.

1.9 AIM OF THE PROJECT

1.9.1 PRELIMINARY STUDIES

The occupation with composting at the Laboratory of Microbiology started in 1990, when questions about the possible emission of fungal spores from a composting site that was situated right next to a hospital were raised. In collaboration with experts from other fields (waste management specialist, allergologist, hygienist), the site, a very extensively managed open-air windrow system, was examined, and the presence of high concentrations of *AF* in the compost (> 10^7 cfu/gDW) as well as in the air (> 10^3 cfu/m^3) during turning with a front end loader detected. Recommendations were issued to move the composting site to another location (TRELLO BEFFA, personal communication). Immunological studies (Ig-specific ELISA tests and lymphocyte transformation tests (LTT)) performed on 15 compost workers chronically exposed to organic dusts showed increased LTT responses, comparable to those of to *AF* allergic patients, but no increase in antibodies (GUMOWSKI *et al.*, 1992).

In 1993, under mandate of the Swiss Federal Office of the Environment, we performed a cross-sectional study, comparing the occurrence and the dispersal of *AF* in eight different composting plants using different composting systems (BEFFA *et al.*, 1993). The results can be summarized as follows (LOTT FISCHER *et al.*, 1995):

AF was detected in all composts, independent of the composting technology, although considerable variations were observed from one site to the other, even if the same composting system was used. Numbers of *AF* were lowest when composting in bioreactors. In general, more molds could be found at the surface of the compost heaps than in the center, where higher temperatures prevented mold growth. Numbers of *AF* in fresh composts varied from not detectable to $1 \cdot 10^6$ cfu/gDW. If the initial material contained more garden waste, numbers of *AF* were higher than if it contained more kitchen waste. In the intermediate compost, when the highest temperatures (up to 60°C at the surface, and up to 70°C in the center) were reached, numbers of *AF* were reduced significantly. In the mature compost, though, a recolonization by molds was often observed. Lower temperatures and storage in huge piles that were not turned favored the development of *AF*, especially at the surface.
When comparing the results from different composting sites that used the same composting technology, two factors seemed to influence greatly the proliferation of *AF*: the turning frequency of the windrows, and the degree of humidity in the compost. In was found that because of the low temperatures in the outer layers of windrows, it was not the core temperature that was decisive for the elimination of molds and other pathogenic or allergenic microorganisms, but it had to be ascertained that 65°C were reached in the whole compost material. Thus, a prerequisite for a good compost management proved to be an efficient compost turning system that allowed rapid turning, a good mixing of the compost and addition water if the compost was too dry.
From observations made in systems that used artificial aeration, it was inferred that good oxygenation of the compost was necessary for an optimal degradation of the organic material, and helped to prevent bad odors that occurred if the compost became anaerobic. However the amount of injected air was not optimized so that it did dehydrate or cool down the compost too much.
AF spores present in composts got dispersed in the air during shredding of the biodegradable waste, turning of the heaps, aeration of the windrows, and to a minor degree during screening of the mature compost. Correlation was found between the amount of molds in the compost and their concentration in the air, although weather conditions and type of turning system influenced the dispersion.

Mold counts were high close to the working areas, but decreased considerably already at a distance of 50 m downwind. Turning of fresh compost released more mold particles than turning of mature composts. This was in accordance with the lower mold densities in the mature composts. *AF* counts in the air around totally closed systems (bioreactors) were insignificant. Concentrations of *AF* in the air at sites treating only small amounts of green waste were lower than at industrially operating sites. There, the concentrations without activity were significantly higher than in places far from composting sites. Every site was only investigated once, presenting therefore only a momentary picture. The influence of seasonal variations, or the performance of a given system with different types of waste (e.g. more wet, unstructured kitchen waste due to extended curbside collection programs) had not been tested.

1.9.2 PRESENT STUDY

On the bases of the observations made in the above mentioned studies, we started in 1994 a three-year project financed by the Swiss Federal Research Foundation (Project no. 5002-038921, Priority Program Biotechnology, Module 5b (Biorisk), project leader: Dr. TRELLO BEFFA) with the title **Composting of organic wastes: optimization of the thermogenic phase to overcome hygienic and public health hazards.** The aims of this study were (project text): a) to understand the conditions of the appearance of hazardous organisms (pathogenic and allergenic molds and bacteria) in compost; b) to evaluate the risk that constitute these; c) to develop procedures that would lead to a better hygienization, especially optimization of the thermogenic phase, and d) to transfer the results to existing composting plants and to evaluate the effects of proposed improvements *in situ*.

Destruction of compost pathogens is closely related to the development of elevated temperatures in the composting cycle. However, recommendations were formulated that temperatures should not exceed 65°C, in order to prevent a "microbial suicide" (DE BERTOLDI *et al.*, 1985). In order to get more insight into the bacterial flora during the thermogenic phase, part of the project work dealt with the ecology and taxonomy of the thermophilic bacteria (MICHEL BLANC, 1997) as well as the growth characteristics and degradation potential of some of these thermophiles (PIERRE-FRANÇOIS LYON).

My part of the project was to elucidate the occurrence, growth and dispersal of potentially pathogenic and/or allergenic microorganisms in compost, to test the effect of thermohygienization (reduction or elimination of pathogens by heat) towards these organisms, and to evaluate the recolonization potential during compost maturation as a function of organic matter degradation (= stabilization).
Our investigations were restricted to *AF* and to Gram$^-$ bacteria as indicators of mesophilic organisms, also with the intention to determine if *AF* could serve as indicator organism. Part of the results about coliform bacteria presented in this thesis are from VERONICA GARCIA (1998), who carried out her diploma work under my direction.
Although an abundance of studies exist about the presence and dispersal of *AF* during composting in different types of systems, and with different starting materials (see Tables 7 and 8), no data are available about the influence of operational characteristics of different composting processes on the growth and dispersion of either fungi or bacteria. The only recommendation given by MILLNER (1995) is to reduce bulk movement of compost, and to prolong the curing time. This is, however, against the very aim of a non-static composting system.

HANSEN et al. (1996) listed the following factors that can be controlled during any composting process, although not every system allows the same control.

Table 10: Controllable factors for composting (HANSEN et al., 1996).

initial substrate /mix	process parameters	process parameters (suite)
organic amendment	type of process	moisture control
C/N ratio / bulking agents	particle size (choice of shredder)	temperature control
percent recycled compost	reactor vessel / windrow size	aeration (frequency / force /
moisture content	mixing equipment	percent recycled air)
inoculation	turning frequency	rotting and curing time

The control of the composition of the initial substrate is normally not feasible : all the material that is delivered to the site has to be processed. Only during winter can wood be stocked, and used as bulking agent in summer, when too much wet material arrives. Nevertheless, the study of the influence of different initial substrates on the composting process can help to foresee measures to take when the starting material varies due to seasons, or when special materials have to be composted.
The type of process, as well as the particle size, mixing equipment and reactor or windrow size (the latter is normally determined by the type of turning machine used) are normally given, and can not be changed unless a site is newly constructed or radically rebuild. However, if serious problems exist, e.g. with bad odors, reconsiderations about the type of process has to be made.
The factors easiest to control are the ones connected with the composting operation itself: turning frequency, moisture and temperature control, aeration and actual composting, and curing time.

Figure 8 presents graphically the influence that these factors have on the organic matter degrading microflora, and thus on microbial activity, which equates to heat output.

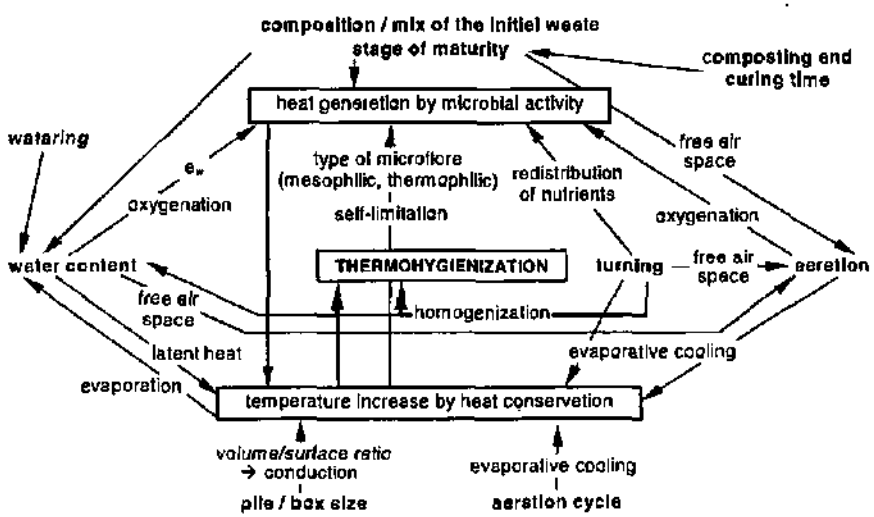


Figure 8: Relationship between temperature and other physico-chemical compost parameters.

The approach of our research was to consider the hygienization of compost as the consequence of high temperature formation through optimal composting conditions. By an accurate description of the composting process and its main parameters (composition of the starting material, size and shape of compost heaps, turning frequency, aeration cycles), it was tried to determine their influence on the composting process, and ultimately on the thermohygienization, by monitoring physico-chemical compost parameters (temperature, gases, pH, humidity, organic matter, etc.).

The main part of the work were field studies, while some laboratory experiments, either in a bench-scale composter (PIERRE-FRANÇOIS LYON) or with pure cultures isolated from composts, served to isolate some factors and to study them separately.

For our study, we were working in close collaboration with several composting industries, representing some of the most frequently used systems in Switzerland. The choice of the partners was made by the system they employed, but also by their willingness to place their installation at our disposal for experiments, and by the geographical location of the site. The types of systems can be classified as follows, in ascending complexity:

- open-air windrow composting with natural aeration
- box composting with or without forced aeration, roofed or inside a building
- trench composting, with forced aeration, inside a building
- in-vessel composting, with forced aeration, inside a building

At the composting sites of our industrial partners, the presence and dispersion of *AF* and coliforms were investigated. In function of the results obtained by a first survey, changes in the compost management were proposed, and, if implemented, monitored.

Field studies have the advantage of giving an insight into real life working situations. On the other hand, parameters such as the exact composition of the starting material, turning and aeration cycles, as well as meteorological conditions are difficult to control. Understandably, the proper functioning of the installation is often given priority over the needs of the experimental procedures. Also, the geographical distance of the sites to our laboratory made a close surveillance of the experiments impossible.

2. MATERIAL AND METHODS

The first part of this chapter deals with the laboratory experiments designed to elucidate certain phenomena that were observed during the field trials. The methods used for the field trials, as well as the experiments carried out at each composting site will be described in the second part.

2.1 LABORATORY STUDIES - PHYSIOLOGY OF ASPERGILLUS FUMIGATUS

The experiments in the laboratory were carried out to determine physiological properties of *AF*, especially its thermoresistance. They also served to compare strains isolated from different composts, and a strain of medical origin. All strains isolated from composts came from the open-air windrow site, and were recovered from compost after turning.
The following strains were tested:

- GR2 isolated from the air, during turning of 12 week old compost
- GR5 isolated from fresh biowaste
- GR6 isolated from a 2 week old compost
- GR7 isolated from a 6 week old compost
- GR8 isolated from a 10 week old compost
- G10 medical strain (spontaneous nitrate reductase$^-$ mutant of strain CBS[17] 144-89, described by JATON-OGAY *et al.*, 1994)

Strains were isolated on Malt Agar, purified, the spores suspended in phosphate buffer[18], and stored frozen at -80°C in 10 % glycerol, as proposed by DENNING *et al.* (1992).

All physiological test were carried out on Malt Extract[19] (1.5 %), either solidified with agar (2 %), or liquid. Because of the higher purity of this malt extract compared to the one used for the enumeration, the agar plates or broth obtained was clear, and allowed thus an easier optical evaluation of growth.

2.1.1 MAXIMUM GROWTH TEMPERATURE

Temperature is the most important factor governing the growth of any microorganisms found in compost. To determine the cardinal temperatures that allowed growth of *AF*, the strains GR5 to GR8 were incubated in a Temperature Gradient Incubator (TGI)[20] on malt extract agar slant tubes, at temperatures ranging from 5.2°C to 59.6°C. The calculated temperature difference between 2 tubes was 1.88°C. In total, 30 tubes were inoculated, each with 10 µl of thawed spore suspensions,

[17] Centraalbureau voor Schimmelcultures, Baarn, NL
[18] pH 7, with the addition of 0.005 % Tween 80
[19] Malt Extract Broth (Oxoid, CM57)
[20] Scientific Industries Inc., New York, USA

and incubated in the TGI. About every 12 hours, the growth and the formation of conidiospores were recorded with the naked eye.

2.1.2 GROWTH RATE

To determine the temperature that allowed maximum growth rates, 10 µl of thawed spore suspensions (GR2, GR5 to GR8) were inoculated in duplicate in the center of 9 mm Petri dishes filled with 25 ml of malt agar. The plates were incubated in desiccators with water on the bottom (100% rH), at 18, 23, 30, 37, 40, 45, 50, 52 and 55°C. The colony diameter was measured every 12-24h.

2.1.3 FROM GERMINATION TO SPORULATION

Deuteromycetes (Fungi imperfecti), to which *AF* belongs, have a life cycle that consists in a vegetative state, during which the fungus grows. Then follows a phase of development of asexual reproduction bodies, the conidiospores. These become disengaged from the parent thallus, and are dispersed. In a suitable new environment, such spores initiate new mycelia (SMITH & BERRY, 1974). In the context of composting, the time that such a life cycle takes is of importance when determining the interval between two turning events: by mixing, material from the center of a compost heap that has already undergone thermohygienization is reinfected with spores formed in the outer, cooler layers. If the life cycle of the fungus is short enough, those spores can germinate, grow, and new spores can be formed, thus leading to a renewed infection and the temperature appropriate.
To test the conditions that allow germination and spore formation of *AF* under laboratory conditions, 10 µl of thawed spore suspensions (GR2, GR7 and G10) were inoculated on microscopic slides covered with a thin agar film (1 ml of either malt extract agar or compost agar[21] per slide (76x26 mm)). The slides were placed on glass beads in sterile Petri dishes with water at the bottom, and incubated covered at 30, 37, 45 and 50°C. Germination was observed microscopically directly on the slides every hour for the first 12 h, then in greater intervals.

2.1.4 THERMORESISTANCE

Hygienization of compost can be achieved by controlling the process in a way that high temperatures are reached in the material. To determine the temperatures that have to be obtained to destroy either *AF* mycelium or *AF* conidiospores, the thermoresistance of these two physiological states of *AF* were tested.

2.1.4.1 AF MYCELIUM

2.5 ml of thawed spore suspensions (strains GR2, GR5, GR8 and G10) were inoculated in malt extract broth under strong agitation (250 rpm) in a baffled Erlenmeyer flask at 37°C overnight. By this way of submerse culture, a homogenous suspension of fine mycelium particles was obtained. Microscopic control showed no presence of conidiospores.
2 ml of this mycelium suspension were added to test-tubes containing 4 ml of sterile malt extract broth that had been pre-heated in a water bath to the temperature at which the thermoresistance was tested. After different exposure times, the tubes were removed from the water bath, and cooled im-

[21] 50 g of 2 week old compost were suspended in 150 ml deionised water, sonicated for 1 minute, agitated 10 minutes at 200 rpm at room temperature, sonicated for 1 minute, and centrifuged 10 minutes at 10'000 rpm. To the supernatant, agar was added to a final concentration of 1.5 %, and autoclaved.

mediately in ice water to <10°C. The temperature of the water bath, and the temperature inside a control tube were monitored continuously.
The tubes were then left at room temperature (not longer than 15 minutes) until the activity of the mycelium was measured by determining its consumption of oxygen with a Clark type polarographic oxygen electrode. The samples (3 ml of either the mycelium suspension that had been incubated, or of unexposed mycelium) were filled in magnetically stirred (stirring speed: 480 rpm) sample chambers, which were placed in a circulating water bath set at 37°C. Before each measurement, samples were heated to the measurement temperature and saturated with air by stirring for 2 minutes without the electrode being inserted. The consumption of oxygen was followed for at least 2 minutes, and the gradient of the resulting curve determined from the moment on that the O_2 consumption was linear. From each measurement, the oxygen used by the malt extract broth alone was subtracted.
The consumption of oxygen (nmol $O_2 \cdot min^{-1} \cdot mg\ DW^{-1}$) was calculated on the base of an oxygen content of the saturated malt extract broth of 199.9 nmol/ml. The dry weight of the mycelium was determined by filtering 50 ml of mycelium suspension through a pre-dried, pre-weighted folded filter, which was then dried under vacuum at 70°C for 24h, and weighted again. From the graphic depiction of the values of O_2-consumption (on a logarithmic scale) versus time of exposure, the slope was calculated. The resulting D-value (1/slope) indicates the time in minutes at a given temperature necessary for a 90 % reduction of the O_2-consumption.

2.1.4.2 AF *CONIDIOSPORES*

Malt agar slants were inoculated with thawed spores suspensions and incubated for one week at 37°C, to obtain heavily sporulated cultures. The conidiospores were collected in the caps of the slant tubes by knocking the tubes gently while they were held upside down. After transfer to a sterile vial, the spores were kept at 4°C until use. The "dry" harvesting of conidiospores had the advantage that only the spores were collected, and that they could be stored for some time.
To prepare the suspensions for the thermal inactivation experiments, the spores were suspended in phosphate-citrate buffer[22] (accordingly to FUJIKAWA & ITOH, 1996) and homogenized for 2 minutes at high speed in the Stomacher Lab-Blender[23] to break up aggregations and chains of spores. After estimating the number of spores with a hemocytometer (average of the count of 20 small squares), the suspension was diluted with buffer to give a final concentration of $2 \cdot 10^6$ spores/ml, and filled in sterile Pyrex test tubes with tight plastic caps, in portions of 3.5 ml. The samples were heated in a water bath that was kept constant at the temperature at which the thermoresistance was tested. The temperature of the water bath, and the temperature inside a control tube were monitored continuously. In order to bring the sample to the designated temperature, it was pre-heated for 2 minutes (temperature raising period, initial spore concentration after the pre-heating period = N_2), after which the temperature holding period followed. After the exposition, the tubes were cooled immediately to <10°C in ice water. They were then kept at room temperature until the viable spore count was carried out by MPN: from dilutions (1:5) in physiological salt solution, 10 x 10 µl per tube were spot-plated on Malt Extract agar. After incubation at 37°C for 48-72h, the number of spots that showed growth were counted, and the MPN / ml spore suspension calculated. The results were presented graphically as the rate N/N_2 of the spore number after N minutes of exposure over the spore number after the pre-heating period (N_2), on a logarithmic scale, versus the time of exposure.
Experiments were carried out with the strains GR2, GR7 and G10.

[22] 0.1 M $Na_2HPO_4 \cdot 12\ H_2O$ (= 35.8 g/l), 1000 ml deionized water, 0.05 ml Tween 80. Adjusted with 0.05 M citric acid monohydrate (10.5 g/l) to pH 7.0, autoclaving.
[23] Seward Medical, London, UK

2.2 FIELD STUDIES - METHODS

Field studies were carried out at the composting sites of our industrial partners, and always comprised the following components:

- Measurement of physico-chemical parameters (temperature, gases) in the compost
- Measurement of bioaerosols generated by the different activities on the site (compost unloading, shredding, turning, screening, etc.)
- Compost sampling, either before or after turning
- Measurement of microbiological parameters (molds, bacteria) in the compost samples
- Measurement of physico-chemical and chemical parameters (water content, pH, nutrient content) in the compost samples

The following subsections will deal with the different kinds of measurements carried out in the course of the field studies, and will be presented in the order mentioned above, as this corresponds to the sequence of activities on each sampling event.

2.2.1 ON-SITE PHYSICO-CHEMICAL MEASUREMENTS IN THE COMPOST

The physico-chemical measurements in the compost give an indication about the progress of the composting process, and allow the determination of the living conditions for the compost microorganisms, either the ones responsible for the degradation of the organic material, or the pathogens. The parameters determined were temperature and gas concentrations. The following devices were used:

2.2.1.1 TEMPERATURE

In function of measuring depth and mode (spot checks or continuos) different thermometers were used.:

- up to 30 cm of depth: Ebro TTX 290S[24] (acc.: ± 0.1°C)
- up to 1.5 m of depth: Sekundenthermometer TM-920C[25] (acc.: max. ± 1% of the measured value)
- up to 6 m of depth: Heumesssonden HMSA 40000 or 6000[26] (acc.: ± 1.5°C)
- continuous measurements: Squirrel Data Logger 1201-170K, with thermistor temperature probes, type CT[27] (acc.: max ± 0.18°C)

[24] Ebro Electronic GmbH, Ingoldstadt, D
[25] Stelzner GmbH, Nürnberg, D
[26] C.M. Heim GmbH, 72218 Wildberg, D
[27] Grant Instruments (Cambridge) Ltd, Barrington, Cambridge, UK

To draw isotherms from the temperature profile measurements, each measuring point was expressed as the x- and y-coordinates in a two-dimensional system, taking the lowest measuring point at the left as 0/0 (x-value, y-value, as the distance in centimeters). The corresponding temperature measured at each point was taken as the z-value. In addition to the temperature profile measurements, the coordinates of the points on the windrow surface were calculated. Their temperatures were considered as being the same as the ambient air (according to FERNANDES *et al.*, 1994, and on the basis of own punctual measurements). From these xyz triplets (the x and y are shown in Figure 9), a mesh grid was interpolated by the inverse distance algorithm, and the new data points depicted as a contour plot. The distance weight value was 5. For the calculations of the temperature iso-curves of triangular windrows, intervals of the mesh were 20 in the x-direction, and 5 (for 75 cm high windrows), 6 (for 80-85 cm high windrows), 7 (for 90-100 cm high windrows) or 8 (for 100-125 cm high windrows), respectively in the y-direction. Measurements that had been made for one half of triangular windrows were considered to be symmetrical for the other half. The program used for these operations was SigmaPlot[28], version 3.0. The contour plots were then manually redrawn, thereby smoothing the contours.

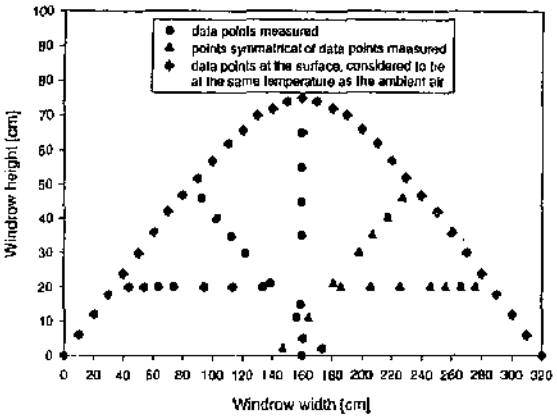


Figure 9: Data points used to calculate iso-curves in a compost windrow.

2.2.1.2 GAS CONCENTRATIONS

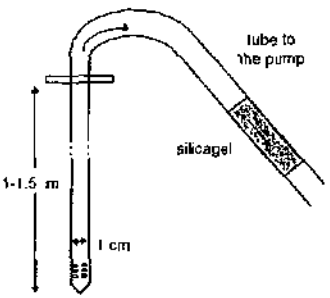


Figure 10: Gas measurement probe.

For gas concentration measurements, two type of apparatus were used: multigas detection instruments (Multiwarn) or gas tubes. Both are fabricated by Drägerwerk AG[29]. Carbon dioxide was measured at the beginning of the project with gas tubes, later on with the newly acquired Multiwarn CO_2/CO.

For the gas measurements, air was sucked through a 1 or 1.5 m long closed steel tube with a number of holes at the end that was inserted into the compost (Figure 10). To avoid damage to the gas measurement instruments, the air was passed through a tube with silicagel to remove excess humidity. The Multiwarns (O_2/H_2S/LEL and CO_2/CO) were branched in parallel, the airflow of each apparatus being 0.5-0.8 l/min. They were calibrated regularly in the laboratory, and were controlled once a year by the manufacturer.

[28] Jandel Scientific GmbH, Erkrath, D
[29] Drägerwerk AG, Lübeck, D

Table 11 gives an overview of the gas measuring apparatuses used, the measuring principle, the measurement range, and the resolution. The error of all Multiwarn sensors is smaller than 5 % of the measured value, and ± 0.2 vol % for the oxygen measurements.

Table 11: Characteristics of the air sampler used.

gas	type of air sampler	measuring principle	measurement range	resolution
oxygen (O_2)	Multiwarn P, O_2-Ex-H_2S	galvanic cell	0-25 vol%	0.1 vol%
hydrogen sulfide (H_2S)	Multiwarn P, O_2-Ex-H_2S	electrochemical	0-200 ppm	1 ppm
methane (CH_4)	Multiwarn P, O_2-Ex-H_2S	catalytic oxidation (heat of reaction)	0-100 % LEL (= lower explosion level of explosive gases) 100 % = 5 % methane	1 % LEL
carbon dioxide (CO_2)	Multiwarn IR, P/CO_2-CO	infrared	0-100 vol% %	*
carbon monoxide (CO)	Multiwarn IR, P/CO_2-CO	electrochemical	0-200 ppm	1 ppm
ammoniac (NH_3)	Dräger tubes ammoniac 2/a and 5/a	chemical	2-30 ppm and 5-70 ppm	-
hydrogen (H_2)	Dräger tubes hydrogen 0.2%/a	chemical	0.2-2 vol%	-
carbon dioxide (CO_2)	Dräger tubes carbon dioxide 0.5%/b, 1%/a and 5%/A	chemical	0.5-10 vol%, 1-20 vol% and 5-60 vol%	-

* 0.01 vol% % in the range of 0.5 vol%
0.1 vol% in the range 4.5-10 vol%
1 vol% in the range 9.5-100 vol%

2.2.2 BIOAEROSOL MEASUREMENTS

For measuring the content of microorganisms in the air, we used different bioaerosol samplers:

- *Surface Air Sampler (SAS) Standard*[30] *with a 90 mm head*
- SAS Super 90[30] with a 90 mm head
- MAS-100[31]

The samplers used present the following advantages, compared to other samplers on the market (Anderson, Reuter Centrifugal Sampler (RCS))

- *light, compact, battery-powered apparatus*
- one-stage samplers, thus a reduced number of Petri-dishes
- quite high air-flow (90-180 liters/min), making possible repeated measurements during the often short moments of bioaerosol generation, e.g. dumping of waste
- utilization of Petri-dishes with a diameter of 9 cm, which allowed the counting of up to 250 fungal colonies, in contrast to the RCS, where particles are impacted on agar strips of 2 cm width

[30] Pool Bioanalysis Italiana (PBI), Milano, I
[31] MBV, Stäfa, CH

The MAS presented several additional advantages:

- use of standard Petri dishes, in contrast to the SAS, where special contact plates filled exactly to the rim with near medium have to be used
- integrated flow monitor that regulates the aspirated air to a constant value of 100 l/min, thus compensating for wind during sampling, as well as varying volumes of agar in the Petri dishes

The characteristics of each sampler are presented in Table 12.

Table 12: Characteristics of the bioaerosol samplers used (JENSEN et al., 1994 and R., MEIER, Novartis Pharma AG, Basel CH, personal communication).

sampler	U [m/s]	d_{50} [µm]	Q [l/min]	no. of holes	minimum sampling volume [l]	upper limit of detection [cfu/m³] [a]
SAS Standard	14.70	2.0	180	487	50	$5.5 \cdot 10^4$
SAS Super 90	8.72	2.7	90	487	10	$3.3 \cdot 10^5$
MAS-100	11.00	1.6	100	400	1	$2.6 \cdot 10^6$

U velocity of air through a hole
d_{50} cut diameter, above which the collection efficiency of the impactor approaches 100 % (see Figure 7)
Q airflow rate
[a] with the minimum sampling volume

The samplers differed in the airflow rate, affecting the minimum sampling volume, and therefore the upper limit of detection. Sampling volume was between 1 and 1000 l, depending on the suspected concentration of microorganisms and the minimum sampling volume of the apparatus.

For the SAS sampler, Omiko® (Oberflächen Mikroorganismen Kollektor)[32] plates filled to the rim (14 ml agar/plate) were used. For the MAS-100, normal Petri dishes plates were used.

If an agar plate contained more than 250 cfu, the result was given as "bigger than the upper limit of detection", calculated as the probable number of germs at the maximum of colonies possible (487 for the SAS, and 400 for the MAS). If sampling volumes higher than the minimum were chosen, a lower detection limit resulted.

The measurements were normally carried out at 1.5 m height from the ground (nose height). An important factor when collecting air spora is that the sampler is held in a position such that the air stream faces the entrance, otherwise concentrations are underestimated (EDMONDS, 1979). Sampling in the open-air was always carried out holding the sampler horizontally. However, it proved to be almost impossible to measure always in the direction of the wind, as it changed direction all the time, due to eddies forming around obstacles.
At each sampling location, three replicate measurements were made.

Outdoors, speed and direction of wind were detected at the location of the bioaerosol measurement, with an anemometer (Windmaster Mark 3)[33].

The choice which sampler to use was made on its availability (the MAS-100 was only purchased in the last year of the project). Often, samplings were carried out simultaneously in different locations, so two samplers were used in parallel.

[32] Manufacturer: Petraplastik AG, Chur, CH
[33] Schiltknecht Messtechnik AG, Gossau (ZH), CH

After exposition, the agar plates were removed, incubated, and the grown colonies counted. To account for the probability of more than one particle passing through a single hole in the sieve plate, but forming only a single colony, the colony numbers were corrected for "Positive Holes" according to the following formula (FELLER, 1968; ANDERSEN, 1958):

$$P_r = N\left\{\frac{1}{N}+\frac{1}{(N-1)}+\frac{1}{(N-2)}+\ldots+\frac{1}{(N-r+1)}\right\}$$

P_r = expected number of culturable particles to produce r positive holes, i.e. the cfu counted
N = total number of holes

In this context, it has to be signaled that the conversion table given in the handbook for the SAS Standard and the SAS Super 90 is wrong (calculations based on 260 holes instead of 487 holes). The corrected table is given in Annex I.

To calculate mean fungal concentrations, the geometric mean of the three replica measurements was used, because several studies (MEHTA & PIERSON., 1996; BUTTNER & STETZENBACH, 1993; RAITKONEN, 1992; LACH, 1985; JONES & COOKSON, 1983) demonstrated that airborne microorganisms showed not a normal, but a log normal distribution. The calculation of the geometric mean allowing no values of 0, 2/3 of the lower limit of detection were used instead, according to DOUWES *et al.* (1996), citing RANINGER (1994).
The results were expressed as colony forming units (cfu) per m^3 (= 1000 l) air.

If very high concentrations were suspected, making direct counting on the agar plate impossible, the agar was, after exposition, removed from the plate, suspended in 100 ml physiological salt solution[34] and homogenized for 1 min. in a Stomacher. From this suspension, decimal dilutions were carried out, and plated on agar.
Growth media, incubation conditions, and identification were the same as for the detection of microorganisms in the compost (see Chapter 2.2.4).

Comparative bioaerosol measurements to those carried out at composting sites were done once a month in locations far away from any composting installations:

- In a city center (Neuchâtel, 430 m above sea level)
- In a rural area (Val-de-Ruz, agricultural area, 750 m above sea level)
- On a mountain pass (Vue-des-Alpes, 1300 m above sea level)
- In a suburban area (Neuchâtel, in front of the University building (UNI Mail), 480 m above sea level)

Measurements were always done against the wind. Air temperature and humidity were detected with a combined thermometer/hygrometer H 270[35].

[34] NaCl, 9 g/l
[35] Dostmann electronic GmbH, Wertheim-Reicholzheim, D

2.2.3 COMPOST SAMPLING

Compost samples were taken for microbiological and chemical analyses, usually at the same locations where temperature and gas measurements had been carried out previously. Sampling at the surface was done by hand, inside the heap with a compost auger (diameter 10 cm, length of head: 25 cm). The given sampling depth indicates the position of the middle of the auger head. If not stated otherwise, about 2 kg of compost were taken, filled in a plastic bag, and refrigerated immediately. Samples were transported cooled to the laboratory, and processed immediately, or stored at 4°C until the following day.

2.2.4 MICROBIAL ANALYSIS

Microbial analysis was carried out to determine the amount of microorganisms present in the compost samples, either of potentially pathogenic or indicator organisms (*AF* and coliforms), or of those responsible for the degradation of the organic matter.

From each well-mixed compost sample, 30 g of wet compost were suspended in 270 ml sterile, deionized water. These suspensions were shaken for 30 minutes at 150 revolutions per minute (rpm) at room temperature, to detach the microorganisms from the compost particles.
From the basal suspension decimal dilutions (1 ml in 9 ml) were carried out, either in physiological salt solution or in nutrient broth[36].
After incubation, the by the naked eye visible colonies were counted, and the weighted mean of colony numbers was calculated according to the following formula:

$$c = \frac{\Sigma_c}{n_1 \cdot 1 + n_2 \cdot 0.1 + n_3 \cdot 0.01}$$

c:	weighted mean of colony numbers
Σ_c:	sum of the colonies of all plates that were considered for calculations
n_1:	number of plates of the lowest dilution analyzable
n_2, n_3:	number of plates of the next higher dilution steps

The Most-Probable-Number (MPN) of organisms was calculated from the number of positive reactions per dilution step by a computer program (HURLEY & ROSCOE, 1983), taking into account the dilution factor.
All results were expressed as colony forming units (cfu) or Most Probable Number (MPN) per dry weight of compost (DWC). All graphs show a logarithmic scale for the y-axis.

[36] NB = Nutrient Broth, 8 g/l (Merck, 5443)

***Aspergillus fumigatus*, total molds and yeasts:** 0.1 (for plates with a diameter of 9 cm) or 1 ml (for plates with a diameter of 12 cm) from 3 successive dilutions in physiological salt solution were plated in parallel on agar plates. The following medium was used: Malt Extract Agar[37] with addition of the antibiotics streptomycin[38] and novobiocine[39]. Incubation was carried out for 24-48 h at 40°C. *AF* colonies were differentiated from other thermotolerant molds and yeasts by their characteristic green-gray color, the columnar shape of the conidial heads and the size of the spores (SAMSON & VAN REENEN-HOEKSTRA; 1988, see also Figure 5). The number of cfu was calculated as the weighted mean of the colonies counted at each dilution. Results were expressed as cfu/gDW of *Aspergillus fumigatus* (*AF*) and of total thermotolerant molds and yeasts (TTM). Lower detection limit was 5-10 cfu/g fresh weight of compost (FWC).

Coliforms, *Enterabacteraceae* and Gram⁻ bacteria: 0.1 ml from 3 successive dilutions in physiological salt solution were plated in parallel on 9 cm diameter agar plates. If not stated otherwise, the spread plate method was employed. The lower detection limit in that case was 50 cfu/g FWC. The following media were used:

MacConkey Agar[40]: Incubation for 24h, counting of red colonies only, 37°C: total coliforms; 44°C: fecal coliforms.
Incubation for 48h, counting of all colonies, 37°C: Gram-negative bacteria.

Modified Tergitol Agar (MTA)[41]: Incubation for 24h at 37°C, counting of red colonies = total coliforms. Lower limit of detection: 50 cfu/gFW.

VRB-Agar[42]: Inoculation in the agar mass, with overlayer, incubation for 24-48h. Only red colonies with a diameter of minimum 0.5 mm were considered. Lower limit of detection: 5 cfu/gFWC.

CC-Agar[43]: Inoculation on the surface or in the agar mass, incubation at 37°C, for 48h. Enumeration of the different groups of bacteria (*Enterobacteraceae*, coliforms, *E. coli*) according to the colony color, as indicated in Table 13.

EC-ID-Agar[44]: Inoculation on the surface or in the agar mass with overlayer, incubation at 37°C, for 48h. Enumeration of the different groups of bacteria (*Enterobacteraceae*, coliforms, *E. coli*) according to the colony color, as indicated in Table 13.

ECC-Agar[45]: Inoculation on the surface or in the agar mass with overlayer, incubation at 37°C, for 48h. Enumeration of the different groups of bacteria (*Enterobacteraceae*, coliforms, *E. coli*) according to the colony color, as indicated in Table 13.

[37] MA = Malt Agar: Malt Extract (Difco, Basingstoke, UK, L39) 20 g/l, Agar agar (Merck, 1.01614) 15 g/l
[38] Streptomycin sulfate (Fluka Chemie, Buchs, CH, 85880) 0.05 g/l
[39] Novobiocin sodium salt (Fluka, 74675) 0.01 g/l
[40] MC = MacConkey Agar (Merck, 5465)
[41] MTA = Modified Tergitol Agar, pH 7.2: Meat Extract (Merck, 1.03979) 5 g/l, Peptone from caseine, pancreatically digested (Merck, 1.07213) 10 g/l, Yeast Extract (Merck, 1.03753) 6 g/l, Lactose 20 g/l, bromothymol blue 0.005 g/l, Agar Agar 14 g/l, with the addition of 2,3,5-triphenyl-tetrazoliumchlorid (TTC), 0.025 g/l and 3,9-diethyl-6-tridecanol hydrogensulfat sodium salt (Tergitol 7 or Imbentin-AGS/35) 0.1 g/l, after autoclaving
[42] VRB = Violet-Red-Bile-Agar (Merck, 1.01406)
[43] CCA = Chromocult® Coliform Agar (Merck, 1.01406), with the addition of cefsulodin sodium salt (Fluka, 22126) 15 mg/l, after autoclaving
[44] EC-ID = *E. coli* Identification Agar (bioMérieux SA, Marcy l'Etoile, F)
[45] ECC = *E. coli*/Coliform Agar (CHROMagar®, Paris, F

LMX-broth[46]: 8 x 0.18 ml from 4 successive 5-fold-dilutions (1 ml in 4 ml) in LMX broth were distributed into sterile 96-hole microtiter plates and incubated for 48-72 h at 37°C. For each dilution, the number of wells showing a change of the indicator from yellow to blue-green were recorded.
For the determination of the *E. coli* MPN, the number of wells showing fluorescence under UV light (366 nm) and being indole positive (appearance of a red color after the addition of 2 drops of the Ehrlich reactive[47] to each well) were taken into account.
The results were expressed as MPN/gDWC. The lower detection limit was 28 MPN/gFWC.

Table 13: Appearance of bacterial colonies on different selective media. ng = no growth.

medium	MacConkey	VRB	TGA	CCA	EC-ID	ECC	LMX
Gram⁻ bacteria	colorless or red	-	colorless to red	ng	white	white	-
Enterobacteraceae	colorless, after 24 h of incubation red	pinpoint, red	red	white	white	white	growth (turbidity)
Coliforms	red	red + halo	red + yellow halo	pink	blue	red	blue-green
Escherichia coli	red	red + halo	yellow + yellow halo	purple	pink	turquoise	blue-green + fluorescent + indole positive

For the identification of strains isolated from the different selective media, commercial systems (API 20[48], API 20NE[48], BIOLOG™ system[49]) were used. Tests were carried out on overnight cultures grown on Nutrient Agar. Inoculation of the test kits, and interpretation of the results was done according to the instructions of the manufacturer. The BIOLOG™ system relies on the ability of the bacteria to oxidize specific substrates distributed in 96 well microliter plates (95 different carbon sources + 1 negative control). Substrate utilization becomes visible by a purple coloration of the well. Prior to identification, the bacterial strain to be tested must be Gram stained, because different plates exist for Gram-negative and for Gram-positive bacteria. Gram reaction was tested (a) by the Gram differential coloration, (b) by the KOH reaction (colonies of Gram-negative bacteria rubbed in a drop of 3 % KOH solution on a glass slide become viscous, while Gram-positive bacteria show no reaction, (GREGERSEN, 1978)), and (c) the aminopeptidase-reaction (colonies of Gram-negative bacteria that are rubbed in a drop of aminopeptidase reactive[50] applied to blotting paper become yellow after about 1 min., while Gram-positives stay colorless).
Oxidase reaction was tested on 24 h old cultures grown on solid media with test sticks[51].

Total heterotrophic microorganisms: 7 x 0.18 ml from 6 successive decimal or 5-fold dilutions in nutrient broth were distributed into sterile microtiter plates and incubated for 48-72 h at 30°C for mesophilic counts or at 50°C or 60°C for thermophilic counts. For each dilution, the number of wells showing growth (turbidity) were recorded, and the MPN calculated.
The results were expressed as MPN/gDWC. The lower detection limit was 28 MPN/gFWC.

[46] Fluorocult® LMX-Broth (Merck, 10620), with the addition of novobiocin (Fluka, 74675) 30 mg/l, after autoclaving
[47] p-dimethylaminobenzaldehyde 4 g, ethanol absolute 380 ml, concentrated HCl 80 ml
[48] bioMériceux SA, Marcy d'Etoile, F
[49] MicroPlate, BIOLOG, Hayward, CA., USA
[50] 4 % solution of L-Alanin-4-nitriloanilide hydrochloride (Fluka 05215) in 50 mM Tris maleate (Fluka 93344), adjusted to pH 7.0 with 5M NaOH
[51] Oxidase Identification Sticks (Oxoid, BR 64)

2.2.5 PHYSICO-CHEMICAL AND CHEMICAL ANALYSIS OF THE COMPOST SAMPLES

In order to follow the progress of the composting process, several physical and chemical parameters were followed in the compost samples. External laboratories performed some of the analyses.

Dry weight (*DW*): 100 g wet compost were pre-dried at 50°C for 24h, and dried at 70°C under vacuum (125 mbar) for another 24h.

pH: 30 g wet compost were suspended in 270 ml deionized water (= 1:9 water extract, basic suspension for the microbiological analysis) and shaken 30 minutes at 150 revolutions per minute. After about 2 hours standing time, pH was measured with a pH-Meter (Digital Ionanalyzer 501[52])

Organic matter (OM), ashes, total nitrogen ($N_{Kjeldahl}$), and total carbon (C) determination were carried out accordingly to the directives of the Federal Research Institute for Agricultural Chemistry and Environmental Hygiene (FAC) (ANONYMOUS, 1995b).

The degree of degradation η, indicating what percentage of the initially present organic matter (OM_0) was degraded after a time t of the composting process, was calculated accordingly to KROGMANN (1994), considering the amount of ash as constant during the whole composting process.

$$\eta(\%) = \frac{100}{OM_0} * \left(1 - \frac{100 - OM_0}{100 - OM_t}\right) * 100$$

Water extractable organic carbon (WEOC) was determined in the following way, according to ZSOLNAY & GÖRLITZ (1994): 5 g air dried compost (at 35°C, during 48h) was extracted in 100 ml deionized water by shaking at room temperature for 1h. The resulting suspension was filtered first through a folded filter, then through a microfilter (0.45 µm). In this filtrate, the DOC (dissolved organic carbon) was determined by catalytic combustion at 780°C (Shimadzu TOC 5000), and the detection of the resulting CO_2 by IR spectroscopy. The laboratory ORLAB carried out these analyses[53].

[52] Orion Research Inc, Cambridge, Mass, USA
[53] Orlab SA, Orbe, Switzerland

3. RESULTS AND DISCUSSION

3.1 LABORATORY EXPERIMENTS - PHYSIOLOGY OF AF

The laboratory experiments were designed to allow a closer look at the influence of one isolated factor - in this case the temperature - on the survival and proliferation of *AF*. As with any laboratory system, it was not possible to reproduce the conditions that prevail in a compost heap: experiments were carried out with *AF* grown on Malt Agar, or with suspensions of mycelium in Malt Extract Broth or spores in physiological salt solution. The different tests were done with strains isolated from composts of different age (GR5: fresh biowaste; GR6: 2 weeks; GR7: 6 weeks; GR8: 10 weeks; GR2: 12 weeks (air during turning)), in order to determine if the time a strain had been exposed to the compost environment had an influence on its physiology, especially on its thermoresistance. However, there is no certainty that an *AF* strain isolated from a mature compost has been in there since the beginning of the process. Infection with material from other heaps could have happened in the course of the process, either by the turning machine, or via the air. The behavior of the compost strains was also compared to that of a clinical strain (G10). For more details, see Chapter 2.1.

3.1.1 GROWTH TEMPERATURE MAXIMA

The results of the growth tests carried out in the Temperature Gradient Incubator (TGI) are presented in Table 14.

First mycelial growth was observed with the naked eye after less than 24 hours of incubation, between 33.3 and 39.0°C and 44.6 and 48.3°C for all the strains tested. Differences between strains isolated from composts of different ages were insignificant, and probably influenced by the moment of the first observation. First spores were observed between 1.2 and 1.7 days of incubation. The maximum temperature at which first sporulation was recorded was generally the same as that of first mycelial growth. Sporulation at the inferior end of the temperature range was slightly delayed. In the course of incubation, mycelial growth up to 50.2°C was quickly established, while growth at the lower temperature happened more slowly. At the end of the observation period (11 days), minimum temperatures at which mycelial growth was recorded was between 14.6 and 16.5°C, and the maximum temperature at 52.1°C. No sporulation occurred at the highest temperature which allowed mycelial growth, except strain G7, which also sporulated at 52.1°C. The minimal temperature at which sporulation was observed, at least in the time span of the observations, was 18.3°C, again with the exception of strain GR7, which also sporulated at 16.5°C.

REISS (1986) indicated a minimum growth temperature for *AF* of 10-12°C, an optimum of 37-43°C, and a maximum of 52-55°C.

Table 14: Temperature range of mycelial growth and sporulation of four AF strains isolated from composts of different age, as a function of incubation time. Incubation was carried out on agar slants, in a Temperature Gradient Incubator (TGI), from 5.2°C to 59.6°C; temperature difference between two tubes (total of 30 tubes) was 1.88°C. For more details, see Chapter 2.1.1. Growth and sporulation were controlled 1-4 times per day visually with the naked eye.

strain	GR5 (from fresh material)				GR6 (from 2 week old compost)			
incubation	mycelial growth		sporulation		mycelial growth		sporulation	
time [days]	min. [°C]	max [°C]	min. [°C]	max [°C]	min. [°C]	max [°C]	min. [°C]	max. [°C]
0.8	39.0	46.5	-	-	39.0	44.8	-	-
1.0	31.5	50.2	-	-	31.5	48.3	-	-
1.7	27.7	50.2	37.1	46.5	24.0	48.3	35.2	46.5
1.8	25.8	50.2	31.5	48.3	24.0	50.2	35.2	48.5
2.0	25.8	50.2	31.5	48.3	24.0	50.2	35.2	48.3
2.5	22.1	50.2	29.6	48.3	24.0	50.2	27.7	48.3
2.7	20.2	50.2	29.6	48.3	22.1	50.2	27.7	48.3
2.8	20.2	50.2	29.8	48.3	22.1	50.2	27.7	48.3
3.0	20.2	50.2	29.6	48.3	22.1	50.2	27.7	48.3
3.5	20.2	50.2	29.6	50.2	20.2	52.1	27.7	48.3
8.6	16.5	52.1	20.2	50.2	18.5	52.1	27.7	50.2
10.6	16.5	52.1	18.3	50.2	16.5	52.1	18.3	50.2

strain	GR7 (from 6 week old compost)				GR8 (from 10 week old compost)			
incubation	mycelial growth		sporulation		mycelial growth		sporulation	
time [days]	min. [°C]	max [°C]	min. [°C]	max [°C]	min. [°C]	max [°C]	min. [°C]	max. [°C]
0.9	33.3	46.5	-	-	35.2	48.3	-	-
1.0	33.3	48.3	-	-	33.3	48.3	-	-
1.1	29.6	50.2	-	-	31.5	48.3	-	-
1.2	29.6	50.2	39.0	46.5	31.5	50.2	37.1	46.5
1.8	25.8	50.2	39.0	46.5	29.6	50.2	37.1	46.5
2.1	25.8	52.1	33.3	48.3	29.6	52.1	31.5	48.3
2.3	25.8	52.1	33.3	50.2	25.8	52.1	29.8	48.3
2.9	22.1	52.1	29.6	50.2	22.1	52.1	29.8	50.2
3.9	22.1	52.1	27.7	50.2	18.3	52.1	29.8	50.2
5.8	22.1	52.1	25.8	50.2	18.3	52.1	29.6	50.2
6.8	22.1	52.1	24.0	50.2	16.5	52.1	25.8	50.2
7.8	22.1	52.1	22.1	50.2	16.5	52.1	24.0	50.2
8.3	14.6	52.1	18.3	50.2	18.6	52.1	18.3	50.2
8.9	14.6	52.1	18.3	52.1	16.5	52.1	18.3	50.2
10.0	14.6	52.1	18.3	52.1	14.6	52.1	18.3	50.2
11.0	14.8	52.1	16.5	52.1	14.6	52.1	18.3	50.2

3.1.2 FROM GERMINATION TO SPORULATION

The detection of the moment of the first mycelial growth and the appearance of the first spores (see Chapter 3.1.1 above) was made with the naked eye. However, at a microscopic level, these events could certainly be observed earlier. To be able to follow germination, mycelial growth and sporulation microscopically, *AF* spores were placed on a glass slide covered with a thin agar layer. The slides were incubated in a humid atmosphere to avoid desiccation, and observed regularly during 24 hours. Incubations were carried out at temperatures below (30°C) and above (50°C) as well as at that of maximum growth (37-45°C). On the one hand, malt agar was used, as in other laboratory experiments, on the other hand an aqueous compost extract solidified with agar (for details, see Chapter 2.1.3), to more closely simulate conditions encountered in compost.

The microphotographs depicted in Figure 11 show the life cycle of *Aspergillus fumigatus*. The cycle started with the dry conidia (Figure 11-1), which had a diameter of 2-4 µm. Upon incubation on agar, the spores started to swell (Figure 11-2), due to the absorption of water, and a number of cytological changes in the spore: division of the nucleus, increase in the quantity of endoplasmic reticulum and number of ribosomes and mitochondria. At the same time, the metabolic rate increased and the enzymatic system got activated. One germ tube then started to form, visible at the moment when it broke through the outer spore wall (Figure 11-3). Its growth continued by apical extension (Figure 11-4 and 11-5), and branching and multilateral hyphal growth out of one spore could be observed (Figure 11-6). At some points, where several spores were close together, the formation of microcolonies (Figure 11-6) was noticed. Generally, development was much advanced in those colonies compared to single spores. Hyphal growth continued until the hyphae from single spores started to touch each other (Figure 11-7). After that moment, first aerial hyphae were observed in the microcolonies (Figure 11-8). From this instant on, the mycelium was visible with the naked eye. Growth out of the single spores continued, to form a dense two-dimensional network of hyphae (Figure 11-9) that later became three-dimensional (Figure 11-10). The next stage was the formation of the conidiophores, first only visible at the edge of a microcolony, by a vertical hyphae, the conidiophore stipe, with a swollen end (Figure 11-11). From the vesicle, uniseriate philaides grew out (Figure 11-12). From these conidiogenous cells, the conidia, e.g. external, asexual spores were formed (Figure 11-13). Young conidial heads with chains containing only a few conidia appeared spherical (Figure 11-14), while mature ones showed the for *AF* typical columnar shape (Figure 11-15). At the end of the cycle, the mature conidia got dispatched from the conidial head (Figure 11-16).

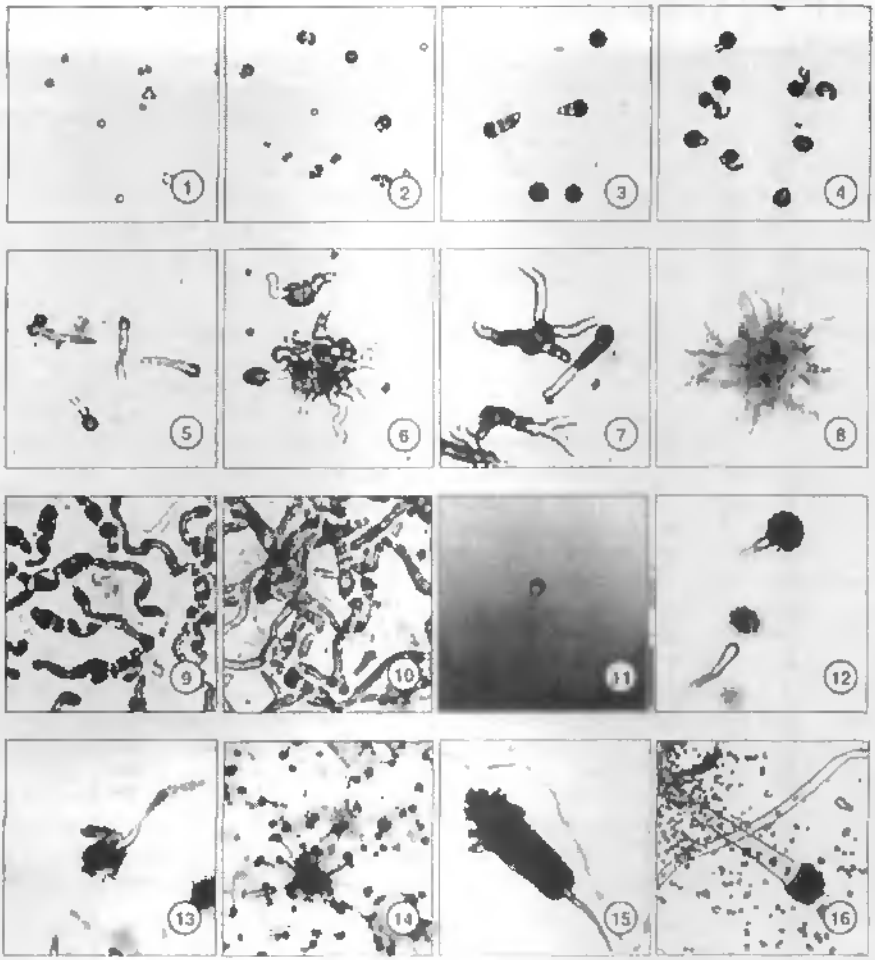


Figure 11: *Life cycle of Aspergillus fumigatus (strain GR2), grown on MEA or Compost Extract Agar, at 37 or 45°C. Microphotographs (bright field) were taken with a Leitz Dialux 20 EB.*
1: normal spores; 2: swollen spores; 3: appearance of first germination tubes; 4: length germination tube > diameter of spore; 5: length germination tube 5-10 x diameter of spore; 6: multilateral germination, appearance of first microcolonies; 7: hyphae growing out from single spores start touching each other; 8: first aerial hyphae in microcolonies; 9: dense two-dimensional net of hyphae; 10: three-dimensional net of hyphae, 11: appearance of first conidiophores at the edge of microcolonies; 12: conidiophores with philaides; 13: conidial heads with first spores; 14: conidial heads with spores, round; 15: conidial heads with spores, columnar; 16: mature spores which get easily dispatched from the conidial head.

Table 15 shows the time that was needed to reach a certain stage of development under the chosen experimental conditions, at the example of *AF* strain GR2.

Table 15: Time [hours] needed for AF strain GR2 to reach a certain stage of development. Incubation was carried out on different media (MEA = malt extract agar; CEA = compost extract agar (1:4 aqueous, clarified compost extract) and at different temperatures in a humid atmosphere (100% rH). Numbers indicating a stage of development correspond to the microphotographs shown in Figure 11. no = no observations made.

agar	temp.	stage of development							
medium	[°C]	1	2	3	4	5	6	7	8
MEA	30	0	4	9	-	10	-	11	12
CEA	30	0	4	7	9	12	-	13.5	-
MEA	37	0	3	7	8	-	9	10	11
CEA	37	0	3	5	7	-	8	9	10
MEA	45	0	-	3	5	7	9	10	11
CEA	45	0	3	7	8	-	9	12	18
MEA	50	0	4	15	-	18.5	-	18	19.5
CEA	50	0	4	13.5	16.5	19.5	-	21.5	nd
agar	temp.	stage of development							
medium	[°C]	9	10	11	12	13	14	15	16
MA	30	13.5	15	18	19.5	21.5	24	-	68.5
CE	30	15	16.5	19.5	21.5	24	-	68.5	
MA	37	12	13.5	-	15	16.5	18	19.5	24
CE	37	11	15	19.5	-	-	21.5	-	68.5
MA	45	12	13.5	16.5	19.5	22	68.5		
CE	45	21.5	24	-	-	-	68.5		
MA	50	-	21.5	no	no	no	no	no	no
CE	50	no	no	no	no	no	no	no	no

It took 3-4 hours for all media and temperatures tested until the spores were activated, visible by a swelling (stage 2). For the appearance of the first germination tubes (stage 3), a difference was observed between the MEA and the CEA: the latter favored the germination stadium, except at 45°C. However, the further stages, e.g. hyphal growth (stages 4-10), were more rapidly attained on the more nutrient rich Malt Extract Agar. The spore formation (stages 11-16) seemed not to be much influenced by the environmental factors: once hyphal growth had attained a certain density, spore formation was induced in any case. The minimum period of vegetative development required before the organism became competent to produce reproductive bodies was very short in *AF*: at the optimal growth temperature, only approximately ten hours passed between the moment of the hyphal outgrowth and the formation of the first spores. Also, the development of reproductive structures seemed not, as in other fungi, be dependent on some limiting nutrient factors, as sporulation happened as well as on the nutrient rich MEA as on the nutrient poor CEA.

As expected, germination was more rapid at the temperatures ideal for growth of *AF* (37 and 45°C), than at 30°C, and especially at 50°C, where the whole development was greatly delayed. It has to be mentioned that at this temperature, incubation could not be prolonged to more that 21.5 hours, because the very fine agar layer on which the fungus was grown dried out, in spite of incubation in 100 % rH.

The same experiment was carried out with the strains G10 and GR7. The course of development was slightly enhanced on MEA in the reference strain G10, and slightly slower in GR7, indicating that some differences existed between the different strains. However, spores were obtained with all strains on all media and at all incubation temperatures (except at 50°C) within 24 hours.

In the view of the development of *AF* in compost, this implies that, under ideal conditions, the mold can undergo one whole life cycle between daily carried out turnings. However, the results of the experiment at the compost installation of Grenchen (see Chapters 3.2.1.2A and B) showed that even at the windrow surface, where temperatures where permissive for *AF* growth, only very low fungal numbers were measured when regular turnings were carried out. This would mean that thermohygienization towards *AF* of the material in the core was complete, and re-infection with *AF* from outside (air, turning machine) was minimal. Also, fungal growth conditions at the surface of compost particles might not have been as good as in the laboratory on agar medium, or that concurrence with other fungi or bacteria or predation by microinvertebrates had inhibited the development of *AF*. Studies in a composting system, e.g. by taking samples at short intervals (every 2 hours) after a turning event, and observation of spore germination, could give information about the behavior of *AF* in the field situation.

3.1.3 GROWTH RATE

A mold colony developing on a solid medium consists of hyphae that grow radially outward from the point of inoculum, with the hyphae at the colony margin being approximately parallel to one another, and at the same distance apart (TRINCI *et al.*, 1994).

The growth of strain GR7 on Malt Agar in a humid atmosphere (desiccator with water at the bottom) at 45°C is depicted as an example (Figure 12). Growth started slowly, to become logarithmic from the 2nd day until the 11th day. After that moment, colony extension slowed down, most probably due to the exhaustion of available nutrients in the medium (TRINCI *et al.*, 1994). The curves had a similar form for all strains and all temperatures (10-60°C) tested, regression coefficients were always close to 1, and the experiments were highly reproducible.

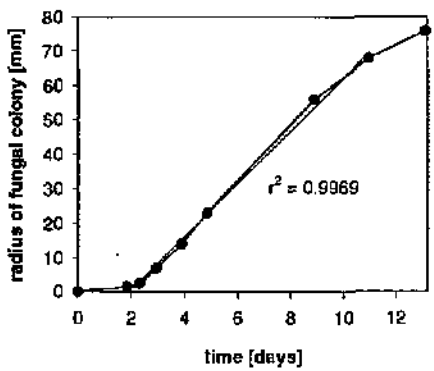


Figure 12: Diameter of an AF colony (strain GR7) as a function of time at 45°C. The fine straight line is the regression line drawn from the part of the graph where growth is linear. r^2 = regression coefficient.

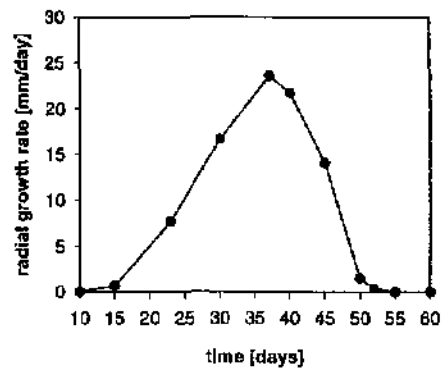


Figure 13: Radial growth rate of mycelium of AF (strain GR7) as a function of temperature.

Figure 13 shows the radial growth rate, calculated from the part of the growth curves where mycelial extension was linear, as a function of temperature. The example of strain GR7 is presented. Temperature of maximal growth rate (37°C), and maximal growth rate (between 22 and 28 mm/day) were almost identical for all the strains tested. The results corroborate those obtained by NESSI (1994) with *AF* strains isolated from garden composts.
Compared with the thermophilic fungus that is second most frequently found in compost, *Scytalidium thermophilum*, *AF* showed a lower temperature optimum for the maximum growth rate (*S. thermophilum*: 46-48°C), but a much higher growth rate (*S. thermophilum*: maximum 12 mm/day, (WIEGANT, 1992)). This could explain the large predominance of *AF* in compost when temperatures are ideal for its growth. The successful colonization of compost by *AF* might also be due to the different modes of reproduction of the two fungi: while *AF* produces a high number of easily dispersible conidiospores (see Figure 5), are the clamydospores of *S. thermophilum*, produced in lesser number, not readily airborne (see Figure 27).
Temperatures that supported maximum growth rate were slightly lower (37°C) compared to the temperatures at which mycelial growth first appeared (between 33.3 and 39.0°C and 44.6 and 48.3°C, Table 14. The difference might be due to the much more exact incubation temperature control in the TGI.

3.1.4 TEMPERATURE RESISTANCE OF *AF*

While growth maxima and growth rate, as well as germination and sporulation play a role for the colonization or the recolonization of compost with *AF*, its thermoresistance is of importance when determining the temperature-time combination needed for its destruction. According to REISS (1986), mycelium gets inactivated in 5-10 minutes in moist heat (60°C), and asexual mold spores have a thermoresistance that is 5-10°C above that of the mycelium. However, several authors (KOTHARY & CHASE, 1984; GEDEK, 1980; HAINES, 1995; see Chapter 1.8.1.2A) reported that spores of *AF* survived at higher temperatures, without giving, though, detailed experimental data. Own experiments were thus carried out to determine the thermal inactivation as well of the mycelium as of the spores of *AF*.

3.1.4.1 MYCELIUM

Normally, thermal inactivation curves of microorganisms are obtained by enumerating surviving cells (cfu or MPN). This is not possible with fungi, because they are modular organisms, e.g. one fungal individual can give rise to more than one colony. To carry out decay experiments with *Scytalidium thermophilum*, WIEGANT (1992) monitored the CO_2 evolution at 45°C, as a function of time after incubation at various temperatures. We adapted this method to monitor the activity of *AF* mycelium by following the oxygen consumption with a Clark type electrode, after exposition to exactly maintained temperatures in a circulating water bath during various lengths of time (see Chapter 2.1.4.1).

Figure 14 shows the oxygen consumption (nmol O_2/ min·mgDW of mycelium), as a function of incubation time, at the example of strain GR2 exposed to 60°C. The oxygen consumption was calculated from the slope of the respiration curves, which were linear over the measuring time (2-10 minutes).

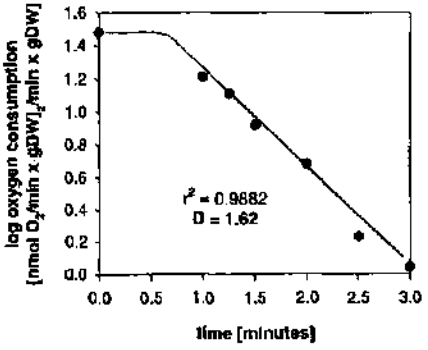


Figure 14: Loss of activity, expressed as oxygen consumption (respiration), in AF mycelium (strain GR2) after incubation at 60°C for different lengths of time. Respiration was carried out at 37°C in Malt Extract Broth. • experimental points; — computed death rate with points after exposition; ----- possible course of the inactivation curve. r^2 = correlation coefficient.

Loss of activity was exponential (continuous line), confirming the theory that thermal inactivation of microorganisms obeys a first order model (SCHLEGEL, 1985). Thus, by plotting the logarithm of the oxygen consumption against the incubation time, a straight line was obtained (Figure 14). The shoulder (dotted line) could be due the small temperature decrease observed after addition of the mycelium suspension to the *tube containing 2/3 of preheated suspension* medium. The D-value, defined as the time required at a given temperature to destroy 90 % (1 log) of the fungal activity, was calculated as the reciprocal value of the slope of the linear part of the inactivation curve. This means that only the points obtained with the samples that were exposed to the temperature were used.

The data points and regression lines for the inactivation of *AF* mycelium (at the example of strain GR2) at different temperatures (40-60°C) are shown in Figure 15. No inactivation was observed at 40°C. With increasing exposure temperatures, inactivation was more and more rapidly attained: above 52°C, activity was reduced to zero in less than half an hour.

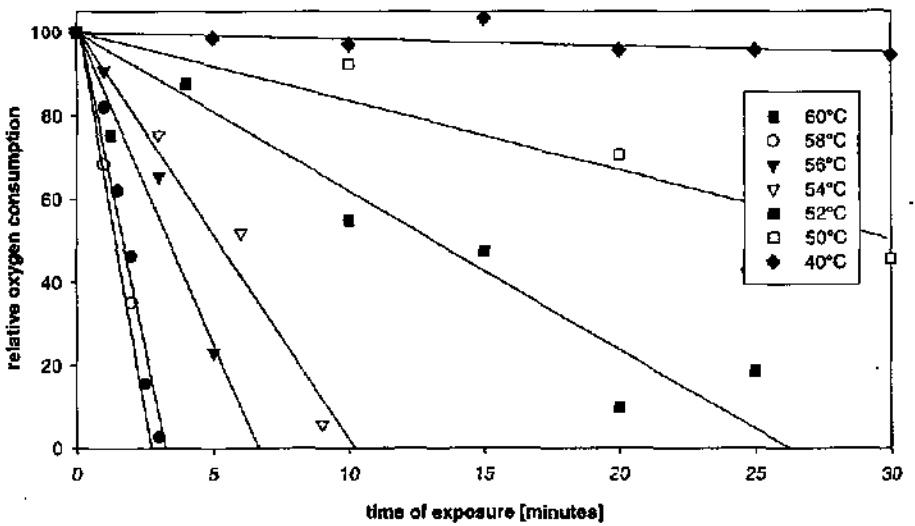


Figure 15: Loss of activity, expressed as relative oxygen consumption (respiration of the non-exposed mycelium = 100) for AF mycelium (strain GR2), after exposition to different temperatures.

Table 16: D-values (minutes for a reduction of activity by 90 %) of AF mycelium. The strains GR2, GR5 and GR8 were isolated from compost, G10 is a medical reference strain.

incubation temp.[°C]	D value [min.]			
	GR2	GR5	GR8	G10
60	1.0	2.6	1.5	1.5
58	1.7	3.7	2.0	1.7
56	4.5	6.7	3.1	3.7
54	10.7	10.8	8.7	7.7
52	14.4	9.9	26.4	12.0
50	27.3	58.5	nd	nd
40	1111.0	nd	nd	nd

Table 16 shows the D-values, calculated from the slopes of the inactivation curves, for the different strains tested. At 60°C, it took only a few minutes to reduce the activity almost completely. With increasing incubation temperatures, the D-value increased. At 52°C, a temperature where growth was still possible (see Figure 13), activity was nevertheless reduced to 10 % after 12-26 minutes of exposure. This could be a transitory response to the temperature change from 37°C (incubation temperature) to the test temperature. Cells cultivated at the optimal growth temperature (37°C, see Figure 13) possessed probably not the same set of enzymes, especially thermoresistant isoenzymes, membrane lipids or other stabilizing molecules than strains cultivated near the upper growth limit. To prove this hypothesis, comparative experiments with cells pre-grown at the test temperature should be carried out. At 40°C, no reduction of the activity was observed.

All the strains tested showed the same pattern, although differences were observed for individual results, without clear tendencies. However, the results show clearly that at elevated temperatures (> 60°C), as often encountered in compost, *AF* mycelium would be inactivated almost immediately, unless the compost matrix would have a protective influence.

3.1.4.2 SPORES

Very little information is available about the thermoresistance of *AF* spores: REISS (1986) indicated that normally asexual mold spores had a thermoresistance that was 5-10°C above that of the mycelium. However, several authors (HAINES, 1995; KOTHARY & CHASE, 1984; GEDEK, 1980) reported that spores of *AF* survived for some time at temperatures as high as 80°C. The only experiments carried out about the thermoresistance of *AF* spores in the context of compost were done by AMLINGER (1993), who found in laboratory experiments that all spores of *AF* were eliminated at temperatures of 64-72°C in a few minutes.

To get more precise details about the thermoresistance of *AF* spores, experiments with 2 compost (GR2, GR7) and 1 medical reference strain (G10) were carried out. The spores ($2 \cdot 10^6$ spores/ml) were suspended in phosphate-citrate buffer (pH 7.0), and exposed in a circulating water bath to exactly maintained temperatures (55°C to 75°C, in intervals of 5°C). In order to bring the sample to the designated temperature, it was pre-heated for 2 minutes (temperature raising period, initial spore concentration after the pre-heating period = N_2), after which the temperature holding period followed. After exposition, the viable spore count was carried out. (For details, see Chapter 2.1.4.2).

Figure 16 presents examples of thermal inactivation curves (ratio N/N_2 of the spore number after N minutes of exposure over spore numbers after the pre-heating period N_2, on a logarithmic scale, versus the time of exposure). At intermediate test temperatures (60°C) the curves showed the form depicted in Figure 16: a shoulder (time lag or lag phase) at the beginning of the heating, an accelerated decline (log phase), and a tail.

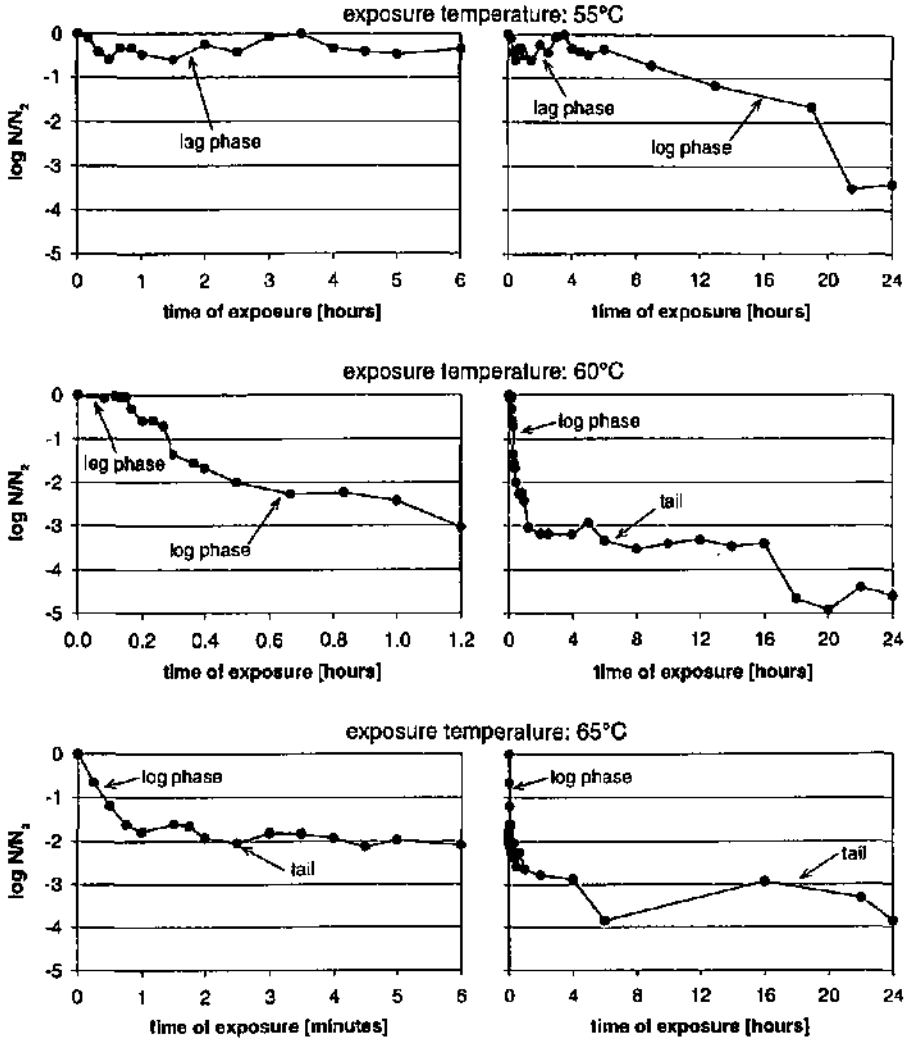


Figure 16: Thermal inactivation curve at 55°C, 60°C and 65°C of spores of AF strain GR2. The data are reunited from 3 different experiments. left: first minutes/hours of the experiment, right: whole length of the experiment (24 hours).

The lag time varied as a function of temperature (several hours for curves obtained at 55°C, absent for 65°C). Tails of the inactivation curves were observed at 60°C and 65°C incubation; at 55°C, no tail phase was reached in the 24 hours the exposure experiment lasted. At 70°C and 75°C, concentrations were below the detection limit (9 MPN) already after the pre-healing (2 min).
Test temperatures had no big influenced on the proportion of surviving spores: this was always between 10^{-3} and 10^{-4}. Data at 65°C were inconclusive; sometimes, a surviving population was observed at the end of the experiment, sometimes not.

Table 17: D-values (time in minutes for the reduction of spore numbers by 90%) for the lag and the tail phase of the different strains tested, as a function of exposure temperature. nd = not detected.

strain	temp.	log	tail
	55°C	667	nd
GR2	60°C	11	703
	65°C	0.6	124
	55°C	539	nd
GR7	60°C	8.9	834
	65°C	0.8	142
	55°C	410	nd
G10	60°C	31	436
	65°C	0.8	313

Estimated D-values (calculated by linear regression of the linear portions of the inactivation curve) for the log as well as for the tail phase are shown in Table 17. The three strains tested showed similar results. At 55°C, the D-value of the log phase amounted to several hours, at 60°C to a dozen of minutes, and at 65°C to less than one minute.

When comparing the D-values at 60°C of the mycelium (Table 16) with those of the spores during the log phase (Table 17), one observes an about 10 fold higher resistance of the latter.

The D-values of the tail phase were always significantly higher than those of the log phase. While almost no inactivation was observed at 60°C, did the exposure to 65°C lead to D-values of approximately 2 hours.

It was often stated that the thermal inactivation of microorganisms obeys a first-order model. However, observation of sigmoid survival curves were frequently made. According to FUJIKAWA & ITOH (1996), tailing in the survival curves of fungal spores is often very pronounced.

The same authors carried out experiments with *A. niger* spores. On the basis of the very tailed thermal inactivation curves observed, they postulated that thermotolerant cells were generated during the heating. In fact, their experimental data fitted closely a thermotolerant subpopulation model:

$$N/N_0 = p^{-k_1 t} + (1-p)^{-k_2 (t-t_d)}$$

N = number of cfu/ml at time t
N_0 = number of cfu/ml at time 0
p = proportion of thermotolerant cells of the total population
k_1 = death rate coefficient for the thermotolerant subpopulation
k_2 = death rate coefficient for the normally sensitive cells
t_d = time lag (shoulder)

The proportion of surviving cells was more or less constant, between $2.6 \cdot 10^{-3}$ and $2.8 \cdot 10^{-4}$ for the different temperatures tested, and could be calculated by $\ln p = 0.0001037T^2 - 0.01187T + 0.337$. k_1 was 0, e.g. the tail was horizontal; k_2 increased when increasing the exposition temperature. The lag time (t_d) was longer at lower temperatures. Spores cultured from the surviving population showed no increased thermotolerance compared to the original population. Reheating of the surviving spores, after a holding period of a minimum of 3 h at room temperature, did gave the same thermal inactivation curves as those of the original population. However, as spore numbers were reduced by 3-4 order of magnitudes at each heating, repeated heating periods (in the experimental set-up: 30 minutes at 3 h intervals) lead to a complete inactivation of the spores.

No explanation was given about the possible mechanisms of thermotolerance acquisition. Often, the production of heat shock proteins is evoked (MADIGAN *et al.*, 1997). Agglomeration of spores during the heating was not observed.

We did not carry out the experiments described above with our *AF* strains. However, the close resemblance of the thermal inactivation curves let suppose that *AF* spores would behave similar to *A. niger* spores. Comparison of the D-values of the log phase showed higher values for *AF* (approx. 10 minutes at 60°C, compared to approx. 1 minute for *A. niger*). This can be explained by the generally higher thermotolerance of *AF* (growth maximum at 52-55°C) compared to *A. niger* (45-47°C) (REISS, 1986).

The thermosubpopulation model (FUJIKAWA & ITOH, 1996) does take into account that thermal inactivation curves of fungal spores have a lag time (t_d). However, it can not describe the exact form of the shoulder. KING *et al.* (1979) calculated nonlogarithmic death curves for *Byssochlamys fulva* and other microorganisms. The formula $(\log N_0 - \log N)^a = kt + C$ linearized the data, the death rate k being the slope of the curve. The value a could be computed from the slope of the curve resulting by plotting log (log N_0-log N) against log t. Calculated data closely fitted experimental data, as well as recalculations of non-logarithmic death curves from the literature.

Thus, to accurately describe the thermal inactivation curves of *AF*, the models of FUJIKAWA & ITOH (1996) and KING *et al.* (1979) have to be combined. Of course, experimental data should be carried out to confirm the validity of such a new model.

Considering the effectiveness of intermittent heating for a complete inactivation of fungal spores that show a tailed inactivation curve, the concept of furthering thermohygienization by frequent compost turning, thereby bringing material repeatedly to the hot center of the heaps, makes sense. Also, observations made during the experiments indicated that tailing of the inactivation curves were only obtained if the exposition temperature was constant, but not when temperatures fluctuated, e.g. when experiments were carried out in an incubator instead of in a water bath. This would mean that *AF* spores in frequently turned composts, where temperatures are never constant, would be much faster inactivated that those in a static pile, where a portion of the spores would survive for a long time, even at higher temperatures. Detailed laboratory experiments would be necessary to verify these observations.

Another point that has to be mentioned is that inactivation experiments were carried out in a liquid medium. The compost matrix might have a protective effect. Trials to repeat the experiments by inoculating spores in sterile compost failed due to the impossibility to get a controlled and uniform heating of the compost.

3.2 FIELD STUDIES - DESCRIPTION OF INSTALLATIONS, EXPERIMENTS CARRIED OUT

Table 18 offers a summary of the most important data of the installations. More details are presented when discussing each site.

Table 18: Main characteristics of the composting installations studied.

type	aeration	turning frequency	dimension of the heaps (H x W x L)	special features of the installation	duration of the process	waste treated per year
windrow	no	daily (5x/week)	triangular 1.2 x 3.5 x 60 m		6 weeks	8'000 t
box	yes	weekly	2.5 x 4 x 15 m		4-6 weeks	3'500 t
box	no	part of the box, 2-3x/week	4 x 5 x 9 m in closed hall	combined • fermentation of kitchen waste • composting of garden waste	6 weeks	12'000 t composting 5'000 t fermentation
trench	yes	daily (5x/week)	1.2 x 2 x 66 m	closed hall, treatment of exhaust air in a biofilter	4 weeks	12'000 t
bioreactor	yes	weekly	3.3 x 2.7 x 2 m	pilot plant to study parameters of a large box composting system on-line measurements of temperature and CO_2	7-8 weeks	14 t / experiment

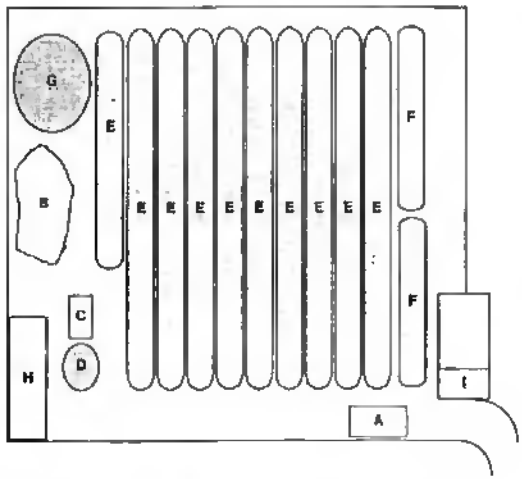
3.2.1 WINDROW COMPOSTING

The main part of the work was carried out at the installation of Grenchen, for one thing because the open-air windrow technology is still the most used system in Switzerland, but also because the short distance to our laboratory allowed a close follow-up of the process. In total, three experiments were conducted in the years 1994, 1995, and 1996, respectively, each consisting of a follow of one or several windrows over the whole span of the composting process. Each experiment lasted at least 6 weeks.

3.2.1.1 OESCRIPTION OF THE INSTALLATION

The composting is done in open-air windrows (3.2 m large x 1-1.2 m high x 40 m long). The windrows are placed on an asphalted surface; the run-off water is collected and treated in the nearby waste water treatment plant. At the beginning of the process, the windrows are turned daily (every work day, from Monday to Friday), after 4-6 weeks, the frequency is reduced to 2-3 turnings/week. Turnings are carried out with a rotative turning machine (type "Sandberger"). If necessary, water (drinking water quality) is added during the turning; windrows in an advanced stage of maturity are covered with a tarpaulin.

The duration of the process is 6-7 weeks for compost destined for agriculture (screened at 40 mm), and up to 6 months for compost destined for horticulture (screened at 20 mm) or potting mixes (screened at 10 mm). After screening, the compost is stocked in big heaps in the open for the agriculture grade, and under roof for the horticulture grade qualities.
The enterprise Vollenweider AG possess several installations of the same type as the one at Grenchen. They have a small laboratory where compost analyses are carried out.



A Scale
B Biodegradable waste
C Shredder
D Freshly shredded material
E Windrows
F Experimental windrows
G 7-8 weeks old compost, screened at 20 mm (agriculture)
H 10-12 week old compost, screened at 10 mm (horticulture) storage under roof
I Laboratory

Figure 17: *Schematic depiction of the composting site in Grenchen.*

Figure 18: *Open-air windrow composting site. In the foreground: turning machine. In the background: mobile screener (left), and shredder (right).*

3.2.1.2 EXPERIMENTS CARRIED OUT

3.2.1.2A GRA 1

The first experiment carried out at Grenchen consisted in a follow-up of one compost windrow treated in the usual manner of the installation = intensive

Duration of the experiment: June to middle of July 1993, over a period of 45 days (= 6.5 weeks).

Starting material: 50 % separately collected kitchen and garden waste (mainly lawn cuttings)
30 % leaves and twigs
10 % wood chips
10 % reject from the screening

Turnings: every work day (Monday to Friday), except on day 2, 35 and 36.

Watering: on day 31, 37, 38, 41 and 42.

Physico-chemical measurements in the compost windrow:

- - 20 cm lateral, at 50 cm height and an angle of 30° = surface
- - 60 cm, lateral, at 50 cm height and an angle of 30° = center

Parameters measured: temperature, O_2, CO_2 (measured with Dräger tubes), CH_4, H_2S, H_2, NH_3
For detailed methods, see Chapters 2.2.1.1 and 2.2.1.2.

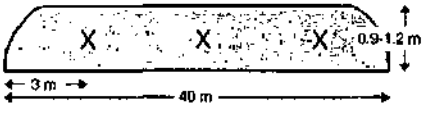


Figure 19: Scheme of the measuring and sampling points (longitudinal section).

Measurements were carried out at three points along the length of the windrow: in the middle, and 3 m from each end (Figure 19). Results are given as the arithmetic mean, with the standard deviation (1σ).

Sampling: at the measuring points, samples were taken as described in Chapter 2.2.3.

The samples were prepared for microbiological and physico-chemical measurements as described in Chapters 2.2.4 and 2.2.5.

Microbiological analyses: *AF*, thermotolerant molds and yeasts, thermophilic bacteria. For detailed methods, see Chapter 2.2.4.

Physico-chemical analyses: pH, dry weight. For detailed methods, see Chapter 2.2.5.

3.2.1.2B GRA 2

The second experiment carried out at Grenchen consisted in a follow-up of two compost windrows, each 25 m long, one treated in the usual manner of the installation (daily turnings (5 x / week) = intensive), and one turned 1 x / week (= moderate intensive).

Duration of the experiment: end of October through middle of December 1994, over a period of 55 days (= 8 weeks)

Starting material: 70 % leaves and twigs
20 % separately collected kitchen and garden waste
10 % reject from the screening

Turnings of the intensively treated windrow: week 1-7: daily, except on day 7 and 28

week 8: Monday, Wednesday, Friday

Physico-chemical measurements in the compost windrow: every 10 cm, as shown in Figure 20:

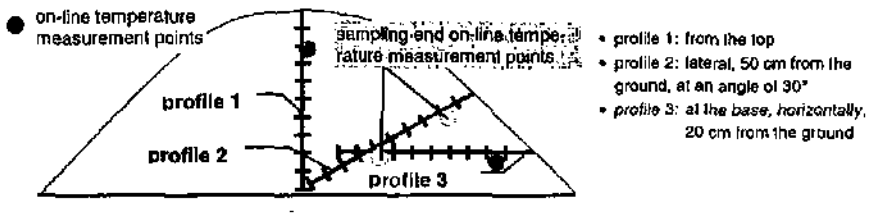


Figure 20: Schematic depiction of temperature and gas measurement profiles, sampling points and on-line temperature measurement points (cross-sectional view).

Parameters measured: temperature, O_2, CO_2 (with the Multiwarn), CO, CH_4, H_2S, NH_3.
The measurements were made in the middle along the length of each compost windrow. Measuring devices were as described in Chapter 2.2.1.1 and 2.2.1.2.

On-line temperature measurements: continuously with the Squirrel Data Logger, registration of values every hour, at the locations shown in Fig. 20:
- - 20 cm from the top
- - 20 cm lateral
- - 60 cm lateral
- - 20 cm at the base

Measuring devices were as described in Chapter 2.2.1.1.

Bioaerosol measurements: 1 x / week, *Aspergillus fumigatus* and thermotolerant molds; 5 m behind the turning machine, in the direction of the wind, with the SAS Standard. For detailed methods, see Chapter 2.2.2.

Sampling frequency:		
	week 1 and 2:	daily (every work day = 5 x / week)
	week 3 and 4:	3 x / week
	week 5 to 7:	2 x / week
	week 8:	1 x / week

At the points shown in Figure 20, compost samples were taken as described in Chapter 2.2.3.

Microbiological analyses: *Aspergillus fumigatus*, thermotolerant molds and yeasts, Gram-negative bacteria (on MacConkey Agar), thermophilic and mesophilic heterotrophic bacteria. For detailed methods, see Chapter 2.2.4.

Physico-chemical analyses: pH, dry weight, organic matter*, ash content*, C/N ratio*, Water Extractable Organic Carbon (WEOC)*. * = analyses carried out by ORLAB. For detailed methods, see Chapter 2.2.5.

3.2.1.2C GRA 3

The third experiment carried out at Grenchen consisted in a follow-up of three compost windrows, each 16.5 m long, one treated in the usual manner of the installation (daily turnings = 5x/week = intensive), one turned once a week (= moderate intensive), and one once a month (= extensive).

Duration of the experiment: from beginning of September through middle of December 1995, over a period of 104 days (= 15 weeks). The windrow that was only turned once a month was left in place until middle of February 1996 (in total 167 days (= 24 weeks) of composting).

Starting material: 50 % separately collected kitchen and garden waste
40 % leaves and twigs
10 % reject from the screening

Turnings of the intensively treated windrow:

week 1 to 6:	every work day (Monday to Friday), except on day 8 and 9
week 7 to 9:	3 x / week, except on day 50 and 57
week 10 to 13:	2 x / week
week 14 and 15:	1 x / week

Physico-chemical measurements: the same as in GRA2, every 10 cm up to 40 cm, then every 20 cm, (see Figure 20). Parameters measured: temperature, O_2, CO_2 (with the Multiwarn apparatus), CO, CH_4, H_2S, NH_3.
The measurements were made in the middle along the length of each compost windrow. Measuring devices were as described in Chapter 2.2.1.1. and 2.2.1.2.

The temperature was measured on-line in the same way as in experiment GRA2. For the monthly turned windrow, temperatures were only monitored at - 20 cm and - 60 cm lateral.

Bioaerosol measurements were carried out 1 x / week, for the detection of *Aspergillus fumigatus* and thermotolerant molds and yeasts.

- in 2 m distance behind the turning machine, with the SAS Super 90
- in 10 m distance to the turning machine, in the direction of the wind, with the SAS Standard

Sampling: the sampling points were the same as for experiment GRA 2 (see Figure 20). Samplings were carried out as described in Chapter 2.2.3.

Sampling frequency:

week 1:	daily (every work day = 5 x /week)
week 2 and 3:	4 x / week
week 4 and 5:	3 x / week
week 6 and 7:	2 x / week
week 8 to 15:	1 x / week
week 16 to 24:	1 x / month

Microbiological analyses: *Aspergillus fumigatus*; total thermotolerant molds and yeasts; coliforms (on VRB-Agar); *Escherichia coli*; thermophilic and mesophilic bacteria. For detailed methods, see Chapter 2.2.4.

Physico-chemical analyses: pH, dry weight, organic matter*, ash content*, C/N ratio*, water extractable organic carbon (WEOC)*, % reject after screening at 30 mm. * = analyses carried out by ORLAB. For detailed methods, see Chapter 2.2.5.

3.2.1.3 RESULTS AND DISCUSSION

3.2.1.3A GRA 1

The first experiment aimed at following physico-chemical and microbiological parameters of one windrow throughout the whole composting process, in order to monitor their evolution without variations due to different starting materials.

Physico-chemical measurements

The physico-chemical results obtained from this first experiment will be discussed in detail, because they show the typical evolution of a well controlled composting process.

TEMPERATURE

The temperature evolution depicted in Figure 21 is very typical for a naturally aerated windrow, and corresponds to the many curves shown in literature (MICHEL *et al.*, 1996; FERNANDES *et al.*, 1994; KROGMANN, 1994; SHUVAL *et al.*, 1991; BIDDLESTONE & GRAY, 1987; GRAY & BIDDLESTONE, 1971a). At the beginning the temperature rose faster at the surface, because there the good oxygenation through diffusion of air from the surface allowed a good activity of aerobic microorganisms. The same was observed by FERNANDES *et al.* (1994), who made extensive temperature measurements in passively aerated static compost piles, and by MILLER *et al.* (1989), who examined phase I mushroom composting stacks.

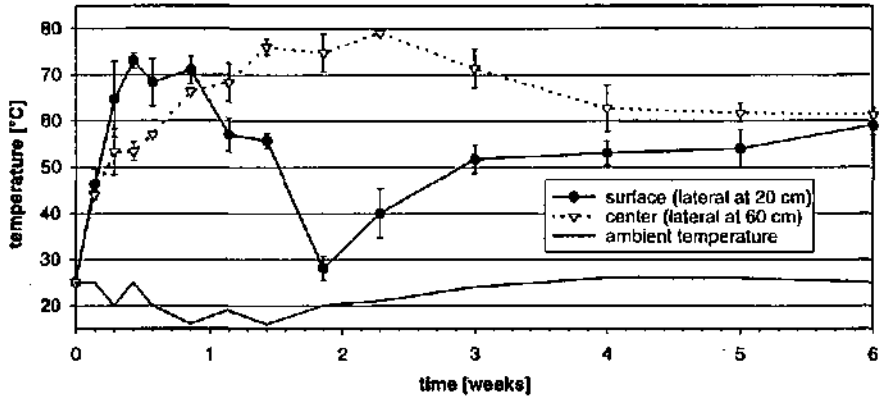


Figure 21: Temperature evolution in an open-air compost windrow. The error bars represent the standard deviation (±1 σ) of measurements at three points along the length of the windrow.

After only three days, the maximum was reached (72°C), and temperatures then started to fall because of heat loss to the environment, and stayed for the rest of the experiment, except for a short period of cold northern winds in the second week, between 50 and 60°C. In the center, temperatures continued to ascend until the 16th day of composting, to reach a maximum of 79°C. Despite the high nutrient content of the starting material (a large percentage of grass clippings and herbaceous garden waste), temperatures fell from the third week on, to be stable around 60°C for the rest of the experiment.

The constant temperatures around 60°C, and the high concentrations of H_2S and CH_4 measured in the center (see Figure 23), are both indicators of anoxic conditions reigning from the 4th week on, promoting the growth of thermophilic anaerobes with a temperature optimum between 55°C and 60°C.
The standard deviation of the measurements executed at three points along the length of the windrow never exceeded 5°C, demonstrating the good homogeneity of the material, and justifying for the following experiments a sole point of measuring and sampling. However, the measurements taken at the two points along the windrow cross-section (surface = depth of 20 cm; center = depth of 60 cm) showed important temperature differences. Between the hot center and the cooler surface, temperatures differed under special circumstances (cooling of the outer parts of the windrow by low ambient temperatures) by more than 40°C. Although the building up of a temperature gradient in a compost windrow is often mentioned, or even demonstrated, in literature (FERNANDES *et al.*, 1994; STENTIFORD, 1993; INBAR *et al.*, 1990; FRICKE, 1988; STENTIFORD *et al.*, 1985; MACGREGOR *et al.*, 1981), the effect of it on variations of microbial activity, thermohygienization or final compost quality was never properly investigated.
To get a better idea about the temperature distribution a open-air compost windrows, extensive temperature measurements along three profiles were carried out during the following experiments (see Chapter GRA2 and GRA3).

GASES

The gas measurements shown in Figure 22 demonstrate the stoichiometric relationship between *oxygen consumption and carbon dioxide production*.

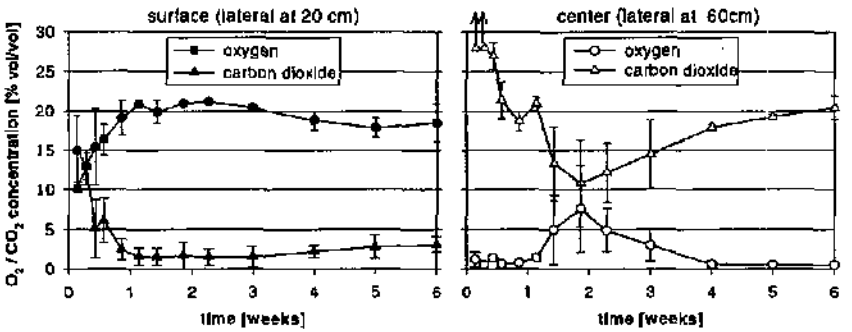


Figure 22: Oxygen and carbon dioxide evolution in an open-air compost windrow. The error bars represent the standard deviation(±1 σ) of measurements at three points along the length of the windrow.

O_2 and CO_2 values summed up to about 21 %, except for the first days of composting, when CO_2 concentrations of more than 28 % indicated an additional CO_2 production by anaerobic processes taking place in the center of the heap (HELM, 1995). Big differences were seen between the well oxygenated compost surface and the less well aerated inner parts of the windrow. Very low interstitial oxygen concentrations, however, are not forcibly a sign of anaerobiosis. In fact, the instantaneous level of oxygen is the expression of a dynamic equilibrium between microbial oxygen uptake and its replenishment by aeration. High rates of both uptake and replenishment, or low rates of both these factors can result in similar oxygen levels (MACGREGOR *et al.*, 1981).

Oxygen uptake and replenishment are coupled: high microbial activity, which equates to a high oxygen uptake, brings about a strong heating of the compost, which drives natural ventilation through the "chimney-effect". The presence of CO_2 over 21 vol. %, of CH_4 and of CO are much better indicators of anaerobic metabolisms. Several explanations can be found for the slight oxygen peak around the second week of composting: it might be due to the strong winds that caused a cooling of the surface (see Figure 21), or to the very high temperatures (> 70°C) measured at that moment, leading either to a reduction of the microbial activity or improving the natural aeration by a more pronounced "chimney-effect". Many authors have demonstrated that composting activity rates decrease at temperatures above 60°C (VON RHEINBABEN, 1993; MCKINLEY & VESTAL, 1984; FINSTEIN *et al.*, 1983; MACGREGOR *et al.*, 1981). When temperatures dropped below 70°C (see Figure 21) after the third week, microbial activity resumed, as shown by the increasing CO_2 and decreasing O_2 concentrations.

Hydrogen sulfide (Figure 23), either stemming from the aerobic degradation of proteins, or from the reduction of sulfate under anoxic conditions, was only detectable in the center of the windrow, as also observed by MILLER *et al.* (1991) when examining stack 1 mushroom compost. After a small peak in the first week, H_2S was measured in higher concentrations (up to 125 ppm) at the beginning of the second week. This corresponds to the order of degradation of the organic substrate present in compost: proteins are only metabolized after the free sugars have been consumed (VON RHEINBABEN, 1995). Degradation experiments by DE BERTOLDI *et al.* (1988), carried out in a bioreactor, showed an important H_2S peak after the first week of composting.
A second big increase in H_2S was observed after the peak temperatures. It seems that the microflora present at temperatures above 70°C did not posses any protein degrading activity any more. This did resume as soon as the temperatures fell below 70°C. Towards the end of the experiment, the hydrogen sulfide stemmed most probably from anaerobic processes, as methane, which is only produced in the absence of oxygen, was also detected.

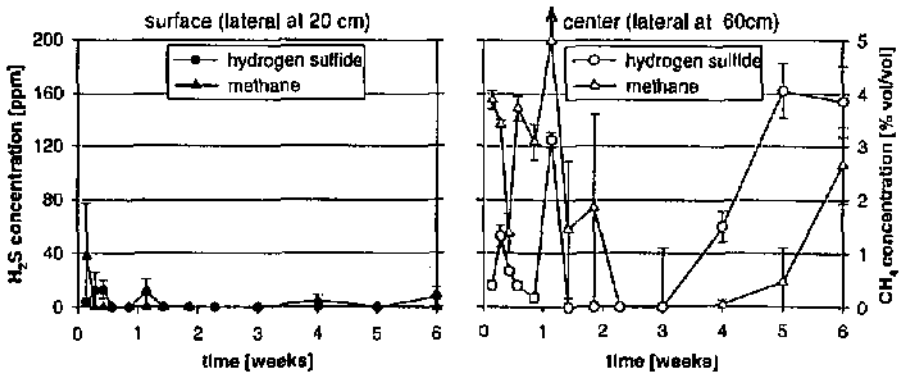


Figure 23: Hydrogen sulfide and methane evolution in an open-air compost windrow, in function of the sampling depth. The error bars represent the standard deviation (± 1σ) of mensurements at three points along the length of the windrow.

The presence of H_2S in concentrations of 40-60 ppm, up to the 40th day of composting, was measured in a compost windrow composed of only kitchen waste (HELM, 1995), indicating that material with a high content of organic matter and little structure material can emit hydrogen sulfide gas for a long time.

Methane (Figure 23) was, not surprisingly, only measured in the center of the heap. Although composting should, by definition, be an aerobic process, oxygen demand during the first intensive phase of composting is so important that anaerobic zones are almost inevitable if no forced aeration occurs (FINSTEIN *et al.*, 1986b).
Metabolites of fermentative processes were also observed at the end of the process. The decrease of the free air space, due to the size reduction of the compost particles, together with an increased microbial activity after the heat peak phase (see Figure 21) might be the cause for anoxic regions in the core of the heap.

The order of the appearance of the gases (first hydrogen sulfide, and then methane) can be explained by a sequential substrate utilization, as a function of redox potential, as shown in Figure 24.

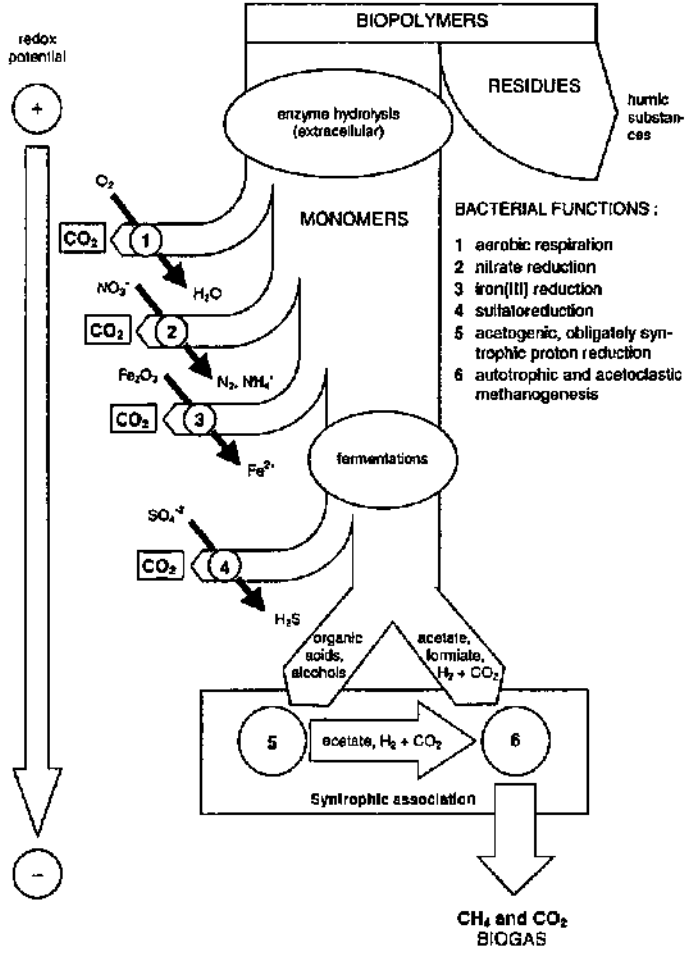


Figure 24: Carbon flow and the succession of bacterial functions in an aerobic/anaerobic environment (from ARAGNO & ULEHLOVA., 1996).

WATER CONTENT AND pH

The decrease of the water content in the compost (Figure 25A) was almost linear up to the 4th week of composting. In the first week, more water was evaporated at the surface, where at that moment temperatures were higher. The further reduction of the water content at the surface was most certainly due to drying by wind. From the 4th week on, water contents were very low, almost at the limit for satisfactory microbial activity (see Chapter 1.6.4).

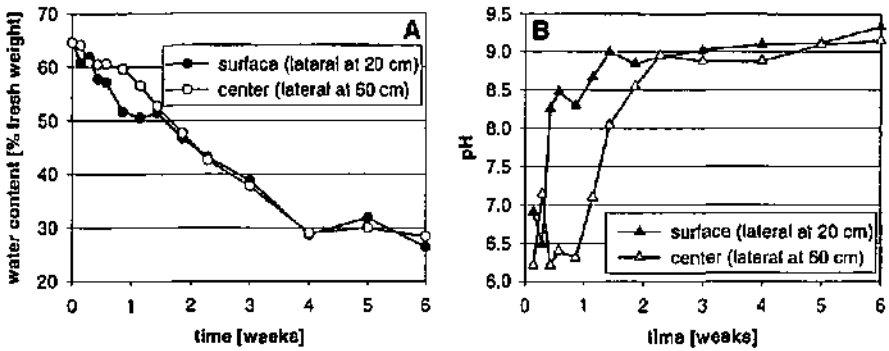


Figure 25. Evolution of water content (A) and pH (B) in compost produced in open-air windrows.

The pH curves (Figure 25B) show the classical evolution of garden and kitchen waste composts described by many authors (ANONYMOUS, 1993, see also Chapter 1.5.4): after an initial slight decrease below pH 7, they increased steadily to reach a final pH value around 9. The increase was delayed in the center, a sign of suboptimal composting conditions. The high temperatures measured inside the windrow most probably inhibited protein degradation, and therefore ammonium formation. Taking into account that the windrow was turned daily, and thus the whole material mixed each day, the differences in pH at the center and at the surface indicate that a stratification, due to varying microbial activity, can built up in less than 24 h.

Microbiological analyses

FUNGI

Concentrations of *Aspergillus fumigatus (AF)* and thermotolerant molds and yeasts (Figure 26) in fresh biowaste were quite high: more than 10^6 colony forming units (cfu) were counted per gram compost (dry weight).

These numbers were quickly reduced during the first week of composting, to stay at or below the detection limit of 10 cfu/g (fresh weight) for *AF*, and 1-2 orders of magnitude more for thermotolerant molds. At the end of the experiment, a slight recolonization was observed. The quite parallel course of the curves from the surface and the center indicate that the short interval between turnings did not allow an important regrowth of *AF* at the surface, where temperatures would have been permissive for its growth. These data confirm an earlier experiment by our research group at the same composting installation, where we found between $1.4 \cdot 10^2$ and $2.3 \cdot 10^3$ cfu/gDW *AF* after 1 day, < 20 after 3 weeks, and between $2 \cdot 10^1$ and $3 \cdot 10^2$ in the sereened compost after 6 weeks of composting (BEFFA *et al.*, 1994). Yeasts were only detected during the first week of composting process (separate data not shown).

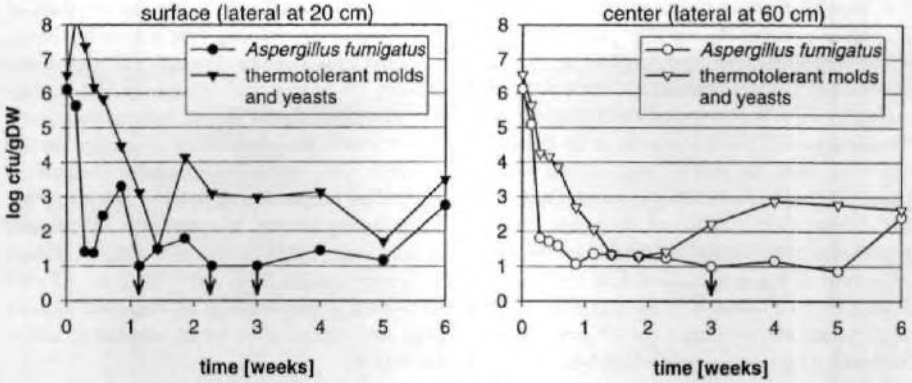


Figure 26: Evolution of AF and total thermotolerant molds and yeasts during composting in an open air windrow. ↓ values below the detection limit of 5-10 cfu/g fresh weight.

Under the culturing conditions chosen (malt extract agar, incubation at 40°C), the mold the most frequently isolated in the temperature phase after the peak, but still above 50°C, was *Scytalidium thermophilum*. In fact, this name was proposed by STRAATSMA & SAMSON (1993) to group the by COONEY & EMERSON (1964) formerly named species *Torula thermophila*, *Humicola insolens*, and *Humicola grisea* Traaen var. *thermoidea*.

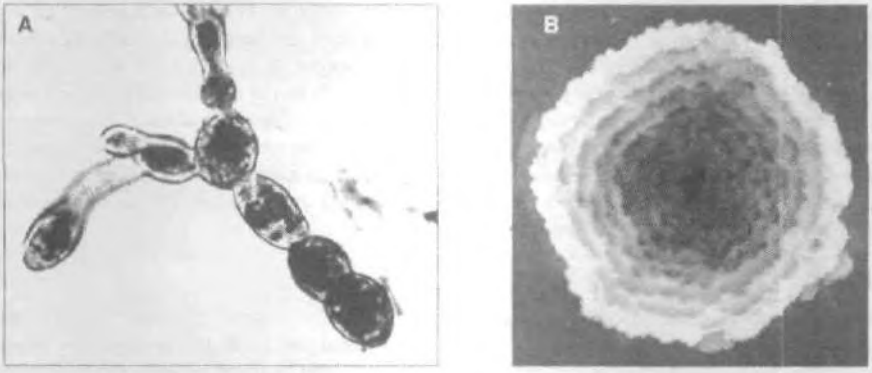


Figure 27: Microscopic (A) and macroscopic (B) photographs of Scytalidium thermophilum isolated from 6 week old compost. Growth on Malt Extract agar (20 g/l) at 40°C. A: 400 x, coloration of clamydospores with methyl blue. B: after 48 h incubation in 100 % rH.

Their description of the species "at 40°C rapidly growing irregular, lobed colonies, first white, soon becoming dark green-gray to black with a white to light gray mycelial overgrowth; globose to ellipsoidal thick walled, darkly pigmented conidia, sometimes called clamydospores (EMERSON, 1968) or aleuriospores (CHANG & HUDSON, 1967) borne singly or in short, intercalary chains" fits exactly the macro- and microscopic observations of the colonies isolated in our laboratory almost exclusively from compost in the phase after the peak temperatures were reached (Figure 27A and B).

The same observations were made by CHANG & HUDSON (1967), who found maximum numbers of *Humicola insolens* during the "plateau" period of the composting process, that means the phase where temperatures stayed constant at about 60°C after the peak heating. Towards the end of the composting process, marked by temperatures near ambient, the numbers of this species got slightly reduced, while a strong recolonization by *AF* was observed, as found in our own experiments. MILLNER *et al.* (1977) reported, in an extensive research about the colonization of sewage sludge and woodchips compost by fungi, a high percentage of *AF* in the 3-week forced-aerated composting phase, while *H. grisea* var. *thermoidea* was the most abundant fungus during the 4 weeks of curing, and *Torula thermophila* and *H. grisea* var. *thermoidea* during storing. It seems that the slightly higher thermoresistance of *Scytalidium thermophilum* (maximum radial growth rate at 45°C, no loss of activity of the mycelium at 53.5°C; WIEGANT, 1992) compared to 37°C and < 50°C for *AF* (see Chapter 3.1.4.1) gives it an advantage for colonization as long as temperatures are high. But as soon as they become propitious for *AF* again, it is the latter who seems to be better adapted to life in compost, might by a higher cellulolytic and xylanolytic activity.

Three out of 19 mushroom workers diagnosed with EEA showed positive serum reactions against *S. thermophilum* (VAN DEN BOGART *et al.*, 1993), indicating that this mold has a week allergenic potential.

EMERSON (1968) also placed *Thermomyces lanuginosus* (≡ *Humicola lanuginosa*) in the so-called *Humicola-Torula* complex, but STRAATSMA & SAMSON (1993) stated that this species can be easily distinguished from *S. thermophilum* by a slow growth and a reddish brown color of the colonies. As this species occurs early in the fungal succession during the composting process (STRAATSMA & SAMSON, 1993), no confusion of the two species should be possible. Nevertheless, CHANG & HUDSON (1967) reported that they found only *AF* and *Thermomyces lanuginosus* in the later stages of composting of wheat straw.

GÖTTLICH (1996) isolated *Talaromyces thermophilus* (anamorph of *Penicillium dupontii*) in high concentrations (up to $8 \cdot 10^5$ cfu/m^3) in the air close to a 14 week old compost, as well as in concentrations of $4 \cdot 10^4$ cfu/m^3 during the screening of mature compost. It might not be astonishing that *Scytalidium thermophilum* was only isolated in low numbers from the air. The quite big and heavy clamydospores rest attached to the mycelium, and get not easily dispersed, although we frequently detected this mold during the handling of compost in or after the peak heat phase. It is surprising, however, that we never isolated *Talaromyces thermophilus* from mature composts. Due to the short incubation time (normally 48 h, never more than 72 h) used in our experiments, this slow growing species (EMERSON, 1968) might have been overlooked.

BACTERIA

Concentration of thermophilic bacteria (growth at 60°C) (Figure 28) was already high (10^8 MPN/gDW) in the starting material, and stayed at about this level during the whole experiment, at the surface as well as in the center. No correlation could be found between the temperature measured at the sampling point, and the concentration of thermophiles. Slightly higher thermophile numbers were observed at the surface than in the center, where temperatures were almost too high to support the growth of Bacilli, the main population enumerated by incubation at 60°C (BLANC *et al.*, 1997). Comparison with data from the literature is difficult, because the term "thermophilic" is defined differently by each author. Also, the method of sample treatment and preparation of the compost suspension plays an important role. By more rigorous methods (ultrasonic treatment, the use of pyrophosphate buffer) as the ones employed in our laboratory (suspension in water, shaking at 250 rpm for 30 minutes) more bacteria get detached from the compost particles (J. WIEGEL, University of Georgia, Atlanta, personal communication).

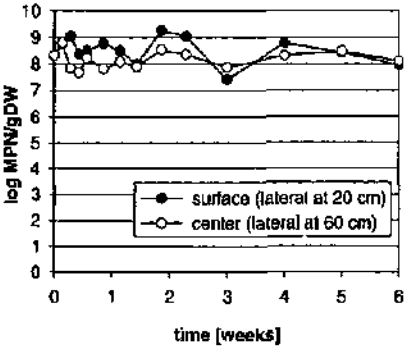


Figure 28: Evolution of thermophilic bacteria during the composting in an open air windrow.

By incubation at 55°C, MICHEL *et al.* (1995) found $5 \cdot 10^5$ thermophiles in fresh material (which had been dried and rewetted for the experiment, though). Concentrations rose to a maximum of $2 \cdot 10^{11}$ MPN/gDW during the thermophilic phase, and then dropped to $2 \cdot 10^{10}$ towards the end of the composting process. LIBMOND & SAVOIE (1993) reported maximum concentrations of $4 \cdot 10^{11}$ thermophiles (cfu/gFW) during the peak composting phase, and $3 \cdot 10^9$ cfu at the end of the process, by incubation at 45°C. ANDREWS *et al.* (1994) showed the presence of 10^6 thermophiles during the hot phase of composting in a tumbler, and between $1 \cdot 10^6$ and $3 \cdot 10^7$ during the maturation phase. The detection of the thermophilic flora was made, though, after a passage at 80°C for 15 minutes prior to plating, and incubation at 55°C, and reports thus most more likely the number of spore-forming thermophiles. Our findings and these by the authors mentioned above are in contrast to data given by DE BERTOLDI & ZUCCONI (1987), who showed a decrease to almost 0 of thermophiles after the peak temperatures. The persistence of thermophiles is not astonishing: most of them are spore-formers of the genus *Bacillus* (BLANC *et al.*, 1997; BEFFA *et al.*, 1996b); their spores can thus be detected throughout the whole composting process. Also, most of them have a wide span of growth temperature.

3.2.1.3B GRA 2 and 3

The results from the experiments GRA2 and GRA3 will be discussed together, because they were designed in the same way, and differed only in the composition of the starting material. They are the subject of a publication in "Waste Management and Research" (LOTT FISCHER *et al.*, 1998). Results from experiment GRA2 were presented at the international symposium "The Science of Composting" as a poster: "Composting of organic garden and kitchen waste in open-air windrows: influence of the turning frequency on the development of *Aspergillus fumigatus*" (LOTT FISCHER *et al.*, 1996).

Physico-chemical measurements

TEMPERATURE

Figures 29 and 30 show the temperature curves registered on-line. Missing data are due to data-logger failure. Ambient temperatures represent values measured during measuring and sampling, between 8:30 and 9:30 am.

Peak temperatures reached in all experimental windrows were the same (around 75°C), but the time to get to this temperature, and the duration of the thermogenic phase (temperature > 60°C) differed considerably, in function of the starting material, and the turning frequency.

In the compost consisting mainly of garden waste (Experiment GRA2), surface temperatures (Figure 29A) rose very rapidly in the first few days of the composting process to temperatures between 70-75°C, and stayed, during the following 3 weeks, for most of the time above 60°C, to decline thereafter. The peak was reached a little bit later in the weekly turned windrow than in the daily

turned one, but temperatures in the former could be maintained at a higher level (> 50°C) during the cooling phase. In general, surface temperatures were, except for the first few days, always below those measured in the center. Depending on the ambient air temperature, but also on wind and rainfall, differences between the center and the surface could be as high as 30°C. The more the composting process progressed, the smaller these differences got.

The effects of the two different turning frequencies become more obvious if one looks at the core temperatures (Figure 29B). In the daily turned windrow, the heat peak was already reached in the second week of composting, but this was only the case in the forth week for the windrow that was turned once a week. It seems that the frequent turnings helped in redistributing the nutrients, and in making the substrate, which contained quite a high percentage of wood, better available for the degrading microflora, by mechanically breaking the wood pieces apart. A factor for the quite fast temperature reduction in the daily turned windrow might also be the low ambient temperatures that provoked a strong cooling during the turnings. HAY & KUCHENRITHER (1990), citing IACOBONI (1983) advised against too frequent turnings in the maturation phase, in order not to cause a decline in temperature.

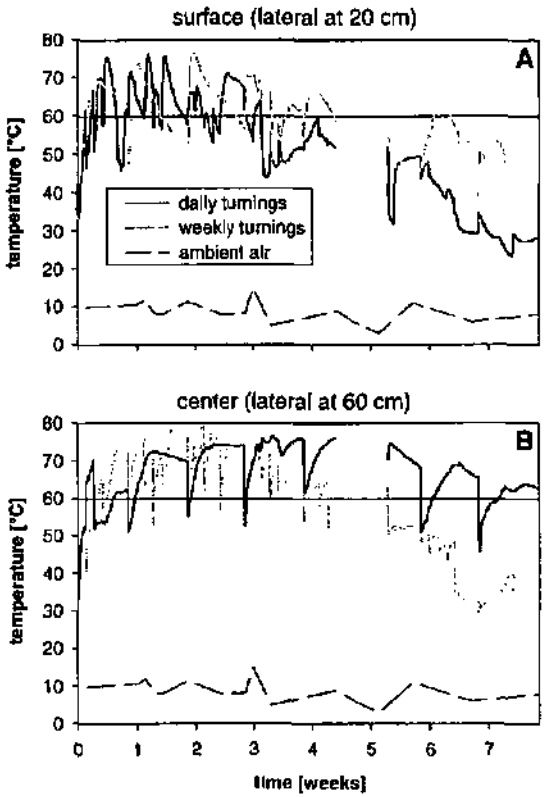


Figure 29: Temperature evolution at the surface (A) and in the center (B) of a daily turned (intensive = 5x/week, on every work day up to week 6, then 2-3x/ week) and a weekly turned open-air windrow. Experiment GRA2 (mostly garden waste, C/N 40:1).

In the windrows made of garden and kitchen waste (Experiment GRA3, Figure 30), the differences between the daily and the weekly turned windrow were less pronounced, the mean temperature of the weekly turned windrow being even slightly higher.
The surface temperatures (Figure 30A) did rise very rapidly in the beginning of the composting process, but did not exceed 60°C any more beyond the 5th week of composting, while core temperatures (Figure 30B) could be maintained above this value for at least 2 months. The collapse of temperatures in the surface material in the 11th and 12th week might be explained by a drying of the compost surface (see Figure 35). Taking into account the reduced turning frequency also for the frequently turned windrow at this moment (2 x/week), the humidity could not be corrected immediately.
The temperature rise at the beginning of the process was a little delayed in the center of the windrow (Figure 30B), probably due to insufficient oxygenation in the quite wet and dense material, and thus to a more important energy storage in form of latent heat. Temperature maxima where reached in the daily turned windrow at the beginning of the third week of composting, in the weekly turned one a few days later. The high nutrient content allowed to maintain the temperatures for almost two months above 60°C, versus only 5 weeks for the garden waste composts (see Figure 29).

When comparing the temperature evolution in the center during the maturation phase (12^{th} to 16^{th} week) of the daily and the weekly turned windrow (Figure 30B), it can be seen that the core temperatures of the former were 10-20°C higher. This indicates that there composting conditions (water and nutrient content) were permissive for optimal microbial activity until the end of the experiment, while the low humidity (Figure 35) in the weekly turned windrow did bring about a premature end to the composting process.
Center temperatures exceeded 60°C in the windrow that was turned once a month (Figure 30B); maximum temperatures were reached after the first turning (see Figure 29). I the surface (Figure 30A), however, the 60°C mark was only surpassed for short moments after the turnings, and fell well below 50°C for the rest of the time. Temperature evolution in the center and at the surface was quite parallel, with a difference of about 20°C.

The temperature curves also showed that the usually practiced composting duration of 6-7 weeks lead to a first stabilization of the compost material by the degradation of the easily metabolizable substances. When treating nutrient rich material, 3-4 months would be necessary for the production of a really stabilized compost that would not heat any more after turnings.

Similar results to the ones shown above were obtained by P. SCHERER (FH Hamburg, personal communication), who investigated the temperature evolution in compost windrows of comparable size, turned every second day with a Sandberger machine. In the windrow consisting of 30 % biowaste and 70 % structure material, maximum temperature in the core (80 cm from the top) reached 70°C in the first week, and fell below 60°C after the 2nd week of composting. In the compost containing 70 % biowaste and 30 % structure material, temperatures rose faster, stayed in the center above 70°C for at least 2 weeks, and descended only after 4 weeks below 60°C. In both treatments, temperature differences of up to 20°C were observed between the center and the surface (lateral at 10 cm).

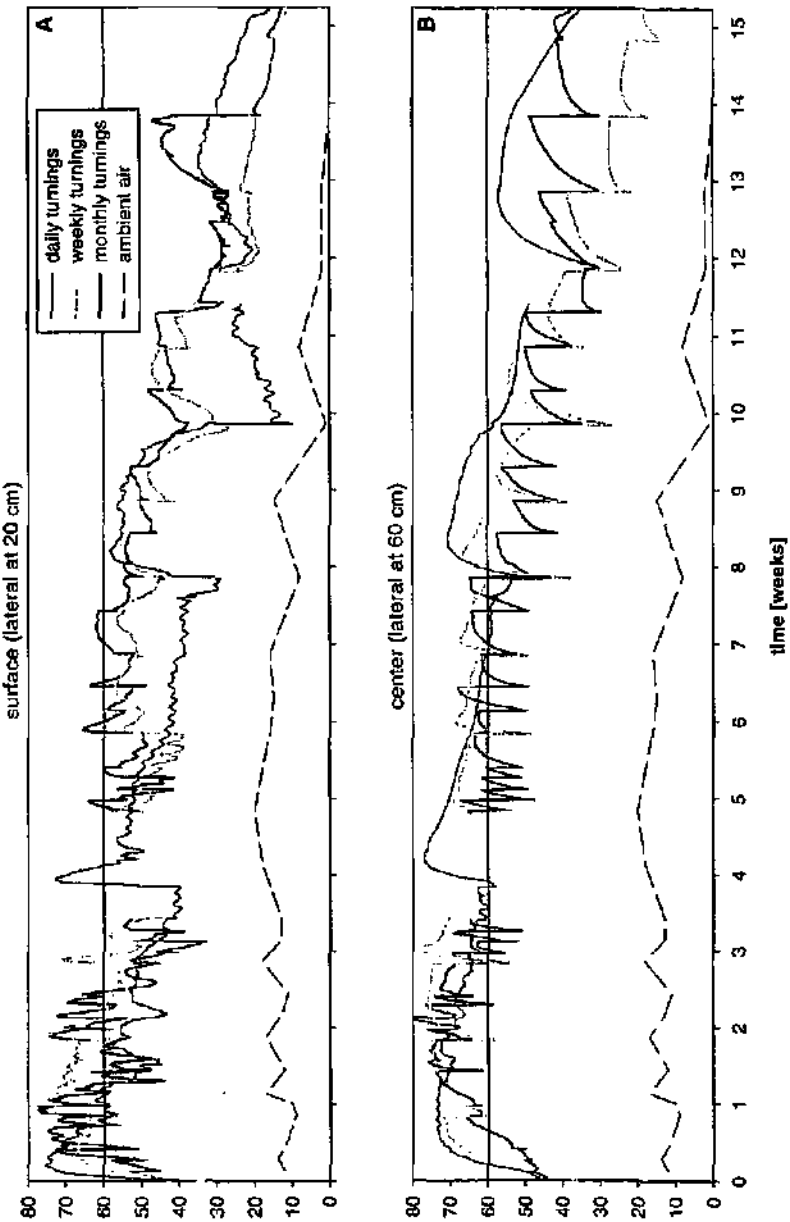


Figure 30: Temperature evolution at the surface (A) and in the center (B) of a daily turned (intensive = 5x/week, on every work day up to week 6, then 2-3x/ week), a weekly turned, and a monthly turned open-air windrow. Experiment GRAZ (garden + kitchen waste, C/N 30:1)

Interesting data were presented by INSAM *et al.* (1996), who compared the temperature evolution in cattle manure compost. When no turning occurred at all, temperatures never exceeded 50°C in none of the zones (upper, outer, inner, lower) of a windrow of comparable size, indicating anaerobic conditions in a large part of the windrow. With 1-2 turnings per week, and the addition of straw (50 or 100 kg / 5 tons of manure), temperatures between 50-65°C were reached in almost the whole mass, with the exception of the outer zone (first 10 cm), where temperatures were clearly lower. These data corroborate the observations of our experiments: turnings are indispensable in open-air windrow composting to 1) mix the material to expose the microorganisms to new nutrient sources, 2) break up the compost particles, augmenting thus the surface for microbial attack, 3) reduce the water content by favoring evaporation creating thus conditions that allow a sufficient supply of air, a prerequisite for aerobic microbial activity, 4) augment the porosity, to compensate for the compaction of the material in the lower layers due to the weight of the material, aiding the convective aeration of the windrow.

Temperature measurements at two points can only give a rough idea about the temperature distribution in a compost windrow. As Figures 29 and 30 show, important gradients built up in the compost heaps. The different temperature zones and their evolution in the course of the composting process are represented in Figures 31 and 33 for experiment GRA2 and GRA3, respectively. Measurements were carried out just prior to turning, representing thus for the weekly or monthly turned compost the situation after a maximum period of non-disturbance.

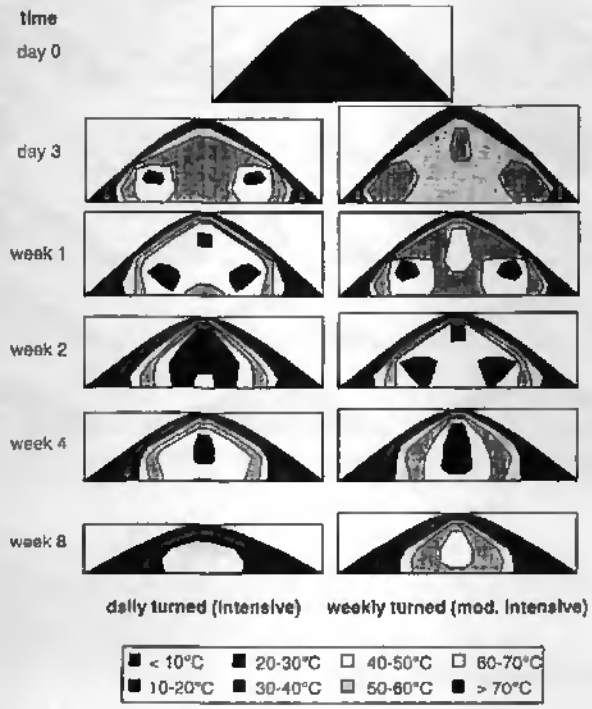


Figure 31: Evolution with time of temperature distribution in daily and weekly turned windrows, expressed as isotemperature curves. Experiment GRA2 (mainly garden waste, C:N 40:1).

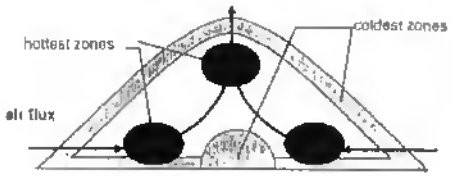


Figure 32: Scheme of air flux in a compost windrow

The material in the upper and the lower outer parts of the triangular windrow was always the hottest (Figure 32), the former representing the zone where all the hot air passed to leave the windrow at the summit (which, by the way, was visible at the surface as a small stripe of wet material, due to the condensation of the vapor), the latter being the zone that was oxygenated best by the influx of fresh air, showing thus maximum heating. The central part at the base received less air, most probably due to compaction of the compost and reduction of free air space (FERNANDES et al., 1994), and warmed thus less. Also, the outer layers were colder through important heat losses to the environment. This confirms the theory the convection could induce significant mass flows in the upper and outer areas of a windrow, but such flows would decrease greatly in the lower or interior areas of the windrow (MILLER et al., 1989).

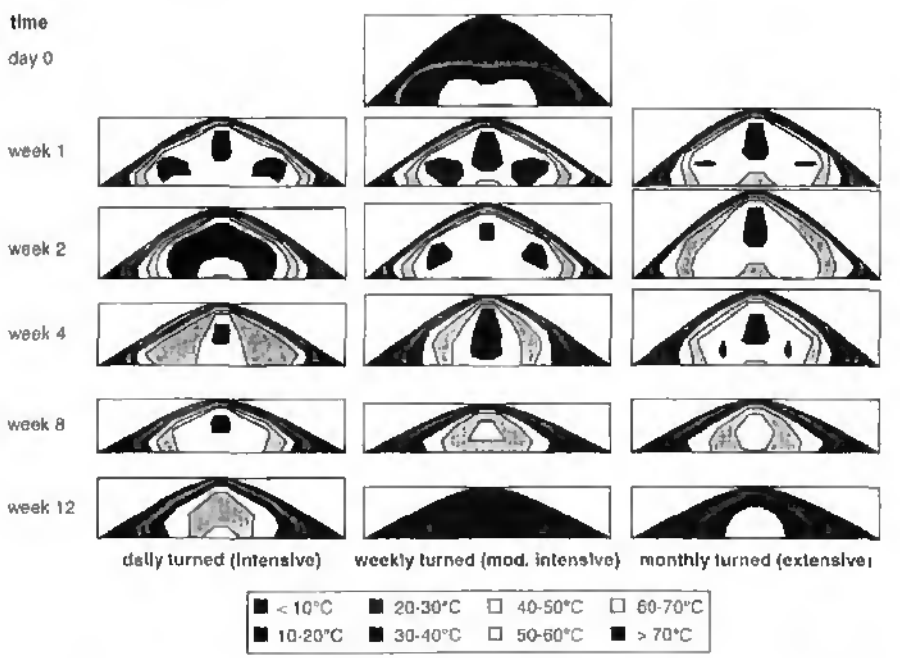


Figure 33: *Evolution with time of temperature distribution in daily and weekly turned windrows, expressed as isotemperature curves. Experiment GRA3 (garden + kitchen waste, C:N 30:1.*

In both experiments, the daily turned windrow had larger zones of very high temperatures (> 70°C), and the stage of maximum temperatures was reached earlier than in the weekly or monthly turned ones. When the starting material consisted mainly of garden waste (experiment GRA2, Figure 31), temperatures in the daily turned windrow were, after 8 week of composting, < 50°C, while with

more kitchen waste (experiment GRA3, Figure 33), core temperatures between 60 and 70°C were maintained. Except for a faster temperature increase in the first week, no big differences could be observed in the weekly turned windrows between the two experiments, corresponding to the temperature curves shown in Figures 29 and 30.

Similar iso-temperature curves were presented by FERNANDES *et al.* (1994), who compared the performance of passively aerated compost systems for the degradation of a mixture of peat and poultry manure. In the beginning, higher temperatures were measured near the pile exterior, while the lower middle part stayed colder. After about 10 days of composting, peak temperatures around 70°C were reached, the iso-curves showing the typical "onion" layering of the temperature zones, with the core being the warmest. After 30 days, temperatures were back to ambient. The only difference was that by comparing two different initial mixtures, the one that contained more organic matter (poultry manure: peat = 4.5:1) showed 5-10°C lower temperatures than the one with less poultry manure (1.5:1). In that case, the extremely high water content (80 %) of the former mixture might have led to an insufficient oxygenation, and thus a slighter self-heating.

To better compare the temperatures reached in the different windrows, Figure 34 presents the percentages that represent the different temperature zones in the heap cross-section for experiment GRA2 and GRA3, respectively. The zones were chosen as follows, as a function of the growth or survival of *AF* (see also Chapter 3.1.4): below 30°C, growth is very slow, but spores and mycelium can of course survive. Between 30 and 50°C, growth of *AF* is very rapid, and heavy sporulation occurs. Between 50 and 60°C, no or only very little growth occurs, but most of the spores survive. Above 60°C, the majority of the spores get killed in a short span of time.
The stage where the biggest part of the windrow (60-70 %) reached temperatures above 60°C was attained very early in the process: for the daily turned windrows after 1 week, for the weekly or monthly turned ones after 2-3 weeks. After the peak, only about 40 % of the whole composting mass was at temperatures above 60°C, and there was always a quite constant part (20-30 %) of the windrow that showed temperatures ideal for the proliferation of *AF*. MÜLLNER *et al.* (1977) calculated the zone of < 60°C, suited for the development or survival of *AF*, as 60 % of the total volume for a free-standing pile, and of 25-36 % for a 6 m wide, 2.5 m high and 25 m long windrow consisting of mixed sewage sludge and wood chips. When calculating the areas of the different temperature zones from the representations of iso-temperature curves shown by HAY & KUCHENRITHER (1990), citing from a report by IACOBONI (1983), it results that only between 4 % (for 5x/week turned windrows) and 15 % (for windrows turned 2-3 x/week) of the windrows were > 60°C. The author's deduction, however, that the small hot zone in the daily turned windrow was solely due to cooling due to the frequent turnings does not take into account that the frequent turnings could have accelerated the degradation process, provoking an exhaustion of the nutritive sources for the degrading microflora, and therefore an accelerating temperature decline. The dimension of the zone below 30°C was greatly influenced by the ambient air temperature, as observed by FERNANDES *et al.* (1994), who found a strong correlation between the temperatures measured in to corner of trapezoid heaps, and the ambient temperature.
The delay in the heating of the compost made of garden and kitchen waste (experiment GRA3) due to the high amount of water is clearly visible, as well as the slow heating of the weekly turned windrow in experiment GRA2.
It is interesting to observe that the absolute highest temperatures (78°C for experiment GRA2, and 82°C for experiment GRA3) were not measured at the moment where the biggest percentage of the windrows was at temperatures above 60°C, but 1-2 weeks later.

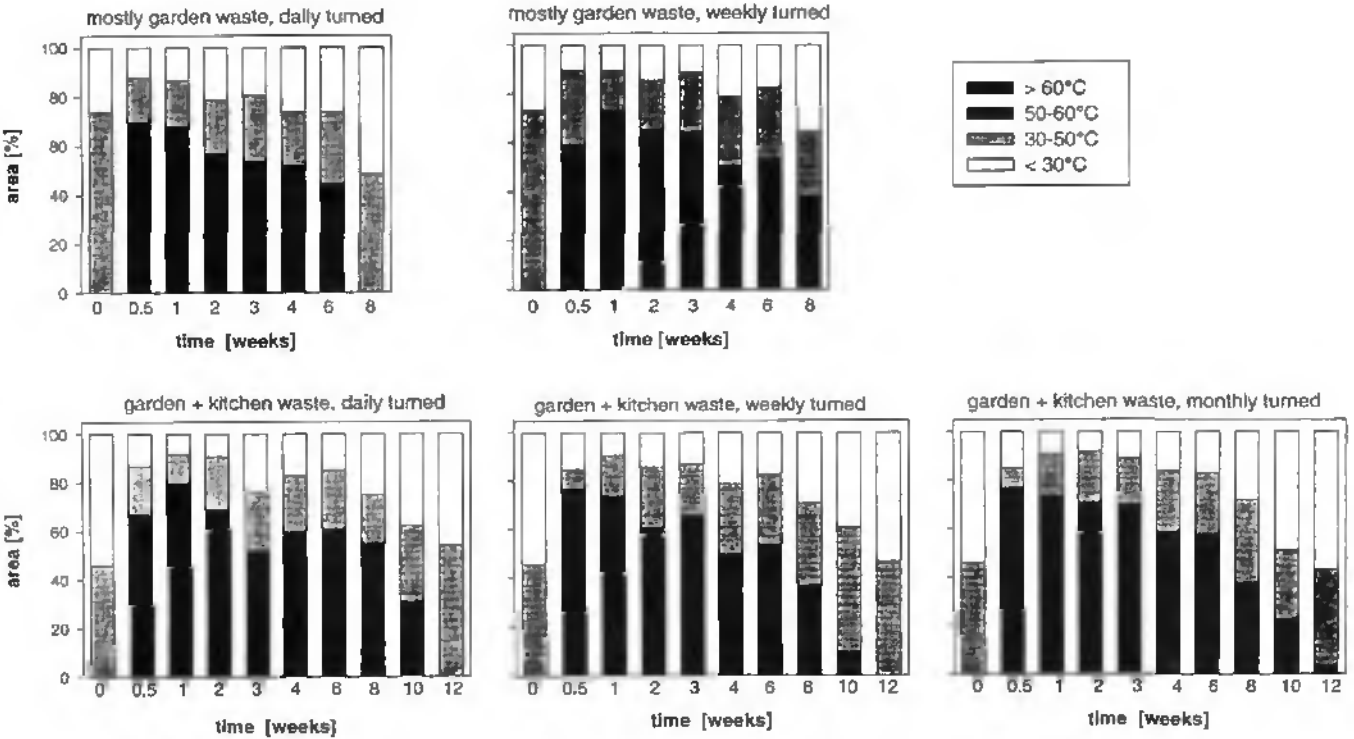


Figure 34: Percentage of different temperature zones in open-air windrows. Experiment GRA2: mostly garden waste; Experiment GRA3: garden + kitchen waste.

When interpreting the results shown in Figures 31 and 33, it has to be taken into account that the iso-curves were drawn after calculations that might not always exactly represent the reality, because temperature measurements were not carried out evenly in the windrow. However, verification of the shape of the temperature iso-curves was obtained by carrying out measurements in a compost windrow of the same dimensions every 20 cm vertically and horizontally (results not shown).
Also, temperatures were considered as being symmetrical for the two halves of the triangular windrows. This was confirmed by measurements by FERNANDES *et al.* (1994) in open-air windrows during the summer months in Ottawa, Canada, and by INSAM *et al.* (1996), for microbial activity measurements taken from the two sides of a cattle manure compost windrow set up during April to June in Austria, but refuted by SCHUCHARDT (1990), who showed asymmetrical temperature distribution in windrows, as a function of wind, set up during winter in Northern Germany. Windrows being placed under a roof did, however, show symmetrical iso-curves. In the situation of our own measurements, temperatures were might only slightly influenced by the wind, as the windrows were placed lengthwise in the direction of the wind. Measurements were carried out on that side of the windrow facing outward of the roting area, and being thus the half that represented the "worst case", because it was more exposed to wind than the other half.

But it is not only the different temperature zones in a windrow that influence the thermohygienization towards potential pathogens, it has also to be taken into account how often the compost is turned, that means the different zones mixed, and also how effective this mixing is. SCHUCHARDT (1990) has executed very interesting experiments to prove the mixing efficiency of a newly developed turning machine: by placing numbered wood pieces at given positions in the windrow, and searching for them after the passage of the turning machine, he could show that they were more or less evenly distributed. From the bottom zone (first 30 cm, representing 31 % of the windrow cross-section), 70 % of the markers got to the hot center zone, while 30 % stayed in the colder bottom zone. From the surface zone (the first 10 cm under the surface, at the top the first 5 cm, representing 17 % of the cross-section), 55 % got to the hot center zone, while the rest (45 %) got to the bottom. From the center (representing 52 % of the windrow cross-section), 42 % stayed in the center, the rest got to the bottom.

WATER CONTENT

The amount of water in a compost is an essential factor for the proper functioning of a composting process, as it influences the uptake of nutrients by the microorganisms, but also the oxygenation of the material. Furthermore, the dryer the compost, the more organic dust, consisting for a large part of microorganisms, is generated when compost is turned (MILLNER *et al.*, 1994).

In experiment GRA2 (Figure 35A), water content initially increased from about 50 % to 55 %, most probably due to the combined effect of the generation of process water by the degradation of organic substrates, and by the supply of rain water. As the composting was carried out in the open, the influence of the weather became more strongly apparent at the surface of the windrow than in the center, where an almost constant humidity of 50-55 % was maintained. At the end of the experiment, the compost was still quite wet (60 % humidity), which could pose problems for the screening, where the ideal humidity should be between 35 and 40 % (RICHARD, 1997).

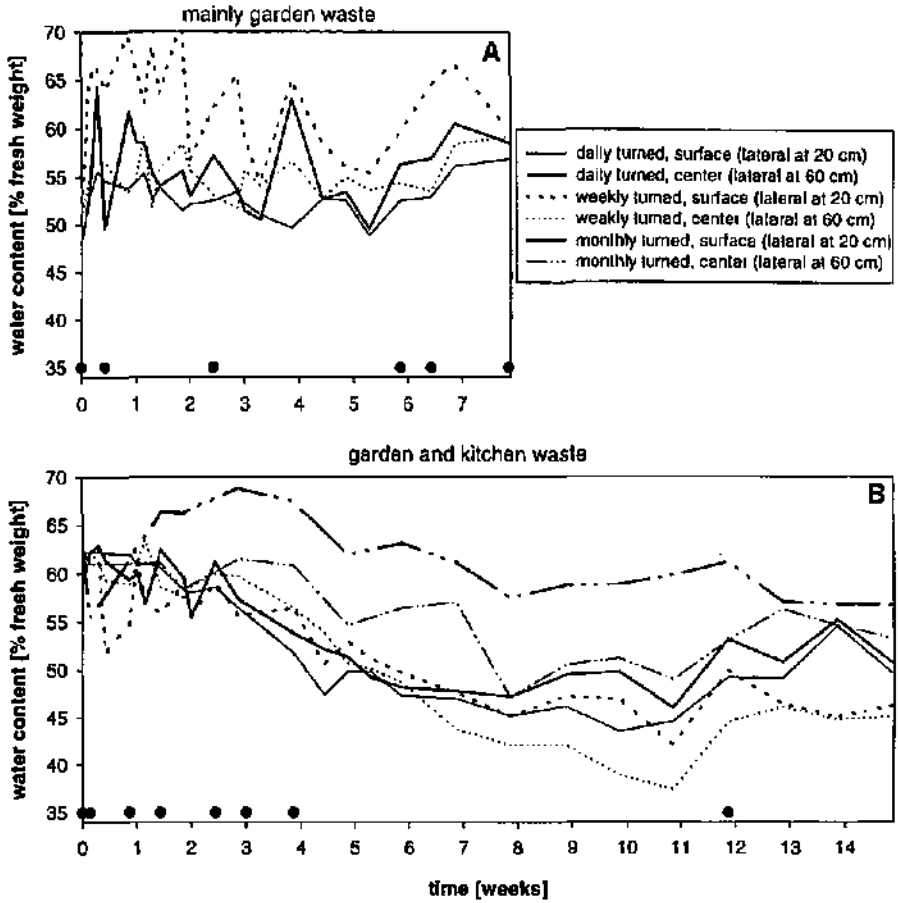


Figure 35: Evolution of water content in differently treated open-air windrows: daily turned (intensive = 5x/week, on every workday up to week 6, then 2-3x/ week), weekly turned (moderate intensive) or monthly turned (extensive). A = experiment GRA2 (mostly garden waste, C/N 40:1); B = experiment GRA3 (garden + kitchen waste, C/N 30:1). • rain during sampling.

When the raw material contained more kitchen waste (experiment GRA3), the initial water content was higher (around 60 %) (Figure 35B). Due to the rainy weather at the beginning of the experiment, no drying was observed during the first two weeks; the surface of the extensively treated windrow became even wetter, because the windrow was not covered with a tarpaulin. The high water content was also remarkable in the daily turned windrow, where the soaked material of the compost base was daily mixed with the material from the rest of the windrow, resulting in an overall very humid compost, which translated in a delayed temperature raise in the center (see Figure 30B). It can be concluded that in very wet weather, too frequent turnings can result in a too intensive wetting and cooling of the compost.

The contrary was observed during dry weather: by addition of water during turning, a sufficient degree of humidity could be maintained in the intensively treated windrow, while the windrow that was only turned once a week got too dry (see week 11), reaching values that were critical for micro-

bial activity. The experiment was carried out during late fall; in the summer months, the problem of dryness would probably be even more pronounced.
Due to the generally lower temperatures in the extensively treated windrow, drying was less intense: even after 6 months of composting, 60 % humidity was measured. Especially the surface stayed too wet all through the experiment.

pH

The rise of the pH is due to the metabolization of organic acids, and the liberation of ammonium, a sign of degradation of proteins. Normally, their breakdown is finished in the first weeks of the composting process, leading to a stabilization of the pH at around pH 8.

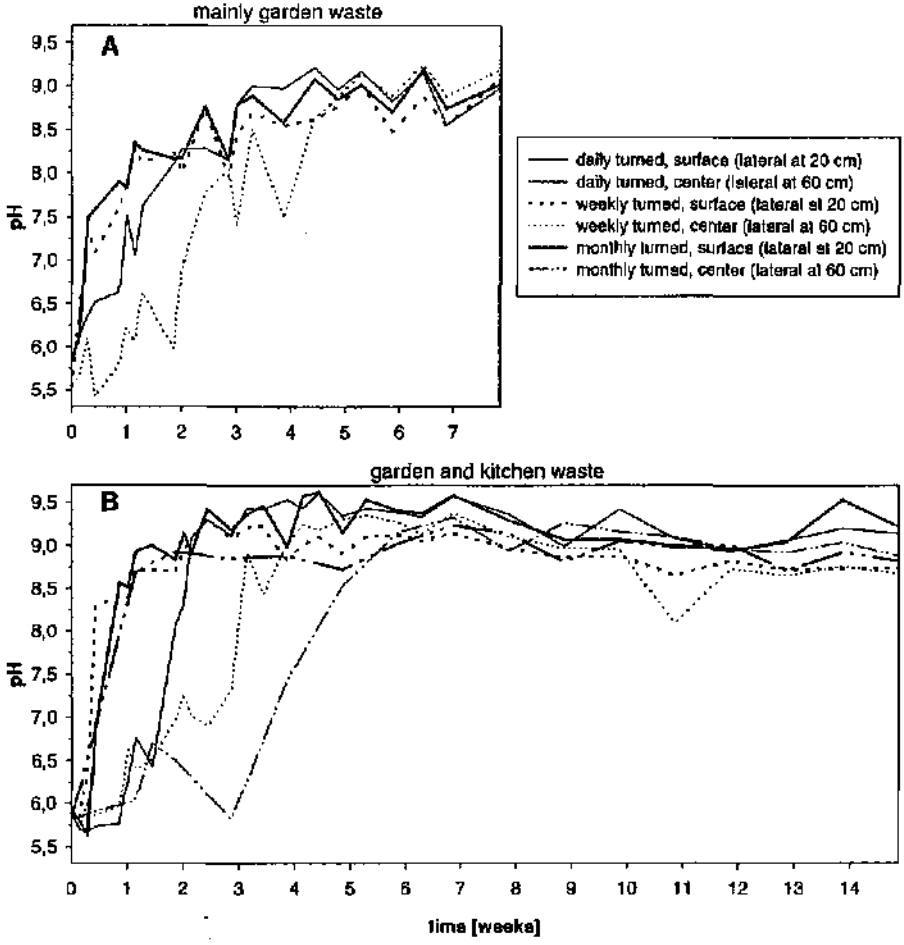


Figure 36: Evolution of pH in differently treated open-air windrows: daily turned (intensive = 5x/week, on every workday up to week 6, then 2-3x/ week), weekly turned (moderate intensive) or monthly turned (extensive). A = experiment GRA2 (mostly garden waste, C/N 40:1); B = experiment GRA3 (garden + kitchen waste, C/N 30:1).

Figure 36 shows the evolution of pH for the two experiments GRA2 and GRA3. While the pH rise was quite rapid in the samples taken at the surface, it was retarded in the ones from the center, confirming the pH measurement made in experiment GRA1. The delay in the pH rise was the more pronounced, the less intensively the windrows were treated. In the monthly turned windrow (Figure 36B), the pH even fell until the 3rd week of composting, a sign that in the core of this windrow, anaerobic conditions were present. The same was observed by ROWE et al. (1996), who interpreted the faster pH rise in the daily turned windrow as a more rapid degradation of organic acids due to a better aeration. The pH rise was also a function of the starting material: the more the initial mix contained easily degradable substances, the faster it happened. In the monthly turned windrow, a slight decrease of the pH from 8.8 to 8.3-8.4 was observed at the end of the 6-month composting period (results not shown).

DEGRADATION PARAMETERS

The main goal of composting is the transformation of a putrescible material into a relatively stable, humus-like end product, suitable for storage and that can be applied without damage to plants. The better the conditions are for microbial activity (nutrient content and availability, oxygenation, moisture, optimal temperature range, pH, etc.), the faster the degradation of the organic matter advances. Any treatment of compost (shredding, turning, aeration) happens in the view of an acceleration of the degradation. In this way, the surface necessary for the composting process can be reduced. According to different authors (ILLMER & SCHINNER, 1997; INSAM *et al.*, 1996; MICHEL *et al.*, 1996; HELM, 1995; FRICKE, 1988; GRAY & BIDDLESTONE, 1971a), turning led to a higher degree of decomposition, or in other words to a more mature compost (CHEN & INBAR, 1993).
Of course, the maturity that a compost should reach is to be defined as a function of its utilization: when it is applied to fields, and undergoes there normally further transformation before sowing or planting, a less mature product is required than when it is used in potting mixes for horticulture.

Several parameters can be measured to follow the maturation of a compost (see Chapter 1.5.3). Various authors proposed to analyze the water soluble fraction of the compost, which should contain to a large percentage those nutrients directly utilizable by the microflora, because the uptake of nutrients from the compost material by microorganisms happens almost exclusively if they are dissolved in water. However, evidence exists that some types of microorganisms are directly sticking to the surface of solid particles; the enzymes that hydrolyze the polymers are located in their membrane, and the uptake happens without solubilization of the monomers (PIERRE-FRANÇOIS LYON, personal communication).
The monitoring of the organic matter content, the C:N ratio and the water extractable organic carbon (WEOC) for the different treatments should show if a more frequent turning led to a faster maturation.

As a function of the composition of the starting material, the initial C/N ratio was higher for the garden waste (40:1, GRA2,) than for the mixture of garden and kitchen waste (30:1, GRA3) (Table 19). After two months of composting, however, the values were very similar (around 18) for the two experiments, and did not change much any more for the rest of the composting process.

The data for the organic matter content showed the same tendencies: from an initial content of 68-72 %, the values dropped to 45-48 % after two month of composting, and stayed at this level, except for the monthly turned windrow in experiment GRA3, where the overall lowest values were reached after 16 weeks of composting. It seems that in this windrow, due to the low temperatures in most of the material, the action of mesophilic organisms, most probably Basidiomycetes lead to an

extensive degradation of the otherwise difficult to degrade lignin-hemicellulose complex. This confirms the theory that degradation is maximal at 45-55°C (STENTIFORD, 1996).
The water extractable organic carbon (WEOC), which contains the fraction of organic material directly utilizable by the microorganisms, decreased more rapidly in the daily turned composts (from 1143 µg/gDW in experiment GRA2, and 1642 µg in experiment GRA3, to 201 and 394 µg after 4 weeks, and 164 µg and 381 µg after 8 weeks of composting, respectively) than in the weekly (272 and 472 µg after 4 weeks, and 220 and 496 µg after 8 weeks) or monthly (663 µg after 4 weeks and 506 µg after 8 weeks) turned ones.

Table 19: Degradation parameters (C:N, organic matter, water extractable organic carbon) for differently treated windrows (daily turnings (5x/week) = intensive; weekly turnings = moderate intensive; monthly turnings = extensive), for experiment GRA2 and experiment GRA3. Samples were taken after turning.

C/N							
Experiment	**turning frequency**	**starting material**	**2 weeks**	**4 weeks**	**8 weeks**	**12 weeks**	**16 weeks**
GRA2	daily	40	31	29	18	nd	nd
GRA2	weekly	40	31	26	20	nd	nd
GRA3	daily	31	23	20	16	20	13
GRA3	weekly	31	22	16	16	15	14
GRA3	monthly	31	nd	19	17	14	12
organic matter [% of dry weight]							
Experiment	**turning frequency**	**starting material**	**2 weeks**	**4 weeks**	**8 weeks**	**12 weeks**	**16 weeks**
GRA2	daily	72	57	58	45	nd	nd
GRA2	weekly	72	60	57	48	nd	nd
GRA3	daily	68	60	53	47	48	42
GRA3	weekly	68	55	48	48	43	43
GRA3	monthly	68	nd	48	47	40	35
Water Extractable Organic C [mg/g dry weight]							
Experiment	**turning frequency**	**starting material**	**2 weeks**	**4 weeks**	**8 weeks**	**12 weeks**	**16 weeks**
GRA2	daily	1143	261	201	164	nd	nd
GRA2	weekly	1143	454	272	220	nd	nd
GRA3	daily	1642	617	394	381	354	264
GRA3	weekly	1642	841	472	496	331	244
GRA3	monthly	1642	nd	663	506	405	295

WEOC showed indeed to be a much more sensitive parameter than C/N or OM to monitor degradation, presenting a distinct evolution for the different treatments, but also for the composts made from different starting materials (Table 19). The more rapid decrease in WEOC for the daily turned windrows, as well in experiment GRA2 as in experiment GRA3, could indicate that through the frequent mixing of material from the surface and the center of the heap, the microbial activity was not at all affected, but rather enhanced, and the degradation accelerated. INSAM *et al.* (1996) found a faster decreasing amount of dissolved organic C in windrows turned 1-2 times/weeks compared to one that was not turned at all.
The composition of the initial material influenced greatly the amount of WEOC: the compost made from mainly kitchen waste (experiment GRA3) contained even after 4 months of composting about

the same amount of WEOC than the one made from mainly garden waste (experiment GRA2) after 2-4 weeks of composting.
The values for the WEOC presented above were about 10 times lower compared to the ones given by GARCIA *et al.* (1990), who listed values between 1.27 and 4.56 % DW (corresponding to 12700 to 45600 µg/gDW) for different starting materials (sewage sludge, grape debris, city refuse), and 0.41 to 1.19 % DW after 90 days of composting. Unfortunately, these authors did not indicate if and how they filtered the sample for analysis, which could have a big influence on the amount of carbon measured. Even if the absolute values can not be compared, tendencies in the course of the composting process were similar, and the fraction of the water-soluble carbon showed the most important decrease compared to other maturity parameters measured (C/N, color of the water extract).
Also, MARUGG *et al.* (1991) found higher values: 7410 µg/gDW in the fresh material (a 1:1:1 (v/v) mixture of grass clippings, brush and leaves), and 2060 µg/gDW after 4 months of composting. They did not indicate, though, by what method they measured total carbon. Data of water soluble organic C in municipal solid waste compost presented by IANNOTTI *et al.* (1994) can also not be interpreted, because these authors indicated mg total water soluble C per ml, without giving the amount of water used for the preparation of the water extract. Astonishingly, they found in the first time an increase of the WEOC, as for other parameters (organic matter, C/N), and only from the 10th day of composting on a steady decrease, until the 12th week of composting, after which only a slight reduction was measured. The authors interpreted the remaining, no more metabolizable organic matter towards the end of the compost maturation process as soluble humic substances.
The quite high WEOC content in the composts made from garden and kitchen waste after 4 months of composting when no more self-heating was observed, an indication that the compost did not contain any easily degradable substances any more, might thus be partially explainable by the presence of a high amount of soluble humic compounds. Maybe the formation of such substances was favored by the high concentration of organic matter in the starting material.

Comparisons of the treatments have to be made with precaution, because part of the water soluble substances can be lost in the leachates, which can originate from compost containing excessive amounts of grass clippings or other highly degradable and very moist materials. FRICKE (1988) indicated for biowaste compost containing a high percentage of kitchen waste a maximum of 15-25 l of leachates per t fresh weight. Soluble substances can also be washed out of uncovered heaps in heavy rains: MARUGG *et al.* (1991) calculated the amount of total carbon in leachates during heavy rain at 600 g / m^2 occupied by a windrow containing 1/3 grass clippings at the beginning of the process, and at 170 g / m^2 after 4 months of composting. In our experiments, important washout should have been avoided by covering the heaps, if the weather forecast announced rain, with a tarpaulin.

Another way of representing the maturation of the compost is to present the rate of degradation (Figure 37) calculated according to KROGMANN, (1994) (see Chapter 2.2.5). In both experiments, more than half of the organic matter was degraded in the first 2-4 weeks of composting, to proceed more slowly for the rest of the experiment to final values between 65 and 75 %.

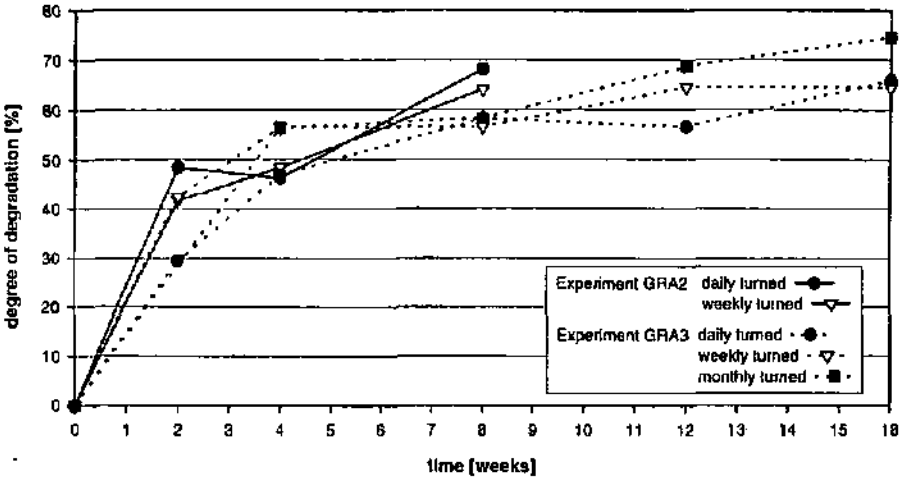


Figure 37: Evolution of the degree of degradation, calculated on the basis of the WEOC measurements (see Table 19) of a daily turned (5x/week); weekly turned or monthly turned open air windrow, for experiment GRA2 and experiment GRA3. Samples were taken after turning.

Microbiological analyses

An crucial feature of industrial composting is the important self-heating of the compost, accelerating on the one hand the degradation process, leading on the other hand to a thermohygienization towards human, animal and plant pathogens and weeds. Among the potential human pathogens and allergens, *AF* has special significance, as this saprophytic mold has its habitat in compost. *AF* concentrations were investigated in the compost, but also in the air during turning, as infection happens through inhalation of *AF* spores. Besides *AF*, bacteria belonging to the Family of *Enterobacteraceae*, especially coliforms, some being considered as indicators of fecal contamination, can be found in compost. It was investigated if coliforms were eliminated in the same order as *AF* in the course of the composting process.

ASPERGILLUS FUMIGATUS

In the compost

For the discussion of the following results, it has to be taken into account that the sampling depths indicated are approximate values (length of the compost auger 25 cm), and that, especially for the samples taking in the center, a slight contamination with material from the outer layers might be possible. The "surface" samples were taken in a depth of about 20 cm, where, at least in the peak heat phase, temperatures were already quite elevated. *AF* might have been present in higher concentrations in a zone between 5 and 20 cm depth, where temperatures and humidity were permissive for its growth.

The initially high *AF* concentrations in the garden waste ($2 \cdot 10^5$-$3 \cdot 10^6$ cfu/gDW, Figure 38), got quickly reduced to low levels in the daily turned windrow after 1 week of composting in the center of the heap (Figure 38B), and stayed at these low concentrations until the core temperature fell below 50°C. At that moment, a recolonization of the compost with *AF* to about 10^3 cfu/gDW was observed. When the turning frequency was reduced to only one turning per week, *AF* elimination was delayed in the initial thermogenic phase. However, due to the continuance of the hot phase, no recolonization up to the end of the experiment was observed.

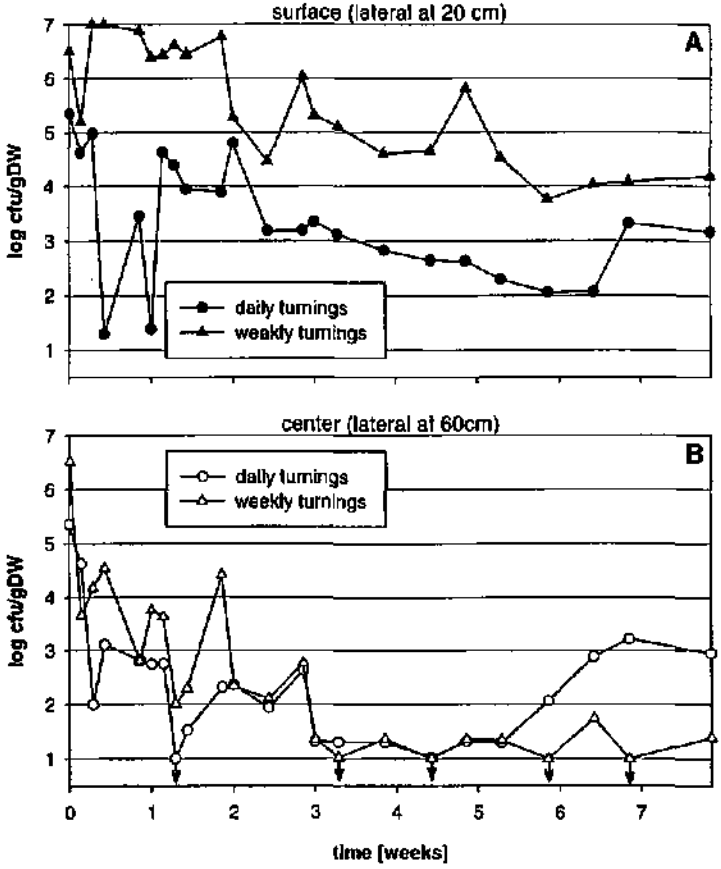


Figure 38: Evolution of Aspergillus fumigatus in experiment GRA2 (mostly garden waste, C/N 40:1) at the surface (A) and in the center (B) of a daily turned (= 5x/week, on every workday up to week 6, then 2-3x/ week) and a weekly turned open-air windrow. ↓ values below the detection limit of 5-10 cfu/g fresh weight.

Daily turnings effected also a better elimination of *AF* in the surface material (Figure 38A), however concentrations never fell, with the exception of the first week, below 10^2 cfu/gDW. With weekly turnings, no values below 10^4 cfu/gDW were measured.

In spite of a slower temperature increase in the daily turned windrow at the beginning of the composting process in experiment GRA3 (garden and kitchen waste; Figure 30) compared to experiment GRA2 (Figure 29A), reduction of *AF* numbers as well as in the center as at the surface was more pronounced (Figure 39). The low oxygen concentrations (most of the time below 5 %, results not shown) measured in this phase of the process might have contributed to the fast elimination of the molds from an initial concentration of $2\text{-}6\cdot10^6$ cfu/gDW to between not detectable and 10^2 cfu/gDW. *AF* concentrations in the daily and the weekly turned composts were quite the same, in agreement with the quite similar temperature evolution in the two treatments at the beginning of the process (Figure 30). The almost identical *AF* counts at the surface (Figure 39A) and in the center (Figure 39B) of the daily turned windrow indicate a good homogeneity of the compost material.
Recolonization started first (from the 4th week of composting on, parallel to the onset of the temperature decline) at the surface of the weekly turned compost, and reached about one order of magnitude higher values than in the compost that was turned daily, indicating that by the less frequent turnings, degradation of organic matter was less intense, and more nutrients were available to the mold after the thermogenic phase. SOARES (1996) showed that the recolonization of compost by *Salmonella* was all the higher as the compost was less mature. In the center, recolonization was observed even before the core temperatures fell below 50°C: from the 8th week on, *AF* numbers started to increase, although the temperatures were for 3 more weeks > 50°C.

Extensive compost management, e.g. one turning per month, did hardly bring about a thermohygienization of the compost at all. In spite of the quite high core temperatures (> 60°C until the 10th week of composting), *AF* concentrations were only reduced slowly, and never got below 10^2 cfu/gDW. At the surface, *AF* numbers remained between 10^4 and 10^5 cfu/gDW for the whole experimental period. Final concentrations (at 22 weeks of composting, results not shown) were 10^3 cfu/gDW, again higher than in the daily or weekly turned composts.
Sudden increases in the core *AF* concentrations, for example after 3 weeks in experiment GRA2, and after 3½ weeks in experiment GRA3, followed temperature decreases at the surface. It seems that the wind and rain cooled a broad layer at the windrow surface, allowing regrowth of *AF* in this zone, and reinoculating thus heavily the center part of the windrow when the compost was turned.

One of the few literature data where the concentrations of *AF* in compost were determined in function of the compost temperature is available from MILLNER *et al.* (1977). *AF* was detected in compost samples from a 21-day old static aerated pile prepared from sewage sludge and wood-chips in temperature zones of 60°C or less, in concentrations between 10^3 to 10^5 cfu/gDW. One zone with temperatures between 62 to 63°C and a very low moisture content of 28 % yielded $2\cdot10^3$ *AF*/gDW. From hotter zones (60-82°C) no *AF* could be cultured. It should be noted, though, that the lower limit of detection was 10^3 cfu/gDW. Concentrations were maximal ($> 5\cdot10^5$ cfu/gDW) in a sample taken from a zone of 46-50°C, and very low (30 %) moisture content. In the cured compost (about 50 days old, 20 days with, 30 days without forced aeration), at a temperature of 45°C, concentrations were lower than in the 3-week old compost, ranging from $8\cdot10^4$ to $4\cdot10^5$ cfu/gDW. Values are thus very similar to those from the monthly turned compost in experiment GRA3. TÖTER (1994) examined the *AF* content of biowaste during composting in static aerated piles: from an initial concentration of $3.5\cdot10^5$ cfu *AF*, and $1\cdot10^7$ cfu yeasts, the amount dropped to $4.6\cdot10^3$ in the hot (70-77°C) center of the pile, while at the cooler surface (48-55°C), concentrations of $1.9\cdot10^5$ cfu/gDW persisted.

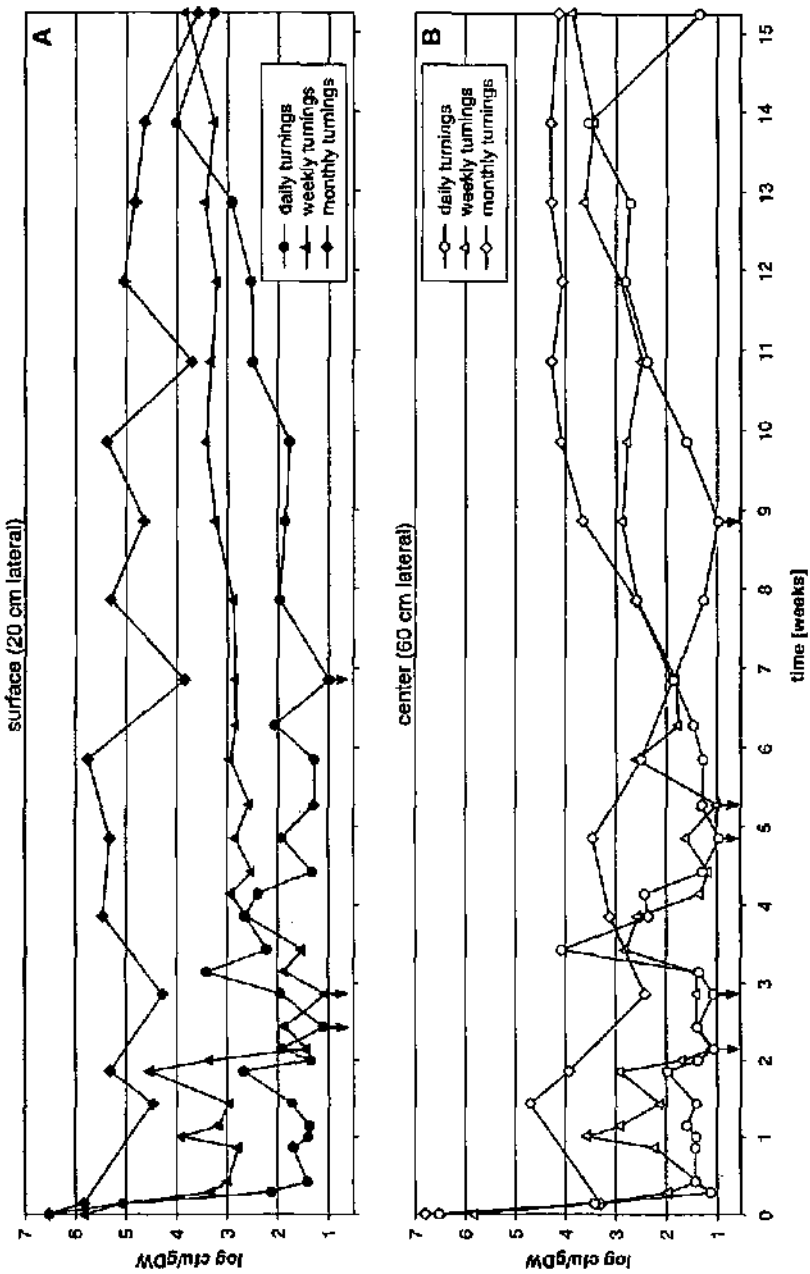


Figure 39: Evolution of Aspergillus fumigatus in experiment CRA3 (garden + kitchen waste, C/N 30:1) at the surface and in the center of a daily turned (= 5x/week, on every work day up to week 6, then 2-3x/ week), a weekly turned, and a monthly turned open-air windrow ↓ values below the detection limit of 5·10 cfu/g fresh weight.

In the air

The potential health hazard of *AF* arises not from the direct contact with the compost, but from the inhalation of mold propagules (spores and pieces of mycelium) generated during the mechanical agitation of compost (FELDMANN, 1995; MILLNER, 1995). In order to test if the reduced *AF* concentrations measured in the composts by an intensive treatment resulted in lesser dispersion, the concentration of *AF* in the air during turning was measured.

Figure 40 shows the concentration of *AF* propagules in the air in 5 m distance to the machine during turning of composts made mainly out of garden waste (experiment GRA2).

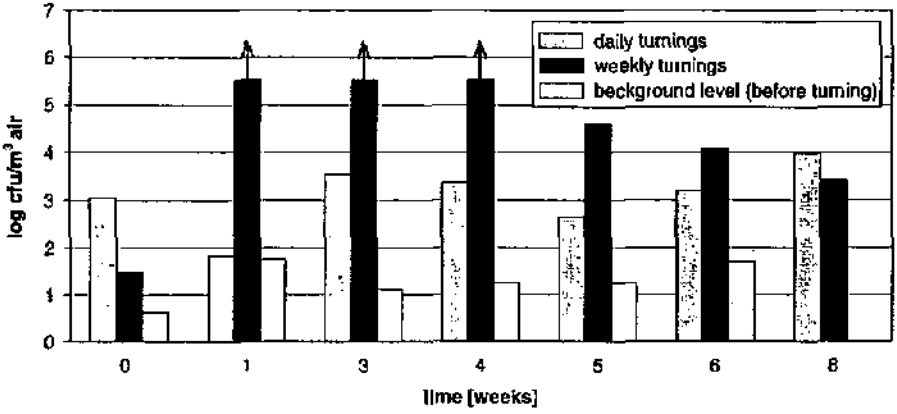


Figure 40: Concentration of dispersed AF propagules (cfu/m³ air) in the air 5 m behind the turning machine, in the direction of the wind. Experiment GRA2 (mostly garden waste, C/N 40:1). ↑ values above the detection limit (3.3·10³ cfu/m³ air).

Up to the 6th week of composting, higher *AF* concentrations were measured when the moderately intensive treated compost was turned. Numbers largely exceeded in the beginning the upper level of detection of $3.3 \cdot 10^3$ cfu/m³ air.
AF concentrations during the turning of the intensively treated windrow (daily turnings) were almost zero in the beginning, when concentrations at both the center and the surface were very low (see Figure 38), rose to a maximum of 10^4 cfu/m³ air in the third week of composting, after which a decline was observed until the 6th week. Then, recolonization began, which translated in again rising *AF* concentrations in the air.

On the basis of the results obtained in the experiment GRA2, it was decided to slightly change the experimental set-up, in that measurements were made for one very close to the turning machine (at a distance of 2 m), in order to avoid wind effects. At the same time, the dispersion at a distance of 10 m, as best as possible in the direction of the wind, was detected. In such a distance to the machine, the presence of people working on the composting site would be possible. As it had been observed in the experiment GRA2 that plates were often completely overgrown, 3 of the 6 replica plates from the measurements 2 m behind the turning machine were treated with the dilution / culturing method (see Chapter 2.2.2), the other 3 were cultured directly.
Results are expressed as the geometric mean of the 3 directly cultured plates; for the dilution/culturing method, the three plates were united to one sample.

From Table 20 it can be seen that the counts made after washing the spores from the agar surface were generally higher; differences could be more than one order of magnitude.

Table 20: Comparison of airborne AF propagules (cfu/m³ air), measured during turning 2 m behind the machine, and detected either by directly incubating the agar plates (direct culturing), or by washing the spores from the agar surface, and plating the dilutions of the washing solution on agar. Experiment GRA3 (garden and kitchen waste). GM = geometric mean, nd = not determined.

	daily turned windrow			weekly turned windrow			monthly turned windrow		
age (weeks)	direct culturing		washing/ culturing	direct culturing		washing/ culturing	direct culturing		washing/ culturing
	GM	range		GM	range		GM	range	
0	$3.2 \cdot 10^2$	$1 \cdot 10^2$-$8 \cdot 10^2$	$6.7 \cdot 10^3$	$3.2 \cdot 10^2$	$1 \cdot 10^2$-$8 \cdot 10^2$	$6.7 \cdot 10^3$	$7.6 \cdot 10^1$	< 100-100	$6.7 \cdot 10^3$
1	$>3.3 \cdot 10^5$	-	$8.1 \cdot 10^4$	$>3.3 \cdot 10^5$	-	$8.0 \cdot 10^6$	nd	-	nd
3	$1.3 \cdot 10^3$	$8 \cdot 10^2$-$3 \cdot 10^3$	$1.0 \cdot 10^4$	$3.6 \cdot 10^3$	$7 \cdot 10^2$-$3 \cdot 10^4$	$4.8 \cdot 10^4$	nd	-	nd
4	$5.3 \cdot 10^3$	$3 \cdot 10^3$-$9 \cdot 10^3$	$2.5 \cdot 10^3$	$9.5 \cdot 10^3$	$4 \cdot 10^3$-$2 \cdot 10^4$	$1.5 \cdot 10^4$	$>3.3 \cdot 10^5$	-	$3.3 \cdot 10^5$
5	$2.9 \cdot 10^2$	$2 \cdot 10^2$-$4 \cdot 10^2$	$3.5 \cdot 10^4$	$2.5 \cdot 10^4$	$2 \cdot 10^4$-$3 \cdot 10^4$	$2.4 \cdot 10^5$	nd	-	nd
6	$4.4 \cdot 10^2$	<100-$6 \cdot 10^3$	$9.1 \cdot 10^4$	$2.2 \cdot 10^4$	$2 \cdot 10^4$-$3 \cdot 10^4$	$6.2 \cdot 10^4$	nd	-	nd
8	$5.9 \cdot 10^3$	$8 \cdot 10^3$-$2 \cdot 10^4$	$6.0 \cdot 10^4$	$1.5 \cdot 10^4$	$1 \cdot 10^3$-$2 \cdot 10^4$	$9.1 \cdot 10^4$	$>3.3 \cdot 10^5$	-	$5.8 \cdot 10^5$
9	$6.7 \cdot 10^3$	$3 \cdot 10^3$-$1 \cdot 10^4$	$8.3 \cdot 10^3$	$2.8 \cdot 10^4$	-	$1.3 \cdot 10^5$	nd	-	nd
11	$1.0 \cdot 10^3$	$3 \cdot 10^2$-$3 \cdot 10^3$	$2.0 \cdot 10^4$	$4.2 \cdot 10^3$	$3 \cdot 10^2$-$2 \cdot 10^4$	$3.3 \cdot 10^3$	nd	-	nd
12	$3.4 \cdot 10^3$	$8 \cdot 10^2$-$1 \cdot 10^4$	$1.7 \cdot 10^3$	$7.5 \cdot 10^3$	$5 \cdot 10^3$-$9 \cdot 10^3$	$4.4 \cdot 10^4$	$1.2 \cdot 10^4$	$7 \cdot 10^3$-$2 \cdot 10^4$	$3.7 \cdot 10^5$
14	$1.1 \cdot 10^3$	$2 \cdot 10^3$-$3 \cdot 10^3$	$1.7 \cdot 10^3$	$3.9 \cdot 10^3$	$4 \cdot 10^2$-$2 \cdot 10^3$	$1.0 \cdot 10^4$	nd	-	nd
15	$1.3 \cdot 10^3$	$2 \cdot 10^2$-$3 \cdot 10^3$	$7.5 \cdot 10^3$	$1.7 \cdot 10^3$	$1 \cdot 10^3$-$2 \cdot 10^3$	$2.5 \cdot 10^3$	nd	-	nd
16	nd	nd	nd	nd	-	nd	$4.6 \cdot 10^3$	3.3-$5.9 \cdot 10^3$	$4.8 \cdot 10^4$
20	nd	nd	nd	nd	-	nd	$7.2 \cdot 10^2$	$4 \cdot 10^2$-$2 \cdot 10^3$	$2.8 \cdot 10^4$

This can be explained by the fact that a certain percentage of spores dispersed in the air are in chains, which yield only one colony by the direct culturing method, but break up when the spores are removed from the agar in the Stomacher. REPONEN *et al.* (1996) found in an laboratory set-up that the generation of spores from an agar surface at 30 % relative humidity (rH) yielded only 38-48 % spore singlets, the rest were chains of two or more spores, including long chains up to 15 spores. At high rHs, as they are encountered during the turning of a compost, however, these spore chains tend to break up: at 95 % rH, MADELIN & JOHNSON (1992) reported up to 85 % single spores. P. SCHERER (FH Hamburg, personal communication) found, when measuring the microorganism dispersion at a composting site, slighly higher values when the gelatin filters where dissolved and the resulting solution plated than when the filters were cultured directly. By collection of bioaerosols during the movement of sewage sludge compost by a front-end loader on a six-stage Andersen Sampler, MILLNER *et al.* (1980) found particles > 4.7 µm, interpreting this as clumps of *AF* spores. BOLEU *et al.* (1995) advised against comparing results obtained by direct culture (any culture plate samplers or filter samplers where the filter is cultured as is) with such from dilution plating (homogenization of agar, liquid impingers or dissolved filters), the latter leading generally to much higher counts because propagules will be separated in individual spores.

For the following comparison of the dispersion in the air of *AF* propagules 2 m behind the machine (Figure 41A) during the turning of the differently treated windrows, only the data obtained after washing of the agar plates will be used, for one thing because they represent better the true particle number encountered, but also because by this method, no upper limit of detection exists, and thus also concentrations $>3.3 \cdot 10^3$ cfu/m³ air could be measured.

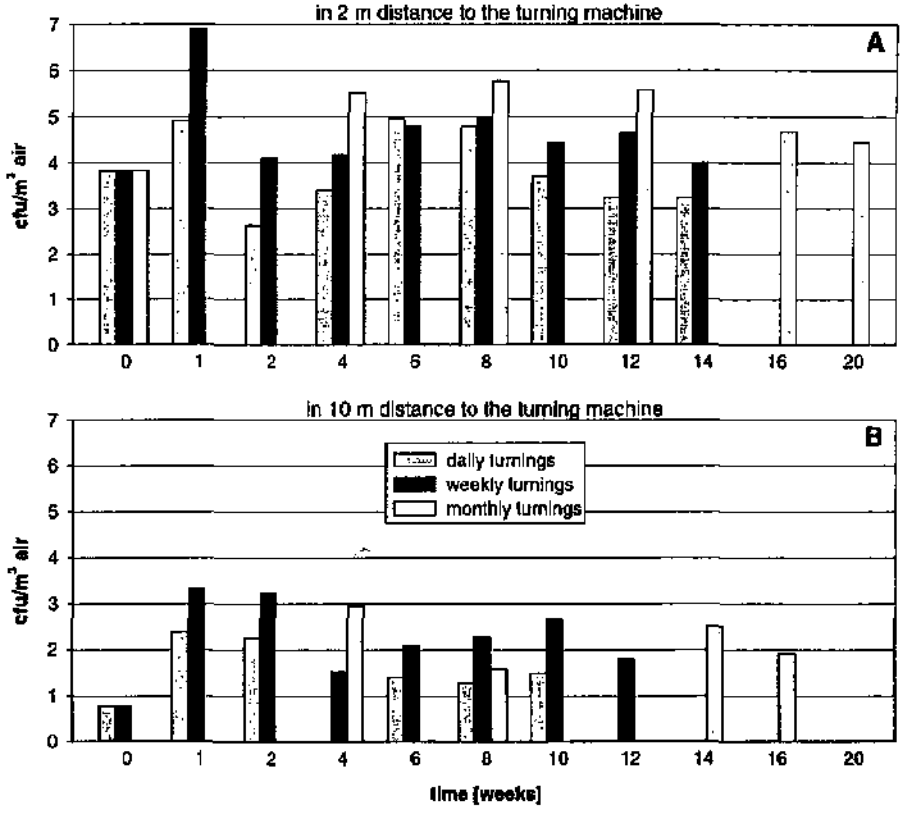


Figure 41: Comparison of airborne AF propagules (cfu/m³ air) measured during turning of differently treated (daily = 5x/week (intensive); weekly (moderate intensive) or monthly (extensive) turned) windrows 2 m behind the turning machine (A), and in 10 m distance in the direction of the wind (B). Cfu's for the measurements at 2 m were obtained by washing the particles from the agar surface, and plating the resulting dilutions again on agar, those at 10 m by direct culturing.

The results of the measurements made in close vicinity to the turning machine showed, as had already been demonstrated in the experiment GRA2, that generation of bioaerosols was smaller, the more frequently the windrows were turned.

Already during the setting-up of the windrows, 10^4 cfu/m³ air *AF* were detected. The concentration even increased, to reach a maximum value of 10^7 cfu/m³ at the first turning of the moderate intensively managed windrow. During the peak of the thermogenic phase of the daily and weekly turned windrows (weeks 2-3), concentrations were slightly lower (10^3-10^4 cfu/m³), to rise again, as soon as the temperatures started to fall. From the 8th week on, less propagules got dispersed, although in the compost, a slight recolonization was observed, and the drying of the material should in principle have favored dispersion. It should be kept in mind, though, that *AF* concentrations were only measured at two locations in the heap (lateral at 20 and 60 cm depth) that are not representative of the entire windrow.

When turning the extensively treated windrow, concentrations between 10^4 and 10^5 cfu/m^3 air were measurable up to the 5th month of composting. Only at the end of the experiment (after 6 months), < 1000 cfu/m^3 got dispersed (results not shown).

Measurements made in 10 m distance from the turning machine (Figure 41B) showed concentrations of airborne *AF* that were 2-3 order of magnitudes lower than those in the immediate vicinity of the machine. Evolution of results was less obvious than when air sampling was carried out very close to the machine. This could be the result of the difficulty to take measurements in the direction of the wind. Determination of direction as well as force of wind showed often a very unstable situation, especially on the composting site itself, where many obstacles led to the formation of local turbulences.

Although the literature is rich with studies about the dispersion of *AF* at composting (see Table 8, Chapter 1.8.1.2A) comparison of the data is very difficult, because every study was unique regarding the type of composting installation investigated, the air samplers used and the culture media employed. Also, it was often not stated in what distance to the mold emitting source the measurements were made, or what type of activity (shredding of raw material, turning, screening) was going on.
The results that can be compared best to ours are data by P. SCHERER (FH Hamburg, personal communication), who investigated the emission of bacteria and fungal spores on a similar composting site, where turnings were carried out every second day with the same type of machine as in our experiments. Air measurements were made in a distance of 2 m to the turner with a Sartorius MD8 sampler, collecting the particles on gelatin filters. *AF* spore numbers were generally much lower (< 10^4/cfu m^3) than in our study, most probably due to the use of a filtration bioaerosol sampler, which tends to lower the viability of spores because of the trauma of flow rate, dehydration and collision (JACOBS, 1994). It could be shown, nevertheless, that the turning of a windrow made up of mostly biowaste (70 %), leading to high composting temperatures, provoked in the beginning of the experiment less emission of *AF* propagules than the turning of a windrow consisting of mainly structure material (30 % biowaste), where core temperatures exceeded only in the first 2 week of composting 60°C. From the 10th week of composting on, when temperatures in both windrows where between 30-40°C, that means in the optimal range for the growth of *AF*, more mold propagules could be measured than during the thermogenic phase; concentrations were even slightly higher in the windrow with a high percentage of biowaste, indicating that the more abundantly available nutrients lead to a higher recolonization.
When considering data from bioaerosol measurements, it should also be kept in mind that the sampling method used only detected the viable organisms, and that laboratory tests carried out with spores of *Penicillium chrysogenum* demonstrated that about 25 % of the spores were not viable (BUTTNER & STETZENBACH, 1993). But, also non-viable spores can act as allergens, in that a structural component of the fungal cell wall, the β-1→3-glucan, is itself an inflammatory agent (DOUWES *et al.*, 1996).

GRAM-NEGATIVE / COLIFORM BACTERIA

Beside *AF*, compost can contain other potential human pathogens that get into the compost via the raw material: enterobacteria like *Salmonella, Shigella, Escherichia coli* or *Klebsiella*, enterococci, *Legionella* and others. The proof of the group of the so-called coliforms, that means Gram-negative, lactose-positive rods which contain many harmless strains, is often used as an indicator for the presence of other, potentially pathogenic fecal bacteria.

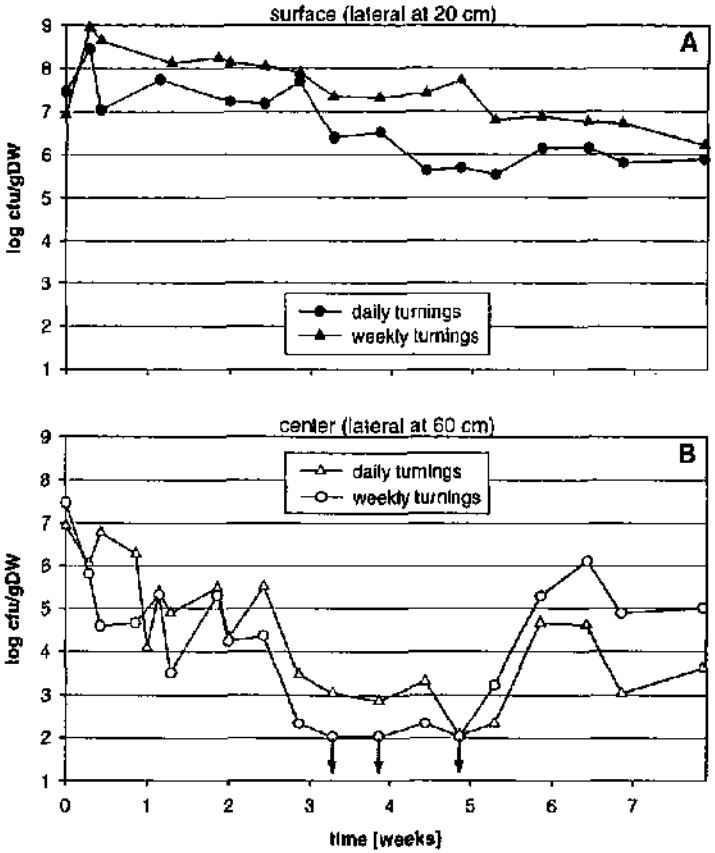


Figure 42: Evolution of Gram-negative bacteria at the surface (A) and in the center (B) of open-air windrows. Experiment GRA2 (mainly garden waste, C:N=40:1). Windrows were turned daily (5x/week = intensive) or weekly (moderate intensive). ↓ values below the detection limit of 50 cfu/g fresh weight. Detection on MacConkey Agar, counting of all colonies.

The data in Figure 42 represent the Gram-negative bacteria. Actually, the selective agar medium used (MacConkey Agar) was supposedly only allowing the growth of coliforms. Tests showed, however, that already after 24 h of incubation at 37°C, 73 % of the colonies were oxidase-positive, most presumably strains belonging to the group of Pseudomonads or Aeromonads. Also, all colonies became already red after a short time of incubation, accordingly to the manufacturer a typical sign for coliforms, even if this was in the beginning more pronounced with the strains belonging to the coliform group. In accordance with the literature (REINTHALER *et al.*, 1997; JAGER *et al.*, 1995; MOTE *et al.*, 1988) all colonies were counted, and the total expressed as Gram-negative bacteria. Tests showed that Gram-positive bacteria (*Bacilli, Staphylococcus aureus*) did not grow on the MacConkey agar medium, but *Pseudomonas aeruginosa* did very well.

When the data shown in Figure 42 are compared to those of *AF*, it stands out that Gram-negative bacteria seemed to survive better than the fungus under the composting conditions. Concentrations were in the center (Figure 42B) only at the moment of the peak temperature reduced to no more detectable. Recolonization set in as soon as the temperatures went down again. At the surface, almost no reduction was observed: concentrations were at the end of the experiment still $> 10^6$ cfu/gDW.

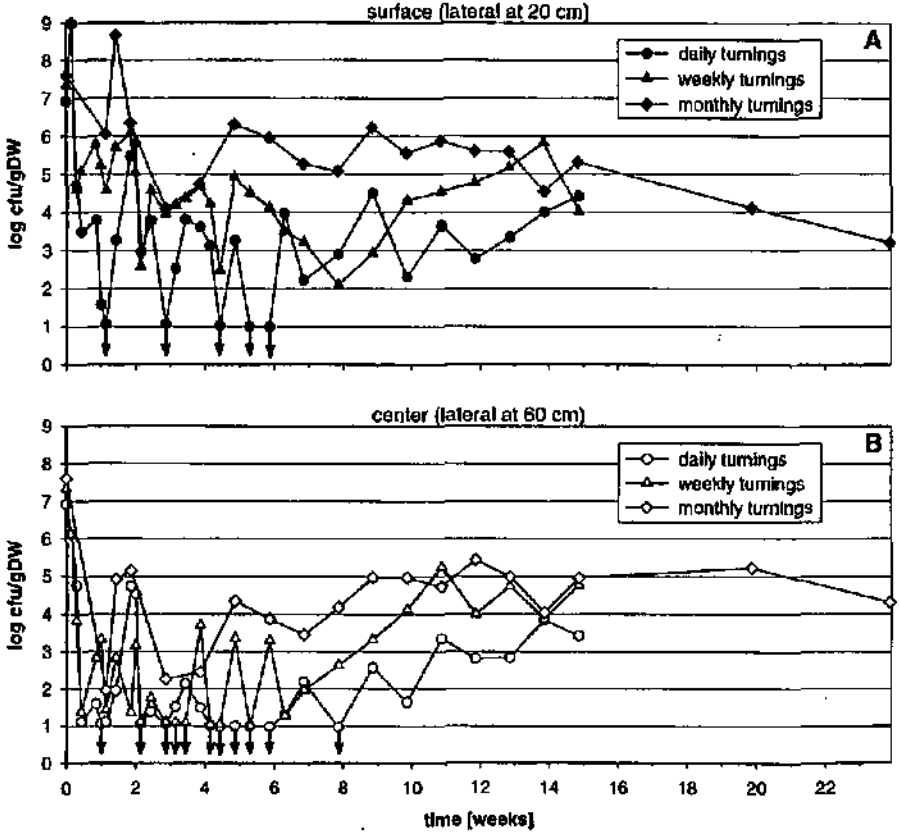


Figure 43: Evolution of coliforms during composting at the surface (A) and in the center (B) of open-air windrows. Experiment GRA3 (garden and kitchen waste, C:N=30:1). Turning frequency: daily (5x/week, intensive); weekly (moderate intensive); monthly (extensive). ↓ values below the detection limit of 10 cfu/gFW. Detection was made on VRB-Agar.

In the following experiment (GRA3), a different selective medium was chosen to detect the coliforms: the Violet Red Bile (VRB) Agar, a medium frequently used for the enumeration of coliforms in food, where the coliform flora is often accompanied by a great number of other Gram-negative bacteria. In order to enhance the selectivity, bacteria were cultured in the agar mass and not on the surface, and after setting, a second layer of agar was poured to cover the first one.

In this way, microaerophilic growth conditions were created that favored the facultative anaerobic coliforms over other, strictly aerobic bacteria. Tests showed that indeed of the 39 strains isolated, 97 % were oxidase-negative, and were identified as belonging to the group of coliforms (genera *Klebsiella*, *Escherichia*, *Enterobacter*, *Citrobacter*).

The curves shown in Figure 42 resemble those for *AF*, with the restriction that the elimination of coliforms was less complete, and that the recolonization, at least for the daily turned windrow, set on earlier, and reached higher final concentrations. It can also be remarked that the fluctuations were more pronounced, which can mean that as soon as conditions were locally a little more favorable, these bacteria were immediately able to develop again, or that inoculation from the outer, cooler layers was at times so intense that it took a while before numbers were reduced again under the influence of high temperatures. As for *AF*, coliform numbers were only gradually reduced in the extensively treated windrow.

When comparing the behavior of Gram-negative bacteria and coliforms, it seems that the former are much more adapted to the life in compost than the latter, which are supposed to only contain species that live normally in the gut of warm blooded animals. To the same conclusions came JAGER *et al.* (1994a), who investigated the fate of Gram$^-$ bacteria and fecal streptococci during composting. In finished compost from different sites (8-19 weeks of processing), they could isolate between $3 \cdot 10^5$ and $2 \cdot 10^8$ Gram$^-$ bacteria and between $2 \cdot 10^4$ and $6 \cdot 10^6$ streptococci per gram compost. While the number of Gram$^-$ bacteria was as high as in the raw material, the number of streptococci was reduced by several order of magnitudes.

Although coliforms are widely used as indicator for a fecal contamination (SCHLEGEL, 1985), not all representatives of this group are of fecal origin. For example *Erwinia*, a plant pathogen, may be well adapted to the life in compost. For this, the detection of *Escherichia coli*, a normal component of the intestinal flora of humans and animals (10^5 to 10^9 cfu/g feces; SCHMIDT, 1994) might give a better indication of the fate of potential fecal contaminants, although recent studies indicate that *E. coli* might survive or even thrive outside the gut (MARA, 1995, cited by P. SCHERER (FH Hamburg, personal communication)).

The graphs in Figure 44 show that *E. coli*, initially present in concentrations between 10^5 and 10^6 MPN/gDW, were, in the case of the daily turned windrow, not detectable any more after 1 week of composting, in the weekly turned windrows after 2 weeks.

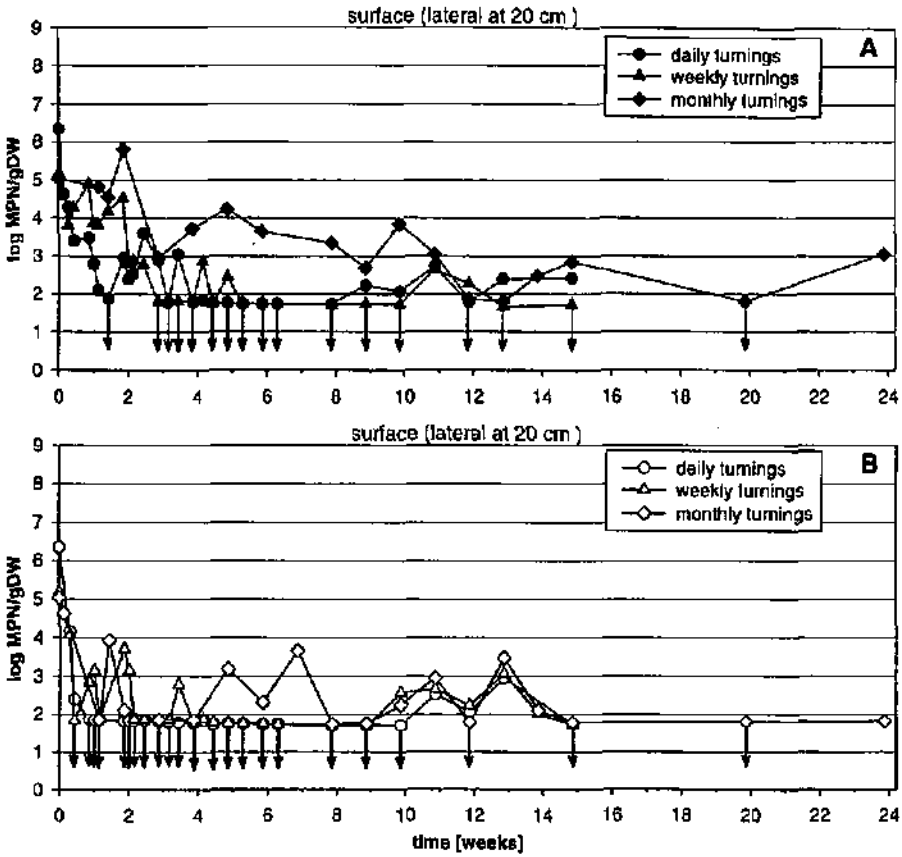


Figure 44: Evolution of E. coli during composting at the surface (A) and in the center (B) of open-air windrows. Experiment GRA3 (garden and kitchen waste.C:N=30:1). Turning frequency: daily (5x/week = intensive); weekly (moderate intensive); monthly (extensive). ↓ values below the detection limit of 28 MPN/g FW. Detection was made by the MPN-method in LMX-broth. Presence of E. coli was admitted if fluorescence and a positive indole-reaction was observed.

Recolonization was weak, not exceeding 10^2 MPN/gDW. In the monthly turned windrow, concentrations between 10^2 and 10^4 MPN/gDW were measurable up to the 16^{th} week of composting. Recolonization in the maturation phase was minimal, probably due to the competitive advantage of the saprophytic microflora present in the compost at this phase (DE BERTOLDI *et al.*, 1983).

The thermal inactivation of microorganisms in a compost heap that is agitated in frequent intervals is described in a mathematical model developed by HAUG (1993). He made the following simplifying assumptions: the compost material is composed of two zones, each of which has a uniform temperature. The temperature in the cooler zone is sublethal and causes no organism destruction, while a uniform lethal temperature occur in the hotter zone. Each time the windrow is turned, there is sufficient mixing energy to cause a random redistribution of the material.

$$n_t = n_0\left[f_l + f_h e^{(-k_d \Delta t)}\right]^N$$

$$f_l + f_h = 1$$

n_t = number of organisms surviving
n_0 = number of organisms initially present
f_l = fraction of composting material in the low temperature, sublethal zone
f_h = fraction of composting material in the high temperature, lethal zone
k_d = thermal death coefficient
Δt = time interval between compost turnings [days]
N = number of pile turnings

The application of the model for daily ($\Delta t = 1$), weekly ($\Delta t = 7$) or monthly ($\Delta t = 30$) turning intervals, assuming an initial concentration of 10^7 organisms, is shown in Figure 45.

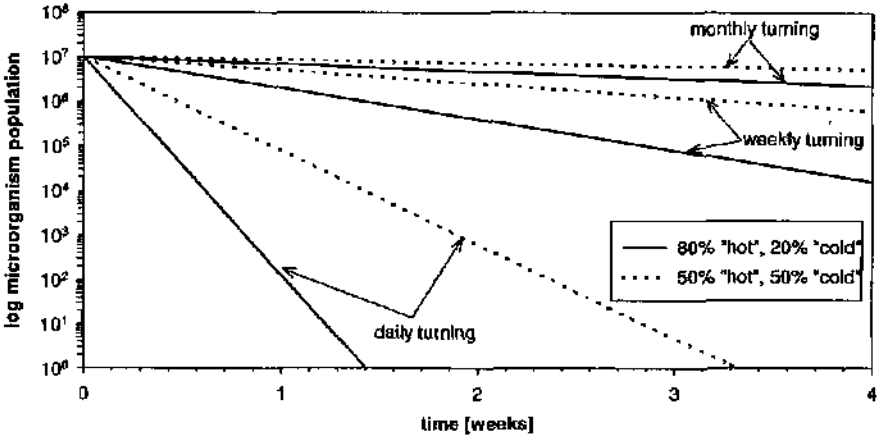


Figure 45: Modelisation of thermal inactivation of potentially pathogenic microorganism in daily, weekly or monthly turned compost heaps, with different percentages of hot zones (complete inactivation of pathogens; $k_d = \infty$) and cold zones (no inactivation of pathogens).

Two different conditions were assumed: a windrow with 80% of the material in the "hot" zone, and one with 50%. Taking the case of *AF* but also other pathogens, which get inactivated at temperatures between 55°C and 60°C, these assumptions represent real situations in the first month of the composting process, that means during the really thermogenic phase. The model shows that the turning frequency greatly influences the inactivation: while it would take in a daily turned windrow about 1.5 weeks in a compost with a large hot zone, and a little more than 3 weeks in one where only half of the material is at temperatures > 55-60°C to reduce the number of potential pathogens form an initial concentrations of 10^7 to 10^0, weekly turnings would lead to reductions of the microbial number of 2.5 or 0.5 order of magnitudes after 3 weeks, respectively, and monthly turnings would effect almost no pathogen reduction.

Comparison with experimental data (Figures 38, 39 and 42-44) is difficult, because the model depicts the situation in the whole composting mass, while in our experiments, data form only two sampling points are available. Even if the tendencies are the same (stronger reduction of *AF* numbers by higher turning frequencies), the thermal inactivation is much more rapid than the model would predict. It has to be considered, though, that samples denoted "surface" were not taken at the surface itself, but in a depth of 20 cm, where in the beginning of the process, temperatures were often above that lethal for *AF* (see Figures 29 and 30). To prove the validity of the model, samplings after turning from the whole windrow section should have been taken.
The model is insofar interesting as that it shows that a number of turnings are necessary to reduce the number of potential pathogens: to completely eliminate them (reduction by a factor of 7 order of magnitudes), 10 turnings are needed in the case of a hot zone comprising 80 %, which really represents an optimal situation, and 23 in the case of a hot zone of 50 %. To reduce an initial number of 10^6 coliforms, a number frequently detected in biowaste, to 10^3, a limit demanded by the US, it would take 4 turnings with 80 % of the material in the hot zone, and 10 turnings when half of the material is in the hot zone. The US law stipulates 5 turnings in the 15 days where temperatures have to be above 55°C (see Chapter 1.3). The 3 turnings prescribed by the Swiss law would reduce an initial number of 10^6 microorganisms by 1 order of magnitude when half of the material is in the hot zone, and by 2 order of magnitudes when 80 % is in the hot zone.
The model is a simplification that does not take into account microbial regrowth in the parts of the windrow that show sublethal temperatures. Also, it was assumed that complete inactivation occurred in the hot zone, which in the case of *AF* spores, might not be true (see Chapter 3.1.4.2). On the other hand, other factors than the temperature (e.g. competition with other microorganisms or antagonistic relationships, natural die-off due to depletion of nutriments or water, UV radiation at the surface of the compost (HAUG, 1993) do also contribute to the elimination of potential pathogens in compost.

MESOPHILIC AND THERMOPHILIC HETEROTROPHIC BACTERIA

The MPN of mesophilic bacteria was quite high in the initial material mix in experiment GRA2 (mainly garden waste, Figure 46); it contained $2 \cdot 10^{11}$ MPN/gDW of compost.
In the center of the daily turned windrow (Figure 46B) they got quickly reduced to about 10^8 during the first weeks of composting, and stayed at about this level for the rest of the experiment. At the surface (Figure 46A), a linear reduction of the MPN was observed up to the 40th day of the experiment, when also 10^8 MPN/gDW were reached; after this, no more changes were noticed.
In the center of the weekly turned windrow (Figure 46B), about the same evolution of mesophiles was seen as in the daily turned one. At the surface, however, 10^8 MPN/gDW were only reached at the end of the experiment, e.g. after 55 days of composting.

The initial concentration of thermophilic bacteria under the chosen growth conditions (incubation in Nutrient-Yeast broth at 60°C) in experiment GRA2 (Figure 47) was lower ($4 \cdot 10^7$ MPN/gDW) than the mesophiles, and rose in both treatments (daily or weekly turnings) in the first two weeks of composting, to reach between 10^9 and 10^{10} MPN/gDW, the daily turnings causing a faster rise. While about the same concentration of thermophiles was observed at the surface and in the center of the daily turned windrow during the thermophilic phase (about 10^9 MPN/gDW), getting reduced to 10^8 towards the end of the experiment, a considerable difference between the center and the surface was seen in the weekly turned windrow, the surface supporting astonishingly more thermophiles than the center. Due to the higher temperatures, concentrations at the end of the experiment were one order of magnitude higher (10^9 MPN/gDW) than in the daily turned windrow.

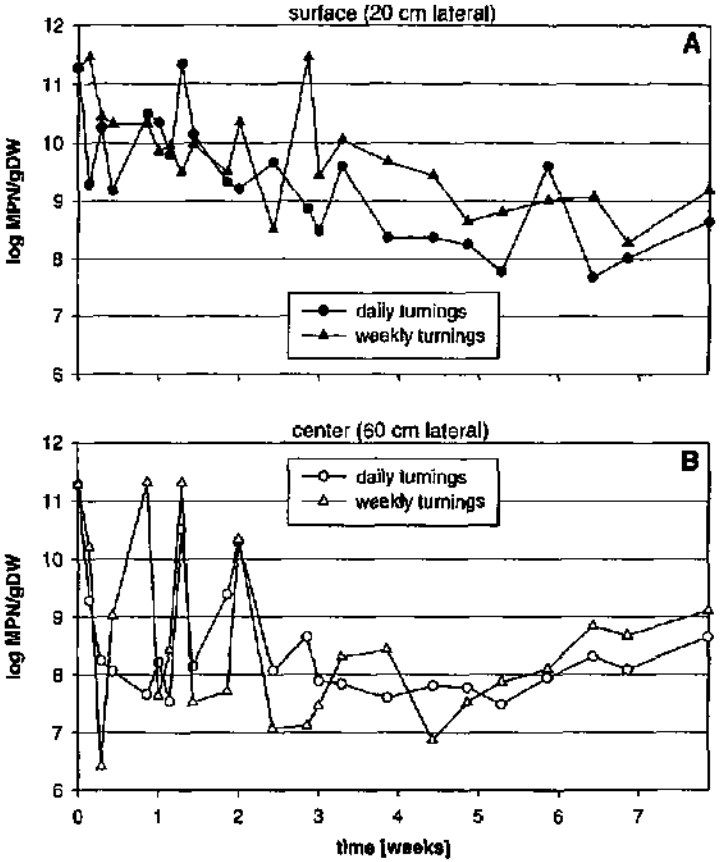


Figure 46: Evolution of mesophilic bacteria (grown in Nutrient-Yeast broth, incubation at 60°C) during composting at the surface (A) and in the center (B) of open-air windrows. Experiment GRA2 (mainly garden waste, C:N=40:1). Turning frequency: daily (5x/week = intensive); weekly (moderate intensive). Detection was made by the MPN-method.

In the experiment GRA3 (Figure 48) the number of mesophilic bacteria was slightly lower in the initial mix (10^{10} MPN/gDW), may be due to self-heating of the very nutrient-rich material already during collection. As in experiment GRA2, numbers got reduced to about $1 \cdot 10^8$ in the center, and to $3 \cdot 10^8$ at the surface for the daily turned windrow, and stayed at about this level for the whole duration of the experiment. In the weekly turned windrow, about the same concentrations were observed, but the numbers were not as constant as in the daily turned one: in the first 3 weeks, reduction was more important, while after the peak heat phase a slight augmentation to $3 \cdot 10^9$ MPN/gDW was observed. It was only the monthly turned windrow that showed differences of about one order of magnitude between the mesophilic counts of the surface and the center: as expected, numbers were higher at the exterior than in the core. The highest mesophilic bacteria concentration was measured at the surface of the monthly turned heap, compared to the other treatments.

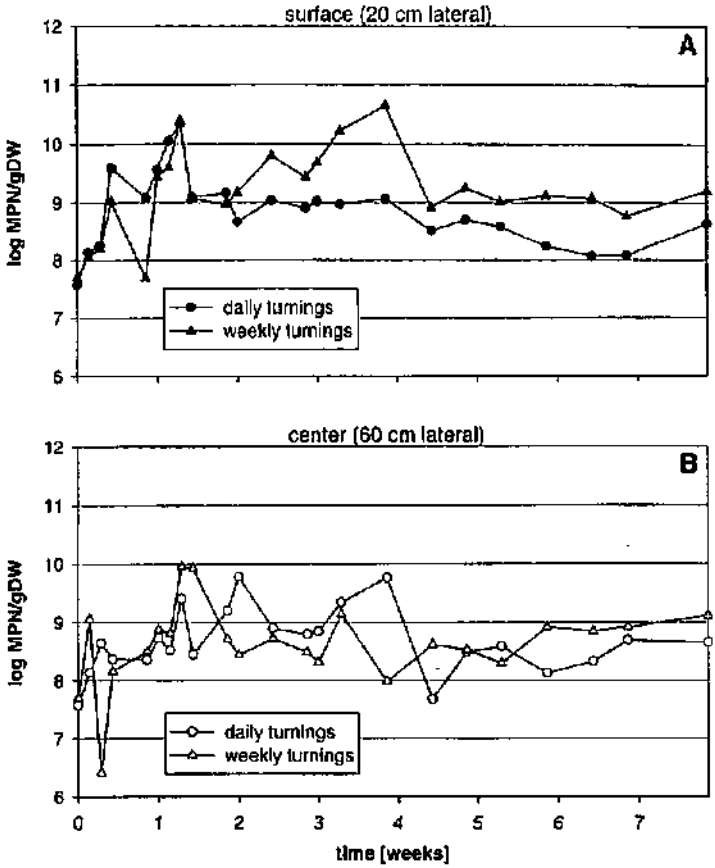


Figure 47: Evolution of thermophilic bacteria (grown in Nutrient-Yeast broth, incubation at 30°C) during composting at the surface (A) and in the center (B) of open-air windrows. Experiment GRA2 (mainly garden waste, C:N=40:1). Turning frequency: daily (5x/week = intensive); weekly (moderate intensive). Detection was made by the MPN-method.

Thermophilic counts were also higher in the initial mix in experiment GRA3 (Figure 49) than in experiment GRA2, an indication that some thermogenesis had already taken place in the material prior to the delivery to the composting installation. The daily turned windrow showed again almost identical values for the material sampled in the center and at the surface; concentrations were maintained at about 10^9 MPN/gDW until the 7^{th} week of composting, and got then reduced to about 10^8, coinciding with the decrease of the temperature below 60°C.

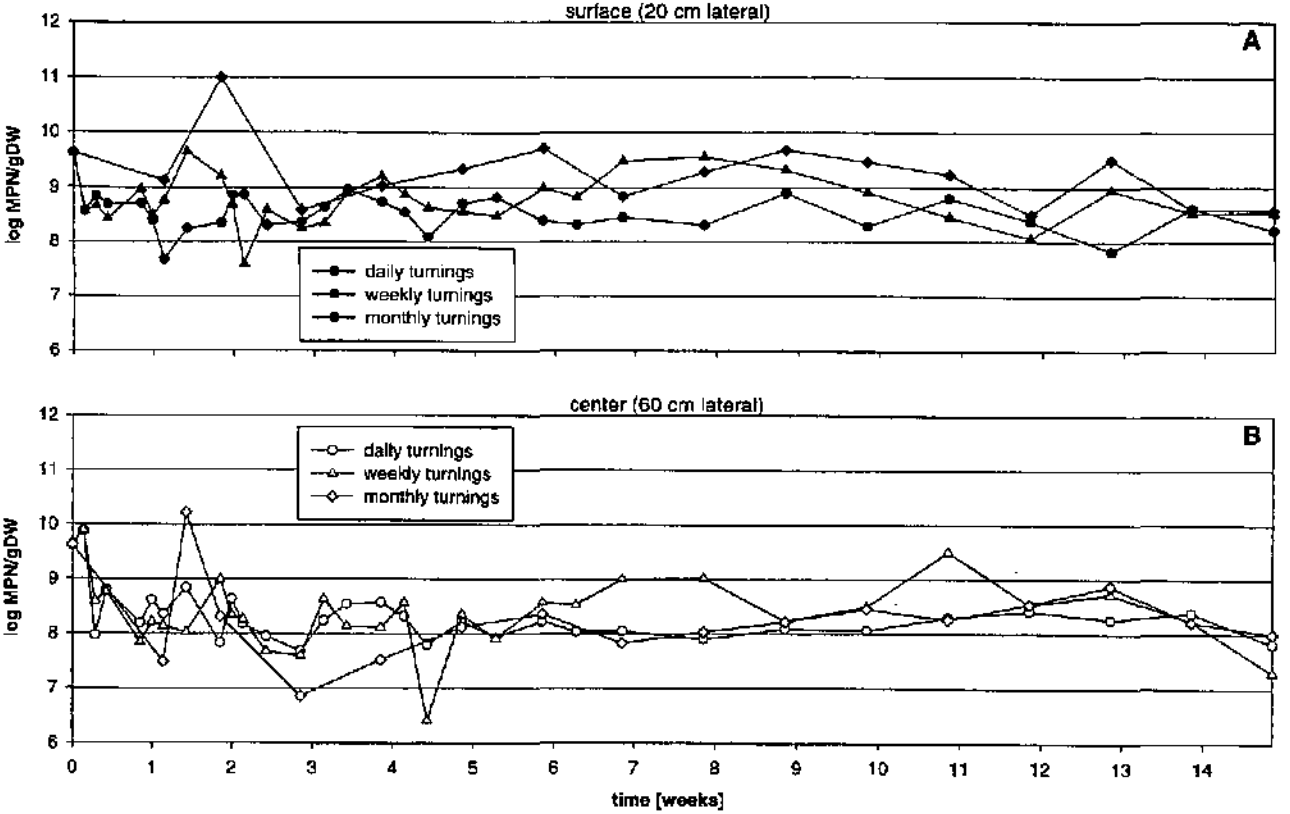


Figure 48: Evolution of mesophilic bacteria (grown in Nutrient-Yeast broth, incubation at 60°C) during composting at the surface and in the center of open-air windrows. Experiment GRA3 (garden and kitchen waste, C:N=30:1). Turning frequency: daily (5x/week = intensive); weekly (moderate intensive); monthly (extensive). Detection was made by the MPN-method.

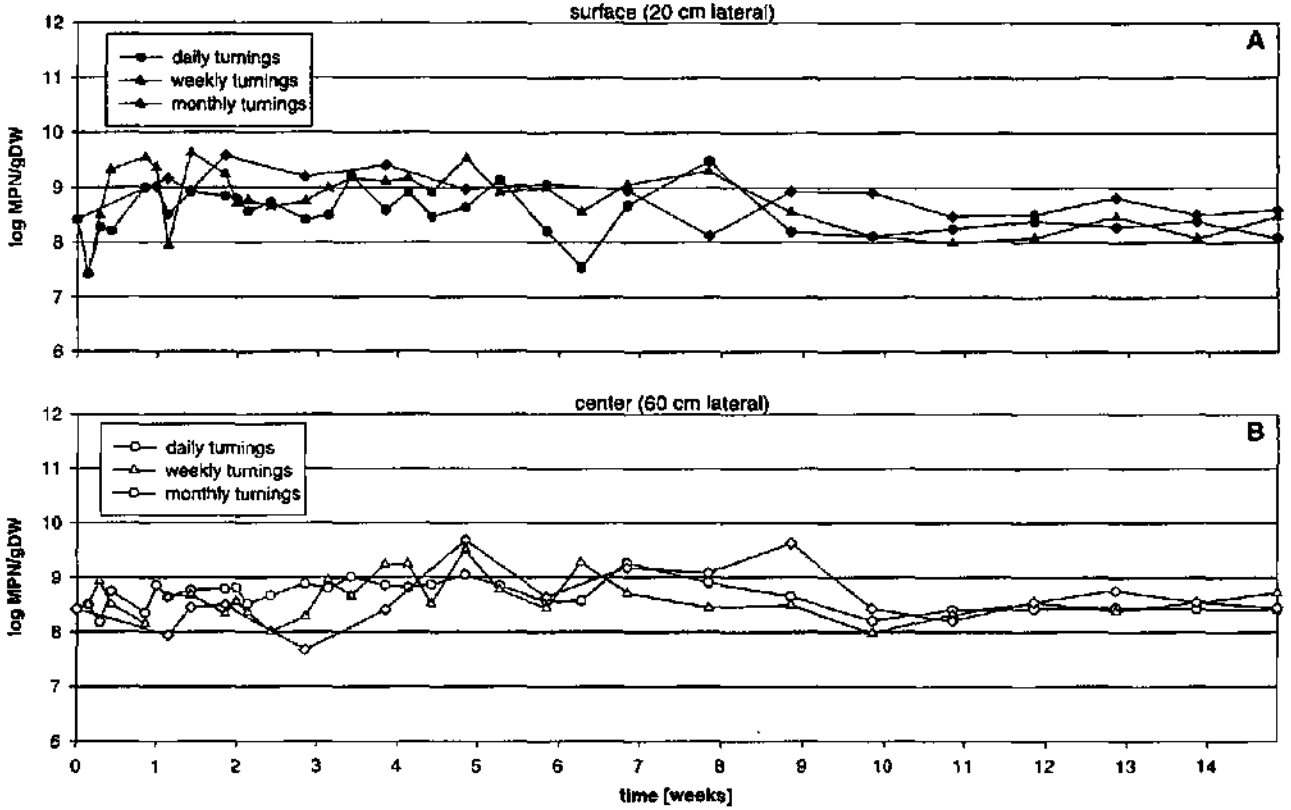


Figure 49: Evolution of thermophilic bacteria (grown in Nutrient-Yeast broth, incubation at 30°C) during composting at the surface (A) and in the center (B) of open-air windrows. Experiment GRA3 (garden and kitchen waste, C:N=30:1). Turning frequency: daily (5x/week = intensive); weekly (moderate intensive); monthly (extensive). Detection was made by the MPN-method.

3.2.1.3C Further practical implications of frequent turnings

Table 21: Percent reject (screening by hand at 30 mm)

composting time [weeks]	turning frequency		
	daily	weekly	monthly
0	22	22	22
4	19	17	nd
6	14	16	nd
10	9	13	nd
12	13	12	24
15	11	11	13
20	-	-	10
24	-	-	7

The elevated turning frequency, besides assuring a good thermohygienization, had further beneficial effects on the composting process: during the treatment of more woody wastes (experiment GRA2), the repeated passage of the turning machine was mechanically breaking apart the wood pieces, providing new surfaces for microbial attack, but also reducing the size of the particles.
MICHEL *et al.* (1996) found that the bulk density of the compost (kg/m^3) in frequently (2-3 times/week) turned yard trimming coropost increased faster compared to only 1x/month turned windrows, and that the percentage of reject during sieving was greatly reduced by the intensive turning regime. The same tendencies were found in experiment GRA3 (Table 21).

We also noticed that the control of compost humidity was possible only by frequent mixings, because an effective addition of water can solely be carried out during turning (KEENER *et al.*, 1994). During very wet periods, though, the turning frequency should be lowered, otherwise the compost gets too humid through the incorporation of the very wet material from the base of the windrow. Also, turnings should be carried out with moderation in very cold weather, especially for windrows in an advanced state of maturation, because of the risk of cooling out the compost too much (HAY & KUCHENRITHER, 1990). The quite low air temperatures (< 5°C) might be the cause for the rapid decline in temperature of the daily turned windrow in experiment GRA2 (Figure 29).

While daily turnings seemed less important for the thermohygienization of material with a low C/N ratio, other factors have to be taken into account when deciding on the turning frequency of biowaste with a high percentage of kitchen waste. With such starting materials, it is important to lower the water content in the fresh compost quickly, because thick interstitial water films hinder the transfer of oxygen into the particles (MILLER, 1996). In consequence, large anaerobic zones can prevail, with the emission of bad odors. In fact, dry matter evolved more quickly in the more frequently turned windrows (compare Figure 35, water content). Moreover, high windrow core temperatures drive natural aeration by the chimney effect, resulting in better oxygenation through higher air velocities (MILLER, 1996), and a more important water loss through evaporation. Also, by the turning, the quite compact material gets fluffed up, which has a beneficial action on the natural air flux inside the windrow.
Of course, beside qualitative, economical aspects as well as the end use of the produced compost (agriculture or horticulture) have to be taken into account when deciding about the optimal management of a composting installation.

3.2.1.3D Current state of management at the composting site

In order to reduce operation costs (personnel, wear and tear of the turning machine), the turning frequency was reduced to 3 turnings per week (Monday, Wednesday, Friday), and the windrows for the production of agricultural grade compost did not get covered with a tarpaulin any more.
In 1997, the enterprise opened, together with other partners, a methanization plant, where biodegradable kitchen, retailer and restaurant wastes can be treated. Upon delivery to the different composting sites owned or operated by the enterprise Vollenweider, the waste gets sorted in a woody fraction, which is shredded and composted immediately, and a wet fraction, which is supplied to the methanization plant. The solids remaining after methanization are returned to the composting sites, where they are mixed with freshly shredded woody material, and composted. The energy produced by burning the biogas from the methanization process is transformed to electricity used for the own requirements of the methanization plant The surplus can be sold as so-called "green electricity" to interested privates at a price 3 times that of normal current. The heat is used in a neighboring cement industry for the heating of their molds.
To enhance the sales of the mature compost to the large public, a company was founded who prepares different mixtures of compost with soil and Chinese reeds, sold in 50 l bags.

3.2.2 BOX COMPOSTING (ROOFED; AERATED)

3.2.2.1 TECHNICAL DATA OF THE INSTALLATION

The installation, depicted schematically in Figure 50, consists of roofed, aerated boxes (15.5 m long x 4 m wide x 3 m high), which can each hold about 150 m^3 compost (volume of the starting material, filling height of 2.5 m). At the end of the composting process, the volume is reduced to 80 m^3 (filling height of 1.3 m). The biodegradable waste (50 % separately collected kitchen and garden waste and waste from a vegetable processing industry, 50 % tree and shrub trimmings; occasionally aerobically stabilized sewage sludge (pressed to 20 % dry weight)) is immediately shredded upon reception, and filled into the box by a system of conveyor belts.

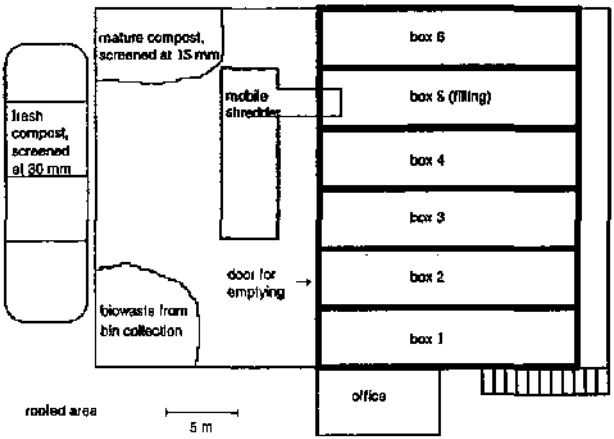


Figure 50: Schematic depiction of the composting site at Tägerwilen.

The composting is temperature controlled by two sensors, which are placed in the central part of each box, at an approximate depth of 50 cm (Figure 51A). The higher of the two temperatures measured by the sensors is taken into account. If the temperature sinks below 50°C, no aeration occurs at all, if it is between 50-70°C, aeration happens at intervals, and above 70°C, aeration is continuos. Most of the time, aeration is in intervals which are varied as a function of the stage of composting: an initial high aeration rate is used, this is progressively reduced with time (variable rate aeration system, LETON & STENTIFORD, 1990). One ventilator is installed per box, with a capacity of 40 m^3 air/min (corresponds to about 60 m^3 air/ $h \cdot t_{fresh\ weight}$ by continuous aeration, calculated on the basis of a mean compost density of 0.5 t/m^3). The compost is turned once a week with a slowly rotating, hollow spiral (diameter 80 cm) with moves on overhead rails, and which has a turning capacity of 20-40 m^3 compost/h (Figure 51B). Water can be added during turning.

After about 6 weeks of composting, the boxes, which are equipped with a door on one side, are emptied with a front-end loader. The compost is screened to 30 mm, and stocked in the open until use (agriculture), being turned, in the case of longer storage, from time to time with a front-end loader. A small fraction of the mature compost gets screened a second time to 15 mm, and gets stocked under roof, for use in horticulture, or to be put in sacks for sale to the general public. The rejects are recycled, after being manually separated from foreign matter (plastics).

Figure 51: *Temperature probes (A), turning system (B), and aeration channels (C) of the box composting system.*

The installation was laid out for the treatment of 2000 t green waste/y, but already in the first year of operation (1993), 2100 t were treated. In the second year, 2700 t got delivered to the site, and because an ongoing increase of waste was noticed, an extension of the installation by adding four boxes more was realized in 1996, being operational after our experiments were terminated.
As a consequence of the surplus of material, the rotting time in the boxes had to be drastically shortened: instead of the initially planned 8-10 weeks of treatment, the compost had to be taken out of the boxes already after 4-5 weeks. Due to the excellent contact that the manager of the installation had with the farmers in the surroundings, the produced compost got most of the time immediately distributed and put on the fields, avoiding an uncontrolled continuation of the composting process outside of the boxes.

3.2.2.2 EXPERIMENTS CARRIED OUT

The objective of the examination of the installation carried out during September 1994 was to obtain first data about the quality of the composting process and that of the end product. The microbiological and physico-chemical analyses should allow a judgment of the process, and serve as base in view of future improvements.
The aeration cycles were as follows: 1 minute aeration every 5 minutes for the up to 2 week old composts (corresponding to a total amount of air of 10 $m^3/h \cdot t_{fresh\ weight}$), 1 minute every 10 minutes for the older composts (5.5 $m^3/h \cdot t_{fresh\ weight}$).

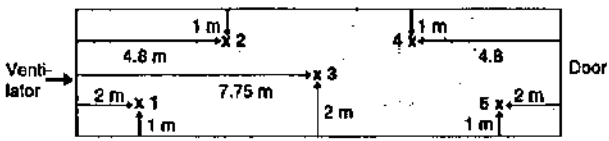


Figure 52: Measuring and sampling points in a compost box.

In order to get an idea about the homogeneity of the compost in the quite large boxes, temperature measurements were carried out vertically at different points, in 6 and 40 day old compost (Figure 52), as well as in the fresh compost stocked in the open and the mature one stocked under roof. Measurements were carried out at 10, 30, 50 and 100 cm depth. For detailed methods, see Chapter 2.2.1.1.

Gas measurements (O_2, CO_2, CH_4, H_2S, NH_3) were performed in each box at the surface (-10 cm) and in the center (-60 cm) of the compost mass, each time in a location with high temperature (60-70°C, "hot") and in one with low temperature (45-50°C, "cold"). For detailed methods, see Chapters 2.2.1.2.

Bioaerosol measurements were carried out in different locations on the site, without any activity, during shredding, aeration of 6, 19 and 40 day old compost, and turning of 40 day old compost. For detailed methods, see Chapter 2.2.2.

Sampling: at the same points where gas measurements were carried out, samples were taken, according to Chapter 2.2.3.

Microbiological analyses: *Aspergillus fumigatus*; thermotolerant molds and yeasts; heterotrophic thermophilic bacteria. For detailed methods, see Chapter 2.2.4.

Physico-chemical analyses: pH, dry weight. For detailed methods, see Chapter 2.2.5.

The aim of the following tests was to determine the influence of the starting material, which varied greatly in function of the time of the year, on the composting process. For this, the installation was visited three times: in summer (June), in fall (November) and in early spring (April). Although care was taken to proceed each time in the same manner, some of the process parameters that were out of the range of our control changed in the course of the experiment. Table 22 gives a summary of the most important changes:

Table 22: Composting conditions and composition of the starting material.

date of sampling	aeration	air supply total [$m^3/h \cdot t_{fresh\ weight}$]	sewage sludge [dry weight per box]	composition of the starting material
June (summer)	• compost 1-2 weeks: 2 min., 2 min. pause	30	2 t	grass cuttings, weeds, herbaceous garden waste, kitchen waste, structure material
	• compost 2-3 weeks: 1 min., 2 min. pause	20	2 t	
	• compost 3-4 weeks: 30 sec., 2 min. pause	12	2 t	
November (autumn)	• in all boxes: 1 min., 15 min. pause	3.8	only in the compost 3-4 weeks: 1.7 t	dead leaves, shrub cuttings, kitchen waste, structure material
April (early spring)	• compost 0-1 weeks: 30 sec., 5 min. pause	5.5	compost 0-2 weeks: 1.2 t compost 2-3 weeks: 4 t	tree cuttings, kitchen waste
	• compost 1-4 weeks: 15 sec., 5 min. pause	2.9		

Temperatures were measured at each occasion, at 5-10 different points vertically (at 10, 50, 100 and 150 cm depth) in the youngest compost (1-1.5 weeks old), in an intermediate compost (2-2.5 weeks old) and in the maturest compost (3.5-4 weeks old). For detailed methods, see Chapter 2.2.1.1.

Bioaerosol measurements were carried out without any activity on the site, and during unloading of biowaste, shredding, aeration, turning or screening, dependent on the activities carried out at the moment of measurement. For detailed methods, see Chapter 2.2.2.

Table 23: Age of the composts sampled [days].

indication	summer	autumn	early spring
young	11	4	5
intermediate	19	13	16
mature	26	24	29
screened	unknown	28	42

Sampling: on the basis of the temperature measurements, compost was sampled from each box in a "cold" (40-50°C) and a "hot" (60-70°C) spot. Samples were also taken from the unshredded biowaste, and, if obtainable, from the screened compost in stock. Table 23 indicates the exact age of each compost sample, as composts of exactly the same age were not available each time. For detailed methods see Chapter 2.2.3

Microbial analyses: *Aspergillus fumigatus*; thermotolerant molds and yeasts; heterotrophic mesophilic and thermophilic bacteria; coliforms (on VRB-Agar), *E. coli* (for sampling in fall and early spring). For detailed methods, see Chapter 2.2.4.

Physico-chemical analyses: pH, dry weight. For detailed methods, see Chapter 2.2.5.

3.2.2.3 RESULTS

3.2.2.3A First examination (September 1994)

Physico-chemical measurements in-situ

Temperature and gas measurements were carried out at the points depicted in Figure 52.

6 DAYS OLD COMPOST

Table 24: Temperature measurements in various depths in 6 day old compost. Measuring points see Figure 52.

	measuring point					
depth	1	2	3	4	5	mean
10 cm	36	64	66	61	27	50.9 ± 18.0
30 cm	50	70	70	74	31	58.9 ± 18.1
50-60 cm	39	69	62	71	35	55.2 ± 17.0
100 cm	36	70	57	66	28	51.6 ± 18.2
mean	40.2 ± 6.6	68.2 ± 2.6	63.9 ± 5.7	67.8 ± 5.9	30.6 ± 3.4	

In the 6 days old compost, the temperature distribution was very heterogeneous (Table 24). The points in the middle of the box (points 2-4) showed mean temperatures > 64°, while the ones towards the two ends of the box, either on the side where the air entered (point 1), or where the door was (point 5), measured only between 30°C and 40°C. This inhomogeneity could have been either caused by cooling of the compost due to the aeration on the one side, and conduction on the other side (see Chapter 1.6.2), or by an inadequate mixing of the starting material (mixture of kitchen waste with shredded brush), the material being filled into the box from one end to the other, and not in horizontal layers. The temperatures differences in the vertical direction were less important: between the hot center (30 and 60 cm depth) and the cooler surface (due to conduction and condensation) and bottom (due to evaporative cooling) up to 13°C were measured.

The gas measurements also indicated that the composting process progressed differently in the different parts of the box (results not shown) in the compost in the cold zone (point 5), the O_2 and CO_2 content were close to that of the ambient air (19.3-19.6 % and 0.5-0.6 %, respectively). Microbial activity was not completely absent, though, as showed the ammoniac (18 ppm NH_3) measured in 10 and 50 cm depth. This means that aeration was largely sufficient to replace the oxygen consumed. In the hot center zone (point 2), much greater microbial activity was detected: already in 10 cm depth, the O_2 content was reduced to 10.5 %, and in - 50 cm it was down to 1.1 %, while the CO_2 mounted to 11 %. Methane was present in concentrations of up to 3.6 % in 50 cm depth, hinting at anaerobic conditions. Gas measurements before and after the aeration event showed an increase of the oxygen content from 2 % to only 11.5 %, insufficient for a correct oxygenation. NH_3 was only detected in very small quantities (1.5 ppm), the high temperatures probably inhibiting protein degradation.

19 DAYS OLD COMPOST

Table 25: Temperature measurements in various depths in 40 days old compost. Measuring points see Figure 52.

	measuring point					
depth	1	2	3	4	5	mean
10 cm	58	66	55	54	42	54.9 ± 8.6
30 cm	63	63	68	54	44	58.4 ± 9.8
50-60 cm	59	58	62	58	44	58.2 ± 7.0
100 cm	56	49	60	43	44	50.3 ± 7.5
mean	59.0 ± 3.1	59.1 ± 7.5	61.3 ± 5.5	52.2 ± 6.4	43.3 ± 0.9	

The temperature distribution was much more homogenous (maximum difference of 17°C between the sampling points) than in the 6 days old compost. Temperatures were generally lower (Table 25). Again, the coldest zones were observed towards that end of the box with the metal door (point 5). In the vertical direction, the same temperature distribution was observed as in the young compost: a cooler surface and bottom, and a hot center.

Gas concentrations (results not shown) in the cold zone (point 5) on the other hand were very similar to the ones measured in the 6 days old compost The hot zone showed very low microbial activity (19.3-19.4 % O_2, 1.4-1.5 % CO_2). This was most probably due to the very low water content (26-33 %, see Table 28). For an optimal composting process, the water content should be between 40-60 %, below 30 %, the microorganisms become severely inhibited (see Chapter 1.6.4).

A second reason for the weak microbial activity could also be an extensive degradation of the organic matter. However, the measurements in the coarsely screened compost showed that the microorganisms continued to degrade the organic matter as soon as the humidity was re-established, due to natural wetting by storage in the open (see following paragraph).

MATURE COMPOST, COARSELY SCREENED, STORAGE IN THE OPEN

Table 26: Temperature and gas measurements in various depths in the coarsely screened compost. nd = not determined.

depth [cm]	O_2 [vol%]	CO_2 [vol%]	CH_4 [vol%]	H_2S [ppm]	NH_3 [ppm]	temp. [°C]
10	9.9	nd	2.70	0	nd	45.7
50	0.3	28	4.95	21	4	68.2
100	nd	nd	nd	nd	nd	54.0

The high temperatures together with the low oxygen and high carbon dioxide concentrations indicate that the screened compost (Table 26) was not yet stabilized; in fact, the short composting time (4-5 weeks) did not allow producing a mature compost. Due to the fine structure of the screened material, natural aeration of the heap was insufficient, demonstrated by the presence of high concentrations of methane and hydrogen sulfide in the inner parts of the heap. It has to be considered, though, that gas concentrations measured at one point do not necessarily indicate a gas production at exactly that location: the methane, for example, measured in 50 cm depth, could not have been produced there, because high temperatures (>60°C) would have inhibited the activity of methanogens. However, it had most probably been produced deeper in the heap, where the temperature (54°C) was optimal for methanogenesis. Due to its mobility, methane can easily migrate through the compost mass. Hydrogen sulfide, being less volatile, would accumulate at the site of production. On the other hand, as it can serve as substrate for sulfate oxidizers, it might not be detected.

MATURE COMPOST, FINELY SCREENED, STORAGE UNDER ROOF

Table 27: Temperature and gas measurements at various depths in finely screened compost. nd = not determined.

depth [cm]	O_2 [vol%]	CO_2 [vol%]	CH_4 [vol%]	H_2S [ppm]	NH_3 [ppm]	temp. [°C]
10	4.1	nd	17	6	nd	40.4
50	0.2	nd	14	12	nd	46.1
100	nd	nd	nd	nd	nd	nd

The finely screened compost (Table 27) had only a weak microbial activity, shown by the only slightly elevated temperatures. Nevertheless, the conditions were practically anaerobic in the lower parts of the heap, although the heap was not very high (about 70 cm). The very fine structure and the storage under roof, against a wall, prevented any air exchange, leading to an accumulation of methane and hydrogen sulfide.

Physico-chemical measurements in the compost samples

Table 28: Physico-chemical characterization and temperatures at the moment of compost sampling.

no	sample	depth [cm]	temp [°C]	H_2O [%]	pH
1	fresh biowaste	-	25	64.9	5.98
2	6 days "cold"	10	28	57.2	7.63
3		60	35	47.2	8.02
4	6 days "hot"	10	64	51.2	8.26
5		60	69	59.3	6.26
6	40 days "cold"	10	44	32.7	8.79
7		60	40	26.4	8.83
8	40 days "hot"	10	64	30.2	8.26
9		60	58	25.5	8.41
10	mature, coarsely	10	46	41.2	8.92
11	screened	60	68	36.6	8.83
12	mature, finely	10	40	35.9	8.33
13	screened	60	46	38.2	8.56

Table 28 presents a physical and chemical description of the composts examined, as well as the exact points where the samples were taken. The water content of initially about 65 % decreased in the course of the composting process down to about 25 %. The strong drying of the compost was due to the aeration, and the only sporadic watering of the material. The influence of a more important heat production with drying effect, due to an enhanced microbial activity, can be observed: the samples taken in the "hot" zones were generally dryer than those taken in the cold zones were. The material in the center of the heap was clearly drier than the one on the surface, where condensation of the evaporated water happened. The screened compost got rehydrated to a water content of about 40 %, again permissive for microbial activity.

The pH of the fresh material was, as expected, quite low (about pH 6), and rose with increasing compost maturation. The delay in pH elevation of the hot compost sample taken at 60 cm depth confirms that anoxic conditions prevailed there.

Microbiological analyses

IN THE COMPOST

Figure 53 presents the concentration of *AF* and total thermotolerant molds and yeasts.

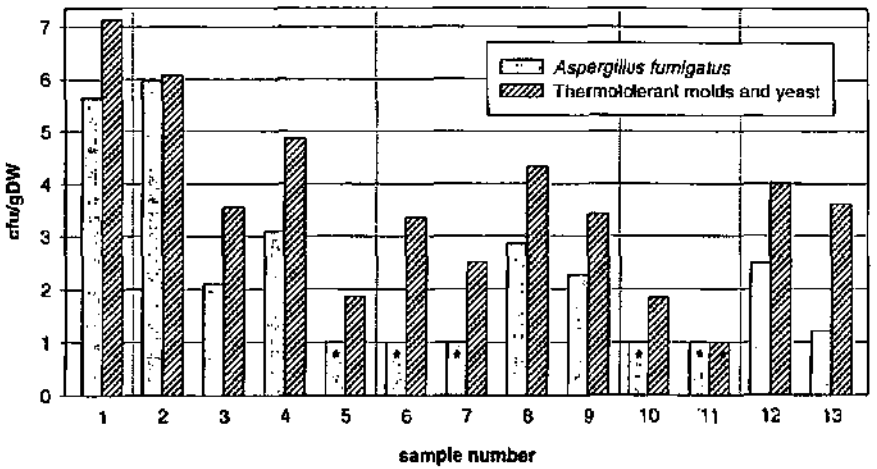


*Figure 53: Concentration of thermotolerant mold and yeast in composts of different maturity and at different temperatures. * = value below the detection limit of 5-10 cfu/g fresh compost.*

1 = fresh biowaste,
2-5 = compost 1 week, (2 = surface, cold; 3 = surface, hot; 4 = center, cold; 5 = center, hot)
6-9 = compost 6 weeks (6 = surface, cold; 7 = surface, hot; 8 = center, cold; 9 = center, hot)
10-11 = coarsely screened, (10 = surface, 11 = center)
12-13 = finely screened, (12 = surface; 13 = center).

Detailed description of the samples see Table 28.

The fresh material (sample 1) contained about 10^6 cfu *AF*, comparable to values that were normally measured in biodegradable waste. The number of other fungi was about one order of magnitude higher, mainly due to the presence of yeasts, which found in the first acid phase of the composting process ideal growth conditions.

The hygienizating effect of raising temperatures could be seen in the 1 week old compost: in the samples 2 and 3, which had been taken at points that had not been heated yet, clearly more *AF* was detectable than the samples 4 and 5, which had been exposed to temperatures exceeding 60°C. *AF* concentrations at the surface where always higher, although temperatures were not very different from the ones in the center. It seems that other factors, such as high concentrations of gases, humidity, or other, for the fungi toxic substances in the depth of the composting mass lead to a inhibition of fungal development.

The 6 weeks old compost showed generally lower fungal numbers than the young compost. More *AF* were measured in the samples that were warmer (58-64°C), but also more humid than in the ones with a very low water content (26-32 %). The activity of *AF*, being among the fungi one of the least resistant to low a_w values, was severely inhibited. Other thermotolerant species seemed to be less sensitive to low water content.

In the coarsely screened compost, where temperatures had increased again, due to rehydration to more than 35 % H_2O, only low *AF* concentrations were detected. The finely screened compost supported only a weak recolonization of *AF*, the quite low temperatures favoring obviously the development of other fungi better adapted to degrade the more recalcitrant substances, such as lignin and cellulose.

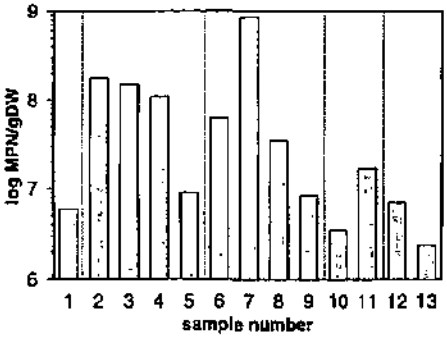


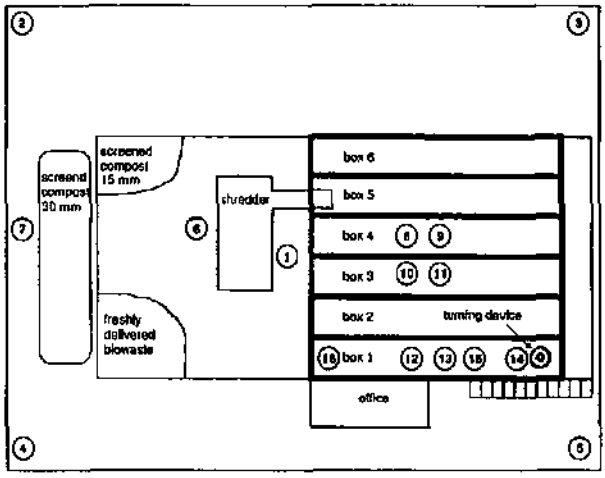
Figure 54: Concentration of thermophilic bacteria (incubation at 60°C in Nutrient-Yeast Broth. Samples are the same as in Figure 53. Detailed description of the samples see Table 28.

The thermophilic flora (Figure 54) was present in high concentrations already in the fresh material. In the first week of composting, the number of thermophiles increased by more than a factor 10, the mean concentration of all the four samples being $7.2 \cdot 10^8$ MPN/gDW. In the 6 week old compost, concentrations were slightly lower ($6.3 \cdot 10^8$), most probably due to a reduced activity in the very dry material, causing a general reduction of temperatures. In the screened compost, further reduction of thermophiles was observed, (coarsely screened: $9.3 \cdot 10^7$, finely screened: $4.2 \cdot 10^7$), an increase in temperature > 60°C, as e.g. measured in the center of the coarsely screened compost, leading to a renewed increase of thermophile numbers.

IN THE AIR

The results of the bioaerosol measurements are shown in Figure 55. The background concentration of either *AF* or total thermotolerant fungi on and around the site was practically zero, and corresponds to the levels measured normally far away from composting installations. The total fungal count was slightly higher on the eastern border of the place, due to the light wind (0.1-0.3 m/s) blowing from the west, carrying some spores away from the composts. It has to be mentioned, though, that the air at the moment of the aerosol measurements was quite humid, and that it rained sometimes slightly. This could have resulted in a washout of spores (STETZENBACH *et al.*, 1992).

By shredding, the spore counts in the air got slightly raised 2 m away from the shredder, but already in 20 m distance, concentrations where in the range of the background concentration. In comparison with other composting sites, where levels up to $6 \cdot 10^4$ cfu/m^3 air had been measured during shredding, the concentrations were very low, probably due to the immediate processing of the delivered waste. We had observed in biowaste put on heaps for a few days before shredding already a strong self-heating, leading, especially at the surface of the heap, to a strong proliferation of molds (BEFFA *et al.*, 1994).
In the boxes, in 50 cm over the compost surface (the aerosol sampler was held vertically towards the compost surface), hundred fold higher concentrations were measured than outside the boxes. Interestingly, concentrations were lower during the periods when the compost was aerated, the spores being probably diluted in the passing airstream. When the aeration stopped, sedimentation of the fungal propagules, which were hold back by the walls and the roof of the installation, lead to again higher spore loads.



background concentration

no.	sampling point	*AF*	TTM
1	middle of the site	6	10
2	south	4	20
3	west	0	4
4	north	0	4
5	east	0	95

in the box, 50 cm above the compost surface

no.	sampling point	*AF*	TTM
8	1 week old compost, without aeration.	65	75
9	ditto, with aeration	50	75
10	3 weeks compost, without aeration	720	770
11	ditto, with aeration	160	260
12	6 weeks old, compost without aeration	90	110
13	ditto, with aeration	40	95

during shredding

no.	sampling point	*AF*	TTM
6	2 m from the shredder	470	590
7	20 m from the shredder	20	25

during turning of 40 days old compost

no.	sampling point	*AF*	TTM
14	1 m from the turner	350	590
15	5 m from the turner	90	220
16	8 m from the turner	25	40

Figure 55: Concentration of Aspergillus fumigatus (AF) and total thermotolerant molds and yeasts (TTM) in the air during various activities on the composting site. Circled numbers indicate the sampling locations. The results are expressed as cfu/m³ air.

The highest concentrations were emitted from the 3 weeks old compost. As shown by the physico-chemical measurements, the 1 week old compost was in large parts not yet very hot, promoting thus little development of thermophilic molds. In the 3 weeks old compost, temperatures were ideal for the massive proliferation of *AF*. The fungal flora at this moment was composed to more than 80 % of *AF*. The 6 weeks old compost showed, due to the drying, in general a weak microbial activity, resulting in a low dispersion.

During turning of the 6 weeks old compost, *AF* concentrations in the immediate vicinity of the turner where about one order of magnitude higher than during aeration, to drop to background level when measurements were carried out at the other end of the box. Taking into account that at certain locations in the compost, up to $7 \cdot 10^5$ cfu/gDW *AF* had been measured (see Figure 53), these concentrations were extremely low, compared to turning of open-air windrows (see Figures 40 und 41).

The turning of the compost by the spiral, where the compost got gently mixed vertically without being displaced, generated very low spore and dust emissions.

On the bases of the results of this first examination, the following weak points of the installation were pointed out, and improvements suggested:

- The very inhomogeneous temperature distribution, leading to an irregular composting process and thermohygienization, could be prevented if the initial material was either better mixed, or filled into the boxes in layers, the mixing being then achieved by the vertical movement of the turner.
- Taking into account the very inhomogeneous temperature distribution, the problem arose of choosing the points where the temperatures for the aeration control were measured. If a point was chosen that was too hot, the compost was aerated too strongly, leading to a further cooling of the zones that had not heated yet. On the other hand, if at the point of temperature control, temperatures were low, not enough air was supplied to the zones with a great microbial activity, leading to anaerobic conditions, with the resulting odor problems.
- LETON & STENTIFORD (1990) criticized the use of only one temperature probe to monitor and control an entire compost pile, consisting of many distinct and varied temperature zones. They proposed a system with several thermocouples for temperature control, however, they neither stated where these should be placed, or what algorithm should compute a mean temperature. As the composting system has two temperature sensors per box, a test could be carried out to determine if a more homogenous temperature distribution in the box would be achieved if the (arithmetic) mean of the two temperatures was used as control for the aeration system. Also, an automatic temperature registration system would allow a better follow-up of temperature evolution, and a more rapid intervention (variation of the aeration cycle, the control temperature for aeration in intervals or continuous, turning and water addition) in the case of problems.
- One problem was also the strong drying of the compost, due to the aeration, as noticed in the 6 weeks old compost. Regular watering at each turning, or even at additional turnings, would replace the humidity lost be evaporative cooling. The rotting time in the boxes being already quite short (due to larger amounts of green waste delivered to the installation than initially planned), any disturbance of the process (inhomogeneity of the material, lack of water, inadequate aeration) should be avoided.
- If the compost has to be removed from the boxes in an unmature stage, it would be preferable not the screen it for storage, in order to allow natural aeration to happen. The windrows should not be higher than 1.5 m, and a minimal distance should be left between the individual rows.

3.2.2.3B Seasonal influance of the sterting material

The input material, as well by its composition as by its structure, can vary widely during the different seasons. HELM (1995) analyzed the biowaste (source separated biodegradable household waste, consisting of kitchen waste, but also the in private gardens arising grass and brush cuttings) and the structure material (tree and brush cuttings from horticulturists and communities) in different seasons (summer, autumn, early spring) delivered to a composting installation in Germany. Although the moisture content of the biowaste did not vary much (around 65 %) in the different seasons, its organic matter content increased from 62 % (of DW) in the summer to 78 % in the early spring material, which contained also the highest amount of nitrogen (2 %), due to the high amount of kitchen waste. The structure material, on the other hand, had the highest water content in autumn (63 %), and the lowest in summer (32 %), the organic matter content being in all seasons about the same (59-63 %).

During the different seasons, the percentage of biowaste and structure material in the source separated household waste varies, too. FRICKE (1988), also in a German study, measured up to 80 % *garden waste during the months May to December, while from January to March, the biowaste consisted almost exclusively of kitchen waste.*

For an optimal composting process, structure material and biowaste have to be combined, in order to obtain a substrate that contains enough nutrients and humidity, but at the same time preserves its structure: tree and brush cuttings without leaves that accumulate in winter can be stored, to be mixed with the often very wet and compact material (grass cuttings) incurring in summer.

Physico-chemical measurements

TEMPERATURE

To control the homogeneity of the material within one box, temperature profiles were carried out over the whole area in 5-10 points. Figure 54 shows the mean values and the standard deviation (σ^{n-1}).

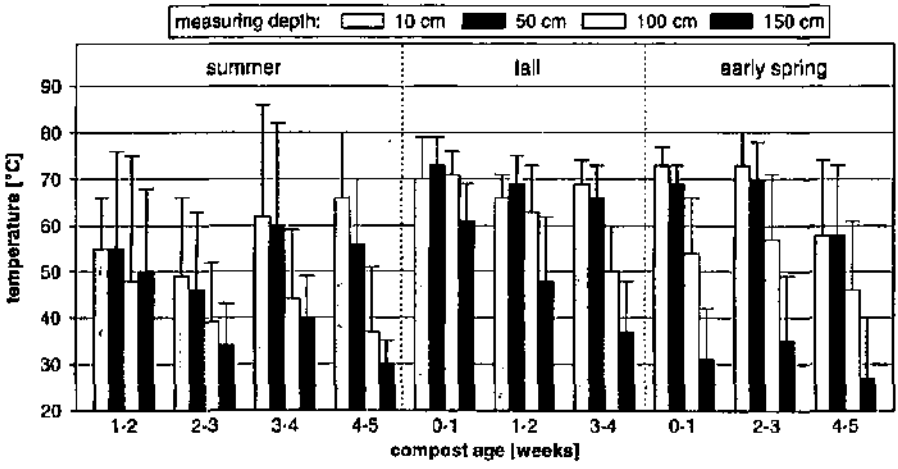


Figure 56: Mean values and standard deviations (σ^{n-1}) of temperature measurements at 5-10 points/box.

The measurements showed again, as observed during the first examination, greatly varying temperatures between the different measuring points, the standard deviation being often > 10°C, the temperature differences amounting up to 40°C. In general, a cooling was noticed at the bottom, where the air was introduced. Due to the simultaneous drying, microbial activity became restricted, leading to a further temperature decline.

Astonishingly, the lowest temperatures were measured in summer, probably linked to the very intensive aeration (0.5-2 minutes aeration, 2 minutes pause), cooling the wet starting material (grass cuttings in large amounts, to which sewage sludge had been added as well, see Table 22). This lead in the lower parts to a strong drying (see Table 29), resulting in very low temperatures, which, e.g. in the 3-4 weeks old material, did not exceed 40°C.

The highest temperatures were measurable in the fall compost. This material seemed, as well by its structure as by its nutrient content, to support ideal conditions for microbial activity, even if no sewage sludge had been added. The long pause between the aeration phases (15 minutes) probably prevented a too strong cooling. The fall material (above all dead leaves) presented also the most homogenous starting material, the standard deviations being very small.

Thanks to the addition of sewage sludge to the early spring material, consisting mainly of wood, quite high temperatures could be maintained: up to a depth 1 m, > 55°C were measured in the fresh and the intermediate compost. Only the mature compost showed, at the bottom, a strong cooling due to the introduction of cold air.

WATER CONTENT

Table 29 presents the water content (in %) of the composts at different seasons.

Table 29: Water content of the biowaste (without addition of sewage sludge), of the compost at different stages (mean value of the two samples taken) and of the screened compost.

age [weeks]	summer	fall	early spring
fresh	71	68	58
0-1	-	67	43
1-2	55	52	-
2-3	51	-	49
3-4	43	46	-
4-5	32	-	45
screened	32	44	48

As the composition of the material let assume, the summer material (prior to addition of sewage sludge) had the highest water content, the early spring material the lowest. During the rotting process, the composts lost water continuously. The screened compost had varying moisture contents. It should be considered though that the storage time was not the same.
In autumn, the composts got watered already at the first turning (after 1-3 days). In this way, a too strong drying, as observed in the early spring, but also in the initially very humid summer material, could be avoided. During the cold season, the air used for ventilation is very dry, being thus able to take up great amounts of water during its passage through the compost

GASES

The correlation between oxygen concentration and temperature is depicted in Figure 57: one can distinguish three groups of data sets: at an oxygen concentration above 15 %, temperatures ranged from 20 to 75°C, the points at the lower end of the temperature scale having the highest oxygen concentration.

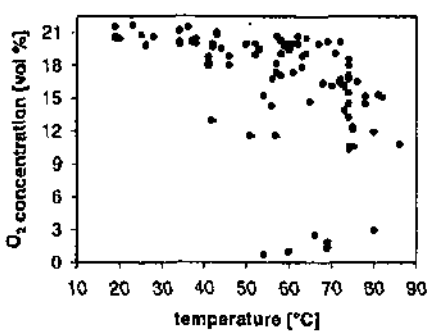


Figure 57: Correlation between temperature and oxygen concentration measured in all composts examined (different ages and different seasons).

At locations where oxygen concentrations between 10-15 % were measured, temperatures laid between 40°C and 82°C, with no clear tendency. In a few points with very low oxygen content (0-5 %), temperatures were comprised between 55°C and 80°C. With increasing oxygen, temperatures increased, too.
The distribution of the temperature/oxygen concentration data resembles the elliptical pattern described by MILLER *et al.* (1989), who measured temperature and oxygen concentrations in naturally aerated mushroom compost phase I stacks. In contrary to those unvented composts, the data presented in Figure 57 are characterized by the almost complete absence of points with very low oxygen content, meaning that in most of the locations, oxygen supply was equal or superior to the oxygen consumption of the microflora.

On the other hand, the large number of points with low temperatures but sufficient oxygen implies an over-aeration, preventing a temperature increase. The almost total absence of anaerobic zones was also shown by the only very occasional detection of methane or H_2S. In consequence, no bad odors were ever noticed at this installation.

Microbiological analyses

IN THE COMPOST

Figure 58A presents the fungal concentrations measured in the different compost samples.

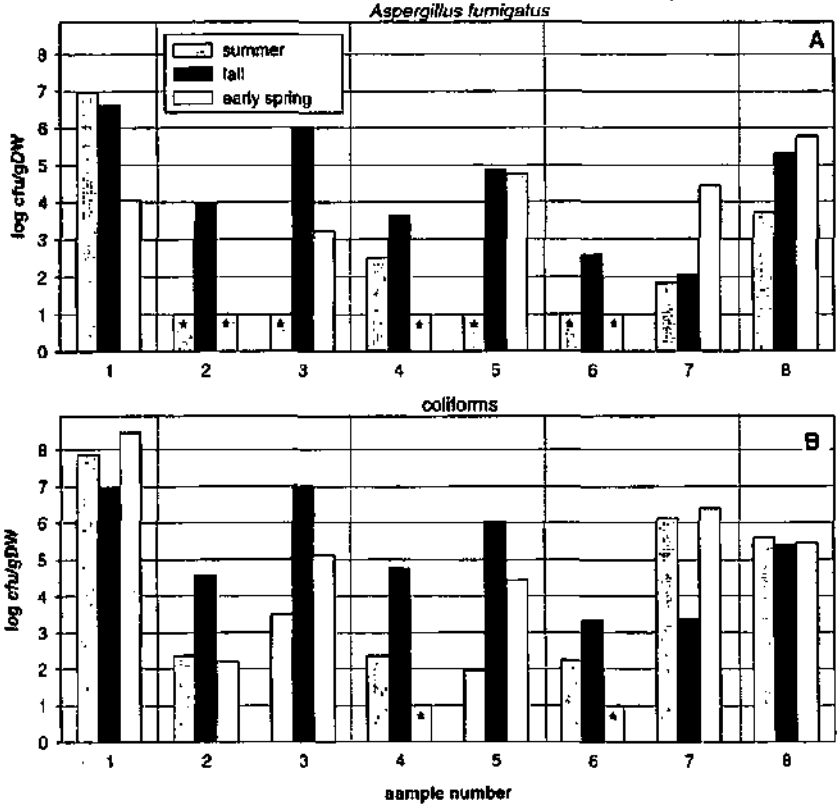


Figure 58: Aspergillus fumigatus (A), and coliforms detected on VRB-agar (B) in composts of different maturity and seasonal provenience.
1 = fresh biowaste
2-3 = young compost, (2 = hot; 3 = cold)
4-5 = intermediate compost, (4 = hot; 5 = cold)
6-7 = mature compost (6 = hot; 7 = cold)
8 = screened compost

*For description of the compost samples, see Tables 23. Columns with an * indicate values below the detection limit (10 cfu/gDW).*

It is clearly visible how the *AF* numbers, initially present in the biowaste in concentrations up to 10^7 cfu/gDW in the summer and fall material, got drastically reduced in the first phase of the composting process. This decrease was usually more pronounced in the samples that had been taken in the hot spots. In the course of the composting process, concentrations got further reduced, except in the early spring material, where in the "cold" samples even an increase in *AF* numbers was observed, leading in the screened compost to higher concentrations (10^6 cfu/gDW) than in the fresh material (10^4 cfu/gDW). It seems that the breakdown of the mostly ligneous material during the composting process promoted fungal colonization. But also in the screened material of the summer and fall composts, a clear recolonization was observed.
No explication could be found why in the fall compost, where the highest temperatures were measured and the material showed the greatest homogeneity, the main reduction of *AF* numbers occurred only in the mature compost (samples no. 6 and 7).

The coliform content of the same compost samples is depicted in Figure 58B. The starting material, especially in summer and early spring, when it contained sewage sludge and kitchen waste, showed high initial coliform concentrations. Generally, the coliforms reacted to compost temperature in the same manner as *AF*, e. g. their numbers got quickly reduced in the "hot" samples. A strong recolonization was already observed in the "cold" sample of the mature compost.
In general, about 10 times more coliforms than fungi were present. It has to be considered, though, that colony forming units of bacteria and molds can not really be compared directly: the first represent one bacterial individual (unitary way of life), while many mold colonies can grow out of the spores or parts of the mycelium (normally the tip of each branch) of the same individual (modular way of life). For example, per conidial head, about 10^4 spores are produced (GIRARDIN, 1993). Furthermore, spores and mycelium do not show the same thermoresistance or requests concerning growth or germination (see Chapters 1.8.1.2A and 3.1.4). However, the quantitative enumeration of molds by counting cfu's can give an appreciation about the activity of the fungi, and is, in the context of composting, where microscopical analyses are very difficult, the only practical way of determining the presence of molds.

IN THE AIR

Because of organizational and meteorological reasons, it was not possible to carry out the same bioaerosol measurements at each sampling date. The results given in Table 31 are the geometric mean of at least 3 measurements.

The bioaerosol measurements showed increased mold concentrations during dumping of the biowaste from the collector truck, during turning of the compost in the immediate vicinity (in the box) to the turning spiral, and during screening. The higher mold concentrations in the fall material were reflected in the raised concentrations in the air during various activities on the site. The mold concentration was about 100 times higher in the middle of the place than at the perimeter, and in 200-1000 m, also downwind from the site, about the same as in normal ambient air.

Table 30: Concentration of AF (cfu/m³ air) at different working places.

working place	season	cfu / m³ air
office	summer	25
cabin of front end loader, during screening	summer	230
sorting band: upwind	fall	670
downwind		∞ (TTM > 24'000)

Table 30 presents the pollution of the air at different working places. The only working place that showed at times very high mold concentrations was the sorting station for the manual removal of contaminants from the screen reject, especially when measurements were carried out downwind.

Table 31: Concentration of AF (cfu/m³ air) during different activities, and in various distances to the emission point, during composting of material of different seasonal provenience. ∞ = Agar plates completely overgrown, AF not countable; TTM = thermotoleram molds total; - = not measured.

activity	distance	summer	fall	early spring
dumping biowaste	1 m	1600-∞ (TTM > $3.3 \cdot 10^5$)	-	360
shredding	1 m	460	-	-
	3 m	45	-	-
	5 m	26	-	-
aeration compost 0-2 weeks	1 m	70	∞ (TTM > 6000)	-
aeration compost 2-4 weeks	1 m	240	3200-∞ (TTM > 6000)	-
turning compost 0-2 weeks	1 m	80	5700	-
	5 m	-	1200-∞ (TTM > $2.4 \cdot 10^4$)	-
turning compost 2-4 weeks	1 m	< 100	-	-
	2 m	20	-	-
screening	1 m	∞ (TTM > $3.3 \cdot 10^5$)	∞ (TTM > $3.3 \cdot 10^5$)	-
	5 m	1300	1300	-
	30 m	600	-	-
background (only aeration)	middle	95	15	40
	perimeter	3-15	-	8-20
	200 m, N	2	-	3
	500 m, S	2	-	< 1

3.2.2.3C Assessment of the performance of the composting system

The conclusions drawn from the analysis of the results of the second series of examinations were confirming the ones obtained after the first check of the installation:

- The temperature distribution in the boxes was very heterogeneous, most probably the result of an inadequate mixing of the initial waste components. Because the turning brings about mostly a vertical mixing of the material, this should be filled in layers into the boxes, so that the different wastes get distributed regularly over the whole area of the box.
- There is a risk that the aeration dries the compost, above all in the lower layers, interrupting the microbial degradation activity. This could be avoided by early and regular watering at each turning. A higher turning frequency (at least twice/week), especially at the beginning of the composting process, where aeration has to be strongest, would allow to better control the water content, and to get a more homogeneous water distribution in the compost.
- The setting of the aeration cycle (duration of the aeration and of the pause between two aeration phases) seemed to be purely empirical. Our punctual measurements did not allow making a final statement which of the different cycles were best suited. No agreement about this subject was also found in literature (see Chapter 1.6.3 about recommend cycles of aeration). More detailed measurements over a longer period of time would be necessary to determine the oxygen need of the degrading microorganisms, and should take into account such factors as age and the composition of the compost, temperature and humidity. Gas kinetic measurements (speed of oxygen consumption after an aeration cycle) would allow answering that kind of questions.

- The sampling at different seasons showed varying results, as well as physico-chemical as microbiological. However, it could not be clearly determined if this was the result of the differing composition of the waste, or of the simultaneous change of other composting parameters (aeration cycles, addition of sewage sludge). To be able to make a clear statement about the influence of the starting material, one should be able to compare composts that are prepared under standardized conditions. These standard conditions were not available, taking into account that the composting installation investigated was in operation only since a short time. As it was not yet extended at the moment of the measurements, the composting time had to be shortened strongly in consequence, in order to be able to process all the accumulating material.
- Generally, a good correlation between the temperatures measured in the compost and the concentration of potentially pathogenic microorganisms was observed: the hotter the compost, the better the hygienization. Only the fall samples showed, in spite of high temperatures, quite high mold and coliform concentrations. Other factors (humidity, oxygen content) might have influenced the thermoresistance of those microorganisms.
- As seen in other composting installations, a clear correlation existed between fungal numbers in compost and those aerosolized during turning and screening.
- The composting time was very short (maximum 5 weeks), resulting on the one hand from the large quantities of green waste that had to be treated at times, on the other hand from the big demand for compost. As the screened compost was never stocked more than a few days on the site, but got put on the fields immediately, this short composting time should not give rise to problems. However, it should be tested if such fresh material is not phytotoxic. With the extension of the installation, the composting time can be prolonged to produce more mature composts, which might be sold at a slightly higher price.
- The addition of sewage sludge has proved to be very effective in wintertime, when mainly woody difficult to degrade materials have to be composted. It would have to be controlled, however, if correct thermohygienization conditions can always be attained. In summer, when the biowaste is already very nutrient rich, and, moreover, very humid, the addition of sewage sludge is not advised, otherwise the initial mixture gets too wet and compact.
- The bioaerosol measurements at the working places showed increased mold concentrations during the manual sorting of foreign objects in the screen reject. During this work, a facemask should be worn, or the working place should be stationed in a manner that the wind comes from behind, and carries the dust away from the person working.

3.2.2.3D Current state of management

At the moment of the redaction of the thesis (summer 1998), the installation was extended to 10 boxes. This resulted in a prolongation of the rotting time in the boxes from formerly 4-6 weeks to 8 weeks. However, the turning frequency got reduced (every 7-10 days), because no additional turning device had been installed. The amount of waste treated per year is momentarily 4000 t, with still rising tendencies. Due to the better be utilization of the capacities, the amortization of the installation, and the low maintenance costs, tipping fees per ton waste delivered to the site could be lowered from initially CHF 150.- to CHF 100.-. The compost containing sewage sludge is delivered for free to the farmers; compost without sludge is sold for CHF 10.-/t (coarsely screened quality), and for CHF 20.- (finely screened quality).

3.2.3 TRENCH COMPOSTING

3.2.3.1 DESCRIPTION OF THE INSTALLATION

The composting installation KEWU, situated at Krauchthal (BE), treats garden, park and kitchen waste of about 100'000 inhabitants from 13 communities, total about 12'000 tons annually. The composting process takes place in aerated trenches. Each of the 11 trenches (66 x 2 x 2 m) can hold approx. 250 m^3 compost. The separately collected biowaste gets tipped, as a function of their composition (kitchen waste, grass clippings; leaves, long grass, garden waste; structure material), into different bunkers. The processing of easily degradable substrates happens within 24 h.

Ferrous foreign objects are sorted out automatically of the coarse shredded material before two further passes through fine shredders (tree and shrub cuttings: < 20 mm; kitchen waste, leaves, grass: 50-75 mm). The material preprocessed in such a way is stocked in separated bunkers, one for green waste, and one for structure material. From there, the materials get mixed, watered (final water content: 60 %) and filled into the trenches. About 14 m^3 (equals about 4 tons of material) can be added per trench per day. Normally, chargings and turnings are carried out every workday (Monday to Friday).

Aeration pipes are laid in the trench floors in a stone bed, over which woods chips are filled. Each trench is divided in five zones (A = fresh material to E = mature compost) that are aerated separately. The aeration takes place either in pre-determined time cycles, or can be temperature controlled by temperature sensors, which are placed at mid-height in the trench wall, in the middle of each zone. The volume of air fed to each zone amounts to 22.5 m^3/min by continuos aeration, corresponding to 54 $m^3/h \cdot t_{fresh\ weight}$, assuming a mean amount of 50 m^3 compost/zone, and a density of the material of 0.5 t/m^3.

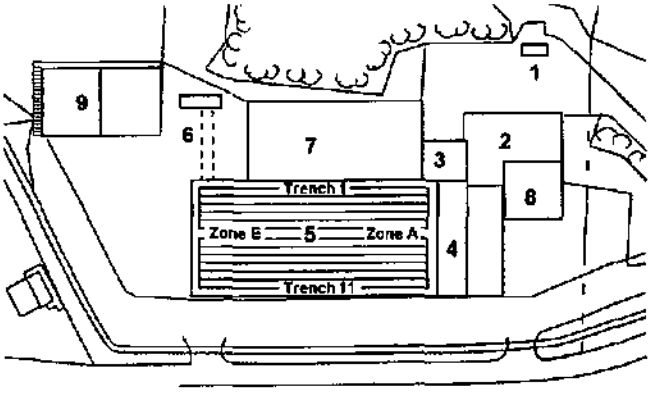


Figure 59: Schematic plan of the installation at Krauchthal (KEWU).

The turning of the compost happens with a special turning machine, which runs on rails on top of the trench walls. Turning starts with the matures compost (zone E). By moving forward, the turner takes the material up, mixes and loosens it, and discharges it behind the machine. With each passage, the compost is displaced by 3.6 m. The oldest material is directly charged on conveyer belts and moved to the screener. During turning, approximately 700 liters of water are added to the center zones (B, C and D). In zone E, a slight dry off is desired, in order to facilitate screening. The passage of the compost through the rotting hall amounts to 30 days if new material is charged every workday. The final product is screened to 20 mm and stocked under roof in a big heap. The compost is mainly used in agriculture.

3.2.3.2 EXPERIMENTS CARRIED OUT

The objective of the examination of the installation carried out during November 1996 (winter material) was to obtain first data about the quality of the composting process and of the end product. The microbiological and physico-chemical analyses should allow a judgment of the process, and serve as base in view of future improvements. A second, identical examination was carried in early June 1997 (summer material), to compare the performance of the installation with different starting materials.

Measurements in the rotting hall were carried out in winter in trench 11, in summer in trench 1.

Physico-chemical measurements (temperature, O_2, CO_2, H_2S, CH_4; for detailed methods, see Chapter 2.2.1) were carried out in zone A (fresh compost, approx. 1 week old), zone C (intermediate compost, approx. 2 weeks old), and zone E (mature compost, approx. 4 weeks old, Figure 59), in five different points (Figure 60), at a depth of 20, 50 and 100 cm. In the winter material, measurements at points 2 and 3 were repeated after turning of the compost. In the summer material, the measurements were only carried out in point 3. The starting material after shredding, the compost stored under roof and the biofilter material were also analyzed

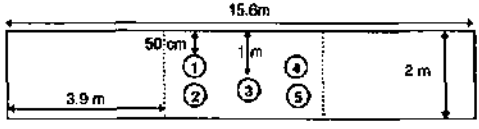


Figure 60: Measuring and sampling points in one zone. The dotted line indicates the boundaries of one batch of material.

During an additional examination in late June 1997, temperature gradients in the trench were determined by carrying out vertical measurements every 20 cm up to a depth of 90 cm, and thereafter every 10 cm. Such profiles were done every 20 cm, over the whole width of the trench. Isotherms were drawn as described in Chapter 2.2.1.1.

Table 32: Sampling points.

no	age [weeks] / zone	depth	no	age [weeks] / zone	depth
1	fresh	-	9	2 / C	mixed
2	1 / A	20	10	4 / E	20
3	1 / A	50	11	4 / E	50
4	1 / A	100	12	4 / E	100
5	1 / A	mixed	13	4 / E	mixed
6	2 / C	20	14	storage	20
7	2 / C	50	15	storage	60
8	2 / C	100	16	biofilter	20

Sampling of compost was done in the zones A, C and E, in point 3 (see Figure 60), before and after turning (Table 32). The turning machine was stopped approx. 20 cm before the sampling point, and backed up approx. 1 m, thus exposing a cross-section from which samples could be gathered horizontally in 20 cm depth, in 20, 50 and 100 cm from the top (Figure 61). As the compost was moved during turning, the sample after turning (mixed sample) was taken 3.6 m behind the point where the other samples had been taken.
Sampling in the stored material was done in 20 and 100 cm depth, with the aid of a compost auger. For detailed methods, see Chapter 2.2.3.

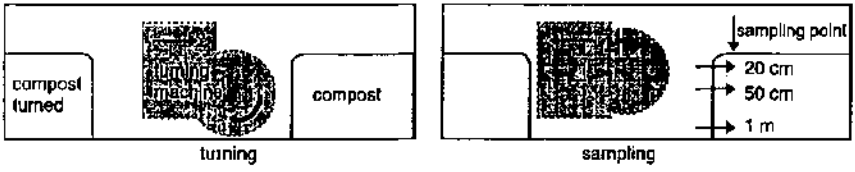


Figure 61: Procedure for sampling, and sampling points.

The stage of maturity and the aeration cycles at the moment of examination were as follows (Table 33). The winter compost had at the moment of measurement not been turned for 3 days, the summer compost for 2 days.

Table 33: Stage of maturity and aeration cycles.

zone	A		C		E	
material	winter	summer	winter	summer	winter	summer
age [days / weeks]	5 / 1	1-4 / 1	15-18 / 2	15 / 2	28 / 4	26 / 4
aeration [min] / pause [min]	1 / 30	2 / 30	1 / 30	2 / 45	1.3 / 63.3	2 / 53
total amount of air [$m^3/h \cdot l_{fresh\ weight}$]	1.74	3.38	1.74	2.40	1.09	1.96

Additionally, the site was examined in May 1997. Physico-chemical measurements (temperature and gases) were carried out in trench 1 at each time 3 points (points 1, 3 and 5, see Figure 60) in the zones A (composted since 1 day), C (since 26 days), and E (since 41 day). Samples were taken after turning.

Bioaerosol measurements were carried out in the rotting hall with and without turning of compost, in the compost storing area, in the office area, and after the biofilter. The SAS Standard and Super 90 were used. For detailed methods, see Chapter 2.2.2.
Bioaerosol determinations were repeated during composting of summer material (late June 1997). The SAS Super 90 and the MAS were used. . For detailed methods, see Chapter 2.2.2.

Measurements were effected at three moments:

- early in the morning, before turning started. However, filling of the trenches in the rotting hall was already in function
- during mid-morning, while composts in the zones C and E were turned
- during the pause at noon

Measurements were carried out in the rotting hall, at the point of entry of air into the biofilter, and on top of the biofilter. Due to the high fungal spore concentrations expected in the samples taken before the biofilter, Petri dishes were not incubated directly, but the agar was detached, and the spores suspended in physiological salt solution by homogenization in the Stomacher Lab Blender (see Chapter 2.2.4).

3.2.3.3 RESULTS

3.2.3.3A Comparison of wintar and summer material

Physico-chemical measurements

WINTER MATERIAL

Figure 62 shows the temperature distribution in trench 11. Before turning, temperatures at the surface or the center of the compost were slightly above or slightly below 60°C. They were lower only at the bottom, especially in the 4 week old compost, most probably caused by a cooling of the instreaming air.

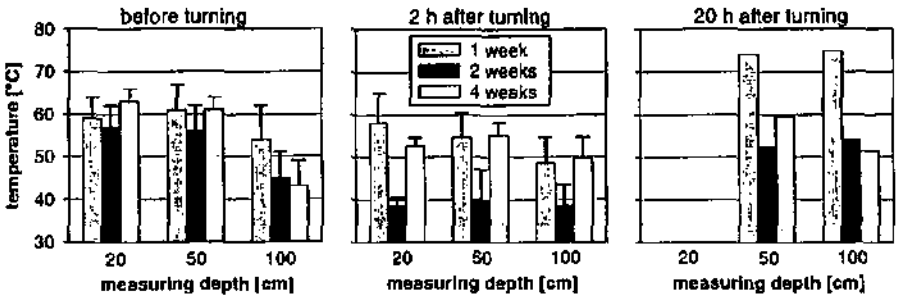


Figure 62: Temperature distribution in trench 11 (winter material). The columns represent means of 5 (before turning) or 2 (2 h after turning) measurements and the standard deviations.

Temperatures got reduced by 5-20°C by the turning of the composting material, especially in the 2 week old material, probably due to the addition of water. 20 h after turning, the stimulating effect of the turning on the microbial activity could be observed: in the 1 week old material, temperature rose in less than 1 day to 70°C, values higher than before turning. The less important temperature increase in the 2 and the 4 week old compost could be an indication that part of the easily degradable substances was already metabolized.

Compared to the other composting systems examined (open-air windrows, box composting in bigger but less frequently turned boxes, see Chapter 3.1.1 and 3.1.2) it stands out that the temperatures were not very high, but they were very homogeneously distributed. The relatively low temperatures might be caused by the starting material (mainly dead leaves), which was not very rich in easily degradable organic substances. The good homogeneity of the material was certainly the result of the fine shredding of the biodegradable waste, the good mixing before filling in the trench, and the frequent turning.

Table 34: Gas concentrations in winter compost. The results before turning are means of 5 measurements.

age	depth	before turning		20 h after turning
[weeks]	[cm]	O_2 [vol %]	CO_2 [vol%]	O_2 [vol %]
1	20	18.4 ± 0.6	10 ± 3	nd
(zone A)	50	18.8 ± 0.9	8 ± 3	16.7
	100	18.0 ± 2.0	8 ± 4	9.4
2	20	19.4 ± 0.6	5 ± 1	nd
(zone C)	50	19.6 ± 0.7	5 ± 2	15.6
	100	20.3 ± 0.3	2.9 ± 0.5	17.7
4	20	18.0 ± 1.0	12 ± 3	nd
(zone E)	50	19.0 ± 2.0	9 ± 2	18.7
	100	20.1 ± 0.4	4 ± 1	18.6

The gas measurements (Table 34) also demonstrated the good homogeneity of the winter material. All sampling sites showed an oxygen concentration > 8 % prior to turning. The air supplied to the compost (1.2-7.2 m^3 air / h t_{FW}) was obviously sufficient to create and maintain oxic conditions. The lower values after turning (10 to 15 % oxygen) were an indication of the boosting of microbial activity by the mixing and watering. Astonishingly, the CO_2 concentrations were very high. The short aeration phäses had might not driven off the carbon dioxide, heavier than air and therefore accumulating at the bottom of the trench. However, the high CO_2 concentration should not influence the microbial activity. Methane and hydrogen sulfide were not detected at all.

Table 35: Temperature and gas concentration in the screened winter compost stored outside of the hall, under roof.

depth [cm]	Temp. [°C]	O_2 [vol %]	CH_4 [vol %]	H_2S [ppm]
20	54	11.4	0.2	0.5
50	60	2.9	0	3.6
100	56	1.4	> 5	2.8
150	48	nd	nd	nd

The situation in the stored winter compost was different (Table 35): already in 50 cm depth, less than 5 % oxygen was measured. In a depth of 1 m, oxygen was further reduced, with the simultaneous presence of methane and hydrogen sulfide. This could indicate that the compost was not yet completely stabilized after the intense, but short rotting time in the hall, and that the degradation process continued in the storage. Furthermore, the piling up of the finely screened material under roof did not allow a sufficient air circulation in the compost, provoking the large anaerobic zones.

SUMMER COMPOST

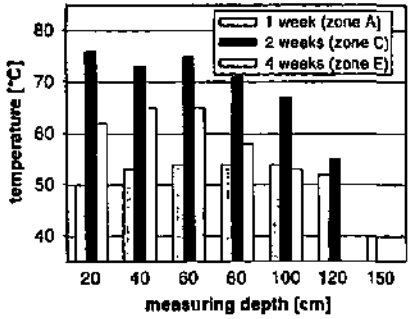


Figure 63: Compost temperatures before turning (summer compost).

Table 36: Gas measurements in summer compost, before turning.

Zone	A (1 week)		C (2 weeks)		E (4 weeks)	
depth [cm]	O_2 [%]	CO_2 [%]	O_2 [%]	CO_2 [%]	O_2 [%]	CO_2 [%]
20	13.5	7.2	13.7	6.9	15.9	4.6
40	15.6	4.6	15.0	6.7	17.0	6.5
60	17.1	4.1	15.3	5.4	18.0	2.7
80	17.2	3.6	14.6	4.8	20.5	1.1
100	17.0	3.4	15.7	4.2	19.5	1.6
120	20.5	0.7	nd	nd	nd	nd

Table 37: Physico-chemical characteristics (temperatures and gas concentrations) of summer compost stored outside of the hall under roof

depth [cm]	temp. [°C]	O_2 [%]	CO_2 [%]	CH_4 [%]	H_2S [ppm]
measurement lateral					
20	40	10.5	13	0.25	0
50	54	0.8	28	0.5	0
100	50	0.5	32	0.8	55
150	40	0.7	33	1	42
measurement from the top					
20	42	18.6	9.5	0.3	0
50	56	6.3	22	3.2	0
100	60	1.9	32	> 5	0
150	51	1.1	36	> 5	0

The summer compost in the zone A showed homogenous, but not very high temperatures (Figure 63). The short composting time (1-4 days), the high water content, and the stronger aeration compared to the winter material could explain this slow temperature rise.
The maximum temperatures of > 75°C were reached after 2 weeks of composting, while at the end of the composting process in the rotting hall, temperatures had come down to approximately 55°C. As in the winter material, temperatures at the bottom of the trench were lower. Due to the generally higher temperatures in the summer material, and the stronger aeration, vertical temperature gradients were more pronounced that in the winter material.

The summer material, rich in easily degradable substances due to large amounts of grass clippings, allowed more intense microbial activities. This resulted not only in higher temperatures, but also in lower oxygen concentrations (Table 36): although aeration was increased, less O_2 was measured. However, concentrations were at no point below 13 vol%. The absence of methane or hydrogen sulfide confirmed that no anoxic zones existed.
Astonishingly, CO_2 concentrations were lower than in the winter material. This could be explained by the longer aeration phases (2 minutes instead of 1 minute), allowing a better removal of the CO_2.

The screened summer compost in storage (Table 37) showed similar temperatures and gas concentrations than the winter material. Conditions were anoxic below a depth of 50 cm, resulting in the production of methane and hydrogen sulfide. Due to the lacking air circulation, CO_2 concentrations of > 30 % were measured in the core of the pile.

Table 38 presents the physico-chemical values of the samples, and, if available, those of the starting material. For discussion of the temperatures, see paragraph above.

Table 38: Physico-chemical parameters of the compost samples.

parameter	temp. [°C]		pH		water content (%FW)		C/N	
sample	winter	summer	winter	summer	winter	summer	winter	summer
fresh biowaste (half green waste, half wood)								
after shredder	-	-	6.3	4.9	59	68	nd	nd
zone A (compost 1 week)								
2 (surface)	57	50	5.3	5.6	59 [a]	61	nd	nd
3 (center)	65	54	6.3	5.4	60 [a]	62	nd	nd
4 (bottom)	59	40	5.8	5.5	58 [a]	64	nd	nd
5 (mixed)	-	-	5.8	5.5	61 [a]	57	28[c]	28
zone C (compost 2 weeks)								
6 (surface)	62	76	7.7	7.7	58 [b]	54	nd	nd
7 (center)	60	75	7.7	7.9	59 [b]	45	nd	nd
8 (bottom)	50	55	8.0	7.8	62 [b]	46	nd	nd
9 (mixed)	-	-	8.1	7.8	59 [b]	50	23[d]	24
zone E (compost 4 weeks)								
10 (surface)	66	62	8.3	8.4	55	41	nd	nd
11 (center)	61	65	8.4	8.6	53	41	nd	nd
12 (bottom)	41	53	8.8	8.6	51	41	nd	nd
13 (mixed)	-	-	8.2	8.5	58	40	20	19
compost screened, storage under roof								
14 (surface)	54	50	8.7	8.0	49	34	nd	nd
15 (center)	58	60	9.0	8.3	41	39	nd	nd
biofilter								
16 (surface)	15	28	4.8	5.3	82	83	nd	nd

[a] H_2O content of the starting material of this batch: 61 %
[b] H_2O content of the starting material of this batch: 62 %
[c] C/N of the starting material of this batch: 28
[d] C/N of the starting material of this batch: 29-39

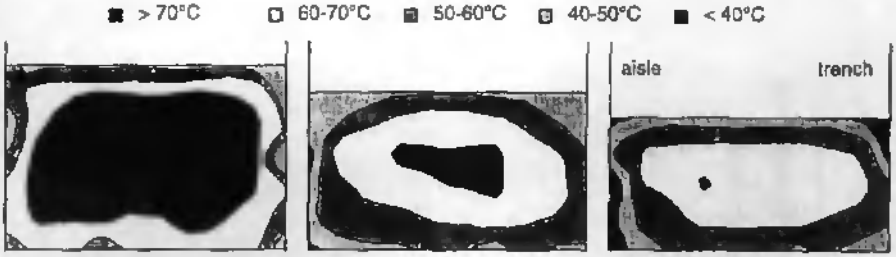
The pH of the fresh winter material amounted to 6.3, was reduced in the first days of composting, and rose until the end of the compost process in the rotting hall to pH 8. During storage under roof, a further increase could be observed up to pH 9. The slow pH increase in the 1 week old compost was most probably caused by the poor degradability of the starting material (a high percentage of dead leaves), leading to a generally slower composting process. The low pH of the biofilter can be attributed to the composition of the filter material (heather).
The pH in the summer material was slightly lower than in the winter material throughout the whole composting process. The most marked differences concerned the starting material (4.9 vs. 6.3), and the final compost (8-8.3 vs. 8.7-9). The low pH in the starting material could be explained by a greater production of acidic intermediate metabolites during the degradation of the high amount of sugars in the grass clippings. The lower pH in the screened compost could have been caused by a less intense protein degradation at the high temperatures measured in the summer material (Figure 63). The different composition might also have lead to a compost with a greater buffering capacity.

The water content of the material at the beginning of the composting process was approximately 60 %, as stipulated by the operators. These optimal values could be maintained during the whole rotting time in the hall. The desired slight drying of the compost for an easier screening was achieved in the zone E; further drying happened during storage. The biofilter was very wet (82 % water), most probably due to the condensation of the humid vitiated air in the filter material.
The water content in summer, although higher in the starting material than in winter, got reduced much more rapidly during the composting process, due to the stronger aeration and the higher temperatures. In the 4 week old compost, and in the material in storage, the water content was limit for a correct composting process.

The C/N ratio in winter varied in the starting material between 28:1 and 39:1. No reduction was observed after the first week of composting; after 2 weeks, it amounted 23, and after a further 2 weeks to 20. The objectives of a C/N ratio of < 15 after 30 days of composting in the rotting hall, guaranteed by the constructor of the installation, was not achieved. It has to be considered, though, that the starting material had had most probably a higher C/N ratio than that required by the constructor (28-30). This type of installation was designed for the composting of sewage sludge, where the initial C/N ratio can be easily adjusted by the admixture of wood chips to the sludge. However, when composting green wastes that change with the seasons, a constant composition of the starting material can not be obtained.
The C/N ratios of the summer material were comparable of that of the winter, although the amount of easily degradable carbon sources might have been higher. Again, the composting time in the rotting hall did not allow producing a material with the required C/N ratio of 15.
The quite similar final quality of the composts made out of the winter and the summer material corroborates observations made by MICHEL *et al.* (1993 and 1996), who compared composts made of different mixtures of grass clippings and brush (like our summer material) to ones made up of mainly dead leaves (as our winter material): they came to the conclusion that the mature composts were quite similar. The compost made with grass clippings contained slightly more nitrogen in form of nitrate, and also more soluble salts, potassium and phosphorous. The ratio leaves:grass:brush did neither influence the temperatures, nor the oxygen concentration, the pH or the water content. Their experiments had been carried out in open-air windrows, where the composting process itself could not be changed in function of the starting material, while in the trench composting system, the differences between the summer and the winter material concerning temperature, water content, and consequently also microorganism concentrations (see following paragraph) were probably influenced by the different aeration regimes.

The drawing up of the isotherms from the temperature measurements carried out in the summer material (second summer measurements, late June 1997) are represented in Figure 64.

In the 3 days old compost, already 85 % of the mass was at temperatures > 60°C, and none of the material was colder than 40°C. A temperature gradient built up from the center almost regularly in all directions. At the bottom and towards the trench wall, some irregularities of the isotherm can be seen, most probably due to the formation of preferential air channels. After 2 weeks of composting, the hot core had already gotten smaller, but still more than 50 % of the material showed temperatures > 60°C. At the end of the composting process in the hall, a still quite warm center zone (44 % of the mass between 60 and 70°C) persisted, surrounded by larger layers of compost material at lower temperatures. All three temperature profile measurements showed a slight asymmetry of the temperature distribution over the width of the trench: the side towards the aisle was slightly colder than that adjoining another trench. However, due to the fact that the composting was carried out in a closed hall, these differences were only minimal.



compost zone A (3 days)

temp. range	%
> 70°C	58
60-70°C	29
50-60°C	12
40-50°C	4
< 40°C	0

compost zone C (2 weeks)

temp. range	%
> 70°C	10
60-70°C	42
50-60°C	39
40-50°C	9
< 40°C	<1

compost zone E (4 weeks)

temp. range	%
> 70°C	<1
60-70°C	44
50-60°C	38
40-50°C	14
< 40°C	4

Figure 64: Temperature isotherms and percentage that represent the different zones in summer composts of different age during composting in trenches (cross-sectional view).

The composting in trenches in a closed hall allowed the development and the maintenance of higher temperatures compared to open-air windrows (see Chapter 3.2.1.3B). The weaker loss of metabolic heat to the environment, also during turning, could account for the higher temperatures. The better oxygenation surely also lead to a more intense microbial activity, as aeration was not used for temperature control, but was just set to maintain oxic conditions in the compost, heat production surpassed heat loss by evaporative cooling. The very high temperatures effected a rapid inactivation of potentially pathogenic microorganisms (see Figure 65). However, the isotherms showed mesophilic zones in the 2 and 4 week old compost, allowing a recolonization by potential pathogens.

Microbiological analyses

IN THE COMPOST

Figure 65 depicts the concentration of *AF*, coliforms, and *E. coli* in winter and summer composts of different age, at different depths in the trench.

The quite low *AF* concentrations that were detected in the fresh winter material ($3.4 \cdot 10^4$ cfu/gDW) might not be precise: enumeration of molds in material with a low pH was often difficult, because yeasts (in this case $4.5 \cdot 10^7$ cfu/gDW, results not shown) dominated on the culture plates, concealing the mold colonies. Coliforms were present in concentrations $>10^6$ cfu/gDW. No *E. coli* were detected in the starting material, consisting only of park waste, and more specifically of dead leaves. This confirms the literature reports that kitchen waste is the major source of *E. coli* (KOZAKIEWICZ & SMITH, 1994).

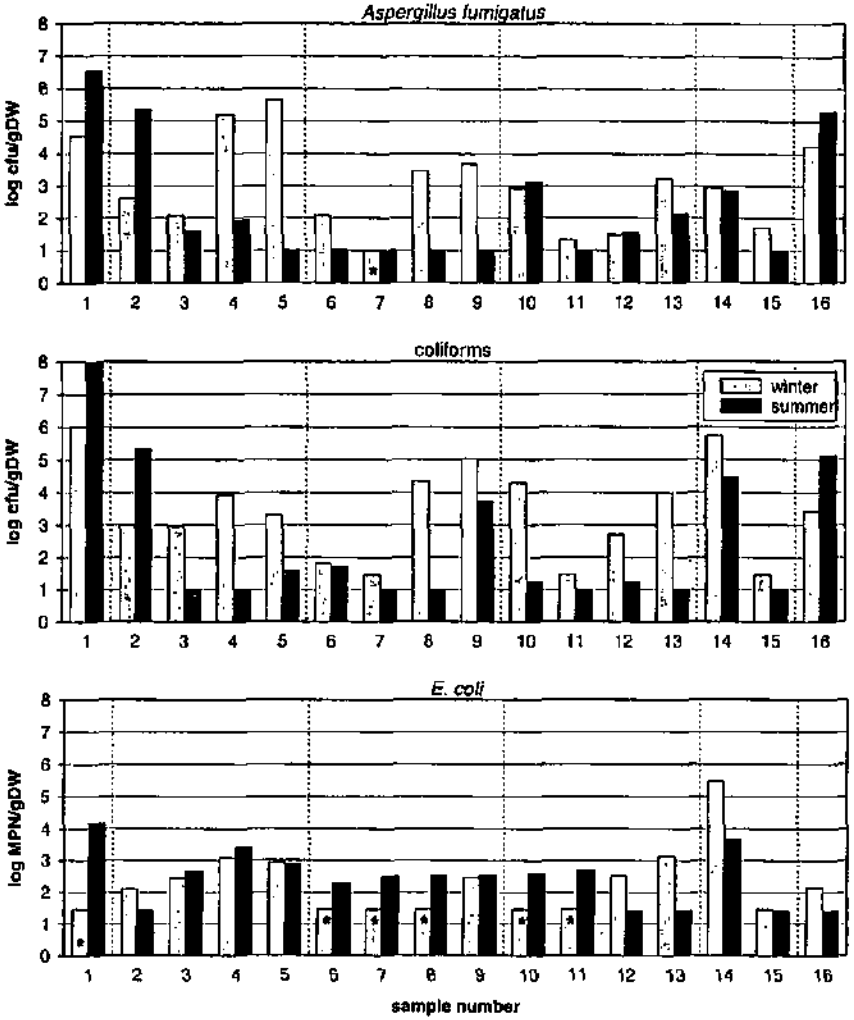


Figure 54 : Concentration of Aspergillus fumigatus, coliforms (detected on VRB-agar), and E. coli (detected in LMX-broth) in winter and summer compost of different age and at different depths in the trench.

1 = fresh green waste
2-5 = compost 1 week (2 = surface; 3 = center; 4 = bottom; 5 = mixed)
6-9 = compost 2 weeks (6 = surface; 7 = center; 8 = bottom; 9 = mixed)
10-13 = compost 4 weeks (10 = surface; 11 = center; 12 = bottom; 13 = mixed)
14-15 = screened compost (14 = surface; 15 = center)
16 = biofilter material

*Columns with an * indicate values below the detection limit (10 cfu/gFW for AF and coliforms, 28 MPN/gFW for E. coli).*

The 1 week old compost showed a reduction of *AF* numbers to 120-440 cfu/gDW in the upper parts of the material, in spite of the not very elevated temperatures (57-65°C, see Figure 62), allowing normally the survival of a fraction of the mold spores. The competition with the degrading microflora contributed most probably to the disappearance of the molds. At the bottom of the trench, at the point of air entry, a proliferation of *AF* was observed, seemingly favored by the provision of oxygen. This proliferation appeared to be important, because after turning, elevated *AF* concentration was also measured in the mixed material.
The coliform numbers showed tendencies similar to *AF*, but did not re-grow in the same manner at the bottom of the trench.
In the 1 week old compost, colonization with *E. coli* to concentrations of 130-1200 cfu/gDW was observed. Either the starting material of this batch had already contained *E. coli*, or they stemmed from other compost batches that contained kitchen waste. Contamination could happen by the turning machine, or by the vitiated air from the preprocessing hall (dumping, shredding) used for the aeration of the composts.
In the course of the composting process, concentrations of indicator microorganisms diminished continuously until the end of the rotting process in the hall. Higher concentrations were always measured in the material sampled at the bottom of the trench, where temperatures were not sufficient for thermohygienization. This resulted in the maintain of *AF* concentrations of 10^3 cfu/gDW, and of coliforms of 10^4 in the compost sampled after turning.
In the material in storage, at the surface of the heap, an important regrowth of coliforms and *E. coli* ($3\text{-}5\cdot10^5$ cfu or MPN/gDW) was observed. *AF* was not able to recolonize the maturing material, being replaced by the thermotolerant fungus *Scytalidium thermophilum* (results not shown), and probably mesophilic species that were not detected because incubations were carried out at 40°C.
The biofilter material carried high concentrations of *AF* (10^5 cfu/gDW). In consideration of the low temperature measured in the filter (15°C), allowing only a very slow growth of the mold (see Chapter 3.1.3), we presume that the *AF* particles detected were most probably spores that were brought in with the vitiated air. They surely survived, but could not germinate.

The fresh summer material contained elevated *AF* quantities, representing almost 100 % of the total thermotolerant molds (results not shown). During the composting process, the values dropped considerably, after all in the zone C (compost of 2 weeks), where temperatures > 70°C were reached. At the end of the rotting time in the hall, the compost was to a lesser degree recolonized by molds. At this moment, *S. thermophilum* was detected besides *AF*.
As in the winter compost, the material in storage showed a recolonization by molds as well as by coliforms at the surface. However, in the depth of the pile, where temperatures around 60°C and low oxygen concentrations (< 2%) were measured, no potential pathogens or indicator organisms were detected.
The biofilter was much more contaminated with *AF* (> 10^5 cfu/gDW) than in winter. The spores stemmed probably not only from the vitiated air, but the mesophilic temperatures measured in the filter (28°C) could have allowed a proliferation of the mold.
Coliform concentrations, very elevated in the starting material (10^8 cfu/gDW), got drastically reduced already in the first week of composting, and were mostly below the detection limit, with the exception of the sample taken after turning in the 2 week old material. Recolonization to 10^4 cfu/gDW was observed at the surface of the screened compost in storage. The biofilter material also contained coliforms in concentrations of approximately 10^5 cfu/gDW. *E. coli* were present in considerable concentrations in the fresh material (10^4 MPN/gDW), and in young compost sampled at the bottom of the trench. Afterwards, no or very few were detected any more. Only the surface material of the compost stored in a pile, and the biofilter contained > $1\cdot10^4$ MPN/gDW.

For comparative reasons, the results for the coliforms obtained with the VRB-agar, and those for *E. coli* with the LMX were shown. Other selective media used for testing the summer material lead to quite different results (Figure 64).

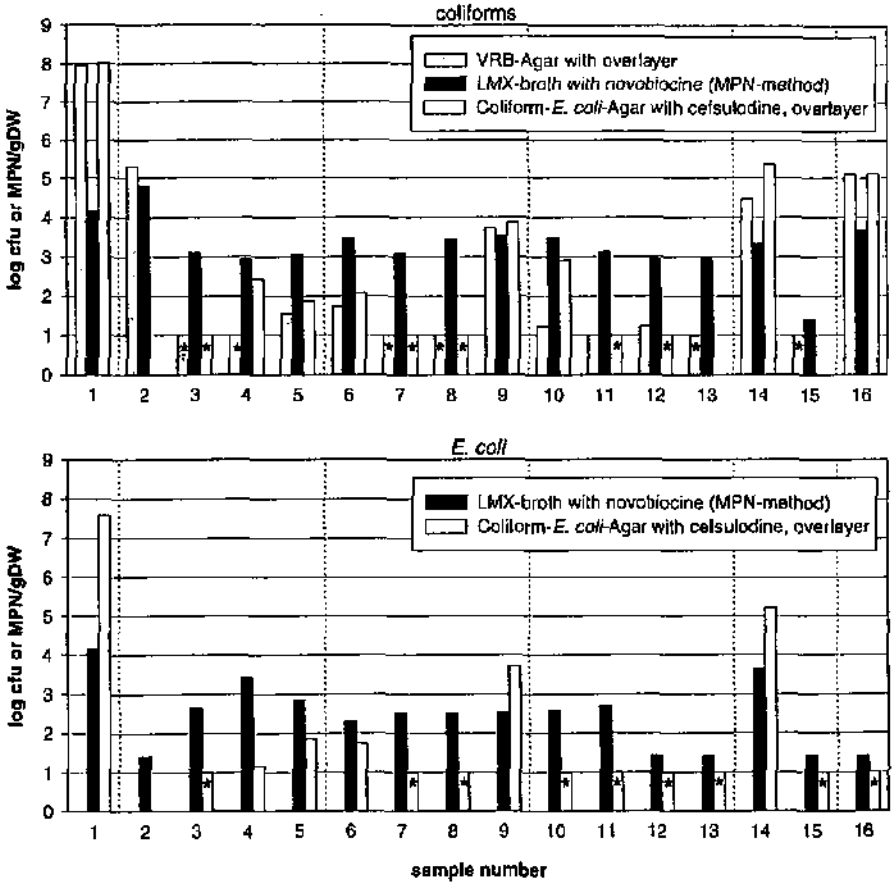


*Figure 55: Comparison of different selective media for the enumeration of coliforms and E. coli in summer compost. Columns with an * indicate values below the detection limit (10 cfu/gFW plate counts (Coliform-E. coli-Agar), 28 MPN/gFW for MPN's (LMX-broth).*

1 = fresh green waste
2-5 = compost 1 week (2 = surface; 3 = center; 4 = bottom; 5 = mixed)
6-9 = compost 2 weeks (6 = surface; 7 = center; 8 = bottom; 9 = mixed)
10-13 = compost 4 weeks (10 = surface; 11 = center; 12 = bottom; 13 = mixed)
14-15 = screened compost (14 = surface; 15 = center)
16 = biofilter material.

Enumeration of cfu's on the two solid media (VRB-Agar and Coliform-*E. coli*-Agar (CCA)) gave quite similar results. Concentrations determined with the LMX-broth were lower in the case of high coliform numbers, but higher in the composts where coliform numbers were greatly reduced due to the elevated temperatures. The same was also observed for the detection of *E. coli*. These differences might be due to a generally better growth of the compost microflora on solid media, or to the different enumeration techniques (plate counting Vs. MPN).

Another explanation for the observed differences might be the better suppression of the accompanying microflora on the solid media, were the growth of strictly aerobic species was impaired by cultivation in the agar mass, and an additional layer of agar (overlayer). Tests had shown that detection was enhanced by inoculating in the agar mass, compared to inoculation of the agar surface (GARCIA, 1998).

Also, the addition of the antibiotic cefsulodine to the chromogenic medium could have influenced its selectivity. Results obtained in the scope of this study by GARCIA (1998) about the enumeration of coliforms in compost had shown that the addition of cefsulodine to the CCA, suppressing *Pseudomonas ssp.* and *Aeromonas ssp.*, allowed to detect one half to one order of magnitude more coliforms and *E. coli* than the CCA without cefsulodine. The presence of a large number of other Gram-negative bacteria can mask the coliforms, and their β-D-galactose activity, used to identify them on the selective medium, can be diminished (BERG & FIKSDAL, 1988). Also, the basic composition of the selective agar might influence the detection of specific bacterial groups. Tests with the different chromogenic media on the market (CCA, Coli-ID, and EEC, description see Chapter 2.2.4) had shown, as well as with single strains isolated from composts, as with compost extracts, a higher recovery rate with the CCA, even without the addition of cefsulodine.

Further research would be needed to define the factors that are responsible for the different results obtained with the various selective coliform media. Of course, when results from different experiments have to be compared, the same medium has to be used.

Table 39: Genera of presumed coliforms isolated with different selective media (MacConkey, VRB, Tergitol, CCA, EC-ID, EC; description of media see Chapter 2.2.4) from composts from different sites and of different maturity (M: a = all stages of maturity; i = intermediate maturity; m = mature, screened compost). From GARCIA, 1998.

Genus	M	number of strains isolated
coliforms		
Citrobacter	m	4 (*C. freundii*)
Enterobacter	a	21 (19 *E. cloacae* (12 *E. agglomerans*)
E. coli	a	12
Klebsiella	a	20 (17 *K pneumoniae. ssp. pn* (13 *K. oxytoca*)
non-coliforms		
Ochrobactrum	i	1 (on MacConkey Agar)
Proteus	a	1 (on MacConkey Agar)
Total		59

Table 39 gives information about the types of coliforms detected in compost. The results represent all strains isolated from the different sites examined. The spectrum of the species found at each site was similar. No difference was also observed between the types of bacteria isolated on the different selective media used, although the limited number of strains only allows indicating tendencies. The main coliform genera were *Enterobacter* (after all *E. cloacae*), *Klebsiella* (after all *K. pneumoniae ssp. pneumoniae*) and *E. coli*, which was discovered in compost of all stages of maturity. This is in accordance with several investigations reported in literature: MÖSE & REINTHALER (1985), cited by ROTH (1994) found in biowaste mainly *Enterobacter sp., Proteus sp., E. coli, P. aeruginosa, Klebsiella sp., Serratia sp.* and *Citrobacter sp.* BRINTON & DROFFNER (1994) reported the presence of species of the family *Enterobacteraceae* and *Pseudomonadaceae* in material that had been composted during 15 days in a closed vessel, *P. aeruginosa* being the most prevalent organism. RÜDEN *et al.* (1994) found in composts from different composting sites (each time identification of 20 strains) in one installation a strong prevalence of *E. cloacae* (70 %), *C. freundii* (25 %) and *K. pneumoniae*

(5 %), at another installation, however, 60 % *Pseudomonas maltophilia*, and 40 % *Aeromonas hwoffi*. ANDREWS *et al.* (1994) detected in compost prepared from sewage sludge the genera *Pseudomonas*, *Serratia*, *Xanthomonas* and *Klebsiella*, but no *E. coli*. CLARK *et al.* (1983) reported the genera *Klebsiella*, *Enterobacter*, *Serratia* and *Pseudomonas* as the most commonly found Gram-negative bacteria in the air of a composting plant.

Citrobacter freundii was only detected in intermediate mature composts. A few non-coliforms were also isolated on MacConkey agar. Most of the non-coliforms detected on the selective coliform-media could not be identified with the API 20NE system, and appear thus not in the results. The presence of *Ochrobactrum anthropii*, normally detected in human clinical material (SWINGS *et al.*, 1992), was confirmed by two further isolations on CCA, which allows the growth of Gram-negative non-coliforms, identifiable by colorless colonies. The non-coliforms isolated from this medium belonged to the genera *Aeromonas* (1 strain), *Flavimonas* (1 strain), *Pseudomonas* (3 strains), *Comamonas* (2 strains), *and Serratia* (2 strains).

If we compare the concentration of molds and indicator bacteria in the composts produced during winter with that produced during summer, we can notice the following tendencies:

- The green and kitchen waste delivered to the site in summer contained much more potentially pathogenic and indicator bacteria than that in winter. However, at the end of the rotting time in the hall, the summer compost had undergone a better thermohygienization, due to the generally higher temperatures.
- The compost in storage, due to the fact that it did not get mixed any more, did show an important proliferation of molds and coliforms in the material at the surface of the pile. This was more pronounced in the winter material; the higher water content might have contributed to this recolonization.
- The biofilter contained more *AF* and coliforms in summer than in winter. For *E. coli* it was the contrary. The same tendencies could be observed in the fresh waste. The principal aerolization of microorganisms has thus to occur when the incoming waste is treated (shredding, mixing, and filling of the boxes) rather then by turning the older material. Also, in summer, an active proliferation of potential pathogens in the filter material could happen, when biofilter temperatures were permissive for microbial growth.

The enumeration of thermophilic bacteria (Figure 67) showed > 10^7 MPN/gDW already in the starting material. The highest concentration of moderate (growth at 60°C, mainly *Bacillus* species (BLANC, 1998) and probably also highly (growth at 75°C, *Thermus* species, BEFFA *et al.*, 1996) thermophilic bacteria was reached in the 2 week old compost. At this stage, the thermophilic flora seemed to be most numerous, but not most active: temperature (Figure 62) and gas measurements (Table 34) both indicated a higher microbial activity in the 1 week old material.

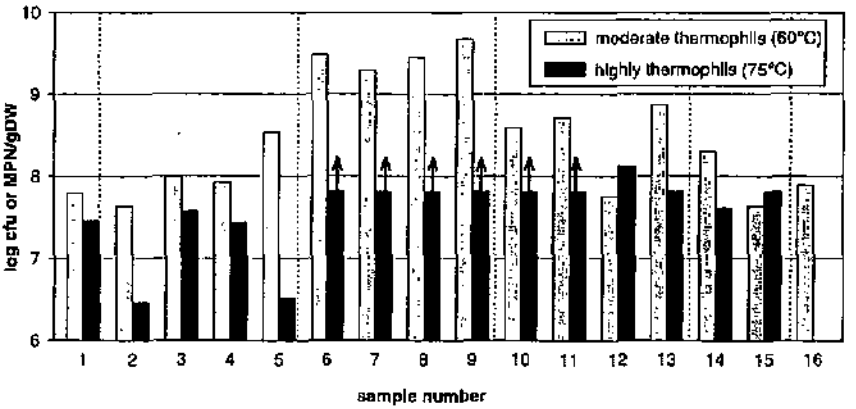


Figure 56: Concentration of thermophilic bacteria in compost (winter material). ↑ values above the detection limit of $6.5 \cdot 10^7$ MPN/gFW. *1* = *fresh green waste*
2-5 = *compost 1 week (2 = surface; 3 = center; 4 = bottom; 5 = mixed)*
6-9 = *compost 2 weeks (6 = surface; 7 = center; 8 = bottom; 9 = mixed*
10-13 = *compost 4 weeks (10 = surface; 11 = center; 12 = bottom; 13 = mixed*
14-15 = *screened compost (14 = surface; 15 = center)*
16 = *biofilter material.*

IN THE AIR

Table 40 shows the results of the bioaerosol measurements carried out inside and outside of the rotting hall, as well as on top of the biofilter. Additionally to the series of measurements carried out in winter and in summer, those executed in spring (month of May) are presented.

In all seasons, very high bioaerosol concentrations were measured in the rotting hall. The air contained several 1000 spores per m^3, even without turnings being carried out. In this case, the source of air contamination was the charging of the trench, and the aeration. Measurements of mesophilic fungi and bacteria effected at the same time showed concentrations of $> 2 \cdot 10^6$ cfu/m^3 air and $3 \cdot 10^4$ cfu/m^3, respectively (GUTZWILLER, Swiss Accident Insurance Institute, personal communication).

The intense mixing of the material by the turning machine resulted in high *AF* spore emissions ($> 10^5$-10^6 cfu/m^3). The turning of the compost in zone C lead generally to lesser spore dispersion than either in zone A (fresh material) or in zone E (compost at the end of the rotting time in the hall). These findings are in accordance with the *AF* concentrations detected in the compost: the intermediate compost, where temperatures exceeded 75°C, contained only few molds, while a recolonization was observed in the more mature material, where temperatures were lower (see Figure 65).

Table 40: Mold spores in the air during different composting activities, and at different seasons. TTM = total thermotolerant molds and yeasts, ∞ = agar plate completely overgrown, no counting possible.

location	activity / zone / age	AF [cfu / m³]		
		winter	summer	spring
in the rotting hall, without turning				
side filling (Zone A)	filling + emptying of trenches	∞ (TTM > $2.8 \cdot 10^4$)	∞ (TTM > $5.3 \cdot 10^4$)	nd
side exit compost (Zone E)	ditto	$2.5 \cdot 10^3$	$2.5 \cdot 10^3$	nd
in the rotting hall, with turning				
1 m from the turner	turning / zone A / 1 week	∞ (TTM > $1.7 \cdot 10^5$)	$2.5 \cdot 10^4$	∞ (TTM > $2.6 \cdot 10^5$)
10 m from the turner	ditto	∞ (TTM > $5.5 \cdot 10^4$)	$1.8 \cdot 10^4$	∞ (TTM > $2.6 \cdot 10^5$)
1 m from the turner	turning / zone C / 2 weeks	∞ (TTM > $5.5 \cdot 10^4$)	$9.0 \cdot 10^3$	∞ (TTM > $2.6 \cdot 10^5$)
10 m from the turner	ditto	$1.5 \cdot 10^3$-∞ (TTM > $5.5 \cdot 10^4$)	$5.0 \cdot 10^3$	$7.3 \cdot 10^4$
1 m from the turner	turning / zone E / 4 weeks	∞ (TTM > $1.7 \cdot 10^5$)	$1.9 \cdot 10^4$	$1.2 \cdot 10^4$-∞ (TTM $2.6 \cdot 10^5$)
10 m from the turner	ditto	$1.7 \cdot 10^3$	$1.3 \cdot 10^5$	(TTM > $2.6 \cdot 10^5$)
in the open				
in the open	screening	70	4	2
in the storage, under roof	none	180	15	nd
in front of the screener	screening	420	13	50
in front of a house next to the site	none	20	1	2
preparation / office				
next to the computer	none	140	14	8
2 m from the truck	dumping of biowaste	$6.4 \cdot 10^3$	∞ (TTM > $1.7 \cdot 10^5$)	nd
50 cm from the conveyor belt	shredding	$8.8 \cdot 10^3$	∞ (TTM > $1.7 \cdot 10^5$)	nd
on top of the biofilter				
left side	see [a b c]	$1.5 \cdot 10^3$	440-∞ (TTM > $4.4 \cdot 10^4$)	30
right side (side of air entry)	ditto	∞ (TTM > $6.6 \cdot 10^4$)	860-∞ (TTM > $4.4 \cdot 10^4$)	120
left side	see [d e f]	230	∞ (TTM > $2.7 \cdot 10^5$)	120
right side (side of air entry)	ditto	510	$1.2 \cdot 10^4$-∞ (TTM > $2.7 \cdot 10^5$)	400

[a] winter: shredding
[b] summer: turning and emptying of trenches
[c] spring: shredding and mixing
[d] winter: none except aeration
[e] summer: shredding, filling of trenches, turnings and emptying of trenches
[f] spring: none except aeration

To investigate the capacity of the filter to hold back fungal spores, additional measurements were carried in late June (Table 41). The measurements were carried out at different moments of the day: *early in the morning when operations started, during turning at mid-morning, and at the end of the* pause at noon.

Table 41: Concentrations of Aspergillus fumigatus (AF) and total thermotolerant molds (TTM) in the rotting hall and before and after the biofilter, at different moments of the day.

Time	early morning (7h15)		turning of compost		at noon (pause)	
sampling point	*AF* [cfu/m³]	TTM [cfu/m³]	*AF* [cfu/m³]	TTM [cfu/m³]	*AF* [cfu/m³]	TTM [cfu/m³]
rotting hall						
middle of zone A	∞	$> 2.6 \cdot 10^4$	$3.6 \cdot 10^4$	$3.7 \cdot 10^4$	$1.3 \cdot 10^3$	$1.3 \cdot 10^3$
middle of zone C	∞	$> 3.3 \cdot 10^4$	$4.6 \cdot 10^3$	$4.8 \cdot 10^3$	820	840
middle of zone E	$3.1 \cdot 10^5$	$3.2 \cdot 10^3$	970	$2.4 \cdot 10^3$	110	160
side filling	∞	$> 3.3 \cdot 10^4$	$1.3 \cdot 10^4$	$1.3 \cdot 10^4$	∞ *	$> 1.3 \cdot 10^{4}$ *
side exit compost	∞	$> 3.3 \cdot 10^4$	$2.1 \cdot 10^4$	$2.1 \cdot 10^4$	$1.3 \cdot 10^{5}$ *	$1.3 \cdot 10^{3}$ *
biofilter						
at the entry of air into the biofilter	$1.4 \cdot 10^6$	$1.4 \cdot 10^6$	$2.2 \cdot 10^5$	$2.3 \cdot 10^5$	$6.7 \cdot 10^4$	$6.8 \cdot 10^4$
on top, left side (side of air entry)	∞	$> 2.6 \cdot 10^4$	$2.0 \cdot 10^4$	$2.0 \cdot 10^4$	580	580
on top, middle	830	$1.5 \cdot 10^3$	$2.1 \cdot 10^3$	$2.1 \cdot 10^3$	130	140

* filling of the trenches had already started

Astonishingly, the highest *AF* concentrations in the rotting hall were measured early in the morning. This could be explained by the fact that at the moment of measuring, the aeration of the rotting hall was still reduced to 30'000 m^3/h (night-time energy saving mode), instead of 60'000 m^3/h during the day. During turning of compost, concentrations in the hall were mostly between 10^3 and 10^4 cfu/gDW, but got reduced during the pause at noon, when neither turnings nor shredding of fresh material were carried out, and the air used for compost aeration entraining thus no spores.

The concentrations of *AF* measured in the air at the entry of the biofilter were a function of those measured in the rotting hall: they were highest in the morning, and lowest during the pause at noon. After the filter, concentrations were reduced to 10 % at the side of air entry, and to 1 % in the middle of the biofilter.

Investigations by VISSIENNON *et al.* (1996) about the emission of fungal spores from biofilters (consisting of woodchips, bark, and mature compost) of composting facilities showed that fungal concentrations after the filter were about 1 order of magnitude lower than in the incoming air if the filter was well maintained. If the filter was too dry, emissions after the filter exceeded even those of the composting process, indicating an active release of spores from the filter.

3.2.3.3B Assessment of the performance of the composting system

The results of the examinations showed a generally effective and highly controllable composting system. In comparison with the other systems tested, the homogeneity of the material, and the only little temperature and gas concentration gradients were striking. Some weak points of the system concerned the aeration system, the temperature control, the rotting time and the storage of the compost after the rotting in the hall:

The bark chips that were filled over the gravel which covered the aeration pipes got crushed and compacted by the turning machine, and were also subjected to microbial degradation. This resulted in a clogging of the aeration channels, necessitating the complete emptying of the trenches, and the replacement of the bark chips already after one year of operation.

The composting system was designed to allow the control of the composting temperature through aeration. However, the temperature probes, which are set into the trench walls, are not indicating a temperature representative of the bulk composting mass, because there is often a gap between the compost material and the trench wall. The aeration was therefore set in fixed cycles determined empirically, the compost temperature having to be measured manually.

The fact that turning and passage through the rotting hall are coupled, e.g. that the compost material is moved on in the trench at each turning, allows little flexibility regarding rotting time. Especially for starting materials that contained a large percentage of difficult to degrade substances, such as dead leaves, the 30 days of composting were not sufficient to produce a ripe compost. A simple prolongation of the rotting time in the hall might not lead to a much maturer product, because the material would be less turned. The results of the experiments at the open-air windrow site showed that frequent mixings did especially favor the composting of more woody materials (Chapter 3.2.1.3B).

The storage of the still active compost in big piles under roof provoked the formation of anoxic zones in the core of the heap. The end products of anaerobic metabolisms (fatty acids, such as lactate, acetate, butyrate, propionate or valeriate) are phytotoxic (ZUCCONI *et al.*, 1985). KIRCHMANN & WIDEN (1994) showed the presence of such acids, especially acetate, in concentrations up to 3 % of dry matter during almost 6 months composting of household wastes, and in lower concentrations (max. 0.8 %) in composts made of household and park waste. Maximum concentrations were measured after 42 days of composting, in windrows that were turned twice a week by a front end loader.

To avoid that the compost gets anoxic during storage, it should be put up in several, smaller windrows, allowing a natural aeration to take place. Ideally, the windrows should be turned from time to time. The installation of an additional aeration system in the storage area, either fix by channels situated in the floor (similar to the system described for the box composting system (see Chapter 3.2.2.1)), or mobile by flexible pipes over which the pile gets constructed, would also remedy the problem of anaerobioses.

The only emission of fungal spores to the environment from the completely closed system was across the biofilter. More detailed measurements should disclose the efficiency of the filter to hold back *Af* spores.

Filling of the trenches and turning of fresh compost led to high spore concentrations in the rotting hall. No fix working places are required in the hall, as the system is fully automated; the different operations are monitored from a computer situated in the office complex. However, for reasons of maintenance, measuring and sampling, the personnel visit the hall daily. For these activities, which normally do not take much time, the wearing of a facial mask is highly recommended, as also stipulated in the report by the Swiss Accident Insurance Institute (GUTZWILLER, personal communication).

3.2.4 COMPOSTING IN A BIOREACTOR

3.2.4.1 DESCRIPTION OF THE INSTALLATION

The composting installation in Uzwil (Figure 68) comprises two units: a partially roofed windrow composting site, where yard wastes are composted, and a closed building housing at pilot scale reactor which serves to test the influence of different starting materials and different parameters on the performance of the composting process. The pilot system allows simulating the conditions encountered in the flat-top windrow (stacking height 2.8 to 3.3 m) composting system Wendelin developed and commercialized by Buhler AG.

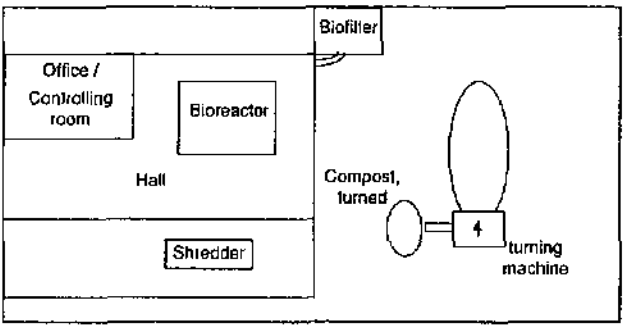


Figure 57: Srhematic plan of the composting site (part with the bioreartor) of Buhler AG, Uzwil. enclosed area.

The experiments in which we participated served to test the behavior of different wastes (in our case biowaste) in a newly developed composting concept (Wendelin AirTec). It is based on a specific temperature controlled aeration management: during the first 4 weeks, the air is supplied to the compost from the bottom by pressure aeration, during the 2 following weeks of maturation from top to bottom by sucking. In the real scale system, which contains composts of different maturity, the vitiated air from the more mature compost aerated by sucking is led through the compost in the intensive stage of rotting aerated by blowing; the two zones are separated by a removable wall. This new concept should allow to produce compost of Rottegrad IV to V in 5 to 6 weeks (BRUNSCHWILER, 1995).
We only participated in the experiments carried out in the pilot scale reactor.

The completely closed, rectangular (2.7 x 3.3 x 2 m) bioreactor holds 20 m^3 = 14 tons of compost. The front of the reactor can be opened for sampling and emptying. The reactor is not thermally insulated. The waste is shredded by a triple-screw shredder, sieved to a particle size of 70 mm, and filled over a layer of wood chips into the bioreactor. The wood chips avoid a clogging of the air ducts with compost particles, and help to better distribute the air into the compost mass. The composting process is temperature controlled, by variation of the aeration intensity. Air can be supplied under pressure to the reactor from the bottom to the top, or be sucked through the composting mass, from top to bottom. Aeration occurs either with fresh ambient air or with air that has already passed through the compost (recycled air). The different aeration regimes will be described for each experiment.

Temperatures were measured and recorded on-line (Figure 69) at the following locations:

- T1 (20 cm from the bottom), T2 (80 cm from the bottom), T3 (180 cm from the bottom), at 80 cm depth, laterally.
- T4 (control), at - 30 cm below the compost surface (= sensor which regulated the aeration).

During the duration of the experiment, the height of the mass in the reactor diminished, so that the position of the lateral temperatures probes in relation to the compost surface changed.
O_2 and CO_2-concentrations were measured and recorded on-line in the air at the outlet of the bioreactor.
The compost mass (weight) and the filling height (by ultrasound) of the reactor were measured and recorded on-line.

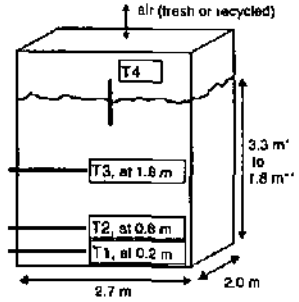


*Figure 58: Locations of the four temperature sensors placed in the compost. * filling height at the start of the experiment ** filling height at the end of the experiment.*

The compost was turned weekly. For this, the bioreactor was emptied with a front-end loader, the compost put up in a windrow in the open air, turned with a specialized machine, and filled again in the bioreactor. The whole turning process took about 2-3 hours. If necessary, water was added to the compost during the turning.

3.2.4.2 EXPERIMENTS CARRIED OUT

Our laboratory participated in the experiments V12 and V13, (organized by Buhler AG) to furnish some information about the presence and number of microorganisms (both potentially pathogenic or indicator microorganisms, and non-pathogenic degradative microorganisms) during composting of biowaste from bin collection (C:N of approximately 20) from a suburban area.

3.2.4.2A Experiment V12

The experiment was started on October 4, 1994. The starting material consisted of source-separated kitchen and garden waste from bin collection. Normally wood chips would have been added to the biowaste, but this was not necessary in this experiment because the starting material contained already enough structure material.
The compost was aerated in the following way:

- Week 1: fixed flow of 10 $m^3/h \cdot t_{fresh\ weight}$ fresh air and about 30 $m^3/h \cdot t_{fresh\ weight}$ recycled air.
- Week 2 to 4: temperature controlled (at 65°C) pressure ventilation (air from bottom of the reactor to the top). The aeration was regulated in the following way: if the average temperature measured over 1 h of registration was 2.4°C above the average of the previous hour, the ventilation was started. The compost was first aerated with recycled air, then, if this did not lower the temperature enough, with fresh air.
- Week 5 to 7: inverse ventilation (sucking ventilation, air from the top to the bottom) with a fixed airflow of 10 $m^3/h \cdot t_{fresh\ weight}$.

Oxygen could not be monitored during the first 2 days of the experiment because the sensor was out of function.
After two weeks of composting, approx. 1.5 m^3 water were added to the compost during the turning to reach again 50-55% humidity.

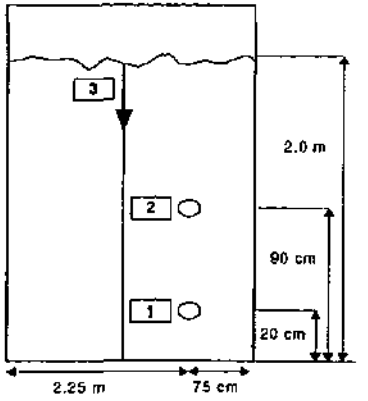


Figure 59: Locations of physico-chemical measurement points.

After 5 weeks of composting, physico-chemical measurements were carried out in the locations shown in Figure 70. For this, the temperature and gas probes were inserted horizontally in holes on the front of the bioreactor intended for this purpose (locations 1 and 2). The profile 3 was executed vertically from the top of the reactor. Filling height at this moment was 2 m. Measurements were carried out from 10 to 120 cm depth. The aeration had been stopped for the duration of the measurements.

The following parameters were determined: temperature; O_2; CO_2; H_2S; CH_4; CO. For detailed methods, see Chapter 2.2.1.

Sampling: compost was sampled at the points shown in Figure 71:

- sample 1: periphery, 20 cm from the bottom
- sample 2: periphery, 1 m from the bottom
- sample 3: center, 20 cm from the bottom
- sample 4: center, 1 m from the bottom

Samples 1 and 2 were taken at the surface, after opening of the bioreactor, samples 3 and 4 were taken when the reactor was partially emptied with a front-end loader, in 1 m depth. Another sample was obtained after turning, from different parts of the windrow. Samples were taken weekly, usually by the personnel of Buhler AG, and sent by express mail to the laboratory, where they normally arrived on the day of sampling. They were cooled immediately to 4°C, and treated the following day.

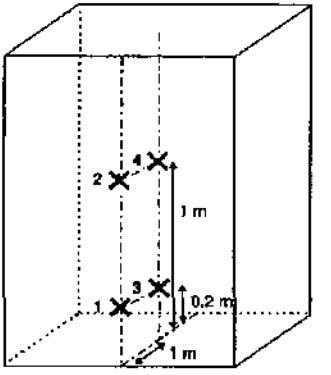


Figure 60: Sampling locations in the bioreactor.

Physico-chemical analyses: pH; dry weight. For detailed methods see Chapter 2.2.3.

Microbiological analyses: *Aspergillus fumigatus*, total molds and yeasts; Gram⁻ bacteria (on MacConkey Agar); heterotrophic mesophilic and thermophilic bacteria. For detailed methods see Chapter 2.2.4.

In addition to the analyses carried out in our laboratory, a number of tests were done by the control laboratory of Buhler AG: organic matter (ashes), total carbon, total nitrogen, C/N, Abbaugrad; Rottegrad (self-heating capacity). For further details, see Chapter 2.2.5.

3.2.4.2B Experiment V13

The experiment was started on February 10, 1995. The starting material consisted of organic kitchen (approx. 50 %) and garden waste (approx. 50 %) from bin collection from a small town. No wood chips were added because the material contained already too much structure material.

The composting procedure was the same as in experiment V12, except that aeration was also temperature controlled during the first week. Between day 7 and day 9, no temperature measurements were recorded because the logger was out of function. Samples for the microbiological tests were only taken in the center of the reactor, at 20 cm and 1 m from the bottom (sampling locations 3 and 4; Figure 71). No physico-chemical measurements or bioaerosol measurements were carried out.

Microbiological analyses: *Aspergillus fumigatus*; total molds and yeasts; Gram⁻ bacteria (on MacConkey Agar); coliforms (on Modified Tergitol-Agar); mesophilic and thermophilic bacteria. From the initial dilution of each sample, parallel dilution series were executed. The results shown are mean values of the two series, the error bars indicating the standard deviation (σ^{n-1}). For detailed methods, see Chapter 2.2.4.

3.2.4.3 RESULTS

3.2.4.3A Experiment V12

Physico-chemical measurements

Table 42: Physico-chemical measurements in 1 week old compost, in the bioreactor, after opening of the door, ventilation stopped, at a height of 1.3 m. nd = not determined.

depth horizontal [cm]	T [°C]	O_2 [vol %]	CO_2 [vol %]	CH_4 [vol %]
10	71	12.7	nd	0
30	67	3.5	nd	2.7
80	nd	10.7	nd	1.4

Some physico-chemical measurements were performed in the 1 week old compost directly in the bioreactor and in the windrow before and after turning.

High temperatures and aerobic conditions were present in the bioreactor (Table 42), with a small amount of methane produced by anaerobic bacteria in the poorly aerated zones located in 30 cm depth, where oxygen concentrations were quite low. The good oxygenation in 80 cm could be an indication for the existence of preferential air channels building up in the bioreactor.

Table 43: Physico-chemical measurements in 1 week old compost taken out of the bioreactor and put up in a windrow for turning. Measurements were performed laterally at a height of 0.5 m above ground. nd = not determined.

depth horizontal [cm]	T [°C]	O_2 [vol %]	CO_2 [vol %]	CH_4 [vol %]
30 minutes after removal from the bioreactor				
20	57	11.1	8	0
75	61	2.3	12	0.3
10 minutes after turning				
20	49	20.9	nd	0
75	51	15.1	10	0
30 minutes after turning				
20	49	20.7	nd	nd
75	51	7.8	17.5	0

The temperature of the compost mass, after putting it up in a windrow for turning, remained relatively high (57-61°C) (Table 43). By the turning, the temperature decreased about 10°C.
A marked increase of the oxygen content was observed 10 minutes after the turning: from 11 % at the surface, and 2.3 % in the center, it rose to 21 % and 15 % respectively. However, 20 minutes later, oxygen concentrations in the core of the windrow had decreased substantially, parallel to an increase of the CO_2 concentration, due to the high microbial activity. These measurements confirm the results obtained at other installations (see Chapter 3.2.1), and described in literature (HELM, 1995; MILLER *et al.*, 1989) that turning only leads to a momentarily oxygenation of compost. The outer layers were always sufficiently oxygenated.

The temperatures and gases were also measured in the 5 week old compost in the bioreactor (Table 44) at the locations depicted in Figure 70.

Table 44: Physico-chemical measurements in 5 week old compost, in the bioreactor, door closed, ventilation on, nd = not determined. Measuring sites see Figure 68.

	Site 1 = 20 cm from the bottom (measurement horizontal)				Site 2 = 80 cm from the bottom (measurement horizontal)				Site 3 = 30cm below the surface (measurement vertical)			
depth [cm]	T [°C]	O_2 [%]	CO_2 [%]	CH_4 [%]	T [°C]	O_2 [%]	CO_2 [%]	CH_4 [%]	T [°C]	O_2 [%]	CO_2 [%]	CH_4 [%]
10	20	20.8	0.04	0	nd	20.8	0.10	0	37	19.6	0.84	0
20	32	20.8	0.07	0	41	20.8	0.18	0.05	41	18.2	1.82	2
30	45	20.3	0.72	0.05	40	20.2	0.60	0.05	51	16.2	3.25	2
40	55	19.5	1.38	0.10	44	19.8	0.92	0.10	58	15.0	4.25	2
50	61	18.5	2.41	0.10	49	18.9	1.52	0.15	65	13.7	5.4	2
60	66	17.2	3.44	0.10	54	18.2	2.04	0.15	72	12.1	6.8	1
70	70	16.3	4.24	0.10	58	17.7	2.41	0.15	77	9.5	8.7	1
80	74	15.5	4.98	0.10	60	17.3	2.63	0.15	79	9.2	9	1
90	76	nd	nd	nd	62	nd	nd	nd	80	8.0	10	1
100	79	nd	nd	nd	63	nd	nd	nd	81	7.8	10	1
mean	57.8	-	-	-	52.3	-	-	-	64.1	-	-	-

Low temperatures (≤ 55°C) were present in the periphery (0-40 cm) of the bioreactor, with higher temperatures at the bottom (site 1) than in the middle of the bioreactor (site 2). The temperatures increased strongly (49-81°C) in the center (50 to 100 cm). Horizontal measurements revealed good aeration without production of CH_4. However, vertical measurements from the top (site 3), with air flow from top to bottom, showed zones with oxygen < 10% and elevated concentrations of methane, indicating the presence of anoxic zones. No formation of hydrogen sulfide (H_2S) was measured at any point (results not shown).

Figure 72 shows the temperature evolution, monitored by on-line measurements.

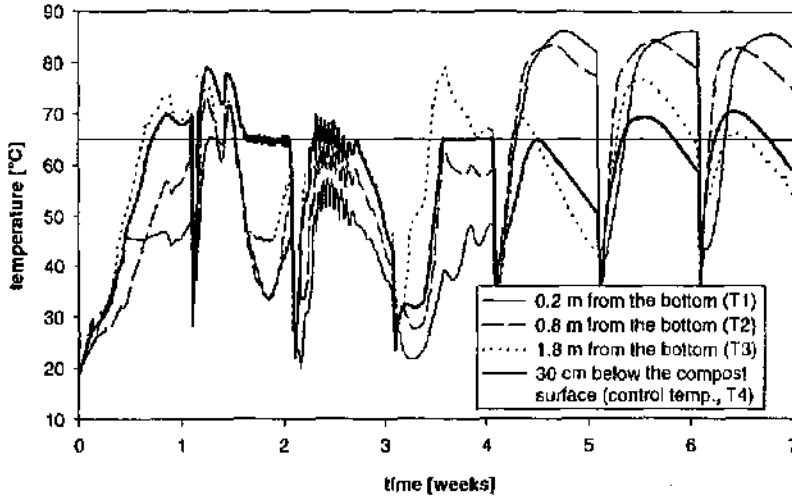


Figure 72: On-line temperature measurements with four sensors placed in the bioreactor. Temperature set point from the beginning of week 2 to the end of week 4: 65°C. Locations of the temperature probes see Figure 69.

During the first week, the rate of temperature increase was slow, and the temperature distribution in the mass heterogeneous (20°C to 45-75°C in 6 days). On the bottom of the reactor, at the point of air entry into the compost, temperatures were not higher than 45°C. After the first turning (on day 8) the temperature increased very rapidly, temperatures exceeding greatly the set temperature of 65°C at the control point (30 cm from the top). Only in the second half of week 2 could the temperature be lowered to 65°C at the top. However, due to the strong aeration, temperatures in the rest of the bioreactor decreased strongly to 35-45°C on day 13, being accompanied by an intensive drying of the material (see Figure 74).

The water added during the second turning generated a fast temperature rise in the whole composting mass (50-70°C), the temperature gradient between T3, which showed the same temperature as the control (T4, 65°C), and the bottom (T1) being only 15°C. Towards the end of the third week, another temperature drop was observed (35-45°C), this time in the whole compost. As the water content was in a range that allowed good composting (45-65 %, see Figure 72), the decreasing temperatures were most probably due to an exhaustion of easily degradable substances.

The forth week of the experiment was characterized by a delayed temperature increase (72 h to reach the desired temperature of 65° at the top of the bioreactor), also a sign that substrate depletion was occurring. Temperatures where higher at the measuring point situated at a height of 1.8 m, reaching up to 80°C, than at the control point (30 cm from the top). Due to the volume reduction of the composting material, the former was just below the surface of the compost, thus above the control temperature. Temperatures at the bottom (T1) did not exceed 50°C. The inversion of the aeration at the beginning of week 5 (sucking, from top to bottom) brought about also an inversion of the temperatures: the highest ones being now measured at the bottom (T1) of the bioreactor (> 80°C), whereas the compost at the top (T3 and T4) was 20-25°C colder. These very high temperatures in the whole composting mass were maintained until the end of the experiment (52-82°C for week 7).

The inversion of the hottest zone was similar to that observed by STENTIFORD *et al.* (1985) during the composting in forced aerated static piles; in the case of negative pressure, the highest temperatures were obtained near the aeration pipes, and with positive pressure near the apex.

Table 45: Evolution of the temperature in compost measured at different locations in the bioreactor prior to sampling. Sampling points: 1 = 20 cm from the bottom, periphery; 2 = 1 m from the bottom, periphery; 3 = 20 cm from the bottom, center; 4 = 1 m from the bottom, center (see Figure 71).

	sampling point				
week	1	2	3	4	mean
1	56	70	53	63	61 ± 8
2	41	62	52	64	55 ± 11
3	33	41	35	35	36 ± 3
4	47	57	57	66	57 ± 8
5	32	41	79	63	54 ± 21
6	48	45	84	69	62 ± 18
7	56	39	83	56	59 ± 18
mean	45 ± 10	51 ± 12	63 ± 19	59 ± 11	

Table 45 shows the temperatures of the compost at the moment of sampling. The temperatures measured in the center of the bioreactor correspond to those measured on-line at the sampling time. Those at the periphery were measured manually by the person taking the samples, prior to sampling.

The temperatures at the periphery (measuring and sampling point 1 and 2) were generally *lower than in the center, rarely reaching 60°C.* This was especially true for the second half of the experiment (weeks 4 to 7). This corroborates the punctual measurements shown in Table 44. It has to be considered, though, that the bioreactor was not insulated. The horizontal temperature gradient would be lesser in the flattop windrow that the bioreactor system is supposed to simulate, because of the much larger dimensions of the windrow (80 m long x 20 m large). However, the temperature gradient observed in the vertical direction, commented in Figure 72, would also build up in the flat-top windrow, with temperatures differences between the top and the bottom as much as 40°C.

Microbial degradation of organic matter results in O_2 consumption and CO_2 production, the latter being a mirror image of the former, as shown in Figure 73.

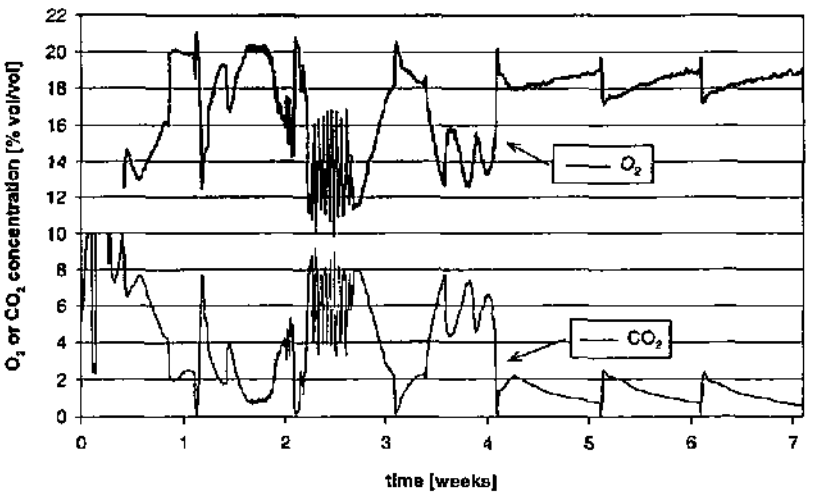


Figure 73: Carbon dioxide and oxygen concentration in the vitiated air.

Unfortunately, due to instrument failure, no O_2 measurements could be carried out during the first phase of the experiment. The high CO_2 concentration, exceeding 10 % (upper limit of detection) are an indication of very intense microbial activity. In the further course of the composting process, the gas concentrations showed an evolution similar to that of the temperatures: when temperatures were high as a result of good microbial activity, CO_2 concentrations were elevated (between 4-6 % in the air exiting the bioreactor), and O_2 concentrations low, respectively. Also, turning was each time strongly stimulating the microbial activity. When temperatures fell, either due to an excessive drying of the composting material, or to depletion of easily degradable substances, could this be observed by gas concentrations approaching those of normal air. The fluctuations in the third and the fourth week were caused by specific aeration cycles, and were reflected also in the measured temperatures, the aeration leading to a cooling of the composting material.

No explanation could be found for the very high temperatures during the second half of the composting process, during sucking aeration (Figure 72): the gas measurements showed in fact only very little microbial activity. Of course, the gas concentrations in an aerated system are a function of the airflow. Calculations of kg CO_2 emitted per kg compost, taking into account the amount of air passing through the compost, showed a reduction of CO_2 production from 1-2 kg/kg compost during the first 4 weeks of the experiment, to 0.15-0.3 kg/kg compost during the second half. This would corroborate the theory that very high temperatures are inhibitory to microbial degradation processes (FINSTEIN & MILLER, 1985). However, it is unclear how these high temperatures could have been generated in the first place: composting mass reduction, volume reduction, organic matter reduction (from 68 % [DW] in the starting material to 54 % by week 4, to a final percentage of 51 %), and Rottegrad all indicated that the degradation was to a large part terminated after 1 month of composting. The little air flow (10 $m^3/h \cdot t_{fresh\ weight}$), together with the quite low water content (35-45°C), preventing heat storage in form of latent heat in the water fraction, might have lead to the elevated temperatures measured in the compost.

Table 46: Evolution of the pH in compost samples taken at different locations in the bioreactor, as well as in the material after sampling. Sampling points: 1 = 20 cm from the bottom, periphery; 2 = 1 m from the bottom, periphery; 3 = 20 cm from the bottom, center; 4 = 1 m from the bottom, center (see Figure 71). 5 = after turning.

	sampling point				
week	1	2	3	4	5
0	4.6	4.6	4.6	4.6	4.6
1	7.6	7.3	5.6	5.9	6.7
2	8.3	7.9	8.1	6.4	8.2
3	8.6	8.8	8.7	8.9	8.7
4	8.3	8.7	8.8	8.7	8.4
5	8.8	8.7	8.7	9.1	8.5
6	8.5	8.8	8.7	8.9	8.4
7	9.0	8.5	8.7	8.7	8.7

The pH (Table 46) increased strongly during the first 2 weeks from 4.6 to about 8, more slowly during the 3rd week (from 8 to 8.5-9) and remained constant thereafter.

After the first week, samples taken at the periphery showed significantly higher pH values (7.3-7.6) than those at the center (5.6-5.9). Taking into consideration that the temperatures after 1 week of composting measured at the periphery (56-70°C) compared to the center (53-63°C) were also slightly higher (Table 45), it seems that the degradation process in the first two weeks was faster at the periphery than in the center of the bioreactor. This might be due to a better oxygenation, the air passing preferentially along the wall, where the compost was less compressed than in the center of the reactor.

The water content (Figure 74) of the fresh organic material was relatively high (72 %) but not unfavorable to microbiological activity.

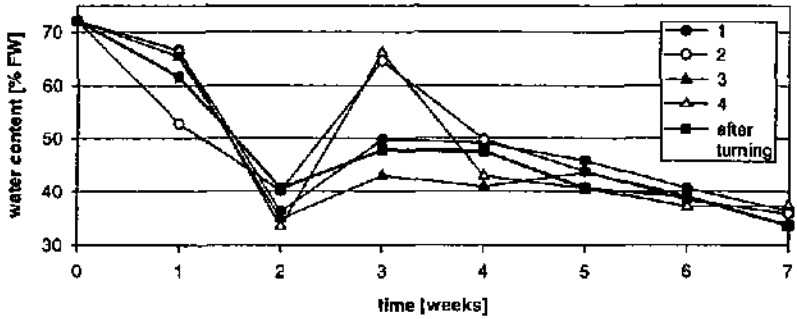


Figure 74: Evolution of the water content (in %) in compost sampled at different locations in the bioreactor, and after turning. Sampling points: 1 = 20 cm from the bottom, periphery; 2 = 1 m from the bottom, periphery; 3 = 20 cm from the bottom, center; 4 = 1 m from the bottom, center (see Figure 71).

The loss of water was very important during the first 2 weeks (72 % to 33-41 %), most probably due to the intensive aeration. Water addition was thus necessary after the second week of composting, leading especially at the top to an again sufficiently humidity; while at the bottom, where the air inlet was, the material dried quickly. The high temperatures during the second half of the composting process effected a decrease of the water content to 34-37 % in the whole mass at the end of the experiment (periphery, center, and after turning) by evaporative cooling. The water content of the material after turning, being closer to the material extracted at the periphery, indicates that conditions in the reactor were in most part rather dry.

Microbiological analyses

IN THE COMPOST

AF numbers (Figure 75) were very high (approximately 10^7 cfu/gDW) in the starting material, and represented about 10-20 % of the total molds and yeasts. The concentration of *AF* in proportion to the total molds increased with the duration of the experiment, so that at the end *AF* represented more than 50 % of the total molds. Yeast numbers were particularly high (more than 50 % of the total fungi) in the fresh material, and disappeared rapidly during the second week of composting (results not shown).

The *AF* concentration was strongly diminished both in the center and in the periphery during the first week of the composting process, even at the bottom, where temperatures did not exceed 45°C. The low pH (between 5.6 and 5.9 in the center, see Table 46) should not affect molds, as they grow normally best in a pH-range of 4.5-6.5 (REISS, 1986).

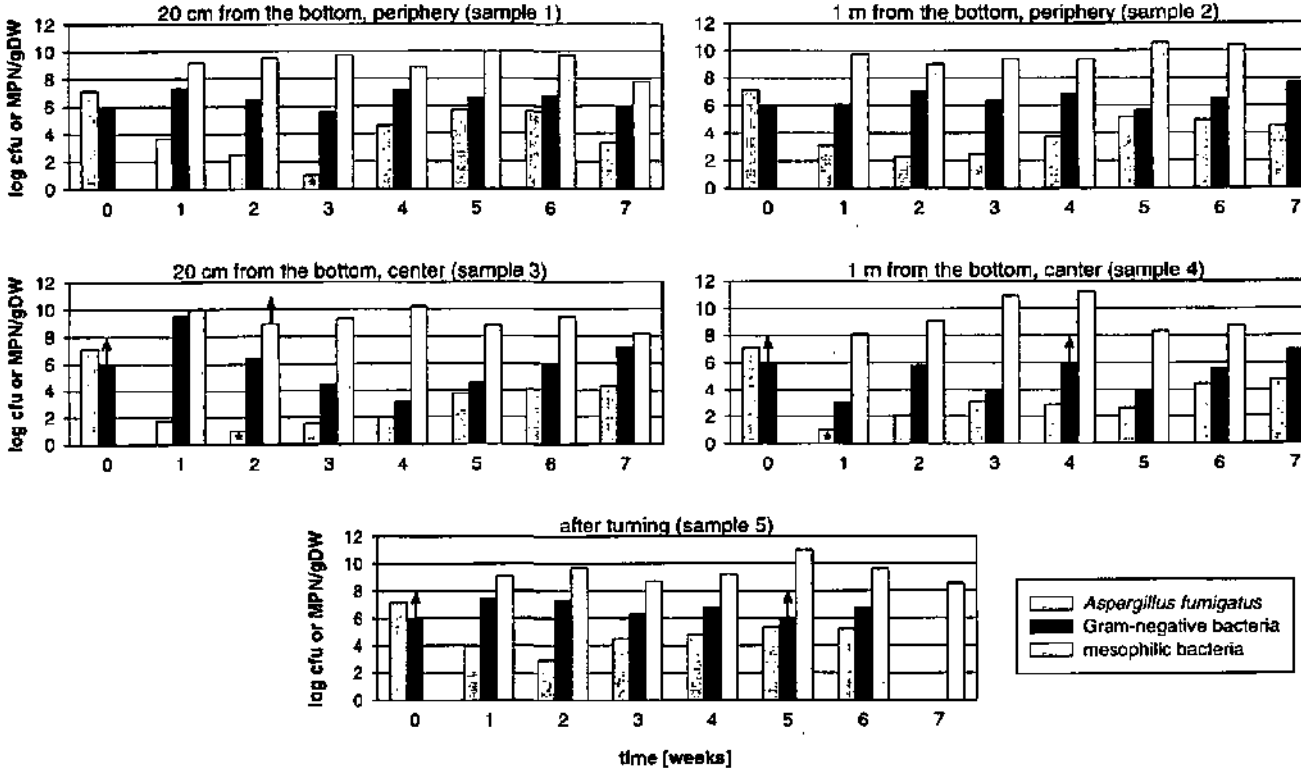


*Figure 75: Evolution of the concentration of Aspergillus fumigatus, Gram⁻ bacteria and mesophilic bacteria measured in compost sampled at different locations in the bioreactor, and in the compost after turning. Bars with an * indicate results below the detection limit (10 cfu/gFW), those with a ↑ above the detection limit.*

The presence of yeasts, which are favored at low pH, competing with the molds, may explain better the rapid disappearance of *AF*. Up to the 4th week of the experiment, *AF* numbers stayed below 10^3 cfu/gDW for all the samples taken in the bioreactor. However, the higher concentrations (up to 10^4 cfu) measured in the compost after turning hint at the presence of zones where the thermohygienization was less marked. From the 4th week on, all the samples showed fungal recolonization, in spite of the very high temperatures (up to 80°C at the bottom of the reactor) measured from the 5th week on, when aeration was in the sucking mode. The quite low water content might have had a protective effect against thermal inactivation. MILLNER *et al.* (1977) had reported in a quite dry compost sample (28 % water content) taken at 63°C *AF* concentrations of $2 \cdot 10^3$ cfu/gDW, while samples exposed to the same temperature, but having a moisture content of > 45 %, yielded no *AF* growth.

The number of Gram⁻ bacteria (Figure 75) was relatively high in fresh organic material ($4\text{-}5 \cdot 10^6$ cfu/gDW), and represented 0.01 to 1 % of the total mesophilic flora. At the periphery, their concentrations remained constant during the whole duration of the experiment ($7 \cdot 10^5\text{-}7 \cdot 10^6$). In the center, the temperatures above 60°C reached at the top in the beginning of the composting process reduced their numbers markedly, while at the bottom, where the temperatures attained were inferior to 50°C, a proliferation of Gram⁻ bacteria was observed. In the course of the composting process, their numbers varied between $1 \cdot 10^4$ and $1 \cdot 10^6$ cfu/gDW at the top, and between $1 \cdot 10^3$ and $1 \cdot 10^4$ cfu/gDW at the bottom, except for the last week of the experiment, where a slight recolonization of the compost was observed.

Compared to *AF*, Gram-negatives were always present at higher concentrations. Their numbers were also less rapidly reduced at the beginning of the composting process. Research by Droffner and co-workers (DROFFNER & BRINTON, 1996; DROFFNER *et al.*, 1995a and b; BRINTON & DROFFNER, 1994; DROFFNER & YAMAMOTO, 1992a, b and 1991) demonstrated by the use of gene probes the presence of Gram⁻ bacteria (*E. coli*, *Serratia marcescens*, *Citrobacter freundii*, *Klebsiella pneumoniae*, *Enterococcus gallarium*, *Enterobacter cloacae*, *Pseudomonas aeruginosa*, *P. pseudoalcaligenes*, *P. alcaligenes*, and *Alcaligenes faecalis*) in active (50-70°C) composts. They presumed that some mesophils have mechanisms for survival and perhaps replication at elevated temperatures. The thermotolerance seemed not only to be caused by phenotypic adaptations to the thermal environment (production of heat shock proteins), but by mutations, involving the expression of a cellobiase and a gyrase, being involved in the DNA supercoiling, and thus the expression of a large number of genes. The ability to hydrolyze cellobiose, an intermediate of the cellulose degradation, could be a selective advantage in a thermogenic compost environment.

Research carried out in the scope of our project (GARCIA, 1998) about thermotolerant coliforms in compost failed to isolate any coliforms on the medium used (Chromocult Coliform Agar, CCA, composition see Chapter 2.2.4). However, strains of *Pseudomonas putida* were isolated that grew up to 65°C. Normally, the accompanying flora (Pseudomonads, Aeromonads) is suppressed by the addition of cefsulodine (5 mg/l) to the CCA, but the thermotolerant *P. putida* strains showed to be resistant to this agent.

The number of mesophilic bacteria (Figure 75; total count in Nutrient Broth at 30°C) was extremely high during the whole duration of the experiment ($6 \cdot 10^7\text{-}10^{11}$ MPN/gDW). Higher concentrations of these bacteria were measured when the temperatures of the compost were between 30°C and 50°C. The persistence of mesophils was most probably due to the fact that *Bacillus*, very abundant in compost (BLANC, 1998) can form spores, which are resistant to high temperatures. The high concentration of total mesophilic and Gram-negative bacteria in the whole mass of compost was clearly shown by the sampling after turning.

Thermophilic heterotrophic bacteria (results not shown) were present at a relatively high concentration ($6 \cdot 10^7$ MPN/gDW) in the fresh organic waste. Their concentration increased, parallel to the increase of the temperature, during the first week, and remained constant thereafter at a level of $10^8\text{-}10^{10}$ MPN/gDW for all samples.

IN THE AIR

The measurements of airborne microorganisms were carried out after 1 week and after 5 weeks of composting

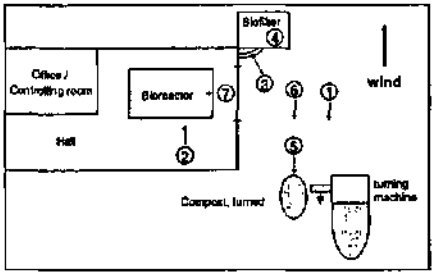


Figure 76: Plan of the composting site, numbers indicating the points where the dispersion of bacteria was measured, arrows indicating the direction of the measurement.

The background noise concentration in the middle of the composting place (without human activities) was relatively low (Table 47), but about 10 times superior to those detected far from composting sites. This pollution was certainly due to the activity on the open-air windrow site, situated right next to the place where the measurements were carried out. For the 1 week old compost, the highest concentration of airborne molds was measured in the hall where the bioreactor stands.

Table 47: Dispersion of Aspergillus fumigatus (AF) and total thermotolerant molds and yeasts (TTM) during various activities, and without activity (bgn = background noise). The numbers correspond to the sampling sites indicated in Figure 76. ∞ = plates completely overgrown, not countable.

No	sampling site	activity	*AF*/m³	TTM/m³
age of compost: 1 week				
2	hall, door closed, 2 m from the reactor	ventilation	$2.4 \cdot 10^3$ - ∞	$2.9 \cdot 10^3$ - > $3.3 \cdot 10^4$
3	air outlet, before the biofilter	ventilation	70	80
4	air after biofilter, 50 cm from the surface	ventilation	50	100
5	during turning, at 2 m, downwind	turning	220	290
age of compost: 5 weeks				
1	middle of place, towards heap	bgn	30	60
5	ditto, putting in windrow with front end loader	putting in windrow	$4.0 \cdot 10^3$	$4.7 \cdot 10^3$
7	during turning, at 1 m, downwind	turning	∞	> $3.3 \cdot 10^5$
8	manual sampling in the bioreactor, with a fork	sampling	∞	> $6.6 \cdot 10^4$

This confirms results from literature that air contamination with microorganisms inside buildings is usually higher than outdoors, due to a reduced air exchange (MARCHAND *et al.*, 1995; CLARK *et al.*, 1983, MARK, 1992). However, concentrations were very low compared to windrow or box composting in closed buildings (see Chapters 3.2.3 and 3.2.5). Investigations by NERSTING *et al.* (1991) about the emission of bacteria, fungi, endotoxins and dust during composting in a indoor reactor showed also only low concentrations of total microorganisms ($5 \cdot 10^2$ to $3 \cdot 10^3$ cfu/m³ air) and endotoxin (0.11-3.49 ng/m³).

Slightly elevated concentrations were measured during the outdoor turning of the compost in 2 m distance to the turning machine. As the machine moved forward quite slowly, taking up the material up front, and dropping it laterally, thereby forming a new windrow, mechanical manipulation was less intense than with the machine used at the windrow composting site (see Chapter 3.2.1.3B), reducing mold spore emission.

The higher concentrations detected after the biofilter indicate a colonization of the filter, confirming the statement of the operation manager that it was not working properly.

For the 5 week old compost, the back ground noise level was about the same, but the concentration during the turning was a lot higher, in parallel with the higher concentrations detected in the compost (see Figure 75).

3.2.4.3B Experiment V13

Physico-chemical measurements

The temperature rise (Figure 77) at the beginning of the experiment was a lot faster and more homogenous than in V12: Temperatures > 60°C were already reached after 50 hours of composting, while in V12 it took four days, at the hottest spot (top) to reach this temperature (Figure 72).

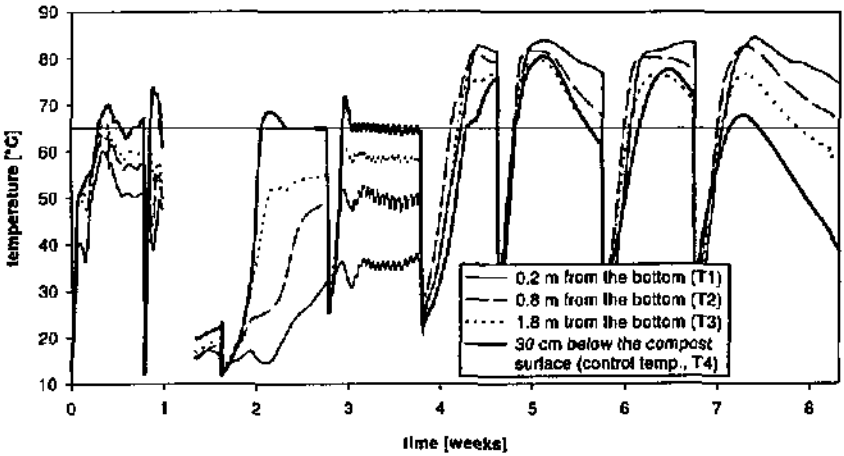


Figure 77: On-line temperature measurements with 4 sensors placed in the bioreactor.

This was most probably due to the different aeration modes: airflow was constant during the first week in V12 (10 m^3 fresh air and 30 m^3 recycled air/t·h), but the compost in V13 had already aerated from the beginning on in the temperature control mode, resulting in a lesser air flow, and thus lesser cooling. The high temperatures were maintained in the first week almost in the whole mass, except at the bottom (T1), where it decreased slightly to 50°C.

After the first turning, a fast temperature rise was observed, but then temperatures declined rapidly to 15-20°C, due to a defect of the temperature control system, resulting in a too strong aeration. Although aeration was completely stopped for three days, no re-heating occurred; however, a drying of the material could be prevented (see Table 48).

After the second turning, and watering of the compost, the temperatures rose again, but very slowly: it took three days for the compost at the top of the bioreactor (T4) to reach the control temperature of 65°C. This temperature was only reached in the very top layer. At 1.8 m (T3), the temperature was 55°C, and the temperatures at the bottom (T1) of the reactor did not exceed 30°C.

In the fourth week, slightly higher temperatures were recorded (T1: 35°C, 1.8 T3: 60°C), although they were in general 5-10°C lower than those at the same stage of composting in V12.

From week 5 on, under inverse aeration, the temperature evolution was similar to that in V12. Very high temperatures (80°C) were maintained in the whole mass for 3 weeks. Only in the last week (week 8) did a significant temperature drop occur, in contrast to V12, where this had already happened in week 7. The late temperature decline was most probably connected to the delay of the degradation process in the second week. Other results (mass reduction, degree of degradation, organic matter content; results not shown) also indicated almost no changes of the material during the first two weeks of the composting process.

Table 48 presents the temperatures read at the sampling zones prior to sampling.

Table 48: Evolution of the compost temperature measured at 20 cm and 1 m from the bottom of the bioreactor prior to sampling, and of the pH measured in the samples taken at the same points were the temperature measurements had been carried out.

weeks	0	1	2	3	4	5	6	7	8
temperature									
20 cm from the bottom (sample 3)	25	52	15	32	37	81	77	84	66
1 m from the bottom (sample 4)	25	61	20	55	58	77	82	71	57
pH									
20 cm from the bottom (sample 3)	5.15	7.55	8.02	7.72	8.28	8.60	8.43	8.08	8.58
1 m from the bottom (sample 4)	5.15	7.82	8.17	7.97	8.30	8.39	8.23	8.66	8.69

Temperatures were for the first 4 weeks higher at a height of 1 m than at 20 cm. Later, from the moment on that the direction of the aeration was inversed (from top to bottom) higher temperatures were observed at 20 cm.

The pH increased strongly during the first week (5.2 to about 8), and slowly thereafter (8.6 at the end). The pH in the compost taken at 20 cm from the bottom of the bioreactor rose slightly slower during the first four weeks than in the one taken at 1 m. It seems that the higher temperatures measured at 1 m (55-60°C) effected a faster degradation. This corroborates the observations made for V12.

Table 49: Evolution of the water content (in %) in compost sampled at the top and at the bottom of the bioreactor. Mean values and standard deviation (σ^{n-1}) are presented. In brackets: no. of 10 gram samples taken.

week	20 cm from the bottom (sample 3)	1 m from the bottom (sample 4)
0	59.8 ± 2.8 (10)	59.8 ± 2.8 (10)
1	40.2 ± 1.6 (5)	50.0 ± 0.8 (5)
2	55.6 ± 1.3 (5)	53.5 ± 2.1 (5)
3	60.0 ± 4.2 (5)	53.3 ± 0.6 (5)
4	57.2 ± 0.4 (5)	53.6 ± 1.8 (4)
5	57.5 ± 0.9 (5)	57.9 ± 0.9 (5)
6	55.2 ± 0.4 (5)	56.9 ± 0.6 (5)
7	52.3 ± 0.7 (6)	48.9 ± 1.1 (5)
8	46.6 ± 1.3 (6)	48.7 ± 0.9 (5)

The water content (Table 49) remained relatively constant during the whole experiment (60 % of water at the beginning to 45-50 at the end), and was at any moment sufficient for a correct composting process. The material was always slightly drier when temperatures were elevated (during the first two weeks at the top, then at the bottom).

To test the homogeneity of the samples regarding their moisture content, 5-10 10 g samples were taken and dried according to the standard procedure (see Chapter 2.2.5). The results showed a standard deviation that varied between 1 and 7 %, the biggest differences, with one exception, being observed in the starting material. The homogeneity of the material being considered sufficient, one 100 g sample was used for dry weight determinations in further experiments.

The differences observed for various other parameters between the two experiments might be due to the different starting material: this contained in V13 more garden waste, resulting in a higher initial organic matter content (73 % in V13 versus 68 % in V12), and pH. However, the less important total OM reduction (12 % in V13 versus 18 % in V12) reached at the end of the experiment showed that part of this organic matter was only difficult to degrade. The higher percentage of woody material led also to an inferior CO_2 production (kg CO_2 x kg compost^{-1} x h^{-1}) compared to V12 (results not shown).

Microbiological analyses

The initial concentration of *AF* (Figure 78) in the starting organic material ($2 \cdot 10^5$ cfu/gDW) was almost two orders of magnitude lower than in V12. This might be due to the higher amount of garden waste in the initial mixture. The fast temperature rise during the first week up to 65°C (Figure 77) reduced the number of *AF* at the top of the bioreactor to $1 \cdot 10^3$ cfu/gDW. At the bottom, however, where temperatures did not exceed 60°C during the first four weeks, their number continued to increase. The same was observed at the top, where 60°C were reached again only at week 3. During the second half of the experiment, the concentration of *AF* began to decline in the whole compost mass, though more strongly at the bottom, where higher temperatures (> 80°C) were measured.
When comparing the evolution of *AF* in V12 and V13, it seems that the generally higher temperatures in V12 had a better hygienization effect.

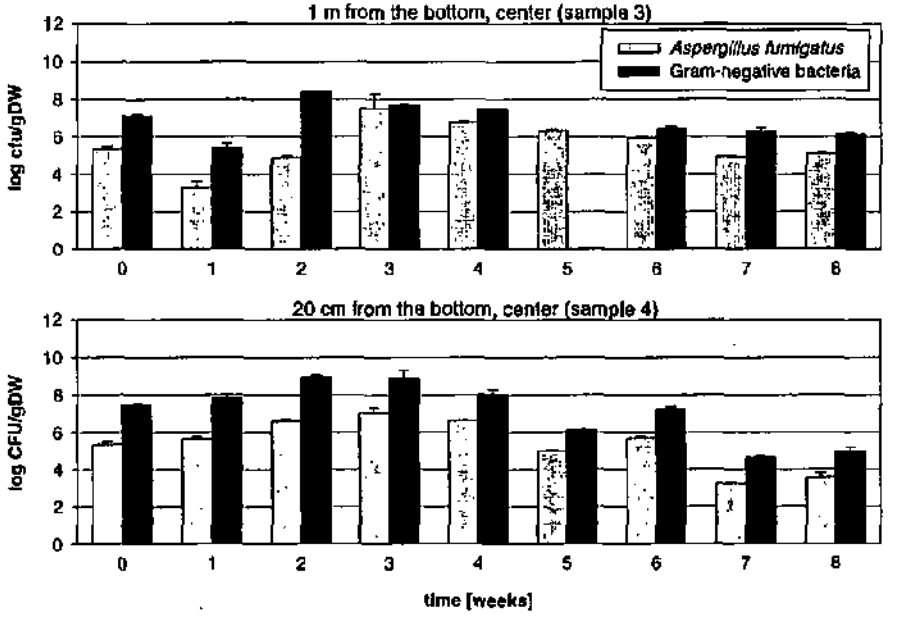


Figure 78: Evolution of the concentration of Aspergillus fumigatus and Gram⁻ bacteria measured in the center of the bioreactor. Error bars are the standard deviation of replica dilution series.

The evolution of Gram⁻ bacteria followed closely that of *AF*, but their concentration was always between 0.5 to 2 order of magnitudes higher than the latter. The results of the fecal coliforms determined on Modified Tergitol Agar, after incubation at 44°C, are not shown. In fact, this presumably selective medium for coliforms showed to support the growth of high numbers of non-coliforms: of 7 colonies, tested after 24 h of incubation, that showed the red color typically of coliforms, only 1 was oxidase-negative, thus presumably a coliform. After a further 24 h of incubation, 9 out of 14 colonies were non-coliforms. Identification of the strains with the API 20E system showed the presence of the coliforms *E. coli*, *K. pneumoniae*, *K. oxytoca*, *P. mirabilis*, *Enterobacter cloacae*, *E. agglomerans*, and with the API 20NE system, the non-coliforms *Pseudomonas ssp.* *Serratia liquefaciens*, *Chromobacter violaceum* and *Flavobacterium multivorum*. No identification of the other oxidase-positive strains was possible.

Thermophilic heterotrophic bacteria (THB, Figure 79) were present at high concentrations ($2 \cdot 10^6$ MPN/gDW) in the fresh organic waste at the beginning of the composting process, similar to the results in V12 (results not shown). THB numbers increased strongly (from 10^8 to 10^{10} MPN/gDW) during the first week of composting. In spite of the relatively low temperatures measured in the compost (16-60°C), their number remained constant during the weeks 2 to 4, and decreased thereafter slowly to reach approximately the initial values at the end of the experiment. The thermogenic phase (60-82°C) of the last three weeks did not favor the proliferation of this group of bacteria. Identifications showed that at an incubation temperature of 60°C, thermophilic heterotrophic *Bacilli* were the dominant genus, while at higher incubation temperature (75°C) bacteria related to the genus *Thermus* could be isolated. In moderately thermogenic (< 60°C) composts of V13 (first weeks) the number of bacteria related to *Thermus* was low (< 10^5 cells/g compost), while their numbers increased strongly (10^9- 10^{10} cells/g compost) at the end of the process, where high temperatures (65-85°C) were measured (Beffa *et al.*, 1996).

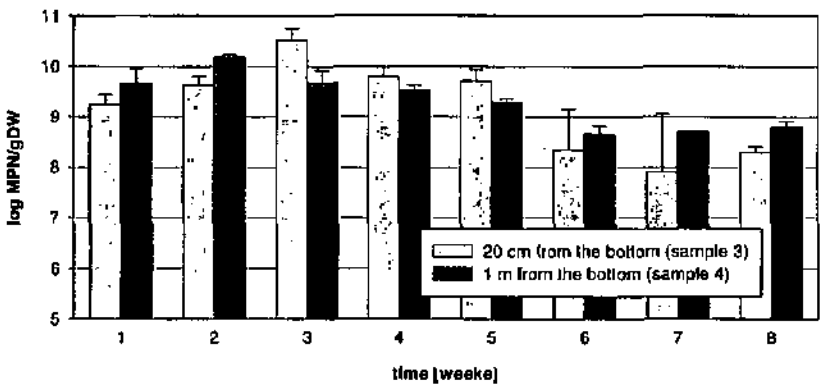


Figure 79: Evolution of thermophilic heterotrophic bacteria during composting in a bioreactor. Error bars are the standard deviation of replica dilution series.

3.2.4.3C Assessment of the performance of the composting system

For both experiments (V12 and V13) two distinct phases of the composting process could be observed: a first phase (week 1-4) with temperature control at 65°C, with blowing aeration from bottom to top and a second phase (week 5 to the end of the experiment) with no temperature control, but a fixed aeration cycle, with sucking aeration from the top to the bottom.

Phase 1 was characterized by rapid, but inhomogeneous temperature increase, temperatures being up to 50°C higher at the top of the bioreactor than at the bottom. STENTIFORD *et al.* (1985) observed in piles aerated with positive pressure the highest temperatures near the apex. A horizontal temperature gradient was also observed, the periphery being much cooler than the center, due to heat loss by radiation, but also to the possible existence of preferential air channels along the wall of the bioreactor. Because of the temperature control by the aeration system, temperatures hardly exceeded 70°C. In both experiments, the composting was slowed down at one moment (V12: week 3, V13: week 2), most probably due to a too strong dehydration and cooling down of the composting material by a surplus of air. Under these inhomogeneous conditions, the destruction of potential pathogens was not assured. The very low temperatures (30-50°C) even allowed the proliferation of indicator bacteria and fungi. The most important physical (mass and volume reduction) and chemical (pH, organic matter, Rottegrad, C/N ratio) changes of the compost material happened during this first phase of the process.

Considering the important temperature gradient and the problems with desiccation, the question arises if it would not be advisable to augment the set point of the temperature control to 70°C. This would lead to a lesser air demand of the system, thus to lower energy consumption, but also to lesser drying of the compost at the bottom part of the bioreactor, and thus to higher temperatures in the lower part of the reactor.

The second phase showed a more homogenous temperature distribution in the bioreactor, although the compost at the bottom of the reactor was hotter than that at the top. This confirms the observation of STENTIFORD *et al.* (1985), who detected, in the case of negative pressure compost aeration, the hot zone in the lower part of the pile, near the aeration pipes. Temperatures in this second phase were considerably higher (70-85°C), most probably due to the diminished aeration that allowed maintaining oxic conditions, but effected no cooling of the material. By the activity of the aerobic microflora, activated by the turning (redistribution of nutrients and water), temperatures rose quickly, accompanied by an augmentation of the CO_2 production (kg CO_2/kg compost). However, the very high temperatures reached seemed to promptly lead to a self-limitation of the microbial community, translated by a temperature decline and a drop in CO_2 production. Microbial activity did not resume, only re-mixing of the material could provoke a new temperature increase. Suppositions are that the availability of easy degradable substrates was the limiting factor for growth of thermophilic organisms. In this context, the polymer (hemicellulose) degradation potential of the highly thermophilic genus *Thermus*, present in very hot composts in high numbers, is the subject of research at our laboratory (PIERRE-FRANÇOIS LYON, personal communication). Also, the importance of a temperature gradient for the composting process was investigated, e.g. the cleavage of polymers and/or the production of enzymes in the colder zones, and consecutive consumption of the monomers by the thermophilic flora, or the polymer hydrolysis by thermostable enzymes in the hot zone. A prerequisite for this would be of course a mixing of the material. The experiments V12 and V13 showed clearly the importance of turning, not only to redistribute nutrients, and to break up preferential air channels in the composting mass, but also to allow rehydration of the compost. More frequent turnings at the beginning of the process, at the moment of strongest aeration, would allow to better control compost humidity.

In spite of the elevated temperatures, the thermo-hygienization towards potentially pathogenic microorganisms was only partial. Normally, temperatures of 65°C-75°C should be sufficient to eliminate or to greatly diminish the concentration of potentially pathogenic microorganisms (HAUG, 1993). As we did not take the samples, a possible contamination during sampling might have happened. Another explanation could be the formation of thermotolerance or thermoresistance in otherwise mesophilic organisms (see discussion of V12 and V13 about coliforms and Gram⁻ bacteria, and Chapter 3.1.4.2 about the thermoresistance of spores). The observed killing effect of high temperatures on mesophilic microorganisms at the beginning of the composting process, but no more towards the end, seems to confirm this "Mutant Theory". Is therefore very important to reach high temperatures (65°C to 70°C) very fast at the beginning of the composting process, in order to avoid that such thermotolerant populations can form. STENTIFORD *et al.*, (1985) proposed for best pathogen inactivation in a static compost pile a hybrid aeration system with an initial negative pressure period producing high core temperatures. At the moment when the set temperature (60-65°C) can no longer be hold, aeration should be reversed, causing the high temperatures to move towards the outer layers. Alternative blowing and sucking aeration might also lead to a vertically more homogenous temperature distribution.

The aim of our participation in the experiments, contrived by the responsible persons of Buhler AG, being to provide some information about the microbial quality of the compost, we had no influence on the experimental arrangement, nor did we had access to data from other experiments or to some proprietary information concerning the temperature control program. Collaboration with Buhler AG ended after these two experiments described above, because they did not continue with the same type of starting material. There was thus no possibility to test some of our propositions for a modified composting process.

The bioreactor was a model at pilot-scale to test the influence of different composting parameters (type of starting material, aeration regime) on the behavior of the composting system and the quality of the compost produced. No information was available how closely the conditions of the large-scale system could be reproduced in the pilot-scale reactor. Certainly, horizontal temperature gradients would be less important if a larger volume would be composted. On the other, the mixing of the material would probably be less intense, as it is left more or less in place. Also, emission of spores, at least at installations that are not in a completely closed hall, would be more substantial. In the case of treatment of the vitiated air, the performance of the biofilter concerning microorganism retention should be examined.

3.2.5 COMBINED METHANIZATION AND BOX COMPOSTING

3.2.5.1 DESCRIPTION OF THE INSTALLATION

The composting and methanization site "Allmig" is situated in the agglomeration of the town of Zug in a special zone (composting zone), about 500 m from the next housing area. The site was opened in 1987, and until march 1994, compost was produced in big flattop open-air windrows. The shredding machine and part of the stocking area were roofed.

The new part of the installation that was put in operation during summer 1994 (Figure 80) allows the combined composting of structured garden and park waste in boxes, together with pre-methanized kitchen waste. The biogas that results from the fermentation is burned in a co-generation power plant. This produces enough electricity (1/3) and heat (2/3) produced to cover the entire power demand of the installation. Of the 18'000 tons of biodegradable waste from 14 communities (about 110'000 inhabitants) treated annually, 6'000 tons are wet kitchen waste that is suitable for methanization (data from 1993). 85 % of the compost produced is used in agriculture.

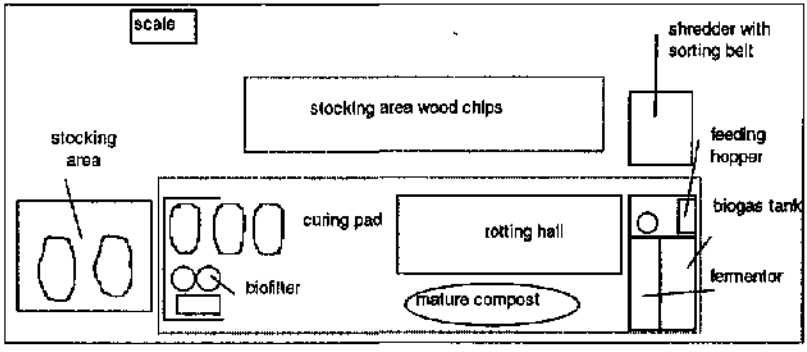


Figure 80: General view of the composting installation Allmig (--- roofed areas).

The source separated biodegradable waste is, prior to the passage through the shredder, fractionated into a wet (➔ methanization) and a woody (➔ composting) component. Before and after shredding, foreign materials are sorted out by hand. The shredded material is poured in the feeding hopper, and from there conveyed to either the fermentation tank or the rotting boxes.

Methanization: The wet waste gets shredded a second time prior to entering the fermentor, where the material stays 18-20 days. The horizontal fermentor has a volume of 450 m^3. 20-30 m^3 kitchen waste can be added daily, and about the same amount of fermentor sludge can be extracted. The methanization takes place at 54-57°C. In the starting phase, the produced biogas contained 50-55 Vol% methane and 45-50 % CO_2, during continuos operation later on, the methane content was raised to 60-68 Vol%.

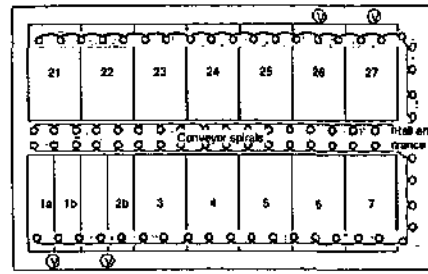


Figure 61: Schematic of the closed rotting hall. Number = box number; V = aeration.

Composting: The composting takes place in boxes, which are in a completely closed hall (see Figure 81). The total 14 boxes are arranged in two rows. Each row has its own filling and emptying system. Two boxes on each row (no 1, 2, 26 and 27) are equipped with an aeration system (positive (blowing) or negative (sucking) pressure). Each box (12 m long, 5 m large, 3 m high) contains 180 m^3 compost, which is composed of 150 m^3 garden and park waste and 30 m^3 methanized kitchen waste.

The filling, turning and emptying of the boxes takes place automatically with a system of conveyer spirals. For turning, the lowest compost layer gets transported out of the box by means of hydraulic scrapers, the material gets mixed, if necessary water is added, and is then filled on top of the heap. In function of the density of the composting material, a layer of 50-100 cm gets mixed per turning. Each box is turned 2-3 times per week. The air in the rotting hall is warmed with the exhaust air of the co-generation power plant. The waste air of the rotting hall is cleaned in a biofilter (counter-current sprinkler system).

After a composting time of 4-5 weeks, the compost is evacuated from the hall, and piled in big heaps for curing during three weeks. During this time, the material is turned once a week with a front-end loader. At the end of the curing time the compost is screened to different sizes, dependent on its end use (agriculture, horticulture (mixed with soil).

All process steps during methanization and composting are computer controlled. Temperatures (1 temperature probe per box, 1 m from the ground, 1.2 m long), air humidity in the rotting hall and compost material movements (filling, emptying, watering, aeration, addition of fermentor material) are monitored on-line. All data is registered, and can be printed out for control.

The installation was classified as a pilot and demonstration site. Under its supervision, the mass and energy balance of the combined system were drawn up during the first two years of operation, operational data got collected, and quality analyses of the end product as well as assessment of the odor emissions in the vicinity of the site were carried out. These analyses were continued without help from the SDE for the following years of operation by the operator himself.

3.2.5.2 FIRST EXAMINATION (OCTOBER 1994)

3.2.5.2A Experiments carried out

The objective of the first examination of the installation carried out during October 1994 was to obtain first data about the quality of the composting process and that of the end product. The microbiological and physico-chemical analyses should allow a judgment of the process, and serve as base in view of future improvements. During this first inventory, the fermentor was not functional yet; the installation was in operation only since 4 months.

Table 50: Measuring and sampling points.

no	samples	box no	depth (cm)
1	1 week	27	100
2	2 weeks	1b	20
3	2 weeks	1b	80
4	3 weeks	21	0
5	3 weeks	21	10
6	3 weeks	21	50

Physico-chemical measurements: the following parameters were measured in composts of different maturity (see Table 50), and at different depths (0 to 300 cm) of the heaps: temperature, O_2, CO_2, CO, CH_4, H_2S. The measurements were made at a distance of 2 m to the middle aisle of the rotting hall, in the middle of the box. As the boxes were not filled very regularly with compost, care was taken to execute the measurements not in a indentation or on a hill, in order to get data that would be representative of the whole box. Measuring devices were as described in Chapter 2.2.1.1 and 2.2.1.2.

Bioaerosol measurements: the concentration of *Aspergillus fumigatus*, thermotolerant molds and mesophilic bacteria was measured at different working places in the installation:

- *in the rotting hall (middle aisle)*
- outside the hall where the compost was stocked, between the different compost heaps
- during the manual sorting of shredded material, next to the person working
- during the manual sorting rejects of the screen, next to the person working
- *in the exhaust air of the biofilter, immediately after the exhaust tube*

Measurements were carried with the SAS Super 90. For detailed methods see Chapter 2.2.2.

Sampling: at the same points where gas and temperature measurements were carried out, compost was sampled as described in Chapter 2.2.3.

Microbiological analyses: *Aspergillus fumigatus*, total thermotolerant molds and yeasts, thermophilic and mesophilic bacteria. For detailed methods, see Chapter 2.2.4.

Physico-chemical analyses: pH, dry weight. For detailed methods see Chapter 2.2.5.

3.2.5.2B Results

Physico-chemical measurements

COMPOST IN THE ROTTING HALL

Table 51: Temperature and gas concentration measurements in different depths in 1 week old compost that was not yet turned (box no. 27).

depth [cm]	temp. [°C]	O_2 [%]	CO_2 [%]	CH_4 [%]	H_2S [ppm]	NH_3 [ppm]
20	52	10.5	16	4.1	0	1
50	46	2.9	> 20	> 5.0	3	1
100	42	1.0	nd	> 5.0	5	nd
200	35	nd	nd	nd	nd	nd
270-300	nd	nd	nd	nd	nd	nd

Compared to the other installations examined (open-air windrows (Chapter 3.2.1), box (Chapter 3.2.2), trench (Chapter 3.2.3) or bioreactor (Chapter 3.2.5)), temperatures in compost, which was nevertheless already since 5 days in the box, were very low (Table 51). The surface, where some oxygen was detected, showed the highest temperatures. In the depth of the box, conditions were completely anoxic, in spite of aeration, leading to a very slow heat production.

For experimental reasons, aeration did not happen with fresh air, but with air that had already been used for the aeration of the box no 26. Figure 62 shows the gas kinetic measurements in box 27.

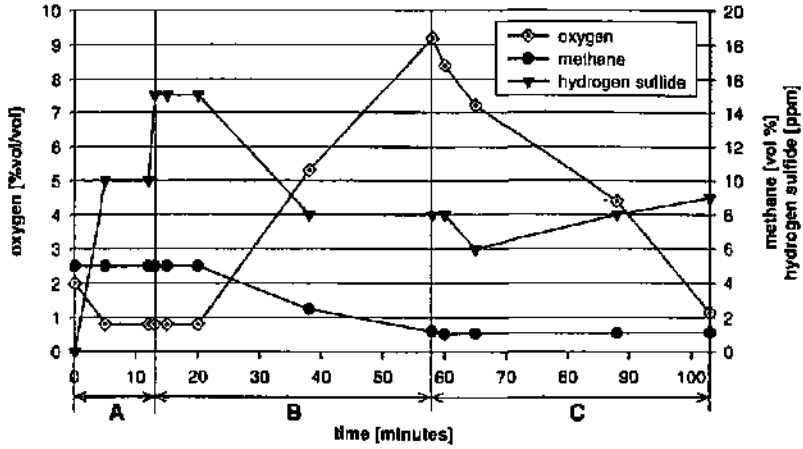


Figure 62: Gas kinetic measurements of O_2, CH_4 and H_2S, carried out in 100 cm depth in the 5 days old compost that was not yet turned. Pressure aeration: 200 m^3/h (= 0.06 $m^3/h \cdot t_{fresh\ weight}$)
Phase A: air from sucking aeration of box 26
Phase B: fresh air
Phase C: no aeration.

The measurements during the phase A showed that the air that had already passed through the box 26 was "used", e.g. it did not contain any oxygen anymore, and was even "enriched" with methane and hydrogen sulfide, both gases stemming most probably also from anaerobic metabolisms in box 27.
When aeration happened with fresh air (phase B), an almost immediate increase in oxygen, and a decrease in CH_4 and H_2S concentrations was observed. Due to the very low aeration power (0.06 $m^3/h \cdot t_{fresh\ weight}$, compared to literature values (see Chapter 1.6.3) and data of the other installations examined (see Chapters 3.2.2 and 3.2.3 and 3.2.4) of tens of $m^3/h \cdot t$), oxygen increased within 40 minutes to only about 10 %.
When the aeration was stopped (phase C), oxygen was consumed by the microflora, and after another 40 minutes, O_2 concentrations again below 1 % were reached. Parallel, hydrogen sulfide was formed, while the very oxygen sensitive methanogens had not yet resumed their activity.

These gas kinetic measurements show the enormous oxygen demand of the microflora in the fresh compost, leading, if no adequate aeration was provided, immediately to anoxic conditions. For this reason, the aeration cycle as it is in used at the present moment in the installation, is one air pulse every 30 minutes.

Table 52: Temperature and gas concentration measurements in different depths in the 2 week old compost that was turned twice (box 1b).

depth [cm]	temp. [°C]	O_2 [%]	CO_2 [%]	CH_4 [%]	H_2S [ppm]	NH_3 [ppm]
50	45	8.8	nd	0.55	0	nd
100	49	1.1	20	> 5.0	14	0
200	56	nd	nd	nd	nd	nd
270-300	40	nd	nd	nd	nd	nd

In the 2 week old compost (Table 52), a hesitant heating of the mass was observed. The temperature gradient was opposite that of the 1 week old compost: the microbial activity led to higher temperatures in the deeper layers of the box. The air supplied to the hall (the box 1b was situated directly beneath the ventilator) cooled the surface. Also in the 2 weeks old material, conditions were anoxic below 1 m depth, in spite of the aeration system installed in this box. A too great cooling of the boxes situated right beneath the ventilator could be avoided through the pre-warming of the air entering the rotting hall with the waste heat of the co-generation power plant. This was planned, but at the moment of the examination not yet realized.

The very low oxygen content in the compost indicated that the turning, carried out at low frequencies (once a week), and including not even the whole mass in the box (at each turning, only about ¼ of the compost at the bottom of the box is evacuated, and, by a spiral system, re-filled at the top), did not lead to a sufficient oxygenation of the material. Much more frequent turnings, and/or the installation of an adequate aeration system would be indispensable.

Table 53: Temperature and gas concentration measurements in different depths in 3 weeks old compost that was turned 6 times (box 6).

depth [cm]	temp [°C]	O_2 [%]	CO_2 [%]	CH_4 [%]	H_2S [ppm]	NH_3 [ppm]
20	42	5.0	16	4.45	0	2
50	51	2.2	nd	> 5.0	2	nd
100	57	0.7	> 20	> 5.0	11	< 2
200	61	nd	nd	nd	nd	nd
270-300	37	nd	nd	nd	nd	nd

In the 3 weeks old compost (Table 53), the highest temperatures were measured in 50-100 cm. In this zone, enough oxygen was available to allow the activity of an aerobic microflora. The heat production in this otherwise not aerated box might have led to a convective air movement, taking air in at the side of the boxes, which were not filled very evenly. Another possibility of air supply might have been the ventilator that supplied air to the hall, and that was situated right above the box. The lower temperatures at the bottom of the box let suppose that there, conditions were again anoxic.

Table 54: Temperature and gas concentration measurements in different depths in 2 weeks old compost that was turned 5 times (box 21).

depth [cm]	temp [°C]	O_2 [%]	CO_2 [%]	CH_4 [%]	H_2S [ppm]	NH_3 [ppm]
20	69	6.5	15	0.8	0	0
50	74	11.7	nd	0.8	0	0
100	75	4.6	nd	1.8	0	nd
200	57	nd	nd	nd	nd	nd
270-300	40	nd	nd	nd	nd	nd

In the 4 week old compost (Table 54), a temperature decrease was observed. However, degradation of organic matter was by far not finished, as showed the measurements of the curing compost (compare Table 55). The temperature decline might be caused by the depletion of the substances that were degradable under anoxic conditions, as most of the measured points showed only very low oxygen concentrations. The low pH (see Table 56) also indicated that conditions were mainly anaerobic during the whole rotting process in the hall.

COMPOSTS ON THE CURING PAD (ROOFED)

The high temperatures that were measured at almost all the locations (Table 55) showed clearly that the compost after the treatment in the hall still contained a great amount of substances degradable under oxic conditions. In fact, the temperature evolution during the 4 weeks of curing resembled that of windrows put up with fresh biodegradable waste (see Chapter 3.2.1): in the 1 week old curing heap, the temperatures were highest at the surface, where oxygenation was optimal. In the course of curing, the temperature front moved towards the center (FERNANDES *et al.*, 1994; MILLER *et al.*, 1989), due to heat storage in the compost material.

Table 55: Temperature measurements in the curing composts (after 4 weeks of composting in the rotting hall).
Measuring point A: from the top, vertical
Measuring point B: 1.5 m from the ground, in an angle of 45°, lateral
Measuring point C: 20 cm from the ground, horizontal.

curing time	1 week			3 weeks			4 weeks		
location	pyramidal heap, 4 m high, 2.5 m diameter at the foot			table pile, 2.5 m high, 10 m long, 4 m wide			table pile, 2.5 m high, 10 m long, 4-3 m wide		
depth [cm]	A	B	C	A	B	C	A	B	C
20	72	76	-	76	45	36	71	66	40
50	65	73	-	74	68	55	71	49	39
100	59	66	-	65	63	64	68	41	41
125	56	53	-	60	-	54	63	-	38

In the 4 week old curing compost, temperature began to fall, in spite the fact that the compost had been restacked several times, normally enhancing bacterial activity. This temperature decline might this time really be due to a depletion of the organic substances

PHYSICO-CHEMICAL CHARACTERISTICS OF THE SAMPLES

Table 56: Physico-chemical characteristic of the samples. For description of the samples, see Table 50.

no	O_2	temp. [°C]	H_2O [%]	pH
1	oxic	47	53.8	5.35
2	anoxic	50	64.2	5.96
3	anoxic	78	61.7	5.85
4	oxic	30-40	63.6	5.78
5	oxic	60	63.8	5.68
6	oxic	74	62.5	7.10

Table 56 compiles the physico-chemical parameter of the samples taken from the boxes in the rotting hall. From each box, samples had been taken at locations that showed different temperature and oxic states.
The pH of almost all the samples was below 6. Only in the 3 week old compost, where temperature and oxygen measurements indicated aerobic degradation, did it rise to pH 7. These low pHs are an indication that anaerobic metabolisms prevailed during the whole process in the rotting hall, leading to the production of organic acids. On the other hand, the low pH delayed protein degradation, which is fastest in the range of pH 7 to 8 (NAKASAKI *et al.*, 1993), and which would have induced a pH rise. Under normal composting conditions, a pH above 7 is observed within 1-2 weeks (see Chapter 1.6.7 and the results of the other installations examined).

The water content of all the samples was very high, mostly above 60 %. While this by itself does not inhibit a correct composting process, it could, in view of the depth of the boxes, lead to compaction of the material at the bottom (see Chapter 1.6.6). However, the turning system, which takes material from the bottom and re-fills it at the top, will counteract this problem. Nevertheless, the constant water content is another indicator that the material did not show much degradation during the rotting in the hall, the low temperatures and the missing or inadequate aeration leading to hardly any evaporating of water.

Microbiology

IN THE COMPOST

Figure 83 shows the concentration of *AF* and thermotolerant molds and yeasts in composts of different temperature and age.

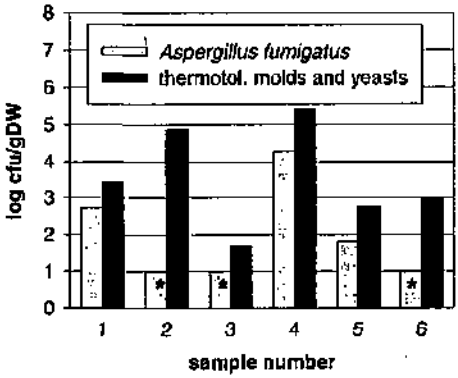


*Figure 63: Concentration of Aspergillus fumigatus and thermotolerant molds and yeasts in composts of different temperature and age. Columns with an * indicate values below the detection limit (10 cfu/gDW).*

1 = 1 week, -100 cm, 47°C, oxic
2 = 2 weeks, -20 cm, 50°C, anoxic
3 = 2 weeks, -80 cm, 78°C, anoxic
4 = 3 weeks, surface, 30-40°C, oxic
5 = 3 weeks, -10 cm, 60°C, oxic
6 = 3 weeks, -50 cm, 73°C, oxic

The *AF* numbers in the 1 week old compost was approximately $1 \cdot 10^3$ cfu/gDW, comparable to concentrations found in other composting system in material of the same age. The higher concentrations of total thermotolerant fungi were attributed to the occurrence of yeasts, which are normally found in high numbers in fresh compost, when pH is low.

The 2 week old compost, almost completely anoxic, did not support the growth or survival of any *AF*. The other thermotolerant fungi, due to the low pH still mainly yeasts, could be found in high concentrations at the surface, where temperatures were permissive for their growth, but only in very small numbers in the depth, where the very high temperature (78°C) severely inhibited fungal growth.

In the 3 week old compost, the oxygen content did again permit mold growth: it was most important at the surface (more than 10^4 cfu/gDW), and declined with increasing compost depth, parallel to increasing temperatures. Still, yeasts were present in high concentrations (approx. 10^3 cfu/ gDW).

Table 57: Mesophilic and thermophilic bacteria in compost of different temperature and age. Sample description see Table 50.

no.	temp. [°C]	O_2	mesophiles [MPN/gDW]	thermophiles [MPN/gDW]
1	47	oxic	$1.7 \cdot 10^8$	$1.4 \cdot 10^8$
2	50	anoxic	$>3 \cdot 10^{10}$	$1.9 \cdot 10^8$
3	78	anoxic	$>3 \cdot 10^{10}$	$5.1 \cdot 10^8$
4	30-40	oxic	$1.7 \cdot 10^8$	$1.7 \cdot 10^9$
5	60	oxic	$>3 \cdot 10^{10}$	$6.6 \cdot 10^8$
6	73	oxic	$>3 \cdot 10^{10}$	$3.2 \cdot 10^8$

Table 57 presents the concentration of mesophilic bacteria (in Nutrient Broth at 30°C). They were present in high numbers: in four of the six samples, their MPN was $>10^{10}$. Thermophilic bacteria (in Nutrient Broth at 60°C) values were contained between $1 \cdot 10^8$ and $1 \cdot 10^9$ MPN/ gDW, except in the 2nd week, where $5 \cdot 10^9$ MPN/gDW where enumerated from the very hot sample (78°C) taken from the depth of the box. Neither the quite low oxygen content nor the low pH did seem to influence greatly the thermophilic numbers (BLANC, 1998), they were present in concentrations comparable to those in other composting systems (see Chapters 3.2.1 to 3.2.5)

IN THE AIR

Table 58: Concentration of Aspergillus fumigatus (AF), total thermotolerant molds (TTM) and mesophilic bacteria (MB) in the air at different locations of the composting site. The results are given as cfu/m³ air. ∞: plate overgrown, no counting possible.

sampling place	*AF*	TTM	MB
in the rotting hall	980	$1.3 \cdot 10^3$	$1.2 \cdot 10^4$
in the storage (roofed)	30	90	$2 \cdot 10^3$ - > $7 \cdot 10^4$
air at the exit of the biofilter	380	490	> $7 \cdot 10^4$
working place: sorting band shredder	$4 \cdot 10^3$ - ∞	$6 \cdot 10^3$ - > $2 \cdot 10^5$	$3 \cdot 10^4$ - > $3 \cdot 10^5$
working place: sorting band screen	90	210	$2 \cdot 10^4$ - > $3 \cdot 10^5$

At all the sampling locations (Table 58), *AF* concentrations were higher than in unpolluted air (0-10 cfu *AF*/m³ air), the highest concentration being measured at the working place of the person who was manually sorting foreign material out of the shredded material.

Higher microbial concentrations were measured in the rotting hall than outdoors. It has to be mentioned though that at the day of sampling, the turning installation was out of order, so no compost movement happened. *AF* constituted 50-80 % of the total fungi, dependent of the sampling location. Mesophilic bacteria were present everywhere in higher concentrations than fungi. No determinations were made concerning the nature of the mesophils, but analyses by REINTHALER *et al.* (1997) of the composition of airborne bacteria in a enclosed table-pile composting installation showed frequent isolations of coliforms (*Enterobacter, Serratia, Klebsiella*), *Xanthomonas, Acinetobacter* and *Pseudomonas*. This confirms that the species frequently found in compost were emitted, and could be found in the air.
The biofilter was holding back about 2/3 of the fungal spores contained in the air of the rotting hall.

3.2.5.3 SECOND EXAMINATION (MAY 1995)

The second examination of the installation, carried out during May 1995, was meant to show the installation in full function. Unfortunately, the fermentor was still in the start-up phase, the sludge produced was a mixture of methanized liquid manure and kitchen waste. Also, it was not possible, as intended, to take samples from the fermentor, and none of the boxes was aerated, due to problems with obstructed ventilation lines. The results given have thus only experimental character. The operator confirms that during the observation time by the SDE (1996 and 1997), and also later on, the fermentor was working very reliably.

3.2.5.3A Experiments carried out

Physico-chemical measurements: the following parameters were measured in composts of different maturity (Table 59), and at different depths (0 to 300 cm) of the heaps. Parameters determined: temperature, O_2, CO_2, CO, CH_4, H_2S. Measurements were carried out vertically from the top of the box.

Additionally to our own measurements, the personnel of the installation had monitored the temperatures in 30 and 100 cm depth every 2-3 days for each box. Date of turning and water addition was also registered. Measuring devices were as described in Chapter 2.2.1.1. and 2.2.1.2.

Table 59: Measuring and sampling points.

no	samples	box no	depth (cm)
1	after shredding	-	-
in the rotting hall			
2	5 days	26	5
3	1 week	22	100
4	2 weeks	5	100
5	2.5 weeks	3	100
6	3 weeks	21	100
in the storage under roof			
7	exit hall (4 weeks)	7+23	50
8	12 weeks, hot	-	30
9	12 weeks, cold	-	10
10	8 weeks, screened	-	20

Bioaerosol measurements: the concentration of *Aspergillus fumigatus* and thermotolerant molds was measured at different working places in the installation (see Figure 85). Measurements were carried with the SAS Super 90. For detailed methods, see Chapter 2.2.2.

Sampling: at the same points where gas and temperature measurements were carried out, compost was sampled as described in Chapter 2.2.3.

Microbiological analyses: *Aspergillus fumigatus*, total thermotolerant molds and yeasts, Gram-negative bacteria (on MacConkey Agar), coliforms (on VRB-agar), thermophilic bacteria. For detailed methods, see Chapter 2.2.4.

Physico-chemical analyses: pH, dry weight. For detailed methods, see Chapter 2.2.5.

3.2.5.3B Results

Physico-chemical measurements

COMPOST IN THE ROTTING HALL

Table 60 presents the mean temperature evolution during the process in the rotting hall, calculated from the data gathered in the three weeks prior to sampling by the personnel of the composting installation, by measuring temperatures every 2-3 days in all the boxes.

Table 60: Temperatures [°C] (mean and standard deviation) of composts in the rotting hall at different depths in the box. n = number of measurements.

compost age [week]	1	2	3
n	11	14	8
sampling depth [cm]			
30	60 ± 6	60 ± 7	62 ± 6
100	50 ± 6	55 ± 5	60 ± 9

Table 61: Temperature [°C] evolution of composts in the rotting hall, at different depths of the box. n = number of turnings, SD = standard deviation.

age	n	depth [cm]						
[weeks]		10	20	50	100	150	200	mean/SD
0.5	0	57	62	54	46	46	48	52 ± 7
1	1	31	54	58	60	50	48	50 ± 10
2	3	59	75	60	55	52	57	60 ± 8
3	4	68	79	74	64	61	61	68 ± 7

Temperatures in 30 cm depth were always higher than in 1 m, were anaerobic metabolisms predominated. Compared to all the other composting installations examined, maximum temperatures were low, never exceeding 70°C.

The temperatures measurements taken at the moment of the second examination of the site (Table 61) showed in the fresh compost values similar to the ones detected during the first examination (see Chapter 3.1.5.2A), although this time none of the boxes were aerated. The highest temperatures were measured at the compost surface, where some oxygen was detected (Table 62). In the depth of the box, conditions were rather anoxic. The methane measured in 50-100 cm depth stemmed most probably from zones deeper down of the heap, where no oxygen was present any more.

The first turning did not lead to a significant temperature increase. The very high CO_2 values, exceeding by far the stoichiometric values of aerobic metabolisms, together with the presence of methane, hinted again at anoxic conditions in large parts of the box.
The increased H_2S concentrations can be interpreted as the onset of protein degradation, leading even in the case of aerobic metabolism to the accumulation of hydrogen sulfide. At the low oxygen concentrations measured, anaerobic sulfate-reducing bacteria could also be active, if the compost contained enough sulfate or free sulfur, produced by the mineralization of proteins or other sulfur containing substrates.

Table 62: Gas measurements in composts of different age, and at different depths in the box.

age [weeks]	depth [cm]	O_2 [vol%]	CO_2 [vol%]	CH_4 [vol%]	H_2S [ppm]
0.5	50	2.5	56	> 5	5
	100	1.8	59	> 5	39
1	50	1.3	50	> 5	22
	100	1.0	57	> 5	100
2	50	1.4	53	> 5	10
	100	0.7	60	> 5	79
2.5	50	1.1	21	> 5	0
	100	3.7	17	6.6	0
3	10	16.2	7.9	> 5	0
	20	7.9	22	> 5	0
	50	2.2	45	> 5	0
	100	1.5	51	> 5	67

In the 2 weeks old compost, temperatures assuring an at least partial thermohygienization were reached in the upper part (10-50 cm) of the box, although oxygenation was insufficient for a correct, aerobic composting process. Temperatures up to 60°C can be reached even under anoxic conditions, the thermophilic anaerobic microflora having its optimum at 55°C. H_2S concentrations were already declining, showing that the degradation of proteins came to an end.

The oxygen levels in the 2.5 weeks old compost, which had been turned just prior to the measurements, had declined to the usual low values already one hour after turning (Table 62). The temperatures in this frequently turned box were on the other hand quite low (results not shown), indicating that the turning system led to an important cooling of the material.

The 3 weeks old compost, which had been turned for the last time 5 days before the measurements, showed in the upper part of the box, where oxic conditions prevailed, temperatures that were sufficient for a thermohygienization. Below 1 m, however, the gas measurements indicated anaerobic metabolisms, and temperatures did not much exceed 60°C. The H_2S was most probably formed by anaerobic, sulfate-reducing bacteria, as proteins had been used up already in the 2nd week of composting.

COMPOST ON THE CURING PAD (ROOFED)

Table 63: Compost on the curing pad, table pile (1.5 m high), approx. 12 weeks old.

depth [cm]	temp. [°C]	O_2 [%]	CO_2 [%]	CH_4 [%]	H_2S [ppm]
lateral					
10	45	20.0	0.85	0	0
30	74	5.7	18	2.35	0
80	52	0.5	22	4.30	10
100	57	1.2	34	> 5	55
top					
50	59	0.5	35	4.90	0
100	61	0.7	36	> 5	48

The curing compost (Table 63) showed temperature and gas gradients typical of an unaerated compost pile: the hottest zone was situated 30 cm below the surface, where heat conduction was low, and heat generation, due to sufficient oxygenation, high. In the core of the pile, conditions were anoxic. Again, H_2S, stemming from sulfate reduction was measured. Temperatures were very high for a 12 week old material, indicating that the composting process had been generally slow, due to the infrequent turning, and the anaerobic conditions.

Table 64: Physico-chemical characteristics of the compost samples. Sample description see Table 59.

sample no.	O_2	temp. [°C]	H_2O [%]	pH
1	-	-	64	6.34
2	oxic	46	48	8.38
3	anoxic	60	58	5.92
4	anoxic	55	56	6.89
5	oxic	48	57	6.94
6	anoxic	64	53	7.66
7	oxic	48	49	7.05
8	oxic	74	42	8.52
9	oxic	45	50	8.17
10	-	-	50	8.89

The pH of almost all the samples was below 7 up to 2.5 weeks of composting (samples 1-5, Table 64), with the exception of the very fresh compost (5 days old) taken at the surface: the good oxygenation and the low temperatures seemed to favor protein degradation. The material reached the for a mature compost typical values of pH >8 only during the curing outside the hall did. Compared to the material sampled during the first examination, the pH, but also the water content, showed an evolution during the treatment time in the rotting hall, even if activities were slower than in the other systems studied. The nature of the starting material, containing in springtime more easy degradable materials than in autumn, and may be also the admixture of the sludges from the bioreactor, probably lead to an enhanced composting process.

Microbiological analyses

IN THE COMPOST

Figure 82 shows the concentrations of *AF*, total thermotolerant molds and yeasts, coliforms and Gram-negative bacteria detected in composts of different maturity.

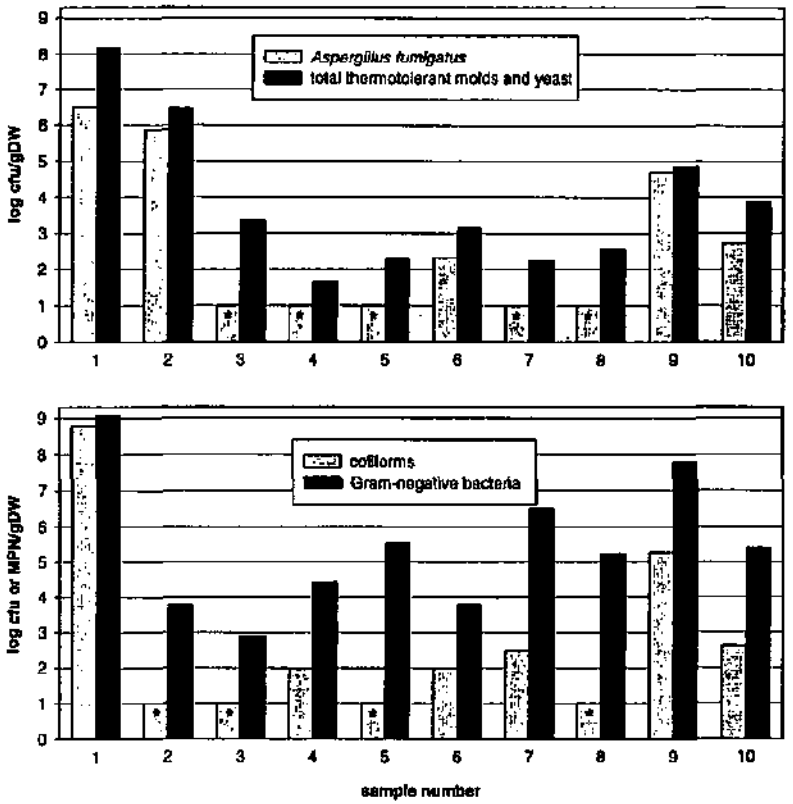


*Figure 82: Concentration of Aspergillus fumigatus, total thermotolerant molds and yeasts, coliforms and Gram-negative bacteria in composts of different maturity. Columns with a * indicate values below the detection limit (10 cfu/gFW). Coliforms (only red colonies) and Gram-negatives (all colonies) were detected on MacConkey Agar. For detailed description of the samples, see Tables 59 and 64.*

1 = fresh green waste; 2 = compost 5 days; 3 = compost 1 week; 4 = compost 2 weeks; 5 = compost 2.5 weeks; 6 = compost 3 weeks; 7 = compost 4 weeks; 8-9 = curing compost (8 = hot; 9 = cold); 10 = screened compost.

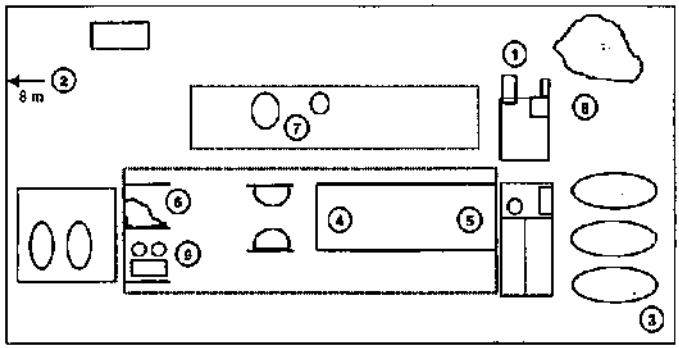
AF was reduced from more than $1 \cdot 10^6$ to not detectable already after 1 week of composting in the rotting hall, due to the rising temperatures, but probably also due to the anoxic conditions. *AF* concentrations remained low in the samples taken in the hall. However, in the curing composts (samples 7 to 10), a recolonization up to $4 \cdot 10^4$ cfu/gDW was observed at the surface of the piles, where conditions were propitious for the development of *AF*.

The fact that *AF* made out almost the entire thermotolerant fungal flora of the curing piles shows that it found an ideal proliferation environment. Under the partially anoxic conditions in the rotting hall, other thermotolerant fungi, mainly yeasts, could persist.
Screening of the compost lead to a reduction of fungal numbers, most probably by removing wood particles that were heavily colonized by fungi.

The starting material, consisting of grass cuttings, fruit and vegetable waste and brush, was especially rich in coliforms and Gram-negative bacteria: it almost contained 10^9 cfu/gDW. Coliform numbers got quickly reduced in the first days of composting in the hall; as with *AF*, a recolonization occurred in the curing compost, but to a lesser degree. The low oxygen concentration could not constitute a major cause for the disappearance of coliforms in the rotting hall; they are by definition facultative anaerobes. They were might not able to compete with other Gram-negative bacteria that survived better, especially in the samples that showed temperatures between 48°C and 55°C (samples 4, 5 and 7).

IN THE AIR

Figure 85 shows the bioaerosol measurements carried out at different locations on the site.



no	sampling site	activity	*AF*	TTM	Colif.	Gram⁻
1	7 m from the screen	none	10	30	< 5	< 5
2	on the access road	none	4	5	< 5	< 5
3	behind the wood chips pile	normal operation	10	14	3	5
4	entrance of the hall, at the height of the grating	turning of 4 week old compost (box 7)	200	290	nd	nd
5	back of the hall, at the height of the grating	turning of 4 week old compost (box 7)	1400	1700	< 100	< 100
6	storage, 1 m from the front-end loader	turning of cured compost	380	1100	nd	nd
7	storage, 1 m from the front-end loader	turning of cured compost	120	810	nd	nd
8	1 m from the sorting belt	sorting	820	1300	nd	nd
9	exit air biofilter	normal site operation	110	160	10	190

Figure 85: Sampling sites, and concentration of Aspergillus fumigatus (AF), thermotolerant molds and yeasts total (TTM), coliforms and Gram-negative bacteria per m^3 air. nd = not determined.

The bioaerosol measurements showed on the perimeter of the place only very slightly elevated *AF* concentrations. Coliforms or Gram⁻ bacteria were not detected at all. In the hall, mold concentrations were 25 times higher when measurements were carried out in the back of the hall, close to the point where fresh air was introduced into the hall and where no turning was carried out, and 175 times higher immediately above a box where 4 week old compost was extracted from the box, and transported by the conveyor system out of the hall. The low air pollution can be explained by the generally low mold concentrations in the compost. The turning system, laid out in such a way that compost does not fall from big heights, nor gets too much agitated during the transport in the channels, also contributes to a low aerosolization. The high humidity in the hall might also have restricted spore emission. Bacteria were only detected in very low concentrations in the vitiated air after the biofilter.
While microorganism concentration in the air of the rotting hall did not seem to constitute a major problem, the air quality was severely impaired by the very high CO_2 concentrations measured, exceeding the 0.5 % limit considered harmful for human health. Also, odors in the hall were very unpleasant, the volatile short chain fatty acids from the anaerobic metabolisms were irritating the eyes, and could hardly be washed off the skin.

3.2.5.3C Assessment of the performence of the system

The temperatures and gas concentrations measured in the composts, as well as the physico-chemical values of the compost samples showed an improved activity of the composting microflora at the time of the second examination in comparison with the first one. The mean temperatures were slightly higher, the pH increased more quickly, and the water content decreased. This could be attributed to the composition of the starting material, the admixture of fermentor sludge, and the slightly intensified turning frequency. The improved activity of the degrading microflora had a positive influence on the potentially pathogenic and indicator microorganisms: they were almost completely eliminated during the composting in the hall by the strong concurrence, the elevated temperatures and the lacking oxygenation (this only for the obligate aerobic molds). Due to the low concentrations of pathogens in the compost, the pollution of the air in the hall with spores was not very important.
The low oxygen and high carbon dioxide and methane concentrations in the boxes indicated that anoxic conditions were present already in a short distance to the compost surface. The missing aeration provoked fermentative metabolisms in large parts of the compost. In addition, the admixture of fermentor sludge might have led to a proper inoculation with anaerobic microorganisms. The strong reheating of the curing compost stored outside the hall was a sign that the material contained still enough easily degradable substances at the end of the 4 weeks in the rotting hall to allow a considerable metabolic activity of aerobic microorganisms.

On the basis of our results, the operators of the installation recognized the necessity to install an aeration system in all the boxes. However, the system with which 4 boxes were already equipped proved to be inadequate, in that the air channels, situated at the bottom of the boxes, were rapidly clogged up with compost particles. The gravel covering the air pipes got compacted by the action of the scrapers that transported the compost out of the boxes. To clean the air channels, the gravel would have to be frequently removed, washed and refilled. The managers decided therefore to develop their own, improved aeration system, and to install it for tests in one box. If the test were successful, all the other boxes would be equipped. HARTSOCK *et al.* (1994) stressed the importance of providing a reliable method for flushing the interior of the aeration piping, otherwise reduction of the airflow may result.

An adequate aeration, together with a intensified turning, in the first 1-2 weeks daily to avoid a too important drying of the material at the bottom (see Chapters 3.2.2 and 3.2.4) should bring about an inactivation of the anaerobic microflora introduced with the fermentor material, and favor the development of aerobic, more and more thermophilic microorganisms. This would lead to a good thermohygienization, and an accelerated degradation, by which the recolonization with pathogens during the curing could be prevented.

It was agreed that further tests at the installation should only be carried out once the new aeration system was installed. Because the installation of the new aeration system was delayed due to technical problems (two patented licenses did not work !), so that further measurements were not possible any more in the scope of this work. Since 1998, all boxes have been equipped with an aeration system, as stipulated in this work; temperature and degradation of organic matter are now evolving according to the operator's standard.

4. GENERAL DISCUSSION AND CONCLUSIONS

The following discussion, considering the results obtained at all the sites investigated, as well as the laboratory experiments, will contain several parts: one concerned with the microbiology of composting, especially the thermohygienization and recolonization with potentially pathogenic and/or allergenic microorganisms. More technical, the advantages and disadvantages of each type of system will be evoked, and the possible managerial measures discussed that could be taken to solve some of the problems encountered. Also, protective actions are proposed in the view of the occupational health of the compost workers.

4.1 GENERAL CONSIDERATIONS ABOUT COMPOST MICROBIOLOGY

Although all the literature concerned with composting states that composting is a microbial process, actual data about the different groups of organisms involved in the process are scarce. This is not astonishing, taking into account the heterogeneity of the substrate, and the ever changing conditions (temperature, water activity, nutrient content, oxygenation), as well as spatially as temporarily, in a compost heap. Furthermore, the detection of the different microbial groups by cultural methods only allows to quantify a small percentage of all the microorganisms present, and depends largely on the cultivation method (BLANC, 1998). Also, these techniques permit only to show the presence of certain types of organisms, but are not suited to reveal if they are also active ; bacterial spores e.g. are detected although they are inactive. Lately, methods were employed that do not rely on cultivation of the microorganisms (analysis of the fatty acids contained in the cell envelop, or of the genetic material) to characterize the microorganisms implied in composting (BLANC, 1998; HELLMANN *et al.*, 1997; HERRMANN & SHANN, 1997; MALIK *et al.*, 1994). They showed important changes in the populations in the course of the composting process from mesophils (to a big percentage fungi) to thermophiles, the latter comprising actinomycetes, Gram-positive bacteria (thermophilic *Bacilli*), anaerobes and methanogens. The cooling phase was characterized by the reappearance of fungi.
These results corroborate with our physico-chemical and microbiological observations made in well managed composts: the mesophilic flora (as well molds and yeasts as coliforms) of the fresh material got quickly reduced, due to the strong temperature increase in the first few days of composting. At the same time, thermophile numbers increased. They remained more or less unchanged for the time the composting processes were monitored (1-2 months), while recolonization with molds and coliforms was observed when temperatures fell below approximately 60°C.
Concerning the population changes within the thermotolerant fungi, at least those that grew on Malt Extract Agar at 40°C, it showed that *AF*, often present in large numbers in fresh biowaste, had to come up against competition from yeasts in the first, acidic phase of the composting process. As soon as the pH started to rise, yeasts disappeared. At the same time, temperatures rose, leading generally to an almost complete disappearance of *AF*. A correlation between compost temperature and *AF* concentration could be seen (see following chapter). In the beginning of the cooling phase, the appearance of *Scytalidium thermophilum*, also a thermotolerant mold, was observed regularly. It was partially displaced by *AF* when temperatures got reduced further. The large predominance of *AF* might also be due to the fact that enumeration of molds concerns mainly spores. *AF* produces

easily detachable conidiospores in high numbers (up to 10^4 spores/individual), while *S. thermophilum* forms intercalary clamydospores in lesser quantities.
Coliforms (considered generally as indicators of a contamination with fecal matter, but also stemming from plant and soil material), and especially *E. coli* were present in large numbers in fresh biodegradable waste. The main genera identified were *Enterobacter*, *Klebsiella*, *Escherichia* and *Citrobacter*. No difference was seen between the composition of the coliform flora in fresh waste, during the process, and in the finished compost. Coliforms had about the same thermoresistance as *AF* at the beginning of the thermogenic phase. However, recolonization at the end of the hot phase set in earlier.

It has to be considered, though, that temperature distribution in the compost material varied greatly, with the consequence that the microbial successions described above happened at different rates in different parts of one and the same compost. Furthermore, in the case of non-static systems, continuos mixing of populations, but also nutrients and enzymes took place. All this makes it very difficult to establish general "rules" of composting microbiology.

4.2 INFLUENCE OF TEMPERATURE/ TEMPERATURE GRADIENT ON THE ELIMINATION OF AF AND INDICATOR BACTERIA (COLIFORMS)

Temperature is considered to be the main factor for the control of potentially pathogenic microorganisms in compost. In Figure 86, the results of all the *AF* concentrations measured (at total of 328 samples) at all the sites examined (with the exception of the site of Uzwil) are united.

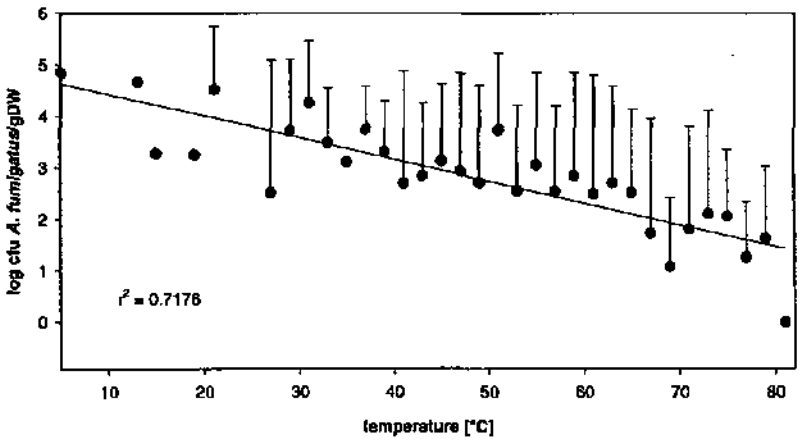


Figure 86: Arithmetic mean and standard deviation (+1 σ) of AF concentrations within temperature intervals of 2°C, as a function of temperature at the point of sampling, in compost from all the composting systems investigated (except Uzwil). Values below the detection limit (10 cfu/gFW) were considered as 1 (log_{10} of 1 = 0).

The linear regression line of the mean *AF* values showed a quite good negative correlation (r^2 = 0.72) between compost temperature and *AF* concentration, although the error bars, indicating the standard deviation within a 2°C temperature interval, were quite important.

These substantial deviations could be due, on the one hand, to an increased thermotolerance by the development of thermoresistance in certain cases, or, on the other hand, to an accelerated suppression of *AF* by other factors than the temperature, such as pH, humidity, anoxy, concurrence with other organisms for nutrients, and the production of fungicidal substances.

From the slope of the regression line, the correlation between compost temperature and *AF* concentration can be quantified as follows: an elevation of the temperature of 10°C would lead to a 2.6 fold reduction of *AF* numbers. Or: to reduce *AF* numbers by 1 order of magnitude, the compost temperature has to be raised by approx. 24°C. However, no indication is given about the duration of the exposure of the compost to these temperatures necessary to effect the reduction of *AF* numbers. As the shortest interval between two samplings was 1 day, this seems to be the minimal time necessary. To exactly determine the thermal inactivation at a given temperature, samplings in intervals < 1 day would have to be carried out.

When comparing the results from the different sites, it seemed that three main factors influenced the survival of *AF* in compost, independent of the type of composting system: the temperature, the water content of the material, and the turning frequency. These factors are interrelated, as shown in Figure 8: compost temperature is the result of heat generated by the metabolic activity of compost microorganisms. To be active, those need nutrients (substrate), water and oxygen. Too much water inhibits the oxygenation at the micropore level. Turnings effect a redistribution of substrate, microorganisms and humidity, and facilitate oxygenation by augmenting the free air space inside the composting mass.
The type of biodegradable waste used for the composting process admittedly influenced temperature evolution, but the water content of the material in the course of the composting process played also an important role. Especially in artificially aerated systems, excessive drying was often severely impairing the process, and thus the temperature evolution (see results from Uzwil, Chapter 3.2.4.3, and Tägerwilen, Chapter 3.2.2.3). The composition of the starting material, changing with the seasons, usually influenced composting only in the first few days: if more easily degradable substances were available, the temperature rise was faster, and the elimination of potentially pathogenic microorganisms quicker. However, very wet waste exhibited a delayed heating, due to clogging of the free air space with water, leading to an insufficient oxygenation (see experiment GRA3, Chapter 3.2.1.3B). Measurements showed in all the systems examined more or less important temperature gradients, caused by the movement of air through the composting mass, either by natural or forced aeration. Temperature control by aeration usually increased the temperature disparities. In heaps or windrows in the open, cooling of the compost at the surface by low ambient air temperature added to the temperatures differences, observed normally in all systems, between the hot center and the cooler peripheral layers. Due to these temperature gradients, only part of the material was exposed to temperatures high enough to lead to a thermal inactivation of potential pathogens: zones hotter than 60°C in the first 4 weeks of composting made out between 20-60 % in open-air windrows, and 40-80 % in indoor composting in trenches.
Therefore, turning of the compost to expose all material to high temperatures proved to be indispensable for a complete thermohygienization, as demonstrated by the results obtained at Grenchen (Chapter 3.2.1). Also, each time that turnings were effected, microbial activity was spurred, on the one hand by the momentarily input of oxygen, on the other hand by a redistribution of nutrients and water. In fact, effective watering of the compost was only possible during turning.

Results of the bioaerosol measurements confirmed the data from literature (see Table 8) that emission of *AF* spores could be very high (up to 10^7 cfu/m^3) when fresh biodegradable waste or young compost was mechanically treated. Emissions were correlated to the concentration of fungi measured in the compost. Also, the manner in which the turning of the compost was carried out greatly influenced the quantities of fungal spores emitted: more gentle, but probably also less effective and certainly slower turning led to the production of less aerosol. When these operations took place in the open, a rapid dilution occurred, so that concentrations already a few meters away from the source of *AF* spore emission was greatly reduced, to be identical to that in normal air (0-10 cfu/m^3) at a few hundred meters distance to the site. In closed installations, which were normally fully automated and necessitated thus in principle no presence of personnel, *AF* concentrations were usually a few thousand cfu/m^3. On the basis of these air measurements, we took the view that health risks existed only for the personnel directly involved in composting operations, but not for the population living in the vicinity of a composting site.

An open question concerns the biofilters: although the few measurements carried out the installations that were equipped with a filter showed a certain retention of fungal spores, more investigations would be necessary to prove their efficiency under different climatic situations. Some results hinted in fact at a proliferation of molds in the filter, making it a spore source instead of a spore sink.

4.3 ADVANTAGES AND DISADVANTAGES OF EACH TYPE OF SYSTEM

In the following, each type of composting system will be discussed in the view of managerial and technical measures that were, or would have to be taken to improve the composting process as a whole, and thus also the thermohygienization.
The discussion is deliberately limited to technical aspects. Of course, economical considerations are also of great importance when decisions about the compost management are made. Another aspect is the required maturity of the end product: managerial measures have to be optimized for the quality demanded by the end user. It does not make sense to invest a lot of money and energy to produce a high quality compost, if this is not necessary for the end use. Further research is necessary to define compost maturity and the quality required for specific end uses (agriculture, horticulture).

4.3.1 OPEN-AIR WINDROWS

Composting in open-air windrows, although relatively inexpensive and technically easy to realize, allows only a limited control of the process. As aeration of the material has to happen by the convection of air inside the windrow, great attention has to be given to a correct mixture of the starting material. The experiment with the starting material consisting to 50 % of each separately collected kitchen and garden waste (C:N of 30:1; experiment GRA3, Chapter 3.2.1.2C) showed indeed only a slow temperature raise at the beginning of the process. It started only really when the water content was reduced to such a degree that the increase in free air volume allowed a sufficient provision of oxygen to the aerobic microflora.
When the starting material contains high amounts of kitchen waste, odor problems occur.

Turning of the compost with a specialized machine was easily practicable, due to the arrangement of quite low, freestanding windrows. This allowed a highly flexible treatment adapted to the stage of maturity of each windrow. The machine was equipped with an efficient system for watering of the

compost, very important especially during the dry summer months. Additionally, spraying of the compost with water before turning will reduce mold spore emissions (MILLNER *et al.*, 1994).
Daily turnings led to a fast composting process and a good thermohygienization, however, operation costs, as well as for the personnel as for maintenance of the turning machine, which showed much wear and tear, were elevated. Because our measurements had shown that the composting of material containing a great percentage of kitchen waste was not very different if it was turned daily or weekly, a reduction of the turning frequency to 3 turnings per week was envisaged.
The more woody material degraded a lot faster when it was turned daily. Biodegradable wastes with a high wood content are typically delivered in winter. During this season, the affluence of material is normally reduced. A prolongation of the process by turnings in a two-day rhythm would therefore not cause any operational problems. Also, too frequent turnings during the cold season showed to cool down the compost in the maturation phase too rapidly. A negative effect of frequent turnings was observed during very rainy periods: in spite of the covering the windrows with the tarpaulin, water accumulated at the base of the heap, leading by mixing to a general wetting of the compost.

4.3.2 COMPOSTING IN BOXES OR TRENCHES, ROOFED OR IN A CLOSED HALL

When composting is carried out in boxes, a new parameter for the control of the process has to be taken into consideration: aeration. Air is blown into the compost to assure a sufficient supply of oxygen to the aerobic microflora. At the same time, moisture is transported out of the compost by the air stream, and heat is withdrawn through evaporative cooling.
Two of the installations examined used pressure induced aeration, although the air quantities and cycles were quite different: at the KEWU, total air was approximately 1-4 m^3/kg $compost_{fresh\ weight}$·h, supplied during 1-2 minutes, with pauses of 30-60 minutes. At the site of Tägerwilen, air volumes were higher: between 2 and 25 m^3/kg $compost_{fresh\ weight}$·h, but supplied in shorter intervals (0.25 to 1 minute every 2-15 minutes). Generally, the fixing of aeration cycles seemed to be highly empirical, and only vaguely adapted to seasonal changes of the type of material to be composted.
One problem encountered in the two systems composting in boxes was the filling height (2-4 m) of the material. Turning of the big masses of compost was slow, and turning frequencies were accordingly low. This led to an inhomogeneous temperature distribution, vertical as well as horizontal, and consequently to an inconsistent composting process, and an insufficient thermohygienization. Also, when composting in boxes, aeration had to be quite strong to reach the top parts of the heaps, with the consequence that the lower layers of compost had a tendency to dry out.
At the site of Baar (box composting without aeration, at the moment of put-in-operation of the installation), almost no degradation of organic matter was observed during the treatment in the boxes. In those composts, *AF* concentration was low, most probably due to the low oxygen content in the material. But as soon as the material was stored in big heaps outside the boxes, strong heating occurred, and high concentrations of *AF* (up to 10^5 cfu / gDW) were measured on the surface of the heaps, as in extensively managed open air windrows. According to the operator, the situation, due to the installation of a well working aeration system, has much improved.

In general, the handling of compost after the actual rotting process, e.g. the thermophilic phase during which the compost was treated intensively (turning, aeration, watering) showed to be unsatisfactory. Although sporadic turnings were carried out at some of the installations, they proved to be insufficient in view of the still high microbial activity in the compost after the relatively short rotting phase, the height of the piles, and the storage under roof, which hindered natural aeration to happen. This uncontrolled continuation of the degradation process lead not only to the renewed

formation of anoxic zones in the center of the piles, with the known negative effects (bad odors, emission of greenhouse gases, formation of phytotoxic substances), but also to the recolonization of the thermo-hygienized compost with potential pathogens. To prevent the observed deterioration of compost quality during curing or storage, the following measures can be taken. At some sites, they have already been realized, or are in the test phase:

- optimization of the actual composting process, and/or prolongation of the rotting phase, in order to produce a compost that is to a high degree stabilized, and would show only little microbial activity any more during curing, and would not allow recolonization by potential pathogens
- curing of unscreened compost, in order to maintain a certain porosity of the material
- putting up of small windrows (maximum 1-1.5 m high), to promote natural aeration by convection
- regular turning of the windrows (minimum 1 x per week)
- installation of an aeration system under the windrows
- direct delivery to the farmers, who use the fresh compost directly, or stock it in small windrows on-site, e.g. at the edge of the field

4.3.3 COMPOSTING IN BIOREACTORS

Composting in a bioreactor allowed in principal the highest degree of control over the process. All input and output parameters could be easily monitored, analyzed and adjusted in function. On the other hand, visual control of the compost and sampling were often not possible. A problem often encountered with bioreactor composting was the homogenous mixing of the material. Because of the large size of reactors, this was not easy to realize. When mixing was absent or insufficient, preferential air channels were formed within the composting mass, leading to an uneven oxygenation. As in box composting, drying out of the compost through superoptimal aeration was frequent. Thermohygienization was thus insufficient, in spite of quite high core temperatures.

Composting in the bioreactor generated no spore emission at all inside the building where the reactor was situated, except for loading and emptying of the reactor.
As with box or trench composting, much attention has to be given to a correct curing and storage of the compost after the treatment in the bioreactor.

4.3.4 BIOFILTERS

Three of the installations where composting was carried out indoors either in boxes, trenches or in a bioreactor were equipped with a biofilter. Spore counts carried out after the filter yielded results of a few up to several 100 cfu/m^3 of air. Taking into consideration that *AF* concentrations measured inside the rotting hall were constantly a few 1000 cfu, or much higher during turning of the composts, it seemed that the biofilters were reducing the amount of spores dispersed into the environment, although they were not completely holding them back.
More investigations should be done about the retention of microorganisms by biofilters that are usually optimized for the elimination of bad odors.

4.4 TECHNICAL AND MANAGERIAL MEASURES TO AVOID HYGIENIC RISKS DURING COMPOSTING OF BIODEGRADABLE WASTES

Besides the specific improvements to implant, dependent of the composting technology used, several general precautionary measures to avoid hygienic risks during composting of biodegradable wastes were proposed in the literature. They will be presented and critically discussed on the basis of our experimental results, and on practical observations made at the composting sites.

In the report of the study preliminary to the one presented in this thesis, we made the following propositions for preventive measures concerning the personal hygiene of the compost workers, and technical amelioration, as well as conceptual considerations when planing a new composting installation (BEFFA et al., 1992):

- to avoid to expose individuals predisposed for the development of fungal infections or allergies (people suffering from asthma, strong allergies or having a general immunodepressed status due to an other underlying health problem) to compost dust
- to preferably employ non-smokers as compost workers
- to close the cabins of front-end loaders and turnings machines and of locals situated on the composting site, and to install air filtering systems in closed cabins and rooms
- to wear face masks during periods of draught, and when working in areas with high bioaerosol concentrations
- to automate the compost process there where large amounts of organic dusts are generated (automatic loading and unloading of the shredder, turning and screening)
- to evaluate the potential for the proliferation and dispersion of potentially pathogenic microorganisms when planing new installations, as a function of the type of system, the enclosure of the rotting area, the geographical situation, and the distance to the nearest residential areas
- to construct composting sites in a sufficient distance to hospitals, homes for convalescent or elderly people, and kindergartens
- to control the concentration of potentially pathogenic microorganisms at and around a composting site when it is put in operation, and if major changes to the composting procedure are made

The first point, although of great importance when trying to reduce the health risks of compost workers, can often not be fulfilled, simply because no medical examinations are carried out prior to the employment of new workers. The closing of cabins of front-end loaders and turning machines is often missing, especially in summer, because of the lack of air-conditioning systems. Especially in enclosed systems, a complete automation of the composting process is important. However, machine failures are frequent, demanding the presence of personnel in highly contaminated areas. composting in the open demands less automation, because mold spores aerosols are readily dissolved in the air.

SUMMERBELL *et al.* (1994) added the following proposals:

- to encourage the education of the population on the microbial processes in biological wastes during collection and processing
- to minimize the interval between collections of biodegradable waste containers (maximum 1 week)
- to develop standardized methods for the detection and monitoring of fungi of priority interest to human and veterinary medicine
- to include *AF* as test organism for the determination of compost quality (hygiene)

- to put a warning on bags in which compost from open-air windrow sites is sold: «May contain *Aspergillus fumigatus*. Not suitable for indoor plants».
- to achieve hygienization of compost by steam sterilization (10 min. at 90°C)
- to elaborate a monitoring program on the cellular and serological immune responses of compost workers to fungi they are most exposed to

The first point might be of importance when considering the health risks of the general population in the context of biowaste collection and processing: the development of potentially pathogenic molds and bacteria does already start at home, during collection of the biowaste. Biowaste containers should not be placed in closed rooms, and their emptying should happen frequently. However, the frequency of collection of waste containers (every week vs. every 2 weeks) does not to seem be of great importance. The proliferation of microorganisms in biowaste is normally so rapid that complete colonization happens in 2-3 days, and no more changes are observed thereafter (GALLENKEMPER *et al.* 1995, GELLENBECK *et al.*, 1994). Potting soils used for indoor plants can be a source of *AF* contamination (STAIB *et al.*, 1987). GARCIA (1998) examined bagged composts (often mixed with peat or other substrates) sold to the general public: they contained in the mean 10^4 cfu *AF*/gDW. However, as have clearly shown our results, it is not only the compost from open-air windrows that contains *AF*. Normally, a strong reduction of *AF* numbers is obtained during the thermogenic composting phase, independent of the system used, if the process is correctly carried out. It is of great importance, though, how the compost is treated after the thermogenic phase, as it is at that moment that recolonization occurs. Sterilization of compost should be avoided, because this leads also to the inactivation of the useful compost flora !

RANINGER (1994) suggested the following points:

- workplaces for the manual sorting of biowaste should be placed in cabins that are aerated overhead with fresh air
- biowaste from source separated collection should be treated daily
- leachates that have not been hygienized should not be used for the watering of compost after the peak heating phase
- the vitiated air from closed composting halls should be eliminated via a biofilter, to minimize the dispersion in particular of staphylococci and *Aspergilli*
- newly built installations should to a large extent be automated
- different front-end loaders should be used for the unloading of fresh waste and the moving of compost, in order to prevent a spreading of pathogenic germs, or cleaning of the machines with high-pressure hot water
- measures of personal hygiene should include: the wearing of protecting clothing (overall), gloves, dust- and bacteria tight mask), disinfecting of hands and forearms, no eating or smoking at the work place, cleaning of the work clothes by the employer
- each compost worker should undergo a medical examination at the moment of employment, in order to detect health problems (allergics, bronchial asthma, immune deficiency, chemotherapy) that could lead to a increased sensibility
- each compost worker should be actively immunized against poliomyelitis, hepatitis A and B, tetanus and diphtheria
- the quality of the air at work places should be controlled regularly
- a minimal distance of 300 m should be kept from the composting site to the nearest development accommodating persons with a reduced immune defense (hospitals, health resorts, old-age homes, schools, kindergartens, etc.) or residential areas
- handling of compost in the peak heat phase as well as dry material should be reduced

As not only the sorting of fresh biowaste leads to emission of spores, but also of the reject from screening (see Tägerwilen, Chapter 3.2.2; and Baar, Chapter 3.2.5), the same should be applied to those work places. If the workstations are outdoors and mobile, the direction of prevailing winds should be considered, in order that the wind carries any bioaerosols away from the person working. Filters of cabins of front-end loaders, turners or similar machines should be serviced regularly.
Concerning biofilters, it should be added that its functioning has to be checked regularly, also to see if the retention of mold spores and bacterial cells is guaranteed, and can be ensured also with seasonal changes of the starting material, but also of the biofilter itself (temperature, humidity).
The use of different front-end loaders, or its cleaning, is difficult to realize, especially with systems where turnings of material of different age are carried out continuously, e.g. at the KEWU, Chapter 3.2.3. Furthermore, contamination happens, at least for fungal spores, also via aerosols.
The wearing of bacteria tight masks should not be a permanent measure, because of their discomfort. If possible, work places should only be in locations where a sufficient air change allows working without masks. However, for maintenance work in closed composting halls, the wearing of a mask should be absolutely recommended, or the activities in the hall have to be interrupted.

Different other authors stressed the importance of preventive measure, such as the initial medial check-up upon first employment of compost workers, to detect a possible genetical predisposition for certain types of diseases. Individuals with increased IgE concentration or impaired lung function or recurring inflammations of mucous membranes should be excluded from this type of work. Regular (annual) medical check-ups should emphasize specially on lung function, total IgE concentration and blood sugar (MARTH *et al.*, 1997). Such examinations are a prerequisite for efficient prevention and treatment of occupational illnesses, in that problems get detected early (VERKOYEN, 1994). However, in this field, information of physicians, but also more research on immunological methods is needed. Just in the case of *AF*, the normally used standard extract for the test of allergic reactions does oot in all cases give a positive result (PIERRE GUMOWSKI, personal communication).

In the view of further epidemiological studies, regular measurements of the microbial pollution of the air at work places in composting plants should be carried out. Research in the special area of detection of bioaerosols in composting plants should aim at giving recommendations about a standardized procedure concerning the type of air sampler to be used, but also the number of repetitive measurements needed to make statistically valuable statements about bioaerosol concentrations (POTTHAST, 1994). At long term, these studies should lead to the fixing of limits for microbial contamination of the air in composting plants on the basis of conclusive microbiological criteria.

5. OUTLOOK / FURTHER RESEARCH

"Composting happens, despite our best efforts to improve it" (unknown)

Composting research is a very fascinating, but sometimes also very frustrating discipline: due to the complexity of the composting process, governed by a number of interrelated factors, many observed phenomena remain unexplained. Also, observations are normally made at a macroscopic level, while the real "composting action" happens at the surface, or inside each compost particle.

In the scope of my small contribution to the composting science, some questions were answered, but many new ones arose. If I would have another four years, it would try to tackle the following ones:

- Temperature is a key factor in industrial composting systems. However, compost heaps never show uniform temperature distribution. A mathematical model would be useful that would calculate a mean temperature of a compost windrow or heap, taking into account the temperature gradient (how many temperature measurement points would be necessary to describe this gradient ?), but also the number of times the material had been mixed. It could then be tested if there is a correlation of this mean temperature to the concentration of potentially pathogenic in compost (measured best after mixing of the material).
- One big unknown was encountered when examining aerated composting systems: the aeration. Each system employed different airflows, and different aeration cycles. Detailed, continuous gas measurements would be necessary to determine the right amount of air, allowing a good oxygenation of the material, without drying it out. On the basis of such measurements, aeration regimes adapted to the starting material and to the maturity of the compost could be worked out.
- A deterioration of the compost quality after the actual rotting process was often observed. Efforts should be made to develop curing processes that would prevent a recolonization by potential pathogens and/or allergens, avoid phytotoxicity, and conserve inorganic substances (nitrate and sulfate).
- Mechanical treatment of compost leads to a massive dispersion of *AF* spores (up to 10^7 cfu/m^3 air). However, already in a short distance to the source, concentrations are reduced to a few hundred spores/m^3. VISSIENNON *et al.*, (1996) explained this by the low dispersion tendency of *AF* spores. I think that just the contrary is the case: *AF* spores are very small, so they will not sediment, but stay airborne. With the rising hot air (60-70°C, if actively composting heaps are turned) spores would thus rise, and then get diluted. Experiments, e.g. by releasing small balloons that have exactly the same sinking speed than *AF* spores (GRABER, FSI, Würenlingen, CH, personal communication), could demonstrate the path of fungal spore dispersion.
- Besides *AF*, the mold most frequently isolated from composts was *Scytalidium thermophilum*. As it has also a faint allergenic action (VAN DEN BOGART *et al.*, 1993), investigations should be made about its ecology, and its dispersion in bioaerosols.
- Biofilters are mainly optimized for the reduction of bad odors in the vitiated air of composting plants. It should also be tested to what extend they can hold back microbial cells. Also, it has to be assured that they do not become the source of microbial cell dispersion.
- Bioaerosol measurements were only carried out for the detection of *AF*. However, actinomycetes and Gram-negative bacteria can also have a pathogenic and/or allergenic action. In addition to measurements of viable microorganisms that of endotoxins and mycotoxins should be effected (SAMSON, Centraalbureau for Schimmelcultures, Delft, NL, personal communication).

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r	Pr	r	Pr	r	Pr	r	Pr	r	Pr	r	Pr	r	Pr	r	Pr	r	Pr
1	1	61	65	121	139	181	226	241	332	301	468	361	657	401	942	441	1144
2	2	62	66	122	140	182	228	242	334	302	471	362	661	402	948	442	1155
3	3	63	67	123	142	183	228	243	336	303	473	363	665	403	953	443	1166
4	4	64	69	124	143	184	231	244	338	304	476	364	669	404	959	444	1177
5	5	65	70	125	144	185	232	245	340	305	478	365	673	405	965	445	1188
6	6	66	71	126	146	186	234	246	342	306	481	366	677	406	971	446	1200
7	7	67	72	127	147	187	235	247	344	307	484	367	681	407	977	447	1212
8	8	68	73	128	148	188	237	248	346	308	487	368	685	408	983	448	1224
9	9	69	74	129	150	189	239	249	348	309	489	369	689	409	988	449	1236
10	10	70	75	130	151	190	241	250	350	310	492	370	693	410	996	450	1249
11	11	71	77	131	152	191	242	251	352	311	495	371	697	411	902	451	1262
12	12	72	78	132	154	192	244	252	354	312	498	372	701	412	908	452	1276
13	13	73	79	133	155	193	245	253	356	313	500	373	706	413	915	453	1290
14	14	74	80	134	157	194	247	254	358	314	503	374	710	414	921	454	1304
15	15	75	81	135	158	195	249	255	361	315	506	375	714	415	928	455	1318
16	16	76	83	136	159	196	250	256	363	316	509	376	718	416	935	456	1334
17	17	77	84	137	161	197	252	257	365	317	512	377	723	417	942	457	1350
18	18	78	85	138	162	198	254	258	367	318	514	378	727	418	949	458	1366
19	19	79	86	139	163	199	255	259	369	319	517	379	732	419	956	459	1383
20	20	80	87	140	165	200	257	260	371	320	520	380	736	420	963	460	1400
21	21	81	88	141	166	201	259	261	373	321	523	381	741	421	970	461	1418
22	22	82	90	142	168	202	261	262	375	322	526	382	745	422	978	462	1437
23	24	83	91	143	169	203	262	263	378	323	529	383	750	423	985	463	1456
24	25	84	92	144	171	204	264	264	380	324	532	384	755	424	993	464	1477
25	26	85	93	145	172	205	266	265	382	325	535	385	759	425	1000	465	1498
26	27	86	95	146	173	206	267	266	384	326	538	386	764	426	1008	466	1520
27	28	87	96	147	175	207	269	267	386	327	541	387	768	427	1016	467	1543
28	29	88	97	148	176	208	271	268	389	328	544	388	774	428	1024	468	1568
29	30	89	98	149	178	209	273	269	391	329	547	389	778	429	1033	469	1593
30	31	90	99	150	179	210	274	270	393	330	550	390	784	430	1041	470	1620
31	32	91	101	151	181	211	276	271	395	331	553	391	789	431	1050	471	1649
32	33	92	102	152	182	212	278	272	398	332	556	392	794	432	1058	472	1679
33	34	93	103	153	183	213	280	273	400	333	560	393	799	433	1067	473	1712
34	35	94	104	154	185	214	281	274	402	334	563	394	804	434	1076	474	1747
35	36	95	106	155	186	215	283	275	404	335	566	395	809	435	1085	475	1784
36	37	96	107	156	188	216	285	276	407	336	569	396	815	436	1095	476	1825
37	38	97	108	157	189	217	287	277	409	337	572	397	820	437	1104	477	1869
38	40	98	109	158	191	218	289	278	411	338	576	398	825	438	1114	478	1918
39	41	99	111	159	192	219	290	279	414	339	579	399	831	439	1124	479	1972
40	42	100	112	160	194	220	292	280	416	340	582	400	836	440	1134	480	2033
41	43	101	113	161	195	221	294	281	418	341	586					481	2102
42	44	102	114	162	197	222	296	282	421	342	589					482	2183
43	45	103	116	163	198	223	298	283	423	343	592					483	2281
44	46	104	117	164	200	224	300	284	425	344	596					484	2402
45	47	105	118	166	201	225	301	285	428	345	599					485	2565
46	48	106	119	166	203	226	303	286	430	346	602					486	2808
47	49	107	121	167	204	227	305	287	433	347	606					487	3295
48	50	108	122	168	206	228	307	288	435	348	609						
49	52	109	123	169	207	229	309	289	438	349	613						
50	53	110	125	170	209	230	311	290	440	350	616						
51	54	111	126	171	210	231	313	291	443	351	620						
52	55	112	127	172	212	232	315	292	445	352	624						
53	56	113	128	173	213	233	317	293	447	353	627						
54	57	114	130	174	215	234	318	294	450	354	631						
55	58	115	131	175	217	235	320	295	453	355	634						
56	58	116	132	176	218	236	322	296	455	356	638						
57	61	117	134	177	220	237	324	297	458	357	642						
58	62	118	135	178	221	238	326	298	460	358	646						
59	63	119	136	179	223	239	328	299	463	359	649						
60	64	120	138	180	224	240	330	300	465	360	653						

r = colony forming units counted
Pr = probable count

ACKNOWLEDMENTS

A PhD thesis is never a solitary exercise, but the sum of uncountable hours of fruitful discussions, practical help and moral support. The following people have contributed in an important way to my work, and I wish to thank them with all my heart for their help and support

- First of all, my "big boss", professor **Michel Aragno**, who welcomed me in his laboratory, and assigned me this very interesting thesis subject, except the fact that I was a double stranger: coming from a different field of microbiology- namely food microbiology – and from a different linguistic area of Switzerland (from the other side of the "Röstigraben !). But I think that things have worked out quite well: I just moved with the compost to the very end of the food chain, getting to know at the same time the vast and passionate field of microbial ecology. What concerns the *Rösti*: they are excellent here, too. The "Röstigraben" does thus not exist !
- My work was part of that of the "compost team", whose head and locomotive is **Trello Beffa**, my "little boss". He was always motivating and encouraging us if the fieldwork was exhausting, or if we lost patience with the lab experiments that would not work.
- **Pierre-François Lyon** is my faithful colleague and friend of the first hours of the "compost team". With his seriousness and his excellent work attitude, I knew that I could always count on him: in the field, in the lab, or for a day out (unfortunately too rare !).
- When I encountered **Michel Blanc** for the first time, I knew immediately that I had not only found a reliable colleague in the lab, an excellent French teacher (all complaints concerning my French have to be addressed to him), an office mate with whom I established an ideal "equilibrated chaos", but a friend for life. I am very pleased that his escape to New Zealand has been only of short duration.
- At the beginning of my thesis, women were a rarity in the lab. I was therefor very lucky to find two women with whom it was easy to work, but with whom I also felt a "feminist complicity": **Nicole Jeanneret**, the stable element of our lab, without her, things would just not have worked out as well, who knows all the little secrets of microbiology, and who is always ready to lend a helping hand. **Catherine Fischer**, whom I unfortunately did see too rarely, because she carried out the most part of her thesis work in the Tessin.
- A good part of my work was carried out in the field, a work often strenuous, and not always very interesting. I like to express my thanks to all my **helpers – often the poor apprentices** – who had to accompany me in rain or shine to the composting places. Many thanks also to the people of the companies with whom we collaborated, and who did their best for that our experiments worked out well. I have especially appreciated the help of **Ueli Calli** of Vollenweider AG, **Hans Buess** of KEWU, **Hans Sgier** of the installation at Tägerwilen, and **Heinz Brotschi** of the installation at Baar.
- Being a Ph.D. student means an investment of 100 % (or more) of his time for research. I did appreciate very much that **Diego** did not only tolerate me when I was coming home stinking after a day out at a composting site, but did not complain about the many evenings and weekends spent home alone, when, instead of coming home "in half an hour" as promised, I stayed many more hours in the lab, because of an experiment that did not work out as planned, or a computer failure. Many thanks also to **my parents and my family** who were always very interested in my work.
- I'd like to thank the members of my thesis committee, **Trello, Mauro Gandolla, Dr. Hans Siegrist** and **Prof. Insam**, who took the time to study my work with a critical eye.
- Finally, many thanks to all my colleagues at the lab and at the University for having made me feel welcome in Neuchâtel, and to the population of Neuchâtel and the Swiss Confederation who has financed, with their taxes, the new Biology building, allowing me to carrying out my research with always one admiring eye on the lake of Neuchâtel and the Alps.