GASEOUS COMPOUNDS AND MICROBIAL PROCESSES INVOLVED IN THE PREPARATION OF THE SUBSTRATE OF AGARICUS BISPORUS

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EEN WETENSCHAPPELIJKE PROEVE
OP HET GEBIED VAN DE NATUURWETENSCHAPPEN

PROEFSCHRIFT

TER VERKRIJGING VAN DE GRAAD VAN DOCTOR
AAN DE KATHOLIEKE UNIVERSITEIT TE NIJMEGEN,
VOLGENS BESLUIT VAN HET COLLEGE VAN DECANEN
IN HET OPENBAAR TE VERDEDIGEN
OP 1 MAART 1991
DES NAMIDDAGS TE 13.30 UUR PRECIES

DOOR

PETRUS JOHANNES LAMBERTUS DERIKX

GEBOREN OP 30 MAART 1958
TE HEYTHUYSEN

Promotores : Prof. Dr. Ir. G.D. Vogels
Prof. Dr. L.J.L.D. Van Griensven

Co-promotor: Dr. H.J.M. Op den Camp

Remain and contemplate awhile
Upon the noble thermophile,
It bears its breast to searing heat
Whilst lesser cells make fast retreat
Despite that stress, it still is able
To maintain its enzymes stable
Through steam and stench, it reigns supreme
In each heated niche of nature's scheme
Whether royal blue of boiling springs
Or biomass of lowly and discarded things
So, salute when you pass the compost pile
Wherein reigns the noble thermophile ...

F. Stutzenberger,
Lett. Appl. Microbiol., 11, 173-178, 1990.

aan mijn ouders

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CONTENTS

CHAPTER 1.

THE CULTIVATION OF *Agaricus bisporus* AND THE PREPARATION OF ITS SUBSTRATE

After being long a favourite table delicacy of epicures, cultivated mushrooms have taken an increased importance as an ingredient in many European and Oriental dishes in recent years. The low caloric value and the high content of unsaturated fatty acids make mushrooms extremely suitable in western diets. As mushrooms contain 19-40 % protein on a dry-weight basis [44] and the protein quality is at least as good as the proteins of vegetables [84], they should desirably be included in the diet of people in developing countries. *Agaricus bisporus*, the white button mushroom, and *Lentinus edodes*, the Shiitake mushroom, account for 70 % and 14 %, respectively, of the total world production of cultivated mushrooms. The United States, France, and the Federal Republic of Germany are responsible for 50 % of total world consumption of *Agaricus* mushrooms. Japan, Hong Kong, and China are the primary consumers of *Lentinus* mushrooms. *A. bisporus* was first domesticated in the Paris region in 1650 and then its cultivation developed into an industry in western countries [81]. Now the mushroom is grown in over 70 countries [8] and the annual production in the Netherlands amounted to 120 million kg in 1988 [87]. *L. edodes* is reported to have been grown in China since 800 years. It was further developed in Japan about 300 years ago. Cultivation of other mushrooms remained limited to local enterprises often producing only on a small scale. For example, in Europe some extensive methods have been developed to grow *Pleurotus ostreatus* (oyster mushroom) and *Coprinus comatus* (inky cap).

The cultivation of *Agaricus bisporus* has become a high-technology industry in western countries. Fundamental research of this mushroom has developed into a branch of science derived from microbiology, fermentation science, and environmental engineering. The nutrients for mushroom growth are provided in composts, which are prepared differently for different kinds of mushrooms. The fungi have long been known as "nature's trashburner" because of their role in decomposition. All fungi decompose organic substrates and without exception agricultural and industrial wastes are used as basic raw materials for the preparation of compost.

Recently, an extended description of the modern technique of the cultivation of mushrooms in the Netherlands has been published [86]. In this chapter attention is focused on the preparation of the substrate for the cultivation of *Agaricus bisporus* in the Netherlands.

The ingredients for the preparation of the mushroom substrate

General

As *Agaricus bisporus* is a heterotrophic organism, it requires complex organic substances as a carbon and energy source. Some information is available about the nutritional requirements for mycelial growth in defined media [82], but little is known of the nutrition of the fungus in compost. The substrate commonly used comprises agricultural wastes, which are partly degraded during the preparation process. To the substrate is usually referred to as mushroom compost.

Straw

Wheat straw (*Triticum aestivum*), rye straw (*Secale cereale*), oat straw (*Avena sativa*) and barley straw (*Hordeum vulgare*) are all suitable for the production of compost. Because of their superior texture wheat straw and rye straw are preferred. The use of herbicides during the production of the straw does not influence the composting process nor the mushroom yield in a negative way [35]. An average composition of wheat straw is given in Table 1. As expected, cell wall components account for up to 82 % of the total weight of wheat straw. The protein and fat content is only low [85].

Table 1: Average composition of wheat straw [13,85].

Component	Content[a]
Cellulose	43 %
Hemicellulose	23 %
Other carbohydrates	8 %
Lignin	16 %
Ash	6 %
Crude protein	3 %

[a]) as percentage of the dry weight.

Horse manure

Straw-rich horse manure is obtained from maneges and stud-farms. In order to avoid undesirable fungal growth in the manure during storage, collection is done every fortnight. The main characteristics of horse manure are summarized in Table 2. Other straw-rich manures, such as pig manure, are also suited for the preparation of mushroom compost but in The Netherlands hardly any other kind of straw-rich manure is available.

Table 2: Main characteristics of horse manure used as the major constituent of mushroom compost [33,35].

	mean	range
Dry matter content[a]	37 %	25 - 45 %
Total nitrogen content	1.3 %	1.0 - 1.5 %
Ammonia content	0.2 %	0.2 - 0.3 %
Bulk density	375 kg/m3	200 - 500 kg/m^3

[a]) values expressed as percentage of fresh weight, all other values are expressed as percentage of the dry weight.

Gypsum

The role of gypsum in mushroom composting is still not fully understood. It is suggested that the addition of gypsum prevents greasiness of the compost. Gerrits [32] found a positive effect of gypsum on the mushroom yield when the ammonia content of the compost is high at filling. Simultaneously this author observed lower pH values in composts supplemented with gypsum.

Chicken manure

Manure from broiler chickens, kept on wood shavings, is generally used as a source of nitrogen. The main characteristics of chicken manure are summarized in Table 3. The choice is mainly determined by the relative ease of handling and low costs of transport, due to the high dry matter content of this manure. Other manures are equally applicable but may demand slight changes either in the recipe or in the processing or both.

Table 3: Main characteristics of chicken manure, used as a constituent of mushroom compost [13,35].

	mean	range
Dry matter content[a]	60 %	30 - 85 %
Ash content	21 %	7 - 36 %
Total Nitrogen content	4.5 %	1.7 - 6.5 %
Ammonia content	1.4 %	0.5 - 2.2 %

[a]) values expressed as percentage of fresh weight, all other values are expressed as percentage of the dry weight.

Water

Water is essential for the composting process, not only as a solvent for all kind of nutrients but also to enable a close contact between the solid substrate and microorganisms [38,53]. For the growth of *Agaricus bisporus* water is also highly needed [37]. Evaporation plays an important role in nutrient transport during the formation of fruit bodies [41]. A water content of the compost at spawning of 66 % has proven to result in an optimal mushroom yield [31]. Fruit bodies themselves contain 90 to 93 % of water on a fresh weight base [33].

The composting process

General

The preparation of mushroom compost is regarded as an essentially aerobic decomposition of complex organic substances by microorganisms [28,38]. The main objective of the composting process is to obtain a selective substrate for *Agaricus bisporus* [52,67,86]. The significance of microorganisms in the composting process for the selectivity of the substrate is reviewed recently [23]. In the Netherlands the process based on the short composting method, first described by Sinden and Hauser [72,73], is used. Because of shortage of straw-rich horse manure to fulfil the total demand for mushroom compost, a recipe based on wheat straw is used as well. The total amounts of the various

ingredients used in the two different recipes are mentioned in Table 4. The sequence of mixing of the ingredients during the outdoor part of the composting process is summarized in Figure 1.

Table 4: Constituents used for the preparation of one ton fresh weight of mushroom compost (dry matter content 28 %). Values mentioned are averages [35].

Constituent	dry matter percentage	fresh weight	dry weight
On basis of horse manure:			
Horse manure	37 %	882 kg	326 kg
Chicken manure	60 %	88 kg	53 kg
Gypsum	80 %	27 kg	22 kg
On basis of wheat straw:			
Wheat straw	85 %	322 kg	274 kg
Chicken manure	60 %	356 kg	214 kg
Gypsum	80 %	27 kg	22 kg

Straw pretreatment

Straw is mixed with chicken manure to bring the total nitrogen content to a level equal to the horse manure. Water is added for a period of 7 to 10 days.

Phase I

Phase I includes two subphases, known as the flat heap and the windrows. At the start of the flat heap treatment the pretreated straw and the horse manure are mixed and water is added over a period of 7 days. At the end of this period an additional amount of chicken manure is added together with gypsum. The material is mixed and stacked into windrows, with a cross-section of 1.8 by 1.8 m. Homogeneity is increased by mixing on day 3, 6, and 8 after stacking.

Phase II

Phase II treatment or peak heating is also subdivided into two subphases: pasteurization and conditioning. During the former part the temperature is allowed to rise to a level of 56 °C and maintained for six hours. During the latter part the temperature is lowered to 45 °C by supplying more fresh air. This level is maintained until ammonia is absent in the effluent air. Usually, Phase II is completed within 7 days and the resulting compost is then ready for inoculation with *Agaricus bisporus*. Phase II can be performed either in growing chambers or in bulk in so-called tunnels [34]. Nowadays, about 50 % of the mushroom compost used in the Netherlands obtains its Phase II treatment in bulk.

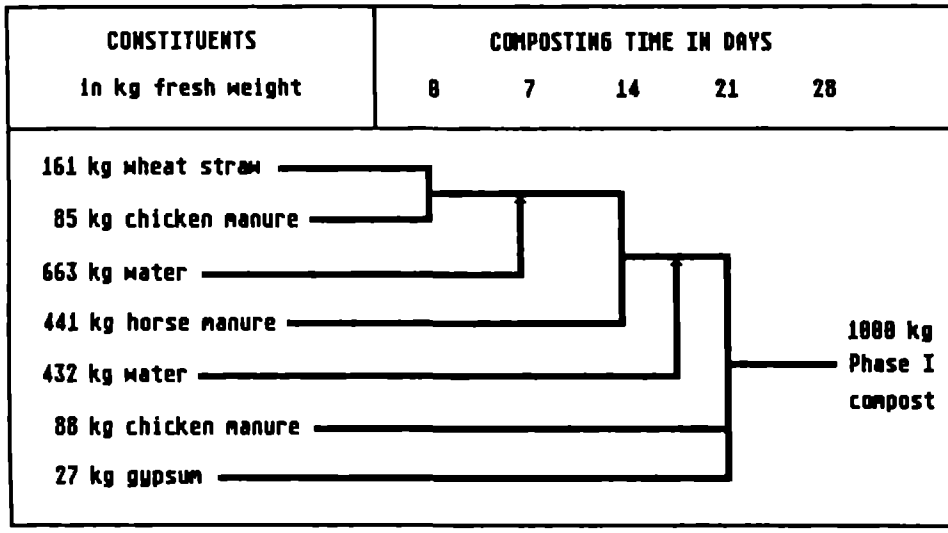

Figure 1: Schematic representation of the outdoor composting process, including straw pretreatment and Phase I composting.

The composting process is performed outdoors on a concrete floor (Phase I) or indoors in tunnels or growing houses (Phase II). As a result contact with the surroundings is limited to gas exchange.

According to Gerrits [31] the main contribution to the ventilation in Phase I is made by the chimney-effect. Because of the microbial activity heat is produced and as a result air moves to the top of the stack. Fresh air enters the stack at the sides. In Phase II gas exchange is enhanced by forced aeration (tunnels) or by reducing the thickness of the compost layer (growing house). Although air velocities between the composting particles may differ widely between Phase I and Phase II, gas exchange inside the straw particles is limited to diffusion in both phases.

The process of air movements serves as a source of oxygen [56,65] and as a drain for gaseous compounds, which are set free during the composting process. The major representative of the latter is carbon dioxide, formed during the breakdown of organic matter both under aerobic and anaerobic conditions. The second important compound to carry away is water. Because the relative humidity of the incoming air is usually less than 100 per cent [41] and the temperature is raised by the composting material, the air tends to take up water during its passage through the composting material. Depending on the balance between the amount of water removed by this process and the amount of material broken down, the water content of the resulting compost will change [55].

Less important in terms of the overall mass balance of the composting process is the amount of ammonia emitted during the preparation process. Although never reported in scientific literature the loss of ammonia during the composting process is believed to be about 2 kg per ton of compost produced [Gerrits, personal communications]. Attention is drawn to the emission of ammonia during the last decade because of its contribution to acidification due to nitrification and undesirable fertilization of natural environments [46].

Besides the components mentioned above, whose chemical identity and behaviour is well established, a less defined group of compounds is emitted by the composting material usually summarized by the word stench. Although the contribution of this group to the mass balance is negligible, its presence may lead to complaints by people living nearby a composting facility [57,61].

Analysis of odorous air

Qualification and quantification of odor are time-consuming and difficult jobs, which can be done either by olfactometry or by analytical chemical techniques. During olfactometric measurements the odor strength of an air sample is determined by presenting serial dilutions of the sample to a panel. The human nose is used as a detection device [5,42]. Besides the odor strength also information on odor quality in terms of pleasant or unpleasant may be obtained. Disadvantages of this method are its time-consuming character and the poorly understood mechanism of interaction of odorant mixtures, mainly as a result of the lack of understanding of the odor perception process itself [1,4,49].

Chemical characterization of malodors are reported frequently [30,60,92,93]. The use of gas chromatography combined with mass spectrography proved to be extremely useful and an impressive number of compounds is identified in various air samples [2,12,66]. Among these, many samples originated from complex biological systems, known to produce offensive odors, e.g. swine and cattle confinements [54,59,70], waste water treatment plants [7,48] and manures [17,63,75,91]. The presence of volatile fatty acids, aldehydes and various volatile sulfur- and nitrogen-containing compounds was revealed. The odor characteristics of the pure chemicals is well documented [6,15,51,69,80,88] and the relation between odor strength and odorant concentration is given by the Weber-Fechner psychophysical power-law [14,50]. In this case the information about odor characteristics of mixtures is also rather sparse. Another disadvantage of the chemical characterization method is the fact that no guarantee can be obtained that chemical analysis of the offensive air has resulted in a complete list of components present in the air. As a consequence of the dilemma described above both methods are presently used, although the results are hard to compare. In the Netherlands only olfactometric measurements are approved by local and government authorities when testing odor emissions by environmental regulations.

Biochemical and microbiological aspects of composting

Composting is an ancient practice. According to Poincelot [64] the Bible already mentions compost several times, but insight in the fundamental

processes occurring during the decomposition of complex organic substances only came available with the introduction of modern analytical chemical techniques. One of the first reports on this subject was made by Waksman et al. in 1939 [89].

During the entire composting process (Phase I and II) the amount of lignin present does not change significantly [29]. The weight loss obtained during composting is a result of breakdown of cellulose and hemicellulose [10,36].

As indicated by Finstein et al. [27] composting is usually viewed as a problem of handling materials and of process configuration, without reference to its microbial underpinnings. As a result, temperature is allowed to reach values far beyond the optimum. As a control parameter for maximum composting rates, temperature should be preferred over e.g. gas composition [27]. The composting process is an example of ecological successions of microorganisms [22,28,39,89]. Initially a largely bacterial flora dominates. On starting with wheat straw viable counts are as low as 10^6 colony forming units/g dry matter, but within a few days this value increases to 10^{10} colony forming units/g dry matter [11]. Among the bacteria present *Bacilli* species are most abundant [25]. At the end of Phase I actinomycetes become dominant, which results in a grey-white coating on the composting material, known as fire-fang [3,25]. Thermophilic fungi show up at the end of Phase II [68] and Straatsma et al. [78] showed the abundance of *Scytalidium thermophilum* at the end of Phase II. Table 5 gives a compilation of microorganisms isolated from mushroom compost. It is meant as an illustration of the variety of species which can be found in the compost. By no means it pretends to be complete; the number and the diversity of organisms found by isolation techniques primarily depends on the choice of the substrate and the culture circumstances. The list would be even longer if isolates, originating from other composting material, were to be included. For an extended description the reader is referred to [64,79].

Table 5. Microorganisms isolated from mushroom compost at different stages of composting and cropping.

Species	Phase I	Phase II	Spawn run	Spent compost	Ref
Bacteria					
Bacillus coagulans	a[a]				[26]
Bacillus licheniformis	x				[90]
Bacillus stearothermophilus	d				[26]
Bacillus subtilis	a				[26]
Chromobacterium sp.	c				[26]
Flavobacterium sp.	a				[26]
Pseudomonas sp.	a				[26]
Serratia sp.	c				[26]
Serratia marcescens	x				[90]
Desulfovibrio desulfuricans	c				[16]
Actinomyceten					
Excellospora flexuosa		x			[83]
Micropolyspora sp.	x				[90]
Nocardia brasiliensis		x			[18,76]
Pseudonorcardia thermophila		x			[18]
Streptomyces albus				x	[47]
Streptomyces diastaticus		x		x	[47]
Streptomyces griseus		x			[47]
Streptomyces rectus		x			[18,76]
Streptomyces sp. (gray)	b				[26]
Streptomyces thermoviolaceus		x			[18,76]
Streptomyces thermovulgaris	b	x			[18,26,76]
Streptomyces violaceoruber		x			[18]
Thermoactinomyces glaucus		x			[18]
Thermoactinomyces thalpophilus		x			[47]
Thermoactinomyces vulgaris	a	x		x	[18,26,47,77]
Thermomonospora alba		x			[83]
Thermomonospora chromagena		x			[47]
Thermomonospora curvata		x			[18,76,83]
Thermomonospora fusca		x			[18,47,83]
Thermomonospora viridis	b				[26]
Thermopolyspora polyspora		x			[18,76]
Fungi					
Absidia sp.	x				[74]
Absidia ramosa	x				[9]
Acremonium sp.			b		[62]
Acremonium butyri			x		[19]
Alternaria tenuis	x				[9,74]
Anixiellla reticulata			x		[19]

Table 5: continued

Aspergillus sp.	x		b,x		[19,62,90,74]
Aspergillus amstelodami	x				[9]
Aspergillus candidus	x		c		[9,62]
Aspergillus flavus		x	x		[19,47]
Aspergillus fumigatus	a,x	x	a	x	[9,18,26,47,62,74,76]
Aspergillus nidulans	x	x			[9,47]
Aspergillus ochraceus			c		[62]
Aspergillus repens	x				[9]
Aspergillus terreus			x	x	[19,47]
Aspergillus versicolor	x	x	c		[9,47,62]
Aurobasidium pullulans	d,x				[9,26]
Beauveria sp.			c		[62]
Botryotrichum piluliferum			x		[19]
Boytrytis cinerea	d				[26]
Cephalosporium sp.	d				[26]
Chaetomium sp.	x				[74]
Chaetomium globosum			c		[62]
Chaetomium olivaceum			b		[62]
Chaetomium rectopilium				x	[21]
Chaetomium thermophile	b,x	x			[9,18,26,76]
Chromelosporium fulvum			x		[19]
Chrysosporium sp.			c		[62]
Chrysosporium luteum		x	x		[19,47]
Chrysosporium selon			b		[62]
Cladosporium herbarium	a,x		c,x		[9,19,26,62,74]
Clitopilus pinsitus	x				[9]
Coprinus sp.	x				[74]
Coprinus cinereus	x				[9]
Coprinus fimentarium			c		[62]
Coprinus megacephagus	x				[9]
Diehliomyces microsporus			c		[62]
Doratomyces microsporus			x		[19]
Epicoccum purpurascens			x		[19]
Fusarium sp.	x		c		[62,74]
Fusarium culmorum	x				[9]
Geotrichum candidum			b,x		[19,62]
Gliocladium roseum			x		[19]
Gliomastix sp.			x		[19]
Humicola sp.	x				[74]
Humicola grisea-thermoidea	a	x	a	x	[18,26,47,62]
Humicola insolens-thermoidea	c	x	a		[18,24,26,76]
Humicola lanuginosa	a,x	x	b		[9,18,40,62,76]
Hypomyces rosellus			x		[19]
Malbranchea pulchella-sulfurea	x	x			[9,20]
Monilia fimicola	d		c		[26,62]
Mortierella sp.			b		[62]
Mucor sp.	a,x		a		[26,62,74]
Mucor hiemalis			b		[62]
Mucor miehie		x			[20]
Mucor pusillus	a	x			[18,26,40,76]
Mycelia sterilia Ct6	x				[9]

Table 5: continued

Myceliophtora lutea			b		[62]
Myriococcum albomyces		x			[20]
Nigrospora sp.				x	[47]
Oedocephalum sp.			b,x		[19,62]
Oidiodendrom tenuissium			c		[62]
Paecilomyces sp.			a	x	[47,62]
Paecilomyces varioti			x		[19]
Papulaspora byssina			c,x		[19,62]
Penicillium sp.	a,x		a		[9,26,62,74]
Penicillium chrysogenum			b		[62]
Penicillium expansum		x			[47]
Penicillium oxalicum			x		[19]
Peziza fulva			c		[62]
Pythium sp.			b		[62]
Rhizomucor pusillus	x				[90]
Rhizopus nigricans			c		[62]
Scopulariopsis brevicaulis			c		[62]
Scopulariopsis fimicola			x		[19]
Scytalidium thermophilum		a			[78]
Sepedonium sp.			b		[62]
Sordaria fimicola			c		[62]
Spicaria sp.			b		[62]
Sporendonema purperascens			c		[62]
Sporotrichum thermophile	x	x			[9,20,74]
Stibella thermophila		x			[18]
Stysanus stemonites	x		a		[9,62]
Talaromyces duponti	x	x			[9,18]
Talaromyces lanuginosa	x				[90]
Talaromyces thermophilus		x			[20]
Thermoascus aurantiacus		x			[20]
Thermomyces lanuginosus		x			[68]
Thermomyces stellatus		c			[62]
Thielavia sepedonium			x		[19]
Thielavia thermophila		x			[20,76]
Torula thermophila		x	a		[20,62,76]
Trichoderma viride	a		c,x	x	[19,26,47,62]
Trichoderma koningii			a		[62]
Trichothecium roseum			b,x		[19,62]
Trichurus sp.				x	[47]
Trichurus spiralis			x		[19]
Verticillium sp.			c		[62]

a) Indication of the appearance of the organism: a = abundant, b = frequent, c = less frequent, d = scarse, x = no indication available.

The formation of most odorous compounds in complex biological syste coupled to breakdown of organic matter. Especially when oxygen become limiting, the ensuing anaerobic conditions result in putrefaction of matter and production of objectionable gases. The involvement of microorganisms in this process is well documented [43,45,71,94,95]. M and Macauley [58] found a positive correlation between odor problems increasing usage of nutrient-rich materials as ingredients for mushrc compost. Excessive initial concentrations of available nutrients did result in an increased nitrogen level of the final compost, implicati higher loss of nitrogen, probably in the form of ammonia, during the composting process.

Outline of this thesis

During the preparation of mushroom compost organic matter is degrac as a result carbon dioxide and water are produced. In the meantime considerable amounts of heat are set free. Odour nuisance and ammonia emission accompany this process. Until now the major part of this bre process is performed outdoors and the composting regime applied, is a of trial and error rather then a product of an in depth understandin microbial processes involved. The investigations described in this th undertaken to enlarge the understanding of the microbial processes wi special respect to the emission of gaseous compounds including odor.

In Chapter 2 the microbiological community, present in the composti material, is studied in terms of biomass content and biological activ

The presence of anaerobic environments in the composting material i investigated in Chapter 3 by measurements of methane in the air evolv the compost stacks. The isolation and characterization of two strains thermophilic methanogenic bacterium from Phase I compost is describec Chapter 4.

In Chapter 5 the air evolving from the compost stacks is analyzed i to elucidate the chemical nature of the major components, responsible pungent character of the odor.

During the course of the investigations plans were developed and re

to perform part of the outdoor composting process in a tunnel-like environment. This process is now known as indoor composting and it was shown that the resulting compost was suitable for mushroom cultivation.

The formation of the previously identified malodorous compounds under laboratory circumstances is studied in Chapter 6 together with the production of these compounds during indoor composting.

In Chapter 7 a mathematical model for indoor composting based on heat and mass balances is presented. Using general microbiological phenomena and parameters, derived from the conventional process, process progress and aeration rates for the indoor composting can be calculated.

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CHAPTER 2.

BIOMASS AND BIOLOGICAL ACTIVITY DURING THE PRODUCTION OF COMPOST USED AS A SUBSTRATE IN MUSHROOM CULTIVATION

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Biomass and Biological Activity during the Production of Compost Used as a Substrate in Mushroom Cultivation

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The production of a suitable substrate for the cultivation of the common white button mushroom, *Agaricus bisporus*, is referred to as composting. High microbiological activity causes temperatures of the composting material to rise as high as 80°C. At stacking, an optimal oxygen consumption rate of 140 μmol of O_2 h^{-1} g (dry weight)$^{-1}$ was found in the compost at 50°C, whereas the oxygen consumption rate of the end product was lower at all temperatures tested. No significant differences were observed between biomass content and mineralization rate of ^{14}C-labeled glutamate of the two composts. Biomass content was shown to be a major function of both temperature and the sampling site position in the stack. On the basis of the results reported here, a minimal composting time of 3.3 days for the phase I process was calculated. Further suggestions are made to reduce the time necessary for the production of a substrate for *A bisporus* considerably.

The commercially cultivated white button mushroom *Agaricus bisporus* (Lange) Imbach and the closely related *A bitorquis* (Quelét) Saccardo are grown on a special substrate, prepared from horse manure, wheat straw, and broiler chicken manure This paper will focus on the outdoor composting process (phase I), whereas for the total preparation of a suitable substrate for *A bisporus* a second treatment (phase II) is needed (10, 13) Traditionally, the substrate for mushrooms is called compost, although the duration of the process is much shorter than the time needed for the preparation of compost from sewage sludge or domestic refuse (1 7, 18) Including a prewetting period of the straw of 14 days, the phase I process only takes 28 days Nevertheless, in many aspects the preparation of mushroom substrate is comparable to the other composting processes mentioned In all of these processes, the breakdown of solid organic matter by microorganisms is the crucial step This can be accomplished either aerobically or anaerobically As we have shown recently, considerable concentrations of methane are present in the air evolving from the composting material (5), indicating the presence of anaerobic microen vironments It was calculated that at least 3 5% of the loss of dry matter was achieved by anaerobic breakdown Although anaerobic microenvironments cannot be avoided in static compost piles, the major part of the loss of dry matter is due to aerobic breakdown, which results in the production of carbon dioxide, water, biomass, and considerable amounts of heat Because of the insulating character of the composting material and the absence of forced air movements, heat is transferred only slowly to the outside of the stacks As a result, a steady increase in temperature is observed, and depending on the dimensions of the stacks, temperatures as high as 80°C are reported (8, 11) Since the majority of the microorganisms isolated so far from this composting material are not extremely thermophilic, it is unlikely that maximum biological activity is exhibited at this high temperature In general, composting is considered to be an aerobic process (11), and as indicated above the contribution of anaerobic breakdown in the process described here is only small Therefore, biological activity can be measured as oxygen consumption rate

The oxygen consumption rates of garbage and sludge mixtures increase logarithmically with temperature from 20 to 70°C, as reported by Schulze (23) This finding was confirmed by Jeris and Regan (16) with mixed refuse for temperatures up to 60°C Above 60°C, a sharp drop in oxygen consumption rates was observed, demonstrating the inhibitory effect of elevated temperatures on the biological activity A similar profile was found by McKinley and Vestal (18), who used the mineralization rate of ^{14}C-labeled glutamate by municipal sewage sludge extracts as a measure for biological activity Optimal activity was found at a range of 35 to 45°C, while the mineralization rate drops to zero at 65°C The formation of biomass during the composting process is believed to contribute to the selectivity of the substrate for *Agaricus* spp and, as these fungi are known to produce lytic enzymes, the biomass will serve as a nutrient source (9, 24) A negative correlation is reported between biomass content and temperature above 45°C (18), another indication that high temperatures may be unfavorable during composting

The aim of the present investigation was to describe the production of compost used as a substrate in mushroom cultivation in terms of changes of biomass content and biological activity and of the correlation of these parameters with temperature

MATERIALS AND METHODS

Compost samples. The compost used in this investigation originated from a large commercial compost farm, where the total outdoor composting process takes 28 days On this farm the recipe of Gerrits (13) is used Wheat straw is the major source of organic matter in the composting material It consists mainly of cellulose hemicellulose, and lignin 36 0, 25 3 an 11 0% of the dry matter, respectively (19) On day 0, wheat straw and broiler chicken manure are mixed and a

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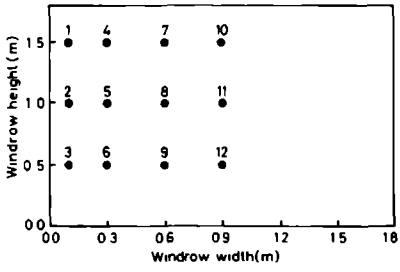

FIG 1 Cross section of a windrow Sampling sites are indicated by numbers

prewetting period of 14 days starts Horse manure is added on day 14 and water is supplemented for 7 more days On day 21 gypsum and additional broiler chicken manure are mixed through the composting material Simultaneously, the material is stacked in windrows with a cross section of approximately 2 0 by 2 0 m and the composting process is continued for 7 days, during which the material is mixed twice to increase homogeneity (13)

Biomass estimations were performed on compost samples obtained at different stages of the process The correlation between temperature of the sampling site and the biomass content was studied in samples taken at different positions through a cross section of the windrow (Fig 1) on day 23 of the process just before the first mixing The temperature of the composting material was measured with an Impac Tastotherm D 700 thermometer prior to the collection of the samples Oxygen consumption and mineralization experiments were performed with compost taken at days 21 and 28 of the process These samples are referred to as "compost at stacking" and "phase I compost,' respectively After collection, the compost samples were transferred immediately to the laboratory in plastic bags without any precaution to prevent cooling Upon arrival, each sample was mixed thoroughly and divided into subsamples for further analyses

Oxygen consumption of compost. A subsample of 100 g (fresh weight) of compost was transferred into a 1-liter serum bottle and incubated in a stove for at least 1 h at the desired temperature Then the gas phase was replaced by air which was conditioned prior to use by passage through plastic tubing (10 m, 6-mm inner diameter) and two water-filled impingers kept at the desired temperature The bottle was closed with a rubber stopper As a trap for carbon dioxide produced by the compost, a cellulose dialysis tubing (Visking, diameter, 1 4 cm, length, 10 cm) containing 5 ml of 4 M sodium hydroxide was attached to the stopper inside the bottle Any contact of the dialysis tubing with the compost was avoided After closing the bottle, a connection was made with one end of a gas burette by inserting a hypodermic needle through the stopper The other end of the gas burette was connected to a water-filled reservoir Volume changes of the gas phase were measured with the water in the gas burette and in the reservoir at the same level (25) Decrease in volume was taken as oxygen consumption

Oxygen consumption of compost extracts. Subsamples of 40 g (fresh weight) of compost were shaken with 1 liter of tap water for 10 min Large solid particles were discarded after decantation of the liquid The liquid phase thus obtained is referred to as compost extract Oxygen consumption in the extract was measured with a biological oxygen monitor model 53 and a bath assembly 5301 (Y S I Incorporated) by the method of Hemrika-Wagner et al (14) No buffer or exogenous substrate was added to the extracts

Mineralization rates of compost extracts. Compost extracts were prepared as described above To 2 ml of extract 0 1 ml of L-[U ^{14}C]glutamate (3 7 kBq, 0 38 nmol) was added in stoppered vials The mixture was incubated at various temperatures The vials were equipped with a disposable center well containing 0 3 ml of ethanolamine-ethylene glycol (1 2, vol/vol) to trap $^{14}CO_2$ produced The evolution of $^{14}CO_2$ was linear with time over at least 120 min Routinely, incubations were terminated after 60 min by the addition of 0 5 ml of 3 M perchloric acid to the medium, followed by a second incubation for 18 h at 4°C to ensure complete volatilization of CO_2 Radioactivity of $^{14}CO_2$ was measured in 10 ml of toluene-methanol (2 1, vol/vol) containing 0 4% Omnifluor by means of a Philips model 4400A liquid scintillation counter All counts (counts per minute) were corrected to disintegrations (disintegrations per minute) by using the channel ratio method Mineralization rates are expressed as nanomoles of glutamate mineralized per hour of incubation per gram (dry matter) of compost extracted

Microbial biomass determination. Biomass formed during composting has been measured in several ways, including direct counts, ATP content and total extractable lipid phosphate content (18, 24) Here, total extractable lipid phosphate estimations are preferred because of the higher reproducibility (18, 26) Total lipids were extracted from wet samples containing approximately 6 g of dry matter Water was added depending on the moisture content of the sample up to a total volume of 20 ml The chloroform methanol extraction procedure of Bligh and Dyer (3) was used After digestion in 30% perchloric acid for 2 h at 180°C, inorganic phosphate was measured colorimetrically at 830 nm, using the molybdate blue reaction (2) Results are expressed as micromoles of PO_4 per gram (dry weight) of compost

Analytical procedures. The gas phase was analyzed as described by Hutten et al (15), using a Pye Unicam model GCV gas chromatograph equipped with a thermal conductivity detector Dry-matter content was calculated after drying the compost samples to constant weight at 70°C, resulting in an average dry-matter content of compost at stacking and phase I compost of 24 8 and 27 4%, respectively

Chemicals. Gases were obtained from Hoek Loos, Schiedam The Netherlands Omnifluor was purchased from New England Nuclear, Dreieichenheim, Federal Republic of Germany L-[U-^{14}C]glutamate was obtained from the Radiochemical Centre, Amersham, United Kingdom All other chemicals used originated from Merck, Darmstadt, Federal Republic of Germany

RESULTS

A representative illustration of oxygen consumption by phase I compost at different temperatures is shown in Fig 2 Sterilized compost (30 min at 121°C), used as a control, showed a negligible change in volume during incubation Gas chromatographic analyses of the gas phase, after cessation of the volume changes of the oxygen uptake experiments with untreated compost, showed the absence of both oxygen and carbon dioxide When the sodium hydroxide solution was replaced by water during the incubation, only small volume fluctuations were observed As a result, only a negligible net volume change over the whole incubation period was measured After incubation, the presence of

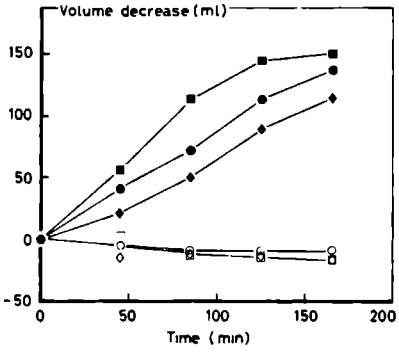

FIG 2 Volume changes obtained by incubation of phase I compost at various temperatures before (closed symbols) and after (open symbols) sterilization ● ○ 40°C ■ □ 55°C ◆ ◇ 60°C

carbon dioxide and the absence of oxygen in the gas phase of this control were confirmed by gas chromatographic analyses

To determine whether the rate of carbon dioxide uptake by the sodium hydroxide solution in the dialysis tubing was sufficient to trap all carbon dioxide released during incubations, empty bottles were flushed with N_2-CO_2 (80 20%, vol/vol) Volume changes caused by carbon dioxide uptake from the N_2 CO_2 atmosphere ranged from 7 to 20 ml/min over the temperature and time range tested (data not shown) As the volume changes during the experiments with untreated compost never exceeded the rate of 2 ml/min, it is concluded that the uptake capacity of the carbon dioxide trap was sufficient From the slope of the oxygen uptake curves (Fig 2), the oxygen consumption rate at a given temperature can be calculated per gram of dry weight The averages of experiments with both compost at stacking and phase I compost are shown in Fig 3 The oxygen consumption rate of the compost at stacking is higher as compared with phase I compost Compost at stacking showed an maximum oxygen consumption rate at 50°C of about 140 μmol of O_2 g (dry weight)$^{-1}$ h ^{1} Above this temperature oxygen consumption rates declined, reaching a level of 55 μmol of O_2 g (dry weight) ^{1} h^{-1} at 70°C Compost samples taken at the end of phase I showed an increase in oxygen consumption rate at temperatures up to 40°C, above 40°C, the oxygen consumption rate remained stable at a level of about 50 μmol of O_2 g (dry weight)$^{-1}$ h ^{1}

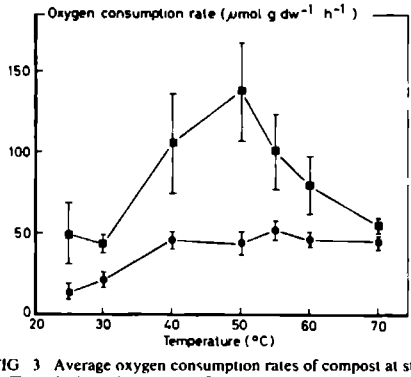

FIG 3 Average oxygen consumption rates of compost at stacking (■) and phase I compost (●) at various temperatures Bars represent standard error of the mean The number of measurements range from 4 to 8 dw Dry weight

TABLE 1 Oxygen consumption rate of aqueous compost extracts after various treatments measured at different temperatures

Treatment and temp (°C)	Oxygen consumption rate (μmol of O_2 g [dry wt] ^{1} h ^{1})			
	Compost at stacking		Phase I compost	
	−KCN	+KCN	−KCN	+KCN
Extraction				
25	28 4	0	29 0	6 6
50	63 5	18 9	49 7	15 2
70	0 4	0	0	0
Extraction + centrifugation (15 min 13 000 × g 4°C)				
25	0	0	2 7	0 4
50	5 3	1 3	1 8	1 2
70	0	0	0	0
Extraction + centrifugation + boiling (15 min)				
25	0	0	0 1	0
50	0 7	0	0	0
70	0	0	0	0

Corrected for oxygen consumption by the electrode

The influence of different treatments on oxygen consumption rates of extracts prepared from compost at stacking and phase I compost is summarized in Table 1 At 25 and 50°C, the same tendency was observed for the oxygen consumption rates of the extracts as for the untreated compost samples, although the absolute value of the extracts are lower After centrifugation, which removes the major part of the microorganisms present, the oxygen consumption rate of both extracts was reduced over 90% at 25 and 50°C After an additional heat treatment (15 min at 100°C), the extracts showed a negligible oxygen consumption rate At 70°C no significant oxygen consumption was observed for both extracts independent of the treatments applied The influence of potassium cyanide known as a strong inhibitor of the terminal step of the cytochrome-mediated respiratory pathway, was studied at a final concentration of 0 5 mM At 25 and 50°C the addition of potassium cyanide reduced the oxygen consumption rate over 70% Subsequent addition of benzohydroxamic acid (12 5 mM) did not alter the oxygen consumption rates significantly (data not shown) Benzohydroxamic acid is known as an inhibitor of the cyanide insensitive respiratory pathway (6 22)

The temperature dependency of the mineralization of ^{14}C labeled glutamate by phase I compost extracts is shown in Fig 4 The highest mineralization rate was observed at a temperature range of 50 to 55°C, with a steep decline at higher temperatures Mean mineralization rates of extracts prepared from compost taken at different stages of the

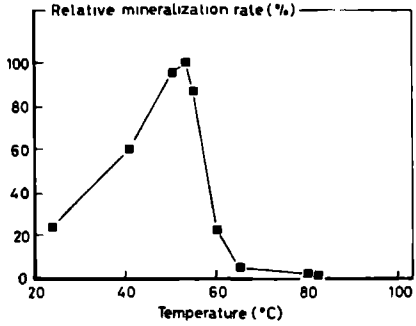

FIG 4 Temperature dependence of the ^{14}C labeled glutamate mineralization rates by phase 1 compost extracts Mineralization rate at 55°C was set to 100%

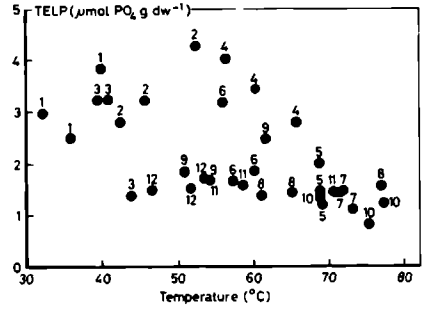

FIG 5 Biomass content versus site temperature of compost samples taken 2 days after stacking Numbers refer to the sampling position as shown in Fig 1 TELP Total extractable lipid phosphate dw dry weight

process are summarized in Table 2 Taking into account the high standard deviations caused by heterogeneity of the material no significant differences were observed

Total extractable lipid phosphate analyses were used as a method to study the biomass content of the composting material Figure 5 shows the biomass content versus the temperature of the sampling site The data of three independent experiments are included In general two levels of biomass content were observed corresponding with the origin of the compost samples Samples taken at locations 1 to 4 (Fig 1) covered the temperature range of 32 to 65°C but, with only one exception, possessed a biomass content higher than 2 3 μmol of PO_4 g (dry weight)$^{-1}$ Temperatures of the other sites were within the range of 43 to 78°C, while the biomass content hardly ever exceeded 2 μmol of PO_4 g (dry weight)$^{-1}$ The average results of biomass estimations performed on samples taken over the entire composting process are presented in Fig 6 From the start of the prewetting period up to day 18 an increase of biomass was observed Later in the process no significant changes in biomass content were measured As a result the end product phase I compost, contained a sixfold-higher biomass content (2 4 μmol of PO_4 g [dry weight]$^{-1}$) when compared with the starting material

DISCUSSION

Microbiological activity and biomass content of the composting material changed during the production of compost used as a substrate in mushroom cultivation Both parameters showed a correlation with temperature Microbiological activity of untreated compost was measured by oxygen consumption The optimal temperature of the oxygen consumption rate of compost at stacking (Fig 3) coincides with the optimal temperature of the mineralization rate of phase I compost extracts (Fig 4) This temperature optimum (50°C) is somewhat lower than the value of 59°C reported by Jeris and Regan (16) for mixed refuse composting, but equals closely the reported optimal temperature for municipal sewage sludge composting (18) Oxygen consumption rates of extracts are lower than those observed with untreated compost This is unexpected since the gas-liquid barrier for oxygen diffusion is omitted and probably due to only partial extraction of the microbial population It is known that cellulolytic microorganisms strongly adhere to fibers (17) Nevertheless, as the same tendency in oxygen consumption rate is observed in extracts compared with untreated compost a practical system is obtained for testing the influence of different treatments or additives or both The dramatic reduction in oxygen consumption rates after centrifugation of the extracts or the addition of potassium cyanide demonstrated the involvement of biological systems in this process That the addition of benzohydroxamic acid had no effect on the oxygen consumption rate indicates that cyanide-insensitive respiration does not play an important role in the observed oxygen consumption (22)

TABLE 2 Mean mineralization rates of [^{14}C]glutamate of various aqueous compost extracts at 55°C

Compost sample	n	Mineralization rate (mean ± SD nmol of glutamate g [dry wt]$^{-1}$ h^{-1})
Day 21	5	6 7 ± 1 9
Day 24	12	7 2 ± 3 3
Day 28	7	5 4 ± 5 3

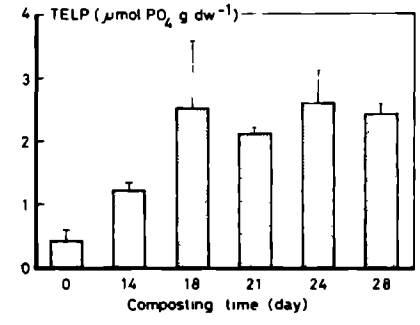

FIG 6 Mean biomass content and standard deviation at different stages during the production of compost used as a substrate in mushroom cultivation TELP Total extractable lipid phosphate, dw, dry weight

Elevated temperature is one of the most extreme environmental stresses to which organisms are exposed (4). Biological activity of compost extracts, measured as oxygen consumption and mineralization of glutamate, dropped to zero at 70°C. In untreated compost an oxygen consumption rate of 50 μmol of O_2 g (dry weight)$^{-1}$ h^{-1} was observed at this elevated temperature. The prolonged biological activity may be due to the poor heat transfer of the composting material, resulting in a lower actual temperature on microscale. Furthermore, chemical reactions cannot be excluded, but until now only vague suggestions are made (21).

Another illustration of the detrimental influence of the high temperature on biological activity is the low biomass content observed in samples originating from high-temperature sampling sites (Fig. 5). Below 65°C, data can be divided into two groups, depending on the position of the sampling site. The first group consists of samples from positions 1 to 4 (Fig. 1) and is high in biomass content, whereas the biomass content of the second group (samples from positions 5 to 12) does not exceed 2 μmol of PO_4 g (dry weight)$^{-1}$. As shown by Randle and Flegg (20), oxygen concentrations in compost tend to drop steeply over a distance as small as 0.5 m during the first days after stacking. Therefore, the low biomass content of the second group is a result of the lack of oxygen in the corresponding part of the stack.

Leaving the prewetting period out of consideration, the actual production of phase I compost takes 14 days or more (10, 13). Temperatures of the composting material as high as 70°C are reached within 1 or 2 days (13). As a result, the composting process will be slowed down due to thermal inactivation of essential microbial enzymes. As the oxygen consumption rate of phase I compost is only 25% of the oxygen consumption rate of compost at stacking, the highest microbiological activity is exhibited immediately after mixing of the constituents. Since compost at stacking and phase I compost do not differ from each other in terms of biomass content (Fig. 6) or mineralization rate of glutamate (Table 2), the observed lower value of oxygen consumption rate is most probably due to depletion of substrate. Readily accessible compounds will first be degraded, and as a result an accumulation of recalcitrant compounds such as cellulose and lignin and biomass in the composting material will occur. From the observations reported here it is highly plausible that the composting process can be shortened. Taking the maximum oxygen consumption rate of 140 μmol of O_2 g (dry weight)$^{-1}$ h^{-1} and assuming that 30% of the dry matter should be degraded during the composting process (12), a minimal composting time of 3.3 days can be calculated. Performing the phase I composting process at a temperature not exceeding 55°C would reduce the composting period by a factor 4 to 5.

Increase of the selectivity of the substrate for *A. bisporus* is a major objective of the composting process described here. During the composting process biomass is formed out of readily accessible compounds (Fig. 6) and *A. bisporus* is able to digest a broad range of substrates, including microbial cell wall compounds (9). But, as the biomass content does not increase during the last 10 days of the process, continuation of the process after day 18 seems to be meaningless. Nevertheless, a continuous succession of microorganisms will occur and an accumulation of killed microorganisms and microbial metabolites which can act as nutrient source for *A. bisporus* cannot be excluded. Additional research is needed to confirm the desired minimal fraction of dry matter loss during the composting process, especially with respect to mushroom yield. Active biomass is assumed to be a key index of compost selectivity, but chemical and physical factors (e.g., straw weakening and increase of the water-holding capacity) may also be important.

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CHAPTER 3.

PRODUCTION OF METHANE DURING THE PREPARATION OF MUSHROOM COMPOST

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PRODUCTION OF METHANE DURING PREPARATION OF MUSHROOM COMPOST

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KEY WORDS: Agaricus spp.; compost preparation; methane production; anaerobic bacteria

INTRODUCTION

The production of compost as a substrate for the cultivation of Agaricus spp. is considered to be an aerobic process. Randle and Flegg (1978) reported that during outdoor composting (Phase I) oxygen concentrations, measured by gas sampling, were greater than 5 % in 86 % of the windrow volume. However, considering the nature of the composting material it seems unlikely that anaerobic conditions (anaerobic pockets) can be avoided. The presence of sulfur crystals in the center of compost piles (Eicher 1981) supports this view.

The end-products of anaerobic degradation are carbondioxide and methane. Methanogenesis, the last step in this degradation, is brought about by strictly anaerobic bacteria. For an extended description of methanogenic bacteria, their ecology and biochemistry, we refer to the review of Archer and Harris (1986).

In this paper we report for the first time the production of methane in the preparation of mushroom compost. To evaluate the role of anaerobic processes we determined the methane production and total counts of anaerobic and methanogenic bacteria at several stages during composting. Furthermore, the isolation of a pure culture of a methanogen from composting material is described.

MATERIALS AND METHODS

Sample collection

Air samples were taken from the air stream escaping from the stacks. To minimize the influence of the wind an open stainless steel barrel (I.D. 0.64 m) was placed on top of the stack. This barrel had an opening at the top (I.D. 2.5 cm) and a small sampling tube at the side, which ended in the center. The total height of the barrel amounted to 1.2 m. After positioning the barrel, the air inside the barrel was allowed to stabilize for about 15 min before air samples were taken by the use of 60 ml syringes. Immediately after sampling 1 ml of ethane was injected into the syringe to serve as

an internal standard. Ventilation rates were calculated from air velocities measured at the top opening of the barrel with a Wallac GGA-23s thermo-anemometer.
Compost samples were collected at different stages of the process on a commercial compost farm. Unless stated otherwise, samples were taken at about 30 cm inside the stacks. The samples were immediately transported to the laboratory in closed plastic bags without any precaution to prevent cooling.

Incubation of compost samples
Compost samples, taken at different places representing the same stage during composting, were mixed thoroughly by hand before subsamples of about 25 g wet weight were placed into 500 ml serum bottles. The bottles were closed with n-butyl rubber stoppers and aluminum screw caps. In case of incubation under a gas atmosphere other than air, the bottle was flushed for 5 min with the desired gas mixture. To all bottles 1 ml of ethane was added to serve as an internal standard. Incubations were performed at 55°C unless indicated otherwise.

Methane analysis
Methane concentrations were determined gaschromatographically according to Hutten et al. (1981).

Most probable number method for counting bacteria
To estimate the number of anaerobic as well as methanogenic bacteria per g dry matter, 100 g of compost were shaken by hand for 15 min with 200 ml of tap water at room temperature. From the resulting liquid phase decimal dilutions were prepared in triplicate in medium. Strict anaerobic techniques described by Balch et al. (1979) were used. The medium for counting both anaerobic and methanogenic bacteria contained besides 300 ml of compost extract per 1 the following components (g/l): $NaHCO_3$, 10.0; NaCl, 2.0; K_2HPO_4, 1.0; KH_2PO_4, 1.0; $(NH_4)_2SO_4$, 0.5; $MgSO_4.7H_2O$, 0.2; $CaCl_2.2H_2O$, 0.2; yeast extract, 0.5; glucose, 0.5; maltose, 0.5; soluble starch, 0.5; pepton, 0.5 and L-cysteine $HCl.2H_2O$, 0.5. The compost extract was prepared by shaking 50 g of compost with 400 ml of tap water for 15 min. The resulting liquid was sterilized (20 min, 120°C) and centrifuged (10,000 x g for 10 min). The supernatant was added to the rest of the medium just before sterilization. The gas phase consisted of H_2/CO_2(80%/20%). Incubations were performed for 5 days at 55°C without agitation. For determining total anaerobic bacteria growth was monitored by turbidity, while methane production served as an indicator for growth of methanogenic bacteria. Calculations were done by the use of the probability tables presented by de Man (1975).

Isolation of methanogenic bacteria
Enrichment of methanogens was performed on Medium 1 of Balch et al. (1979) modified by Kiener et al. (1983) in 100 ml serum bottles. The procedures followed were essentially the same as described by Blotevogel et al. (1985). Cultures were screened for methane production and coenzyme F_{420}-fluorescence as reported by Doddema and Vogels (1978).

RESULTS

Methane measurements at the commercial compost farm
The methane concentrations measured in stationary air at several stages of

the Phase I composting process are summarized in Table 1, together with the calculated molar production rates. The methane concentration in the atmosphere is about 1.5 ppm (Ehhalt, 1979). The ppm values measured are the net result of methane production and methane consumption since oxidation of methane by methane oxidizing bacteria (Dalton, 1981) can not be excluded. Highest concentrations were recorded above the flat heap after about 3 days and above the "young" windrows (day 20-22).
From the results (Table 1) an average concentration of 915 ppm for all windrow samples was calculated, with a mean air flow of 6.90 $m^3/m^2.h$. Assuming a degradation of 30% of total solids (taken as cellulose) during Phase I we calculated that at least 3.3% of it will be brought about by anaerobic processes resulting in methanogenesis.
To get some information about the numbers of anaerobic bacteria present during the composting process we counted total anaerobic bacteria and

Table 1. Methane concentrations in air escaping from the stacks at several stages of the outdoor composting process.

	Day	n	CH_4 concentration* ppm	Air flow $m^3/m^2.h$	CH_4 production $mol/m^2.d$
Straw bath	(0-11)	13	103 (16-278)	7.09	0.73
Flat heap	(12-15)	27	583 (27-2515)	6.80	3.96
,, ,,	(16-19)	21	4895 (77-44742)	5.54	27.12
Windrows	(20-22)	23	2249 (13-7420)	6.11	13.74
,,	(23-25)	16	89 (5-480)	6.80	0.60
,,	(26-28)	22	409 (26-1790)	7.78	3.18

*Concentrations are given as mean values. Values between brackets indicate the range.

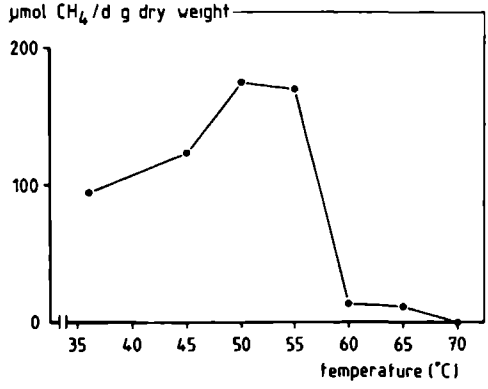

Fig. 1. Methane production rates from windrow samples as a function of incubation temperature.

methanogens using the most probable number (MPN) method. Counts of methanogens ranged from 10^5-10^8/g compost (dry weight) for samples taken from straw bath, flat heap or windrows, while for total anaerobic bacteria values of 10^7-10^9/g compost (dry weight) were calculated.

Experiments under laboratory conditions

By incubation of mixed windrow samples (day 20-28) at various temperatures the optimum temperature for methane production was determined under laboratory conditions (Fig. 1). The temperature optimum was found to be 50-55°C with a slow decrease in methane production at lower temperatures (35-45°C) and a sharp decrease at higher temperatures (60-65°C). No methane production was recorded above 70°C.

The gas atmosphere in the sealed bottles (air; H_2/CO_2, 80%/20% or N_2/CO_2, 80%/20%) appeared to have no effect on initial methane production rate, however under H_2/CO_2 (80%/20%) production stopped after several days.

Samples taken from the outside of a compost stack produced amounts of methane comparable to samples taken from the inside.

Methane production was measured in sealed bottles under standard conditions (air, 55°C) with samples of composting material taken at different stages of the outdoor composting process (Fig. 2). Straw bath samples showed low methane production. Highest values were recorded with flat heap samples and samples from "young" windrows (day 22). This is in agreement with the in situ results presented in Table 1. During Phase I (windrows) we observed an overall decrease in methane production towards the end (day 28). Rather low production rates were measured directly after turning of the windrows.

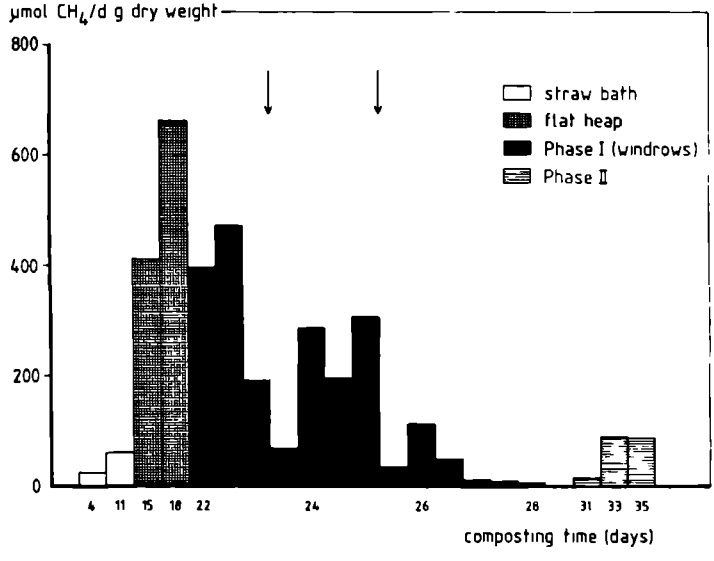

Fig. 2. Methane production rates during the composting process. Samples were taken and incubated as described in Materials and Methods. Arrows indicate turning of the windrows.

For comparison we determined methane production using samples taken at some stages during Phase II. At the start of Phase II (Fig. 2, day 31) values were comparable with values at the end of Phase I (day 27-28). Until the end of Phase II there was an increase in methane production (day 33 and 35). With full-grown compost no methane production was observed.

Isolation of methanogenic bacteria

From a Phase I compost extract a non-motile, rod-shaped methanogenic bacterium was isolated and brought into pure culture on solid incubation medium. Fig. 3 shows a transmission electron micrograph of the isolated methanogen. The isolate occurred as single cells (0.3 x 1.8 µm) and sometimes in chains of 2-4 cells.

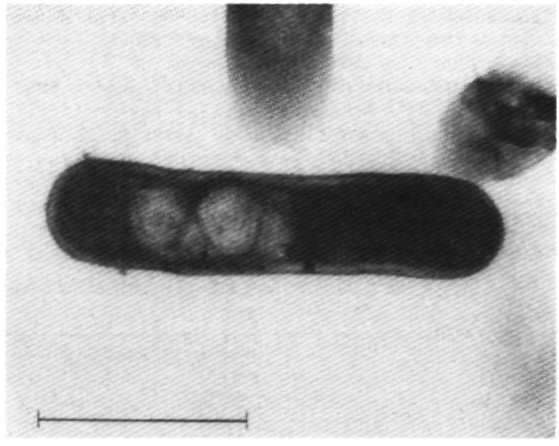

Fig. 3. Transmission electronmicrograph of a methanogenic bacterium isolated from Phase I compost. Bar represents 1 µm.

DISCUSSION

From the results presented it is evident that methane production occurred at all stages of the composting process (Phase I and Phase II). A good correlation exists between direct measurements (Table 1) and experiments under laboratory conditions (Fig. 2). Since methanogenesis is brought about by strictly anaerobic bacteria, anaerobic conditions must occur in the composting material. Samples taken from the outside of the compost stacks were as potent in methane production as samples taken from the inside and the presence of air did not affect methanogenesis in sealed bottles. Therefore we assume anaerobic conditions occur in pockets inside straw fibers. The presence of these anaerobic micro-environments do not exclude the previously proposed anaerobic core of windrows (Eicher, 1981).

The results presented now do not allow a determination of the exact contribution of anaerobic bacteria during the preparation of compost. Since methane is a final product of anaerobic processes, the results indicate that at least 3.3% of the degradation is brought about by anaerobic bacteria. The MPN-method revealed rather high counts of anaerobic bacteria, comparable to those reported for aerobic bacteria (Fermor et al., 1985). The major bacterial species involved in Phase I are members of the genus Bacillus (Fermor et al. 1985). Some of these species isolated from compost are capable of growing under anaerobic conditions (Claus and Berkeley, 1986) and may therefore contribute to the high numbers of anaerobic bacteria.
Full-grown compost did not produce methane. Methanogenic bacteria are at this stage either inactivated or consumed. The latter is supported by Fermor and Wood (1981) who proved that Agaricus bisporus can grow on heat-killed bacteria as sole source of carbon and nitrogen.
A methanogenic bacterium was isolated. It produced high amounts of methane and showed typical fluorescence of coenzyme F_{420} (Doddema and Vogels, 1978). Its shape indicates that the isolated bacterium may belong to the family Methanobacteriaceae. More characteristics, e.g. pH-optimum, temperature optimum, substrate specificity and cofactor composition have to be determined in order to get more information about the exact taxonomic place of the isolated strain. This work is in progress.

SUMMARY

The production of compost as a substrate for the cultivation of Agaricus spp. is considered to be an aerobic process. This paper reports for the first time the production of methane during the Phase I composting process. Analysis of the air present in and emerging from straw bath, flat heap and windrows revealed the presence of 90-4900 ppm methane. During Phase I (windrows) a mean production of 5.9 mol methane/m^2 per day was measured. Total counts of anaerobic and methanogenic bacteria determined by the most probable number method were 10^7-10^9 and 10^5-10^8/g compost (dry weight), respectively. Laboratory experiments with compost samples in sealed bottles revealed a optimum temperature for methane production of 50-55°C. Samples taken at several stages of the composting process all produced methane in amounts varying from 10 to 650 µmol/day per g dry weight. Only full-grown compost did not produce methane. From windrow samples a methane bacterium was isolated and brought into pure culture. The results indicate that methane is formed during all phases of the outdoor composting process. Since methanogenesis is brought about by strictly anaerobic methanogenic bacteria it is evident that anaerobic fermentation is part of the process. The occurrence of anaerobic micro-environments inside straw particles is discussed.

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CHAPTER 4.

ISOLATION AND CHARACTERIZATION OF THERMOPHILIC METHANOGENIC BACTERIA FROM MUSHROOM COMPOST

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Isolation and characterization of thermophilic methanogenic bacteria from mushroom compost

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Key words *Methanobacterium*, Methanogenic bacteria, Mushroom compost, Thermophile

1 SUMMARY

During the first stage of the preparation of mushroom compost oxygen is believed to be readily available However we measured methane in the evoking air above the compost piles and were able to isolate thermophilic methanogenic bacteria from this compost The isolates grow only on H_2 and CO_2 as energy and carbon source and do not require complex factors for growth On the basis of nutritional and morphological characteristics these methanogens were identified as strains of *Methanobacterium thermoautotrophicum*

2 INTRODUCTION

Worldwide production of edible mushrooms is increasing year after year In 1985 the production of *Agaricus bisporus*, the most cultivated mushroom in the western world, exceeded a total of 1 228 000 tons [1] Due to its high, readily digestible protein content and its low caloric value this mushroom fits well in the western diet

Compost used as a substrate for the cultivation of *Agaricus* spp is produced in a two stage process [2] The first stage (Phase I) is done outdoors After wetting, the constituents are thoroughly mixed and placed in windrows with a cross-section of two by two meters for 7 days The second phase (Phase II) is performed indoors under strictly controlled conditions [3]

During Phase I the temperature in the windrows gradually increases to an average temperature of 63°C at the end of Phase I Due to the inhomogeneity of the composting material, and the heat lost on the outer surface, temperature gradients of 25 to 80°C are observed It was shown that thermophilic bacteria are abundant [2] Although no forced aeration is applied in the windrows, Phase I is considered to be aerobic By means of gas sampling, oxygen concentrations are shown to be higher than 5% in 86% of the windrow volume [4]

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However, we found up to 45000 ppm of methane in the evoking air above the compost piles [5] Methane together with carbon dioxide form the main end products of the anaerobic breakdown of organic matter [6] Since only methanogenic bacteria produce methane in reasonable amounts we expected thermophilic methanogenic bacteria to be present in the composting material

The aim of this investigation was to isolate and characterize thermophilic methanogenic bacteria from mushroom compost

3 MATERIAL AND METHODS

3 1 Total counts, enrichment and anaerobic techniques

Mushroom compost was obtained from a commercial farm Immediately after sampling the compost was transported to the laboratory in closed plastic bags No precautions were taken at this stage to prevent cooling nor to exclude oxygen Compost samples were taken at the end of Phase I Total counts of thermophilic methanogenic bacteria were estimated using the most probable number method at an incubation temperature of 55°C

Enrichment was performed by incubating 25 g of Phase I mushroom compost and 5 ml water in sealed bottles at 55°C Methane production was recorded daily After one week 1 ml of liquid was transferred into 100 ml serum bottles, filled with 20 ml of medium 1 [7], modified according to Kiener and Leisinger [8] Bottles were incubated at 55°C under a H_2/CO_2 (80%/20%, v/v) atmosphere in a rotary shaker (200 rpm) Growth on solid medium was performed by adding 2% (w/v) agar to the medium Plates were incubated in anaerobic jars at 55°C without agitation under the desired atmosphere The isolation of the methanogenic bacteria was performed according to Blotevogel et al [9], using strict anaerobic techniques [7]

3 2 Determination of bacterial growth and methane

Optical density measurements at 600 nm in a Zeiss-M4 QIII-spectrophotometer served as indication of growth Methane was determined according to Hutten et al [10], using ethane as an internal standard

3 3 Microscopy

The methanogenic bacteria were detected and viewed by epifluorescence with a Leitz Dialux microscope equipped with a Ploemopak K 2 3 illuminator for incident light [11] Fixation of cells for scanning electron microscopy was done with 2 5% glutaraldehyde 2-Methoxy-ethanol was used for dehydration The cells were dried following the method of critical point drying of Horridge and Tamm [12] and subsequently coated with gold Scanning electron micrographs were taken with a JEOL type JSM T-300 Preparation and fixation for transmission electron microscopy was performed according to Doddema et al [13] Electron micrographs were taken using a Philips 300 electron microscope

3 4 Cofactor analysis

Methanogenic cofactors were extracted and analyzed by HPLC using 7,8-didemethyl-8-hydroxy-5-deazariboflavin (FO) as an internal standard [14,15]

3 5 DNA base composition

Bacteria were disrupted by passage through a French pressure cell at 138 MPa. DNA was isolated and purified from the cell homogenate by the method of Beji et al [16] The thermal denaturation (melting point) performed by the standard procedure of Marmur and Doty [17] was used to determine the mol% G + C

4 RESULTS

4 1 Methane production and total counts

Phase I compost contained up to 2×10^8 thermophilic methanogenic bacteria per gram dry matter On the compost farm we measured an average of 410 ppm methane in the evoking air at the end of Phase I, which implies a production of 25 µmol methane per gram dry matter per day Under laboratory conditions Phase I compost samples produced about 6 µmol methane per gram

dry matter per day Production of methane went on for over three weeks

4 2 Enrichment and isolation

Wet mounts of the cultures taken after one week were examined by fluorescence microscopy Rod-shaped, coccoid and sarcina-like methanogens were recognized by their autofluorescence Rod-shaped bacteria formed the majority among the fluorescent bacteria High amounts of non-fluorescent bacteria were present To reduce the growth of the latter penicillin G (80 μg/ml) and vancomycin (20 μg/ml) were added after transfer to liquid medium After one week of incubation the relative amount of methanogenic bacteria had increased significantly, as shown by fluorescence microscopy In an anaerobic glove box [10] culture samples were streaked onto agar plates, containing medium 1 plus 2% (w/v) agar The plates showed single colonies of the same appearance after one week of incubation at 55 °C under H_2/CO_2 Purity was tested by transferring a single colony to liquid medium 1 with the omission of acetate and formate No growth was observed after 3 weeks of incubation at 55 °C under a N_2/CO_2 (80%/20%, v/v) atmosphere Cultures in liquid medium 1 produced high amounts of methane under H_2/CO_2 atmosphere A good correlation was found between the methane production and the increase of the optical density of the culture at 600 nm (data not shown) Therefore we used the methane production rate as a parameter to determine the doubling time in the following experiments With fluorescence microscopy only rod-shaped bacteria were visible, showing a strong fluorescence All these observations indicated a pure culture The isolate was named strain CNC-1 During the above described procedure we isolated another methanogen, showing only in two aspects a difference with the isolate described so far The colonies on agar plates showed a brighter surface and the susceptibility to sodium chloride was much higher A concentration of 0 2 M increased the doubling time of this isolate to 8 hours This isolate is referred to as strain CNC-2 The results shown hereafter were obtained with strain CNC-1

Although high amounts of methane were observed from enrichments on acetate as sole carbon source we were not yet able to isolate an aceticlastic methanogen

4 3 Cell and colony morphology

Cells stained Gram-positively and were rod-shaped and $0\,3 \pm 0\,05 \times 1\,8 \pm 0\,4$ μm in size Cells showed the tendency to form chains of 2 or sometimes 4 cells (Fig 1A) Neither filaments nor motility were observed using light microscopy Multiplication occurred by binary fission (Fig 1D) Colonies were yellowish green, round, convex and smooth on agar plates, with entire edges Within 7 to 9 days a colony diameter of 2 to 3 mm was attained Scanning electron micrographs showed a smooth outer surface with no evidence of flagella (Fig 1B) The only internal structures visible on ultrathin sections were mesosome-like bodies (Fig 1C), which may be artefacts of preparation [18]

4 4 Growth substrates and factors

H_2 and CO_2 were the only substrates used by the isolate for growth and methane production No growth was observed and no methane was formed after two weeks of incubation with formate, acetate, methanol, methylamines and CO as substrate The isolated bacteria grew well on medium 2 of Balch [7] No decrease in methane production was observed on transferring the bacteria six times on this medium From this it is concluded that no complex factors are needed for growth and the isolated bacterium is capable of autotrophic growth

4 5 Effect of temperature, salt and pH on growth and methane production rate

Optimal temperature for growth was determined under static conditions The total amount of methane produced during 7 h of incubation served as an indicator for growth The isolated bacterium grew with an optimal doubling time of about 5 h at temperatures between 65 and 70 °C No growth was found above 80 °C (Fig 2) In a rotary shaker at 250 rpm a doubling time of 1 2 h was found at 55 °C

Sodium chloride concentrations up to 0 7 M in the medium had no significant influence on the methane production rate At a concentration of 1

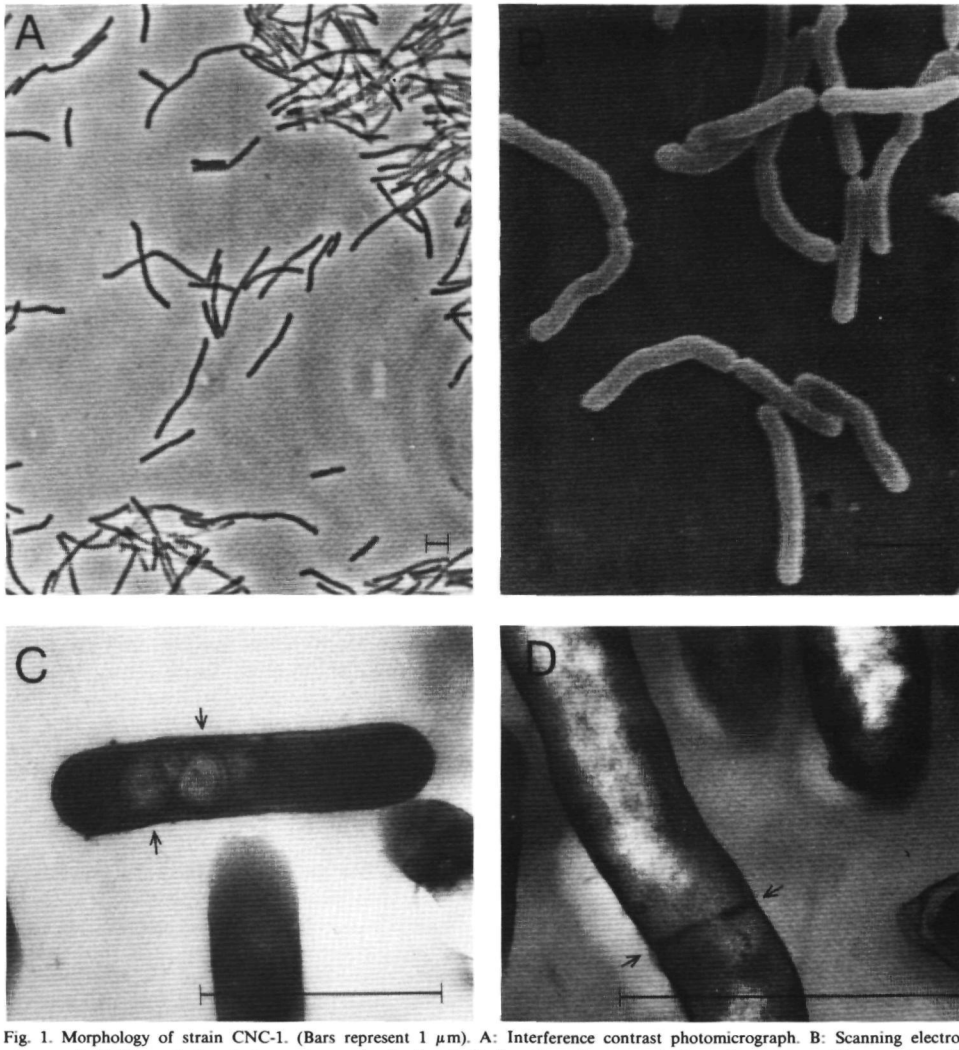

Fig. 1. Morphology of strain CNC-1. (Bars represent 1 μm). A: Interference contrast photomicrograph. B: Scanning electron micrograph. C: Transmission electron micrograph of a longitudinal section; the arrows indicate the mesosome-like structures. D: Transmission electron micrograph showing a bacterium during binary fission (arrows).

M growth was strongly retarded (Td 55 h). By changing the amount of sodium hydrogen carbonate in the buffer different pH values of the medium were obtained. Although the influence of different concentrations of sodium ions seemed to be minor, the total concentration of sodium ions was adjusted with sodium chloride to 0.57 M.

The highest growth rates were obtained at pH

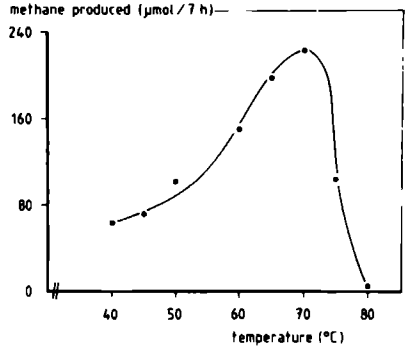

Fig 2 Methane production after 7 h of incubation as function of temperature Points represent the means of triplicate experiments.

values between 6.75 and 6.95. Good growth was even observed at a pH above 8.5 and as low as pH 6.3 (Fig. 3). In the presence of SDS (sodium dodecyl sulfate) growth was slightly inhibited at a concentration of 0.015% (w/v), and was completely absent at 0.03% (w/v), but no lysis occurred up to 0.03% (w/v) SDS.

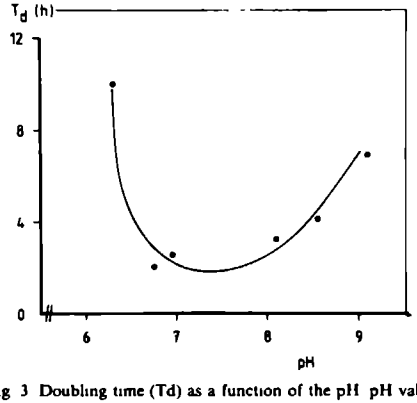

Fig 3 Doubling time (Td) as a function of the pH pH values are registered prior to the experiment in parallel bottles and in the incubated bottles immediately after the experiment Changes in pH were always less than 0.2. The mean value is taken as the pH of incubation.

Table 1

Cofactor content of the isolated methanogen *Methanobacterium thermoautotrophicum* strain ΔH

Compound	Cofactor content (μmol/g protein)	
	New isolate	*M thermoautotrophicum*
Coenzyme F420-3 [a b]	0 06	0 26
Coenzyme F420-2 [a]	1 11	2 01
Coenzyme F420-2 [c]	1 09	2 80
Methanopterin [c]	92 1	117 2
Vitamin B_{12}-HBI [c d]	0 19	0 07

[a] as determined by fluorometric detection at 405-470 nm (excitation-emission wavelengths)
[b] the number of glutamate residues in the side chain is indicated by −3 and −2
[c] as determined with UV-spectrophotometer at 250 nm
[d] HBI 5-hydroxybenzimidazolylcobamide

4.6. DNA base composition and cofactor analysis

The mol% G + C of both the isolated bacterium and *Methanobacterium thermoautotrophicum* strain ΔH were determined, revealing 48.1 and 47.0 mol% G + C, respectively. The cofactor analysis was performed with HPLC-system V for the isolated bacterium and *M. thermoautotrophicum* strain ΔH, grown under identical conditions [15]. A striking resemblance between the two strains is found (Table 1).

5. DISCUSSION

Until now methanogens are isolated from places where the absence of oxygen is obvious, e.g. thermal volcanic environments, sewage sludge digesters, sediments and rumen [6,19]. Nevertheless methanogenesis is also reported for environments where oxygen production occurs under light [20,21]. Here we report the isolation of a methanogen from mushroom Phase I compost, an environment, where oxygen is believed to be readily available. The high number of methanogenic bacteria found in Phase I compost implies that this population is about equal in number to the aerobic thermophilic population [2].

In pure culture, methanogens are more sensitive to oxygen in comparison to their natural environment. Here they often are associated with faculta-

tively anaerobic bacteria providing protection against oxygen. Exposure to air reduced the number of colonies formed on plate dramatically as shown by Kiener and Leisinger [8]. For *Methanobacterium thermoautotrophicum* strain Marburg no viable bacteria were found after less than one day of exposure The presence of methane in the evoking air of the windrows is a strong evidence for the occurrence of anaerobic environments [5] The isolation of a methanogenic bacterium from the composting material confirms this view Although oxygen may be present on macro scale throughout the windrow, anaerobic micro niches may occur due to a high metabolic activity of aerobic and facultatively anaerobic bacteria

The isolated bacteria show no significant morphological and nutritional differences with *M thermoautotrophicum* strain ΔH [22] Further evidence for their similarity was obtained from the mol% G + C and the cofactor content, determined after growth under similar conditions. The difference found in mol% G + C amounts to 1 1%. According to Schleifer and Stackebrandt [23] the difference should be at least 5% to distinguish between two different species Moreover Touzel et al. [24] summarized different mol% G + C values including *M thermoautotrophicum* strains ΔH and Marburg Depending on the method used the values for *M thermoautotrophicum* strain ΔH ranged from 48 6 to 52%. Differences in cofactor content between our strain and strain ΔH are small compared with the values of a variety of different strains of methanogenic bacteria [14].

On the basis of these similarities the isolates should be regarded as strains of *M thermoautotrophicum* and they will be indicated as *M thermoautotrophicum* strain CNC-1 and *M thermoautotrophicum* strain CNC-2.

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CHAPTER 5.

ODOROUS SULFUR COMPOUNDS EMITTED DURING THE PRODUCTION OF COMPOST USED AS A SUBSTRATE IN MUSHROOM CULTIVATION

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Odorous Sulfur Compounds Emitted during Production of Compost Used as a Substrate in Mushroom Cultivation

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Large scale composting facilities are known to cause environmental problems, mainly through pungent air emitted by composting material. In air samples taken above stacks set up to prepare compost used as a substrate in mushroom cultivation, several volatile compounds were identified by means of the coupled techniques of gas chromatography and mass spectrography. Among the compounds identified, sulfur containing compounds [H_2S, COS, CH_3SH, CS_2, $(CH_3)_2S$, $(CH_3)_2S_2$, and $(CH_3)_2S_3$] are the most conspicuous in causing a nuisance. Quantification of these compounds was performed by concentrating a relatively small air sample on Tenax GC. The sampling method appeared to be very useful under field conditions. During the composting process, the concentration of the volatile sulfur compounds in emitted air ranged from 1 to 35 μmol/m^3. The highest concentrations were obtained at the end of the outdoor process. Total sulfur emission amounted to 8.3 mg of sulfur per kg (fresh weight) of compost. The end product still contained 2.58 g of sulfur per kg (fresh weight) of compost. Suggestions about the origin of the volatile sulfur compounds are made.

Among the edible fungi *Agaricus* spp. constitute more than 75% of the total world production. In The Netherlands 115,000 tons of mushrooms were produced in 1987 (15). The substrate for the cultivation of *Agaricus* spp. is a specially prepared compost. Straw, rich horse manure and chicken manure are the main constituents. As a result of the steadily growing demand for compost, the amount of horse manure is no longer sufficient and therefore wheat straw has become a more important basic constituent. The process of composting has been described in detail in previous papers (6, 22).

During phase I of the composting process, microbial degradation of readily available organic compounds is stimulated by thorough wetting and mixing of the constituents. On day zero, prewetted wheat straw and horse manure are mixed and set up in a large pile. On day 8, gypsum and chicken manure are added. After thorough mixing, the material is placed in stacks (approximately 1.8 by 1.8 m in cross section) for 7 more days. In 2 weeks, the outdoor process is completed, resulting in a dark brown product.

In the course of phase I, the temperature rises gradually to an average of 63°C at the end of phase I. Because of heat loss at the outside of the stacks, temperature gradients range from 25 to as high as 80°C in the inner parts of the stacks. As a result of these temperature gradients, air moves through the stacks and water vapor and malodorous compounds are transported to the environment. During phase I, considerable amounts of ammonia are released as a result of ammonification (21), but the identities of other compounds present in the air are still unknown. Miller and Macauley (13) compiled a list of compounds implicated in odors from different composting materials. Among these, especially sulfur- or nitrogen-containing volatile compounds are known to cause a nuisance at very low concentrations (23). The present paper deals with the identification and quantification of several volatile sulfur compounds present in air emitted from stacks during phase I of the composting process performed to produce a substrate for mushroom cultivation.

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MATERIALS AND METHODS

Sampling site. Samples of effluent air above compost stacks were taken. A bottomless stainless steel barrel (1.20 m tall, inner diameter 0.64 m) was placed on top of the stacks to minimize the influence of wind (Fig. 1). The barrel had an opening at the top (inner diameter 2.5 cm) and a small sampling tube at the side which ended in the center. After positioning of the barrel, the air inside was allowed to stabilize for at least 15 min. Air temperature and velocity were measured at the top opening with a Wallac GGA 23S thermoanemometer.

Sulfur determination. To measure total organic and inorganic sulfur contents, compost samples were dried at 70°C and milled to pass through a 5-mm (aperture diameter) sieve. For total sulfur analysis, samples were degraded as described by Tabatabai and Bremner (19). For inorganic sulfur analysis, samples were treated in the same way after being ashed at 550°C. Sulfate was measured by the inductively coupled plasma technique (3). Organic sulfur content was taken as the difference between the total and inorganic sulfur contents. Standard addition of methionine and $CaSO_4 \cdot 2H_2O$ to samples resulted in recovery of 80 ± 10 and 100 ± 11%, respectively, of the total sulfur estimations. When inorganic sulfur was estimated, the same additions yielded recoveries of 2 ± 8 and 97 ± 9%, respectively. This proves that organic sulfur is completely volatilized by the ashing procedure.

Qualitative analysis of air samples. Large volume (30-liter) air samples were obtained via the top opening of the barrel with a Dupont P 4000 pump. Air was sucked through charcoal tubes (70 by 4 mm [inner diameter]) consisting of two compartments. The first contained 100 mg of charcoal and the second contained 50 mg and served as a control to test overloading (25). Tubes were positioned near the center of the barrel and connected with Teflon tubing to the pump.

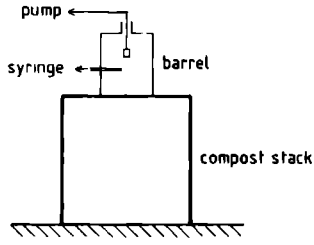

FIG. 1. Schematic diagram of the air sampling procedure used in this study. For more details, see the text.

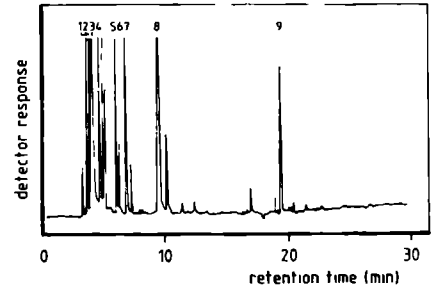

FIG. 2. Typical chromatogram obtained with system A by injection of 1 μl of the carbon disulfide phase after desorption. Unequivocally identified peaks are numbered as follows: 1, propanone; 2, dimethyl sulfide; 3, carbon disulfide; 4, butanone; 5, 3-methylbutanone; 6, benzene; 7, 2-pentanone; 8, dimethyl disulfide; 9, dimethyl trisulfide.

Contact of the air sampled with any material before entering the tubes was avoided. The sample rate was adjusted to 500 ml/min and was checked before and after sampling. Tubes were closed with Teflon plugs immediately after sampling and stored at 4°C until analyzed. The charcoal was removed from the tubes, and compounds bound to the charcoal were desorbed with a minimal amount of carbon disulfide at room temperature for at least 1 h. Alternatively, benzyl alcohol was used in the same way to examine the occurrence of carbon disulfide in the air samples (10). Liquid samples obtained in this way were injected into a Hewlett-Packard 5790A gas chromatograph using either a flame ionization detector or a VG analytical 7070E mass spectrograph. Details of the gas chromatographic system are summarized in Table 1 (system A). The temperature of the mass spectrograph source and the ionization energy were 190°C and 70 eV, respectively.

Quantitative analysis of air samples. Small-volume (60-ml) air samples were taken from the side opening of the barrel with a Monoject syringe equipped with a Teflon piston. The sampling procedure used was that of Tangerman (20). Excess water was removed by passing the sample through a tube containing $CaCl_2 \cdot 2H_2O$. Thereafter, the sample was passed through a tube (80-mm length, 4-mm inner diameter, 6-mm outer diameter) containing 200 mg of Tenax GC (80/100 mesh). To trap volatile compounds, the Tenax tube was cooled in liquid nitrogen (−196°C). The Tenax tubes were kept at −196°C until analysis was performed. Tangerman (20) showed a negligible loss of volatile sulfur compounds on storage of the tubes for 1 week at −196°C. Analyses were performed within 24 h on a Packard 438A gas chromatograph equipped with a packed glass column. The specifications of the two different systems are summarized in Table 1 (systems B and C). The special design of the inlet system of the gas chromatograph enabled replacement of a Tenax tube within seconds (20). As suggested in the literature (4, 18), the flame photometer was calibrated for every compound separately with authentic compounds, a range of 40 pmol to 1.7 nmol was used. Dilutions were made by using the single-rigid-chamber method as described by Barratt (2). To avoid adsorption, all glassware was treated with dichlorodimethylsilane (5% [wt/vol] in toluene) before use.

Chemicals. Dimethyl trisulfide was purchased from Eastman Kodak Co., Rochester, N.Y. All other chemicals originated from E. Merck AG, Darmstadt, Federal Republic of Germany.

RESULTS

Qualitative analysis of air samples. Figure 2 shows results of a typical qualitative analysis with system A (Table 1). Similar analyses were performed by using the mass spectrograph as a detector. The resulting mass spectra were compared with those of authentic compounds to identify the components. As additional evidence, retention times were checked with authentic compounds by using three gas chromatographic systems (Table 2). Analysis of the second section of the charcoal tube showed no signals other than

TABLE 1. Gas chromatographic systems used for identification and quantification of volatile compounds emitted during production of compost used as a substrate in mushroom cultivation

System	Packing	Column specifications				Gas chromatograph specifications			Temp program specifications				
		Length (m)	Outside diam (mm)	Inside diam (mm)	Nitrogen flow (ml/min)	Detector	Detector temp (°C)	Injector temp (°C)	Initial temp (°C)	Initial time (min)	Temp rise (°C/min)	Final temp (°C)	Final time (min)
A	CP Sil 5 CB	25	0.45	0.32	15	FID[a]	300	270	40	10	10	200	10
B	20% SE-30 on Chromosorb PNAW (60/80 mesh)	1.50	6.0	4.0	90	FPD[b]	190	190	50	3	35	190	3
C	Carbopack B HT100 (40/60 mesh)	2.00	6.0	4.0	90	FPD	190	190	80	1	30	140	27

[a] FID, Flame ionization detector.
[b] FPD, Flame photometric detector.

TABLE 2 Physical properties and retention times of authentic compounds identified in the air above compost stacks during production of a substrate for mushroom cultivation

Compound	Boiling point (°C)	100% recognition level (ppm [μl liter])	Odor index at 20°C[a]	Retention time (min)		
				System A	System B	System C
Hydrogen sulfide	−60.7	1	1.70×10	x	0.46	0.49
Carbonyl sulfide	−50.2	[e]	—	x	0.49	0.78
Ammonia	−33.35	55	1.67×10^5	x	x	x
Methanethiol	6.2	0.035	5.33×10^7	x	1.12	1.15
Dimethyl sulfide	37.3	0.1	2.76×10^6	4.01	4.04	2.98
Carbon disulfide	46.3	0.3	1.60×10^6	4.15	1.52	3.31
Propanone	56.2	300	7.20×10	3.71	x	x
Butanone	79.6	6	1.54×10^4	4.84	x	x
3 Methylbutanone	94.5	5	—	6.35	x	x
2 Pentanone	102.0	10	2.00×10^3	7.22	x	x
Dimethyl disulfide	109.7	0.006	—	10.11	5.39	7.86
Dimethyl trisulfide	165[e]	10 [e]	–	19.35	7.10	26.1

These data are from references 23 and 24

[a] Defined as the ratio between the vapor pressure at 20°C and the 100% recognition level both expressed in parts per million (microliters per liter) (23)

[b] x not determined or not detectable with the system used

[c] — Data not available in the literature

[d] Datum from reference 16

[e] Datum from reference 5

those resulting from carbon disulfide which was used in the desorption procedure The carbon disulfide used contained small amounts of benzene (data not shown) Application of benzyl alcohol as a desorption solvent revealed the presence of carbon disulfide in the air sampled As expected the benzene signal at 6 63 min was not found in this analysis The physical and odorous properties of the components identified are given in Table 2 As shown by the values of the odor index, the sulfur compounds were much more readily detected by the human nose than for example ammonia or 2 pentanone For that reason, our attention was focused on the volatile sulfur compounds To investigate the presence of other volatile sulfur compounds which were not trapped on charcoal tubes because of their low boiling points small volume air samples were concentrated on Tenax GC at −196°C Subsequent analyses on systems B and C revealed the presence of hydrogen sulfide carbonyl sulfide and methanethiol in the samples (Table 2) Identification of these compounds was based on their retention times on two different systems in combination with the use of the sulfur specific detector Besides the volatile sulfur compounds mentioned no unidentified peaks were observed

Quantitative analysis of air samples. All of the volatile sulfur compounds mentioned in Table 2 were measured quantitatively in one run on a gas chromatograph equipped with a flame photometric detector using system C (Fig 3) A plot of the logarithm of the peak area versus the logarithm of the amount of sulfur injected showed a linear relationship over the range of 40 pmol to 1 7 nmol The slope of these calibration curves approached the theoretical value of 2 (20)

To estimate the total amount of volatile sulfur compounds emitted during preparation of the mushroom substrate air samples were taken at different stages of the phase I composting process Mixing of prewetted straw with horse manure occurred on day zero Air samples showed distinct changes in the concentrations of the volatile sulfur compounds (Fig 4) During the first 5 days of the process dimethyl sulfide was emitted at a 10 times higher concentration than any of the other compounds After day 5 the dimethyl sulfide concentration increased by a factor of only about 2 but the concentrations of the other volatile sulfur compounds increased by 1 order of magnitude The total emission of volatile sulfur compounds per kilogram of compost produced was calculated on the basis of the concentrations and the air flow rates through the compost stacks The results are presented in Table 3 Dimethyl sulfide was the main component emitted during the first half of the process After day 10 its contribution was exceeded by methanethiol carbon disulfide and dimethyl disulfide Emission of dimethyl trisulfide was small and remained rather constant during the entire process The total loss of sulfur as volatile sulfur compounds amounted to 8 2 mg of sulfur per kg (fresh weight) of compost

Total organic and inorganic sulfur analysis (n = 10) of the product produced by the phase I composting process revealed 0 33 ± 0 81 and 2 24 ± 0 60 g of sulfur per kg (fresh weight) respectively

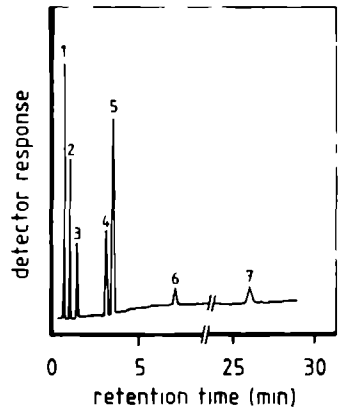

FIG 3 Chromatogram obtained with system C by direct injection of 0 1 ml of a calibration gas mixture containing 63 pmol of hydrogen sulfide (peak 1) 63 pmol of carbonyl sulfide (peak 2) 63 pmol of methanethiol (peak 3) 100 pmol of dimethyl sulfide (peak 4) 125 pmol of carbon disulfide (peak 5) 85 pmol of dimethyl disulfide (peak 6) and 40 pmol of dimethyl trisulfide (peak 7)

TABLE 3 Emission of volatile sulfur compounds during production of the compost used as a substrate in mushroom cultivation

Processing period (days)	Mean air flow rate ($m^3 m^{-2}$ per h)	Concn (μmol kg [fresh wt] of product) of						
		H_2S	COS	CS_2	CH_3SH	$(CH_3)_2S$	$(CH_3)_2S_2$	$(CH_3)_2S_3$
0–3.5	4.56	0.3	1.1	2.0	1.6	9.6	0.6	0.7
3.5–7.0	3.97	0.3	1.3	1.7	0.7	6.8	1.3	1.0
7.0–10.5	4.93	11.2	9.8	2.9	10.2	12.0	1.3	0.8
10.5–14.0	7.19	11.1	11.9	24.3	19.8	13.4	26.9	1.6

DISCUSSION

The use of a charcoal adsorption technique, common in environmental and industrial hygiene (25), appeared to be effective in concentrating a large range of different compounds present in air emitted from stacks set up to prepare a mushroom substrate. Application of different desorption solvents provides special advantages depending on the analytical system used. The sensitivity of the flame ionization detector for carbon disulfide is extremely low, as shown by the rather small solvent peak in Fig. 2. Therefore the appearance of carbon disulfide in the beginning of the chromatogram does not interfere with the other components present. However, when a mass spectrograph is used as a detector, elution of the solvent used causes a disturbance of the high vacuum of the mass spectrograph, prohibiting mass spectrographic analysis until the vacuum is restored. When special interest is taken in the identity of components in the beginning of the chromatogram, the use of benzyl alcohol as a desorption solvent is highly favorable. Use of benzyl alcohol in system A led to a large solvent peak at a retention time of about 13 min.

Among the compounds identified (Table 2), volatile sulfur compounds exhibited the highest odor index, caused by a high vapor pressure at 20°C and a threshold as low as 10^{-5} ppm (10^{-5} μl/liter). Organoleptic qualification of the odor of volatile sulfur compounds is often as foul or pungent (13, 23). Therefore, volatile sulfur compounds have a high nuisance potential, even at very low concentrations. Although the charcoal technique may be used for quantification purposes as well, large air sample volumes and elaborate sample preparation are required. Use of a sulfur sensitive detector and a sophisticated sampling technique allowed reduction of the air sample volume to 60 ml. No further sample preparations were necessary, and the Tenax sampling tubes could be reused several times without loss of efficiency.

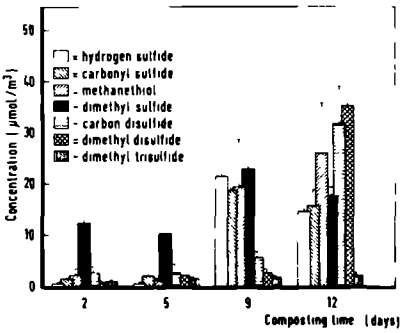

FIG. 4. Concentrations of volatile sulfur compounds in air emitted from compost stacks. The bars represent means of six independent sampling sites, and the standard errors of the means are indicated. Analyses were performed in triplicate at each sample site.

Both the air flow rate through the stacks and the measured concentrations of most volatile sulfur compounds increased during the composting process. This implies an increase in the net release of these compounds by the composting material.

However, the total amount of sulfur (8.2 mg of S per kg [fresh weight]) emitted as volatile sulfur compounds was small compared with either the total organic (0.33 ± 0.81 g of S per kg [fresh weight]) or the total inorganic (2.24 ± 0.60 g of S per kg [fresh weight]) sulfur content of the end product. On the basis of the sulfur analyses of the basic constituents (straw, horse manure, chicken manure, and gypsum) and the amounts needed to obtain 1 kg of phase I compost, organic and inorganic sulfur contents of 0.27 ± 0.41 and 3.09 ± 0.36 g of S per kg (fresh weight) were calculated. As a result of the high standard deviations, no significant loss of either organic nor inorganic sulfur was observed. The high inorganic sulfur content was due to addition of gypsum to the manure mixture (8). Nevertheless, even when gypsum is omitted, sufficient sulfur is present in the composting material to account for the emission reported here. Wheat straw, as the major constituent of the composting material (8), contains 1.3 ± 0.4 mg of S per g of dry matter ($n = 10$). Although its contribution on a sulfur basis is the lowest of all of the constituents used, wheat straw alone accounts for 0.15 ± 0.03 g of inorganic and 0.02 ± 0.05 g of organic sulfur per kg (fresh weight) in the end product. As emission of volatile sulfur compounds cannot be avoided by elimination of sulfur from the material, changes in the process will be necessary to reduce environmental problems.

Production of volatile sulfur compounds from various biologically active systems, including soils (7), breweries (14), and algal mats (26), has been reported. The involvement of microorganisms has been reported (12), and production of volatile sulfur compounds has been stimulated by addition of sulfur containing amino acids (1). Further studies are needed to resolve the agents and substrates involved in the production of volatile sulfur compounds during production of the compost used as a substrate in mushroom cultivation. To control the production of these compounds and handle the air emitted, indoor composting would be an attractive alternative. Although reports about indoor composting date from the early seventies on (11, 17), only recently were promising results obtained (9).

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CHAPTER 6.

EVOLUTION OF VOLATILE SULFUR COMPOUNDS DURING LABORATORY SCALE INCUBATIONS AND INDOOR PREPARATION OF COMPOST USED AS A SUBSTRATE IN MUSHROOM CULTIVATION

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ABBREVIATIONS

COS, carbonyl sulfide; DMDS, dimethyl disulfide; DMS, dimethyl sulfide; DMTS, dimethyl trisulfide; FW, fresh weight; MT, methanethiol.

SUMMARY

Volatile sulfur compounds are known to be produced during the preparation of compost used as a substrate in mushroom cultivation. Because they cause odor problems attempts are undertaken to reduce the production of these compounds. The influence of temperature and various additions on the production of volatile sulfur compounds from composting material were tested on laboratory scale. The production of H_2S, COS, CH_3SH and $(CH_3)_2S$ was proven to be a biological process with an optimal temperature which coincides with the optimal temperature for biological activity. The formation of CS_2 and $(CH_3)_2S_2$ was shown to be an nonbiological process. The emission of volatile sulfur compounds during the indoor preparation of mushroom compost appeared to be remarkably reduced (about 90 %) as compared to the conventional outdoor process. Introduction of this indoor composting process would result in a significant reduction in environmental pollution.

INTRODUCTION

Recently we reported the evolution of volatile sulfur compounds during the preparation of compost, used as a substrate for the edible mushroom *Agaricus bisporus* [6]. The smell of many of these compounds is described as pungent [18,25] and therefore the emission of volatile compounds by composting facilities is a source of complaints by people living in the surroundings and furthermore an environmental stress. The production of volatile sulfur compounds from many different biological systems was reported, e.g. surface-ripened cheeses [8], the ruminant intestinal track [20] and marine environments [5,15,16]. The role of microorganisms in these processes has been reviewed in detail [4,13]. Sulfur containing amino acids, mainly methionine and to a lesser extent cysteine, were shown to be the precursors [2,15]. In the marine environment dimethyl sulfonium propionic acid, an

osmoregulatory compound in algae, is the precursor for the production of DMS [5].

Furthermore, the breakdown of volatile sulfur compounds by various bacteria was demonstrated [14,19,21,23]. Since compost stacks are a very complex environment it may be anticipated that simultaneous production and consumption of volatile sulfur compounds occurs. Reduction of the emission of these compounds can be achieved by decreasing the production rate or by increasing the degradation rate or both. Further insight in the precise origin of the volatile sulfur compounds and the influence of the process parameters of the composting process on the production and consumption rate of these compounds is needed to develop a composting regime, which would result in a minimal emission of volatile sulfur compounds.

The outdoor composting process presently in use was described in detail previously [6,11]. The constituents are mixed and placed in piles for about two weeks. No temperature or aeration control is performed during this outdoor stage (Phase I). Phase I is followed by an indoor treatment of the composting material in bulk (Phase II). In this phase, both temperature and aeration are under close control [10]. The resulting material is suitable for inoculation with the edible mushroom, *A. bisporus*. Recent research revealed that the duration of Phase I could be reduced by one week. Omission of the treatment in windrows during Phase I, followed by a standard Phase II treatment resulted in a substrate from which a normal yield was obtained. This indoor preparation of mushroom substrate is called indoor composting [12].

The aim of these investigations is to extent the knowledge on the production of volatile sulfur compounds during the composting process. Therefore the effects of the incubation temperature and different additions on the production of these compounds by composting material under well-defined laboratory conditions were studied and the amount of volatile sulfur compounds emitted during the newly developed indoor composting process was quantified.

Compost samples

Compost samples were obtained from a commercial composting facility. Compost samples taken at the end of Phase I and at the end of Phase II will be referred to as Phase I compost and Phase II compost, respectively. Subsamples were randomly picked from different stacks representing the same stage of composting and thoroughly mixed prior to analysis or compost incubations.

Compost incubations

Subsamples (10 - 100 g FW) were incubated in sealed 0.5 or 1 l serum bottles for 24 hours at the temperatures indicated. Addition of methionine and potassium sulfate was performed by mixing the chemicals as dry powder among the compost (3.4 mmol.kg FW^{-1}). The occurrence of sulfate reduction was tested under waterlogged conditions [3], with the use of 0.05 M phosphate buffer pH 7.2. Sodium molybdate, a specific inhibitor of sulfate reduction [1,22], was added to a final concentration of 20 mM. Sterilization was performed at 121 °C for 1 hour. Efficiency of sterilization is checked by recording oxygen consumption and carbon dioxide production versus time. After treatment a neglectable oxygen consumption and carbon dioxide production was observed within 24 hours (data not shown) indicating the complete inactivation of the aerobic microorganisms.

Indoor composting

Indoor composting was performed in the experimental tunnels described by Gerrits [10,12]. Straw-rich horse manure, used as the basic constituent, was prewetted for 5 days and then gypsum and chicken manure were added, 25 and 100 kg per 1000 kg FW, respectively. After mixing well the material was placed in containers containing 1000 kg FW each. Four containers occupied one tunnel corresponding to 825 kg $FW.m^{-2}$. Immediately after filling the temperature was allowed to raise up to 56 °C and kept at this level for 8 hours. Then the temperature was lowered and maintained at 45 °C for six days. In total the indoor composting process took 160 hours. Temperature was controlled by regulating the supply of fresh air, no external heating was used. During the entire process an air recirculation rate of 140 $m^3.m^{-2}.h^{-1}$ through the composting material was maintained. As shown by Gerrits [12],

mushroom yields from indoor compost did not differ from yields obtained from conventionally prepared compost.

Analytical procedures

Dry weight content of the compost samples was calculated on basis of the weight loss after drying at 105 °C. The gaschromatographic analysis of air samples and calibrations with authentic volatile sulfur compounds were essentially the same as described previously [6]. Injections were performed either direct or after concentration of a large volume of the gas phase (60 ml) on Tenax tubes [6,24]. Oxygen and carbon dioxide were analysed by means of a gas chromatograph equipped with a thermal conductivity detector [7].

Chemicals

Dimethyl trisulfide was purchased from Eastman Kodak Co., Rochester, N.Y. All other chemicals originated from E. Merck AG, Darmstadt, Federal Republic of Germany.

RESULTS

Phase I compost samples were incubated at different temperatures and the production of volatile sulfur compounds was measured after 24 hours. Because of slight fluctuations in dry matter content of the different compost samples used the results are expressed as μmol.kg dry matter^{-1}.day^{-1}. The average results are represented in Fig. 1. Maximum production of H_2S and MT were 10 to 1000 times higher (Fig. 1A) as compared with the maximum production of the other volatile sulfur compounds (Fig. 1B). All the compounds, except CS_2 and DMDS, show a maximum production between 56 and 70 °C. Over the temperature range tested CS_2 and DMDS showed an increasing production with temperature. For Phase II compost samples a similar tendency was observed (data not shown). To test the involvement of biological processes in the production of volatile sulfur compounds compost samples were sterilized prior to incubation. The production was measured for Phase I and Phase II compost samples at 56 °C and 45 °C, respectively. In Table 1 the results are presented together with these obtained with untreated compost, which served as a control. The production of all volatile sulfur compounds, except CS_2 and DMDS, dropped dramatically upon sterilization for both composts. DMTS was not

found in measurable quantities in any sample.

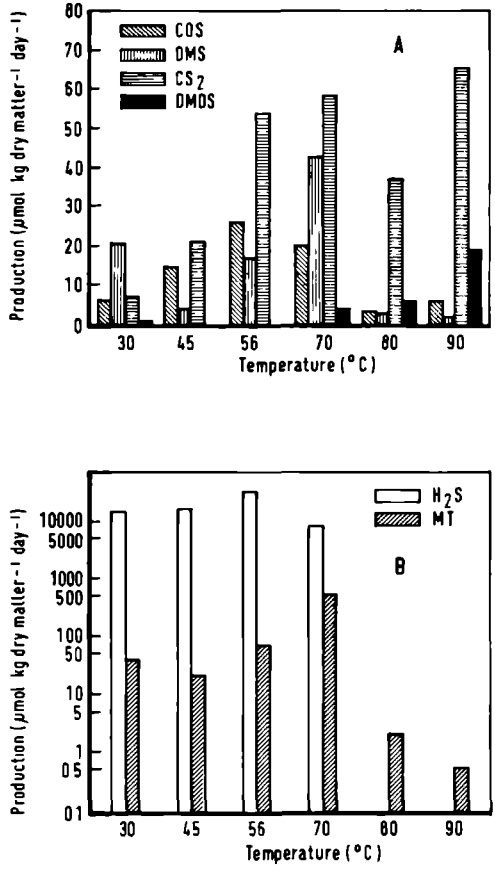

Figure 1. Temperature dependence of the production of volatile sulfur compounds by Phase I compost (25 g FW per 0.5 l bottle).

Table 2 shows the results of the additions of an organic or inorganic sulfur compound on the production of volatile sulfur compounds by Phase I compost. The addition of potassium sulfate resulted in a slight increase in the production of H_2S and DMS, while the addition of methionine resulted in a 27-fold increase in the production of MT. Simultaneously the production of

Table 1. The production of volatile sulfur compounds by Phase I and Phase II compost (25 g FW per 0.5 l bottle) with or without sterilization.

Compost sample	Sterilization (1 h, 121 °C)	Incubation temperature	Production rate[a] H_2S	COS	MT	DMS	CS_2	DMDS	DMTS
Phase I	-	56	31300	26	69	17	54	0	0
Phase I	+	56	0	6	0	1	49	9	0
Phase II	-	45	9300	16	140	20	12	0	0
Phase II	+	45	1	1	0	0	13	4	0

Values are means of three experiments

[a]) Values expressed as μmol.kg dry matter^{-1}.day^{-1}

DMDS increased about three times (Table 2). To elucidate whether the high production obtained for H_2S was due to the presence of sulfate reducing bacteria, sodium molybdate was added to a final concentration of 20 mM in a phosphate buffer (50 mM, pH 7.2) (Table 3). Blank incubations were performed with a equal amount of buffer alone. The amount of buffer was sufficient to create a waterlogged situation for all the compost particles, so intense contact between the inhibitor and the microorganisms is ensured. The

Table 2. The relative influence of additions on the production of volatile sulfur compounds by phase I compost at 56 °C (25 g FW per 0.5 l bottle).

addition	Relative increase in production rate[a] H_2S	COS	MT	DMS	CS_2	DMDS	DMTS
Sulfate	2.0	nd[b]	1.8	3.9	nd	1.0	nd
Methionine	1.5	nd	27	1.0	nd	2.8	nd

[a]) Values expressed as the ratio between the production obtained with and without addition

[b]) nd = not determined

Table 3. Influence of sodium molybdate on the production of volatile sulfur compounds by Phase II compost at 45 °C, measured in a waterlogged situation.

Addition	Production rate[a]						
	H_2S	COS	MT	DMS	CS_2	DMDS	DMTS
Phosphate buffer 0.05 M, pH 7.2	1050	1	108	3	1	0	0
Phosphate buffer + Molybdate 20 mM	0	3	42	5	0	0	0

[a]) Values expressed as μmol.kg dry matter^{-1}.day^{-1}

influence of the addition of buffer alone is small as can be seen when the results are compared with those for Phase II without buffer addition (Table 1). No H_2S was found in incubations to which sodium molybdate was added. Furthermore the production of MT was slightly reduced. Another indication that anaerobic processes play an important role in the production of volatile sulfur compounds came from time dependent measurements of the oxygen concentration in bottles with various amounts of Phase I or Phase II per bottle. These experiments revealed that oxygen was completely used within 6 hours when 100 g FW of compost was present. In the presence of 10 g FW compost oxygen was still present after 24 hours at a level of about 10 % (data not shown). The production of volatile sulfur compounds during these incubations are shown in Fig. 2. The observed production of H_2S and MT were at least 3 orders of magnitude higher under anaerobic conditions. A similar tendency was observed for COS, DMS and CS_2 although the effect did not exceed one order of magnitude.

Temperature has proven to be an important parameter in the observed production of volatile sulfur compounds on laboratory scale (Fig. 1). During indoor composting temperature is kept at values below the optimum temperature for the production of volatile sulfur compounds. To test the influence of the indoor composting regime on the total production of volatile sulfur compounds air samples were collected during three composting trials at different intervals and analysed for volatile sulfur compounds. In Fig. 3 concentrations in the effluent air from one trial are presented as a function of the process time. DMS was found at highest concentrations over the entire

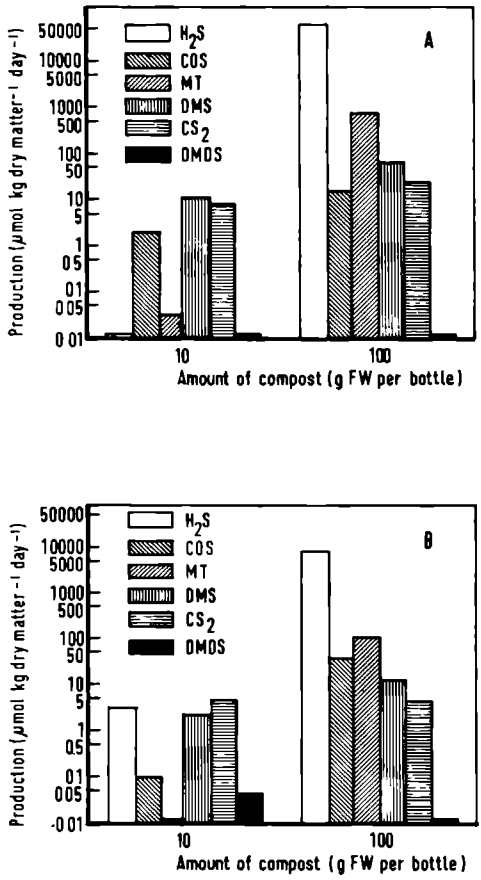

Figure 2. Production of volatile sulfur compounds compost, starting with 10 or 100 g FW of composting material in 1 l bottle. A: Phase I compost, B: Phase II compost

process. MT was only detectable during the first two days, whereas DMDS and DMTS were already below the detection limit (<0.3 $\mu mol.m^{-3}$) after one day. No H_2S was measured in any of the air samples. From the concentrations of volatile sulfur compounds and the fresh air supply rate total production per

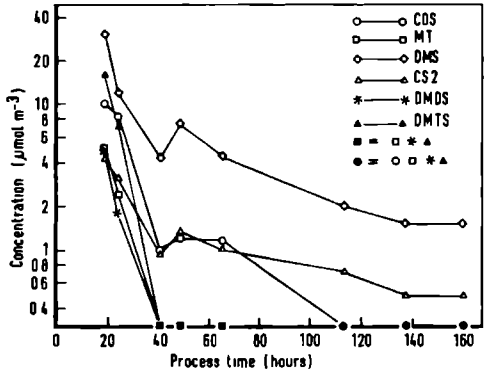

Figure 3. Concentrations of volatile sulfur compounds in the effluent air of an indoor composting process versus process time. A typical pattern is shown. Air samples were analysed in triplicate.

compound was calculated and averages of three independent trials are presented in Table 4. The total emission of sulfur during the indoor composting process amounted to 23 µmol S.kg FW^{-1}. The main contribution to the emission of sulfur is made by DMS (45%).

Table 4. Mean production of volatile sulfur compounds during indoor preparation of a substrate for *Agaricus bisporus*.

	H_2S	COS	MT	DMS	CS_2	DMDS	DMTS
indoor composting	0	3.2±1.2	0.6±0.5	10.2±3.6	2.4±0.6	0.5±0.4	1.1±1.3
windrows[b]	22.3	21.7	30.0	25.4	27.2	28.2	2.4

[a]) Values expressed as µmol.kg FW^{-1} of the end product.

[b]) Values from [6]

The optimal temperature of the production of H_2S, COS, MT and DMS coincides with the optimal temperature (50 - 56 °C) of biological activity determined by oxygen consumption and glutamate mineralization [7]. The biological origin of these compounds is further confirmed by the observation that a heat treatment strongly decreased their production (Table 1). The production of CS_2 and DMDS increased with increasing incubation temperature indicating an nonbiological formation. In several other complex biological systems similar observations were made [2,16,20]. The microbial origin of H_2S production is further proved by the abolishment of the production of H_2S by molybdate, a specific inhibitor of sulfate reducing bacteria [1,22] (Table 3). Since calcium sulfate is a constituent of the composting mixture [11] the availability of sulfate is never the limiting step in the formation of H_2S (Table 4). Although chicken manure, another constituent [11], is relatively rich in protein [17] an increase in the production of MT was observed on the addition of methionine. The same was reported for other systems [2,3,8,13]. Apparently, anaerobic conditions favour the formation of volatile sulfur compounds. In accordance the presence of oxygen strongly reduced the production (Fig. 2). Previously considerable amounts of volatile sulfur compounds were shown to be produced during the composting process in windrows [6]. In this study we show that during indoor composting the concentration of volatile sulfur compounds decreased dramatically (Fig. 3). Presumably, the high recirculation rate of the air through the composting material during indoor composting prevents the occurrence of an anaerobic zone in the inner part of the composting stack [9] and in this way contributes to a reduction in the production of volatile sulfur compounds [17]. Even when anaerobic micro-environments cannot be avoided completely, the recirculation of the emitted air enables a close contact between volatile sulfur compounds formed and the composting material, possibly enhancing breakdown. Preliminary experiments in which small amounts of H_2S were added to compost, showed a rapid disappearance of H_2S (data not shown). Whether this is due to sorption to the composting material or to microbial breakdown or both is unclear and subject of further research.

Recently, a total emission of volatile sulfur compounds of 256 μmol S.kg FW^{-1} was found during the outdoor preparation of compost used as a substrate in mushroom cultivation [6]. Highest emissions (217 μmol S.kg FW^{-1}) were

observed at the end of the process, when the material was stacked into windrows. On the contrary, the total emission of sulfur during the indoor composting process amounts to 23 μmol S.kg FW^{-1}. Replacing the windrow section of the outdoor process by the indoor process described here will result in a total emission of sulfur during the preparation of mushroom substrate of 62 μmol S.kg FW^{-1}. This overall reduction of 76 percent would implicate a significant decrease in environmental pollution caused by composting facilities. Furthermore, the indoor preparation of mushroom substrate enables a better control of process parameters, resulting in a more constant quality of the end product and moreover, mushroom yields comparable to those obtained with traditionally prepared substrate.

LITERATURE

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CHAPTER 7.

A MODEL OF INDOOR COMPOSTING BASED ON HEAT AND MASS BALANCES

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SUMMARY

The preparation of substrate for the cultivation of the edible mushroom, *Agaricus bisporus*, is referred to as composting. During this process organic material is broken down and a considerable amount of heat is produced. The process can be optimized in terms of mushroom production capacity by reducing loss of dry matter and shortening the process time. Conventionally, compost preparation takes place outside, causing emission of ammonia and odour nuisance. Indoor composting is a promising alternative, which also allows control of environmental pollution.

On basis of heat and mass balances calculations are described with a mathematical model for indoor composting. Values known from the conventional composting process are used as input parameters and the composition of the resulting material is predicted, together with process characteristics such as process time and aeration rate. Weight loss and process temperature were shown to have a major effect on all output parameters. It is demonstrated that the process time can be greatly reduced as compared to the conventional process.

INTRODUCTION

The edible white button mushroom, *Agaricus bisporus*, is cultivated on specially prepared compost. The goal of compost preparation is focused on the nutritional needs of the fungus and substrate selectivity [1,2]. Therefore, this process differs remarkably from composting processes applied to municipal waste and sewage sludge, in which volume and mass reduction are the major goals [3].

The preparation of mushroom compost consists of two parts, Phase I and Phase II, based on methods proposed by Sinden and Hauser [4]. Phase I is performed outdoors in large stacks, where a rapid breakdown of organic matter occurs. Phase II takes place indoors under controlled conditions and is characterised by pasteurisation and conditioning of the compost [1,5].

Although economically important improvements were obtained, little is known about the microbial processes underlying the composting process. Especially during Phase I, which is performed in windrows, little or no control of the process parameters is possible. As a result the temperature rises to values

as high as 80 °C and oxygen becomes limiting, at least in the central core of the windrows [6]. Moreover, the evolving air is allowed to enter the environment freely, causing both odour nuisance and environmental pollution as a result of ammonia deposition [7]. In order to meet the environmental regulations, indoor composting has been considered as a possible alternative.

Here the outdoor process is reduced to mixing and wetting of the constituents and the actual composting process takes place in tunnel-like fermentation rooms [8]. Performing the composting process in a controlled environment allows control of the process and treatment of the emitted air. Both temperature and oxygen levels can be used to regulate the aeration rate through the compost. Until now the determination of process parameters has remained limited to bench-scale experiments for practical reasons [3,9,10]. The translation of the laboratory results into terms of tonnes and cubic metres for practical purposes is afflicted with several pitfalls. Therefore, mathematical models may provide better interpretation of experimental results and could allow prediction of system interactions [10,11]. Finger et al. [12] described a model for windrow composting, based on oxygen as the rate limiting reactant. A good correlation was found between the predicted and measured temperature profiles for the static composting process but attempts to increase the composting rate by forced aeration failed. This failure is probably due to the formation of channels through which the majority of the air escapes sideways instead of covering the distance to the top of the stack. Performing the composting process in a tunnel-like environment will overcome this problem. In the model developed and presented here, general microbiological phenomena are combined with parameters derived from the outdoor composting process [1,6,13,14]. Predictions are made for the progress of the indoor composting process and the influence of several input parameters is studied.

DESCRIPTION AND DEFINITION OF THE MODEL

The model describes a composting process performed in a fermentation room, known as a tunnel [15]. This enables the aeration of the compost with a variable amount of air (see Fig. 1). Throughout the calculations the assumption is made that time does not play a limiting role in heat or mass transfer processes. As a consequence the effluent air is considered fully

saturated with water at a temperature equal to the temperature of the composting material. Moreover, no heat is supposed to be lost due to conductive heat transfer through the walls, as its contribution is only small in field-scale facilities [3,16].

At the start of the process, composting material, with similar composition as in the conventional outdoor process at the stage of stacking [1], is placed in the tunnel. It is assumed that all material in the tunnel undergoes the same variations in temperature and material composition and that no shrinking of height takes place. Furthermore, it is supposed that after heating the material is kept at a constant temperature. In the model this temperature is 50 °C, at which an optimal aerobic and anaerobic conversion

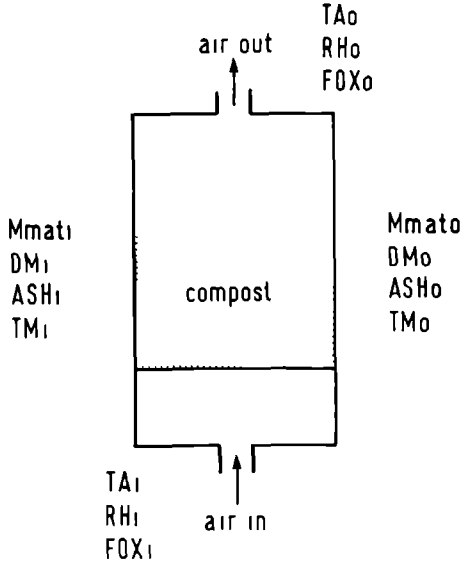

Figure 1: Schematic representation of the composting process in a tunnel-like environment. Symbols associated with the start of the process are indicated at the left, where symbols referring to the end of the process are placed on the right sight of the diagram. For explanation of the symbols see Table 2.

was found in laboratory experiments [6,13]. The heat balance is calculated on basis of changes relative to the starting composting material and the air at inlet conditions. Due to microbial activity organic matter is degraded. The organic matter is considered to be cellulose because of the relative

abundance of this component in starting material and its dominant contribution to the loss of weight during the composting process [17]. The first step in the breakdown of cellulose is its hydrolysis to glucose [18]:

$(C_6H_{10}O_5)_n + H_2O \quad ---> \quad (C_6H_{10}O_5)_{n-1} + C_6H_{12}O_6 \quad \Delta H^0 = -28.2$ kJ/Mol

Further breakdown can be achieved either aerobically or anaerobically according to the following reaction equations:

Aerobic: $C_6H_{12}O_6 + 6O_2 \quad ---> \quad 6CO_2 + 6H_2O \quad \Delta HO = -2803$ kJ/Mol

Anaerobic: $C_6H_{12}O_6 \quad ---> \quad 3CO_2 + 3CH_4 \quad \Delta HO = -133$ kJ/Mol

From the fact that no volatile fatty acids were found in the composting material, it is concluded that if any fermentative intermediates are formed, these are degraded to the level of carbon dioxide or methane. Therefore, the overall breakdown process can be summarized by the above mentioned reaction equations. As can be seen from the enthalpy changes, considerable amounts of heat are generated during the breakdown. The precise amount of heat generated depends on the amount of organic matter broken down during the process and the relative contributions of the aerobic and the anaerobic pathways to the process. As demonstrated recently biomass content does not change

Table 1. Constants[a].

Symbol	Value	Units	Description
Dair	1290	$g.m^{-3}$	Density of dry air at 0 °C
Haer	$2.803.10^6$	$J.Mol^{-1}$	Molar heat of combustion of glucose
Han	$1.33.10^5$	$J.Mol^{-1}$	Molar heat of glucose released during complete anaerobic breakdown
HCair	1.004	$J.g^{-1}.K^{-1}$	Heat capacity of dry air
HCdex	1.22	$J.g^{-1}.K^{-1}$	Heat capacity of dextrine
HCwater	4.184	$J.g^{-1}.K^{-1}$	Heat capacity of water
HCwatvap	1.841	$J.g^{-1}.K^{-1}$	Heat capacity of water vapour
HVwater	2379	$J.g^{-1}$	Heat of evaporation of water at 50 °C
MV	0.02241	$m^3.Mol^{-1}$	Molar gas volume at 0 °C and 1 atmosphere
MWglu	180.16	$g.Mol^{-1}$	Molecular weight of glucose
MWwater	18.0153	$g.Mol^{-1}$	Molecular weight of water
qhydro	28170	$J.Mol^{-1}$	Energy released during the hydrolysis of 1 Mol glucose from cellulose
R	8.31	$J.Mol^{-1}.K^{-1}$	Gas constant

a) Data taken from [21]

significantly during the composting process [13], therefore the rates of the breakdown processes are assumed to be constant during the entire process period.

The time needed to complete the process is estimated on basis of the oxygen consumption rate and the methane production rate, as determined previously [6,13]. These rates were determined with samples taken from composting material processed in the conventional way. The values must be regarded as suboptimal determinations of the oxidative and methanogenic capacities of the composting material.

MATHEMATICAL DEFINITION OF THE MODEL

The explanation of symbols and values used in the model are summarized in Tables 1 and 2.

Table 2. Parameters and their description, used in the calculations.

Symbol	Value[a)]	Units	Description	Source[b)]
Aer		$m^3.m^{-2}.h^{-1}$	Mean aeration rate during the process	
ASHi	0.20	w/w	Ash content of the starting material on dry matter base	
ASHo		w/w	Ash content of the end material on dry matter base	
BD	5.10^5	$g.m^{-3}$ c)	Bulk density of the material	
DMi	0.24	w/w	Dry matter content of the starting material	
DMo		w/w	Dry matter content of the end material	
Faer		w/w	Fraction converted by aerobic microorganisms	
FOXi	0.21	v/v	Oxygen fraction in inlet air	
FOXo		v/v	Oxygen fraction in outlet air	
Fcon	0.3	w/w	Fraction of dry matter lost during composting	[1]
H	2.0	m	Height of the compost stack	
Mmati	1	g	Mass of the starting material	
Mmato		g	Mass of the end material	
MPR	$7.9.10^{-6}$	Mol.g $dw^{-1}.h^{-1}$	Methane production rate of the starting material at 50 °C	[6]
Mwato		g	Mass of water at the end of the process	
Mwate		g	Mass of water evaporated during the process	
OCR	$1.4.10^{-4}$	Mol.g $dw^{-1}.h^{-1}$	Oxygen consumption rate of the starting material at 50 °C	[13]

Table 2. Continued

Psati		Pa	Saturation pressure of water in inlet air	
Psato		Pa	Saturation pressure of water in outlet air	
Qheat		J	Energy required for heating the material to the end temperature	
Qhydro		J	Energy produced during hydrolysis of cellulose	
Qprod		J	Total energy produced during breakdown of glucose	
RHi	0.81		Relative humidity of the inlet air	[14]
RHo	1.00		Relative humidity of the outlet air	
TAi	15	°C	Temperature of the inlet air	
TAo	50	°C	Temperature of the outlet air	
Tcon		h	Time required for the desired conversion	
TMi	15	°C	Temperature of the starting material	
TMo	50	°C	Temperature of the material at the end of the process	
Vair		m^3	Volume of inlet air required to face the process settings	
Vairmin		m^3	Volume of inlet air required to face the oxygen demand	

a) Values of Symbols not mentioned in this table are calculated using the formulas represented in Table 3.

b) Values mentioned without reference originate from the author.

c) Amounts of material are expressed on fresh weight base unless stated otherwise, dw = dry weight.

In Table 3 the formulas used in the model are arranged as mentioned in the text below. Limitations, mainly resulting from common sense considerations, are mentioned at the end of Table 3. Most calculations presented in this model are based on heat and mass balance equations of the general form:
Amount in - Amount out = Amount used - Amount formed
This results in an overall heat balance: 0 - Qair,out = Qheat - Qprod - Qhydro Qair,out represents the amount of heat required to bring the air from the inlet conditions to the outlet conditions. Contributions to this term are made by the heat required for heating the inlet air and the water already present in the air at inlet conditions. Furthermore included is the heat used for the evaporation of water up to the level of total saturation of the air. Qprod and Qhydro are governed by the fraction of the dry matter lost during the process (Fcon). From the heat balance the amount of air necessary to face the process settings (Vair) is calculated. With the need for oxygen by the

Table 3. Formulas, sources and limitations.

$$Vair = \frac{Qprod + Qhydro - Qheat}{\frac{MWwater.HVwater}{R}.\left(\frac{RHo.Psato}{TAo + 273} - \frac{RHi.Psati}{TAi + 273}\right) + \frac{1}{TAi + 273}.\left(273.Dair.HCair + \frac{RHi.Psati.MWwater.HCwatvap}{R}\right)} \quad A)$$

$$Qprod = \frac{Mmati \,.\, DMi \,.\, Fcon}{MWglu - MWwater} \,.\, (\, Faer \,.\, Haer + (1 - Faer) \,.\, Han \,)$$

$$Qhydro = \frac{Mmati \,.\, DMi \,.\, Fcon}{MWglu - MWwater} \,.\, qhydro$$

$$Qheat = Mmati \,.\, (\, DMi \,.\, HCglu + (1 - DMi) \,.\, HCwater \,) \,.\, (\, TMo - TMi \,)$$

$$Psati = 101330 \,.\, \exp\left(12 - \frac{4014}{TAi + 234.6}\right) \qquad [22]$$

$$Vairmin = \frac{Mmati \,.\, DMi \,.\, Fcon \,.\, Faer \,.\, 6 \,.\, MV \,.\, (Tai + 273)}{(MWglu - MWwater) \,.\, Foxi \,.\, 273}$$

$$Mwato = Mmati \,.\, (1 - DMi) + \frac{Mmati \,.\, DMi \,.\, Fcon \,.\, MWwater \,.\, (6 \,.\, Faer - 1)}{MWglu - MWwater} - Mwate \quad B)$$

$$Mwate = \frac{MWwater \,.\, Vair}{R} \,.\, \left(\frac{RHo \,.\, Psato}{TAo + 273} - \frac{RHi \,.\, Psati}{TAi + 273}\right)$$

$$Mmato = Mmati \,.\, DMi \,.\, (1 - Fcon) + Mwato$$

$$DMo = \frac{Mmati \,.\, DMi \,.\, (1 - Fcon)}{Mmato}$$

$$Tcon = \frac{Fcon}{(MWglu - MWwater) \,.\, (OCR/6 + MPR/3)}$$

$$Faer = \frac{OCR/6}{OCR/6 + MPR/3}$$

$$Aer = \frac{Vair \,.\, H \,.\, BD}{Tcon \,.\, Mmati}$$

Table 3. Continued

$$FOXo = \left(\frac{FOXi \cdot Vair \cdot 273}{(TAi + 273) \cdot MV} - \frac{Mmati \cdot DMi \cdot Fcon \cdot Faer \cdot 6}{MWglu - MWwater} \right) \cdot \frac{(Tai + 273) \cdot MV}{Vair \cdot 273}$$

$$ASHo = \frac{ASHi}{1 - Fcon} \quad [17] \quad ^{C)}$$

A) Vair ≥ Vairmin

B) Mwato ≥ 0

C) Fcon < 1 - ASHi

process a minimal amount of air is needed (Vairmin). This is used as the lower limit for Vair.

The amount of water in the end material (Mwato) can be calculated from the mass balance for water, taken into account the amount of water present in the starting material and the incoming air, the amount of water used for the hydrolysis and formed during aerobic breakdown of glucose and the amount of water present in the outlet air. The dry matter content of the resulting material (DMo) is calculated on basis of the mass balance of the dry matter and the amount of water present in the end material.

The time required to achieve the chosen fraction of conversion (Tcon) is calculated with the use of the oxygen consumption rate and the methane production rate. Alternatively, the fraction of conversion can be calculated at a given process time. The relative contribution of the aerobic breakdown process (Faer) can be calculated from both rates. Using the bulk density of the material (BD) and the height of the stack (H), the mean aeration rate over the process period (Aer) is calculated, expressed as $m^3.m^{-2}.h^{-1}$ at 20 ^oC. The fraction of oxygen present in the outlet air (FOXo) follows from the mass balance equation of oxygen, neglecting the amount of oxygen present in the free air space of the material. Finally the ash content of the end product (ASHo) results from the assumption that during the composting process no net change in the total inorganic matter occurs [17].

COMPUTER FACILITIES

All calculations included in the model are performed in a spreadsheet-environment. Both LOTUS-123 on IBM-like computers and LDW-power (Logical Design Works, inc.) on ATARI 1040 ST computers, with 1 Mbyte internal memory or more, are used.

RESULTS AND DISCUSSION

The model presented here enables the prediction of a number of parameters characteristic for the resulting material after a period of composting under defined circumstances. In Table 4 the results are presented for the standard

Table 4. Predicted values of output parameters of the model, using the values of the constants and the input parameters as mentioned in Table 1 and 2.

Symbol	Value	Unit
Aer	66.0	$m^3.m^{-2}.h^{-1}$
ASHo	0.286	w/w
DMo	0.270	w/w
Faer	0.899	w/w
FOXo	0.198	v/v
Mmato	0.622	g
Mwato	0.454	g
Mwate	0.341	g
Psati	1710	Pa
Psato	12400	Pa
Qheat	122	J
Qhydro	12.5	J
Qprod	1120	J
Tcon	71.3	h
Vair	0.00470	m^3
Vairmin	0.00027	m^3

setting of the input parameters (See Table 2) using the formulas from Table 3. None of the limitations mentioned in Table 3 influenced the calculations with the standard setting of the input parameters.

Using the formula of Tcon the progress of the composting process can be calculated by increasing the time stepwise, while keeping the other input parameters kept constant. The effect of time on the most important output parameters is shown in Fig. 2. In the beginning the amount of heat produced is not even sufficient to heat the material to the desired end temperature. As a consequence Vair is set to Vairmin (0.00027 m^3) and the oxygen concentration in the outlet air will drop to zero. After about 10 hours the surplus of heat is carried off by fresh inlet air, simultaneously sufficient oxygen is provided to create a sharp increase in FOXo (Fig.2). The mean aeration rate gradually reaches its maximum of 70.6 $m^3.m^{-2}.h^{-1}$. The changes in DMo and ASHo are only small at the start of the process, but as Tcon reaches 100 h, both output parameters increase sharply. Assuming that the process continues at the same speed the total amount of water original present will be evaporated after about 160 hours of composting (DMo = 1).

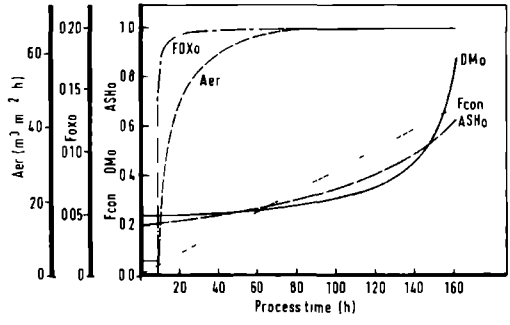

Figure 2: Influence of Tcon on DMo, ASHo, Vair, FOXo and Aer. Other parameters are kept constant at values indicated in Table 2. Note the difference in the scale.

No physical meaning can be attributed to this value as microbiological activity will be neglectable at a value of 0.65 or more for DMo [19]. Therefore, at values beyond 140 for Tcon the predicting value of the model is only small, but the results are presented as an illustration to what prolonged composting will lead to.

In order to investigate the influence of changes in the values of the input parameters a sensitivity analysis is performed. The value of the depending output parameters is calculated as a function of changes in the input parameter. The results are expressed relative to the value of the output parameter as shown in Table 4. The relative changes in the input parameters and the corresponding relative changes in the output parameters are summarized in Table 5. As anticipated from the formulas in Table 3, the influence of some input parameters is straight forward, for instance the influence of H and BD on Aer. On the contrary, the prediction of the influence of changes in other input parameters may be not that simple. As can be seen from Table 5 Vair is mainly governed by TAo and TMo, but their fluctuations are kept as small as possible in practical process operation. More important is the effect of DMi, Fcon, and RHo on Vair. Other input parameters have only a small influence on Vair.

The process time is a linear function of Fcon and is dominated by OCR. Because of an antagonistic effect of OCR on Vair and Tcon, the linear effect of OCR on Aer seems somewhat unexpected. The influence of the other input parameters is as expected because of their influence on Vair and Tcon, thus resulting in a major role for TAo and TMo. DMo is strongly influenced by DMi and less by Fcon and RHo. The amount of resulting material (Mmato) is mainly governed by DMi and Fcon, all other input parameters have only a small effect if any on Mmato. FOXi is the only input parameter which influences FOXo in a noticeable way.

The model presented does not include any recirculation of the air (See Fig. 1). This implicates that heat and mass transfer processes are assumed to take place within the time of one passage of the air through the composting material. To enable these processes large differences in both temperature and moisture content between composting material and inlet air are required as a driving force. Because of this, gradients in temperature and moisture content in the composting material across the tunnel are very likely to develop. To reduce these gradients recirculation is used already during Phase II treatment in bulk [15]. Application of recirculation does not disturb the

Table 5. Sensitivity analyses of Vair, Tcon, Aer, DMo, Mmato and FOXo on basis of relative changes in the values of the input parameters. Results are given as a relative change to the corresponding value at standard settings (See table 4).

Input parameter	-50%[a]	-10%	5%	10%	50%	-50%	-10%	5%	10%	50%	-50%	-10%	5%	10%	50%
	Vair					Tcon					Aer				
BD											-50	-10	5	10	50
DMi	-57	-11	6	11	57						-57	-11	6	11	57
Fcon	-56	-11	6	11	56	-50	-10	5	10	50	-12	-1	1	1	4
H											-50	-10	5	10	50
MPR	6	1	-1	-1	-5	5	1	-1	-1	-5	0	0	-0	-0	-0
OCR	-10	-1	1	1	4	82	10	-4	-8	-31	-50	-10	5	10	50
RHi	-5	-1	1	1	3[b]						-5	-1	0	1	3[b]
RHo	84	10									84	10			
TAi	-8	-2	1	2	12						-8	-2	1	2	12
TAo	407	28	-11	-21	-65						407	28	-11	-21	-65
TMi	-3	-1	0	1	3						-3	-1	0	1	3
TMo	450	31	-12	-22	-68						450	31	-12	-22	-68

Input parameter	-50%	-10%	5%	10%	50%	-50%	-10%	5%	10%	50%	-50%	-10%	5%	10%	50%
	DMo					Mmato					FOXo				
DMi	-63	-16	9	18	132	34	7	-3	-7	-34	-2	-0	0	0	0
FOXi											-54	-11	5	11	54
Fcon	-10	-2	1	3	20	34	7	-3	-7	-34	-1	-0	0	0	0
MPR	3	1	-0	-1	-2	-3	-1	0	1	2	0	0	-0	-0	-0
OCR	-5	-1	0	0	2	5	1	-0	-0	-2	-0	-0	0	0	0
RHi	1	0	-0	-0	-0[b]	-1	-0	0	0	0[b]	-0	-0	0	0	0[b]
RHo	-9	-1				12	1				4	1			
TAi	-2	-0	0	0	2	2	0	-0	-0	-2	-0	-0	0	0	1
TAo	-7	-1	1	1	6	6	1	-1	-1	-6	6	2	-1	-2	-16
TMi	-1	-0	0	0	1	1	0	-0	-0	-1	-0	-0	0	0	0
TMo	-3	-0	0	0	-0	2	0	-0	-0	-0	6	2	-1	-2	-18

a) Relative change in the value of the input parameter as tabulated in Table 2

b) Relative change calculated with a RHi = 1

principle of the calculations of the model. As the only consequence for the model Vair, Vairmin and Aer should be seen as net values at the inlet of the system. The practical implication of recirculation is a much higher electric power input, because as the air velocity increases the pressure drop across the material increases according to a power law of the air velocity [20].

The process time is calculated on basis of OCR and MPR, both measured under laboratory conditions with fresh substrate. The assumption that these rates remain constant during the entire process is based on the observation that the biomass content of the composting material does not change significantly during the process [13]. In the conventional process high temperatures will slow down the microbial process. This inhibitory condition is prevented by indoor composting. Therefore, in our opinion the conclusion is permitted that the composting process can be performed in a remarkably shorter time as compared to the two weeks used now.

When Phase I is replaced by indoor composting performed under controlled conditions as stated above, the opportunity arises to combine Phase I and Phase II. A slightly lower process temperature would be optimal for growth of *Scytalidium thermophilum*, a fungus used as an indicator for compost selectivity [2]. A further reduction of the overall process time may lead to a lower loss in organic matter during composting. Additional research is needed to validate the model described above and to optimize the indoor composting process. The latter implicates a maximum yield of *A. bisporus* achieved by maximum compost selectivity and a minimum loss of organic matter during the composting process.

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The cultivation of edible mushrooms has developed into a modern industry in western countries. This counts in particular for the white button mushroom, *Agaricus bisporus*. The substrate used for its production is prepared by a composting process of mainly agricultural waste materials. Both the recipe and the composting regime are results of trial and error rather than based on in depth understanding of the nutritional requirements of *A. bisporus* or the microbiological underpinnings of the composting process. Nevertheless, the substrate obtained is highly suitable for the cultivation of mushrooms.

In the Netherlands the preparation of the substrate is concentrated on two locations, resulting in a weekly production of over 10,000 tons compost at the largest site. The combination of this large scale production process and the use of manures as ingredients, has given rise to complaints about malodors in the neighbourhood. The research project described in this thesis was undertaken to elucidate the chemical nature of the malodor and to enlarge the understanding of the microbial processes involved in the composting process and more specific in the production of the malodor.

The chemical nature of the malodor was resolved by concentrating large quantities of air evolving from the compost stacks on acivated charcoal. After extraction with various solvents, samples were analysed by means of gas chromatography and the single components were identified with a mass spectrometer. As a result eight different components were identified, all ketones or volatile sulfur compounds. Especially the members of this last group of compounds (carbon disulfide, dimethyl sulfide, dimethyl disulfide and dimethyl trisulfide) are known for their low recognition levels and the pungent character of their smell. By sulfur-specific analysis of air samples additional volatile sulfur compounds were found (hydrogen sulfide, carbonyl sulfide and methanethiol). Concentrations of volatile sulfur compounds in the air evolving from the composting stacks ranged from 1 to 35 $\mu mol/m^3$. In general the concentration of the volatile sulfur compounds tended to increase with increasing composting time. In all stages the concentration of dimethyl sulfide was highest. The total emission of sulfur as volatile sulfur compounds amounted to 8.3 mg of sulfur per kg (fresh weight) of compost produced. Because of the high sulfur content of the ingredients relative to the amount of sulfur mentioned above, it is very unlikely that changes in the

recipe alone can cause a significant reduction of the emission of volatile sulfur compounds during the composting process.

The reduced character of the volatile sulfur compounds found, indicated the local absence of oxygen in the composting material. An additional evidence for the occurrence of anaerobic processes was obtained by measurements of methane in the air evolving from the compost stacks. Methane concentrations up to 4.5 % (v/v) were found. From the total emission of methane during the composting process it was calculated that at least 3.3 % of the total breakdown was performed by anaerobic microorganisms. From the composting material two strains of thermophilic methanogenic bacteria were isolated and characterised. Both strains proved to belong to the species *Methanobacterium thermoautotrophicum*. This species was first described in 1972 but its isolation from such an aerobic environment was not reported before.

One of the major aims of the composting process is to increase the selectivity of the substrate for *A. bisporus*. The breakdown of readily accessible compounds with the simultaneous formation of biomass are believed to contribute to this selectivity. The presence of anaerobic environments in the composting material implies a suboptimal production of biomass, as the energy yield under anaerobic conditions is remarkably lower than under aerobic conditions. By means of biomass estimations, based on the quantification of total extractable lipid phosphate, it was demonstrated that the distribution of biomass coincided with places favourable for optimal biological activity both with respect to temperature and oxygen supply. Oxygen consumption rates and mineralisation rates of glutamate were shown to reach a maximum between 50 to 55 ^{o}C. As temperature reaches values up to 80 ^{o}C in the compost stacks it may be clear that the breakdown of organic matter is considerably slowed down by thermal inactivation of enzymes. In order to tackle problems associated with the emission of malodorous compounds during the outdoor part of the composting process, changes have been made in the composting regime. The thoroughly mixed material is transferred to a fully climatized fermentation room known as a tunnel. Calculations, based on mass and heat balances, showed that temperature could be kept under control by adjusting the supply of fresh air, without creating anaerobic conditions on macroscale. This process is now known as indoor composting and the resulting substrate has been shown to be equally suitable for mushroom cultivation. Due to the optimal process conditions the duration of composting is reduced by one week. An additional advantage of the indoor composting

system is the reduction of the total emission of volatile sulfur compounds during this process by a factor ten. Until now it is unclear whether this is the result of lower production rates, due to better optimized process conditions, or an enhanced breakdown as a result of recirculation of the air through the composting material.

During the conventional outdoor process considerable amounts of ammonia were released by the composting material. Although it is expected that similar quantities of ammonia will be released during indoor composting, highly efficient systems are available to remove the ammonia from the effluent air. Therefore, the introduction of indoor composting for the preparation of the substrate for *A. bisporus* implies a major contribution to the reduction of environmental pollution by composting facilities.

De bereiding van het substraat voor de champignon is een proces dat ontwikkeld is op basis van jarenlange ervaring. Aldus is men gekomen tot een produkt, waarop de champignon uitstekend groeit en dat binnen de wereld van de champignonteelt bekend staat als champignonkompost. In hoofdstuk 1 wordt ingegaan op de gebruikte ingrediënten en de procesvoering tijdens de kompostering.

Het gebruik van de term kompostering kan aanleiding geven tot verwarring met afvalverwerkingstechnieken. Daarbij staat een volume- en massareduktie voorop, terwijl het gevormde eindprodukt in het algemeen slechts een geringe marktwaarde vertegenwoordigt. Bij de bereiding van champignonkompost ligt de nadruk op het verkrijgen van een selektief substraat en volume- en massareduktie zijn daaraan ondergeschikt. De marktwaarde van de champignonkompost is aanzienlijk hoger dan die van andere kompostsoorten.

Uiteraard zijn er ook een aantal overeenkomsten tussen beide processen. Zo treedt bij elke kompostering afbraak van organisch materiaal op onder invloed van mikro-organismen. Daarbij is zuurstof nodig en worden onder andere kooldioxyde en warmte gevormd. Ter illustratie van de soortenrijkdom in een komposthoop is een tabel opgenomen met mikro-organismen, die uit champignonkompost geïsoleerd zijn (HS 1). Hoewel de veranderende omstandigheden in een komposthoop aanleiding kunnen zijn voor verschuivingen in de populatieopbouw van de aanwezige biomassa, is met behulp van biomassametingen aangetoond dat na circa twee weken er geen toename meer optrad in de totale hoeveelheid biomassa (HS 2). Wel bleek dat de biomassa op dat tijdstip nog niet homogeen over de komposthoop verdeeld was, hetgeen aangeeft dat er plekken in de komposthoop zijn, die gunstig zijn voor biomassa-ontwikkeling en plekken die daarvoor minder geschikt zijn. Een belangrijke parameter, die hierop van invloed is, is de temperatuur. Door middel van laboratoriumonderzoek aan verse kompostmonsters werd een optimum in biologische aktiviteit bij een temperatuur van 50 tot 55 ^{o}C gevonden. Een ander bewijs voor het voorkomen van plekken met minder gunstige omstandigheden voor snelle aangroei van biomassa werd verkregen door de lucht boven komposthopen te onderzoeken. Hierin werden aanzienlijke hoeveelheid methaan gevonden (HS 3). Methaan kan alleen gevormd worden onder zuurstofloze omstandigheden. Onder deze omstandigheden verkrijgen de mikro-organismen slechts weinig energie uit de omzetting van organische stof met als gevolg dat hun groei trager is dan die van mikro-organismen die organische stof omzetten onder gelijktijdig gebruik van

zuurstof. Gezien de hoge temperaturen die in de komposthopen kunnen voorkomen (70-80 °C is geen uitzondering), was het interessant om na te gaan welke methanogenen in champignonkompost voorkomen. Na isolatie en karakterisatie van twee reinkweken bleken beide tot de reeds eerder beschreven thermofiele bakteriesoort *Methanobacterium thermoautotrophicum* te behoren (HS 4). Nieuw is wel hun isolatie uit een omgeving die op makroschaal als zuurstofrijk omschreven wordt. Deze schijnbare tegenspraak is te verklaren door de hoge biologische aktiviteit in het komposterend materiaal. Het idee is dat een strodeeltje bedekt is met een aantal lagen bakterien. De zuurstof, die de hoop binnendringt, wordt geheel opgebruikt door bakteriën in de buitenste lagen, zodat onderliggende lagen volledige verstoken blijven van zuurstof.

Naast het reukloze methaan en kooldioxyde zijn er in de uittredende lucht boven een komposthoop ook verbindingen aanwezig, die een sterk prikkelend effekt op de neusslijmvliezen uitoefenen. Aangezien de pH van de kompost in het algemeen tussen 7 en 8 ligt is het niet erg waarschijnlijk dat vluchtige vetzuren verantwoordelijk zijn voor de stank. Pogingen om vluchtige vetzuren aan te tonen in het komposterend materiaal zijn nooit suksesvol geweest. Omdat het vermoeden bestond dat de verbindingen, die verantwoordelijk zijn voor de stank, slechts in zeer lage koncentraties in de lucht voorkomen, werd de lucht eerst gekoncentreerd voordat analyses uitgevoerd werden (HS 5). Met behulp van een gaschromatograaf gekoppeld aan een massaspektrometer werd de identiteit van een aantal stoffen vastgesteld. Van de stoffen, die in de hoogste koncentratie aangetroffen werden, bleek een opvallend aantal tot de zwavelhoudende stoffen te behoren (met name: dimethylsulfide, dimethyldisulfide, dimethyltrisulfide en koolstofdisulfide). Van een groot aantal stoffen uit deze kategorie is bekend dat ze een bijzonder sterke geur bezitten. Zo is dimethylsulfide reeds herkenbaar bij een koncentratie van 1 volumedeel per 10 miljoen delen lucht. Bij nadere analyses, waarbij speciaal naar zwavelhoudende vluchtige verbindingen gekeken werd, werden ook waterstofsulfide, carbonylsulfide en methaanthiol gevonden. Aangetoond werd dat met name het laatste deel van de buitenkompostering (de dijken) een grote bijdrage levert tot de emissie van zwavelhoudende verbindingen. Op grond van zwavelanalyses van het komposterend materiaal werd duidelijk dat de hoeveelheid zwavel die op deze manier uit de kompost ontwijkt zo klein is ten opzichte van de totaal aanwezige zwavel, dat het onwaarschijnlijk is dat verandering van de receptuur alleen leidt tot een belangrijke reduktie in de emissie van bovenbedoelde zwavelhoudende verbindingen. Uit laboratoriumonderzoek volgde

dat de temperatuur een belangrijke faktor vormde bij het ontstaan van de vluchtige zwavelverbindingen (HS 6). Gaandeweg het onderzoek werd het steeds duidelijker dat kleine ingrepen in de huidige buitenkompostering niet konden leiden tot de gewenste vermindering van uitstoot van milieubelastende stoffen. Bovendien voltrekt de buitenkompostering zich onder, voor mikrobiologische processen, allerminst ideale omstandigheden. Zowel de zuurstofvoorziening als de temperatuur staan een optimale omzettingssnelheid in de weg. Om die reden zijn plannen uitgewerkt om een deel van de buitenkompostering in geklimatiseerde ruimtes uit te voeren. Dit proces is intussen bekend als "indoorkompostering". Een belangrijk voordeel voor de procesvoering is de betere kontrole op de voortgang van het proces. Verder kan de uitstoot van milieubelastende stoffen gereduceerd worden door het toepassen van afgasreiniging. Voor het verwijderen van ammoniak uit de lucht zijn zeer efficiente systemen voorhanden. Bij het indoorproces bleken aanzienlijk minder vluchtige zwavelverbindingen uitgestoten te worden dan bij het buitenproces (HS 6). Of dit een gevolg is van een verlaagde produktie of een verhoogde afbraak van deze verbindingen bij indoorkompostering is nog niet duidelijk. Op grond van kennis van het traditionele buitenproces en van enkele mikrobiologische processen is een model voor de indoorkompostering opgesteld, waaruit volgt dat de gewenste omzetting van organisch materiaal onder optimale omstandigheden binnen enkele dagen te realiseren is (HS 7). Dit betekent een aanzienlijke tijdwinst ten opzichte van het huidige proces.

Naar aanleiding van de in dit proefschrift beschreven resultaten kan gekonkludeerd worden dat:

a) omzettingen in de dijkfase geen aanleiding geven tot een netto biomassaaangroei maar wel leiden tot een afname van de aanwezige hoeveelheid organische stof.
b) anaerobe processen gedurende het gehele komposteringsproces een rol spelen.
c) vluchtige zwavelverbindingen voorkomen in de lucht boven het komposterend materiaal.
d) de vorming van vluchtige zwavelverbindingen met name plaats vindt uit organische zwavelverbindingen.
e) de emissie van vluchtige zwavelverbindingen bij indoorkompostering aanzienlijk lager is dan bij het konventionele proces.
f) door optimalisatie van de procesvoering tijdens de indoorkompostering een belangrijke tijdwinst geboekt kan worden.

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CURRICULUM VITAE

Petrus Johannes Lambertus Derikx werd geboren op 30 maart te Heythuysen. Na het behalen van het VWO-diploma aan de St. Ursula scholengemeenschap te Roermond volgde hij aan de Katholieke Universiteit in Nijmegen een opleiding in de chemie. In 1980 legde hij hierin het kandidaatsexamen af (studierichting S2). Het doktoraalprogramma omvatte het uitgebreid bijvak Biochemie (Prof. H. Bloemendal) en het doktoraalhoofdvak Chemische Mikrobiologie (Prof. G.D. Vogels). In deze periode werd het examen Deskundigheid Stralingshygiëne (niveau 3) met sukses afgelegd. De doktoraalopleiding werd in september 1985 afgesloten.

Vanaf september 1985 tot september 1989 is hij als wetenschappelijk onderzoeker verbonden geweest aan het Laboratorium voor Mikrobiologie van de Katholieke Universiteit te Nijmegen. Hier werkte hij aan een samenwerkingsprojekt tussen het Proefstation voor Champignoncultuur te Horst en bovengenoemd laboratorium. Het in dit proefschrift beschreven onderzoek vormt hiervan het resultaat.

Sinds 1 september 1989 is hij als wetenschappelijk onderzoeker verbonden aan de afdeling Milieutechniek van het Instituut voor Mechanisatie, Arbeid en Gebouwen (I.M.A.G.) te Wageningen.