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MICROORGANISMS AND ENDOTOXIN IN EXPERIMENTALLY GENERATED BIOAEROSOLS FROM COMPOSTING HOUSEHOLD WASTE*

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Abstract: Industrial compost made from source separated household waste was investigated. The objective was to estimate the microbial risk potential of bioaerosols over the progressing stages of outdoor pile composting. Bulk samples of 75 kg were collected from piles after 1, 5 and 9 weeks of composting, and bioaerosols were generated experimentally in a rotating drum tester. Samples of bioaerosols and composted materials were quantified for total counts of microbial cells by epi-fluorescence microscopy, viable counts of microorganisms and endotoxin. Five groups of microorganisms were cultured in parallel: mesophilic bacteria, actinomycetes and fungi (25°C), *Aspergillus fumigatus* and thermophilic actinomycetes (55°C). Furthermore, the bulk samples were analysed for nine other groups of viable microorganisms. In general, the concentrations were increasing ($p < 0.05$) over time of pile composting. The microflora was dominated by bacteria and actinomycetes to a total of 10^9-10^{11} cells/g bioaerosol or composted materials. In bulk samples the maximum concentrations of viable bacteria reached 2.1×10^9 cfu/g and the thermophilic actinomycetes 1.8×10^7 cfu/g. The maximum viable concentrations in bioaerosols were 1.7×10^7 cfu/g for bacteria and 1.5×10^7 cfu/g for thermophilic actinomycetes. Maximum endotoxin levels were equivalent to 14 µg/g and 110 µg/g in bioaerosols and bulk samples, respectively. The composition of the microflora differed from most other compost studies by low fungal concentrations, and *Aspergillus fumigatus* was only found sporadically in levels close to the limit of detection. Thermophilic actinomycetes were the predominant source for airborne spores which should be emphasized in risk assessments for this particular type of compost. Personal sampling is recommended to clarify the occupational exposure.

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Key words: industrial compost, household waste, biowaste, bioaerosols, bacteria, actinomycetes, fungi, endotoxin, microbial risk potential.

INTRODUCTION

Composting of municipal solid waste is a disposal alternative which is becoming more popular than landfilling and incineration because of the benefits of transforming the waste into agricultural useful products. Depending on the composition of the microflora of bacteria, actinomycetes and fungi in the basic material, bioaerosols generated from the processing and handling of composted materials may constitute a potential health risk to exposed individuals [16, 22, 27]. Several case studies of occupational exposure to bioaerosols from compost and waste indicate that microbiological agents may cause either respiratory diseases or gastrointestinal symptoms [18, 19, 21, 29, 30]. Most of the case studies are referring to compost of wooden materials from gardens and parks or to compost of household waste mixed with garden waste or sewage sludge. Few data exist of compost made

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of source separated household waste as the main basic material [10, 13, 14], and few data of occupational exposure to this type of bioaerosols are available [23]. In general, the biodegradable household waste fraction is rapidly decomposed by microorganisms, but the development of the microflora during the composting processes is not known.

Outdoor composting of municipal solid waste is under the influence of changes in climatic conditions [2]. The composting of household waste involves aerobic decomposition of organic materials containing high quantities of easily biodegradable carbohydrates, proteins and lipids. Depending on the composting technique, the transformation into compost may last from a month up to about half a year, and composting of garden waste takes longer due to the high content of lignin and cellulose in the wooden materials [22].

The primary aim of the present study was to estimate the relative microbial risk potential in bioaerosols from compost made of source separated household waste. Changes of the microflora in bioaerosols and bulk samples were examined during the progressing stages of outdoor composting. In the study the composting process in a recovery plant established for industrial pile composting, took place within three months.

MATERIALS AND METHODS

Industrial composting technique. The fraction of household waste in the study consisted of biodegradable organic materials (biowaste) separated at the households. The leavings came mainly from vegetables, bread, meat etc., but wet tissues, napkins, flowers and faeces from small pets also formed a considerable part. At a household, the biowaste was collected in thin plastic bags and subsequently stored outside the house in a plastic container or a paper sack. The biowaste was collected every two weeks and delivered at an industrial composting plant recyling biowaste of 10,000 tonnes per year.

At the plant, the biowaste was tipped from the collecting truck into a floor pit from which the waste was transported into a grinding mill by a conveyer. Here the thin plastic bags were shredded and the biowaste was liberated. To obtain more structure in the final product, straw from stables and paper were added in ratios of 86:10:4 for biowaste, straw and paper, respectively. Subsequently, the waste was transferred to a 500 m^3 rotating cylindrical reactor (length 40 m; diameter 4 m) for a precomposting period ranging from 4 to 7 days. By volume the reactor was filled to approx. 40% with biowaste which moved forward by slowly rotation. A strong aeration (5,000 m^3/hour) during the storage in the reactor resulted in an inside temperature of 40–45°C. After precomposting, the waste was transferred to an outdoor area for stacking in piles of a length of 30–40 m with base and height of approx 1.5 m. Under the middle of the baseline of each pile, a canal was placed for drainage of percolate produced during the composting processes.

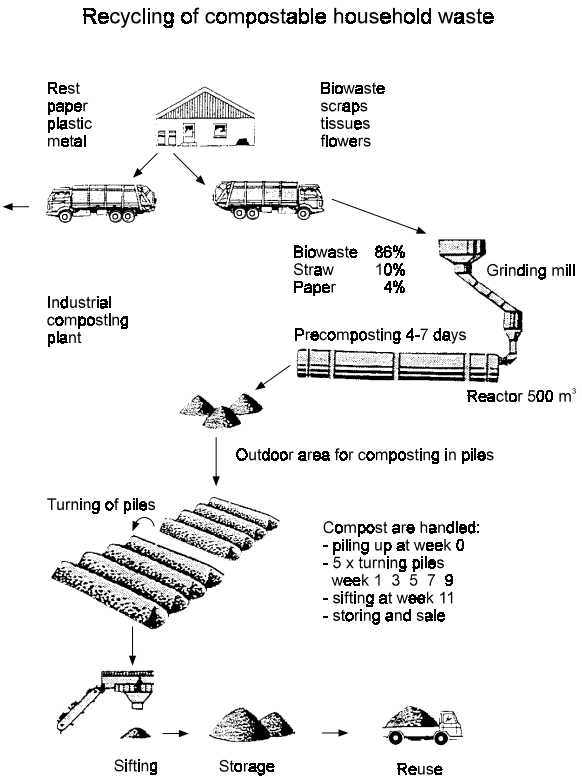

Figure 1. Flow diagram of the recycling processes for presorted household waste during industrial pile composting.

For aeration of the compost the piles were turned mechanically every two weeks. Piles were turned five times: after week 1, 3, 5, 7 and 9, respectively. After 11 week of pile composting, the compost was sieved by a revolving screen and stored for later re-use. Figure 1 illustrates the flow diagram of the recycling of compostable household waste. As a control of the composting process, the temperature in the piles was measured daily with spear-shaped thermometers of 1 m length. To ensure elimination of most of the pathogenic microorganisms, the temperature of every pile should be above 55°C for at least two periods of 14 days. According to the plant, this requirement was always met, and the temperature inside the piles often reached 60–70 °C.

Collection of bulk samples. Samples of compost were examined at the same time where occupational work tasks during the pile composting were performed. Bulk samples of approx. 80 kg compost were collected just after the piles were turned for aeration on week 1, 5 and 9 representing the start, the middle and the end of the pile composting period. The different stages of compost were sampled from three independent piles. After collecting a sample at the plant the compost was stored in paper sacks at 20°C until next day. Before the experimental generation of bioaerosols subsamples of approx. 2 kg were collected for analysis. In addition, bulk samples of 2–3 kg compost collected just after precomposting (week 0) and after sieving (week 11) were analysed in the same way as the samples from the piles.

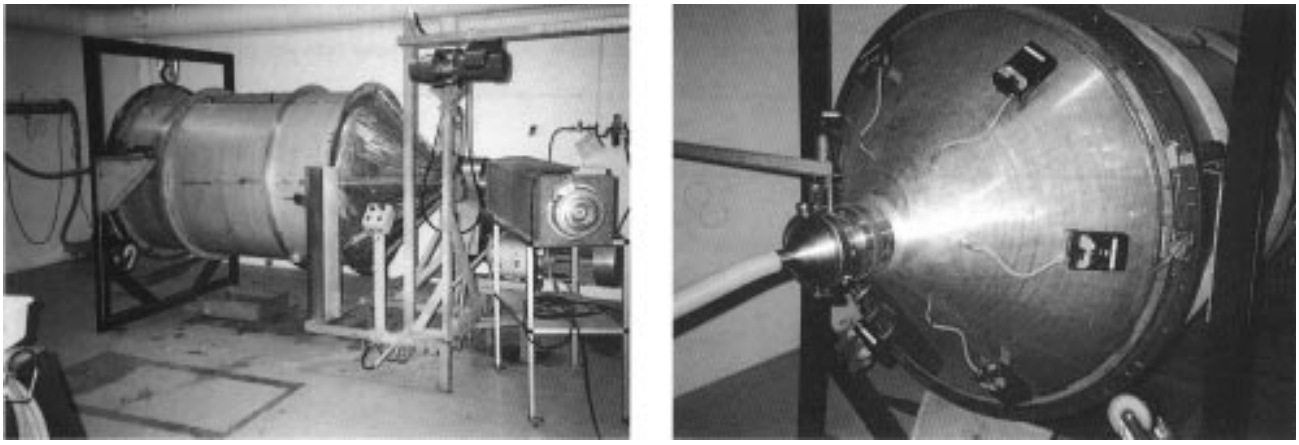

Figure 2. Rotating drum dustiness tester of a volume of 3.3 m^3.
Left: Experimental generation of bioaerosols is done by rotation along the horizontal axis during strong aeration by a flow of sterile filtered air drawn through the drum.
Right: The drum seen from the outlet. The pumps and small tubes are connected to the six filter cassettes placed symmetrically inside the drum.

Generation and collection of bioaerosols. Compost samples of 75 kg were loaded into a 3.3 m^3 rotating drum of stainless steel with conical ends (Fig. 2). The drum was designed by upscaling the Warren Spring Laboratory rotating drum tester [1]. Inside the drum was fitted with 8 longitudinal vanes to lift the compost as the drum was rotated along the horizontal axis. A flow of sterile filtered air (420 l/min) was delivered at the inlet of the drum and exhausted at the outlet by a vacuum pump. For the generation of bioaerosols, a sample of 75 kg compost was rotated at 15 rpm for 240 min. From preliminary pilot studies, where varying quantities of compost (50 and 75 kg) were tested, a generation time of 240 min was required in order to obtain sufficient materials for the microbiological analysis of the bioaerosols. After each run, the compost was removed and the inside surface of the drum was thoroughly cleaned and disinfected.

Bioaerosols were sampled by 6 filter cassettes placed symmetrically in a circle 0.2 m from the outlet of the drum. Two of the filters positioned opposite each other were used for sampling of dust (total particulate matter) while the other four filters were used for sampling of microorganisms. Polycarbonate filters (25 mm, 0.4 µm; Nuclepore, Cambridge, MA, USA) mounted in closed-face field monitors with a 4.4 mm inlet (Nuclepore) were used for collection of microorganisms at an airflow of 2.0 l/min (inlet velocity 2.1 sec/m). Dust was collected on filters of cellulose acetate/nitrate (25 mm, 8 µm; Millipore, Bedford, MA, USA) mounted in closed-face field monitors (Millipore) with a 5.6 mm inlet at an airflow of 2.0 l/min (inlet velocity 1.3 m/sec).

Sampling and preparation. After each experiment, the polycarbonate filters were extracted by adding 5 ml sterile 0.05% Tween 80 solution to the filter cassette and shaking (500 rpm) for 15 min at room temperature. For the microbiological analysis, the extraction fluid of two samples at opposite positions in the drum was pooled in order to obtain sufficient material for the microbiological analysis. The strategy of pooling the samples was done according to previous testing of the drum in generation of aerosols from crushed limestone [1]. The microorganisms were quantified by a modified CAMNEA-method [25] which includes determination of the total number of microbial cells by epifluorescence microscopy (total counts) and by plating and counting the culturable number of microorganisms (viable counts). Samples for viable counts were cultivated immediately while samples for total counts by microscopy were frozen at -80°C for later analysis.

The mass of bioaerosols (total dust) collected on filters of cellulose acetate/nitrate was determined by weighing the filters before and after the sampling (limit of detection 40 µg). In advance the filters were equilibrated at constant air temperature and humidity for at least 24 hours. Subsequently to the gravimetric analysis, the filters were extracted with 10.0 ml non-pyrogenic water by orbital shaking (300 rpm, 15 min) at room temperature. The samples were frozen at - 80°C for later analysis for content of endotoxin.

Counts of micoorganisms. Total counts of microorganisms were performed by epi-fluorescence microscopy at a magnification of 1250 times. For bulk samples 1.0 ml of appropriate dilutions were stained with 0.3 ml 0.01% acridine orange in acetate buffer (bioMérieux, Marcy l'Étoile, France) for 30 seconds and filtered through a dark polycarbonate filter (25 mm, 0.4µm; Nuclepore, Cambridge, MA, USA). The numbers of microbial cells were counted in forty randomly chosen fields and the concentrations were given as cells per g compost (limit of detection: 3.5×10^3 cells/g). For bioaerosols two double samples of 2.0 ml were analysed and the concentrations were calculated as cells per g dust (limit of detection: 1.4×10^4 cells/g).

Table 1. Concentrations of microorganisms and endotoxin in bulk samples from composting household waste. Figures represent mean values (GM) of three samples collected from independent piles at the same composting stage, and range is indicated in brackets. According to Duncans multiple test for grouping the minimum and maximum are indicated by the capitals A and B, respectively. A jump from one capital to another indicates a statistically significant change ($p < 0.05$).

	week 0	week 1	week 5	week 9	week 11
Total counts $\times 10^6$ cells/g	A 15000 (7800-30000)	54000 (30000-73000)	33000 (23000-46000)	B 110000 (72000-170000)	86000 (47000-170000)
Bacteria 25°C $\times 10^6$ cfu/g	A 0.57 (0.026-4.0)	B 72 (7.4-370)	B 40 (18-190)	B 360 (210-710)	B 620 (380-1600)
Actinomycetes 25°C $\times 10^6$ cfu/g	A 0.0003 (<lod-0.002)	A 0.0004 (<lod-0.008)	0.006 (<lod-0.16)	0.011 (0.006-0.032)	B 0.48 (0.02-35)
Actinomycetes 55°C $\times 10^6$ cfu/g	A 0.028 (0.005-0.12)	0.25 (0.005-6.1)	4.5 (3.8-5.5)	B 11 (7.6-20)	B 18 (5.6-91)
Fungi (moulds) 25°C $\times 10^6$ cfu/g	A 0.0004 (0.0002-0.0007)	A 0.0002 (<lod-0.0006)	A 0.0004 (<lod-0.0015)	A 0.0002 (<lod-0.0004)	B 0.004 (0.002-0.024)
A. fumigatus 45°C	<lod	<lod	<lod	<lod	<lod
Endotoxin units $\times 10^6$ EU/g	0.13 (0.066-0.23)	0.14 (0.10-0.21)	A 0.039 (0.024-0.052)	B 1.7 (1.1-2.4)	B 1.2 (0.83-2.1)
Bacteria 37°C $\times 10^6$ cfu/g	A 1.5 (0.97-2.6)	150 (17-1000)	60 (29-180)	800 (460-12000)	B 2100 (330-60000)
Gram-negative bacteria $\times 10^6$ cfu/g	A 0.0009 (0.0002-0.0027)	0.09 (0.0044-1.1)	0.69 (0.049-2.9)	1.1 (0.22-5.0)	B 11 (0.94-130)
Pseudomonas $\times 10^6$ cfu/g	¤ A 0.0001	0.020 (0.0008-0.29)	0.003 (<lod-0.035)	0.011 (<lod-0.18)	B 8.5 (1.6-23)
Coliforms $\times 10^6$ cfu/g	¤ A 0.0001	B 0.73 (0.19-6.6)	B 0.48 (0.02-3.0)	B 2.1 (0.19-7.3)	B 12 (2.0-140)
Bacillus $\times 10^6$ cfu/g	A 0.35 (0.15-0.67)	B 32 (1.1-980)	B 7.2 (1.6-73)	B 79 (42-170)	B 70 (36-260)
Lactobacillus $\times 10^6$ cfu/g	B 0.57 (0.018-6.4)	B 6.9 (0.42-200)	B 3.1 (0.040-46)	¤ A 0.0001	¤ A 0.0001
Faecal streptococci $\times 10^6$ cfu/g	A 0.0002 (<lod-0.001)	B 4.3 (0.17-60)	B 0.96 (0.16-21)	B 0.10 (0.025-0.82)	B 0.39 (0.12-2.3)
Micrococcus $\times 10^6$ cfu/g	A 0.018 (0.002-0.51)	A 0.013 (<lod-0.54)	0.67 (0.13-13)	0.68 (0.17-1.5)	B 24 (9.6-74)
Yeast $\times 10^6$ cfu/g	0.002 (0.0002-0.012)	0.81 (0.059-18)	0.005 (0.0002-0.35)	0.004 (<lod-0.080)	0.010 (0.0059-0.025)

A - minimum, Duncans test; B - maximum, Duncans test; lod - limit of detection (200 cfu/g); ¤ - fixed value = ½ lod (100 cfu/g).

The counting of viable microorganisms was carried out by plating 0.1 ml of ten-fold dilutions of the extraction fluid onto different media. For compost as well as for samples of bioaerosols five groups of microorganisms were enumerated in parallel: Mesophilic bacteria (25°C) and thermophilic actinomycetes (55°) were cultivated on Nutrient agar (Oxoid, Basingstoke, England) with actidione (cycloheximide; 50 mg/l) to prevent fungal growth, and mesophilic actinomycetes (25°C) on 10% strength of the same media. Fungi (moulds) were determined on Dichloran Glycerol agar (DG18; Oxoid), the mesophilic fungi were incubated at 25°C and the thermophilic fungus, *Aspergillus fumigatus*, at 45°C. In addition, the bulk samples of the compost were analysed for nine other groups of microorganisms: Bacteria 37°C (Blood agar; Oxoid) with cycloheximide (50 mg/l), Gram-negative bacteria 25°C (Nutrient agar; Oxoid) with cycloheximide (50 mg/l) and penicillin G (30 mg/l), *Pseudomonas* 25°C (Pseudomonas agar; Oxoid), coliform bacteria 37°C (Mc Conkey agar; Oxoid), *Bacillus* 37°C (Bacillus cereus agar; Oxoid), *Lactobacillus* 37°C (Rogosa agar; Oxoid), faecal streptococci 37°C (Kenner Faecal Streptococcus agar; Oxoid), and *Micrococcus/ Staphylococcus* 25°C (Kranep; Merck, Darmstadt, Germany). Yeast 25°C was cultured on Malt Extract agar (Oxoid) with streptomycin (30 mg/l) and penicillin G (30 mg/l) to prevent growth of bacteria. The counts of colony forming units (cfu) were expressed as cfu per g compost or cfu per g bioaerosol. The limit of detection was 200 cfu/g compost and 2500 cfu/g bioaerosol.

Before each experiment, the efficiency of the cleaning and disinfection of the drum was checked by swab testing internal surfaces at a detection limit of 50 cfu per 25 cm^2 or along a 80 cm seam of a vane. The swab was resuspended in 5 ml saline (0.85% NaCl, 0.1% peptone) plated and incubated 7 days for the detection of bacteria and fungi at 25°C.

Endotoxin analysis. Endotoxin analyses were performed in duplicate using the kinetic Limulus Amebocyte Lysate test (kinetic-QCL endotoxin kit; BioWhittaker). A standard curve obtained from an *Escherichia coli 055:B5* reference endotoxin was used to express the concentrations in endotoxin units (EU). The concentration of endotoxin was calculated as EU per g compost or EU per g bioaerosol. The concentrations can be converted into ng endotoxin (1 ng endotoxin = 15.5 EU).

Other measurements. To evaluate the microbial growth conditions during the composting period, the bulk samples were analysed for pH, content of water (H_2O %) and water activity (a_w). The measurements were carried out according to standard procedures: electrodical pH-measuremets, gravimetric analysis of the content of water before and after drying (110°C, 16 hours), and hygroscopic determination of the water activity.

Statistical methods. Hypothesis of age dependent differences and correlations were tested using SAS software (release 6.08; SAS Institute, Inc.). Before analysis of variance including Duncan's multiple range test (PROC UNIVARIATE; SAS), the concentrations of the microbiological parameters were logarithm transformed (log_{10}) and tested for normal distribution.
The concentrations presented in the text, Tables 1 and 3 are representing the geometric means (GM) of three independent experiments. For the analysis of variance, few data were set to half the limit of detection (¤) but if all values of a parameter were below the limit of detection (lod), statistical analysis was omitted and the concentrations were given as < lod.

RESULTS

Bulk samples of different composting stages. The concentrations of microorganisms and endotoxin from different composting stages are shown in Table 1. Total counts of microorganisms analysed by microscopy demonstrated the maximal concentrations. The content of microorganisms showed a dominance of bacteria (rods, spheres) and a great number of spores from actinomycetes (spheres < 1 µm). A significant increase in the concentrations occurred between week 0 and week 9 of composting indicating a relative slow and moderate rise during the whole period of composting.

Viable counts which were performed for 14 different groups of microorganisms showed various patterns. In general, the highest concentrations were found for bacteria cultured on the basal nutrient medium. For bacteria (37°C), the significant increase took place throughout the composting period (between week 0 and week 11). In contrast, the significant increase for bacteria (25°C) occurred within the first week of composting (between week 0 and week 1). A pattern similar to that of bacteria (37°C) was observed for Gram-negative bacteria and *Pseudomonas* spp., although both the parameters demonstrated much lower concentrations. The concentrations of coliform bacteria, *Bacillus* spp. and faecal streptococci showed a development line similar to that of bacteria (25°C). The significant increase of micrococci/stapylococci occurred with a slight delay between week 1 and week 11 of composting. The only decline of concentration over time was obtained for *Lactobacillus* spp. where the significant change occurred after 5 weeks, and subsequently the concentrations were below the limit of detection.

Among the microorganisms capable of airborne spore generation, a significant increase in thermophilic (55°C) and mesophilic (25°C) actinomycetes occurred steadily over composting time (between week 0-1 and week 9-11). In general, the concentrations of fungi were low but among the viable counts of fungi, yeast formed the major part. The concentrations of yeast reached a maximum after 1 week, but a statistically significant increase was not found. Moulds (fungi 25°C) demonstrated a significant increase at the end of the composting period (between

Table 2. pH, water content (H_2O %), and water activity (a_w) in bulk samples of composting household waste. Figures indicate the range of three samples from each stage.

week	pH	H_2O %	a_w
0	5.29–7.87	56.6–59.2	0.98–0.99
1	7.08–7.77	53.6–57.4	0.97–0.98
5	7.04–8.01	55.2–58.5	0.98
9	8.15–8.48	48.5–52.9	0.98
11	8.21–8.48	41.3–49.4	0.97–0.98

week 9 and week 11) but the concentrations were mostly just above the limit of detection at 200 cfu/g. The thermophilic fungi *Aspergillus fumigatus* were only found sporadically in two out of fifteen bulk samples (week 1: 200 cfu/g and week 9: 800 cfu/g) which was reported as < lod in Table 1. In general, the moulds constituted the lowest concentrations of the measured viable parameters.

The concentration of endotoxin varied throughout the time of composting with a significant increase from a minimum at week 5 to a maximum at weeks 9–11. Converted to µg endotoxin, the minimum and maximum concentration of endotoxin was 2.5 and 110 µg endotoxin per gram compost, respectively.

Table 3. Concentrations of microorganisms and endotoxin in bioaerosols generated from composting household waste. Figures represent mean values (GM) of three samples collected from the same composting stage, and range is indicated in brackets. According to Duncans multiple test for grouping the minimum and maximum are indicated by the capitals A and B, respectively. A jump from one capital to another indicates a statistically significant change ($p < 0.05$).

	week 1	week 5	week 9
	A		B
Total counts $\times 10^6$ cells/g	670 (330-970)	1200 (730-1600)	3500 (1400-6100)
Bacteria 25°C $\times 10^6$ cfu/g	7.9 (0.77-180)	13 (12-15)	17 (3.3-77)
	A		B
Actinomycetes 25°C $\times 10^6$ cfu/g	0.05 (<lod-0.08)	0.10 (<lod-0.16)	0.26 (0.16-0.66)
	A	B	B
Actinomycetes 55°C $\times 10^6$ cfu/g	0.46 (0.19-1.4)	11 (2.9-27)	15 (3.8-75)
Fungi (moulds) 25°C	<lod	<lod	<lod
A. fumigatus 45°C	<lod	<lod	<lod
Endotoxin units $\times 10^6$ EU/g	0.06 (0.02-0.21)	0.21 (0.09-0.62)	0.12 (0.05-0.30)

A - minimum, Duncans test; B - maximum, Duncans test; lod - limit of detection (2500 cfu/g).

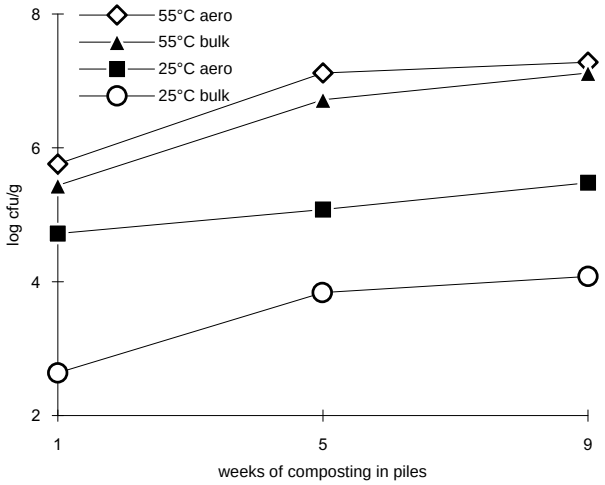

Figure 3. Actinomycetes in bioaerosols and in bulk samples over time of composting. The level of thermophilic actinomycetes (55°C) exceed that of mesophilic actinomycetes (25°C). The concentrations in the bioaerosols (aero) were above the concentrations found for bulk samples (bulk) of compost.

The results of the pH measurements, water content and water activity are shown in Table 2. The pH-values increased rapidly during the start of the composting period reaching a stable weak alcaline level after one week. The content of water in the bulk samples decreased gradually over time from a maximum of 60% to a minimum of 40%, but for all bulk samples of compost, the water activity was between 0.97 and 0.99.

Bioaerosols of different composting stages. The concentrations of microorganisms and endotoxin in the experimentally generated bioaerosols are shown in Table 3. The highest concentrations were found for the total counts of microorganisms which increased significantly over time. Concentrations of bacteria (25°C) and thermophilic actinomycetes (55°C) constituted the major part of the viable counts of the bioaerosols. The increase in bacteria (25°C) between week 1 and 9 was not significant which was consistent with the results from the bulk samples. Concentrations of thermophilic (55°C) and mesophilic (25°C) actinomycetes showed a significant increase over time. For the bioaerosols, all concentrations of fungi (25°C) and *Aspergillus fumigatus* were below the limit of detection which corresponds to approx. 2500 cfu per gram bioaerosol. The concentration of endotoxin in bioaerosols did not change significantly over time. Converted to µg endotoxin, the concentrations ranged from 4 to 14 µg endotoxin per gram bioaerosol.

Distribution between bioaerosols and bulk samples. A comparison was made between paired data for concentrations of total counts, mesophilic bacteria and actinomycetes (25°C), and thermophilic actinomycetes (55°C). The relation between bioaerosols and bulk samples was calculated as the ratio of:
concentration in bioaerosol/concentration in bulk sample.

Table 4. Concentration of microorganisms in bioaerosols related to the concentration in bulk samples of composting household waste. Range of ratios from paired data of total counts and viable counts of bacteria and actinomycetes. Due to observations below the limit of detection for mesophilic actinomycetes, only one ([1]) and two ([2]) ratios were calculated for week 1 and week 5, respectively.

	week 1	week 5	week 9	Maximum deposit
Total counts	0.02-0.03	0.07-0.12	0.05-0.24	Compost
Bacteria 25°C	0.01-1.0	0.16-2.2	0.04-0.50	Compost
Actinomycetes 25°C	16[1]	2-23[2]	12-330	Bioaerosol
Actinomycetes 55°C	0.4-88	7-13	1-36	Bioaerosol

The ratio expresses whether the concentration of a specific parameter in the bioaerosols is higher or lower compared to the source, i.e. the compost bulk sample. Figure 3 illustrates the geometric means (GM) for actinomycetes in bioaerosols and in bulk samples of compost, and Table 4 presents the range of ratios calculated for each parameter. In contrast to the analyses of total counts and of viable counts of bacteria (25°C), the ratios for the two groups of actinomycetes were well above 1. This indicated that the actinomycetes or their spores were particular prone to be airborne during the experimental conditions used in the study.

DISCUSSION

The objective of the study was to estimate the microbial risk potential during the composting processes of source separated household waste. Although an experimental design cannot simulate a realistic microbial emission during occupational handling of compost, the relative potential was estimated at the same time when the compost workers were handling the compost, e.g. at stacking and turning the piles or sieving the composted materials.

Health hazards related to occupational exposure to bioaerosols are reported for different environments mostly in connection to high concentrations of organic dust. Sometimes the causal microbiological agents are isolated but often the causes of the diseases are difficult to assess because of the lack of a clear dose-response relationship. In other cases, a mixture of different agents may cause a disease, e.g. the compost worker's syndrome which is probably due to exposure to high concentrations of spores from fungi (e.g. *Aspergillus fumigatus*) and thermophilic actinomycetes (e.g. species of *Thermoactinomyces* and *Thermomonospora*) and, perhaps endotoxin [4, 17, 18, 22, 30]. From studies in the agricultural environment, cases of organic dust toxic syndrome (ODTS) were demonstrated for Swedish farmers inhaling fungal spores of $10^{10}/m^3$ air while allergic alveolitis was associated with long time exposure to spore concentrations of $10^9/m^3$ [19, 20]. A case study of ODTS and allergic alveolitis was reported from a wood composting facility where "worst case" levels showed similar exposure to fungal spores together with high concentrations of Gram-negative bacteria and endotoxin [30]. In sawmills long time exposure to moulds above 10^6 spores/m^3 caused respiratory symptoms [9]. Among specific pathogens, the thermophilic fungus *Aspergillus fumigatus* has been reported as a common allergen and an opportunistic pathogen [7, 8, 22]. Studies of exposure to endotoxin from Gram-negative bacteria have been performed in the cotton industry. From dose-response experiments and experience from case studies, Rylander *et al.* [28] suggested a concentration of endotoxin below 100 ng/m^3 as a general acceptable level for avoidance of acute airway symptoms. Castellan *et al.* [3] found a level of approx. 10 ng/m^3 as the maximum exposure limit without a significant response. The dose-response relationship is very complicated, but in general, acute symptoms are demonstrated at higher exposure concentrations than is required for chronic effects from long time exposure. At present, no occupational exposure limits for exposure to microorganisms have been adopted.

No data is available on the development of microorganisms in composting of biodegradable household waste. Therefore the results of the present study have to be compared to studies on composts made of different basic materials and at different stages of the composting process. In the present study the rotating drum technique was used to characterize the source of bioaerosols in details. The advantage of using the drum for the experimental generation of aerosols was to achieve reproducible results for statistical use. Another substantial advantage was that the continuous generation of bioaerosols made it possible to obtain sufficient materials for the analysis. However, there are also some limitations in this approach. Using an experimental design in a laboratory scale is not immediately comparable with field studies of personal exposure. The experimental approach does not reflect a true situation of occupational exposure because of the artificial technique for the generation of the aerosols. It should be emphasized that the concentrations found in these experiments are only applicable for a relative risk assessment of the measured bioaerosols.

In general, composting processes of organic materials cause generation of ammonia due to the decomposition of the nitrogen containing part of the substances. The release of ammonia is observable as an increase in pH into a weak alkaline reaction. Outdoor pile composting of municipal solid waste in a Spanish study was evaluated by changes in physical and chemical parameters, showing that the pile sizes had a substantial influence on the composting processes, and excessively large piles did not allow adequate oxygenation of the inner materials resulting in an acidic compost [2]. In the present study, the early increase in pH and the persistent weak alkaline reaction of the compost (pH: 7.08–8.48 after one week and forward) indicates sufficient conditions for the aerobic composting processes. According to this, all samples from the independent piles were regarded as properly composted material, and the data obtained from the experiments were considered as being representative for this type of

composting when the process is in control. Another essential growth factor for microorganisms is content of water in the materials. In the study, the water percentage in the compost ranged from a maximum of approx. 60% to a minimum of 40% with a decline over time which was to be expected due to natural drying and drainage of the percolate. On the other hand, the water activity was constant at 0.97–0.99 during the whole period of pile composting, which ensured potentially favourable growth conditions for a broad spectrum of microorganisms.

From the examination of bulk samples and bioaerosols, the analysis of total counts by microscopy demonstrated concentrations at levels about 10^9–10^{11} cells per gram. Compared to the corresponding highest viable parameters, considerable differences in the concentrations were found. After eleven weeks of pile composting, the concentration (GM) of total microorganisms (total counts) in bulk samples amounted to 40 and 140 times the viable counts of bacteria (37°C) and bacteria (25°C), see Table 1. A similar comparison demonstrated an even larger disparity for the bioaerosols (Tab. 3). Here the concentration of total counts was 200 and 230 times the concentration of bacteria (25°) and actinomycetes (55°C), respectively. The substantial loss of the viability in the bioaerosols is probably due to the generation of aerosols and the sampling technique used. A strong agitation of the materials and the collection on filters over long time will most likely affect the viability of the microorganisms [5, 11]. Griffiths *et al.* [11] summarized a number of problems in microbiological investigations of bioaerosols. For characterizing airborne microorganisms it was emphasized, that total counts by microscopy is an important parameter because the culturing methods (viable counts) severely underestimate the total population of microorganisms in bioaerosols. Furthermore, the viability is of minor importance to workplace monitoring because any allergic or toxic effect occurs whether the microorganisms are viable or not [11]. In the present study, the total amount of microorganisms counted by microscopy should therefore be considered an essential parameter, especially for the risk assessment of the bioaerosols.

The advancing ageing of the compost was associated with an increase in the concentration of microorganisms in the bioaerosols. After eleven weeks of composting, the concentration of microorganisms (total counts) was 5 times above that at week one. This increase in the microbial potential of the bioaerosols may reflect a greater risk of occupational exposure at the end of the composting period.

The raw materials for compost in the present study was mainly derived from source separated household waste (86%). Unlike composting materials from pure vegetable sources, the content of animal food and the faecal contribution from napkins and small pets are likely to supply the compost with a considerable part of the intestinal microflora which may involve a number of infectious species of microorganisms. Some specific biological active agents, e.g. endotoxin from Gram-negative bacteria may also be of some importance [8, 16, 20]. The concentrations of microorganisms (total counts) in compost and in bioaerosols of approx. 10^9–10^{11} cells/g were comparable to concentrations found in percolate derived during storage of identical waste fractions in containers [24] and in bulk samples of compostable waste kept in storage systems similar to that of the actual composting plant [1]. In general, the concentration in the compost ranged from approx. 30 to 80 times the concentration in the bioaerosols, but for both type of samples a significant increase ($p < 0.05$) occured over time. The content of the mesophilic bacteria in compost (bacteria 37°C: 1.5×10^6 cfu/g - 2.1×10^9 cfu/g; bacteria 25°C: 5.7×10^5 cfu/g - 6.2×10^8 cfu/g) was consistent with the levels found in a German study of the microbial quality of compost from biowaste including napkins (bacteria 37°: 3.3×10^5 - 4.7×10^9 cfu/g) [14]. In a case study of respiratory disorders (ODTS and allergic alveolitis) in connection with exposure to bioaerosols from wooden compost, even higher concentrations of mesophilic bacteria were reported for the bulk samples of compost (bacteria 35°C:1.8×10^9 cfu/g - 1.9×10^{10} cfu/g) and the microflora involved was entirely Gram-negative bacteria [30].

The concentrations of faecal bacteria (coliforms and faecal streptococci) increased significantly ($p < 0.05$) within the first week of composting from a level close to the limit of detection up to a stable level of 10^5–10^7 and 10^5–10^6 cfu/g, respectively. A preliminary study reported on the reduction of infectious matters during the composting processes at the actual plant [13]. For bulk samples initial concentrations of coliforms and faecal streptococci of approx. 10^7–10^8 cfu/g were observed followed by a reduction of approx. 3 $\log_{10}$ units during the precomposting process. A reduction of faecal indicators during the thermal composting processes was also found in other studies [7, 12]. For the present study, the concentrations of the coliforms and the faecal streptococci after one week and forward were consistent with the general level demonstrated in comparable compost fractions [14] but a reduction over time was not observed. The analyses of the faecal microflora were only based on counts of colony forming units on two selective media. The statistically persistent level after one week may be due to a subsequent growth of these faecal indicators even if potential pathogenic species in advance had been eliminated by the thermal composting process. Although the influence of the present faecal indicators are not entirely explained, emphasis on the faecal microflora should be taken in risk assessments of work with this particular type of compost.

Over time of composting, a fluctuating concentration was observed for content of endotoxin in the bulk samples and in the bioaerosols. This fluctuation was not in agreement with the steady increase seen for Gram-negative bacteria in bulk samples. In compost, the minimum was found at the middle of the composting period but for the bioaerosols, the maximum was found at the same stage. This pattern was not immediately intelligible. Converted to nanogram endotoxin, the range in the concentrations of compost (2.5–110 µg/g) and of

bioaerosols (4–14 µg/g) was comparable or slightly below the concentrations found in percolate (10–300 µg/ml) [24]. Regarding endotoxin as an important biological agent in occupational bioaerosol exposure [3, 16, 28], it should be stressed that compost of household waste may involve a considerable hazard for personal exposure depending on the dustiness of the materials.

The warm environment during the composting process (up to 60–70°C) will favour the thermotolerant microflora [17, 22]. Actinomycetes were cultured at two temperatures for determination of the mesophilic (25°C) and the thermophilic (55°) species. Each group of actinomycetes was only enumerated on one type of media, which may cause an underestimation for species who did not obtain favourable conditions of growth. After 9 weeks, the concentrations in bioaerosols were 2.6×10^5 cfu/g and 1.5×10^7 cfu/g for mesophilic and thermophilic actinomycetes, respectively. These concentrations were close to converted levels from English studies on the microbial emissions from compost in which levels associated with allergic alveolitis were exceeded [6, 17]. Surprisingly, the thermophilic fungus *Aspergillus fumigatus* was only found in two out of fifteen bulk samples, and for these two samples the concentrations were close to the limit of detection. The low concentrations of fungi in the compost were reflected in the bioaerosols where neither mesophilic fungi (25°) nor the thermophilic *Aspergillus fumigatus* was detected. The absence of fungal spores in the air samples was inconsistent to other studies of microbial emissions from compost made of domestic waste [7, 17, 22]. Lacey *et al.* [17] often found concentrations of fungal spores above $10^6/m^3$, and *Aspergillus fumigatus* was present in the bioaerosols in large numbers. In a case study Weber *et al.* [30] found concentrations of fungi up to 10^9 cfu/g in wooden compost and, when converted into w/w %, concentrations up to approx. 10^8 cfu/g bioaerosol in air samples. In other studies of exposure to bioaerosols from compost or domestic waste, airborne spores from fungi and actinomycetes also constituted a substantial part of the analysed microflora in the bioaerosols [4, 15]. In the present study, the small contribution of fungi to the microflora was probably due to the composition of the basic materials. The low concentrations of fungi found gave evidence to suggest that airborne spores from fungi (moulds) are not predominant in composting of separated biodegradable household waste.

The ability of microorganisms to become airborne is dependent on several conditions including the materials where they grow and the physical characteristics of the species. The more humid, fine-grained and homogeneous quality the basic material is, the less airborne dust is emitted during agitation [26]. In general, bacteria often stick to particles or to surfaces while airborne spores from moulds and actinomycetes are more easily spread in the air [5, 11]. From a previous study of experimentally generated bioaerosols from household waste, a high weight loss of waste stored in paper sacks was observed [1]. The dustiness was correlated to the weight loss of the waste and extremely high concentrations of fungal spores, especially of *Aspergillus fumigatus*, became airborne. For the present study, actinomycetes in the bioaerosols were by far the most dominant type of airborne microorganisms. The existence of airborne spores from actinomycetes at the size of approx. 1 µm and below may easily cause deposition in the alveolar lung tissue [16, 22]. The concentrations of actinomycetes found in the experimentally generated bioaerosols were higher than or at the level of the concentrations in the compost. After five weeks of composting the thermophilic actinomycetes and the mesophilic bacteria formed the highest level of the viable microorganisms in the bioaerosols. Therefore, in a risk assessment of workers composting household waste special attention should be given to the thermophilic actinomycetes.

CONCLUSION

The microbial potential of bioaerosols increased over time in industrial pile composting of biodegradable household waste. Total counts of microorganisms in bulk samples of compost, as well as in bioaerosols from this compost, were comparable to the concentrations in percolate from source separated household waste. The microflora was dominated by bacteria and actinomycetes while fungi were almost absent. The thermophilic actinomycetes were the predominant source for airborne spores. For risk assessments of workers composting source separated household waste, particular emphasis should be put on airborne thermophilic actinomycetes and the faecal microflora in the compost. In characterizing microbial exposure, total counts of microorganisms by microscopy seemed to be the most reliable parameter for this particular occupational setting. Personal sampling is recommended to clarify the occupational exposure.

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