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Sampling of High Amounts of Bioaerosols Using a High-Volume Electrostatic Field Sampler

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For studies of the biological effects of bioaerosols, large samples are necessary. To be able to sample enough material and to cover the variations in aerosol content during and between working days, a long sampling time is necessary. Recently, a high-volume transportable electrostatic field sampler for collection of fine particles has been described. The aim of this study was to investigate whether this sampler can be used for collection of high amounts of authentic bioaerosols that can subsequently be used for biological analysis. The investigation was carried out at a biofuel plant in a straw storage room and in a boiler room over two seasons. The sampled dust was quantified in terms of mass and characterized regarding microbial components and compared with dust sampled by Gravikon and GSP samplers. For the electrostatic field sampler, a prefilter was used to remove large objects. The prefilter was characterized for particle penetration and this testing indicated that the prefilter did not remove particles up to 10 μm, and therefore respirable dust was sampled by the electrostatic field sampler. Using the electrostatic field sampler in the straw storage and in the boiler room, 330 and 315 mg dust (net recovery of the lyophilized dust) was sampled during a period of 7 days, respectively. The sampling rates of the electrostatic field samplers were between 1.34 and 1.96 mg dust per hour, the value for the Gravikon was between 0.083 and 0.108 mg dust per hour and the values for the GSP samplers were between 0.0031 and 0.032 mg dust per hour. The standard deviations of replica samplings and the following microbial analysis using the electrostatic field sampler and GSP samplers were at the same levels. The exposure to dust in the straw storage was 7.7 mg m^{-3} when measured by the electrostatic field sampler and 11.8 mg m^{-3} when measured by the GSP inhalable dust sampler. The quantity (amount per mg dust) of total fungi, *Aspergillus fumigatus*, total bacteria, endotoxin and mesophilic actinomycetes sampled by the electrostatic field samplers and the Gravikon samplers varied within the same season by a factor smaller than four. The quantities of some microbial components were higher in the dust collected with all samplers in March than in August. In conclusion, by using the electrostatic field sampler, it was possible to sample replicas of large authentic aerosol samples that can be used, e.g. biological analysis.

Keywords: aerosol sampling; bacteria; biofuel; electrostatic field sampler; endotoxin; fungi

INTRODUCTION

Bioaerosols in occupational environments are in general a complex mixture that may include viable and dead microorganisms, as well as components and metabolites of microorganisms. They are known to cause different health effects such as hypersensitivity, toxic reactions, irritation, inflammatory responses and infections. The allergenic, toxic and inflammatory responses can be caused by non-viable as well as by viable fungal spores. Maintaining culturability of spores during sampling may be of importance if the fungi are to be identified by traditional cultivation methods or if infectious fungi are to be studied. Furthermore, culturability may be of importance because germination of some spores of some fungal species is seen to increase allergen

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release (Mitakakis *et al.*, 2001). Some microorganisms and microbial components are shown to have an adjuvant effect (Pirie *et al.*, 2003; Huttunen *et al.*, 2004; Instanes *et al.*, 2006). Therefore, it is important to study the health effects of natural bioaerosols.

Different principles such as impingement, impaction, electrostatic precipitation and filtration have been used to sample microorganisms in the working environment. Some of these samplers, e.g. the Andersen sampler and the electrosampler, collect aerosols on an agar medium (Yao and Mainelis, 2006), and thereby it is not possible to use the aerosols for other purposes than cultivation. For studies of biological effects of bioaerosols, large samples are usually necessary. Furthermore, a long sampling time is often important due to variations in microbial components in the air during and between working days (Fishwick *et al.*, 2001; Augustowska and Dutkiewicz, 2006; Jo and Kang, 2006; Madsen, 2006). A long sampling time may also be necessary to obtain a large sample in cases where the aerosol concentration is low. Some samplers such as some impactors have been developed to sample for a very short time, and others, e.g. filters samplers, have been developed to sample for a whole working day (Pasanen, 2005). Recently, a high-volume transportable electrostatic field sampler for collection of fine particles has been described (Sharma *et al.*, 2007a). This sampler has the advantage of simple operation, a high volume flow and it can sample airborne dust for days to weeks.

The aim of this study was to investigate whether the transportable electrostatic field sampler (volumetric flow rate 3500 l min^{-1}) can be used for collection of high amounts of authentic aerosols including microorganisms and microbial components, which can subsequently be used for biological analysis. The investigations were carried out at a biofuel plant because people working at biofuel plants are exposed to high concentrations of bioaerosols (Madsen, 2006). To evaluate the sampling of bioaerosols with the electrostatic field sampler, we also sampled respirable and inhalable dust by other well-known samplers used in occupational environments. We sampled respirable dust with a Gravikon (volumetric flow rate 375 l min^{-1}) that was a development of the Occupational Institute for Safety at Work in Germany for stationary sampling at occupational environments. The Gravikon has been used to sample bioaerosols, e.g. at waste handling facilities (Fischer *et al.*, 1999). We also used a GSP sampler [conical inhalable sampler (CIS) sampler with a volumetric flow rate of 3.5 l min^{-1}] to sample inhalable dust and this sampler is commonly used for occupational monitoring throughout Europe (Kenny *et al.*, 1997) and the sampler has been used in bioaerosol studies, e.g. (Ronald *et al.*, 2007). The dust sampled by the three different samplers was quantified in terms of mass and characterized regarding content of fungi, bacteria, actinomycetes, endotoxin and β-glucan. To test the electrostatic field sampler with dust of different compositions and during different exposure levels, we sampled over two seasons as these factors are sometimes dependent on the season (Nielsen *et al.*, 1997; Oppliger *et al.*, 2005; Madsen, 2006) and finally we also sampled dust in two different areas.

MATERIALS AND METHODS

Airborne dust was sampled at a biofuel plant situated in Denmark on Zealand. The dust sampling was performed in March and August 2006. Two working areas at the biofuel plant were included in the study: a combined straw storage and straw reception (in the following called straw storage) and the combined boiler and straw feeding room (in the following called the boiler room). These areas were chosen because people worked in these areas and we expected the dust to contain both particles from the straw, from the vehicles unloading the straw, forklift trucks and from the combustion process. The characterization of the electrostatic field sampler regarding penetration of particles through the prefilter used was performed at the plant in March 2007.

Characterization of the prefilter used in the electrostatic field sampler

The sampler is described in detail in Sharma *et al.*, 2007a. The sampler is based on a commercial electrostatic office air cleaner (Look-Fair, Vicenza, Italy) maintained by a cross fan. It consists of 17 plates ($\sim$11 $\times$ 17 cm) with a total filter area of 1.6 m^2. The collection efficiency window of the plates is >90% in the particle size range 30 nm–2.5 μm and >3 μm the efficiency dropped linearly and was 50% between 4 and 5 μm. In this study, we used a prefilter made of synthetic fibres and classified as filter-class G, (Look-fair), which filtrates coarse particles. The aim with this prefilter was to remove coarse objects such as pieces of straw etc. We did not know the detailed filtration characteristics of this prefilter and therefore we tested it in the biofuel plant in the straw storage, where particle samples were collected. Testing was done during 8 days. Efficiency was determined by measurements with a Scanning Mobility Particle Sizer (SMPS+C, Model 5400, Grimm, Ainring, Germany) and an Aerosol Particle Sizer (APS-3321; TSI Inc., MN, USA), by comparing results from the first day and after 8 days of sampling. On the first day there was no delivery of straw, but on the eighth day there was a delivery of straw in the straw storage. Testing method was similar as in Sharma *et al.*, 2007b. The only modification was that to obtain the collection efficiency, the prefilter was mounted and removed after each scan in the present study. The scanning mobility particle sizer (SMPS) measures the number concentration of particles from 0.01 to 0.875 μm (mobility diameter). However, owing to counting statistics,

only data from 0.0139 to 0.492 μm were used. Measurements were conducted every alternate 6 min and 47 s (normal scan mode), resulting in 10–12 paired datasets for each day. The aerosol particle sizer (APS) measures the number concentration of particles from 0.542 to 19.81 μm (aerodynamic diameter). However, owing to counting statistics, only APS data up to 10 μm were used. Measurements were conducted every alternate 3 min, resulting in 15–20 paired datasets for each day. During the first day of testing, measurements with the APS were carried out in both the morning and in the afternoon.

Sampling by the electrostatic field sampler and extraction of dust

The electrostatic field sampler was used for collection of dust with a sampling time of 9 days in March and for 7 days in August 2006. In March, one sampler was placed in the straw storage at the height of 1 m above ground level and one sampler in the boiler room about three floors up. In August, one sampler was placed in the straw storage and three samplers in the boiler room.

Particle extraction of the plates was done according to Sharma *et al.*, 2007b but the amount of extraction suspension was also quantified. Part of the suspension (2 ml) was used for quantification of microbial components. Subsequently, the particle samples were lyophilized and gently retrieved using a glass spatula, then weighed.

To get an impression of what was retained in the prefilters, these prefilters were tapped off on clean papers until no more visible dust was released. This dust was weighed and characterized for content of microbial components. We do not know the recovery on the dust extraction from the prefilters and thus these data should be used with caution. The conversion factor from the dust sampled to lyophilized dust was as follows: 16 mg dust weighted 1.0 mg after lyophilization.

Sampling by GSP samplers and extraction of dust

Bioaerosols were sampled as described in Madsen, 2006. GSP samplers (CIS by BGI, Waltham, MA, USA) were placed 1 m above ground level for 3 h in March and for 7 h in August in the straw storage and in the boiler room. The samplers were mounted with Teflon filters (pore size 1 μm) for endotoxin analysis and gravimetric analysis and polycarbonate filters (pore size 0.8 μm) for quantification of total and colony-forming units of microorganisms and for β-glucan. The flow was 3.5 l min^{-1} and this was checked every hour. A total of 12 air samples were collected in the two working areas. The dust on the Teflon filters was extracted in 6.0 ml pyrogen-free water with 0.05% Tween 20 by orbital shaking (300 rpm) at room temperature for 60 min and centrifuging (1000 g) for 15 min, and the supernatant was used for endotoxin assay. The dust on polycarbonate filters was extracted in 10.0 ml sterile 0.05% Tween 80 and 0.85% NaCl aqueous solution by shaking for a 15-min period (500 rpm) at room temperature.

A part of the particle sample was lyophilized and gently retrieved using a glass spatula, then weighed. The conversion factor from the dust sampled to lyophilized dust was as follows: 15 mg dust weighed 1.0 mg after lyophilization.

Sampling by the Gravikon and extraction of dust

The Gravikon VC 25 (GSM, Neuss-Norf, Germany) dust collector was placed 1 m above ground level in the straw storage. The Gravikon was mounted with a sampling head for respirable dust and cellulose acetate filters (pore size 8 μm). The airflow was 375 l min^{-1}. During sampling, the filter resistance reached the maximum permitted value due to dust deposition and the equipment was switched off automatically and the timer recorded the total sampling time. Consequently, the filter was replaced twice during this sampling period in March and the total sampling period was about six-and-a-half days in March and 3 days in August 2006.

The dust on the filters was extracted as described for the GSP samplers but in 300.0 ml extraction solution. Part of the dust suspension was used for endotoxin assay. NaCl aqueous solution (0.85%) was supplied to the other part of the suspension and it was used for quantification of microorganisms and β-glucan. A part of the particle samples were lyophilized and gently retrieved using a glass spatula, then weighed. The conversion factor from the dust sampled to lyophilized dust was as follows: 10 mg dust weighed 1.0 mg after lyophilization. This conversion factor for respirable dust was also used for dust sampled by the electrostatic field sampler.

Measurements of particle size distributions

During the sampling period of 9 days in March 2006, a particle counter (GRIMM model 1200) was measuring particle concentrations and sizes (0.75–20 μm) >1-min intervals in the boiler room. An APS (APS-3321; TSI Inc.) was measuring the number concentration of particles from 0.542 to 19.81 μm (aerodynamic diameter) >1-min intervals in the straw storage. Numbers of particles of different size categories relative to numbers of particles between 0.75 and 1.0 μm were calculated.

Particle release of straw

A rotating drum with horizontal axis and a volume of 3.3 m^3 was used to agitate straw. Straw (3.0 kg) was placed onto the bottom of the drum, which was then

rotated at 7 rpm for 5 min as described in Madsen *et al.*, 2006. An isokinetic probe downstream of the drum delivered a subsample (1.9 l min^{-1}) to a particle counter (GRIMM model 1200) measuring particle concentrations and sizes >6-s intervals. High efficiency particulate air (HEPA)-filtered replacement air was supplied upstream of the drum and in excess to ensure ambient pressure inside the drum. Numbers of particles of different size categories relative to numbers of particles between 0.75 and 1.0 μm were calculated.

Quantification of microorganisms (CAMNEA)

Microorganisms were quantified using a modified CAMNEA method (Palmgren *et al.*, 1986). The number of fungi cultivable on Dichloran Glycerol agar (DG 18 agar, Oxoid, Basingstoke, UK) at 25°C was counted. In addition, agar plates were incubated at 45°C to quantify cultivable *Aspergillus fumigatus*. Estimates were made, firstly of the number of bacteria cultivable at 25°C on nutrient agar (Oxoid) with actidione (cycloheximide; 50 mg l^{-1}) and secondly of mesophilic actinomycetes (25°C) and thermophilic actinomycetes (55°C) cultivable on, respectively, 10 and 100% nutrient agar with actidione (cycloheximide; 50 mg l^{-1}).

The total numbers of bacterial cells and fungal spores were determined after staining in 20 ppm acridine orange (Merck) in acetate buffer for 30 s with subsequent filtration through a dark polycarbonate filter (25 mm, 0.4 μm; Nuclepore, Cambridge, MA, USA). Fungi and bacteria were counted at a magnification of 1250 times using epi-fluorescence microscopy (Orthoplan; Leitz Wetzlar). The numbers of microorganisms were determined in 40 randomly chosen fields or until at least 400 cells were counted.

Quantification of endotoxin and β-glucan

The supernatant was analysed (in duplicate) for endotoxin using the kinetic Limulus Amboecyte Lysate test (Kinetic-QCL endotoxin kit, BioWhittaker, Walkersville, MD, USA). A standard curve obtained from an *Escherichia coli* O55:B5 reference endotoxin was used to determine the concentrations in terms of endotoxin units (EU) (12.0 EU ≈ 1 ng). Dust suspensions were used for quantification of β-glucan using the kinetic Fungitic G Test (Seikagaku Co., Tokyo, Japan). The results were expressed as nanogram per milligram dust.

Treatment of data

Where three repeats of dust sampling were performed, standard deviation of the log-normal-transformed quantities of microbial data was calculated. The data were then back-transformed and the standard deviation and medians presented. To investigate if the prefilter used for the electrostatic field sampler had a statistically significant effect on the particle number concentration measured with the SMPS and APS, the paired datasets (the particle number concentration before and after the filter) for each channels were analysed by a paired *t*-test or a non-parametric test (signed rank test) if the criterion of normality failed.

RESULTS

Numbers and size distribution of particles

Most of the particles released from straw and most of the particles present in the boiler room and the straw storage had an aerodynamic diameter of <10 μm. Most of the particles released from straw had aerodynamic diameters between 2.0 and 7.5 μm.

Numbers of particles of different size categories relative to numbers of particles with aerodynamic diameters between 0.75 and 1.0 μm were calculated. The results show that straw released many particles >1 μm relative to the numbers of particles between 0.75 and 1.0 μm compared to what was measured at the biofuel plant. Furthermore, the ratio between the numbers of particles >15 μm relative to the numbers of particles between 0.75 and 1.0 μm was larger for particles measured in the straw storage than for particles measured in the boiler room (Fig. 1).

Penetration of particles through the prefilter of the electrostatic field sampler

Figures 2 and 3 show the particle number concentrations before and after the prefilter in the submicrometer range up to 0.875 μm on the first day and Day 8, respectively. Figure 2, shows the first day of testing and there was no statistically significant difference in the particle number concentration before or after the filter in the whole size range up to 0.492 μm. Figure 3 shows the eighth day of testing and the particle number concentration of four adjacent channels are pooled because of large standard deviations for each channel. A comparison of the paired datasets of before and after the filter showed that there was no statistically significant difference in the particle number concentration before and after the filter both for any channel up to 0.492 μm or for the pooled data. This indicated that the prefilter did not remove particles in the particle size range from 0.0139 to 0.492 μm. On the first day (Fig. 2), there was a maximum mode at ∼0.2 μm, whereas on Day 8 the particle size distribution was markedly different and the standard deviations were also higher compared to Day 1. This may be due to higher activity on Day 8, where straw was delivered. Figures 4 and 5 show the particle number concentrations before and after the prefilter in the aerodynamic diameter range up to 10 μm. There was no statistically significant

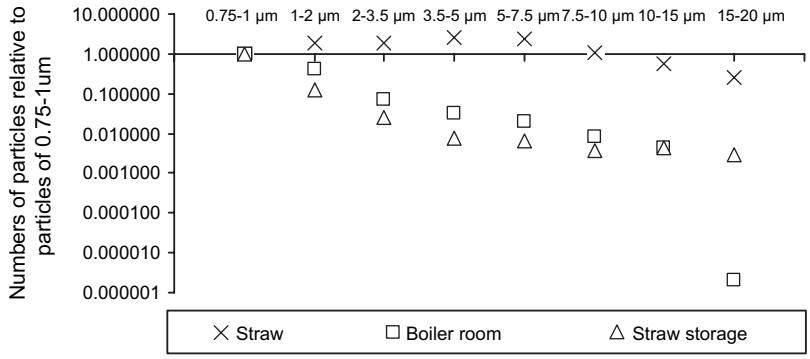

Fig. 1. Number of particles of different size categories relatively to numbers of particles with aerodynamic diameters between 0.75 and 1 µm present in the boiler room and in the straw storage for 9 days in March 2006 and released from a straw sample. Particles in the boiler room and particles released by the straw have been measured using a GRIMM particle counter and particles in the straw storage have been measured using an APS.

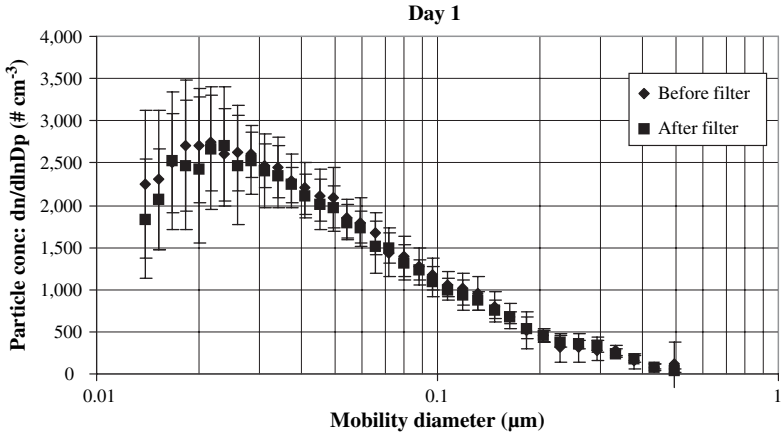

Fig. 2. Performance of the prefilter on Day 1, determined by the SMPS. Error bars represent standard deviations.

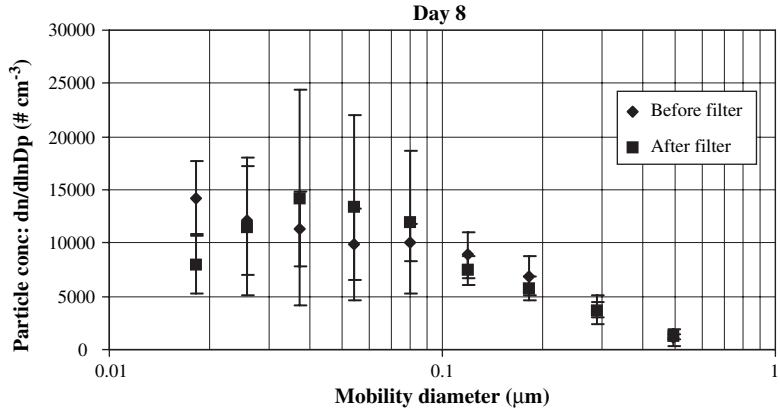

Fig. 3. Performance of the prefilter on Day 8, determined by the SMPS. The number concentration of four adjacent channels is pooled. Error bars represent standard deviations.

difference in the particle number concentration before or after the filter of particles up to 10 µm. The distributions from Day 1 are fairly similar compared to the distribution from Day 8 with a maximum mode of about 0.9–1.0 µm. The main difference was higher particle number concentrations on Day 8, which may be due to higher activity when straw was delivered.

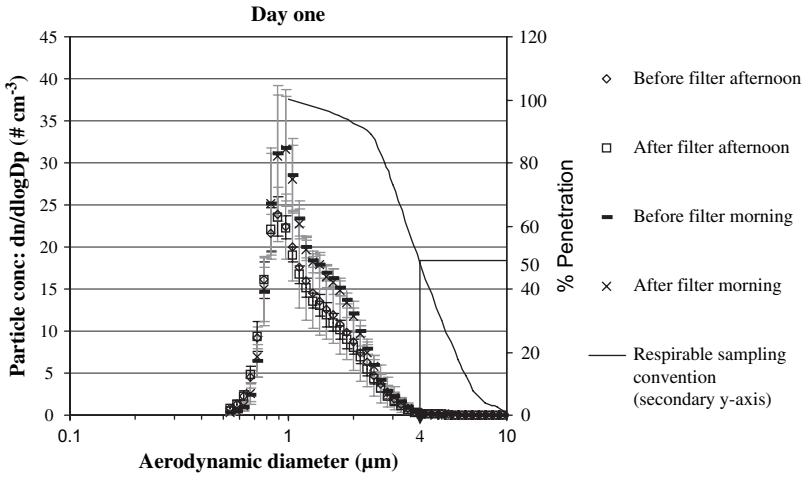

Fig. 4. Performance of the prefilter on Day 1 in the morning and afternoon, determined by the APS. Error bars represent standard deviations. The respirable sampling convention is also plotted (secondary *y*-axis).

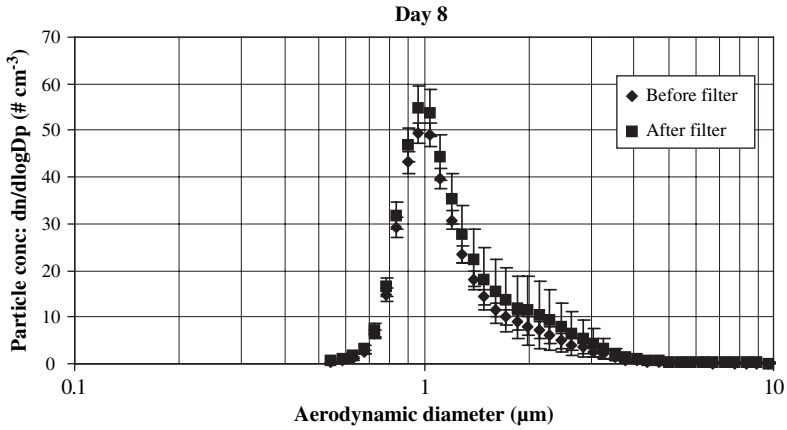

Fig. 5. Performance of the prefilter on day 8, determined by the APS. Error bars represent standard deviations.

Characterization of dust sampled by the three aerosol samplers

All amounts of dust are presented as net recovery of the lyophilized dust. Using the electrostatic field sampler in the straw storage, up to 330 mg airborne dust was sampled. From the prefilter from March, 78 mg dust was released. In the same area in March, a total amount of 130 mg dust was sampled by the Gravikon on the three filters used. In the boiler room, up to 410 mg dust was sampled by the electrostatic field sampler (Table 1). An amount of 39 mg dust was released from the prefilter after sampling in March. The amount of dust sampled per hour (sampling rate) was calculated and the electrostatic field sampler had the highest sampling rate, while the GSP samplers had the lowest rate. The sampling rate of the GSP samplers was higher on the sampling day in August than on the sampling day in March. The exposure measured in the straw storage by the electrostatic field sampler was also higher in August than in March. In March, the exposure was 7.7×10^{-3} mg m^{-3} and in the same period by the Gravikon 3.7×10^{-3} mg m^{-3} (Table 1). The concentration of inhalable dust measured during a working day in March by the GSP sampler was 11.8×10^{-3} mg m^{-3}. In August, dust was also sampled by the three kinds of samplers and it should be noted that the Gravikon only sampled for 3 days while the electrostatic field sampler sampled for 7 days. Furthermore, a high activity with unloading of straw should be noted during the day of sampling in August with the GSP sampler.

The quantity of total fungi, *A. fumigatus*, total bacteria, endotoxin and mesophilic actinomycetes sampled by the electrostatic field samplers and the Gravikon samplers varied within the same season by a factor smaller than four. The quantities of thermophilc actinomycetes were lower compared to the

Table 1. Sampling period, amount of sampled dust, standard deviations and quantities of fungal components (unit per mg lyophilized dust) in the biofuel dust sampled by electrostatic particle field samplers, Gravikon and GSP samplers

Sampler	Place	Period	*n*	Dust				Total fungi per mg		Mesophilic fungi per mg		*Aspergillus fumigatus* per mg		β-Glucan per mg	
				mg	S*	mg h^{-1}	mg $m^{-3} \times 10^{-3}$	Number $\times 10^4$	S*	cfu $\times 10^4$	S*	cfu	S*	mg $\times 10^4$	S*
Electrostatic, March	Boiler room	9 days and nights	1	410		1.90[a]	10.8	290		2.4		2030		11	
Electrostatic, March	Straw storage	9 days and nights	1	290		1.34	7.7	550		1.2		1220		49	
Gravikon[b], March	Straw storage	9 days and nights	1	13		0.083	3.7	160		9.5		700		940	
GSP[a], March	Boiler room	Day with low activity	3	0.0093	1.03	0.0031	13.1	3450		1.22 21	1.23	28050	1.17	180	1.11
GSP[a], March	Straw storage	Day with low activity	3	0.0073	1.06	0.0032	11.8	4800		1.20 60	1.18	21000	1.12	765	1.13
Electrostatic[a], August	Boiler room	7 days and nights	4	315	1.14	1.88	10.7	110		1.22 1.2	1.78	1530	1.36	1.4	1.24
Electrostatic[a], August	Straw storage	7 days and nights	3	330		1.96	11.2	100		4.6		2180		6.1	
Gravikon, August	Straw storage	3 days and nights	1	0.78		0.108	4.8	120		3.0		430		22	
GSP[a], August	Boiler room	Day with high activity	3	0.053	1.14	0.0073	37.0	1875		1.36 4.5	1.04	7800	1.19	74	1.13
GSP[a], August	Straw storage	Day with high activity	3	0.23	1.02	0.032	150	1575		1.07 74	1.04	15750	1.02	150	1.07

[a]Median quantities are presented.
[b]The filter was replaced twice and the total sampling period was 6.5 days.

other microorganisms and it was very different in the samples from the different samplers. The quantities of total fungi, β-glucan, total bacteria and mesophilic actinomycetes were higher in the dust collected with all samplers in March than in August (Tables 1 and 2). The quantities of total fungi, *A. fumigatus*, endotoxin and bacterial quantities were higher in dust sampled by the GSP sampler than by the Gravikon and by the electrostatic field samplers (Table 2). The standard deviations for triplica samples were calculated. The standard deviations for the amount of sampled dust, β-glucan, total bacteria and endotoxin were 1.25 or smaller for samples from the electrostatic field samplers and the GSP samplers. Microorganisms were also present in the dust retained in the prefilters (data not presented). The endotoxin quantity (EU per mg dust) was higher in the prefilter dust than in the dust sampled on the collection plates of the electrostatic sampler in both the boiler room (59820 EU per mg in March) and straw reception (62490 EU per mg in March). The quantities of all microbial components except endotoxin and total bacteria were higher in the prefilter dust from the straw reception than in the prefilter dust from the boiler room. For example, the quantity of total fungi were 4200 $\times$ 10^4 spores per mg prefilter dust from the boiler room and 19 500 $\times$ 10^4 spores per mg prefilter dust from the straw reception.

The relative cultureability of fungal spores sampled by the electrostatic field samplers was 0.2–4.6% and the same values for fungal spores sampled by the GSP samplers (for 3–7 h) were 0.2–5.4% and for spores sampled by the Gravikon 2.5–5.9%.

DISCUSSION

We tested an electrostatic field sampler for collection of bioaerosols. By using the electrostatic field sampler with the chosen prefilter, it was possible to sample and extract high amounts of bioaerosols and subsequently to quantify the microbial components. The standard deviation of three replicas of dust samples from the 9 days of sampling in the boiler room was 1.25. This value can be considered as low in comparison with other standard deviations of environmental samples (Limpert *et al.*, 2001). The standard deviations were also low for most microbial components. It was, however, highest for thermophilic actinomycetes and this may be because they were present in low quantities, thus causing greater uncertainty in the quantification. It may also reflect a more uneven distribution of the organism.

Most of the particles released from the straw and most of the particles present in the boiler room and the straw storage had aerodynamic diameters of $<$10 μm. For the electrostatic field sampler, we used a prefilter to avoid the capture of large objects such as pieces of straw. The particle number concentrations

Table 2. Quantity of bacterial components (unit per mg lyophilized dust) in the biofuel dust and standard deviations (s*) sampled by electrostatic field samplers, Gravikon and GSP samplers

Sampler	Place	Total bacteria		Endotoxin		Mesophilic actinomycetes		Thermophilic actinomycetes	
		Number $\times 10^4$	S*	EU	S*	cfu $\times 10^4$	S*	cfu $\times 10^4$	S*
Electrostatic, March	Boiler room	130		424		5.8		1.3	
Electrostatic, March	Straw storage	89		468		13		0.22	
GSP, March	Boiler room	4800	1.27	3300	1.11	50	1.20	13	2.01
GSP, March	Straw storage	4500	1.22	9990	1.12	180	1.26	23	1.88
Gravikon, March	Straw storage	250		1100		9.5		0.024	
Electrostatic, August	Boiler room	41	1.25	212	1.25	1.4	1.18	0.42	1.25
Electrostatic, August	Straw storage	16		426		0.55		0.33	
GSP, August	Boiler room	3105	1.15	41445	1.10	9.0	1.77	0.96	2.56
GSP, August	Straw storage	4410	1.25	50640	1.12	71	1.17	36	2.47
Gravikon, August	Straw storage	36		480		1.0		0.19	

before and after the prefilter used in this study were not significantly different in the particle size range up to 10 μm. The electrostatic unit consists of 17 plates [electrostatic precipitation (ESP) plates], running at 2.7 kV, which are placed after the prefilter. The collection efficiency of the ESP plates is ∼50% at 5 μm (Sharma *et al.*, 2007b) and consequently the 50% collection efficiency of particles with the prefilter used in this study was ∼5 μm, which is in the respirable range (Fig. 4, where the 50% cut-off for the respirable convention is at 4 μm). The prefilter used in an earlier study resulted in collection of fine particles (Sharma *et al.*, 2007a). The Gravikon used in the present study also collected respirable dust. The sampling capacity of the electrostatic field sampler was ∼60 times higher than the Gravikon and thereby proving to be useful when large amounts of samples are required.

The ratio of the number of released particles relative to the released particles with sizes between 0.75 and 1 μm was higher for straw studied alone, than found at the biofuel plant during the 9 days in March 2006. This indicates that some of the submicrometer airborne particles found at the plant are not of straw origin but may be from, e.g. the vehicles transporting the straw or the fumigation process. The submicrometer fraction is also sampled by the electrostatic field sampler and the constituents of this fraction will contribute to the dust samples. Furthermore, relatively more of the large particles (15–20 μm) were present in the straw storage than in the boiler room (Fig. 1), probably due to the particle release from straw during handling in the straw storage. The higher concentration of particles >15 μm in the straw storage is also in accordance with the higher amount of dust retained in the prefilter in the storage than in the boiler room.

The particle size distribution in the submicrometer range (measured by SMPS) changed significantly from Day 1 to Day 8 in March 2007. There was much higher activity and more dust sources in the straw storage on Day 8, where diesel vehicles delivered straw. After unloading straw, the truck bodies and the floor were cleaned with brooms, which generated dust. Traffic sources, including diesel exhaust emissions from the trucks, would mainly influence the ultrafine size range in the particle size distribution (Ketzel *et al.*, 2004), which in the present study was the situation between Day 1 and Day 8. However, cleaning after unloading the straw would be expected to influence the fine and coarse range in the particle size distribution, but surprisingly there was no marked difference in the size distribution of Day 1 and Day 8. Hence, higher activity in the storage hall by diesel trucks, unloading of straw and cleaning of truck bodies and the floor only had an impact in the submicrometer size range.

Total fungi, *A. fumigatus*, endotoxin and total bacteria were found in highest quantities in dust from the GSP samplers. Even though only few particles >10 μm were present in the air at the biofuel plants, some dust was retained in the prefilter. We have measured the quantities of microbial components in this dust (data only sparsely presented). These measurements showed the highest quantities of endotoxin and total bacteria in the retained dust. The observation that only a small part of the bacteria was of respirable size is in accordance with a study at a composting plant (Kenny *et al.*, 1999). Total fungi were present in higher quantities in the prefilter dust and GSP dust than in dust from the Gravikon and the electrostatic field sampler. This shows that many fungi also are present as particles larger than the respirable size. This is in accordance with a study at a composting plant, where ∼70% of the sampled fungi were of the thoracic size and only ∼30% of respirable size (Kenny *et al.*, 1999). Fungal spores are larger than bacterial spores or cells and a study of airborne agricultural dust showed that sampled fungal spores had mean aerodynamic diameters of between 3.7 and 18.9 μm (Lee *et al.*, 2006). In spite of that, the ratio

between bacteria in inhalable versus respirable dust was in this study up to 276 and for fungi up to 17. Thus, in biofuel dust, fungal spores seem to be more often present as single spores or as small clusters of spores of respirable size, compared to bacteria which seem more often to be present in larger clusters or associated with large particles.

The relative culturability of fungi sampled by the electrostatic field sampler, the Gravikon and the GSP sampler was at the same level, even though the sampling times differed between 3 h and 9 days. In other studies, the relative culturabilities of fungal spores sampled by filter sampling methods have been very different between different environmental samples (Durand *et al.*, 2002; Madsen *et al.*, 2004). Sampling time in both filter sampling and sampling by electrical fields has been shown to negatively affect the culturability of microorganisms which have first been cultivated in the laboratory and then aerosolized (Wang *et al.*, 2001; Yao *et al.*, 2005). On the other hand, a field study performed at a composting facility showed no effect of sampling duration (Durand *et al.*, 2002). The cultivability of spores in the field samples in this study did not seem to be noticeably affected by the very long sampling time of up to 9 days.

CONCLUSIONS

The study showed that it was possible to sample large amounts of dust with the electrostatic field sampler and extract the dust and quantify microbial components. The prefilter for the electrostatic field sampler was characterized for penetration and there was no significant difference in the particle number concentration before and after the prefilter. Consequently, the electrostatic field sampler collected respirable particles due to the collection efficiency of the electrostatic plates. The comparison of the sampler with the GSP sampler and the Gravikon showed that the electrostatic field sampler collected the highest amounts of dust. The sampling capacity was between 1.3 and 2.0 mg lyophilized dust per hour during 7 or 9 days of sampling in the straw storage, which was $\sim$ 60 times and 420 times higher than the Gravikon and GSP sampler, respectively. The standard deviations of the dust sampling and of the subsequent microbial analysis were at the same levels for the electrostatic field samplers and the GSP samplers. The quantities of some microbial components were higher in the dust collected with all samplers in March than in August. The dust from the electrostatic field sampler showed the same level of relative culturability of fungi as the GSP samplers and the Gravikon. Overall, the electrostatic field sampler proved to be suitable for sampling of bioaerosols, where simple operation and high amounts of authentic sample material were the main advantages.

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