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# Identification of Work Tasks Causing High Occupational Exposure to Bioaerosols at Biofuel Plants Converting Straw or Wood Chips

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## 1. Introduction

A bioaerosol is a suspension of airborne dust that contains living organisms or was released from living organisms such as fungi, bacteria, actinomycetes, pollen and other plant material. Exposure to bioaerosols containing high concentrations of fungi, actinomycetes, or endotoxin from bacteria may cause various deleterious health effects mainly on the airways (Douwes et al. 2003). The respiratory disorders caused by bioaerosol components can be dependent on the exposure levels (Rylander et al. 1985; Eduard et al. 2001). For example, a 60 spore m<sup>-3</sup> increment in concentration of the fungus *Epicoccum* has been found to be associated with increased incidence of morning cough (Neas et al. 1996). At biofuel plants, large quantities of biofuel are handled and exposure to bioaerosol components and other particles occurs (Madsen 2006) which may cause respiratory disorders. An epidemiological study shows that the exposure level to microorganisms has an impact on the occurrence of respiratory symptoms among biofuel workers (Schlünssen et al. 2010). Inflammation in relation to respiratory disorders has been evaluated in mice exposed to airborne dust collected in two working areas at a biofuel plant. The study indicates that dust from a biofuel plant, at doses corresponding to two weeks of observed human endotoxin exposure, results in a strong inflammatory response. The airborne dust from the straw storage hall at the biofuel plant induced a stronger inflammatory response than dust from the boiler room and had the highest concentration of most microbial components (Madsen et al. 2008). In contrast, airborne dust collected from a boiler room at a straw plant were more toxic in terms of mutagenicity, PAH (polycyclic aromatic hydrocarbons) concentration and ability to generate reactive oxygen species than dust generated from straw and wood chips and than airborne dust sampled in a straw storage hall (Cohn et al. 2010). Also particles from combustion of dried animal drugs are described to be highly oxidative (Mudway et al. 2005).

Occupational exposure to bioaerosol components and the inflammatory potential of these bioaerosols are different in different working areas at the plants (Madsen 2006; Timm et al. 2009). Furthermore the microbial dustiness of different biofuels (straw, wood chips, wood pellets and wood briquettes) differs (Madsen et al. 2004) and also depends on the storage method and period (Sebastian et al. 2006). Some people at biofuel plants work for a whole day in the straw storage and this can cause a high exposure. The straw shredding area has

also been identified as a high risk exposure area (Madsen 2006). The aim of this chapter is to identify factors influencing exposure to bioaerosols in straw storage halls and to reveal the impact on the exposure of different attempts to reduce exposure, e.g. sealing of a straw shredder. Empirical data showing the influence of opening outdoor gates while straw is unloaded are presented. Furthermore the impact of the quality of the biofuel handled in the straw reception on the human exposure is studied as well as the impact on the exposure of the water content of the handled straw.

## 2. Methods

### 2.1 The biofuel plants

The study included 18 biofuel plants situated all over Denmark. To make this study comparable with earlier publications of studies on the same plants, the same names as used in these previous papers have been used. Thus 13 plants are called a number between 4 and 24 as in another study (Madsen and Nielsen 2010), and five other plants are called plant A,B,C,D and E, also as in another study (Madsen 2006). The plants generated energy using straw or wood chips as the fuel. Airborne dust was sampled in working areas in combined straw receiving and storage halls, which in the following are called straw storage halls. At plants A and E, airborne dust was sampled in areas where work with wood chips was performed and at plants B, C and D dust was sampled where work with straw was performed. At 11 of the plants straw was received on both days of sampling; up to 36 trucks arrived per day with straw. On receipt, the water content in the received straw was measured using a straw bale moisture probe by the people working at the plants. Results varied between 8.1 and 24.0 percentage by dry weight and averages at each plant and each day varied between 10.2 and 15.2 (Madsen and Nielsen 2010). During unloading of straw the gates in the straw storage halls were sometimes open, allowing outdoor air in, and sometimes they were closed. After unloading the straw, the truck body was usually cleaned using a vacuum cleaner or brooms.

### 2.2 Sampling of airborne dust at the biofuel plants

Measurements were performed in the early spring, late autumn and winter season in 2000 to 2006 during two to four working days. The stationary sampling and the measurement of concentrations and aerodynamic diameters ( $d_{ae}$ ) of particles were performed 1.5 m above floor level. 'Total dust' has been defined as the dust collected by a sampler with an entry velocity of 1.25 m/s (Kenny and Ogden 2000); 'total dust' was sampled at plant numbers 4 to 24 using 25 mm closed-face cassettes (Millipore holder; Millipore, Bedford, MA, USA, with an inlet velocity of 1.25 m/s). The samplers were fitted with Teflon filters (pore size 1.0  $\mu\text{m}$ ) for endotoxin, pH and gravimetric analysis and with polycarbonate filters (pore size 1.0  $\mu\text{m}$ , GE Water & Process Technologies) for other analysis.

Personal dust monitoring at all plants and stationary sampling at plants A to E was conducted using GSP inhalable samplers (CIS by BGI, INC Waltham, MA) as described in (Madsen 2006). The samplers were mounted with Teflon filters (pore size 1.0  $\mu\text{m}$ ) for endotoxin and gravimetric analysis and with polycarbonate filters (pore size 1.0  $\mu\text{m}$ ) for other analysis.

After sampling, the filters were transported carefully to the laboratory, and different microbial analyses were performed (**Table 1**). All results are presented as time-weighted averages.

An APS (APS-3321; TSI Inc., USA) or a particle counter (GRIMM model 1200) measured the number concentration of particles from 0.75 to 19.8  $\mu\text{m}$  (aerodynamic diameter abbreviated  $d_{ae}$ ) over one minute intervals in straw storage halls. Data are included in this chapter for measures at plants 14, 15, 16 and 18. The theoretical aspiration of the APS is near 100% for particles as large as 20  $\mu\text{m}$  (Peters et al. 2006). These particle data are used to show the variation in particle concentration as a function of work task and to study the effect of open versus closed gates during unloading of straw. Arrows are drawn in the figures pointing at the time where a certain task starts or occurs.

### 2.3 Dustiness of biofuel collected at the plants

To measure the microbial dustiness of biofuels handled at biofuel plants in autumn and spring, biofuels were sampled at plants A, B, C, D and E in autumn 2000 and spring 2001. The wood chips were sampled from chips craves and the straw carefully sampled from the floor in the straw storage hall immediately after it fell from the bales during unloading from trucks. Consequently one straw sample represents many straw bales. Subsequently the biofuel samples were stored at 9-15°C for 15 hours before the microbial dustiness was studied. The study was performed in triplicate.

A rotating drum was used to generate airborne dust. The dust generator was a rotating drum with horizontal axis and a volume of 3.3  $\text{m}^3$  as described previously (Breum et al. 1999; Madsen et al. 2004). The biofuel (3.0 kg) was loaded into the bottom of the drum, which was then rotated (7 rpm, 5 min). A vacuum pump attached downstream of the drum maintained an airflow of 420  $\text{l min}^{-1}$  through the drum; excess HEPA-filtered replacement air was supplied at the opposite end of the drum, ensuring ambient pressure inside the drum. Dust for microbial analysis was sampled on filter cassettes with teflon filters in closed-faced field monitors (25 mm dia., 8  $\mu\text{m}$ ; Millipore, Bedford, USA) with a 5.6 mm inlet at an airflow of 1.9  $\text{l min}^{-1}$  (1.25  $\text{m s}^{-1}$  inlet velocity), and with polycarbonate filters (25 mm dia., 0.4  $\mu\text{m}$ , Nucleopore, Cambridge, MA, USA) with a 4.4 mm inlet at an airflow of 1.9  $\text{l min}^{-1}$  (2.07  $\text{m s}^{-1}$  inlet velocity) in closed-faced field monitors.

The data on microbial dustiness was used to study the impact of quality of biofuels on the exposure measured at biofuel plants.

### 2.4 Gravimetric analysis and extraction of dust

The mass of the dust collected on the Teflon filters was determined by weighing the filters before and after dust sampling. Before weighing, the filters were equilibrated at constant air temperature and humidity for 20-24 hours. The dust on the Teflon filters was extracted in 10.0 ml pyrogen-free water with 0.05% Tween 20 by orbital shaking (300 rpm) at room temperature for 60 min and centrifuging (1000g) for 15 min. 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.

### 2.5 Determination of endotoxin, NAGase activity and pH

The supernatant from the Teflon filters was analysed (in duplicate) for endotoxin using the kinetic Limulus Amoebocyte Lysate test (Kinetic-QCL endotoxin kit, BioWhittaker, Walkersville, Maryland, USA) as earlier described (Madsen 2006). A standard curve obtained from an *Escherichia coli* O55:B5 reference endotoxin was used to determine the concentrations in terms of endotoxin units (EU) (10.0 EU $\approx$ 1.0 ng). pH was measured in the

supernatant from the dust suspensions from the Teflon filters using a pH meter (PHM220 LABpHmeter, Meterlab).

To quantify the activity of NAGase (EC3.2.1.30) in the supernatant from the polycarbonate filters, the release of *p*-nitrophenol from the substrate *p*-nitrophenol-N-acetyl- $\beta$ -D-glucosaminide (Sigma Chemical Co. USA) was estimated (Madsen and Neergaard 1999). Activities are expressed as pmol sec<sup>-1</sup> per m<sup>3</sup> air.

Measured component	Unit	Description
Bacteria:		
Bacteria	cfu (colony forming units)	Bacteria able to grow on an agar medium
Mesophilic actinomycetes	cfu	A group of bacteria (Gram positive) able to grow on an agar medium at 25°C
Thermophilic actinomycetes	cfu	A group of bacteria (Gram positive) able to grow on an agar medium at 55°C
'Total bacteria'	Number	Living and dead bacteria counted by microscopy
Endotoxin	EU (Endotoxin units)	Endotoxin is a cell wall component from Gram negative bacteria
Fungi:		
Fungi	cfu	Fungi (moulds) able to grow on an agar medium
'Total fungi'	Number	Living and dead fungal spores counted by microscopy
<i>Aspergillus fumigatus</i>	cfu	A living thermotolerant fungal species (mould), able to grow at 45 °C
NAGase	pmol/sec	An enzyme (a chitinase) mainly produced by fungi

Table 1. Measured microbial components

## 2.6 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, England) at 25 °C was counted. In addition, DG 18 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, Basingstoke, England) with actidione (cycloheximide; 50 mg l<sup>-1</sup>) and secondly of the number of mesophilic actinomycetes and thermophilic actinomycetes (55 °C) cultivable on respectively 10% and 100% Nutrient agar with actidione (cycloheximide; 50 mg/ l). The numbers of microorganisms are expressed as cfu (colony forming units) per m<sup>3</sup> air.

The total numbers of fungal spores and bacteria were determined after staining with 20 ppm acridine orange (Merck) in acetate buffer for 30 sec with subsequent filtration through a polycarbonate filter (25 mm, 0.4 $\mu$ m; Nuclepore, Cambridge, MA, USA). Fungi and bacteria were counted at a magnification of x1250 using epi-fluorescence microscopy (Orthoplan;

Leitz Wetzlar). The numbers of fungi were determined in forty randomly chosen fields or until at least 400 cells were counted and are presented as number per m<sup>3</sup>.

## 2.7 Treatment of data

The influence of using a broom versus a central vacuum cleaner (plants 6 and 15), the influence of water content in straw (plants 4, 6, 7, 9, 11, 12, 15, 20, 21, 23 and 24), the influence of sealing a straw shredder (plant 18) and the influence of open versus closed gates (plant 18) on exposure was compared inside the plants. The influence of quality of biofuel (plants A, B, C, D, and E) was studied with plants as random effect. All analyses were performed in SAS 9.1.

Different numbers of trucks with straw arrived and unloaded straw at the straw storage halls over the two days of sampling at 11 biofuel plants. To be able to compare the exposure level on two days of sampling at the same plant, we balanced the exposure level with the number of trucks arriving with straw. Subsequently, the effect of water content in the handled straw on the exposure to 'total dust', *Aspergillus fumigatus*, thermophilic and mesophilic actinomycetes was calculated on the log-transformed data using Proc Mixed, with the biofuel plants as the random effect.

Pearson's correlation coefficients were calculated for the log-transformed data of concentrations measured at the biofuel plants and compared with the microbial dustiness of biofuels measured using the rotating drum. The effect of microbial dustiness of biofuels, kind of biofuel and season on the exposure to 'total dust', endotoxin, fungi and bacteria was calculated on the log-transformed data using Proc Mixed, with the biofuel plants as the random effect. The effect of kind of biofuel and season on the microbial dustiness of biofuels in terms of 'total dust', endotoxin, fungi and bacteria was calculated on the log-transformed data using Proc Mixed, also with the biofuel plants as the random effect.

The number of airborne particles measured during straw unloading with open versus closed gates and data concerning cleaning using a broom versus a vacuum cleaner were compared using Proc Anova. Data on exposure as affected by sealing a straw shredder were analysed using Proc GLM with pair-wise comparisons.

## 3. Results and discussion

### 3.1 Variation in particle exposure through day and night

Particle concentration was measured over three-and-a-half days in March 2006 in a straw storage hall. Results showed an increasing concentration in the morning after the start of work and a decreasing concentration in the afternoon after the end of the working day (about 16:00) (**Figure 1**). **Figures 2** and **3** also show low particle concentrations in the morning before working hours start between 6:30 and 7:00. The last day of exposure measured at **Figure 1** is a Friday, when people at the plant stopped working earlier (about 12:00), and the particle concentration also decreased earlier. During the night, particles were also aerosolised due to the automatic straw feeding (**Figure 1**). In the figure only particles with a  $d_{ae}$  between 0.97 and 7.7  $\mu\text{m}$  are shown, as fungi is typically present in the air as particles with a  $d_{ae}$  between 2 and 5  $\mu\text{m}$ , and bacteria as particles with a  $d_{ae}$  between 1 and 8  $\mu\text{m}$  (Madsen et al. 2009). Many particles had an  $d_{ae}$  between 0.54 and 0.97  $\mu\text{m}$  but particles with this  $d_{ae}$  and  $d_{ae}$  between 0.97  $\mu\text{m}$  and 7.7  $\mu\text{m}$  mainly followed the same pattern (**Figure**

3). However in periods with low activity such as before 7:00 and between 12:15 and 13:00, there was a high number of particles with a  $d_{ae}$  between 1.0 and 7.7  $\mu\text{m}$  compared to particles with a  $d_{ae}$  0.54 between 0.97 $\mu\text{m}$ .

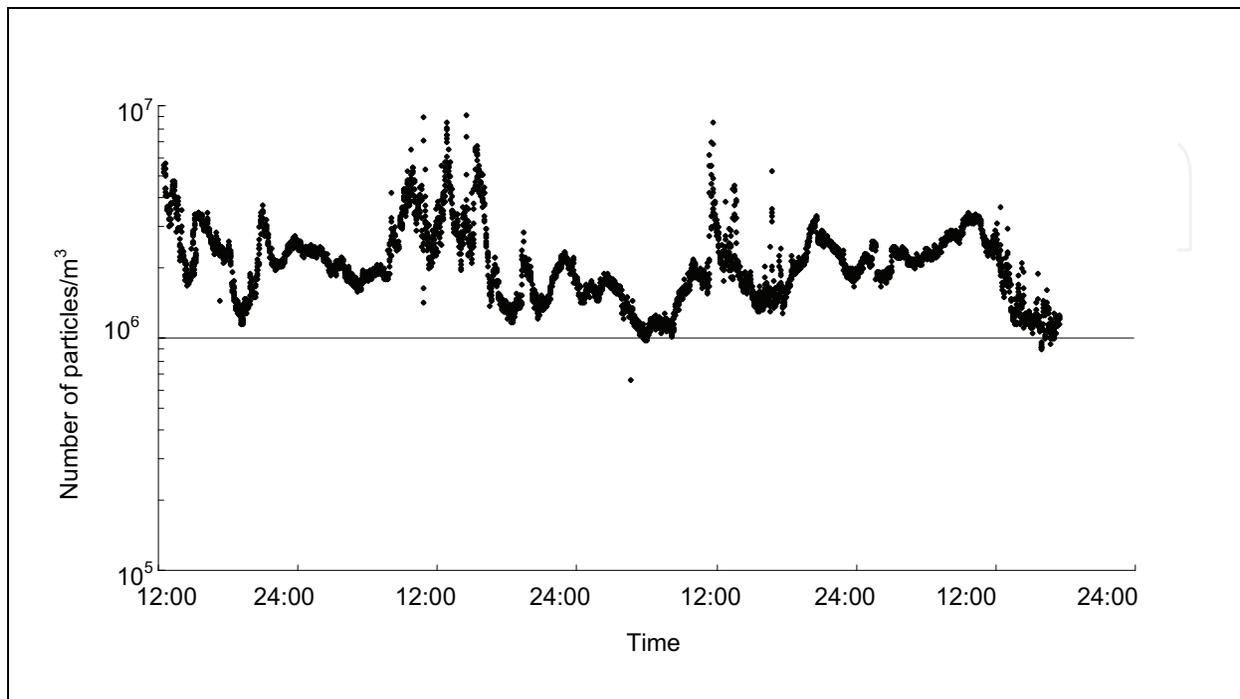


Fig. 1. Concentrations of airborne particles ( $0.97 < d_{ae} < 7.7 \mu\text{m}$ ) in a straw storage hall at plant 14 as a function of time of the day. The measurements have been performed using an APS and the period shown is from a Tuesday in March 2006 at 12:00 and until midnight the following Friday

### 3.2 Unloading straw

At 92% of the biofuel plants, the engine of the trucks or tractors was shut off immediately after entering the straw storage hall. The first step in the unloading of straw was at most plants to remove a net covering the straw. Removal of the net caused an increase in particle exposure (example in **Figure 4**). Next the straw was removed using forklifts, cranes at the plant or, more rarely, cranes on the truck. Unloading of straw causes an increase in concentration of airborne particles (example in **Figures 2, 3 and 4**). At some plants the straw was unloaded and placed in the right place in one step (as in **Figures 1 and 3**), in some other plants it was done in more than one step (example in **Figures 2 and 4**). The extra reorganising of bales of straw can cause an extra exposure period which can cause a more than ten-fold increase in particle concentration, lasting for up to an hour. Based on these measurements it is suggested to explore the possibilities of reducing exposure by organising the unloading of straw and the subsequent straw feeding so that it is not necessary to move the straw bales once they have been unloaded.

### 3.3 Exposure as affected by open or closed gates

To assess the influence of open versus closed gates during unloading of straw, particle concentrations were measured in a period of four minutes before unloading the straw and

during the first four minutes of unloading, when a big gate to the outdoor environment was either closed or open. When the gate was closed during unloading at plant 18, the particle concentration increased during the first four minutes of straw unloading by a factor of 2.9 to 4.4 (dependent on the particle size). When the gate was open, the concentration only increased by a factor 1.5 to 2.7 (**Table 2**). At plant 15 the highest increase in particle concentration (7.5 times) was found during unloading of the first load of straw in the morning and with closed gates (**Figure 3**).

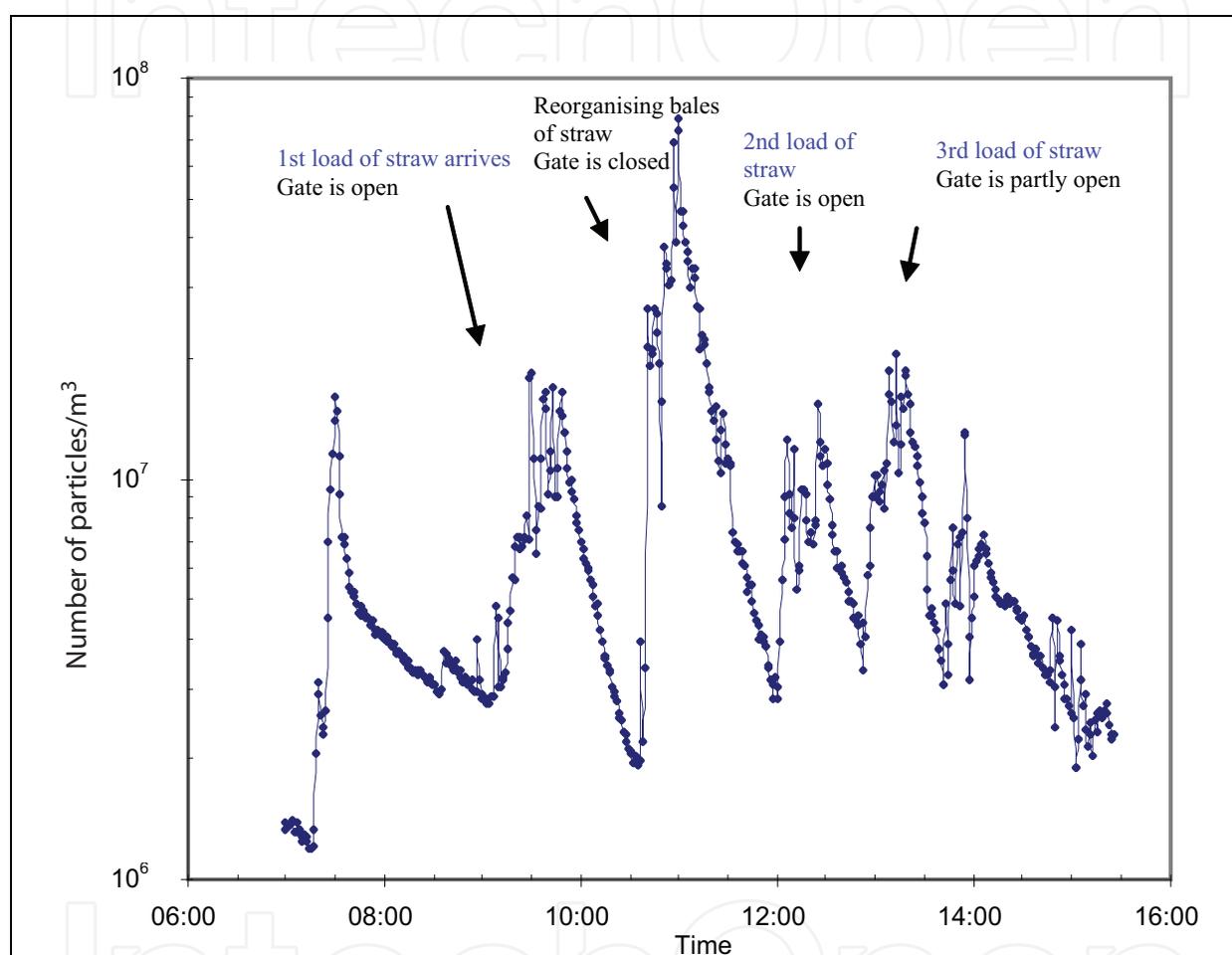


Fig. 2. Concentration of airborne particles ( $1.0 < d_{ae} < 7.5 \mu\text{m}$ ) in a straw storage hall at plant 18 as a function of time of the day. In the period measured, the three loads of straw were received and the gate was sometimes open and sometimes closed. The measurement was performed using a Grimm particle counter between 7:00 and 15:30

The half life period is the period from termination of unloading of straw and until the particle concentration has fallen by 50% of the difference between the peak and the level before unloading commenced. The clearance period is the period from termination of unloading of straw and until the particle concentration is at the same level as it was before unloading the straw. The half-life period and clearance period were lower when the outdoor gate was open than when it was closed (**Table 3**). The difference was significant for particles with  $d_{ae}$  of  $[0.75-1.0]$  ( $p=0.041$ ),  $[1.0-2.0]$  ( $p=0.044$ ),  $[5.0-7.5]$  ( $p=0.047$ ) and  $[7.5-10.0]$  ( $p=0.0108$ ) but not for particles with  $d_{ae}$  of  $[2.0-3.5]$  ( $p=0.121$ ) and  $[3.5-5.0]$  ( $p=0.64$ ).

Particle sizes* $d_{ae}$ in $\mu\text{m}$	Number $\times 10^3/\text{m}^3$		Increase-factor	
	Closed	Open	Closed	Open
]0.75-1.0]	5600	2900	2.9	1.5
]1.0-2.0]	4900	1700	3.3	1.5
]2.0-3.5]	1200	430	3.7	1.5
]3.5-5.0]	3800	1100	4.3	1.8
]5.0-7.5]	550	120	4.2	2.6
]7.5-10.0]	36	8.4	4.2	2.2

\*Measured using a Grimm particle counter

Table 2. Effect of open versus closed gates during unloading of straw at plant 18. Median concentration of particles during the first four minutes of unloading of straw and increase-factor in particle concentration in these four minutes of unloading relative to the preceding period

These data show that when opening the gates to the outdoor air, a dilution of the indoor bioaerosols occurs rather than an aerosolisation of settled dust or of particles on biofuels. The concentrations of bioaerosol components in the outdoor air in other industrial or urban areas (Nikkels et al. 1996; Nielsen et al. 2000; Park et al. 2000; Madsen 2006) are also described to be much lower than inside the biofuel plants. Opening gates could therefore be an obvious measure to reduce bioaerosol exposure.

Particle sizes* $d_{ae}$ in $\mu\text{m}$	Half-life period				Clearance period			
	Closed		Open		Closed		Open	
	Minutes	SD	Minutes	SD	Minutes	SD	Minutes	SD
]0.75-1.0]	51	12.7	14	1.4	>70	-	22	2.5
]1.0-2.0]	51	12.9	14	0.71	>70	-	24	2.1
]2.0-3.5]	46	12.8	12	0.74	>70	-	27	1.8
]3.5-5.0]	29	4.2	8	1.1	>70	-	31	2.1
]5.0-7.5]	22	4.6	8	1.4	69	4.3	32	1.8
]7.5-10.0]	19	4.1	7	1.8	65	4.8	34	2.5

\*Measured using a Grimm particle counter

Table 3. Half-life period and clearance period for concentrations of airborne particles from termination of unloading of straw at plant 18 when gates were closed (n=4), or when gates were open (n=2)

### 3.3 Cleaning

During or after the removal of the straw the body of the truck was cleaned. During unloading of bales of straw, pieces of straw were dropped on the floor, and the floor was sometimes cleaned using vacuum cleaners or brooms or other methods. In the example in **Figure 3**, straw is unloaded using forklifts and the truck body and floor are cleaned using a vacuum cleaner. During the cleaning of the floor the particle, concentration increased when using vacuum cleaners, brooms and compressors (**Figures 3 and 4**).

At two biofuel plants exposure to bioaerosol components was measured during the cleaning of the truck body using either brooms or central vacuum cleaners. The exposure levels to the different bioaerosol components were different at the two plants and the levels are presented separately in **Tables 4 and 5**. The personal exposure to different bioaerosol components was higher when cleaning the truck body using a broom than when using a vacuum cleaner (**Table 4 and 5**).

Bioaerosol components	Fraction (%)	Average exposure/m <sup>3a</sup>
Endotoxin	77	147 EU
Inhalable dust	80	0.21 mg
'Total number of fungal spores'	29*	2.5 x10 <sup>5</sup> number
<i>Aspergillus fumigatus</i>	30*	738 cfu
NAGase	58*	0.38 pmol/sek
'Total number of bacteria'	20*	5.5x10 <sup>5</sup> number
Mesophilic actinomycetes	56*	1377 cfu
pH	77	4.78 no unit
Particles d <sub>ae</sub> ]0.75-1.0]	28*	3.3x10 <sup>7</sup> number
Particles d <sub>ae</sub> ]1.0-5.0]	34*	8.6x10 <sup>6</sup> number
Particles d <sub>ae</sub> ]5.0-7.5]	85	4.8x10 <sup>5</sup> number
Particles d <sub>ae</sub> ]7.5-10]	34*	7.8x10 <sup>4</sup> number

<sup>a</sup>Exposure when the vacuum cleaner and not the broom was used. The exposure was measured for two persons during 2x2 days. Figures marked by an asterisk (\*) were significantly different using a broom compared with a central vacuum cleaner

Table 4. Fraction (%) of personal exposure to bioaerosol components and particles in the straw storage hall at plant 15 using a broom for cleaning compared with using a central vacuum cleaner

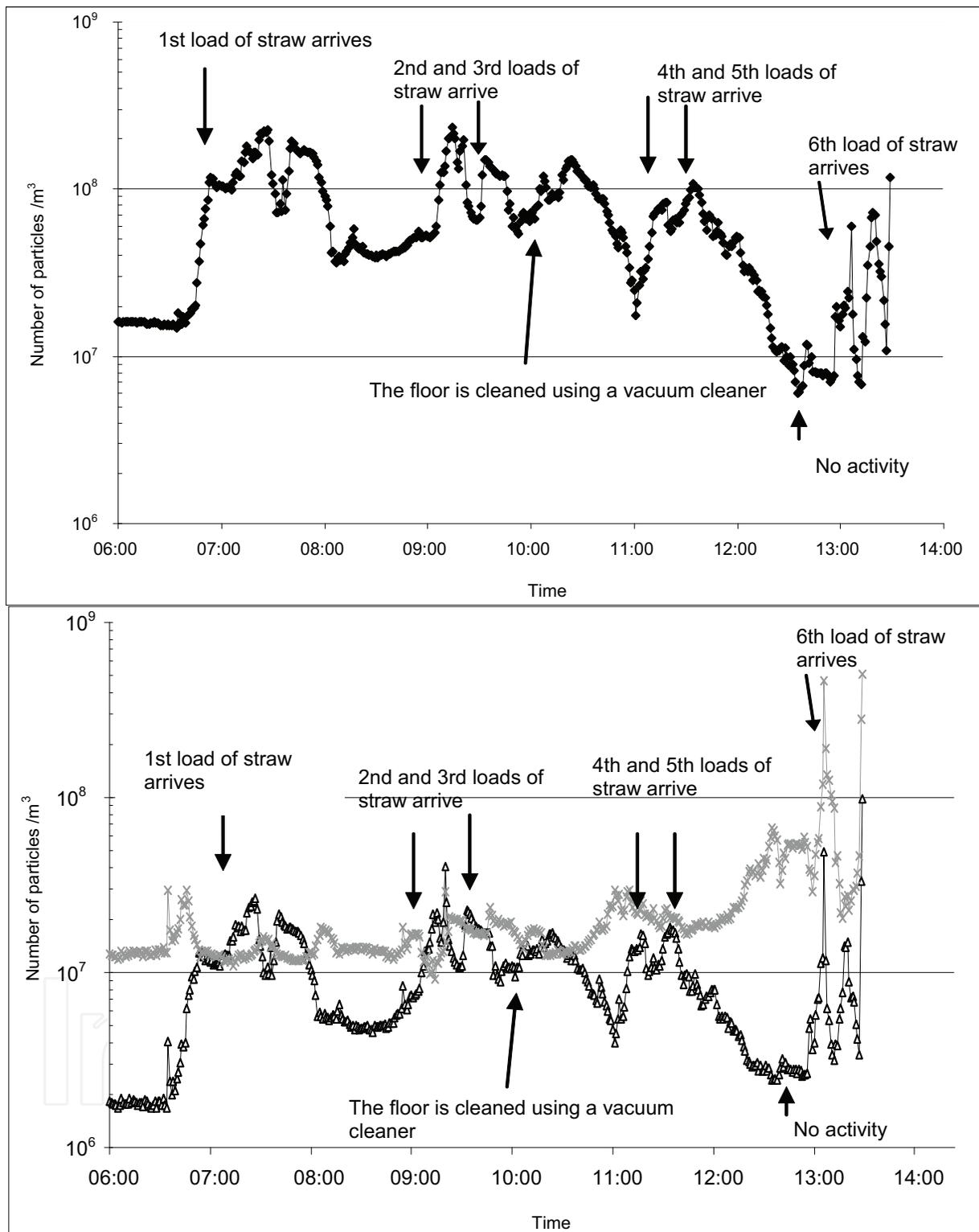


Fig. 3. Concentration of airborne particles ( $0.54 < d_{ae} < 7.7 \mu\text{m}$ , top figure and  $0.97 < d_{ae} < 7.7 \mu\text{m}$ , bottom figure, black symbols) in a straw storage hall as a function of time of the day. The grey symbols are the relation between large and small particles ( $((0.97 < d_{ae} < 7.7 \mu\text{m} / 0.54 < d_{ae} < 0.97 \mu\text{m}) * 100,000)$ ). Six loads of straw were received and the gate was mainly closed. Unloading took between 15 and 20 minutes, and floor cleaning 25 minutes. The measurement was performed at plant 15 in autumn using an APS

When using a broom to clean at plant 15 the personal exposure was above a calculated “no effect level” (NOEL) of 150 EU/m<sup>3</sup> (Smid et al. 1992a; Smid 1993) and when using a central vacuum cleaner it was below the proposed NOEL (Table 4). At plant 6 the exposure to endotoxin was low compared to what has been found in straw storages earlier (Madsen 2006) and compared to plant 15 in this study, and it was lower than the suggested NOEL both when using a vacuum cleaner and brooms. Dust exposure was below the Danish Occupational Exposure limit (OEL) of 3 mg/m<sup>3</sup> (Danish Working Environment Authority (Arbejdstilsynet) 2007) both with and without use of the vacuum cleaner. When using the vacuum cleaner, the exposure to dust was reduced to 80% and 23% respectively of what it was when using brooms at the two plants. A study of sawmill workers has indicated that the lowest exposure causing symptoms in the throat is 3 × 10<sup>5</sup> fungal spores/m<sup>3</sup> (Alwis et al. 1999; Eduard, 2009). Exposure to fungi was reduced at both plants by using the central vacuum cleaners, but it still reached or exceeded this level. Exposure to the fungus *Aspergillus fumigatus* was not higher than a NOEL (Fogelmark et al. 1991) in both situations. Exposures larger than 2 × 10<sup>4</sup> cfu of thermophilic actinomycetes m<sup>-3</sup> have been suggested as a TLV (threshold limit value) (Dutkiewicz et al. 1994). This value was exceeded when using the broom but not when the central vacuum cleaner was used (Table 5). The pH of the dust suspensions seems to be affected by the presence of microorganisms – with a higher pH when more microorganisms were present. Mouldy hay causing farmers lung disease has earlier been described to be less acid than non-problematic hay (Gregory and Lacey 1963).

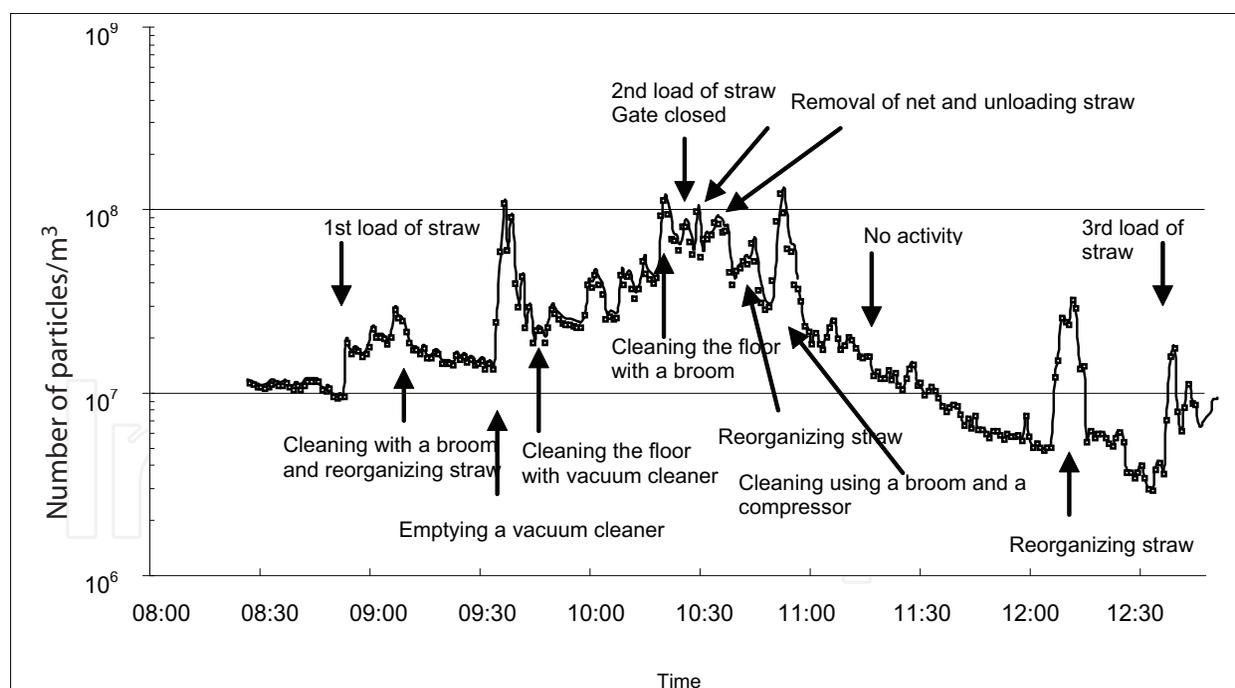


Fig. 4. Concentration of airborne particles ( $0.54 < d_{ae} < 7.7 \mu\text{m}$ ) in a straw storage hall at plant 16 as a function of time of the day. In the period measured, three loads of straw were received. The unloading was performed using forklifts and it took between 6 and 10 minutes. After unloading the straw, the bales of straw were reorganized. Between 9:48 and 10:25 cleaning activities were performed. The measurement was performed in spring 2004 using an APS

Bioaerosol components	Fraction (%)	Average exposure/m <sup>3a</sup>
Endotoxin	133	29 EU
Inhalable dust	23*	0.32 mg
'Total number of fungal spores'	11*	2.4 x10 <sup>5</sup> number
<i>Aspergillus fumigatus</i>	55*	1452 cfu
NAGase	9.3*	0.42 pmol/sek
'Total number of bacteria'	14*	7.4x10 <sup>5</sup> number
Mesophilic actinomycetes	3.1*	2807 cfu
Thermophilic actinomycetes	1.2*	3608 cfu
pH	79	4.57 no unit

<sup>a</sup>Exposure when the vacuum cleaner and not the broom was used. The exposure was measured during 2x2 days. Figures marked by an asterisk (\*) were significantly different using a broom compared with a central vacuum cleaner.

Table 5. Personal exposure to bioaerosol components in the straw storage hall at plant 6 using a broom for cleaning compared with using a central vacuum cleaner

The concentration of stationary measured particles of different sizes was also higher when cleaning the truck body using a broom than when using a vacuum cleaner (**Table 4**). Together the particle and bioaerosol exposure suggest that the exposure in the straw storage hall can be reduced by using a vacuum cleaner rather than a broom.

### 3.4 Exposure as affected by quality of the biofuel

To study the impact of the quality of biofuels on the exposure, exposure levels were compared with microbial dustiness of biofuels collected at biofuel plants. Correlation coefficients (*r*) between exposure in a working area and the microbial dustiness of the biofuel handled in the same area were 0.88 ( $p < 0.0001$ ), 0.77 ( $p < 0.0021$ ), 0.66 ( $p < 0.0001$ ) and 0.68 ( $p < 0.024$ ) for respectively endotoxin, cfu of bacteria, inhalable dust, and cfu of fungi (**Figure 5**). Statistical analysis showed that the quality of the biofuel when measured as dustiness in terms of endotoxin ( $p < 0.0001$ ), bacteria ( $p < 0.0001$ ), fungi ( $p < 0.0001$ ) and dust ( $p < 0.0001$ ) all had a significant effect on the exposure level. Also the season had a significant effect on the exposure to bacteria ( $p = 0.0003$ ), fungi ( $p < 0.0001$ ) and dust ( $p < 0.0001$ ), but not to endotoxin ( $p = 0.19$ ). In contrast the kind of biofuel handled (wood chips or straw) had no significant effect on exposure.

When the effect of season and kind of biofuel on the microbial dustiness of biofuels was studied separately (with plant as a random effect), significant effects of season on dustiness in terms of fungi ( $p = 0.011$ ) and dust ( $p = 0.0093$ ) but not of bacteria ( $p = 0.19$ ) and endotoxin ( $p = 0.79$ ) were found. The kind of fuel (straw versus wood chips) had a significant effect on dustiness in terms of bacteria ( $p = 0.0014$ ), endotoxin ( $p < 0.0001$ ) and dust ( $p < 0.0001$ ) but not of fungi ( $p = 0.10$ ). This higher dustiness of straw than of wood chips in terms of bacteria, endotoxin and dust supports earlier work (Madsen et al. 2004).

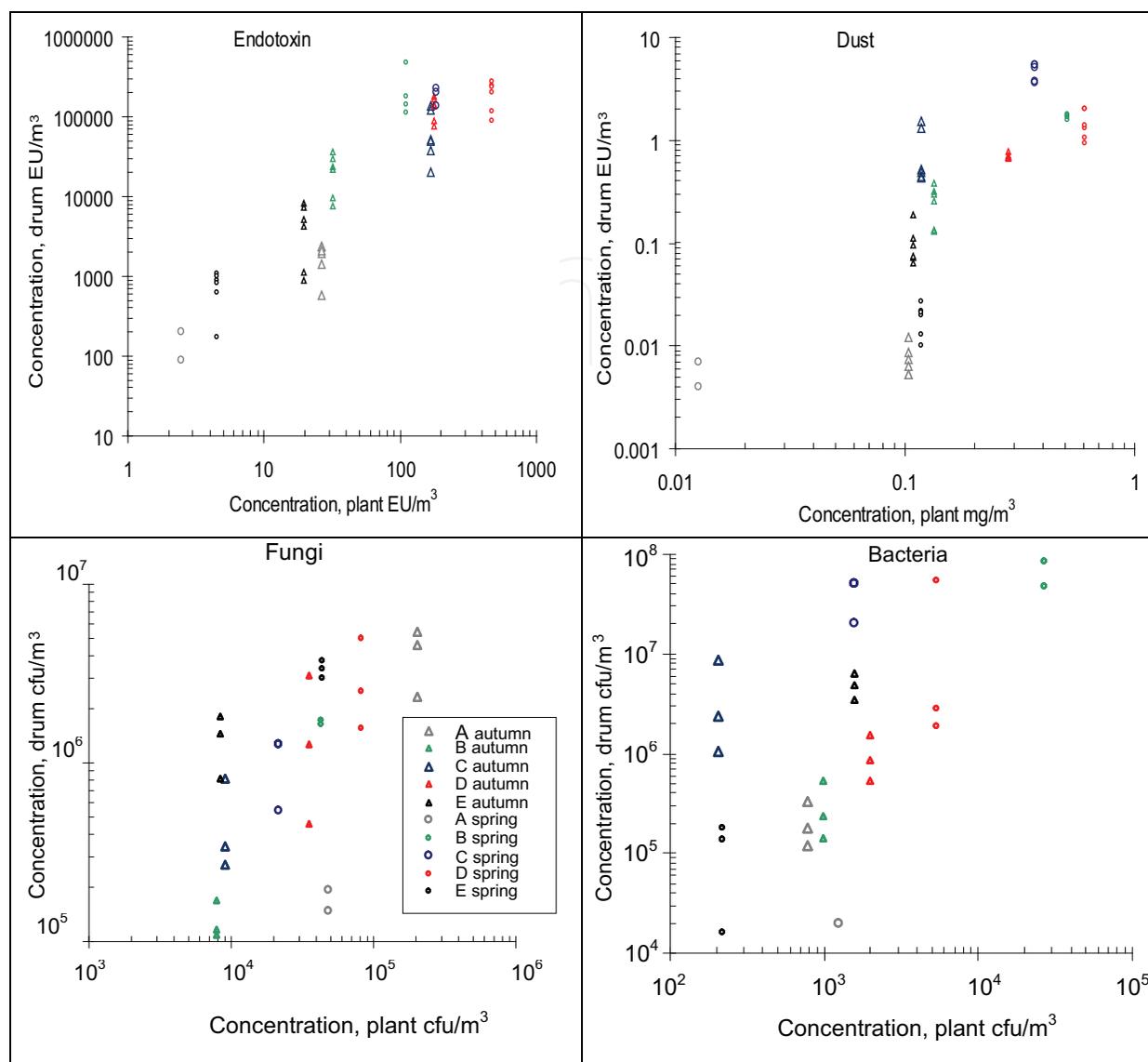


Fig. 5. Concentration (units/m<sup>3</sup>) of endotoxin, dust, fungi and bacteria in the air at five biofuel plants (A, B, C, D and E) versus concentration of these components released from straw or wood chips in a rotating drum. At plants B, C and D measurements were performed in a straw storage hall and at plants A and E measurements were performed where wood chips were unloaded

These positive associations between microbial dustiness on the exposure and the plant show an impact of the quality of the biofuel handled on the personal exposure. Checking the quality of straw and wood chips and rejecting problematic biofuel could thus be a measure to reduce exposure. There is however no easy way to evaluate the quality of biofuels regarding microbial dustiness, but the 'history' of the biofuel may give a hint about the quality of the biofuel. Thus 'storage history' may give a hint about the quality, as storing biofuels over summer outdoors increases their microbial dustiness (Sebastian et al. 2006). In a straw storage hall, higher exposure to dust, fungi, actinomycetes and bacteria is found in spring than in autumn (Madsen 2006); and as this study shows, there is a higher dustiness of biofuels in terms of fungi and dust in spring than in autumn. Furthermore the location

where the biofuel sample is taken should also be considered, as samples taken from the inner part of a biofuel pile are dustier than samples taken from the surface (Sebastian et al. 2006). The kind of biofuel handled (e.g. wood chips, bark chips, straw or wood pellets) (Thörnqvist and Lundström 1982; Madsen et al. 2004; Madsen 2006) and the size of wood chips (Pellikka and Kotimaa 1983) should also be considered, as these factors have been shown to affect the microbial dustiness or the exposure. Furthermore storage of wood for chips as log stacks, rather than as wood chips, also affects the microbial dustiness (Thörnqvist and Lundström 1982) and could thus be considered when predicting the potential microbial dustiness of a material.

In relation to storage of biofuels, microorganisms and CO<sub>2</sub> formation should also be considered. Transport of logs and wood chips in confined spaces can result in rapid and severe oxygen depletion and CO<sub>2</sub> formation, possibly caused by microbial activity (Svedberg et al. 2009).

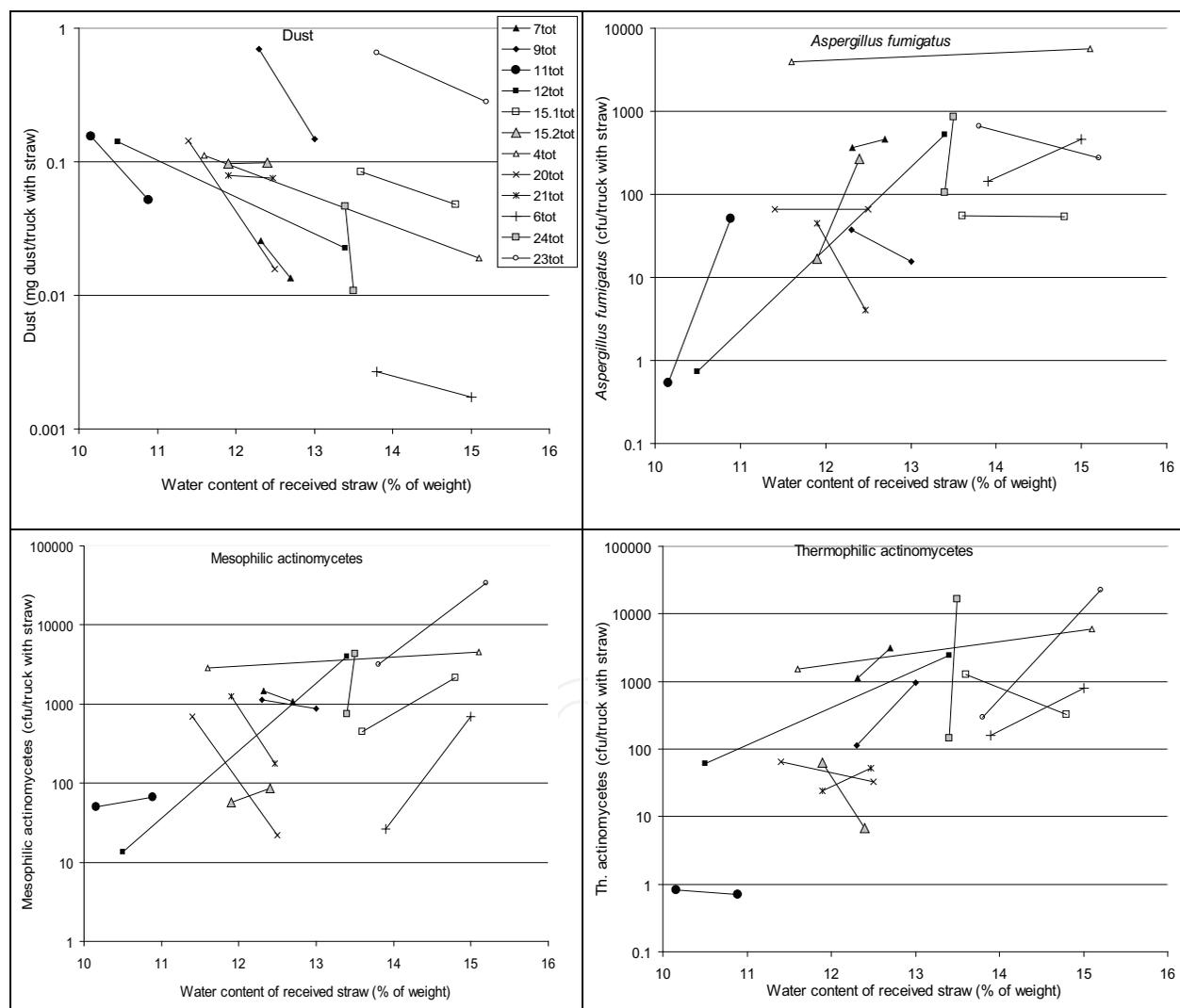


Fig. 6. Exposure to 'total dust' (mg/m<sup>3</sup>/number of trucks with straw) and *Aspergillus fumigatus*, mesophilic and thermophilic actinomycetes (cfu/m<sup>3</sup>/number of trucks with straw) as a function of water content (%) in the straw received during the two days of bioaerosol sampling at straw storage halls at plants 7, 9, 11, 12, 15, 4, 20, 21, 6, 24 and 23

### 3.5 Water content in straw as an indicator of subsequent exposure

Water content in straw is usually measured by people working at the plants using straw bale moisture probes at reception of straw. Therefore whether water content of straw can be used as an indicator of a subsequent exposure level when working with the straw has been investigated.

At some plants (measurements from 24 days at 11 plants), straw was received on all days of bioaerosol sampling at each plant. The exposure to 'total dust', *Aspergillus fumigatus*, thermophilic and mesophilic actinomycetes per number of trucks with straw arriving at the straw storage hall was measured. Furthermore water content in the straw received at each plant was measured. The exposure to 'total dust' ( $p=0.0137$ ) was lower on the days when the water content of the straw received was highest (**Figure 6**). Hence small increases in water content in the straw caused a lower exposure to dust. For *Aspergillus fumigatus* ( $p=0.0112$ ) and mesophilic actinomycetes ( $p=0.0427$ ) a significant effect of water content on exposure was also seen, although this association was opposite, with increasing water content associated with increasing exposure (**Figure 6**). For thermophilic actinomycetes ( $p=0.0536$ ) no significant association was seen between exposure and water content of straw.

As for 'total dust' a higher water content in straw is also seen to cause a lower exposure to endotoxin. Water content in straw is seen to affect both the concentration, exposure level and size distribution of endotoxin-containing particles (Madsen and Nielsen 2010). The microorganisms measured, *Aspergillus fumigatus*, thermophilic actinomycetes and mesophilic actinomycetes, are living microorganisms, while endotoxin is from both living and dead Gram negative bacteria, and dust contains both living and dead microorganisms and other particles. The water content of an organic material may both affect the particle release and growth or sporulation of microorganisms. The effect of water content on dustiness of some materials, such as coal, is reviewed by (Hjemsted and Schneider 1996). Previous studies have shown that 'total dust' and endotoxin on the one side, and *Aspergillus fumigatus*, thermophilic actinomycetes and mesophilic actinomycetes on the other side are differently associated with biofuel (straw and wood chips), while actinomycetes and fungi seem to be more easily released from biofuel than other bacteria and endotoxin (Madsen et al. 2006). This may partly explain why *Aspergillus fumigatus* and actinomycetes were also easily released from the more wet straw.

Water content of straw is affected by the relative air humidity (rh); straw incubated at 20 °C and an rh of 54.4% has been shown to obtain a content of 11.8 % water, while straw stored at a rh of 81.3% has been shown to obtain a content of 17.7 % water (Lawrence et al. 2009). The water activity ( $a_w$ ) level that limits the growth of the majority of bacteria is below 0.90  $a_w$  and for fungi below 0.70  $a_w$ . A water activity of 0.7 corresponds to a moisture content of 13%-15% in straw (Summers et al. 2003). Thus the water content in the bales of straw with the highest water content may have supported growth of some actinomycetes and fungi.

The average water content in the straw at the 11 biofuel plants was between 10.2 and 15.2% and none of the bales of straw was discarded or rejected because of high water content. This and the former study show that increasing water content may cause a higher exposure to both mesophilic and thermophilic actinomycetes and *Aspergillus fumigatus* and at the same time a lower exposure to dust and endotoxin.

### 3.6 Exposure before and after sealing a straw shredder

The concentration of airborne endotoxin ( $p=0.049$ ), 'total number of microorganisms' ( $p=0.016$ ) and NAGase ( $p=0.026$ ) in the straw shredder room was significantly higher before than after sealing a straw shredder (Figure 7). The concentration of airborne dust ( $p=0.061$ ) and 'total number of fungi' ( $p=0.065$ ) tended to be higher in the straw shredder room before than after sealing the straw shredder.

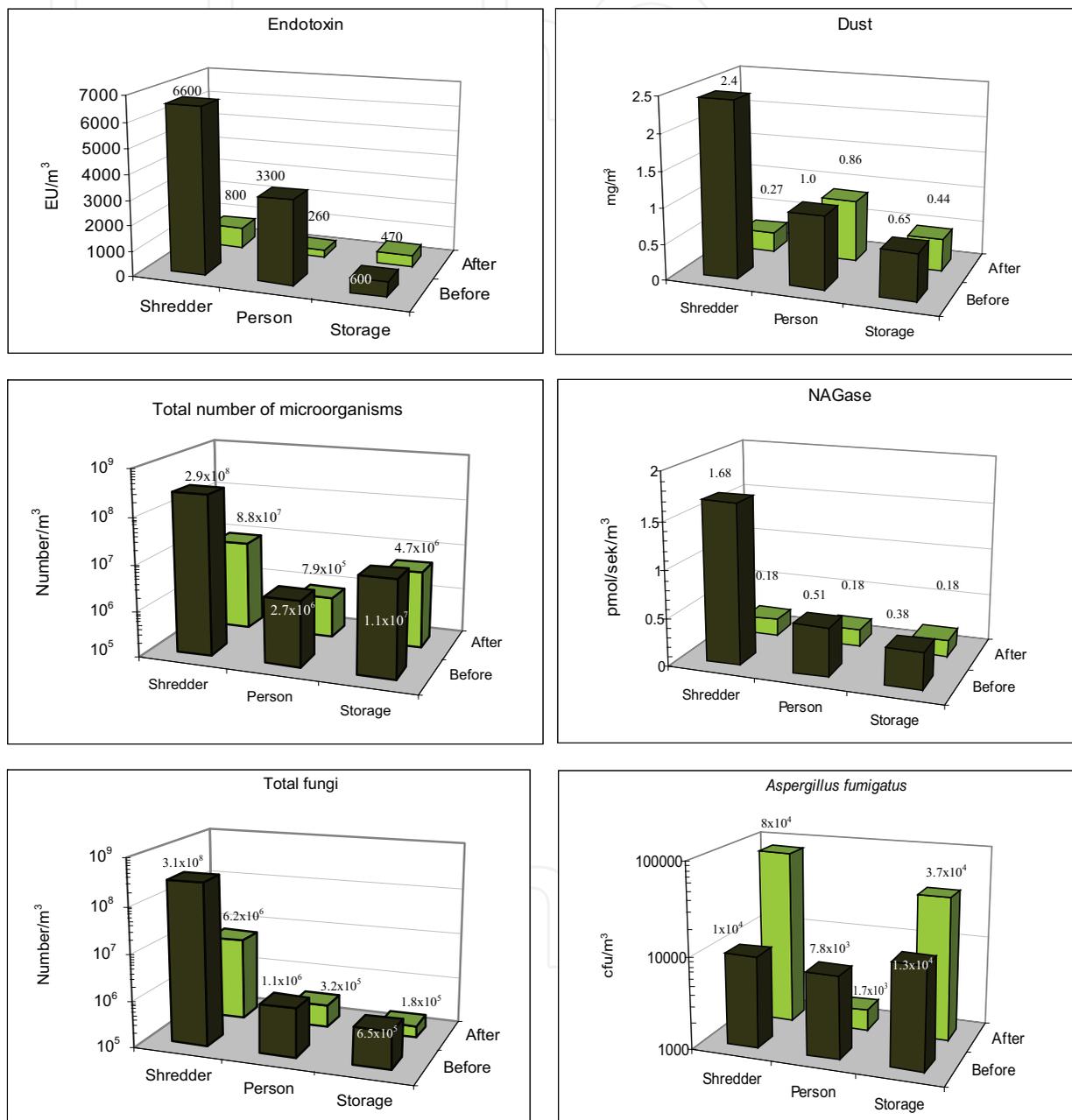


Fig. 7. Exposure to bioaerosol components before and after sealing a straw shredder at plant 18. 'Shredder' is stationary measurements in the straw shredder room; 'Person' is a personal exposure measurement of a person working in the straw storage hall and in the straw shredder room; 'Storage' is a stationary measurement in a straw storage hall next to the straw shredder room

Also the personal measured exposure and the concentration in the adjacent room – the straw storage hall – was affected positively by sealing the straw shredder.

Both before and after sealing the straw shredder, the concentration of endotoxin in the straw shredder room was considerably higher than the calculated NOEL of 150 EU/m<sup>3</sup>. The personal exposure to endotoxin was also considerably lower after sealing but it was still higher than the NOEL. Also the exposure to dust was reduced significantly after sealing the straw shredder, and after sealing the dust concentration in the shredder room was lower than the Danish OEL.

In contrast to the other bioaerosol components, the concentration of *Aspergillus fumigatus* was significantly higher in the straw shredder room ( $p=0.0045$ ) after sealing than before sealing. This may reflect differences in the quality of the straw in the two periods of exposure measure, because *Aspergillus fumigatus* is not always present in straw as it is a thermotolerant fungus, which is only predominant when heat is developed in a stored material like straw.

#### 4. Conclusion

By measuring exposure to bioaerosol components using personal and stationary samplers and particle counters repeatedly at the same plant, it was possible to identify factors affecting the exposure level. Variations in concentrations of airborne particles were found through a day at biofuel plants. At some plants the straw was unloaded and placed in the right place in one step, in other plants this was done in more steps. The extra reorganising of bales of straw caused an extra increase in particle concentration lasting for up to an hour. It is suggested to explore the possibilities of reducing exposure by organising the unloading of straw and the following straw feeding so that reorganising the straw bales is not necessary.

In straw storage halls, unloading straw caused increased particle exposure. Using a broom to clean a truck body during and/or after unloading straw caused a higher exposure than cleaning using a central vacuum cleaner. Cleaning the straw storage hall caused a high exposure and cleaning using a compressor caused a peak exposure. It is recommended to investigate whether cleaning in the straw storage hall during the day between unloading deliveries of straw causes higher exposure than cleaning at the end of the day.

Open versus closed gates during straw unloading also affected the exposure significantly. Open gates caused a lower exposure, and from the data in this study it is suggested to open the gates while unloading straw. The water content in straw also influences the exposure level. While increasing water content causes a decreasing dustiness, the concentration of mesophilic actinomycetes and *Aspergillus fumigatus* in the dust increased, causing an increasing exposure to these living microorganisms.

The quality of biofuel, measured as microbial dustiness, had a significant effect on the exposure, with increasing microbial dustiness causing higher exposure. Consequently exposure may be reduced by using biofuel of high quality. The history of the biofuel may give information about its quality because quality is affected by the season and period and method of storage. Thus, higher dustiness, in terms of fungi and dust, is found in spring than in autumn. Furthermore straw has a higher dustiness, in terms of endotoxin, bacteria and dust, than wood chips.

Sealing a straw shredder caused a significantly lower exposure to bioaerosol components and can thus be recommended if a high exposure is found in this area.

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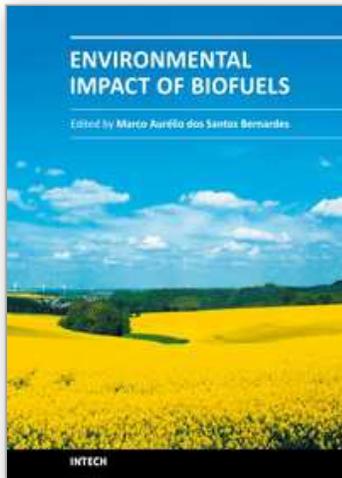
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## **Environmental Impact of Biofuels**

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This book aspires to be a comprehensive summary of current biofuels issues and thereby contribute to the understanding of this important topic. Readers will find themes including biofuels development efforts, their implications for the food industry, current and future biofuels crops, the successful Brazilian ethanol program, insights of the first, second, third and fourth biofuel generations, advanced biofuel production techniques, related waste treatment, emissions and environmental impacts, water consumption, produced allergens and toxins. Additionally, the biofuel policy discussion is expected to be continuing in the foreseeable future and the reading of the biofuels features dealt with in this book, are recommended for anyone interested in understanding this diverse and developing theme.

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