Exposure to microbial components can cause respiratory problems. The exposure levels to microbial components at biofuel plants are not known. Therefore, exposure to inhalable airborne fungi, bacteria, actinomycetes, endotoxin and NAGase was measured using personal and stationary samplers at five Danish biofuel plants in autumn and spring. The personal exposure levels to endotoxin (median = 55 EU m\(^{-3}\)), thermophilic actinomycetes (median = 1.3 \times 10^4\) colony forming units (cfu) m\(^{-3}\)), total bacteria (median = 48 \times 10^4\) cells m\(^{-3}\)) and total fungi (median = 21 \times 10^4\) spores m\(^{-3}\)) were, in general, high at the five biofuel plants. At straw reception areas, higher exposure to most microbial components was found in spring than in autumn. Endotoxin was found in higher concentrations at straw plants than at wood-chip plants, while the opposite was measured for \textit{Aspergillus fumigatus}. Some tasks were associated with exposures to microorganisms and endotoxins at much higher levels than the suggested occupational exposure limits. For example, people working with a straw shredder for at least 30 min during a working day were exposed to a median endotoxin exposure of 23 775 endotoxin units (EU) m\(^{-3}\). People working with estimating the water content in wood chips and repairing the chips cranes for at least 30 min during a working day were exposed to a median value of \textit{A. fumigatus} of 6.7 \times 10^4\) cfu m\(^{-3}\) and a median value of fungi of 70 \times 10^4\) spores m\(^{-3}\). Consequently, this working environment may cause respiratory disorders in the people working at the plant. Differences in exposure levels were seen between the plants and this may partly be due to differences of the process equipment, tasks and the biofuel handled.

\textit{Keywords:} aerosols; agriculture allergens; bioaerosols; endotoxin; inhalable dust; microbial exposure; wood dust

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\textbf{INTRODUCTION}

Several governments have encouraged the use of biofuel, because biofuel constitutes an important sustainable energy resource. At biofuel plants, large quantities of straw or wood waste are handled and exposure to microorganisms, endotoxins and dust occurs, which may cause respiratory disorders. For example, work with mouldy hay, straw and wood chips has caused organic-dust toxic syndrome (Rask-Andersen, 1989). Respiratory disorders caused by microorganisms are dependent on the exposure levels (Rylander \textit{et al.}, 1985; Eduard \textit{et al.}, 2001). The levels of exposure to microorganisms and endotoxin at biofuel plants are not known, though it is known that fungi have caused respiratory problems in people handling wood or wood chips (van Assendelft \textit{et al.}, 1985; Belin 1987; Kolmodin-Hedman \textit{et al.}, 1987; Halpin \textit{et al.}, 1994) and endotoxin has caused respiratory problems, e.g. in agriculture (Heederik \textit{et al.}, 1991). In the wood-processing industry, dose–response relationships have been found between personal exposure to endotoxin and work-related symptoms (Alwis \textit{et al.}, 1999). Therefore, personal exposure levels to microorganisms, endotoxins and dust have been estimated at five Danish biofuel plants over two seasons (Table 1). Included in the study are plants where straw and/or wood chips were used as energy sources. To measure personal exposure during a working day, the employees were monitored for exposure. To measure the exposure levels in different working areas, stationary aerosol samplers were placed in different working areas.
There are no internationally accepted threshold limit values (TLVs) or occupational exposure limits (OELs) for microorganisms and endotoxins, but to be able to analyse the results of exposures in this study, suggested TLVs or OELs from other papers are summarized below. Suggested TLVs or calculated 'no effect values' for inhalable or total endotoxin exposure in different environments are between 30 and 800 endotoxin units (EU) m$^{-3}$ (Haglind and Rylander, 1984; Rylander et al., 1985; Castellan et al., 1987; Kennedy et al., 1987; Smid et al., 1992; Smid 1993; Donham and Cumro, 1999; Donham et al., 2000). In this study the results are mainly related to the TLV of 150 EU m$^{-3}$ suggested by Smid et al. (1992a, b) and Donham et al. (2000). For cultivable thermophilic actinomycetes (Dutkiewicz et al., 1994) suggested a TLV of $2 \times 10^4$ colony forming units (cfu) m$^{-3}$. A Dutch study group has suggested that fungal and bacterial concentrations $>10^4$ cfu m$^{-3}$ should be considered a threat to workers' health (Heida et al., 1995). Eduard et al., (2001) have shown that symptoms in the eyes and nose increased after exposure to fungal spores at the level $2 \times 10^4$ to $5 \times 10^5$ m$^{-3}$ and cough symptoms after exposure to concentrations of $5 \times 10^5$ to $17 \times 10^5$ fungal spores m$^{-3}$, and based on that study the results in this paper are related to a value of $10^5$ fungal spores m$^{-3}$. For single fungal species as *Aspergillus fumigatus*, Heida et al. (1995) have suggested a TLV of 500 cfu m$^{-3}$.

<table>
<thead>
<tr>
<th>Day 1 - autumn</th>
<th>Fuel</th>
<th>Bark chips with salt water</th>
<th>Straw (12–22%, avg. = 15.6)*</th>
<th>Straw</th>
<th>Straw (11.1–14.9%, avg. = 12.5) and forest chips</th>
<th>Different kinds of chips including forest chips</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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<td>2</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td></td>
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<td>7</td>
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<tr>
<td></td>
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<td>93.2</td>
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<td>19 (230 900 kg)</td>
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<table>
<thead>
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<th>Straw (12–23%, avg. = 15.4)</th>
<th>Straw</th>
<th>Straw (12.1–13.4%, avg. = 12.5) and forest chips</th>
<th>Different kinds of chips including forest chips</th>
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<tr>
<td></td>
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<td>11</td>
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</tr>
<tr>
<td></td>
<td>Temp. (°C)</td>
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<td>14.0</td>
<td>8.8</td>
<td>15.7</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>RH (%)</td>
<td>94.1</td>
<td>97.8</td>
<td>91.9</td>
<td>73.1</td>
<td>98.0</td>
</tr>
<tr>
<td></td>
<td>N trucks (amount)</td>
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<table>
<thead>
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<th>Day 1 - spring</th>
<th>Fuel</th>
<th>Forest chips</th>
<th>Straw (12–20%, avg. = 13.4)</th>
<th>Straw (11.3–15.9%, avg. = 12.8) and forest chips</th>
<th>Different kinds of chips including forest chips</th>
</tr>
</thead>
<tbody>
<tr>
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<td>2</td>
<td>1</td>
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</tr>
<tr>
<td></td>
<td>Nst</td>
<td>3</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Temp. (°C)</td>
<td>8.4</td>
<td>13.1</td>
<td>5.5</td>
<td>9.8</td>
</tr>
<tr>
<td></td>
<td>RH (%)</td>
<td>74.2</td>
<td>72.3</td>
<td>52.6</td>
<td>86.4</td>
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<td></td>
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<td>15 (113 040kg)</td>
<td>9 (82 180 kg)</td>
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<table>
<thead>
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<th>Fuel</th>
<th>Industry chips</th>
<th>Straw (12–22%, avg. = 13.5)</th>
<th>Straw (12.8–17.1%, avg. = 14.8) and forest chips</th>
<th>Very wet chips</th>
</tr>
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<tbody>
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<td>1</td>
</tr>
<tr>
<td></td>
<td>Nst</td>
<td>3</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Temp. (°C)</td>
<td>9.2</td>
<td>11.5</td>
<td>4.7</td>
<td>7.8</td>
</tr>
<tr>
<td></td>
<td>RH (%)</td>
<td>74.5</td>
<td>70.2</td>
<td>40.6</td>
<td>74.9</td>
</tr>
<tr>
<td></td>
<td>N trucks (amount)</td>
<td>0</td>
<td>14 (97 240 kg)</td>
<td>2 (Unknown)</td>
<td>7 (64 830 kg)</td>
</tr>
</tbody>
</table>

Data in parenthesis indicate amount of straw received. Information about water content of wood chips was not available.

*Water content of the straw received.

Np, Numbers of personal monitoring.

Nst, Numbers of stationary monitoring.

There are no internationally accepted threshold limit values (TLVs) or occupational exposure limits (OELs) for microorganisms and endotoxins, but to be able to analyse the results of exposures in this study, suggested TLVs or OELs from other papers are summarized below. Suggested TLVs or calculated ‘no effect values’ for inhalable or total endotoxin exposure in different environments are between 30 and 800 endotoxin units (EU) m$^{-3}$ (Haglind and Rylander, 1984; Rylander et al., 1985; Castellan et al., 1987; Kennedy et al., 1987; Smid et al., 1992; Smid 1993; Donham and Cumro, 1999; Donham et al., 2000). In this study the results are mainly related to the TLV of 150 EU m$^{-3}$ suggested by Smid et al. (1992a, b) and Donham et al. (2000). For cultivable thermophilic actinomycetes (Dutkiewicz et al., 1994) suggested a TLV of $2 \times 10^4$ colony forming units (cfu) m$^{-3}$. A Dutch study group has suggested that fungal and bacterial concentrations $>10^4$ cfu m$^{-3}$ should be considered a threat to workers’ health (Heida et al., 1995). Eduard et al., (2001) have shown that symptoms in the eyes and nose increased after exposure to fungal spores at the level $2 \times 10^4$ to $5 \times 10^5$ m$^{-3}$ and cough symptoms after exposure to concentrations of $5 \times 10^5$ to $17 \times 10^5$ fungal spores m$^{-3}$, and based on that study the results in this paper are related to a value of $10^5$ fungal spores m$^{-3}$. For single fungal species as *Aspergillus fumigatus*, Heida et al. (1995) have suggested a TLV of 500 cfu m$^{-3}$. The time-weighted averages (TWA) of exposure to endotoxin, thermophilic and mesophilic actinomycetes, cfu bacteria and fungi, and total numbers of fungi and bacteria, and the enzyme NAGase are if possible related to the suggested TLVs and to publications of exposures in other environments.
MATERIALS AND METHODS

The biofuel plants

The investigation was carried out at five Danish biofuel plants. Energy was generated by the plants using both straw and different kinds of wood chips. Measurements were performed for each plant in autumn 2000 and in spring 2001 and each time over two successive working days (Table 1).

At Plants A, D and E wood chips were used. At Plants A and E the wood chips were tipped into indoor wood-chip pits by trucks outside the buildings. The truck drivers were primarily involved in this process. At Plant D the chips were tipped outside the plant and the employees at the plant were involved in the process. Main tasks at plants using chips were repairing the chips cranes, office work, cleaning conveyor belts, maintenance of machines and buildings, and measurement of the water content in wood chips.

At Plants B, C and D, straw was received in indoor storage areas. The employees at the plants were involved in measuring the water content of the straw and in unloading the bales of straw using trucks and cranes. The unloading of straw from one truck body took ~15 min, and the process was typically terminated by vacuum cleaning or sweeping the truck body, followed by vacuum cleaning or sweeping the plant floor. Main tasks at straw plants were truck driving (unloading and moving of straw bales), cleaning the floor of the straw reception and office work. At Plant B work also included fixing straw shredders. Measurements of the amount of straw received and the water content in the straw received were obtained from the plants (Table 1), but similar data were not available concerning wood chips received.

Personal sampling of inhalable aerosols

Personal dust monitoring was conducted using GSP inhalable samplers (CIS by BGI, INC Waltham, MA). The samplers were mounted with Teflon filters (pore size 1 μm) for endotoxin and NAGase analysis, and polycarbonate filters (pore size 1 μm) for quantification of total number and cultivable units (cfu) of bacteria and fungi. While personal dust monitoring was being performed, technicians recorded the duration of the different tasks being carried out by the persons monitored. Results are presented as TWA for 5–7 h of measurements.

Sampling of inhalable dust in working areas

Inhalable bioaerosols were sampled (GSP samplers) for ~7 h in different working areas. These areas included both areas where fuel was present (e.g. storage areas) and areas with no fuel (e.g. offices). In addition, outdoor references were sampled upwind the plants on each sampling day.

Extraction of dust

The dust on Teflon filters was extracted in 10.0 ml pyrogen free water with 0.05% Tween-20 by orbital shaking (300 r.p.m.) at room temperature for 60 min and centrifuging (1000 g) for 15 min, and the supernatant was used for endotoxin and NAGase 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 r.p.m.) at room temperature.

Determination of endotoxin by the limulus method

The supernatant was analysed (in duplicate) for endotoxin using the kinetic Limulus Amboecyte Lysate test (Kinetic-QCL endotoxin kit, BioWhittaker, Walkersville, Maryland, USA). A standard curve obtained from an Escherichia coli O55:B5 reference endotoxin was used to determine the concentrations in terms of EU (12.0 EU ≈ 1 ng).

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, agar plates were incubated at 45°C to quantify cultivable A. fumigatus. Estimates were made, first, of the number of bacteria cultivable at 25°C on nutrient agar (Oxoid, Basingstoke, England) with actidione (cycloheximide; 50 mg l⁻¹) and, second, of mesophilic actinomycetes and thermophilic actinomycetes (55°C) cultivable on 10 and 100% nutrient agar with actidione (cycloheximide; 50 mg l⁻¹), respectively. The total numbers of bacterial cells and fungal spores were determined after staining in 20 p.p.m. 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). 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 NAGase

We measured exposure to NAGase, which is an enzyme mainly produced by fungi (Madsen, 2003). To quantify the activity of NAGase (EC3.2.1.30), the release of p-nitrophenol from the substrate p-nitrophenol-N-acetyl-β-D-glucosaminide (Sigma Chemical Co. USA) was estimated (Madsen and...
Neergaard, 1999). Appropriate controls without either the enzyme or the substrate were run simulta-
nuously. The plates were incubated at 50°C. Reactions were terminated and a yellow colour deve-
loped following the addition of 50 μl 0.4 M Na₂CO₃
to each well. Absorbance was measured at 405 nm. The background absorbance of each dust suspension
was subtracted from the measured absorbance of each sample. One unit of enzyme activity is defined
as the amount of enzyme that releases 1 μmol of p-nitrophenol ml⁻¹ enzyme min⁻¹. Activities are
expressed as pmol s⁻¹ m⁻³ air.

Temperature and relative humidity

Where outdoor reference airborne dust was sam-
ples, measurements of temperature and relative humidity (RH) were performed during the whole
sampling period (5–7 h) using Gemini Data loggers
(Tinytag TGP-1500, Plus Data Logger, UK). Using
the Gemini Data loggers temperature and RH were
also measured in some selected working areas.

Statistics

The tasks were different at the different plants, but
all plants had an area for biomass reception, and measurements were performed in these areas in
autumn and spring, making comparison between
seasons possible. Hence, analysis of variance was
used to describe factors (plant and season) assumed
to affect the exposures using PROC GLM in SAS
with α = 0.05.

RESULTS

Exposure to endotoxin

Personal exposure to inhalable endotoxins was
between 2 and 119000 EU m⁻³ (Table 2) and 34%
of the subjects measured were exposed to >150 EU
m⁻³. The highest exposure was found for a person
who worked with a straw shredder at Plant B for 90
min out of 6 working hours. The median exposure for
people working with straw shredders for at least 30
min of a working day was 23775 EU m⁻³ (average =
41 891; n = 4). The lowest exposure was found for a
person working partly in an office and partly as a
painter at Plant E.

In the working areas, the median endotoxin concen-
tration was 66 EU m⁻³ (average = 429; max = 21 000;
 n = 88). Table 3 shows endotoxin concentrations in
different working areas, with the highest concentra-
tion close to a straw-bale transporter in a room with a
straw shredder.

Significantly different amounts of endotoxin were
found in the biomass receiving areas at the different
plants (P < 0.0001) (Table 4), with the highest con-
centrations at Plants C and D.

Exposure to fungi and NAGase

Personal exposures to concentrations >10⁴ cfu m⁻³
of mesophilic fungi were found for 81% of the people,
and 68% of the people were exposed to concentra-
tions of fungal spores >10⁴ m⁻³. The highest expo-
sure to cfu fungi, total fungi and NAGase was found
for a person who worked with a straw shredder at
Plant B for 90 min out of 6 working hours. The tasks
to estimate water content in wood chips and
repairing the chips cranes also seemed to cause a
high exposure to fungi. Thus subjects working with
these tasks (n = 7) for at least 30 min of a working
day had median exposures of 17 × 10⁴ cfu of meso-
philic fungi m⁻³ (average = 48 × 10³), 6.7 × 10⁴ cfu
of A. fumigatus m⁻³ (average = 26 × 10³), 70 × 10⁴
of fungal spores m⁻³ (average = 195 × 10³) and
a NAGase activity of 11.2 pmol s⁻¹ m⁻³ (average = 14.3).

A. fumigatus was found in most areas and the high-
est exposure was found for a person who cleaned a
chip pit at Plant A for 1 out of 7 working hours and did
office work for the remaining 6 h. The second highest
exposure was found for a person who cleaned a chip
conveyor belt at Plant E for 90 min out of 7 working
hours (Table 2).

The median concentration of fungal spores in the
working areas was 12 × 10⁴ spores m⁻³ (average =
47 × 10³; max. = 711 × 10³). In areas where chips,
especially bark chips, were handled, high concentra-
tions of fungi were found (Table 3 and 5). Significant
differences (P < 0.0001) were found between the
plants concerning concentrations of the different
fungal measurements in the biomass reception
(Table 5).

Exposure to bacteria including
actinomycetes

All the subjects were exposed to concentrations of
bacteria above 10⁶ cells m⁻³. The highest personal
exposures to the different measured bacteria were
found at Plant B (Table 2) for people working with
straw shredders for at least 30 min per 7 h working
day. The highest level of personal exposure to
bacteria including actinomycetes at Plant E was for
a person cleaning a chip conveyor belt for 90 min out
of 7 working hours. At the two Plants A and E, where
only wood chips were handled, the exposure to ther-
mophilic actinomycetes was, in contrast to at straw
plants, significantly higher than the exposure to
mesophilic actinomycetes (Table 2).

Bacteria were found in high concentrations in all
working areas where wood or straw were handled
(Tables 3 and 4). The median exposure in all working
areas (n = 88) was 118 × 10⁴ bacterial cells m⁻³
(average = 146 × 10⁴) and 2.3 × 10⁴ cfu
bacteria m⁻³ (avg. = 7.2 × 10⁴). The median
concentrations of mesophilic and thermophilic
Table 2. Personal exposure* (TWA m$^{-3}$) to microbial components

<table>
<thead>
<tr>
<th>Plant</th>
<th>n</th>
<th>Max</th>
<th>Median</th>
<th>Average</th>
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<td>47 4.7</td>
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<td>9.9 0.71</td>
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<td></td>
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<td>64 25 119</td>
<td>2.16</td>
<td>19 1.8</td>
</tr>
<tr>
<td>B</td>
<td>8</td>
<td>119 000 281</td>
<td>81</td>
<td>1.44</td>
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<td>0.88</td>
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<tr>
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<td>5485 64</td>
</tr>
</tbody>
</table>

* Data are pooled for autumn and spring. Me, Mesophilic; Tot, Total; A.fum, A. fumigatus; Th, Thermophilic; act, Actinomycetes.

Endotoxin: Concentrations $>150$ EU m$^{-3}$ are in bold according to suggested TLVs (Smid et al., 1992; Donham et al., 2000).
Me. fungi: Concentrations $>10^4$ cfu m$^{-3}$ are in bold according to a suggested TLV (Heida et al., 1995).
Total fungi: Concentrations $>10^5$ spores m$^{-3}$ are in bold according to the observation of Eduard et al. (2001) that symptoms of the eyes and nose increase after exposure to fungal spores at the level $2 \times 10^4$ to $5 \times 10^5$ m$^{-3}$.
NAGase: No suggested TLV available.

A. fumigatus: Concentrations $>500$ cfu m$^{-3}$ are in bold according to a suggested TLV (Heida et al., 1995).
Bacteria: Concentrations $>10^4$ cfu m$^{-3}$ are in bold according to a suggested TLV (Malmros et al., 1992; Heida et al., 1995).
Total bacteria: No suggested TLV available.
Th. actinomyctes: Concentrations $>2 \times 10^4$ cfu m$^{-3}$ are in bold according to a suggested TLV (Dutkiewicz et al., 1994).
Me. actinomycetes: No suggested TLV available.
Table 3. Average concentrations of microbial components (TWA m^{-3}) in different working areas measured in the autumn

<table>
<thead>
<tr>
<th>Plant</th>
<th>Area of assessment</th>
<th>n</th>
<th>Endotoxin (EU)</th>
<th>Me. fungi × 10^5 (cfu)</th>
<th>Tot. fungi × 10^5 (N)</th>
<th>NAGase (pmol s^{-1})</th>
<th>A.fum × 10^5 (cfu)</th>
<th>Bacteria × 10^5 (cfu)</th>
<th>Tot. bacteria × 10^5 (N)</th>
<th>Th. act. × 10^5 (cfu)</th>
<th>Me. act. × 10^5 (cfu)</th>
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<tr>
<td>A</td>
<td>Bark chips pit</td>
<td>2</td>
<td>214</td>
<td>25</td>
<td>147</td>
<td>0.780</td>
<td>7.4</td>
<td>16</td>
<td>123</td>
<td>61</td>
<td>7.5</td>
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<td></td>
<td>Bark chips screw conveyor</td>
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<td>34</td>
<td>280</td>
<td>3.34</td>
<td>22</td>
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<td>Bark chips crane</td>
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<td>0.6</td>
<td>36</td>
<td>398</td>
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<td>30</td>
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<td>170</td>
<td>22</td>
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<td>Ash container</td>
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<td>6</td>
<td>0.33</td>
<td>4.4</td>
<td>0.391</td>
<td>0.32</td>
<td>0.34</td>
<td>1.4</td>
<td>0.11</td>
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<td>2</td>
<td>3</td>
<td>1.5</td>
<td>4.6</td>
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<td>0.78</td>
<td>0.045</td>
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<td>15</td>
<td>134</td>
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<td>B</td>
<td>Straw shredder</td>
<td>4</td>
<td>268</td>
<td>3.2</td>
<td>17</td>
<td>1.37</td>
<td>1.7</td>
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<td>794</td>
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<td>1.7</td>
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<td>Straw bale transporter in straw shredder room</td>
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<td>10 520</td>
<td>2.1</td>
<td>6.4</td>
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<td>Removal of bale string</td>
<td>2</td>
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<td>0.395</td>
<td>1.1</td>
<td>3.6</td>
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<td>Staircase in straw reception</td>
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<td>7</td>
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<td>3.2</td>
<td>0.277</td>
<td>0.48</td>
<td>0.82</td>
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<td>Bd</td>
<td>Bd</td>
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<td>0.11</td>
<td>Bd</td>
<td>Bd</td>
<td>Bd</td>
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<tr>
<td>C</td>
<td>Straw store, no activity</td>
<td>4</td>
<td>9.5</td>
<td>0.18</td>
<td>2.0</td>
<td>0.190</td>
<td>Bd</td>
<td>0.042</td>
<td>1.60</td>
<td>0.036</td>
<td>0.13</td>
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<tr>
<td></td>
<td>Straw store, with activity</td>
<td>4</td>
<td>521</td>
<td>0.74</td>
<td>8.2</td>
<td>0.441</td>
<td>0.056</td>
<td>0.66</td>
<td>52</td>
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<tr>
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<td>1.4</td>
<td>0.44</td>
<td>0.50</td>
<td>0.192</td>
<td>0.027</td>
<td>0.055</td>
<td>0.079</td>
<td>0.055</td>
<td>Bd</td>
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<tr>
<td>D</td>
<td>Straw feeding</td>
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<td>132</td>
<td>1.4</td>
<td>16</td>
<td>0.972</td>
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<td>0.19</td>
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<td>0.341</td>
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<tr>
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<td>Assessment of chips humidity</td>
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<td>2.0</td>
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<td>2.6</td>
<td>2.74</td>
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<td>6.1</td>
<td>0.061</td>
<td>2.3</td>
<td>0.780</td>
<td>Bd</td>
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<td>118</td>
<td>0.0323</td>
<td>0.0029</td>
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<td>9.7</td>
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<td>0.830</td>
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<td>Under chips conveyor belt</td>
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<td>426</td>
<td>88</td>
<td>189</td>
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<td>59</td>
<td>168</td>
<td>82</td>
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<td></td>
<td>Engine room</td>
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<td>3.7</td>
<td>0.176</td>
<td>1.7</td>
<td>0.624</td>
<td>0.0531</td>
<td>0.076</td>
<td>3.7</td>
<td>0.18</td>
<td>0.354</td>
</tr>
</tbody>
</table>

Max average exposure on all plants | 10 520 | 88 | 398 | 3.34 | 32 | 59 | 794 | 170 | 22 |

Me, Mesophilic; Tot, Total; A.fum, A.fumigatus; Th, Thermophilic; act, Actinomycetes.
Bd = below detection level. For details please see footnotes to Table 2.
actinomycetes in all working areas were 1.0 \times 10^4 (avg. = 4.2 \times 10^4) and 1.8 \times 10^4 cfu m^{-3} (avg. = 20 \times 10^4; n = 66), respectively.

Significant differences were found between the plants concerning concentrations in the biomass receiving areas of cfu bacteria (\(P = 0.0006\)), total bacteria (\(P = 0.025\)), and mesophilic actinomycetes (\(P < 0.0001\)), but not concentrations of thermophilic actinomycetes (\(P = 0.0799\)) (Table 4).

**Seasonal differences**

In areas receiving biomass, significantly higher concentrations of all microbial components (\(P \text{ between }<0.0001 \text{ and } 0.024\)) except endotoxin (\(P = 0.51\)) were found in spring than in autumn (Tables 4 and 5). This could, however, be caused by the amount of straw received. Therefore, the data of concentrations in straw receiving areas at Plants B and D were divided by the amount of straw received and the comparison performed again. After this division, the differences between seasons were significant (\(P < 0.0001\) and 0.044) for cfu of bacteria, mesophilic and thermophilic bacteria, NAGase and total numbers of fungi, and again the highest exposure was in spring. Similarly, in an indoor straw storage at Plant C, all microbial components, except endotoxin, were higher in spring than in autumn (\(P \text{ between }<0.001 \text{ and } 0.035; n = 4\)). For personal measurements the trend was the same, but the difference was only significant for cfu bacteria (\(P = 0.034\)).

**Temperature and humidity**

Outdoor temperatures and RHs are mentioned in Table 1. The temperature and RH were very different in different working areas at the same plant. The data in this paragraph are from autumn Day 1 and are averages for a working day. At Plant A, the RH and temperature were 18% and 37°C close to the chips screw conveyor and 95% and 10°C in the chips receiving area. At Plant B the RH and temperature were 71% and 16°C, respectively in the straw receiving area. At Plant C the RH and temperatures were 84% and 8°C and 13% and 43°C in the straw feeding area and ash cellar, respectively. At Plant D the RH and temperatures were 71% and 17°C, respectively, in the straw reception. At Plant E the RH and temperatures were 33% and
30°C respectively, in the room for assessment of water content in wood chips and 27% and 31°C in the boiler room.

**DISCUSSION**

The exposure levels to endotoxin, actinomycetes and fungi were, in general high, at the five biofuel plants when compared with suggested exposure limits. The personal exposures measured were all higher than background levels measured at the same plant (Table 6). In this study the highest exposures to endotoxin were mainly associated with work with straw. Endotoxin exposure during work with a straw shredder was up to 119 000 EU m⁻³ and a dose-response study of exposure to endotoxin in cotton dust has shown that exposure to 56 200 EU m⁻³ for 4 h causes fever, tight chest and breathing difficulties (Rylander et al., 1985). Exposure to endotoxins at levels of ~80 EU m⁻³ also affects lung function (Rylander et al., 1985; Castellan et al., 1987; Milton et al., 1996), and in this study most of the workers handling straw or wood chips were exposed to concentrations >80 EU m⁻³.

The exposures to endotoxins at the biofuel plants studied were much higher than exposures found earlier in the wood processing industry (Alwis et al., 1999). Dennekamp et al. (1999); Radon et al. (2001), Monso et al., (2002), Rongo et al. (2004) and Smit et al. (2005) have used the same or equivalent sampling and endotoxin quantification method as in this study. They found median or geometric mean exposure values of 2.8, 21, 27, 91 and 2060 EU m⁻³ for greenhouse workers, lumber mill workers, wastewater treatment workers, wood industry workers and poultry farmers, respectively. Thus the median personal exposure found in this study (55 EU m⁻³) at biofuel plants was considerably higher, for example, than that for greenhouse workers and considerably lower than that for poultry farmers.

The most exposed person at the biofuel plant was, however, exposed to more endotoxin than the most exposed person at the poultry farms.

Personal exposures to thermophilic actinomycetes were found in concentrations of up to 1509 × 10⁴ cfu m⁻³, and a suggested TLV for thermophilic actinomycetes of 2 × 10⁷ cfu m⁻³ was exceeded in 13 of the 32 measured people. The high exposures to thermophilic actinomycetes were mainly associated with work with a straw shredder and with different working processes with wood chips. The average concentration of thermophilic actinomycetes found at the biofuel plants was higher than that found in animal houses (Dutkiewicz et al., 1994) and in cigar and cigarette factories (Reiman and Uitti, 2000). Similarly, the average exposure to actinomycetes was higher than that found at an English hemp-processing plant (Fishwick et al., 2001).

All the people studied were exposed to concentrations of bacteria higher than the TLV suggested by (Heida et al., 1995; Malmros et al., 1992). In some working areas, the concentration of cultivable bacteria was much higher than the maximum exposure found, for example, in Swiss sawmills (Oppliger et al., 2005), and the average exposure was much higher than that in Finish cigarette and cigar factories (Reiman and Uitti, 2000). The median personal exposure to total bacteria was lower than in animal houses (Radon et al., 2001).

Personal exposures to concentrations >10⁴ cfu of mesophilic fungi m⁻³ were found for 81% of the people, and 68% of the people were exposed to concentrations of fungal spores >10⁷ m⁻³. Eduard et al. (2001) have shown that symptoms in the eyes and nose increase after exposure to fungal spores at the level 2 × 10⁴ to 5 × 10⁵ m⁻³ and coughing increases after exposure to concentrations of 5 × 10⁴ to 17 × 10⁵ fungal spores m⁻³. Consequently fungi can be expected to cause health effects in employees at biofuel plants. The exposure levels to fungi were higher
than the levels found in the wood processing industry (Alwis et al., 1999) and in Swiss sawmills (Opplinger et al., 2005) and lower than that ascertained for pig farmers and poultry farmers (Radon et al., 2001). It should, however, be noted that the people in this study also carried the sampling pumps while doing work in the office and repairing buildings. The enzyme NAGase, produced by most fungi, has only been quantified in a few studies, which are not directly comparable with this study. However, in a study with mouldy and non-mouldy schools, NAGase was found in concentrations of up to 6.3 pmol s\(^{-1}\) m\(^{-3}\) (Madsen, 2003; Madsen and Würtz, 2005), which is, as expected, a lower maximum value than that found in this study.

During a working day the employees worked in different areas at the biofuel plants and, thus, were exposed to wide temperature and humidity changes. Temperature and humidity changes may have an effect on the comfort of the employees (Fanger, 1970), and it may also affect the development of respiratory diseases (e.g. Curson, 1993; Smedje et al., 1997).

Work with bark chips caused a higher exposure to microorganisms than work with forest chips. Similarly, high concentrations of airborne endotoxin were found at a biofuel plant where bark chips were used (de Davila and Bengtsson, 1993) and at a paper factory where a machine was debarking logs (Prazmo et al., 2003). Furthermore, work with straw often caused a higher exposure to endotoxins than work with wood chips. This is in accordance with a study showing that straw releases more endotoxin and LPS per kg biomass than forest wood chips (Madsen et al., 2004). In contrast, fungal concentrations and exposures to fungi were not in general higher at straw plants than at wood-chips plants. This is also in accordance with an earlier comparison of releases of microbial components from forest wood chips and straw (Madsen et al., 2004). At wood-chip plants more thermophilic actinomycetes relative to mesophilic actinomycetes were found and, furthermore, \textit{A. fumigatus} was more abundant at the wood-chip plants. This may be due to self-heating of wood chips causing growth of thermophilic and thermotolerant microorganisms. \textit{A. fumigatus} has previously been found on wood chips (Millner et al., 1977; Passman, 1983) and to be released from both wood chips and straw (Madsen et al., 2004). \textit{A. fumigatus} is allergenic and can be infective (Yocum et al., 1976).

Outdoor references showed different concentrations of microbial components, and the highest concentrations may be partly related to the transport or storage of biomass around the plants. The median reference level of endotoxin was 2.2 EU m\(^{-3}\), which is at the level of earlier measurements in Scandinavia (Madsen, 2006). The median reference level of cfu fungi was 320 cfu m\(^{-3}\), which is in accordance with reference measurements performed in studies in the USA and Germany (Shelton et al., 2002; Herr et al., 2003). The reference level of cfu bacteria was slightly lower than that in the German study. In contrast to Herr et al. (2003), we found thermophilic actinomycetes in some of our reference measurements, and this may be due to transporting biomass around the plants and storing the woods chips outside. On the other hand measurements of other microbial components were not higher than expected for reference measurements.

In a straw storage facility, on days when no straw was transported, there was a higher airborne concentration of actinomycetes and fungi relative to concentrations of bacteria compared with days when straw was transported. Furthermore, high bacterial concentrations relative to fungal concentrations were found in areas where straw was mechanically handled roughly, such as in straw-shredder areas. This indicates that the composition of the exposure is dependent on how the biomass is handled and it is in accordance with an earlier study showing that actinomycetes and fungi seem to be released more easily than bacteria are released from straw (Madsen et al., 2006). The task causing the highest exposure to all microbial components, except \textit{A. fumigatus}, was work with a straw shredder. The highest exposure to \textit{A. fumigatus} was related to work with cleaning of a chips pit. No single or two working areas caused the highest average exposure to all microbial components. Thus the highest average concentration of airborne endotoxin was found in a room with a straw transporter and a straw shredder, while the highest concentrations of mesophilic fungi, \textit{A. fumigatus} and cfu bacteria were found under a chips conveyor belt. The highest concentrations of total fungi, thermophilic and mesophilic actinomycetes were found in a working area with a chips crane, while the highest concentration of NAGase was found in an area with a bark chips screw conveyor. The highest concentration of total bacteria was found in an area with a straw shredder. Work in a straw reception area could also in general cause high exposures, especially to bacterial components. Consequently the employees should try to restrict time spent in these areas to as short a time as possible. Work in an office at plant A also seemed to cause an elevated exposure to microbial components and this is of importance because exposure in the office is not expected by the workers and it may be possible to reduce this exposure by simple interventions.

Even though only a total of 36 samples were collected in areas with biomass reception in autumn and spring significant seasonal differences were seen. Thus exposure for most microbial components, except endotoxin, was higher in straw receptions and in straw storage facilities in spring than in autumn. At Plant B the straw received had a lower
water content in spring than in autumn, and this may affect the release of microorganisms from the straw handled. In addition the straw used in spring is expected to be older than the straw used in the autumn and may thus contain more microbial components due to growth during the storage period. This phenomenon has been revealed for the farmer’s lung, which tends to be more prevalent during the spring than during other seasons (Terho et al., 1980). Seasonal differences in exposure have previously been seen in some other environments, e.g. concentrations of airborne cfu bacteria in swine confinement building were higher in summer than in winter (Duchaine et al., 2000) and exposure to β-glucan of household waste collectors was higher in warm months than in cold months (Thorn, 2001).

CONCLUSIONS

High exposures to microorganisms and endotoxins were found, and, consequently, this working environment may cause respiratory disorders to the people working at the plant. Differences in exposure levels were seen between the plants, due partly to differences in the process equipment, tasks and the biofuel handled. Since differences were seen between plants with different process equipment, it is important when planning new biofuel plants to select the equipment causing lowest possible exposure to microorganisms and endotoxins. Handling forest wood chips or industry chips caused lower exposure to microbial components than bark chips and should, thus, be preferred. Endotoxin was found in higher concentrations at straw plants than at wood chips plants, while the opposite was measured for *A. fumigatus*. In straw reception areas, exposure to most microbial components was higher in spring than in autumn.

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REFERENCES


