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Exposure to Endotoxins and Microbes in the Treatment of Waste Water and in the Industrial Debarking of Wood

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Exposure to microbes is very common in occupations ranging from farming (Heederik et al. 1991, Ojanen 1992) to animal feed (Smid et al. 1992), sugar production (Jensen et al. 1993, Dawson et al. 1996) and modern biotechnology (Crook 1996). Microbial agents are therefore an important cause of occupational diseases (Burrell 1994, Salkie 1994). The nature of the diseases is determined by infectious, irritative, toxic or allergenic properties of microbes (Lacey and Dutkiewicz 1994). Practical approaches have been conceived to obtain a general idea of the exposure to biological factors (Eduard 1996) but the interpretation of the results is not simple in the absence of a specific allergy to a quantifiable allergen. Special emphasis has been laid on the endotoxin exposure from the dead and viable gram-negative bacteria (Palchak et al. 1988, Hollander et al. 1993) because of its great toxic potential (Johanssen 1993). While appealing in its simplicity, the endotoxin hypothesis is, however, only a partial explanation of the ill health in the exposure to complex biological dusts (Shahan et al. 1994).

Finland has an extensive saw mill and pulp and paper industry. To maintain the massive production volumes, extensive raw material storages have to be maintained. The processes themselves produce also great amounts of waste suspended in the process water. Despite of high automation degree in the industry, most of maintanance work and the treatment of waste water are difficult to automate completely, so manual workers are always needed. Their exposure to biological agents has been a major problem to the occupational health care system, and this survey was therefore undertaken to characterize and quantify the risks in the modern industry. To our knowledge, there are very few studies concerning microbe exposure in pulp or paper industry.

MATERIALS AND METHODS

Three wood-processing plants were studied. One produced only pulp with the sulfate process, and the other pulp with a further production of newsprint, carton and fine paper. The principal pulping method in the last-mentioned plant was

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mechanical with an added fraction of sulfate method-produced chemical pulp. The pulp production capacity of the first plant was 300,000 metric tons per year; the newsprint production amounted to 375,000 metric tons, and the fine paper to 120,000 metric tons annually. The third plant had semi-chemical sulfite process with the further production of fluting. The production was 180,000 metric tons per year.

Spruce and birch were used as raw materials in the plants. At the start of the process, the wood was debarked in an automatic large-scale slasher unit combined with a wet-drum debarking technique. At each stage, the bark waste was transported by conveyor belt continuously towards the bark storage field in the vicinity of the debarking unit. Because of the wet debarking technique, the primary bark was pressed dry before its storage or combustion with the sludge from the waste water treatment plants. The waste water treatment plants operated according to the aerobic activated sludge principle. Therefore, the plants housed tons of microbial sludge and wood-derived organicals. Air was fed through the pool bottoms and by pressing it in to the water with compressors through the surface. After the aeration stage, the bacterial sludge was returned from the sedimentation basins to the aeration, and the remaining organic sediment was dried with centrifugal presses, mixed with dried bark and burnt to produce energy for the process.

Endotoxin samples were collected on fiber glass filters (nominal pore size 0.3 μm as tested with calibrated aerosol). The filters were sterilized in an autoclave at 121°C for 20 min before sampling. The filters were purchased from Machery Nagel Corp. (Batch NN 85/200) and the aseptic filter holders from Millipore (MAWG 037 AO). Air was sampled with a SKC pump for 60 min at a velocity of 2.5 l/min. Because the workers visited the exposure producing sites on regular basis several times during the work shift, the samplers were placed 1.5 m from the floor in the debarking units, near the centrifugal presses in the sludge treatment, at the aeration basin, and in the vicinity of the chip storage outside the plant. For control sites, air was sampled in the pulp baling rooms, bale storage, at the dry end of the paper machine, and in the paper storage. Samples were taken in the control rooms of the debarking units, in the control rooms of internal control sites, and for outdoor controls in the open air outside the plants away from the actual production.

Air sampling for suspended microbes were taken with a six-stage Andersen sampler as described previously by Kotimaa (1990) at the same sites.

The endotoxin concentrations were determined with a Limulus amebocyte lysate test (Schimberg et al. 1992) using a modification described in detail elsewhere (Sarantila et al. 1999). The standard curves were established with *E. coli* 0111:B reference toxin (KabiVitrum). To correct for analytical interference, aliquots of the test samples were spiked with 0.4 endotoxin unit/ml. The results in series within ± 10 % of the spiked sample targets were accepted. The filter and reagent

blanks included in each analysis showed no endotoxin activity. For calculation, the equivalence of 1 endotoxin unit corresponding to 0.1 ng was used.

Airborne microbes were cultured as follows. Mesophilic fungi were cultured on Hagen agar with added streptomycin. The plates were incubated for a week at 20°C. Similar samples were taken for thermophiles, followed by incubation for 5 days at 40°C. Xerophilic fungi were taken on malt extract agar which contained 10 % sodium chloride. These plates were incubated at 20°C for a week. Mesophilic bacteria were cultured on tryptone glucose extract agar at 20°C for a week, and the thermophilic actinomycetes on a half strength nutrient agar at 55°C for 3 days. The fungal colonies were counted with the positive hole correction method (Andersen 1958) and they were identified using common mycological procedures.

RESULTS AND DISCUSSION

The highest endotoxin concentration (375 ng/m^3) was measured in the dry debarking unit on the servicing bridge during actual running of the machinery (Table 1). In general, the highest concentrations in debarking were found on the servicing bridges and the lowest on the floors. In sludge treatment, the highest endotoxin concentration (124 ng/m^3) was found near the sludge centrifuges, whereas the highest value near the aeration basin was 42 ng/m^3 during the fall period. Low endotoxin levels were detected at the control sites and in the general outdoor analyses (Table 1).

Table 1. Occurrence of endotoxins and microbes

Site	N	Endotoxins (ng/m^3)	Microbes			
			N	Fungi (cfu/m^3)	Mesophilic bacteria (cfu/m^3)	All microbes (cfu/m^3)
Exposing worksites	93	21 (0.001-375)	59	660 (370-145,000)	6,300 (200-82,000)	14,000 (1,000-170,000)
Internal controls	27	2 (0.001-3)	19	130 (20-3,500)	150 (2-1,200)	770 (40-15,000)
Outdoor controls	33	1 (0.001-3)	24	92 (2-3,500)	420 (120-1,200)	930 (2-6,800)

Each figure represents the geometric mean with the range in parentheses. The exposing worksites include debarking, sludge treatment, and bark pile. The internal controls include pulp baling and storage, dry end of paper machine, and paper storage.

Table 2. Detected fungi

Species	Debarking	Sludge treatment	Internal controls	Basins	Outdoor controls
Penicillium spp.	+	+	+	+	+
Aspergillus fumigatus	+	+	+	+	+
Rhodotorula glutinis	+	+	+	+	+
Cladosporium sp.	+	+	+	+	+
Aureobasidium pullulans	+*	+	+	+	+
Aspergillus sp.	+*	+	+	+	+
Alysidium sp.	+	-	+	+	+
Sporobolomyces sp.	+	+**	-	+	-
Paecilomyces variotii	+	+	+	-	+
Geotrichum candidum	+	+	+	-	-
Scolecobasidium sp.	+	+	-	-	-
Basipetospora sp.	+	+	-	-	-
Hyalodendron sp.	+	+**	-	+	+
Mucor sp.	+	+	-	+	-
Cephalosporium sp.	+	+	-	+	+
Trichoderma viride	+	+	-	+	+
Monocillium sp.	-	+	-	-	-
Olpitrichum sp.	+	+	-	+	+
Rhinocladiella sp.	+	+	+	-	-
A. niger	+	+	+	-	-
Calcarisporium sp.	+	+	-	+	-
Oidiodendron sp.	+	+	-	-	-
Rhizopus nigricans	+	-	-	-	-
Alternaria alternata	+	+	+	+	-
Botrytis cinerea	+	+	+	+	+
Hansfordia sp.	+	+	+	-	-
Phialophora sp.	+	+**	-	+	-
Humicola sp.	+	+**	-	-	+
Ulocladium sp.	+	-	+	-	-
Oedocephalum sp.	-	-	+	-	-
Gilmaniella sp.	-	-	+	-	-
Trichothecium sp.	-	+	-	-	-
Tritirachium sp.	-	+	-	-	-
Chalara sp.	-	+	-	-	-
Gliomastix sp.	-	+**	-	-	-

+ sign denotes the presence and a - sign an absence of the species.
*mainly detected in debarking, ** mainly detected in sludge treatment.

Table 3. Seasonal variation in the occurrence of major fungi

Site	Species	Summer	Fall	Winter
Debarking hall	*Penicillium* sp.	++++	+++	+
	A. fumigatus	++	++	-
	Rhodotorula glutinis	++	+++	++
	Cladosporium sp.	-	++	-
Debarking control room	*Penicillium* sp.	++	-	-
	A. fumigatus	++	++	-
	Rhodotorula glutinis	-	-	-
	Cladosporium sp.	-	-	-
Sludge treatment	*Penicillium* sp.	+++	++	+
	A. fumigatus	++	+	-
	Rhodotorula glutinis	++	+	-
	Cladosporium sp.	-	+	-
Sludge control room	*Penicillium* sp.	-	-	-
	A. fumigatus	-	-	-
	Rhodotorula glutinis	-	-	-
	Cladosporium sp.	-	-	-
Basins	*Penicillium* sp.	-	+	-
	A. fumigatus	-	+	-
	Rhodotorula glutinis	-	-	-
	Cladosporium sp.	-	-	-
Basin exteriors	*Penicillium* sp.	-	++	-
	A. fumigatus	+++	+++	-
	Rhodotorula glutinis	-	-	-
	Cladosporium sp.	-	++	-
Outdoor control	*Penicillium* sp.	-	-	-
	A. fumigatus	-	-	-
	Rhodotorula glutinis	-	-	-
	Cladosporium sp.	-	-	-

+ indicates the presence of >1,000 cfu/m^3 in 25% of the samples, ++ >1,000 cfu/m^3 in 26-50% of the samples, +++ >1,000 cfu/m^3 in 51-70% of samples and ++++ >1,000 cfu/m^3 in more than 71% of the samples.

However, endotoxin concentrations in pig farming (Heederik et al. 1991) are similar to those in our study, the highest values suggesting the risk of organic dust toxic syndrome (Rylander 1997).

The total microbe concentration followed the pattern of endotoxin exposure in general (Table 1). Recent comprehensive analyses of the fungal exposure in the pulp and paper industry cannot be found in the literature for comparison.The fungal spore concentrations are about the same as in wood chip handling or tobacco industry (Kotimaa 1990) or in beet sugar refining (Jensen et al. 1993). Closer analysis revealed a great variety of fungal species (Table 2). The greatest microbe concentration occurred in the summer, and it generally diminished in the winter (Table 3), which finding is in agreement with the results reported elsewhere (Adams 1964, Lehrer 1983, Southword 1974). *Penicillium* spp., *Aspergillus fumigatus* and *Rhodotorula glutinis* and other yeasts were typical of debarking and sludge treatment (Table 3). The *Penicillium* species accounted for 60% of spore concentrations, and *A. fumigatus* and yeasts some 10% each, correspondingly. While the overall fungal counts diminished during the winter, this relationship remained constant.

The highest *Penicillium* spore counts (97,000 cfu/m^3) were found on the servicing bridges of the debarking units in the summer, whereas their number on the floor at the same time was 21,000 cfu/m^3 The *A. fumigatus* spores on the bridge amounted to 25,000 cfu/m^3. The same fungi were also detected in the sludge treatment, although their numbers tended to be smaller than in the debarking (Table 3).
In industrial environments described here, processes emit microbes which are then transported to cleaner areas and are found also outdoors.

The mechanisms behind microbe-induced respiratory ill health of the workers is largely unknown. Less is known about the role of mixed microbial and chemical exposures which are possible, because sulfur dioxide and sulfides are also found in the pulp production (Kangas et al. 1984) and in the sludge treatment (Kangas et al. 1986). This prospect does not, of course, exclude sporadic cases of asthma due to spore allergens, so that control of exposure is still a priority.

Exposure to fungal spores, bacteria, and endotoxins in pulp industry was demonstrated in this study. High concentrations of these biological components indicate potential health hazard to pulp workers.

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