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Anna Ławniczek-Wałczyk, Małgorzata Gołofit-Szymczak
Marcin Cyprowski, Rafał L. Górny

EXPOSURE TO HARMFUL MICROBIOLOGICAL AGENTS DURING THE HANDLING OF BIOMASS FOR POWER PRODUCTION PURPOSES

NARAŻENIE NA SZKODLIWE CZYNNIKI MIKROBIOLOGICZNE W PROCESIE PRZETWARZANIA BIOMASY DO CELÓW ENERGETYCZNYCH

Central Institute for Labour Protection – National Research Institute / Centralny Instytut Ochrony Pracy – Państwowy Instytut Badawczy, Warszawa, Poland
Department of Chemical, Aerosol and Biological Hazards, Biohazard Laboratory / Zakład Zagrożeń Chemicznych, Pyłowych i Biologicznych, Pracownia Zagrożeń Biologicznych

Abstract

Background: Despite numerous benefits related to the utilization of biomass as an alternative source of energy, the handling of biomass creates a risk for the power industry workers of exposure to harmful microbiological agents. The purpose of this study was to evaluate the exposure of the workers to such agents at a power plant co-firing biomass with coal. This assessment was based on quantitative and qualitative characteristics of bioaerosols, supplemented with the analysis of biomass samples. **Material and Methods:** Air samples were collected with both MAS and Andersen six-stage impactors. Two different kinds of biomass samples used in the co-firing technological process were collected: sunflower seed peel pellet and wood chips. Bacterial and fungal concentrations were assessed in the air and biomass samples, and isolated microbial colonies were identified to the genus and/or species level. **Results:** Bacterial and fungal concentrations at workplaces ranged between 5.1×10^2 cfu/m^3 and 2.0×10^4 cfu/m^3, and between 2.2×10^2 cfu/m^3 and 2.3×10^4 cfu/m^3, respectively. The highest concentrations were determined at workplaces related to reloading, screening and biomass transport via conveyor belts to silos. Fungi representing the genus *Aspergillus*, including *A. fumigatus*, *A. niger*, *A. flavus* and Gram-negative rods of the genus *Citrobacter*, *Pseudomonas* and *Rahnella* prevailed in the air at all investigated workplaces. Bacterial and fungal concentrations in biomass samples amounted to 1.8×10^6 cfu/g and 1.1×10^6 cfu/g, respectively. The qualitative analysis revealed that the composition of species in the biomass samples was similar to that observed in the air at workplaces. **Conclusions:** Workers engaged in the biomass combustion technology are exposed to bioaerosol containing potentially pathogenic bacteria and fungi. Med Pr 2012;63(4):395–407

Key words: power plants, occupational environment, biomass, bioaerosol, occupational exposure

Streszczenie

Wstęp: Mimo licznych korzyści płynących z wykorzystania biomasy jako alternatywnego źródła energii proces jej przetwarzania niesie ze sobą ryzyko związane z narażeniem pracowników sektora energetycznego na szkodliwe czynniki mikrobiologiczne. Celem niniejszej pracy było określenie wielkości narażenia na te czynniki na stanowiskach pracy w elektrociepłowni, w której biomasa wykorzystywana jest jako paliwo do współspalania z miałem węglowym. Oceny tej dokonano w oparciu o ilościową i jakościową charakterystykę mikroflory powietrza, uzupełnioną analizą próbek biomasy. **Materiał i metody:** Próbki powietrza pobierano impaktorem MAS oraz 6-stopniowym impaktorem Andersena. Do analizy pobrano również próbki 2 rodzajów biomasy używanej do współspalania: pelety z łusek słonecznika i zrębki drewna. Zarówno w próbkach powietrza, jak i z biomasy określano stężenie bakterii i grzybów, a wyizolowane mikroorganizmy identyfikowano do rodzaju lub gatunku. **Wyniki:** Stężenia bakterii i grzybów w powietrzu na badanych stanowiskach mieściły się w zakresie odpowiednio: od $5{,}1\times10^2$ jtk/m^3 do $2{,}3\times10^4$ jtk/m^3 i od $2{,}2\times10^2$ jtk/m^3 do $2{,}0\times10^4$ jtk/m^3. Najwyższe stężenia odnotowano na stanowiskach związanych z przeładunkiem, przesiewaniem i transportem biomasy na taśmociągach do silosów. W powietrzu na wszystkich badanych stanowiskach pracy dominowały grzyby z rodzaju *Aspergillus*, w tym *A. fumigatus*, *A. niger*, *A. flavus*, oraz Gram-ujemne pałeczki z rodzaju *Citrobacter*, *Pseudomonas i Rahnella*. Średnie stężenia bakterii i grzybów w próbkach biomasy wynosiły odpowiednio: $1{,}8\times10^6$ jtk/g i $1{,}1\times10^6$ jtk/g. Ich analiza jakościowa wykazała podobny skład gatunkowy do zaobserwowanego w powietrzu na badanych stanowiskach. **Wnioski:** Pracownicy zatrudnieni przy spalaniu biomasy są narażeni na bioaerozol zawierający potencjalnie chorobotwórcze bakterie i grzyby. Med. Pr. 2012;63(4):395–407

Słowa kluczowe: elektrociepłownie, środowisko pracy, biomasa, bioaerozol, narażenie zawodowe

Authors' address: Biohazard Laboratory, Department of Chemical, Aerosol and Biological Hazards, Central Institute for Labour Protection – National Research Institute, 16 Czerniakowska Street, 00-701 Warszawa, Poland, e-mail: anlaw@ciop.pl
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INTRODUCTION

Biomass is an energy source considered to be one of the oldest and most frequently energy sources used by mankind. As defined in Directive 2009/28/EC of 23 April 2009 on the promotion of the use of energy from renewable sources, biomass is the biodegradable fraction of products, waste and residues of biological origin from agriculture (including vegetal and animal substances), forestry and related industries, including fisheries and aquaculture, as well as the biodegradable fraction of industrial and municipal waste. Energy production from biomass allows for a reduction in greenhouse gas emissions, and an increase in energy security in Poland (1). Despite numerous benefits related to the utilization of biomass as an alternative source of energy, the processing of biomass creates a risk of exposure of power industry workers to harmful microbiological agents. One of the most common applied biomass energy technologies is co-firing with coal. To this end, most biomaterials need to be pre-treated prior to combustion by chipping, grinding, drying and densification into baling, pelleting, extrusion or briquetting (2). Therefore, the character of work during biomass handling for energy purposes is often associated with a long-term presence of workers in the environment which is strongly biologically contaminated. Organic dust contains two groups of harmful agents: plant raw material components (e.g. plicatic acid, waxes, resins and plant tissue particles), and bacterial and fungal microorganisms developing there (3–7). Among the biologically active compounds of microbial origin, particular attention is currently paid to bacterial endotoxins present in bioaerosols, and (1–3)-β-D-glucans of fungal origin, since they belong to the most conservative structures of microorganism, selectively recognizable by the immune system cells (8).

According to the relevant literature, long-term exposure to harmful microbial agents contained in dust may lead to the occurrence of hypersensitivity pneumonitis (HP), chronic obstructive pulmonary disease (COPD), asthma, chronic bronchitis, bronchial hyperresponsiveness, organic dust toxic syndrome (ODTS), and numerous non-specific inflammatory reactions (e.g. irritation of mucous membranes, conjunctiva and skin) (3,7–14).

Despite numerous studies on the exposure to harmful biological agents present in organic dust, in Poland there is an apparent lack of comprehensive characteristics of biological hazards associated with biomass handling. In the European Union Member States, assessments of exposure to harmful biological agents occurring in a form of bioaerosols during biomass handling have so far been performed to a very limited extent.

The aim of this study was to evaluate the exposure of the workers to harmful microbial agents at the workplaces associated with biomass handling at a power plant co-firing biomass with coal. This assessment was based on quantitative and qualitative characteristics of bioaerosol, supplemented with an analysis of the samples of biomass used for co-combustion with pulverised coal.

MATERIAL AND METHODS

Measurement and analysis of bioaerosols

The tests were conducted during the summer (August–September) on the premises of a power plant in which forest and agricultural biomass is co-combusted with pulverised coal. For the purpose of this research, 10 sampling stations were designated, including: various stages of preparing raw material (biomass) for the co-combustion technological process (workplaces in the technological line, E1–E7), a control laboratory (where biomass quality was tested for its physical parameters, E8–E9), and a position for sampling the "background" (E10). The complete list of measuring points is provided in Table 1. Due to the high risk of explosion and fire at the measuring positions, samples of air were collected using a MAS air sampler (a 100 Eco model, Merck, Darmstadt, Germany). In locations where technical measures so permitted (E1, E7–10), additional samples of air were collected using a 6-stage Andersen impactor (a 12–15 model, Westech Instrument Inc., Marietta, GA, USA). The duration of sampling, and the flow velocity of the air stream were, respectively: for the Andersen impactor – 5 min, and 28.3 l/min; for the MAS air sampler – 1 min and 100 l/min. During the tests, the measuring instruments were placed at a height of 1.5 m above the ground in order to collect bioaerosol samples from the workers' breathing zone. In both cases, the capture surfaces were Petri dishes filled with appropriate microbiological growth media: for mesophilic bacteria, it was TSA agar with 5% sheep blood (Tryptose Soya Agar, bioMérieux, Marcy-l'Etoile, France); for Gram-negative bacteria, EMB agar (Eosin-Methylene blue Agar, bioMérieux); for thermophilic bacteria, 50% TSA agar (50% component concentration TSA agar, BTL, Łódź, Poland); and for fungi, it was MEA (Malt Extract Agar, BTL). The growth media

for bacteria contained added actidione (50 mg/l, BTL), which inhibits the growth of yeasts and fungi, while the malt extract agar contained added chloramphenicol, which inhibits the growth of bacteria (100 mg/l, BTL). The incubation conditions for microbiological air samples, for the groups of microorganisms under research, were as follows: (a) mesophilic bacteria, including Gram-negative bacteria: 1 day (37°C) +3 days (22°C) +3 days (4°C); (b) thermophilic bacteria: 6 days (55°C); (c) fungi: 4 days (30°C) +4 days (22°C). The concentrations of live microorganisms (both bacterial and fungal) were expressed as a number of colony forming units (cfu) on a microbiological growth medium, present in 1 m^3 of sampled air (cfu/m^3). The microorganisms isolated from the air samples were identified down to the genus and/or species level. The identification of bacteria was performed based on the morphological analysis, and by employing the following biochemical tests: API 20 E, API 20 NE, API Coryne, API Staph, API 50 CH (bioMérieux). The identification of fungal microorganisms was performed based on the observations of macro- and microscopic characteristics of colonies, carried out on the basis of available taxonomic keys (15–17). For the identification of yeasts, the API 20 C AUX biochemical test (bioMérieux) was used.

The use of the Andersen impactor also allowed for the separation of bacterial and fungal aerosols depending on the size of aerodynamic diameters of the examined particles. This enabled the determination of the area of their theoretical deposition in the human respiratory system and, on that basis, carrying out a forecast assessment of the potential consequences of inhalation exposure to the body.

The measurements of bioaerosols were taken in duplicate, twice during the summer, at each of the designated sampling stations. The total numbers of air samples collected using the MAS air sampler and Andersen impactor were, respectively, 40 and 20.

Analysis of biomass samples

In order to examine the potential source of biological agents present in the air, samples of biomass used in the co-combustion technological processing were collected to be analysed. Two types of biomass were sampled for the analysis: sunflower seed peel pellets and wood chips. Two weighed amounts of 10 g each were prepared from the biomass samples, and subsequently transferred to flasks containing 90 ml of NaCl (at the concentration of 0.85%). The samples were shaken for 1 hour. Following sedimentation of the biomass solid particles, a series of dilutions (from 10^{-1} to 10^{-9}) was conducted for each of the examined suspensions; subsequently, they were inoculated (in triplicate) at an amount of 1 ml of a sample tested onto Petri plates filled with a medium appropriate for each group of the examined microorganisms. Incubation and identification of microorganisms were performed similarly as in the case of the analysis of bioaerosol. The obtained results were expressed per 1 g of the tested sample of biomass (cfu/g).

Table 1. Sampling points at the power plant
Tabela 1. Punkty pomiarowe na terenie elektrociepłowni

Sampling station Stanowisko pomiarowe	
number numer	name nazwa
E1*	biomass truck unloading station / miejsce rozładunku biosurowców
E2	loading chamber / komora przeładunkowa
E3	biomass stockpile / miejsce składowania biomasy
E4	biomass screener /przesiewacz biomasy
E5	conveyor belt tunnel / tunel taśmociągu
E6	top part of biomass storage silo / górna część silosu na biomasę
E7*	conveyor belt above boiler / przenośnik nad kotłownią
E8*	laboratory – an analysis division / laboratorium – pomieszczenie analizatorów
E9*	laboratory – sample preparation room / laboratorium – pokój przygotowania próbek
E10*	outdoor background / tło zewnętrzne

* Measurements performed using the Andersen impactor as well / Pomiary dodatkowo wykonane za pomocą impaktora Andersena.

Measurements of the air microclimate parameters

In parallel to the measurements of bioaerosol at the examined workplaces, measurements were taken of both the relative humidity and temperature of air using a thermo-/hygrometer (Conrad Electronic GmbH, Hirschau, Germany).

Statistical analysis

The obtained measurement data were statistically processed based on the Student's *t*-test, the univariate analysis of variance (ANOVA) supplemented by the Scheffé's post-hoc test, and the Pearson's correlation analysis using the "STATISTICA data analysis software system" package, version 7.1. 2006 (StatSoft, Inc., Tulsa, OK, the USA), with p-values < 0.05 adopted as statistically significant.

RESULTS

Quantitative analysis of bacterial and fungal aerosols

The concentration values for bacterial and fungal aerosols in outdoor air and at the workplaces located at the power plant obtained using the MAS impactor are presented in Table 2.

The concentrations of the bacterial aerosol ranged from 5.1×10^2 to 2.3×10^4 cfu/m^3 at the workplaces in the technological line, and from 5.6×10^2 to 3.3×10^3 cfu/m^3 in the laboratory. The tests showed statistically significant differences in the concentration levels for that group of microorganisms between individual sampling stations (ANOVA: $p < 0.01$). The occurrence of significantly higher concentrations of bacteria in the air at the workplaces in the technological line, compared to those in the laboratory was found (Scheffé's test: $p < 0.05$). The highest concentrations of bacteria (a mean of 1.6×10^4 cfu/m^3) were recorded at the E4 station (biomass screener); equally high concentration levels of bacteria, in excess of 1.0×10^4 cfu/m^3, were found at the E2 station (loading chamber) (Scheffé's test: $p < 0.5$). The lowest concentrations of bacterial aerosol were recorded at the E3 station (biomass stockpile) – 5.9×10^2 cfu/m^3.

During the measurement sessions, the concentration of fungal aerosol was at the level of 2.2×10^2–2.0×10^4 cfu/m^3 at the workplaces in the technological line, and 1.1×10^3–8.0×10^3 cfu/m^3 in the laboratory. Similarly as in the case of bacterial aerosols, statistically significant differences in the concentration levels for fungi between individual workplaces were found (ANOVA: $p < 0.01$). Again, the most significant differences in the concentrations between the workplaces in the technological line and the laboratory were demonstrated (Scheffé's test: $p < 0.01$). The contamination with fungal aerosol in the air of the examined power plant reached the highest level at the E2 station (1.9×10^4 cfu/m^3). The lowest concentration of fungi in the air was found in the laboratory room E9 (3.2×10^3 cfu/m^3).

Table 2. Bacterial and fungal concentrations in outdoor air and at the power plant obtained with the MAS impactor
Tabela 2. Stężenia bakterii i grzybów na stanowiskach pracy w elektrociepłowni oraz w powietrzu zewnętrznym zmierzone za pomocą impaktora MAS

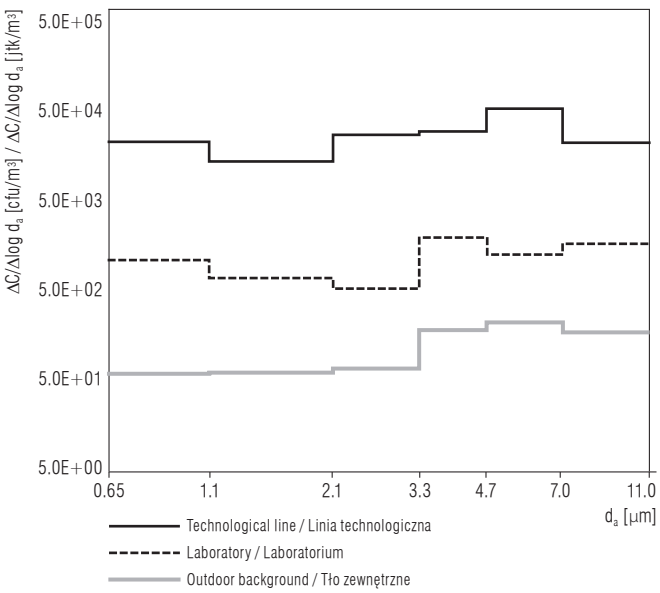
Sampling station Stanowisko pomiarowe	Samples Próbki [n]	Bacteria [×10^3 cfu/m^3] Bakterie [×10^3 jtk/m^3]			Fungi [×10^3 cfu/m^3] Grzyby [×10^3 jtk/m^3]		
		M	SD	range zakres	M	SD	range zakres
E1	4	9.01	7.14	2.82–18.85	6.07	8.33	1.55–18.55
E2	4	14.00	8.41	8.05–19.95	19.35	0.71	18.85–19.85
E3	4	0.59	0.09	0.51–0.68	8.06	8.99	1.10–18.89
E4	4	16.84	8.76	5.81–23.53	18.93	0.09	18.89–19.02
E5	4	6.88	8.44	0.79–18.90	6.53	8.73	0.22–19.01
E6	4	2.52	0.69	2.03–3.00	17.35	2.12	15.85–18.85
E7	4	2.01	0.01	2.00–2.02	4.65	0.99	3.95–5.35
E8	4	2.08	0.94	1.30–3.29	4.09	3.07	1.13–8.05
E9	4	0.67	0.15	0.56–0.77	3.17	0.61	2.74–3.60
E10	4	0.27	0.12	0.18–0.41	0.37	0.50	0.07–0.94

E1–E10 – abbreviations as in Table 1 / objaśnienia jak w tabeli 1.
M – mean / średnia; SD – standard deviation / odchylenie standardowe.

A comparison of the measurement results for the workplaces (E1–E9), and for the background (E10) revealed that the concentrations of both the bacterial and fungal aerosol at the workplaces were significantly higher than the concentration values for the background (*t*-test: p value from $p < 0.001$ to $p < 0.05$, and from $p < 0.0001$ to $p < 0.05$, respectively for bacteria and fungi).

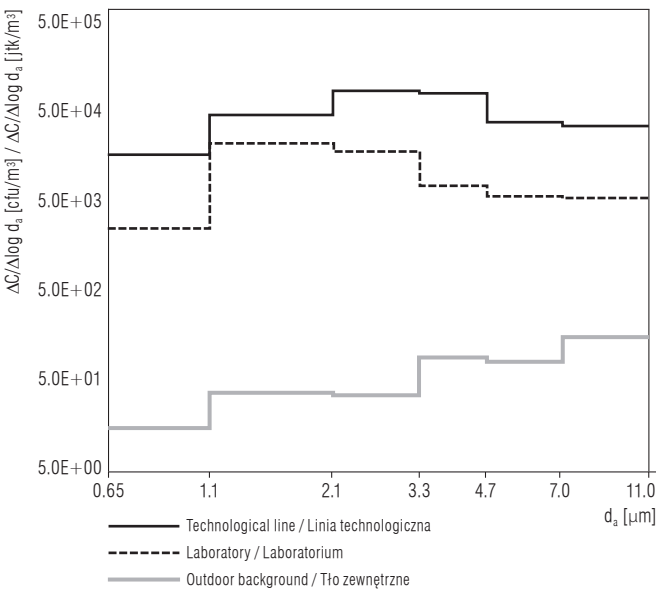
The measurement results for bioaerosols, obtained using the Andersen impactor, are presented in Figures 1 and 2. The univariate analysis of variance showed a diversity in the observed concentration levels for bacteria at individual workplaces, but the differences were not statistically significant. The highest concentrations of bacteria were recorded at the stations in the technological line (a mean of 3.2×10^4 cfu/m^3; standard deviation (SD) of 3.2×10^3). The lowest levels for that aerosol were measured in the laboratory rooms (a mean of 1.4×10^3 cfu/m^3, SD of 6.3×10^2). At the positions where the measurement of the background was taken (outside the examined power plant), the concentrations of bacterial aerosol ranged from 0.8×10^2 cfu/m^3 to 2.2×10^2 cfu/m^3 (a mean of 1.5×10^2 cfu/m^3, SD of 0.9×10^2). While analysing the concentrations of fungi with account taken of the three abovementioned groups of measurement positions, statistically significant differences were found between them (ANOVA: $p < 0.05$). Similarly as in the case of bacterial aerosols, the highest concentrations were found at the workplaces in the technological line (a mean of 5.9×10^4 cfu/m^3, SD of 6.2×10^3) (Scheffé's test: $p < 0.05$), while the lowest concentration values for that aerosol were observed in the laboratory rooms (a mean of 1.3×10^4 cfu/m^3, SD of 1.7×10^4). The concentrations of fungal aerosol in the background ranged from 0.7×10^2 cfu/m^3 to 0.9×10^2 cfu/m^3 (a mean of 0.8×10^2 cfu/m^3, SD of 0.1×10^2). A comparison of the measurement results for the background and the examined rooms showed that the concentration values for the fungal aerosol at the workplaces were higher than those obtained for the background, but those relationships were not statistically significant.

A comparison of the results in scope of the measurement of bacterial and fungal aerosols, taken using both the air sampler and the impactor, revealed that the concentrations of bioaerosols obtained using the Andersen impactor were approx. 4–10 times higher than those obtained using the MAS air sampler, however, the differences were not statistically significant.



d$_a$ – aerodynamic diameter / średnica aerodynamiczna; DC/Dlog d$_a$ – bacterial concentration in aerodynamic particle size ranges expressed in a log form / stężenie bakterii w poszczególnych przedziałach średnic aerodynamicznych cząstek wyrażonych w postaci zlogarytmowanej.

Fig. 1. The size distribution of bacterial aerosol at the workplaces and in the outdoor air at the power plant obtained with a six-stage Andersen impactor
Ryc. 1. Rozkłady ziarnowe aerozolu bakteryjnego na stanowiskach pracy w elektrociepłowni oraz w środowisku zewnętrznym, zmierzone 6-stopniowym impaktorem Andersena



Abbreviations as in Fig. 1 / Objaśnienia jak w ryc. 1.

Fig. 2. The size distribution of fungal aerosol at the workplaces and in the outdoor air at the power plant obtained with a six-stage Andersen impactor
Ryc. 2. Rozkłady ziarnowe aerozolu grzybowego na stanowiskach pracy w elektrociepłowni oraz w środowisku zewnętrznym, zmierzone 6-stopniowym impaktorem Andersena

Analysis of particle size distribution for bacterial and fungal aerosols

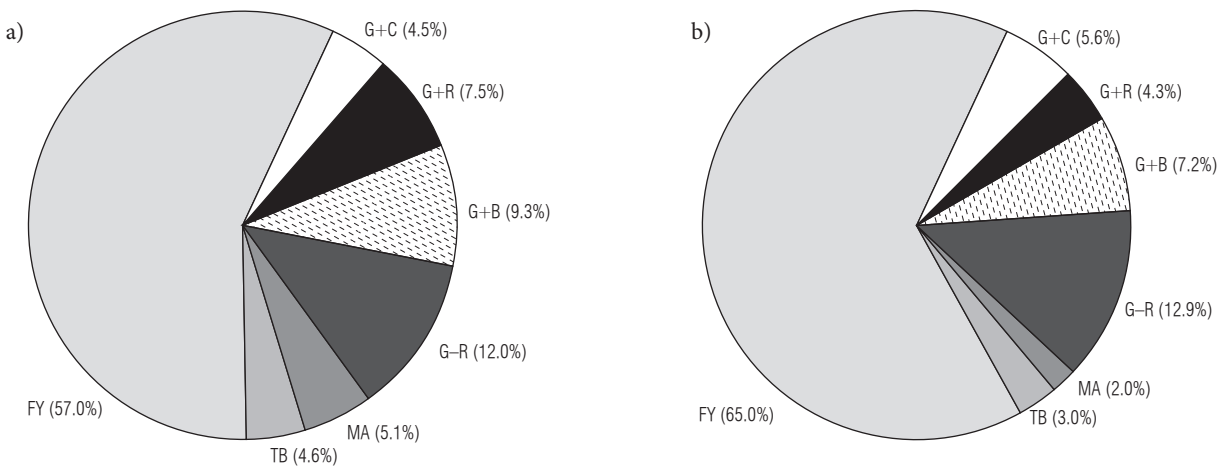
The use of the 6-stage Andersen impactor during the tests allowed for the supplementation of the results of the concentration measurements with the data on the particle size distribution of the air microflora at selected stations in the technological line (E1, E7), the laboratory (E8 and E9), and in the background (E10) (Figures 1 and 2).

Based on the analysis of the particle size distribution curves for bacterial aerosol (Fig. 1), it may be concluded that both in the outside air and at the workplaces in the technological line, the concentrations of bacteria were the highest within the range of diameters of 4.7–7.0 μm, which indicates the presence of bacteria in a form of medium-sized bacterial or bacteria-and-dust aggregates. The courses of both curves were similar, but the concentrations at the workplaces in the technological line were significantly higher than those in the outside air (t-test: $p < 0.05$). It was found from the courses of the particle size distribution curves for bacterial aerosol in the laboratory rooms that the highest concentrations of bacterial aerosol occurred within the ranges of diameters of 3.3–4.7 μm and > 7.0 μm, which indicates the presence of bacteria in a form of both small and large bacteria and dust aggregates.

The particle size distribution of the fungal aerosol in the outdoor environment and at the workplaces is presented in Figure 2. The analysis of the particle size distributions of the fungal particles in the outside air indicates the presence of those microorganisms mainly in a form of large fungal or fungi-and-dust aggregates (> 7 μm). It follows from the analysis of the course of the distribution curve for the technological line that the fungal aerosol reached its maximum concentrations there within the range of 2.1–4.7 μm. Therefore, fungal microorganisms were present there both in a form of single spores and small aggregates. On the other hand, in the laboratory rooms, the concentrations of fungi were the highest within the range of 1.1–3.3 μm, which indicates that most of those microorganisms were present there as single spores.

Qualitative analysis of bacterial and fungal aerosols

The percentages of the identified groups of bacterial and fungal microorganisms in the air at the examined workplaces are presented in Figure 3. The above data include the measurements taken with the applied measuring instruments, i.e. the MAS impactor and the Andersen impactor. The performed qualitative analysis of the air samples collected on the premises of the power plant demonstra-ted a significant diversity of the microflora (Table 3). In total, both at various stages of the biomass co-combustion technological process and in the laboratory, 46 bacteria species belonging to 26 genera, and 35 fungi species belonging to 20 genera were identified, including 30 spe-



G+C – Gram-positive cocci / ziarniaki Gram-dodatnie; G+R – nonsporing Gram-positive rods / pałeczki Gram-dodatnie niezarodnikujące; G+B – Gram-positive bacilli / laseczki Gram-dodatnie; G–R – Gram-negative rods / pałeczki Gram-ujemne; MA – mesophilic actinomycetes / mezofilne promieniowce; TB – termophilic bacteria / termofilne bakterie; FY – yeasts and filamentous fungi / drożdże i grzyby nitkowate.

Fig. 3. Percentage distributions of microbial groups identified in the air at the workplaces at the power plant: a) technological line b) laboratory
Ryc. 3. Udział procentowy zidentyfikowanych grup mikroorganizmów bakteryjnych i grzybowych w powietrzu badanych stanowisk pracy w elektrociepłowni: a) linia technologiczna, b) laboratorium

cies of filamentous fungi and 5 species of yeasts. Despite significant differences in the concentration values for bacteria and fungi at the workplaces in the technological line and in the laboratory, the analysis demonstrated no differences in terms of the qualitative composition of the microbial aerosols between the workplaces in the technological line and the laboratory.

The analysis of percentages of the identified microorganisms showed that in the air microflora at all the examined positions, fungi from *Aspergillus* genus were predominant, including *A. fumigatus*, *A. niger*, *A. flavus* and *A. carbonarius* (33–95%). The second most numerous group consisted of Gram-negative bacilli (1–32%) from *Citrobacter* (2 species), *Pseudomonas* (4 species) and *Rahnella* (1 species) genera. Moreover, Gram-positive bacilli, Gram-positive nonsporing rods, Gram-positive cocci, mesophilic actinomycetes and termophilic bacteria were isolated from the air samples; their percentage in relation to the total microflora was, respectively for the technological line and the laboratory: 9.3% and 7.2%, 7.5% and 4.3%, 4.5% and 5.6%, 5.1% and 2% and 4.6% and 3%.

In the microflora of the outside air, filamentous fungi were predominant (62%). The qualitative comparison of fungal microorganisms present in the air at the workplaces at the power plant with those present in the outside air demonstrated the presence of analogous strains. Among the bacteria, Gram-positive nonsporing rods and Gram-positive cocci were predominant. The percentages of those bacteria groups in relation to the total microflora were 12% and 11%, respectively. No thermophilic microorganisms were isolated from the outside air samples.

Table 3. Microorganisms identified in the air at the power plant and in biomass samples
Tabela 3. Mikroorganizmy występujące w powietrzu elektrociepłowni oraz w próbkach biomasy

Air samples Próbki powietrza	Biomass samples Próbki biomasy
Gram-positive cocci / Ziarenkowce Gram-dodatnie	
Aerococcus viridians, *Kocuria varians*, *Micrococcus* spp., *Staphylococcus* (*S. aureus**, *S. cohnii* ssp. *cohnii*, *S. epidermidis, S. xylosus*)	*Micrococcus* spp.
Nonsporing Gram-positive rods / Pałeczki Gram-dodatnie niezarodnikujące	
Arthrobacter spp., *Brevibacterium* (*B. linens*, B.* spp.), *Corynebacterium* (*C. auris, C. xerosis, C.* spp.*), *Cellulomonas* spp., *Microbacterium* spp.	*Corynebacterium* spp.*, *Cellulomonas* spp., *Microbacterium* spp.
Gram-positive bacilli / Laseczki Gram-dodatnie	
Bacillus (*B. cereus*, *B. circulans*, *B. licheniformis*, *B. megaterium*, *B. mycoides*, *B. subtilis**, *B.* spp.), *Paenibacillus* spp.	*Bacillus* (*B. circulans*, *B. licheniformis*, *B. megaterium*, *B.* spp.)
Gram-negative rods / Pałeczki Gram-ujemne	
Aeromonas hydrophila, *Burkholderia cepacia*, *Citrobacter* (*C. younga, C.* spp.), *Enterobacter cloacae**, *Pantoea* spp., *Pseudomonas* (*P. aeruginosa*, P. luteola, P. oryzihabitans*, *P.* spp.), *Ochrobactrum anthropi, Rahnella aquatilis*	*Citrobacter* (*C. younga, C.* spp.), *Enterobacter cloacae**, *Pseudomonas luteola, Rahnella aquatilis*
Mesophilic actinomycetes / Mezofilne promieniowce	
Actinomyces spp., *Nocardia* spp., *Rhodococcus* spp., *Streptomyces (S. albus, S. griseus, S.* spp.*)	*Nocardia* spp., *Rhodococcus* spp., *Streptomyces* spp.*
Thermophilic bacteria / Termofilne bakterie	
Streptomyces (S. thermophilus, S. spp.*), *Geobacillus stearothermophilus, Bacillus licheniformis*	*Streptomyces* (*S.thermophilus, S.* spp.*), *Geobacillus stearothermophilus*
Filamentous fungi / Grzyby nitkowate	
Absidia spp., *Acremonium strictum*, *Alternaria* (*A. alternata, A.* spp.), *Aspergillus* (*A. carbonarius A. flavus*, *A. fumigatus**, *A. niger*, *A. sydowii*, *A.* spp.), *Cladosporium* spp., *Emericella nidulans, Epiccocum nigrum, Eurotium herbarum, Fusarium* (*F. solani*, *F.* spp.), *Geomyces pannorum, Geotrichum* spp., *Mucor* (*M. plumbeus, M. racemosus, M.* spp.), *Penicillium* (*P. glabrum*, *P. olsoni*, *P. sclerotiorum*, *P. viridicatum*, *P.* spp.) *Phoma herbarum, Rhizopus* (*R. stolnifer*, *R.* spp.), *Trichoderma* (*T. koningii, T. viride*)	*Aspergillus* (*A. flavus*, *A. fumigatus**), *Mucor plumbeus, Penicillium* spp., *Rhizopus stolnifer*
Yeasts / Drożdże	
Candida spp., *Cryptococcus laurentii*, *Rhodotorula* (*R. rubra, R.* spp.), *Sporobolomyces roseus*	*Cryptococcus laurentii*, *Sporobolomyces roseus*

* Microorganisms classified by the Ordinance of the Minister of Health of 22 April 2005 into group 2 according to the level of the risk of infection (32) / Mikroorganizmy zakwalifikowane do grupy 2. zagrożenia według Rozporządzenia Ministra Zdrowia z dnia 22 kwietnia 2005 r. (32).

Quantitative and qualitative analysis of biomass samples

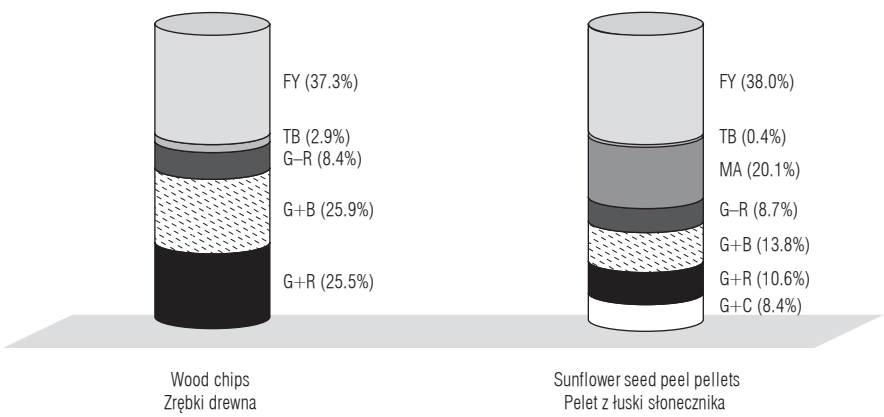
The concentration values for individual groups of microorganisms in the samples of biomass (sunflower seed peel pellets and wood chips) are presented in Table 4. The concentrations of live microflora in the analysed samples were at the following levels: for the bacteria, from 9.5×10^4 cfu/g to 4.1×10^6 cfu/g, and for the fungi, from 3.3×10^4 cfu/g to 2.1×10^6 cfu/g. The highest concentrations of bacteria and fungi were recorded in the samples of wood chips (a mean concentration of, respectively, 3.5×10^6 cfu/g and 2.1×10^6 cfu/g).

The detailed results of the qualitative analysis of the bacterial and fungal microflora isolated from the biomass samples are presented in Table 3 and in Figure 4. In total, 19 bacteria species belonging to 14 genera, and 7 fungi species, including 5 filamentous fungi species and 2 yeast species, were isolated from the biomass samples. In both examined types of biomass, the predominant group of microorganisms was composed of the filamentous fungi and yeasts (37–38%) from respectively, *Aspergillus* and *Cryptococcus* genera. In the wood chip samples, the second most numerous group of microorganisms were Gram-positive bacilli belonging to *Bacillus* genus, Gram-positive nonsporing rods from *Cellulomonas* genus, and Gram-negative rods belonging to *Citrobacter* genus, whose percentages in relation to the total microflora were, respectively, 25.9%, 25.5% and 8.4%. The percentage of termophilic bacteria (from *Streptomyces* and *Geobacillus* genera) was at the level of 2.9% in relation to the total microflora. In the wood chip samples, neither Gram-positive cocci nor mesophilic actinomycetes were found. Among the microorganisms isolated from the sunflower seed peel pellets, the second most numerous group of microorganisms was mesophilic actinomycetes belonging to *Streptomyces* genus (20.1%). In the examined samples of that type of biomass, Gram-positive nonsporing rods from *Cellulomonas* genus, and bacilli from *Bacillus* genus were also numerous (10.6% and 13.8%, respectively). Moreover,

Table 4. Bacterial and fungal concentration in biomass samples
Tabela 4. Stężenia bakterii i grzybów w próbkach biomasy

Type of sample Rodzaj próbki	Samples Próbki [n]	Bacteria [×10⁵ cfu/g] Bakterie [×10⁵ jtk/g]			Fungi [×10⁵ cfu/g] Grzyby [×10⁵ jtk/g]		
		M	SD	range zakres	M	SD	range zakres
Wood chips / Zrębki drewna	4	35.31	5.40	29.91–40.71	21.03	0.44	20.57–21.43
Sunflower seed peel pellets / Pelety z łusek słonecznika	4	0.99	0.04	0.95–1.02	0.61	0.28	0.33–0.89

Abbreviations as in Table 2 / Objaśnienia jak w tabeli 2.



Abbreviations as in Fig. 3 / Objaśnienia jak w ryc. 3.

Fig. 4. Percentage distributions of microbial groups identified in biomass samples: wood chips and sunflower seed peel pellets
Ryc. 4. Udział procentowy zidentyfikowanych grup mikroorganizmów bakteryjnych i grzybowych w poszczególnych próbkach biomasy: zrębkach drewna i peletach z łusek słonecznika

Gram-negative rods belonging to *Pseudomonas* genus, Gram-positive cocci from *Micrococcus* genus, and thermophilic bacteria from *Streptomyces* genus were isolated from the pellet samples; their percentages in relation to the total microflora were, respectively, 8.7%, 8.4% and 0.4%. A comparison of the microorganisms present in the biomass samples with those isolated from the air revealed the presence of analogous strains.

Analysis of the air microclimate parameters

The averaged values of the temperature and relative humidity of the air at the examined sampling stations are presented in Table 5. The correlation analysis revealed no statistically significant relationships between the air microclimate parameters and the concentrations of microorganisms.

bioaerosol (exceeding 1.0×10^4 cfu/m^3) were recorded at the stations associated with reloading, screening and transporting biomass on belt conveyors to silos.

As mentioned above, unlike in the case of other working environments, there are only few studies concerning the levels and the distribution of airborne microorganisms at power plants. The test results published so far describe the exposure to biological agents mainly among the workers employed during the harvesting and processing of crops for biofuel production (18), and at workplaces associated with wood processing (7–13,19–22). The quantitative analysis of bioaerosols conducted by Madsen (6) at five Danish biomass combusting power plants revealed that the concentrations of bacterial and fungal aerosols at those workplaces were at the levels of, 2.3×10^4 cfu/m^3 and 1.7×10^5 cfu/m^3,

Table 5. Air temperature and relative humidity values measured at the power plant
Tabela 5. Temperatura i wilgotność względna powietrza na stanowiskach pomiarowych w elektrociepłowni

Sampling station Stanowisko pomiarowe	Samples Próbki [n]	Temperature Temperatura [°C]			Relative humidity Wilgotność względna [%]		
		M	SD	range zakres	M	SD*	range zakres
E1	6	17.65	7.28	11.18–24.00	54.55	16.72	39.00–69.00
E2	6	19.25	5.45	14.30–24.80	51.03	10.51	40.01–59.97
E3	6	18.15	8.42	10.40–25.60	52.55	13.43	39.24–65.00
E4	6	16.37	8.22	8.91–23.63	61.50	12.76	49.13–72.98
E5	6	17.01	8.24	9.21–24.22	62.01	13.44	47.81–73.96
E6	6	15.12	10.62	5.84–24.30	59.02	5.23	52.04–63.00
E7	6	15.91	0.14	15.88–16.00	57.75	6.13	49.10–61.91
E8	6	22.65	1.03	21.80–23.93	49.59	14.46	36.19–61.95
E9	6	20.95	1.25	19.50–22.10	52.02	11.66	39.89–61.79
E10	6	16.67	7.33	10.20–23.00	59.75	14.75	45.59–72.63

Abbreviations as in Tables 1 and 2 / Objaśnienia jak w tabelach 1 i 2.

DISCUSSION

The presented results are among the few attempts at carrying out an assessment of the workers exposure to biological agents during biomass handling for energy purposes. Microbiological analysis of the air, conducted at the workplaces at the power plant, showed that the main occupational hazard for workers is the organic dust generated during the processing and the use of various types of biomass. The highest concentrations of

respectively. The control measurements carried out in Poland at three power plants by Gołofit-Szymczak and Ławniczek-Wałczyk (23) showed that the concentration values for bacteria and fungi at those workplaces ranged from 2.3×10^2 cfu/m^3 to 2.9×10^4 cfu/m^3, and from 1.9×10^2 cfu/m^3 to 6.3×10^4 cfu/m^3, respectively. Considering the above mentioned data, it may be concluded that the concentration values determined at the examined workplaces fell within the range of values "normally" observed at such facilities.

When interpreting the results of measurements taken at the workplaces in the technological line, reference values for the concentrations of bacteria and fungi in the "industrial settings polluted with organic dust", recommended by the Biological Agents' Expert Group of the Interdepartmental Commission for Maximum Admissible Concentrations and Intensities for Agents Harmful to Health in the Work Environment at the Central Institute for Labour Protection – National Research Institute (24), were used due to significant emissions of organic dust. The quantitative analysis of bioaerosols at all the examined workplaces, conducted using the MAS impactor revealed that the obtained mean concentration values for bacteria and fungi did not exceed the recommended reference values, which equal for bacteria 1.0×10^5 cfu/m^3, and for fungi 5.0×10^4 cfu/m^3. The results obtained at the E1 and E7 sampling stations through the use of the Andersen impactor indicate that the recommended reference values were only exceeded for the fungi.

The examined laboratory rooms can barely be considered typical locations contaminated with organic dust, neither are they public utility facilities in the traditional sense, since they are only accessible to a limited number of personnel, and the work performed there may be associated with massive emissions of biological agents to the air. Due to their nature, these are rooms where hygiene requirements should be strictly observed, and their purity in terms of dust and microbiological contamination should be high. So far, no reference values for such facilities have been developed in Poland. However, when applying the suggested admissible concentrations of microorganisms for the workplaces at public utility facilities (24), it may be concluded that at the stations where the measurements were taken using the Andersen impactor, the recommended reference values were exceeded for both fungi (5.0×10^3 cfu/m^3) and Gram-negative rods (2.0×10^2 cfu/m^3).

The analysis of the collected data revealed that the concentration values for live microorganisms, obtained from the measurements taken using the Andersen impactor at the workplaces in the technological line, were higher than those noted using the MAS impactor, however, the differences were not statistically significant. This is due to the fact that the Andersen impactor's efficiency of capture is high, amounting to 50% for the particles of diameters within the range of 0.6–7.2 μm (25), while the MAS-100 Eco impactor efficiency of capture is at the level of 60% for the particles of diameters larger than 2 μm (26). The conducted analysis shows that both types of impactors used in this research may be useful for the quantitative and qualitative analysis of the bacteria and fungi occurring at the examined working environment. However, the Andersen impactor is able to capture and separate live particles of bioaerosol much more precisely than the MAS impactor.

The qualitative analysis of the microbial aerosol, conducted at the examined workplaces, allowed for carrying out an assessment of potential health hazards caused by its impact on humans. The most numerous group of microorganisms within the examined environment included filamentous fungi and yeasts. Their occurrence was associated with the presence of large amounts of organic matter of plant origin, which (with appropriate humidity and temperature) is a natural space of development for numerous mould species. Among those, the predominant ones were fungi of *Aspergillus* genus, including *A. fumigatus*, *A. niger*, *A. flavus* and *A. carbonarius*. Their presence in high concentrations may result in the appearance of allergic reactions and various non-specific adverse health outcomes. All the above-mentioned species have the ability (under certain conditions) to produce harmful mycotoxins, including aflatoxin (produced by *A. flavus* strains), which exhibits clinically confirmed carcinogenicity, as well as ochratoxin A (*A. niger*, *A. carbonarius*) and others. All of them exhibit significant allergenic effects. Moreover, *Aspergillus fumigatus* is characterised by significant infectious effects (it can induce e.g. pulmonary aspergillosis) (11,17,27,28). *Penicillum viridicatum* species are also able to produce mycotoxins (certain strains produce ochratoxin A). Other species of that genus, identified in the collected samples (*P. olsonii* and *P. sclerotiorum*) are fungi commonly occurring in the environment (mainly in the soil) (11,17). A numerous group among the identified fungi were natural plant pathogens represented by *Eurotium*, *Epicoccum*, *Fusarium* and *Rhizopus* genera, as well as fungi which may indicate an ongoing biological decay of plants (i.e. the genera *Mucor* and *Absidia*). In addition, in the examined samples, some fungi were present which exist naturally in the soil (*Phoma* and *Geomyces* genera) (17). It should be emphasised that fungal spores may exist in rooms for a long time, e.g. on equipment, construction materials and structural elements of buildings, while maintaining the ability to survive for as many as over a dozen years. Immunoreactive particles of fungal origin also include (1–3)-β-D-glucans which are water-insoluble cell wall components. Exposure to (1–3)-β-D-glucans in the working environment may result in the occur-

rence, in the exposed persons, of airway inflammation, hypersensitivity pneumonitis (HP) and probably numerous other non-specific reactions (8).

The second most numerous group of isolated microorganisms were Gram-negative rods of *Citrobacter*, *Pseudomonas* and *Rahnella* genera. The presence of that group of bacteria, usually susceptible to desiccation, may indicate dampness of the biomass to be combusted. Those bacteria occur most frequently in the water, soil and on decaying plants. All the identified species are opportunistic human pathogens and may pose a hazard to persons with an impaired immune system (3,7,8,20,21). Particular attention should be paid to the blue pus rod (*P. aeruginosa*) which can induce severe lung and urinary tract infections. That bacterium is known for its frequent resistance to antibiotics. Like all Gram-negative bacteria, it can release from its cells bacterial endotoxins – pro-inflammatory acting lipopolysaccharides which in high concentrations have an adverse effect on the functioning of the respiratory system (8,29). Moreover, *P. aeruginosa* is able to produce a potent exotoxin A as well as enterotoxins. Some potential for production of potent enterotoxins is also exhibited by *Aeromonas hydrophila*. The species of *Pantoea* genus are among those components of organic dust which pose the greatest hazard to the respiratory system of workers exposed to the inhalation, since they exhibit significant allergenic properties which may induce hypersensitivity pneumonitis and asthma. Their endotoxic properties are the main cause of febrile reactions frequently observed in workers exposed to the inhalation of organic dust (3,7,8,20,21).

A major hazard to workers at power plants where biomass is combusted may also be posed by the thermophilic and mesophilic actinomycetes identified in the samples of air and plant raw material. Inhalation of spores of that group of bacteria may result in the occurrence of HP and other disorders of the respiratory system (3,7).

The performed microbiological analysis of the biomass samples demonstrated that biomaterials used for co-combustion with coal are a source of bacterial and fungal aerosol released to the air. The concentrations of microorganisms in those materials exceeded 3.5×10^6 cfu/g for bacteria and 2.1×10^6 cfu/g for fungi. The quantitative analysis of microorganisms in the two types of biomass indicates that the pellets have higher resistance to biodegradation than the traditionally used wood chips. The qualitative analysis of the biomass samples indicated a species composition similar to that observed in the air. The predominant group was composed of fungi, including *Aspergillus fumigatus*, representing over 37% of the total microflora. The obtained concentration values for microorganisms were similar to the ranges observed by other researchers in that type of samples (5,23,30),

The use of the 6-stage Andersen impactor for the testing allowed for obtaining information on the particle size distribution of the air microflora. The distributions obtained revealed that, at the examined workplaces, microorganisms were present in the air in a form of bacteria as bacterial or bacteria-and-dust aggregates (formed mainly by Gram-negative rods of *Citrobacter*, *Pseudomonas* and *Rahnella* genera), and fungi mostly as single spores and small aggregates (formed mainly by moulds from *Aspergillus* genus). Based on the data on the particle size distributions, it may be concluded that the largest "load" of microorganisms may reach the following areas of the human respiratory system: for bacteria – throat, trachea and the main bronchi; for fungi – pulmonary bronchi and the terminal bronchioles. Interactions between the aerosol particles and the human body cells are, to a large extent, dependent on the location of their deposition. Particles deposited in the trachea area may cause asthmatic reactions, while those settled in the lower respiratory tract sections may induce allergic inflammation (31).

As a result of the measurements taken, in the air at the examined workplaces the presence of the following was found: 8 bacteria species (*B. subtilis*, *B. linens*, *Corynebacterium* spp., *E. cloacae*, *P. aeruginosa*, *S. aureus*, a thermophilic species of *Streptomyces* spp. and a mesophilic species of *Streptomyces* spp.), and 1 fungal species (*A. fumigatus*), classified into risk group 2 according to the Ordinance of the Minister of Health of 22 April 2005 (32). This means that workers at the power plant may be exposed to direct contact with biological agents of occupational hazard, which are potentially pathogenic.

It should be emphasised that the extent and type of contamination at the workplaces associated with biomass handling depend on both the type of the raw material used and the conditions of transport and storage (2,5,30,33). The obtained results indicate that storage of densificated biomass at unsheltered storage sites may lead to the loss of its energy value due to an increase in the moisture content of the raw material, and to the development of bacterial and fungal microflora, which in turn leads to an increased exposure of workers to harmful biological agents.

CONCLUSIONS

The results of this study indicate that both the workers employed in the technological line for biomass combustion and the laboratory workers are exposed to bioaerosols containing potentially pathogenic bacteria and fungi.

Based on the taken measurements of biological agents at various stages of biomass co-combustion at the examined power plant, it may be concluded that the concentrations of bioaerosols do not exceed the suggested admissible values for that type of working environment. However, particular attention needs to be paid to the laboratory rooms in which most rigorous hygienic requirements should be met.

The qualitative analysis of the air microflora at the designated workplaces indicated the presence of bacterial and fungal microorganisms which, according to the Ordinance of the Minister of Health, are classified into risk group 2. In persons with an impaired immune system, direct contact with those microorganisms may cause adverse health effects. For instance, contact with fungi and actinomycetes may be a cause of the occurrence of allergic reactions.

The observed contamination with biological agents should provide the basis for taking appropriate (under the existing legislation) preventive measures aimed at the protection of workers' health against harmful biological agents.

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