

Microbial contamination level of air in animal waste utilization plants

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Abstract

Objective. The aim of this research was evaluation of microbial contamination of air within and in the vicinity of animal waste disposal plants.

Materials and method. Air samples were analyzed to determine total bacterial and fungal counts as well as microbial species composition. Measurements of climate conditions (temperature, humidity, air motion) and total dust concentration were also performed.

Results. Total numbers of bacteria and fungi surpassed the threshold limit values for production halls. The most abundant bacteria detected were those consisting of physiological microflora of animal dermis and mucosa. Fungal species composition proved to be most differentiated in the air beyond the plant area. *Aspergillus versicolor*, a pathogenic and allergenic filamentous fungus, was isolated only inside the rendering plant processing hall.

Conclusions. The measurement results showed a low sanitary-hygienic state of air in the plant processing halls and substantial air pollution in its immediate vicinity.

Key words

animal waste utilization plants, microbial contamination

INTRODUCTION

Safe processing of animal by-products in utilization plants eliminates epidemiological hazards, protects the natural environment against pathogens and facilitates obtaining meat and bone meal (MBM), as well as rendered animal fat. Hence, the entire utilization process of inedible animal products that starts with the fallen stock collection and ends with incineration, has an important sanitary-hygienic, pro-ecological and economic function. However, at the same time, the operation of rendering plants can be considered hazardous to the environment. Such facilities, despite veterinary supervision and regulations of environmental protection agencies, can create a serious danger for sanitary-epidemiological well-being. The processed waste may be a source and also a growth medium for microbial development. One of the main routes of bacteria and fungi transmission is through the air, but this is not a natural environment for bacterial development. The presence of microbes in the air is only temporary and associated with the effect of other factors, e.g. air motion, dust, temperature, humidity and an outdoor air quality which is a source of microflora [1].

OBJECTIVE

The objective of the study was to assess the microbial contamination of air within and in the immediate vicinity of a plant for animal waste disposal.

MATERIALS AND METHOD

The study was conducted in a processing plant which, just like 60% of disposal plants registered, belongs to small utilization facilities. Three-month plant productivity is 500–600 tons of waste. The common wastes disposed of in the plant are animal cadavers or slaughter offals (ca.150 tons/month) or animal by-products (ABPs). The ABPs are classified into three categories: 1) – 52.2%, 2) – 21.8% and 3) – 26.0%, and according to the EU regulations, animal by-products are not segregated; therefore, they constitute Category I, i.e. specified risk material. The majority of tissue processed come from meat industry (92.4%), from slaughterhouses and butchers, the poultry industry (3.4%) and finally, the dairy industry (2.2%).

Technological process for animal waste management in the plant comprises the following stages: collection of animal waste, transportation, waste weighing, cleaning, washing, disinfection of transportation means after unloading, animal waste crushing into particles smaller than 5 cm in the dissection room, sterilization of offal in destructors, separation of MBM from the fat, storage of animal meal in a store hall and fat in containers, and finally, the sale of the end products.

In accordance with the law, the sterilization of raw material should be carried out at 133°C, under 3 bar pressure for 20 minutes, and the material crushed down to a uniform particle size of 5 cm. In the storage hall, meat and bone meal is stored in 500–700 kg bags (BIG-BAG) placed on pallets, and fat in specially designed airtight containers.

To completely avoid cross-contamination from raw animal by-products to sterilized derivative products, the architectural design of the plant provides a physical separation between the 'clean' and 'dirty' area. The plant also has its own waste water biotreatment system in which all process water is treated before being discharged.

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Assessment of air microbial contamination included the determination of total bacterial and fungal counts and their identification. The study was conducted in the spring-summer season (May-July) and autumn-winter period (October-December) in 10 successive series. Air samples were collected inside the plant and at 4 points 100 m from its boundary, in the 'dirty' areas: 1) in the dissection room, in the 'clean' areas – 2) destructor hall, 3) 10 m from the waste water treatment plant, and 4) beyond the plant – 100 m from the plant boundary.

Evaluation of air microbial contamination was performed in accordance with the Polish Standard PN-EN 13098 (2007) [2] using the aspiration procedure, an air sampling pump GilAir 5 (Sensidyne, Inc., Clearwater, USA). Airborne microorganisms were collected on sterile polycarbonate filters in filter cassettes (37 mm, 0.8 mm; Millipore Ireland B.V., Carrigtwohill, Ireland) with the pump calibrated to an airflow of 2.0 l/min. The measuring sets were calibrated before each sampling procedure using a Gillibrator 2 calibrator (Sensidyne, Inc., Clearwater, USA). The equipment was placed at the height of 1.5 meters above the floor. The sampling took 4 hours and were collected in 2 repetitions.

The total number of bacteria and microscopic fungi were estimated by the dilution plate method, with surface inoculation onto suitable growth media: tryptic soy agar (TSA) with 5% sheep blood to determine total bacterial count, and malt extract agar medium (MEA) for total fungal numbers. The air samples inoculated onto the TSA medium were incubated for 7 days at 37°C (1 day), 22°C (3 days) and 4°C (3 days), and onto the MEA medium at 30°C (4 days) and 25°C (3 days).

After completion of the incubation, the microbe colonies grown on each medium were macro- and microscopically assayed. The number of morphological types was then established and a concentration in 1 m³ of the air sample estimated and expressed in colony forming units [CFU/m³]. The bacterial isolates were identified using the microscopic method and biochemical assays, i.e. API tests (bioMérieux, Warsaw, Poland).

The concomitant measurements of climate conditions were taken and included temperature-humidity indices measured with a thermohygrometer (RT811E, Technik, Warsaw, Poland), and air motion with an anemometer (A-1200M1, OBRAIUP, Lodz, Poland). Total dust concentration was estimated with a gravimetric method via an aspirator (224-PCEX8, SKC, Dorset, England). Airborne particulate mass were collected on a glass fibre filter (Whatman GF) with 37 mm diameter (Whatman Int. Ltd., Kent, England) with the pump calibrated to an airflow of 4.0 l/min.

The obtained results were analyzed statistically and characterized with arithmetical mean (M) and the range of values determined. Statistical analysis was conducted according to the nonparametric Mann-Whitney test with statistical software SAS v. 9.1.

RESULTS

The study was carried out in the spring-summer season when the mean air temperature reached 17.7°C, with the peak value of 23.5°C (Tab.1), and in the autumn-winter period with the outdoor temperature ranging between 1.5°C – 7.5°C. The observed differences were statistically significant $p < 0.1$. In the sampling site 100m from the plant boundary,

Table 1. Macroclimate and dustiness around the plant

Sampling locations	Temperature [°C]	Humidity [%]	Air motion [m/s]	Dust concentration [mg/m ³]
100 m of the plant	13.4 (1.5–23.1)	74.0 (51.5–88.0)	2.6 A (0.1–23.0)	2.1 (0.5–4.8)
Summer	20.5 A (17.7–23.1)	68.6 (51.5–82.6)	0.4 (0.1–0.7)	2.9 (1.6–4.8)
Winter	6.3 A (1.5–7.5)	79.0 (75.6–88.0)	4.8 (0.2–23.0)	1.2 (0.5–4.3)
10 m of the sewage treatment facility	14.0 (4.2–23.5)	71.1 (52.7–83.3)	1.5 A (0–13.5)	2.2 (0–3.4)
Summer	21.6 B (18.3–23.5)	64.1 A (52.7–73.4)	0.1 a (0–0.1)	1.3 a (0–3.1)
Winter	6.4 B (4.2–7.3)	78.0 A (76.1–83.3)	2.9 a (0.1–13.5)	3.0 a (1.5–3.4)

a – values denoted with the same letters in columns are different at $p < 0.05$
A, B – values denoted with the same letters in columns are different at $p < 0.01$

Table 2. Average concentration of bacteria and fungi in outdoor environment around the plant

Sampling locations	Total bacteria [CFU/m ³]	Total fungi [CFU/m ³]
100 m of the plant	4.7×10 ⁶ (5.2×10 ² – 4.3×10 ⁷)	2.2×10 ⁵ a (4.0×10 ¹ – 5.6×10 ⁵)
Summer	9.3×10 ⁶ A (8.3×10 ⁴ – 4.3×10 ⁷)	2.9×10 ⁵ (1.7×10 ⁴ – 5.6×10 ⁵)
Winter	6.0×10 ³ A (5.2×10 ² – 2.5×10 ⁴)	2.5×10 ⁴ (4.0×10 ¹ – 5.0×10 ⁴)
10 m of the sewage treatment facility	2.0×10 ⁷ (5.2×10 ² – 1.5×10 ⁸)	1.4×10 ⁴ a (0 – 5.8×10 ⁴)
Summer	4.0×10 ⁷ (8.3×10 ³ – 1.5×10 ⁸)	2.0×10 ⁴ (0 – 5.8×10 ⁴)
Winter	2.5×10 ⁵ (5.2×10 ² – 8.3×10 ⁵)	2.8×10 ³ (0 – 8.3×10 ³)

a – values denoted with the same letters in columns are different at $p < 0.05$
A – values denoted with the same letters in columns are different at $p < 0.01$

Table 3. Microclimate and dustiness inside the plant

Sampling locations	Temperature [°C]	Humidity [%]	Air motion [m/s]	Dust concentration [mg/m ³]
Dissection room	16.1 (2.4–31.6)	64.0 (32.0–88.8)	0.3 A (0.1–0.6)	2.5 (0.7 – 4.7)
Summer	26.0 A (20.7–31.6)	48.8 A (32.0–61.3)	0.4 a (0.2–0.6)	2.0 (0.7 – 4.7)
Winter	6.2 A (2.4–7.4)	79.1 A (76.1–88.8)	0.2 a (0.1 – 0.3)	3.1 (3.0 – 3.3)
Destructor hall	22.5 (16.1–30.3)	50.3 (34.0–61.1)	0.1 A (0 – 0.1)	2.5 (1.8 – 4.4)
Summer	27.1 B (24.1–30.3)	46.1 (39.0–52.7)	0.1 (0 – 0.1)	2.8 a (1.8 – 4.4)
Winter	17.9 B (16.1–22.7)	54.6 (34.0–61.1)	0.1 (0.1 – 0.1)	2.2 a (2.1 – 2.8)

a – values denoted with the same letters in columns are different at $p < 0.05$
A, B – values denoted with the same letters in columns are different at $p < 0.01$

significant differences were also noted while comparing the values of summer and winter measurements of humidity and air motion. The higher temperature values, with a maximum of 31.6°C, were determined in the closed spaces (Tab. 3). The temperature in the dissection room was close to those noted within the sewage treatment plant.

Table 4. Average concentration of bacteria and fungi in indoor air of the plant

Sampling locations	Total bacteria [CFU/m ³]	Total fungi [CFU/m ³]
Dissection room	1.9×10 ⁶ (3.2×10 ² – 1.0×10 ⁷)	2.0×10 ⁴ (0 – 3.3×10 ⁴)
Summer	3.7×10 ⁶ A (3.3×10 ⁵ – 1.0×10 ⁷)	2.2×10 ⁴ (0 – 3.3×10 ⁴)
Winter	7.2×10 ³ A (3.2×10 ² – 3.3×10 ⁴)	1.7×10 ⁴ (8.0×10 ¹ – 3.3×10 ⁴)
Destructor hall	1.2×10 ⁶ (3.6×10 ² – 1.1×10 ⁹)	3.6×10 ⁴ (0 – 1.7×10 ⁵)
Summer	2.3×10 ⁶ a (5.8×10 ⁴ – 1.1×10 ⁹)	4.8×10 ⁴ (0 – 1.7×10 ⁵)
Winter	8.7×10 ³ a (3.6×10 ² – 2.5×10 ⁴)	3.8×10 ³ (0 – 7.6×10 ³)

a – values denoted with the same letters in columns are different at p<0.05

A – values denoted with the same letters in columns are different at p<0.01

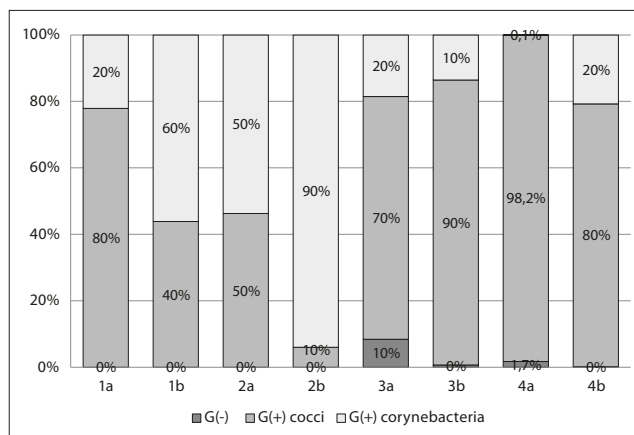
Beyond the plant area, increased air motion was recorded, especially in the autumn-winter season when mean air velocity increased by 2.2 m/s (p<0.05), compared to the summer period. In the confined spaces, significantly higher (mean by 0.2 m/s) air velocity values were established in the dissection room (p<0.01). High air dust concentration was determined in the destructor hall in summer and within the sewage biotreatment device area in winter. In the destructor hall in summer, a significantly higher concentration of total dust was found, compared to the season with lower temperatures (p<0.05).

Total bacterial and fungal numbers showed a difference dependent on the sampling location and season (Tab. 2, 4). In the summer period in all of the studied sites, the concentration of microorganisms, both bacteria and fungi, was higher compared with the autumn-winter season. Irrespective of season, a higher microbial contamination rate was detected in the air of the destructor hall. The highest numbers of bacteria were determined in the dissection room (p<0.01) and destructor hall (p<0.05) during the higher air temperature period.

Smaller fluctuations were observed when comparing the fungal contamination levels, with the highest fungal load detected in the atmospheric air 100 m from the plant boundary (p<0.05). At the other sites, fungal bioaerosol concentration was at a similar level, and declined slightly in the autumn-winter season.

The percentage of each microbial group changed along with the sampling location and season of the year (Fig. 1). Gram-negative bacteria was not detected in the air. However, within the sewage biotreatment unit area in the winter period, Gram-positive corynebacteria dominated, which accounted for as much as 94% of all isolated bacteria. At the same site in summer, Gram-positive cocci shared an increase of up to 46%.

In the production halls, Gram-positive cocci constituted most of the recovered bacteria and their share grew during the higher temperature period. Only in the dissection room, the Gram-positive cocci count in the summer season was lower by 13%, compared to the winter period. The in-plant air assessment showed the presence of Gram-negative bacteria, which were most abundant in the dissection room during the summer period where they made up 8% of all bacteria identified in the air.

**Figure 1.** Percentage of each bacterial group in the examined air. Sampling locations: 100 m from the plant – 1a) summer, 1b) winter; 10 m from the sewage treatment facility – 2a) summer, 2b) winter; dissection room – 3a) summer, 3b) winter; destructor hall – 4a) summer, 4b) winter

From the collected air samples, 12 different taxa were isolated, within which 14 species were determined (Tab. 5). The most ubiquitous were Gram-positive bacteria – cocci and corynebacteria (4 and 5 taxa, respectively).

Bacteria from the genera *Micrococcus*, *Brevibacterium* and species *Staphylococcus xylosus* were recovered from the air

Table 5. Bacterial species and genera identified in air samples collected on the plant

Genera/species	100 m of the plant	10 m of the sewage treatment facility	Dissection room	Destructor hall
G(-) bacteria				
<i>Neisseria meningitidis</i> (I)	-	-	-	+
<i>Neisseria subflava</i>	-	-	-	+
<i>Haemophilus parainfluenzae</i> (I)	-	-	+	+
<i>Sphingomonas paucimobilis</i>	-	-	+	+
G(+) cocci				
<i>Staphylococcus</i> spp.	+	+	-	+
<i>Staph. xylosus</i>	+	+	+	+
<i>Staph. epidermidis</i>	+	-	+	+
<i>Staph. saprophyticus</i>	-	-	+	+
<i>Staph. sanguinis</i>	-	+	+	+
<i>Kocuria varians</i>	+	+	-	-
<i>Kocuria kristinae</i>	+	+	-	-
<i>Micrococcus</i> spp.	+	+	+	+
<i>Micrococcus luteus</i>	+	+	-	-
<i>Aerococcus viridans</i>	+	+	-	+
Gram(+) corynebacteria				
<i>Cellulomonas</i> spp.	+	-	+	+
<i>Corynebacterium</i> spp. (AL)	-	+	+	+
<i>Corynebacterium strictum</i>	-	+	+	-
<i>Corynebacterium urealiticum</i>	+	+	-	-
<i>Arthrobacter</i> spp.	-	+	+	+
<i>Mycobacterium</i> spp. (I, AL)	-	+	-	+
<i>Brevibacterium</i> spp. (AL)	+	+	+	+

(+) - present; (-) absent; Activity: AL- allergenic, I- infectious

Table 6. Fungal species and genera identified in air samples collected on the plant

Genera/species	100 m of the plant	10 m of the sewage treatment facility	Dissection room	Destructor hall
<i>Penicillium</i> spp. (AL, T)	+	+	+	-
<i>Penicillium chrysogenum</i>	+	-	-	-
<i>Aspergillus versicolor</i> (AL,T)	-	-	+	-
<i>Aspergillus clavatus</i> (AL)	-	+	-	-
<i>Fusarium</i> sp (T)	+	-	-	-
<i>Cladosporium</i> spp. (AL)	+	+	+	-
<i>Cladosporium macrocarpum</i>	+	+	+	+
<i>Ulocladium chartarum</i>	+	-	-	+
<i>Acremonium</i> spp.	-	-	+	-
<i>Acremonium chorticola</i>	+	-	-	-
<i>Acremonium strictum</i>	+	+	-	+
<i>Botryotrichum</i> spp.	-	-	+	-
<i>Phomopsis</i> spp.	+	+	+	-
Yeasts	+	-	+	+

+ - present; - absent; Activity: AL – allergenic; T – toxic

samples collected from all studied locations. Two species of bacteria belonging to the genus *Neisseria* were isolated only in the air of the destructor hall. *Staphylococcus saprophyticus*, *Haemophilus parainfluenzae* and *Sphingomonas paucimobilis* were present exclusively in the air in the plant halls, while *Kocuria* sp. and *Micrococcus luteus*, *Aerococcus viridans* and *Corynebacterium urealiticum* were detected only in the atmospheric air.

As for fungi, 9 taxa were isolated – 7 species (Tab. 6). Atmospheric air was found to be most differentiated with regards to fungal species. The following were isolated only in the air samples collected outside the plant: *Penicillium chrysogenum*, *Aspergillus clavatus*, *Acremonium chorticola* and the genus *Fusarium*. Only in the dissection room air, the presence of *Aspergillus versicolor*, as well as fungi from the genus *Acremonium* and *Botryotrichum* was determined. *Cladosporium macrocarpum* were recovered from all the sampling locations.,

DISCUSSION

The animal processing plant under investigation is an environment in which the presence of bacteria and fungi is inevitable but, as the study indicates, occupational exposure of the workers to the bioaerosol level determined may result in adverse health effects because of the very poor air quality. The measured concentrations exceeded the threshold values set for the production halls [3]. In both the 'dirty' and 'clean' areas, i.e. the dissection room and destructor hall, the bioaerosol concentration surpassed the threshold value of occupational exposure limits defined by Malmros [3], as well as the hygiene standards presented by Dutkiewicz et al. [4] for an organic dust-contaminated workplace.

Destructors are sterilizers; hence, the action of destruction should not be a source of contamination. A high rate of indoor air microbial contamination in the destructor hall was likely to have been caused by the dustiness from the

close proximity of the processed material storage hall and the secondary development of bacteria on the stored material. The level of determined contaminants was also affected by the season, primarily by humidity and air temperature. In the period of higher temperatures, doubling of the bacteria population occurred in the dissection room, compared to the winter season. Similar high levels of bacterial contamination were also established in farm buildings [5, 6], but were much lower in the sewage treatment facility area [7, 8].

From the bio-ecological standpoint, a weighty problem is that air pollutants emitted into the atmosphere by plants for dead animal utilization also deteriorate the local air quality. Monitoring around the processing plant showed that the bacterial and fungal contamination rate at the sewage treatment unit persisted at a high level, the same as in the production halls. The air samples taken 100 m from the plant boundary indicated only slightly lower concentrations. At this sampling location, total bacterial and fungal concentrations reached 4.7×10^6 CFU/m³ and 2.2×10^5 CFU/m³ that compared to the standards set for atmospheric air PN-89/Z-04111/02 [9] and PN-89/Z-04111/03 [10], evidenced strong biological pollution of the air.

The air microflora composition in processing plants relies largely on the state of the raw material, its sanitary-hygienic state, hygiene storage conditions and the level of the plant technology. Most microorganisms introduced with animal material, as well as those developing on the stored organic matter, are saprophytic bacteria. Therefore, bacteria from the animal physiological flora of dermis and mucous membranes dominated, i.e. those from the genus *Staphylococcus*, *Micrococcus* and *Corynebacterium*. Ellis et al., [11] report that during the early stage of meat spoilage, mainly Gram-positive bacteria develop, usually represented by micrococci followed by lactic acid bacteria. A specific bacterium identified in the production hall air was *Staphylococcus saprophyticus* – a leading cause of human urinary tract infection. However, this species was not isolated from the atmospheric air samples, which implies that raw material undergoing the utilization process may be its major source.

Of the Gram-negative bacteria isolated solely in the air of the production halls, two species of pathogenic bacteria producing severe symptoms of infections identified – *Neisseria meningitidis* and *Haemophilus parainfluenzae*. Most probably their presence was not caused by plant activity because, as indicated, humans are the natural reservoir of these bacteria.

Among the in-plant air samples, bacteria from the genus *Corynebacterium*, *Mycobacterium* and *Brevibacterium* were also identified and classified into risk group 2, according to the Ordinance of the Ministry of Health of 20 April 2005 on hazardous biological agents [12]. These bacteria may therefore pose a health threat to the workers.

Another important agents increasing occupational risk of workers in the examined plant were moulds, and the fungal aerosol load determined largely exceeded the level determined even in the farm buildings and the waste water treatment facility [13, 14]. Species composition of the identified fungi was more differentiated in the outside air, but only the air samples collected in the confined space showed the presence of *Aspergillus versicolor* which has well known toxic and allergic properties. Fungi occurring in the air of the halls contaminated with enzootic substances, fungi from genus *Penicillium*, *Aspergillus* and *Cladosporium* identified

in the plant, may induce allergic reactions, asthma, and conjunctivitis, or contribute to the development of mycoses, especially systemic mycoses. Due to their small size, large numbers of the spores released are easily deposited in the lower airways.

Moulds are not only a source of mycotoxins but also volatile organic compounds (among others, aldehydes, ketones) which produce systemic poisonings as well as immunotoxic glucans (1,3-beta-D-glucans), glucose polymers being a component of the fungal cell wall. These molecules are responsible for the development of organic dust toxic syndrome (ODTS) and chronic bronchitis. The most common response to exposure to fungal metabolites is immunosuppression, irritation of mucous membrane of the upper airways, leading to chronic inflammation of the respiratory tract [15, 16].

Summing up, the utilization process of animal by-products, regardless of their source, poses a potential threat to human health and the environment. The threat reduction should involve the implementation of appropriate control measures concerning the proper location of processing plants, and essentially the complete hermetization of the utilization process in order to prevent the spread of potentially hazardous bioaerosol.

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