

© Copyright by Polish Academy of Sciences and Institute of Environmental Engineering of the Polish Academy of Sciences, Zabrze, Poland 2019

# Microbiological hazards in closed facilities at sewage treatment plants

## Michał Polus\*, Zbigniew Mucha

Cracow University of Technology, Poland

\*Corresponding author's e-mail: mpolus@vistula.wis.pk.edu.pl

Keywords: bioaerosols, sewage treatment plant, microbiological hazards.

Abstract: In this work microbiological air pollution at several commune sewage treatment plants (capacity up to 15,000 PE) was investigated. The bioreactors in all plants had a covered construction. The air samples were taken indoors as well as outdoors (both on the windward and leeward side) during different seasons. The samples were collected using the collision method. The presence of indicator organisms in the samples was determined according to the Polish Standards. Identification of individual indicators was performed on solid selective--differentiating substrates. To verify the presence of bacteria from Salmonella, Shigella, coliforms and enterococci species, the colonies observed on the MacConkey substrate were then sifted onto SS and Endo substrates. At all facilities (with one exception) the average CFU for the total number of bacteria and fungi did not exceed 1000/m<sup>3</sup>, which is the limit set by the Polish Standards for a pollution-free atmospheric air. Bacteria and fungi concentrations, observed at windward and leeward sides of all plants, were relatively low (<100 CFU/m<sup>3</sup> and <1000 CFU/m<sup>3</sup>, respectively) and comparable. A sewage collection point had only a slight impact on the bioaerosol emission. The concentration of microorganisms in the immediate vicinity of covered reactors (aeration chambers) was rather low and remained below the limits sets by the Polish Standards at three facilities. The CFU of individual indicators, measured in rooms accessible for the personnel, was comparable to the CFU in technological rooms. However some indicators, e.g. a number of Actinomycetes, were significantly higher and reached >100 CFU/m<sup>3</sup>, which means significant air pollution. Similarly, the CFU of hemolytic bacteria had nonzero values. The only place where higher concentrations of bioaerosol were found was the centrifuge room, where digested sludge was dewatered. The number of fungi stayed below the limits there, but the amount of heterotrophic and hemolytic bacteria exceeded the limits and reached the values of ~10000 CFU/m<sup>3</sup> and 800 CFU/m<sup>3</sup>, respectively; it means that the personnel working in this area is exposed to microbiological agents.

### Introduction

In recent years, construction of many sewage treatment plants has been financed by the EU funds. These are continuous flow activated sludge sewage treatment plants, SBR (Sequencing Batch Reactor) treatment plants as well as different types of membrane biological reactors MBR (Mikosz and Mucha 2014, Mucha and Kurbiel-Swatek 2016).

Selection of a proper treatment process and its configuration should take into account broadly defined sustainability criteria, including: minimization of a negative environmental impact of applied solutions, a rational use of natural resources and a social acceptance of the proposed solutions (Jóźwiakowski et al. 2016).

Emission of bioaerosols into the air seems to be an inevitable consequence of operation of a biological sewage treatment plant. The bioaerosols may contain bacteria, fungi and other microorganisms, including human and animal parasites and viruses (Korzeniewska 2011, Teixeira et al. 2013). Formation of bioaerosols is primarily associated with aeration in biological rectors. The bacteria concentration in the bioaerosol is about 10-9 times lower while for the filamentous fungi it is about 10<sup>-6</sup> lower than in sewage (Bauer et al. 2002). Another source of bioaerosols are sewage pump stations, mechanical treatment units, sludge stabilization chambers and devices for dewatering and thickening of sewage sludge (Han et al. 2018). Typically, air samples collected at the sewage treatment plant contain predominately species of conidial fungi of the genera Aspergillus, Penicillium and Alternaria, as well as Cladosporium and Trichoderma; yeasts and yeast-like fungi such as Candida, Cryptococcus, Geotrichum and Rhodotorula usually do not exceed 30%. The genera Salmonella, Shigella, Escherichia, Klebsiella, Serratia, Enterobacter and Proteus are most commonly found in municipal sewage and therefore detected in bioaerosols (Grisoli et al. 2009, Kacprzak et al. 2005, Korzeniewska et al. 2009, Ławniczek-Wałczyk et al. 2018). In recent years, there have been numerous reports on the presence of Archaea in the biocoenosis of activated sludge (mainly the genera Halobacterium and Methanolobus), hence their presence in bioaerosols from sewage treatment plants

can be expected, however the risk to their exposure remains unknown (Anielak et al. 2015).

Inhalation of air containing fungal spores can cause a series of bronchial and pulmonary diseases of allergic origin, alveolitis, asthma or rhinitis (Croft et al. 2016). The main pathogens responsible for these diseases are representatives of the genera *Aspergillus, Candida, Fusarium, Trichosporon* and *Scedosporium*. It can be assumed that about 10% of the described species of conidial fungi pose a threat to human health (Enoch et al. 2017, Richardson and Lass-Flörl 2008).

Emission of bioaerosols varies with the size of the treatment plant, and may be local (no environmental pressure) or have a broader environmental impact. The risk varies with the treatment plant capacity, the type of treatment technology used, atmospheric conditions and work/operation schedule (Gotkowska-Płachta et al. 2013, Korzeniewska 2011). For example, at many small and medium plants, the rooms where subsequent stages of sewage treatment are carried out are integrated with the personnel facilities in one building.

An occupational risk related to work at the sewage treatment plant is difficult to assess since some of bioaerosol components (bacterial endotoxins, allergens, viruses) are undetectable by standard microbiological methods or the measured concentrations of microorganisms are underestimated. The methods based on cultures on selective--differentiating substrates, although commonly used, have a number of disadvantages such as: poor reproducibility, selection of certain species against the others and inability to detect microorganisms nonculturable on microbiological media (Douwes et al. 2003). The methods completely ignore other toxic or allergenic components of bioaerosols, e.g. dead cells. In turn, other methods (e.g. flow cytometry, DAPI staining, FISH or combinations of these techniques) are currently too complicated and expensive to be used on a routine basis (Chen and Li 2005, Lange et al. 1997).

Since most of works that have been carried out on emission of bioaerosols by sewage treatment plants focused on big objects located in larger urban agglomerations (Gotkowska-Płachta et al. 2013, Teixeira et al. 2013) and there is not much data available on small and medium installations (below 15,000 PE and 2000  $m^3/d$ ) the authors focused on evaluation of the health

risk resulting from emission of bioaerosols to enclosed spaces of such sewage treatment plants. All microbiological indicators provided for in Polish legislation were included.

### Materials and methods

#### Characteristics of sewage treatment plants

Four sewage treatment plants, located in the south of Poland, with a daily capacity below 2000 m3/d and PE below 15,000 were selected for the study. Two of them worked as flow--through reactors with activated sludge (facilities I and II), two other (facilities III and IV) were SBR type reactors with a cyclic operation mode. Two facilities (II and III) had biological reactors encased by a reinforced concrete ceiling with inspection openings. In facility I, the biological reactor was placed in a hall with gravitational and mechanical ventilation while in facility IV a closed "tower" type of steel biological reactor was used; the reactor was elevated 10 m above the ground level. At three wastewater treatment plants (I, II, III), technological units were located in the same buildings as personnel facilities and control rooms. One facility (IV) had closed steel reactors adjacent to the separate building, with no connection to the personnel facilities.

Biological treatment is preceded by a preliminary mechanical treatment. It constitutes a compact device (screens and grit chamber) at facilities I, II and IV or a fine screen and a vertical grit chamber at facility III. All these units have been housed in closed and ventilated rooms. Each of the treatment plants employs an installation for supplementary chemical phosphorus precipitation with iron sulphate.

Sludge produced at four plants undergoes aerobic stabilization in separate chambers and then is dewatered in filter presses (I, II, III) or in a centrifuge (IV).

The sewage treatment plants accept mainly municipal sewage and waste from slurry tankers (except for facility II). Only facility III additionally treats wastewater from a tannery, while facility II from local slaughterhouses. Only one of the sewage treatment plants (II) does not meet the effluent quality standards due to hydraulic and pollution overloads and is planned for expansion.

The sewage treatment plants characteristic is given in Tab. 1.

Parameter	Facility					
Farameter	1 11		III	IV		
Capacity Q/PE	800/6160	450/3400	433/5060	1250/14950		
Loading	low-loading	overloaded	low-loading	low-loading		
Type of sewage	ype of sewage municipal municipal + slurry tankers + slaughterhouses		municipal + slurry tankers + tannery	municipal + slaughterhouse + slurry tankers		
Mechanical treatment	compact device (screens and grit chamber) indoors		fine screen indoors; vertical aerated grit chamber outdoors, open basin	vertical screen on a channel + aerated grit chamber, indoors		
Biological treatment	iological treatment multi-phase multiphase activated activated sludge, sludge reactors covered with a ceiling		SBR covered with a ceiling	steel enclosed SBR		
Chemical treatment	precipitation with iron sulphate (PIX)					
Sludge processing	Aerobic digestion, press			Aerobic digestion, centrifuge		

#### Table 1. Characteristic of the studied sewage treatment plants

### Material sampling

The air samples were taken indoors (reactor, mechanical treatment unit, sludge dewatering station, personnel facilities/control rooms) and outdoors, near the fence (on the windward and leeward side). The samples from biological reactor covers were also collected at facilities II and III. The presence of the following indicator organisms was analyzed in the samples (as provided by the Polish Standard):

- total number of mesophilic heterotrophic bacteria,
- total number of filamentous fungi and yeast yeast-like fungi,
- hemolytic staphylococci type  $\alpha$  and  $\beta$ ,
- Pseudomonas fluorescens,
- Actinomycetes,
- bacteria from the Enterobacteriaceae family.

Air samples of a volume of 25–100 liters were collected by collision method using a microbiological air sampler MAS 100 NT (Merck, Germany) on Petri dishes with a diameter of 90 mm. The sampler was equipped with a 300×0.6 mm perforated cover. The samples were collected in 2–3 replicas at different atmospheric conditions and in different seasons (November 2016 – April 2018). Simultaneously with the air sampling, the temperature and relative humidity were measured at each stand. The substrates and incubation conditions are summarized in Tab. 2. All substrates were provided by BTL Ltd., Poland. The medium for growing the *Actinomycetes* was supplemented with ampicillin (50  $\mu$ g/ml) to block a potential bacterial growth.

The values of individual microbiological indicators were expressed as the corrected number of colony forming units (CFU) in a cubic meter of air. The CFU correction was made based on the assumption that an increase in the number of microorganisms in the air results in a higher probability of several cells entering through the same hole into the probe. The conversion was made based on the formula (Macher 1989):

$$CFU = N \cdot \left[\frac{1.075}{1.052 - \frac{N}{300}}\right]^{0.483}$$

where: N – number of colonies.

The total number of filamentous fungi was determined on two different substrates, assuming the higher value as the more accurate one. In the case of MacConkey's colony growth, the material was also screened onto SS and Endo media (to verify the presence of bacteria from the genus *Salmonella*, *Shigella*, coliforms and enterococci bacteria).

### Results

The mean values of the CFU, measured on windward and leeward sides of all objects, did not exceed the limits set by the Polish Standard (<1000 CFU/m<sup>3</sup> and <3000 CFU/m<sup>3</sup>) for the total number of mesophilic heterotrophic bacteria and fungi in pollution-free air (Tab. 3, Fig. 1). There are no significant differences in the bacteria and fungi concentrations on windward and leeward sides, except for facility IV (152 vs 28); the facility has a sewage collection point located on its leeward side. The lowest concentrations of bacteria were found in the vicinity of facility III, which is located in a vast, open space, away from residential areas.

The number of the CFU of hemolytic bacteria and *Pseudomonas* rods determined outdoor was usually zero. Occasionally, the CFU was reported at the level of 1-10 CFU/m<sup>3</sup> so below 25/50 CFU/m<sup>3</sup>, which stands for moderate air pollution, according to the Polish Standards.

The concentrations of heterotrophic bacteria and fungi in the immediate vicinity of biological reactors covered with ceilings (II and III) and located in the closed room (I) are usually higher than the background concentrations (next to the treatment plant), however they do not exceed the allowable values for work rooms (Tab. 4) or for atmospheric air (Tab. 5).

Also bacteria and fungi concentrations in the personnel and dispatch rooms exceeded the background values at all plants; they were comparable to the ones observed in technological rooms (reactor, mechanical treatment and dewatering) but stayed below the limit values for indoors. Assuming that employees spend a significant part of their workday in these rooms, the results were confronted with the recommendations regarding a respirable fraction of microorganisms at residential premises (Tab. 4) and not a single violation was reported. The highest concentration of bacteria was found in the personnel room of facility I (up to 2205 CFU/m<sup>3</sup>) despite its immediate vicinity to the biological reactor hall (up to 300 CFU/m<sup>3</sup>). The low bioaerosol concentration in the biological reactor hall can be probably attributed to good ventilation of the hall, which cannot be said about the personnel room. Some

Indicator	Medium Temp. [°C]		Time [days]
total number of bacteria	nutrient agar	37	1–2
total number of fungi	Czapek's agar 26		5
total number of fungi	wort agar	26	5
hemolytic staphylococci	Blood 5% agar	37	1
P. fluorescens	King B agar	28	5–7
Actinomycetes	Pochon's agar	28	5–7
Enterobacteriaceae	MacConkey's agar	37	1
coliforms	ENDO agar	37	1
Salmonella & Shigella	SS agar	37	1–2

Table 2. Culturing conditions of the microbial growth

## Microbiological hazards in closed facilities at sewage treatment plants

#### **Table 3.** Values of CFU/m³ at individual locations on the premises of the studied facilities; the CFU range and the mean/median value (in brackets)

		bacteria	fungi	hemolytic staphylococci	Pseudomonas fluorescens	Actinomycetes
Facility I	Windward	5–93 <b>(34 / 20)</b>	200–1100 (628 / 585)	0–7 (1 / 0)	0–3 (1 / 0)	0–35 (7 / 0)
	Leeward	20–107 (43 / 25)	273–1180 (634 / 450)	0–7 (2 / 0)	0–0 (0 / 0)	0–10 <b>(3 / 0)</b>
	Mechanical treatment (compact screen/grit chamber)	73–475 <b>(282 / 290)</b>	290–540 ( <b>432 / 467)</b>	3–13 <b>(7 / 6)</b>	0–7 (3 / 3)	7–35 (15 / 10)
	Reactor hall	100–300 <b>(185 / 127)</b>	255–1220 <b>(874 / 1147)</b>	0–30 (19 / 27)	0–20 (5 / 0)	7–113 <b>(50 / 33)</b>
	Sludge dewatering (press)	305–793 <b>(496 / 420)</b>	345–1147 <b>(748 / 753)</b>	7–47 (28 / 33)	0–13 (5 / 3)	0–33 (14 / 7)
	Personnel facility/control room	120–2205 <b>(818 / 533)</b>	367–950 (642 / 673)	5–43 (17 / 13)	0–0 (0 / 0)	7–43 ( <b>32 / 40</b> )
	Windward	0–260 <b>(88 / 5)</b>	40–473 <b>(271 / 300)</b>	0–20 (8 / 5)	0–0 (0 / 0)	5–13 <b>(8 / 7)</b>
	Leeward	0–167 <b>(59 / 10)</b>	175–720 <b>(401 / 307)</b>	0–0 (0 / 0)	0–0 (0 / 0)	0–40 (13 / 0)
Facility II	Mechanical treatment (open screens hall)	15–80 <b>(52 / 60)</b>	50–400 ( <b>210 / 280)</b>	0–13 (6 / 5)	0–0 (0 / 0)	0–7 (2 / 0)
	Reactors covered with a ceiling	25–307 <b>(213 / 307)</b>	60–873 <b>(598 / 860)</b>	7–20 (14 / 15)	0–0 (0 / 0)	10–153 (77 / 67)
	Sludge dewatering (press)	50–273 <b>(141 / 100)</b>	65–1220 (673 / 627)	0–25 (8 / 0)	0–0 (0 / 0)	0–60 (29 / 27)
	Personnel facility/control room	147–935 <b>(443 / 247)</b>	265–773 <b>(544 / 593)</b>	7–60 (31 / 27)	0–0 (0 / 0)	20–340 (136 / 47)
	Windward	0–20 (8 / 5)	300–1740 (816 / 407)	0–7 (2 / 0)	0–5 (2 / 0)	0–5 (2 / 0)
	Leeward	0–20 (9 / 7)	240–1153 <b>(569 / 313)</b>	0–0 (0 / 0)	0–0 (0 / 0)	0–10 <b>(3 / 0)</b>
ty III	Mechanical treatment (screens)	113–800 <b>(371 / 200)</b>	310–1100 (606 / 407)	7–13 (10 / 10)	0–5 (2 / 0)	0–10 <b>(6 / 7)</b>
Facilit	Reactors covered with a celling	10–13 <b>(12 / 13)</b>	345–1967 <b>(908 / 413)</b>	0–0 (0 / 0)	0–7 (2 / 0)	0–20 (8 / 5)
	Sludge dewatering (press)	295–440 (367 / 368)	375–1313 <b>(844 / 844)</b>	53–60 (57 / 57)	0–5 (2 / 2)	5–20 (12 / 12)
	Personnel facility/control room	173–555 <b>(303 / 180)</b>	90–1047 ( <b>421 / 128)</b>	0–0 (0 / 0)	0–0 (0 / 0)	0–27 (16 / 20)
Facility IV	Windward	13–45 <b>(28 / 27)</b>	333–380 ( <b>357 / 357)</b>	0–13 (7 / 7)	0–0 (0 / 0)	0–13 <b>(6 / 5)</b>
	Leeward	5–360 (152 / 135)	193–633 (370 / 284)	0–20 (11 / 13)	0–0 (0 / 0)	0–20 (6 / 4)
	Mechanical treatment (grit chamber)	22–2920 (772 / 367)	7–620 (236 / 180)	0–13 (6 / 5)	0–7 (1 / 0)	0–20 (6 / 0)
	Blowers room next to reactors and a sludge dewatering site	0–780 <b>(284 / 124)</b>	67–387 <b>(220 / 213)</b>	0–47 (15 / 6)	0–0 (0 / 0)	0–5 (2 / 0)
	Sludge dewatering (centrifuge)	4515–9460 (6940 / 7050)	90–753 <b>(521 / 750)</b>	245–827 (487 / 460)	0–0 (0 / 0)	0–67 (17 / 5)
	Personnel facility/control room	47–1150 (334 / 233)	20–418 <b>(214 / 213)</b>	0–20 (5 / 0)	0–0 (0 / 0)	0–13 <b>(5 / 5)</b>



M. Polus, Z. Mucha

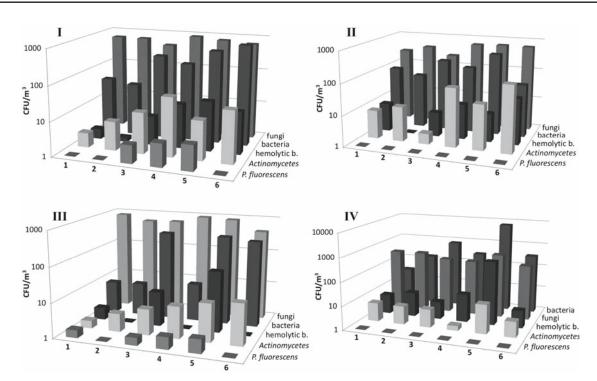


Fig. 1. Concentrations of bioaerosols at investigated facilities (I–IV). 1 – windward; 2 – leeward; 3 – mechanical treatment site; 4 – bioreactors; 5 – sludge dewatering station; 6 – control room

Table 4. Propositions of acceptable concentrations of microorganisms in enclosed spaces (PN-EN 13098:2007)

	Acceptable concentration [CFU/m <sup>3</sup> ]				
	work rooms contam	inated with organic dust	residential premises and public utilities		
	total number	respirable fraction	total number	respirable fraction	
Mesophilic bacteria	100 000	50 000	5 000	2 500	
Gram-negative bacteria	20 000	10 000	200	100	
Actinomycetes	20 000	10 000	200	100	
Fungi	50 000	25 000	5 000	2 500	

Table 5. Evaluation of atmospheric air pollution with bacteria and fungi (PN89 Z-04111/02, PN89 Z-04111/03)

	hastaria	fungi	hemolytic bacteria		Pseudomonas	Actinomycostop
	bacteria	fungi	α	β	fluorescens	Actinomycetes
Pollution-free	<1000	<3000	0	0	0	<10
Moderately polluted	1000–3000	3000–5000	<25	<50	<50	10–100
Heavily polluted	>3000	>5000	>25	>50	>50	>100

indicators (actinomycetes or hemolytic bacteria) were found even higher in these rooms, e.g. at facilities I and II, where there is a direct passage between the personnel rooms and technological halls (bioreactor, press, screen/grit chamber). The number of filamentous fungi in personnel/control rooms was comparable to the values found in technological rooms, except for facility IV, where the personnel room is housed in a separate building.

Although higher CFU values could also be expected there due to an intensive bioaerosol formation but not such a thing was observed. At facilities I and II, with a full or partial enclosure of reactors, the CFU for bacteria and fungi was comparable to the readings in other rooms. The CFU measured on the reactor plane (non-roofed facility III) was similar to CFU on windward and leeward sites of the sewage treatment plant.

A high emission of bioaerosol by the sludge dewatering unit was observed at facility IV, with particularly high CFU values for bacteria and hemolytic staphylococci. These concentrations are strongly related to the centrifuge operation; after several hours of its inactivity the CFU drops to the values comparable with the background values (Fig. 2). Similar tests were conducted by the authors at two other sewage treatment plants using the same and different type of centrifuge and the reported concentrations were comparable to those found in press rooms. The higher concentrations observed in the studied facility were probably caused by poor tightness of the equipment.





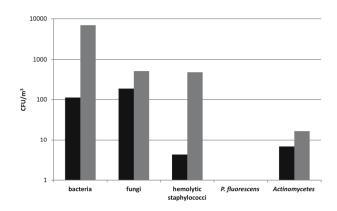


Fig. 2. Concentration of bioaerosol in a centrifuge room (IV) measured during its operation (gray bars) and a few hours after turning off (black bars)

### Discussion

Microbial concentrations measured indoors of four sewage treatment plants are relatively low, comparing to the data reported by other authors. The CFU/m<sup>3</sup> values of bacteria in technological and personnel rooms stay in the range of  $1\cdot10^2$ – $3.9\cdot10^4$  (mean ~  $7.6\cdot10^3$ , median ~  $4.8\cdot10^3$ ), and for filamentous fungi:  $1\cdot10^2$ – $1.4\cdot10^4$  (mean ~  $5.3\cdot10^3$ , median  $1.7\cdot10^3$ ) (Bauer et al. 2002, Gotkowska-Płachta et al. 2013, Han et al. 2018, Korzeniewska 2011, Ławniczek-Wałczyk et al. 2018).

The lack of up-to-date and unified methods for microbiological evaluation of air pollution in enclosed spaces (indoors) makes data interpretation difficult. The Polish Standards are largely outdated (1989) and limited only to methodological recommendations (sampling methods, definition of microbiological indicators, recommended culture media) that often do not keep up with current technologies. According to microbiological indicators (PN89 Z-04111/02; PN89 Z-04111/03), air categories refer only to atmospheric air and include three possible options: pollution-free, moderately polluted and heavily polluted air (Tab. 5).

Therefore, the CFU values measured indoors, in places occasionally available for the personnel, require some further interpretation. In Poland, the recommendations proposed by Krzysztofik (1992) for selected closed rooms have been used; according to these references the acceptable numbers of CFU/m<sup>3</sup> of heterotrophic bacteria, hemolytic bacteria and fungi for residential premises are: ~ 1500 CFU/m<sup>3</sup>, ~ 50 CFU/m<sup>3</sup> and ~ 200 CFU/m<sup>3</sup>, respectively. Assuming these values, the personnel facilities of all four treatment plants meet the recommendations for heterotrophic and hemolytic bacteria. However, the CFU of filamentous fungi is higher (421–642 CFU/m<sup>3</sup>) at facilities I–III. At facility IV, where the control room is located outside the reactor building, sludge stabilization unit and the centrifuge room, the number of fungi slightly exceeds the limit (214 CFU/m<sup>3</sup>).

The recommendations of the Team of Experts for Biological Agents of Interdepartmental Committee for NDS and NDN (PN-EN 13098: 2007) are more liberal (Tab. 4) and all indoor measurements for both personnel and technological rooms meet their criteria.

The lack of appropriate regulations in Polish legislature prompts to compare the results with recommendations

effective in other countries. The American Industrial Hygiene Association assumes that there is no safe level of pathogenic microorganisms in the air, therefore CFU/m<sup>3</sup> for pathogenic bacteria and fungi should be zero (AIHA 1995). Assuming that a number of pathogens in the air is equal to a number of hemolytic bacteria, the average numbers of CFU/m<sup>3</sup> measured in the control rooms of all four facilities (0-31 CFU/m<sup>3</sup>) meet the AIHA criteria for clean air. On the other hand, according to the recommendations of the Commission of the European Communities, small and very small air pollution in closed residential and non-industrial premises requires that the values of CFU/m3 of bacteria and fungi do not exceed 100-500 and 100-200, respectively (CEC 1993). Hence, the air pollution with bacteria and fungi is moderate, according to these regulations. Only the sludge dewatering room with a centrifuge (facility IV) shows a high or even very high bacterial pollution (>2000 CFU/m3). The recommendations of the World Health Organization are similar to the one presented by AIHA, i.e. a clean air should not contain any pathogenic or toxicogenic fungi such as Aspergillus fumigatus (WHO 1998). Since species identification is not always possible, the WHO considers the fungi concentration of 150 CFU/m<sup>3</sup> as the acceptable one (with a dominance of Cladosporium sp. and other phytopathologies up to 500 CFU/m<sup>3</sup>). Looking at these recommendations, slight exceedances of fungi in the air were observed in all rooms.

Apart from the discrepancies in the recommendations presented here, it is important to keep in mind that various methods of air sampling and various techniques of culturing microorganisms were used in these analyses. All this makes the interpretation of the results rather difficult and not straightforward.

### Conclusions

- Emission of bioaerosols to the environment from municipal sewage treatment plants with a closed type construction is small.
- Exposure to air next to enclosed bioreactors, even during aeration, does not pose a significant threat to the plant personnel.
- Most personnel rooms meet the recommendations for residential areas in terms of the number of bacteria.
- Slightly higher values of microbiological indicators are found in sludge dewatering stations with filter presses.

- M. Polus, Z. Mucha
- A centrifuge for stabilized sludge dewatering emitted high concentrations of bioaerosols (single case).
- The bacteria Salmonella, Shigella, coliforms or enterococci were only occasionally found during the study.
- Perhaps it would be reasonable to adopt more strict recommendations for hemolytic bacteria, as they are a measure of pathogenic microorganisms; some countries do not allow their presence indoors (e.g. American Industrial Hygiene Association).
- Construction of closed (enclosed) and therefore more expensive sewage treatment plants can be carried out in special protection areas, e.g. located close to residential buildings.

### Acknowledgement

Publication supported by the Polish Ministry of Science and Higher Education as part of the program of activities disseminating science from the project "Organization of the First International Science Conference - Ecological and Environmental Engineering", 26-29 June 2018, Kraków.

### References

- AIHA (1995). Biosafety Reference Manual 2nd Edition, American Industrial Hygiene Association Biosafety Committee ISBN 978-0-932627-65-0.
- Anielak, A.M., Beńko, P., Bochnia, T., Łuszczek, B., Polus, M. & Żaba, T. (2015). The effectiveness of muncipal sewage treatment with Archaea in sequence biological reactors and Bardenpho systems, Przemysł Chemiczny, 95, 2, pp. 314-319. (in Polish)
- Bauer, H., Fuerhacker, M., Zibuschka, F., Schmid, H. & Puxbaum, H. (2002). Bacteria and fungi in aerosols generated by two different types of wastewater treatment plants, Water Research, 36, 16, pp. 3965-3970.
- CEC (1993). Biological particles in indoor environment, Commission of the European Communities, EUR14988EN, Luxemburg.
- Chen, P.S. & Li, C.S. (2005). Bioaerosol characterization by flow cytometry with fluorochrome, Journal of Environmental Monitoring, 7, 10, pp. 950-959.
- Croft, C.A., Culibrk, L., Moore, M.M. & Tebbutt, S.J. (2016). Interactions of Aspergillus fumigatus conidia with airway epithelial cells: a critical review, Frontiers in Microbiology, 7, 7, p. 472.
- Douwes, J., Thorne, P., Pearce, N. & Heederik, D. (2003). Bioaerosol health effects and exposure assessment: progress and prospects, The Annals of Occupational Hygiene, 47, 3, pp. 187–200.
- Enoch, D.A., Yang, H., Aliyu, S.H. & Micallef, C. (2017). The changing epidemiology of invasive fungal infections, Methods in Molecular Biology, 1508, pp. 17-65.
- Gotkowska-Płachta, A., Filipkowska, Z., Korzeniewska, E., Janczukowicz, W., Dixon, B., Gołaś, I. & Szwalgin, D. (2013). Airborne microorganisms emitted from wastewater treatment plant treating domestic wastewater and meat processing industry wastes, Clean - Soil, Air, Water, 41, 5, pp. 429-436.
- Grisoli, P., Rodolfi, M., Villani, S., Grignani, E., Cottica, D., Berri, A., Picco, A.M. & Dacarro, C. (2009). Assessment of airborne microorganism contamination in an industrial area characterized by an open composting facility and a wastewater treatment plant, Environmental Research, 109, pp. 2135–2142.
- Han, Y., Wang, Y., Li, L., Xu, G., Liu, J. & Yang, K. (2018). Bacterial population and chemicals in bioaerosols from indoor environment: Sludge dewatering houses in nine municipal wastewater

treatment plants, The Science of the Total Environment, 15, 618, pp. 469-478.

- Jóźwiakowski, K., Mucha, Z., Generowicz, A., Baran, S., Bielińska, J. & Wójcik, W. (2015). The use of multi-criteria analysis for selection of technology for a household WWTP compatible with sustainable development, Archives of Environmental Protection, 3, pp. 76-82.
- Kacprzak, M., Neczaj, E. & Okoniewska, E. (2005). The comparative mycological analysis of wastewater and sewage sludges from selected wastewater treatment plants, Desalination, 185, pp. 363-370.
- Korzeniewska, E., Filipkowska, Z., Gotkowska-Płachta, A., Janczukowicz, W., Dixon, B. & Czułowska, M. (2009). Determination of emitted airborne microorganisms from a BIO - PAK Wastewater Treatment Plant, Water Research, 43, pp. 2841-2851.
- Korzeniewska, E. (2011). Emission of bacteria and fungi in the air from wastewater treatment plants - a review, Frontiers in Bioscience (scholar edition), 1, 3, pp. 393–407.
- Krzysztofik, B. (1992). Air microbiology. Wydawnictwa Politechniki Warszawskiej, Warszawa 1992. (in Polish)
- Lange, J.L., Thorne, P.S. & Lynch, N. (1997). Application of flow cytometry and fluorescent in situ hybridization for assessment of exposures to airborne bacteria, Applied and Environmental Microbiology, 63, 4, pp. 1557-1563.
- Ławniczek-Wałczyk, A., Cyprowski, M., Gołofit-Szymczak, M., Wójcik-Fatla, A., Zając, V. & Górny, R.L. (2018). Assessment of fungal aerosol exposure at selected workplaces contaminated with organic dust of different origin, Medycyna Pracy, 69, 3, pp. 269-280. (in Polish)
- Macher, J.M. (1989). Positive-hole correction of multiple-jet impactors for collecting viable microorganism, American Industrial Hygiene Association Journal, 50, 11, pp. 561-568.
- Mikosz, J. & Mucha, Z. (2014). Validation of design assumptions for small wastewater treatment plant modernization in line with new interpretation of legal requirements, Ochrona Środowiska, 1, pp. 45-49.
- Mucha, Z. & Kurbiel-Swatek, K. (2016). Analysis of membrane reactors applications for municipal wastewater treatment plants in current operation and research experience, Przemysł Chemiczny, 95, 2, pp. 236–240. (in Polish)
- PN89 Z-04111/02. Determination of the number of bacteria in the atmospheric air by aspiration and sedimentation sampling. Polski Komitet Normalizacji Miar i Jakości, 1989. (in Polish)
- PN89 Z-04111/03. Determination of the number of microscopic fungi in the atmospheric air by aspiration and sedimentation sampling. Polski Komitet Normalizacji Miar i Jakości, 1989. (in Polish)
- PN-EN 13098:2007. Air at the workplace guidelines for suspended microorganisms and endotoxins measurements, Interdepartmental Committee for NDS and NCS for human health in the Workplace, 2007. (in Polish)
- Richardson, M. & Lass-Flörl, C. (2008). Changing epidemiology of systemic fungal infections, Clinical Microbiology and Infection, 14, pp. 5–24.
- Teixeira, J.V., Miranda, S., Monteiro, R.A., Lopes, F.V., Madureira, J., Silva, G.V., Pestana, N., Pinto, E., Vilar, V.J. & Boaventura, R.A. (2013). Assessment of indoor airborne contamination in a wastewater treatment plant, Environmental Monitoring and Assessment, 185, 1, pp. 59-72.
- WHO (1988). Indoor air quality: biological contaminants, ISBN 92 890 1122 X, World Health Organization report, 1990.
- Wlazło, A., Pastuszka, J.S. & Łudzeń-Izbińska, B. (2002). Evaluation of exposure to bacterial aerosol of workers of a small wastewater treatment plant, Medycyna Pracy, 53, 2, pp. 109-114. (in Polish)



### Zagrożenia mikrobiologiczne w zamkniętych obiektach oczyszczalni ścieków

Microbiological hazards in closed facilities at sewage treatment plants

Streszczenie: W pracy poddano obserwacji stopień zanieczyszczenia mikrobiologicznego w pomieszczeniach czterech oczyszczalni ścieków obsługujących poniżej 15000 RLM, posiadających obiekty socjalne i sterownie zintegrowane z obiektami technologicznymi. Ponieważ większość publikowanych dotąd prac skupiała się na dużych obiektach, autorzy starali się monitorować i oceniać stężenie mikroorganizmów w pomieszczeniach zamknietych małych obiektów. Próbki powietrza z poszczególnych pomieszczeń (reaktor, stacja mechanicznego oczyszczania, stacja odwadniania osadu, pomieszczenia socjalne/sterownie) oraz na zewnątrz obiektów pobierano metodą zderzeniową na podłoża mikrobiologiczne przewidziane przez Polską Normę. Oznaczano wszystkie wymagane wskaźniki mikrobiologiczne. We wszystkich obiektach (z jednym wyjątkiem) średnia liczba jtk/m<sup>3</sup> bakterii i grzybów nie przekraczała 1000/m<sup>3</sup>, a więc była poniżej limitu ustalonego w Polskich Normach dla czystego powietrza atmosferycznego. Stężenia bakterii i grzybów obserwowane na nawietrznych i zawietrznych stronach wszystkich oczyszczalni były stosunkowo niskie (<100 jtk/m<sup>3</sup> i <1000 jtk/m<sup>3</sup>). Punkt odbioru ścieków miał tylko niewielki wpływ na emisję bioaerozolu. Stężenie mikroorganizmów w bezpośrednim sasiedztwie reaktorów krytych (komór napowietrzania) było raczej niskie i pozostawało poniżej limitów określonych przez Polskie Normy. Wartość poszczególnych wskaźników, mierzona w pomieszczeniach dostępnych dla personelu, była porównywalna z jtk/m<sup>3</sup> w pomieszczeniach technologicznych. Jednak niektóre wskaźniki, np. liczba promieniowców były podwyższone i przekraczały 100 jtk/m³, co oznacza znaczne zanieczyszczenie powietrza. Podobnie liczba jtk/m<sup>3</sup> bakterii hemolitycznych była niezerowa. Jedynym miejscem, w którym znaleziono wyższe stężenia bioaerozolu, było pomieszczenie wirówki do odwadniania osadu. Liczba bakterii heterotroficznych i hemolitycznych osiągnęła wartości odpowiednio ~10000 jtk/m<sup>3</sup> i 800 jtk/m<sup>3</sup>. Oznacza to, że personel pracujący w tym obszarze jest narażony na działanie czynników mikrobiologicznych. Obecność bakterii z rodzajów Salmonella, Shigella, bakterii grupy coli czy enterokoków stwierdzano tylko sporadycznie.