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THE VARIABILITY OF THE CONCENTRATION OF BIOAEROSOLS ABOVE THE CHAMBERS OF BIOLOGICAL WASTEWATER TREATMENT

ZMIENNOŚĆ STĘŻENIA BIOAEROZOLI NAD KOMORAMI BIOLOGICZNEJ OCZYSZCZALNI ŚCIEKÓW

Abstract: The article presents the results of research over microorganisms (psychrophilic and mesophilic bacteria and microscopic fungi) found in wastewater in denitrification and nitrification chambers and specifies the proportion of these microorganisms in bioaerosol at various levels above wastewater level (20, 50 and 100 cm). In the denitrification chamber (anoxic) in 1 cm^3 of sewage there were on average $30.35 \cdot 10^6$ CFU of mesophilic bacteria, $72.88 \cdot 10^6$ CFU of psychrophilic bacteria, and $37.3 \cdot 10^5$ CFU of microscopic fungi. In the nitrification chamber, where the oxygen concentration ranged from 0.37 to 2.32 mg $O_2 \cdot dm^{-3}$ of wastewater, the number of microorganisms was lower. In 1 cm^3 of wastewater there were on average $20.2 \cdot 10^6$ CFU of mesophilic bacteria, $51.76 \cdot 10^6$ CFU of psychrophilic bacteria, and $15.22 \cdot 10^5$ CFU of microscopic fungi. In sewage bioaerosols above these chambers, higher numbers of psychrophilic bacteria than mesophilic ones and microscopic fungi were reported. At the same time differences in the number of microorganisms at different heights above the surface of wastewater could be observed in bioaerosol, as well as between the chambers of the bioreactor. It was found that most frequently the amount of microorganisms decreased with height. The percentage emission ratio (*ER*) of microorganisms in bioaerosols coming from wastewater accounted for only a fraction of a percent and ranged from $1.13 \cdot 10^{-8}$ % (microscopic fungi over the denitrification chamber) to $24.53 \cdot 10^{-9}$ % (psychrophilic bacteria over the denitrification chamber). It was found that the process of mixing, aeration of wastewater, have an effect on the emission of microorganisms.

Keywords: microbial ecology, pollution microbiology, microbe, microbe interactions, growth and survival bacteria

Introduction

Within the area of each wastewater treatment plant, there is a direct habitat and spread of different groups of microorganisms, including pathogens. Some examples of the habitat are: influent and effluent municipal wastewater, screenings, sand and sewage sludge. For a human, the most dangerous is a direct contact with wastewater. It is also worth remembering that at various stages of wastewater treatment many microorganisms are

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rising into the atmosphere, and thereby create harmful bioaerosols. Bioaerosols, or biological aerosols, are biological air pollution comprising mostly vegetative forms of bacteria, spores, sclerotia, fragments of mycelia, conidia, viruses, pollen and toxins [1-4]. These elements can pose a pathogenic danger to humans, animals and plants. The composition, size and concentration of particles constituting the bioaerosol are associated with the source of emission, a mechanism of spreading in the air, and environmental conditions prevailing in the environment of the emission source [5-8].

Sewage treatment plants emit microbial contamination in the form of bioaerosols to the atmosphere mainly from wastewater. Turbulence and intense flow of sewage, wastewater aeration and meteorological conditions (especially wind speed, temperature and humidity) are considered to be the factors conducive to the formation and emission of bioaerosols. Wastewater treatment technology, the type and quantity of aeration, the process of wastewater aeration and the type and concentration of microorganisms present in wastewater are essential in the release of bioaerosols [9-11].

The microorganisms can penetrate into the atmosphere from wastewater under favorable conditions if their concentration in wastewater exceeds 10^3 cells in 1 cm^3 [12, 13]. In typical municipal wastewater the value is repeatedly exceeded, so the areas near sewage treatment plants are most vulnerable to the adverse effects of bioaerosols. At the same time, all employees of a sewage treatment plant and people who live nearby are exposed to harmful bioaerosols. Biological aerosols containing potentially pathogenic microorganisms are transported by convection currents of the air and can be the source of numerous diseases occurring among humans and animals. In addition, if the environment to which microorganisms are issued, promotes the growth and survival of bioaerosols components, there may be a higher concentration of air pollution, and thus a real threat to human health [3, 14-17].

Aerosols which are released to the atmosphere contain numerous microorganisms, and are mainly formed during biological wastewater treatment with activated sludge. Therefore, high concentration of bioaerosols in ambient air is observed mainly over mixed and aerated chambers of bioreactors, where droplets are formed with a large variability of diameters, which directly affects the velocity of descent. Large droplets and splashes fall almost immediately on the surface of the liquid (treated wastewater), causing additional spattering of it. The droplets with diameters of 50 to 100 μm (the rate of descent of about 0.3 $m \cdot s^{-1}$) and smaller come into being then. The developing droplets raised into the air quickly evaporate, reducing their diameter. Finally, aerosol particles, which are emerging in the surrounding of sewage treatment plants, have different sizes (from 2.0 to 10.0 μm), which change depending on the system of aeration and mixing sewage, as well as micro-climatic conditions, particularly temperature and humidity. Airborne bioaerosol particles are usually several times larger than single bacteria cells, therefore, a lot of cells of microorganisms may theoretically be floating in a single particle. Bioaerosol particle size also determines the speed of its descent, and also affects the range of its delivery by the wind. Particles having the smallest diameter (to 5.0 μm) create the so-called respirable fraction, which is of great importance to health because it is characterized by a longer maintenance in the air, and by a quick and easy penetration into the human lung alveoli [18-20]. The bioearosols components, which are airborne or dustborne, may also penetrate into the body through mucous membranes or skin. They can also be carried by insects but are rarely foodborne. Biological aerosols can cause irritation, allergic reactions, infections, infectious diseases,

and even toxic reactions. The ingredients of bioaerosols, therefore, pose a serious threat to health in the workplace [21-24].

The publication presents the test findings of bioaerosol and microorganisms in wastewater. The aim of the study was to determine the degree of the emission of microorganisms into the atmosphere from the wastewater bioreactor chambers of the mechanical-biological Municipal Sewage Treatment Plant in Srem (Poland, Wielkopolska Region), which discharges effluent into the river Warta. On the basis of the studies on the abundance of microorganisms in wastewater in the denitrification and nitrification chambers, and in the air, it was possible to identify and calculate the percentage emission ratio (*ER*). Mesophilic bacteria, psychrophilic ones and microscopic fungi were analyzed during the study.

Materials and methods

The study was conducted at the Wastewater Treatment Plant in Srem. The aim of the study was to determine the degree of microbial emission to the atmosphere from the wastewater bioreactor chambers of a mechanical-biological treatment plant. During the study, an average daily flow of wastewater (Q_{da}) was 5200 $m^3 \cdot d^{-1}$ with the equivalent number of inhabitants (ENI) of 84000.

After a mechanical cleaning, the wastewater is pumped into two biological reactors of the Bardenpho system with the following chambers: a phosphorus removal chamber (dephosphatation), and denitrification and nitrification ones, which are in the shape of cylindrical tanks and made of reinforced concrete with the diameter of 30.98 m and the active depth of 5.20 m. Additionally, the system is equipped with a predenitrication chamber preceding it, and which is supplied with recirculated sludge from the secondary settling tanks, and up to 30 % of waste treated mechanically after the initial settlers. The removal of nutrients takes place in the bioreactor through the creation of changeable conditions, such as anaerobic, anoxic and aerobic. In the process of the biological sewage treatment, microorganisms which form the typical activated sludge are used. Oxygen probes, redox potential probes, and the ones which measure the concentration of nitrate and ammonia are used in the process of monitoring and control of the biological reactors. The test bioreactor parameters are summarized in Table 1.

Table 1

Parameters of the biological reactor

Parameter	Unit	Chamber			
		Predenitrification	Dephosphatation	Denitrification	Nitrification
Dissolved oxygen	[g · m^{-3}]	0.0	0.0	0.0-0.5	0.5-2.5
pH value	[-]	> 6.5	> 6.5	> 6.5	> 6.5
Retention time	[h]	0.3	0.5-1.0	6.0	13.0
Chamber capacity *V*	[m^3]	92	367	1046	1708

Microbiological examination of bioaerosol and sewage was carried out in three periods: summer, winter and spring. The concentration of bioaerosols emitted to the atmosphere was investigated with the impact method by using microbial air sampler MAS-100 Eco by Merck. Due to differentiated aeration and mixing of wastewater in the bioreactor, air samples were taken for microbiological analysis from the central part of the bioreactor above the sewage surface of the nitrification and denitrification chambers. In

each of these chambers a test stand was set, located at the height of 20, 50 and 100 cm above the sewage surface, which enabled direct sampling of air (bioaerosol) at the designated altitude.

In addition, a control stand was set approximately 100 m away from the boundary of the sewage treatment plant, on the windward side, which represented the so-called "background research". Each time the temperature and relative humidity of the air, as well as the wind speed and its direction were also studied (by Anemo psychrometer AZ8911 - AZ Instrument Corp.). The studies of bioaerosol were conducted at seven test stands altogether. The stands 1, 2 and 3 were set above the denitrification chamber, respectively, at the heights of 20, 50 and 100 cm above the surface of the wastewater, the stands 4, 5 and 6 were located above the nitrification chamber, also in the respective heights of 20, 50 and 100 cm above the surface of the wastewater, while at the control stand (No. 7) air samples of 120 cm above the ground were collected.

In order to assess the bioaerosol emission from the nitrification and denitrification chambers, the overall number of mesophilic and psychrophilic bacteria, and microscopic fungi were examined. The mesophilic and psychrophilic bacteria were grown on R2A Agar medium, and the microscopic fungi on Sabouraud dextrose agar medium (manufactured by Oxoid). At the particular test stands 25 or 50 dm^3 of air were collected, using a MAS - 100 Eco sampler, onto Petri dishes with prepared medium. In order to compare the results of research, each time three series of air samples at the particular test stand were taken. Mesophilic bacteria were cultured for 48 up to 120 hours at 37 °C, psychrophilic for 72 up to 120 hours at 22 °C, and microscopic fungi for 168 hours at 26 °C. After the period of culture, the grown colonies of microorganisms were counted, and their number was corrected according to the Feller's table. Then, the results were converted to the volume of 1 m^3 of air (CFU - colony forming unit in 1 m^3) and the final result was given as the mean value.

From the same biological reactor chambers (nitrification and denitrification), in which the bioaerosol was tested, sewage samples were also collected from the near - surface layer into the sterile bacteriological bottles. In the laboratory, appropriate dilutions and microbiological cultures were made using the same media (plunged deep into the Petri dishes). After a period of incubation (similar to culture of microorganisms present in the bioaerosol) grown mesophilic and psychrophilic colonies, and microscopic fungi were counted, and the results were reported as CFU in 1 cm^3 of sewage.

In order to assess the degree of the emission of microorganisms from the wastewater into the air, the so-called percentage emission ratio *ER* was calculated. It is expressed by the formula (1), in which the percentage of bioaerosols in the air coming from the wastewater, calculated with the same volume of 1 m^3 has been given (modification of the formula given by Malakootian et al. [17]):

$$ER = \frac{A}{W} \cdot 100\% \tag{1}$$

where: *ER* - the percentage of emission ratio, *A* - concentration of bioaerosols bacteria (fungi) in 1 m^3 of air, *W* - concentration of bacteria (fungi) in 1 m^3 of wastewater.

The statistical analysis was performed using the Statistica v. 10 software (StatSoft). Statistical significance was defined as $p < 0.05$ or $p < 0.10$.

Results and discussion

Microbiological analysis of the wastewater taken from the bioreactor showed that in the denitrification chamber the number of all analyzed microorganisms (mesophilic and psychrophilic bacteria, and microscopic fungi) was almost always higher than in the nitrification chamber (Table 2). In the denitrification chamber, the largest number of the mesophilic bacteria ($37.3 \cdot 10^6$ CFU $\cdot$ cm^{-3}) and the psychrophilic ones ($88.5 \cdot 10^6$ CFU $\cdot$ cm^{-3}) was observed during the winter collection, and the microscopic fungi ($47.7 \cdot 10^5$ CFU $\cdot$ cm^{-3}) during summer. In the nitrification chamber, however, the maximum number of the mesophilic bacteria ($31.1 \cdot 10^6$ CFU $\cdot$ cm^{-3}) and microscopic fungi ($20.8 \cdot 10^5$ CFU $\cdot$ cm^{-3}) occurred during the summer collection, and the psychrophilic ones ($78.6 \cdot 10^6$ CFU $\cdot$ cm^{-3}) in spring time.

Table 2

Average count and standard deviation (*SD*) of mesophilic and psychrophilic bacteria and fungi in 1 cm^3 wastewater in denitrification and nitrification chambers

Season	Chamber	Wastewater [CFU · cm^{-3}] Mean ± *SD*		
		Mesophilic bacteria	Psychrophilic bacteria	Microscopic fungi
Summer	Denitrification	$36.2 \cdot 10^6 \pm 29 \cdot 10^5$	$45.3 \cdot 10^6 \pm 31 \cdot 10^5$	$47.7 \cdot 10^5 \pm 23 \cdot 10^4$
	Nitrification	$31.1 \cdot 10^6 \pm 36 \cdot 10^5$	$25.8 \cdot 10^6 \pm 35 \cdot 10^5$	$20.8 \cdot 10^5 \pm 24 \cdot 10^4$
Winter	Denitrification	$37.3 \cdot 10^6 \pm 20 \cdot 10^5$	$88.5 \cdot 10^6 \pm 42 \cdot 10^5$	$36.5 \cdot 10^5 \pm 61 \cdot 10^4$
	Nitrification	$62.0 \cdot 10^5 \pm 91 \cdot 10^4$	$50.9 \cdot 10^6 \pm 55 \cdot 10^5$	$67.0 \cdot 10^4 \pm 58 \cdot 10^3$
Spring	Denitrification	$17.6 \cdot 10^6 \pm 97 \cdot 10^4$	$84.8 \cdot 10^6 \pm 37 \cdot 10^5$	$27.7 \cdot 10^5 \pm 16 \cdot 10^4$
	Nitrification	$23.3 \cdot 10^6 \pm 15 \cdot 10^5$	$78.6 \cdot 10^6 \pm 42 \cdot 10^5$	$18.3 \cdot 10^5 \pm 21 \cdot 10^4$

Table 3

Average count and standard deviation (*SD*) of mesophilic and psychrophilic bacteria and fungi in 1 m^3 of air above the denitrification and nitrification chambers

Season	Chamber	Air [CFU · m^{-3}] Mean ± *SD*			
		Station	Mesophilic bacteria	Psychrophilic bacteria	Microscopic fungi
Summer	Denitrification	1	$6.7 \cdot 10^3 \pm 1.9 \cdot 10^3$	$1.1 \cdot 10^4 \pm 1.5 \cdot 10^3$	$1.4 \cdot 10^3 \pm 1.2 \cdot 10^2$
		2	$6.0 \cdot 10^3 \pm 1.2 \cdot 10^3$	$7.1 \cdot 10^3 \pm 9.1 \cdot 10^2$	$1.7 \cdot 10^3 \pm 0.7 \cdot 10^2$
		3	$2.0 \cdot 10^3 \pm 2.7 \cdot 10^2$	$4.0 \cdot 10^3 \pm 3.7 \cdot 10^2$	$1.4 \cdot 10^3 \pm 1.0 \cdot 10^2$
	Nitrification	4	$2.6 \cdot 10^2 \pm 0.4 \cdot 10^2$	$3.4 \cdot 10^3 \pm 4.2 \cdot 10^2$	$1.0 \cdot 10^3 \pm 1.0 \cdot 10^2$
		5	$1.4 \cdot 10^2 \pm 0.4 \cdot 10^2$	$2.7 \cdot 10^3 \pm 0.9 \cdot 10^2$	$1.4 \cdot 10^3 \pm 0.9 \cdot 10^2$
		6	$2.2 \cdot 10^2 \pm 0.6 \cdot 10^2$	$2.9 \cdot 10^3 \pm 2.2 \cdot 10^2$	$1.4 \cdot 10^3 \pm 1.2 \cdot 10^2$
Winter	Denitrification	1	$8.0 \cdot 10^2 \pm 1.3 \cdot 10^2$	$3.2 \cdot 10^3 \pm 3.7 \cdot 10^2$	$1.2 \cdot 10^3 \pm 1.1 \cdot 10^2$
		2	$6.3 \cdot 10^2 \pm 0.7 \cdot 10^2$	$3.1 \cdot 10^3 \pm 3.8 \cdot 10^2$	$4.1 \cdot 10^2 \pm 0.6 \cdot 10^2$
		3	$9.7 \cdot 10^2 \pm 1.4 \cdot 10^2$	$2.6 \cdot 10^3 \pm 1.7 \cdot 10^2$	$7.3 \cdot 10^2 \pm 0.5 \cdot 10^2$
	Nitrification	4	$5.8 \cdot 10^2 \pm 0.6 \cdot 10^2$	$1.7 \cdot 10^3 \pm 1.7 \cdot 10^2$	$8.4 \cdot 10^2 \pm 0.6 \cdot 10^2$
		5	$8.6 \cdot 10^2 \pm 1.3 \cdot 10^2$	$4.1 \cdot 10^3 \pm 2.8 \cdot 10^2$	$9.0 \cdot 10^2 \pm 0.2 \cdot 10^2$
		6	$4.0 \cdot 10^2 \pm 0.7 \cdot 10^2$	$3.0 \cdot 10^3 \pm 0.7 \cdot 10^2$	$4.4 \cdot 10^2 \pm 0.2 \cdot 10^2$
Spring	Denitrification	1	$1.7 \cdot 10^3 \pm 2.2 \cdot 10^2$	$5.6 \cdot 10^3 \pm 2.2 \cdot 10^2$	$3.0 \cdot 10^3 \pm 1.7 \cdot 10^2$
		2	$1.0 \cdot 10^3 \pm 1.8 \cdot 10^2$	$4.6 \cdot 10^3 \pm 2.3 \cdot 10^2$	$2.6 \cdot 10^3 \pm 0.5 \cdot 10^2$
		3	$8.0 \cdot 10^2 \pm 2.0 \cdot 10^2$	$5.5 \cdot 10^3 \pm 3.2 \cdot 10^2$	$2.2 \cdot 10^3 \pm 1.0 \cdot 10^2$
	Nitrification	4	$5.3 \cdot 10^2 \pm 0.6 \cdot 10^2$	$3.5 \cdot 10^3 \pm 1.5 \cdot 10^2$	$1.9 \cdot 10^3 \pm 1.1 \cdot 10^2$
		5	$1.0 \cdot 10^3 \pm 1.0 \cdot 10^2$	$2.9 \cdot 10^3 \pm 2.0 \cdot 10^2$	$1.9 \cdot 10^3 \pm 1.0 \cdot 10^2$
		6	$4.0 \cdot 10^2 \pm 0.5 \cdot 10^2$	$4.6 \cdot 10^3 \pm 3.5 \cdot 10^2$	$2.0 \cdot 10^3 \pm 0.2 \cdot 10^2$

It can therefore be concluded that at the stage of the wastewater treatment and its flow from the denitrification chamber into the nitrification chamber, the number and types of

microorganisms are changing, which is related to, among others, the degree of oxygenation and content of nutrients.

On the basis of the results of bioaerosol present in the air, it can be observed that there are differences in the number of microorganisms at different heights above the surface of the wastewater, as well as between the chambers of the bioreactor (Table 3). Taking into account those two examined chambers of the bioreactor, it can also be observed that the emission of microorganisms into the atmosphere from the denitrification chamber is higher than from the nitrification one. This is undoubtedly related to the concentration of microorganisms that occurs at different stages of wastewater treatment.

In all research periods a greater number of psychrophilic bacteria than mesophilic ones, and microscopic fungi was observed in the bioaerosol coming from both chambers of the bioreactor. The results of the studies on the abundance of microorganisms at different heights (20, 50 and 100 cm) above the surface of the wastewater prove that similar relationships cannot be observed in all research periods. There were the following variants observed: with an increasing height the number of microorganisms decreased (e.g. most mesophilic and psychrophilic bacteria over the denitrification chamber), it partly increased with height (e.g. from time to time microscopic fungi over the nitrification chamber) and fluctuated (e.g. periodically mesophilic and psychrophilic bacteria over the nitrification chamber). Rarely, however, the number of the tested microorganisms reached the maximum value at the highest tested height of 100 cm.

While analyzing the number of microorganisms detected in the air, it can be stated that the highest concentrations of bacteria and microscopic fungi in bioaerosol did not coincide with the periods in which these microorganisms were present in the largest concentrations in the sewage. It can be assumed that some of the microorganisms that reside in wastewater do not survive in the air, or the emission level in the air is influenced by, e.g. microorganisms' mass and the droplet size of bioaerosol, turbulence intensity, flow rate, or the concentration of oxygen. Table 4 summarizes the percentage of the emission ratio *ER* of the mesophilic and psychrophilic bacteria, and microscopic fungi in addition the results of the Kruskal-Wallis ANOVA and multiple comparison test.

The results of the Kruskal-Wallis ANOVA test in *ER* comparison between bacteria and fungi revealed a statistically significant *ER* difference between bacteria and fungi ($p < 0.05$ for the 14 trials and $p < 0.10$ for the 4 trials). However, multiple comparison test indicates that significant differences on *ER* level occur more frequently between mesophilic bacteria and microscopic fungi ($p = 0.02$ or 0.03 for 10 trials) and less frequently between psychrophilic bacteria and microscopic fungi (4 trials). Between mesophilic and psychrophilic bacteria there are no statistically significant differences on the *ER* level, regardless of the study period, the type of the chamber and stand.

The comparison of emission ratio between the chambers (stands 1,4; 2,5; 3,6) was made on basis of Mann-Whitney test calculations (level of significance $p < 0.10$). In the vast majority of tests (20 calculations) there was noticed a statistically significant *ER* difference between the denitrification chamber and the nitrification chamber ($p = 0.08$) and in 7 trials differences are not statistically significant (stands 3,6 - spring, summer concerning psychrophilic bacteria, stands 2,5 - spring, concerning mesophilic bacteria and fungi and stands of 1,4 - winter, related with the psychrophilic bacteria and spring related with microscopic fungi).

ER comparison between dates of the research were done on the basis of Friedman ANOVA test. The research period, irrespective of the type of bacteria, fungi, the chamber

and the stand has a significant influence on the level of *ER*. The result of Friedman ANOVA test for all micro-organisms indicated a significant statistical difference for emission ratio between the periods of the study (p = 0.05 for 15 trials and p = 0.10 for 3 trials).

Table 4

The percentage of bacteria and fungi in the air to the number of microorganisms' in wastewater (the percentage of emission ratio) in addition the results of the Kruskal-Wallis ANOVA and multiple comparison test for comparing the *ER* for mesophilic and psychrophilic bacteria and fungi

Season	Chamber	Station	Percentage of emission ratio			The Kruskal--Wallis ANOVA test		The multiple comparison test (p_w)		
			Mesophilic bacteria: air/wastewater [%]	Psychrophilic bacteria: air/wastewater [%]	Fungi: air/wastewater [%]	*H*	*p*	MB vs PB	MB vs F	PS vs F
Summer	Denitrification	1	$18.66 \cdot 10^{-9}$	$24.53 \cdot 10^{-9}$	$2.94 \cdot 10^{-8}$	6.49	0.04^{*}	0.41	0.03^{*}	0.89
		2	$16.67 \cdot 10^{-9}$	$15.56 \cdot 10^{-9}$	$3.52 \cdot 10^{-8}$	5.96	0.05^{**}	1.00	0.30	0.05^{**}
		3	$5.42 \cdot 10^{-9}$	$8.71 \cdot 10^{-9}$	$2.96 \cdot 10^{-8}$	7.20	0.03^{*}	0.54	0.02^{*}	0.54
	Nitrification	4	$0.84 \cdot 10^{-9}$	$13.19 \cdot 10^{-9}$	$4.82 \cdot 10^{-8}$	7.20	0.03^{*}	0.54	0.02^{*}	0.54
		5	$0.45 \cdot 10^{-9}$	$10.55 \cdot 10^{-9}$	$6.94 \cdot 10^{-8}$	7.20	0.03^{*}	0.54	0.02^{*}	0.54
		6	$0.71 \cdot 10^{-9}$	$11.10 \cdot 10^{-9}$	$6.84 \cdot 10^{-8}$	7.20	0.03^{*}	0.54	0.02^{*}	0.54
Winter	Denitrification	1	$2.14 \cdot 10^{-9}$	$3.57 \cdot 10^{-9}$	$3.29 \cdot 10^{-8}$	7.20	0.03^{*}	0.54	0.02^{*}	0.54
		2	$1.68 \cdot 10^{-9}$	$3.45 \cdot 10^{-9}$	$1.13 \cdot 10^{-8}$	7.20	0.03^{*}	0.54	0.02^{*}	0.54
		3	$2.61 \cdot 10^{-9}$	$2.88 \cdot 10^{-9}$	$2.01 \cdot 10^{-8}$	5.42	0.07^{**}	1.00	0.11	0.16
	Nitrification	4	$9.43 \cdot 10^{-9}$	$3.38 \cdot 10^{-9}$	$12.63 \cdot 10^{-8}$	7.20	0.03^{*}	0.54	0.54	0.02^{*}
		5	$13.98 \cdot 10^{-9}$	$8.06 \cdot 10^{-9}$	$13.53 \cdot 10^{-8}$	7.20	0.03^{*}	0.54	0.54	0.02^{*}
		6	$6.50 \cdot 10^{-9}$	$5.85 \cdot 10^{-9}$	$6.62 \cdot 10^{-8}$	5.60	0.06^{**}	1.00	0.22	0.08^{**}
Spring	Denitrification	1	$9.39 \cdot 10^{-9}$	$6.62 \cdot 10^{-9}$	$10.64 \cdot 10^{-8}$	7.20	0.03^{*}	0.54	0.54	0.02^{*}
		2	$5.76 \cdot 10^{-9}$	$5.41 \cdot 10^{-9}$	$9.43 \cdot 10^{-8}$	5.60	0.06^{**}	1.00	0.22	0.08^{**}
		3	$4.55 \cdot 10^{-9}$	$6.48 \cdot 10^{-9}$	$7.90 \cdot 10^{-8}$	7.20	0.03^{*}	0.54	0.02^{*}	0.54
	Nitrification	4	$2.28 \cdot 10^{-9}$	$4.43 \cdot 10^{-9}$	$10.50 \cdot 10^{-8}$	7.20	0.03^{*}	0.54	0.02^{*}	0.54
		5	$4.28 \cdot 10^{-9}$	$3.63 \cdot 10^{-9}$	$10.14 \cdot 10^{-8}$	6.49	0.04^{*}	0.89	0.41	0.03^{*}
		6	$1.71 \cdot 10^{-9}$	$5.81 \cdot 10^{-9}$	$10.87 \cdot 10^{-8}$	7.20	0.03^{*}	0.54	0.02^{*}	0.54

H - the value of designated Kruskal-Wallis statistics test, *p* - the level of test probability statistics calculated using the Kruskal-Wallis test, p_w - the level of test probability for multiple comparison test, MB vs PB - level of test probability between mesophilic bacteria (MB) and psychrophilic bacteria (PB), MB vs F - the level of test probability between mesophilic bacteria (MB) and fungi (F), PS vs F - the level of test probability between psychrophilic bacteria (PB) and fungi (F), * - Statistically significant, $p < 0.05$, ** - Statistically significant, $p < 0.10$

Friedman ANOVA test results used for comparison to the *ER* between the stands revealed that psychrophilic bacteria in the nitrification chamber in the summer and in the denitrification chamber during the winter the level of emission ratio between stations is similar (no significant statistical difference). The results were similar in case of microscopic fungi in the nitrification chamber in the spring. In other cases, the level of *ER* between the stands is very diverse depending on the date of examination, the chamber, the type of bacteria and fungi (p = 0.05 to 0.10).

Bearing in mind the fact that the microorganisms from the wastewater penetrate the air as a result of an intense turbulence, overflow and aeration of the wastewater, as well as the effect of wind and convective air movement on the border between liquid and gas phase

(wastewater / air), it has been calculated that the percentage of the microorganisms in the bioaerosols coming from the sewage (percentage emission ratio) is only a fraction of a percent. Over the denitrification chamber the percentage of the emission ratio of mesophilic bacteria reached an average value of $7.43 \cdot 10^{-9}$ % (ranging from $1.68 \cdot 10^{-9}$ to $18.66 \cdot 10^{-9}$ %), psychrophilic ones $8.58 \cdot 10^{-9}$ % (from $2.88 \cdot 10^{-9}$ to $24.53 \cdot 10^{-9}$ %), and microscopic fungi, $4.87 \cdot 10^{-8}$ % (from $1.13 \cdot 10^{-8}$ to $10.64 \cdot 10^{-8}$ %). Whereas, the *ER* of mesophilic bacteria averaged $4.46 \cdot 10^{-9}$ % (from $0.45 \cdot 10^{-9}$ to $13.98 \cdot 10^{-9}$ %), psychrophilic $7.33 \cdot 10^{-9}$ % (from $3.38 \cdot 10^{-9}$ to $13.19 \cdot 10^{-9}$ %) and microscopic fungi $9.21 \cdot 10^{-8}$ % (from $4.82 \cdot 10^{-8}$ to $13.53 \cdot 10^{-8}$ %) over the nitrification chamber.

In the denitrification chamber, an intense turbulence and wastewater mixing take place and anoxic conditions are maintained. In the nitrification chamber, in contrast, fine-aeration and mixing of wastewater are applied, so that the oxygen concentration in summer was 1.00 to 1.50 mg $O_2 \cdot dm^{-3}$, in winter 1.82 to 1.87, and in the spring 0.37 to 2.32 mg $O_2 \cdot dm^{-3}$. In the reported dates fluctuations in the flow of wastewater (respectively 121.0, 129.0 and 118.0 $m^3 \cdot h^{-1}$) were noted.

Comparing the results of the microbiological tests of the air to the bioreactor operating parameters, it can be seen that in the spring, when the concentration of dissolved oxygen in the oxygen chamber (nitrification) was greater than in the summer, an increase in microbial emissions into the atmosphere was also reported. In the winter, however, despite the high flow rate of wastewater, there was no increased emission of microorganisms recorded, and it could have been caused by a negative effect of low temperature on the survival of bacteria and fungi of bioaerosols. At the same time, an increase in the emission of the microorganisms to the atmosphere with increased dissolved oxygen concentration and flow rate is noticed. At that time, there is also a more turbulent motion of the liquid in the reactor, mixing, cracking of air bubbles and spraying of sewage, which greatly facilitates aerosols formation and their emission.

Table 5

Microclimate parameters during sample collection (φ - relative air humidity [%], v - average wind speed [$m \cdot s^{-1}$], t - air temperature [°C])

Sample collecting location	Collecting season		
	Summer	Winter	Spring
Denitrification chamber	φ = 41.0 % v = 0.2 m · s^{-1} t = 25.4 °C	φ = 26.1 % v = 1.6 m · s^{-1} t = –6.7 °C	φ = 33.3 % v = 1.0 m · s^{-1} t = 19.4 °C
Nitrification chamber	φ = 28.6 % v = 0.4 m · s^{-1} t = 26.4 °C	φ = 29.0 % v = 1.2 m · s^{-1} t = – 6.8 °C	φ = 34.6 % v = 0.8 m · s^{-1} t = 20.0 °C
Research background	φ = 38.0 % v = 0.2 m · s^{-1} t = 27.0 °C	φ = 46.0 % v = 4.0 m · s^{-1} t = –8.0 °C	φ = 30.1 % v = 1.5 m · s^{-1} t = 19.8 °C

Weather conditions are one of the factors affecting the emission of bioaerosols to the atmosphere. On the basis of the received microclimate measurements (Table 5) it can be concluded that the lowest concentration of the microorganisms in the air was found usually in winter, when the temperature was about seven degrees centigrade below zero. Such low temperatures lead to death of certain microorganisms of biological aerosol due to crystallization of water in the cells. Similar conclusions can be drawn with regard to the increased temperature in the summer, which can also inhibit the process of the survival and

proliferation of certain microorganisms in the atmosphere as a result evaporation of water from the cells. Therefore, an increase in relative humidity increases the chances of survival of the microorganisms suspended in bioaerosols.

Reported concentrations of microorganisms in the bioaerosol in the background research (stand no. 7) are generally lower than in the immediate vicinity of the bioreactor chambers. In all test dates, the mesophilic bacteria were at the lowest number, while the psychrophilic bacteria were at the highest (Table 6).

Table 6

Average count and standard deviation (*SD*) of mesophilic and psychrophilic bacteria and fungi in 1 m^3 of air in a control stand (background research)

Season	Air [CFU · m^{-3}] Mean ±*SD*		
	Mesophilic bacteria	**Psychrophilic bacteria**	**Microscopic fungi**
Summer	$0.8 \cdot 10^2 \pm 0.3 \cdot 10^2$	$24.1 \cdot 10^2 \pm 5.3 \cdot 10^2$	$5.9 \cdot 10^2 \pm 1.3 \cdot 10^2$
Winter	$6.1 \cdot 10^2 \pm 1.0 \cdot 10^2$	$15.2 \cdot 10^2 \pm 2.3 \cdot 10^2$	$3.0 \cdot 10^2 \pm 0.2 \cdot 10^2$
Spring	$6.5 \cdot 10^2 \pm 0.7 \cdot 10^2$	$34.3 \cdot 10^2 \pm 1.5 \cdot 10^2$	$2.0 \cdot 10^3 \pm 2.3 \cdot 10^3$

Throughout the whole period of the research, the measured wind speed in the chambers of the bioreactor was usually lower than in the background research. This was due to the fact that the bioreactor chambers are shielded with housing which extends about 100-110 cm above the sewage surface. This construction makes it easy to adjust the level of the sewage in the bioreactor, but also partly limits the transfer of bioaerosols surrounding areas with the help of wind.

A wastewater aeration system plays an important role in the emission of microorganisms to the air. The works of various authors suggest that for reasons of air safety, more favourable is fine-bubble aeration, at which a bioaerosol emission level is lower [19, 25, 26]. Whereas, in anaerobic or anoxic chambers there is an intense mixing of wastewater, overflowing and turbulence, which causes the formation of considerable amount of harmful bioaerosols. It was observed, among other, by Sanchez-Monedero et al. [14] who noted high concentrations of bioaerosol ($27.01 \cdot 10^2$ CFU · m^{-3}) over the denitrification chamber and Prazmo et al. [27]. Similar relations were also recorded in the tested sewage plant in Srem.

Bauer et al. [1] reported that during aeration of sewage, when bubbles reach wastewater - air interface, the air bubbles will burst. The microorganisms contained in the air bubbles and the ones concentrated in the surface microlayer of the wastewater are thus lifted up to the air in the form of drops of bioaerosol at the height of 15 cm. They found that the concentration of microorganisms in the bioaerosols generated by a mechanical mixing system was higher than in the aerosols generated by a fine-bubble aeration system [25, 28, 29]. Bauer et al. [1] on a small, sheltered pilot station with a capacity of 1.6 m^3 and 2.0 m^2 of an area of a wastewater surface, they found in 1 m^3 of sewage from $1.3 \cdot 10^{15}$ to $2.9 \cdot 10^{16}$ of bacteria. In 1 m^3 of air (aerosol collected at the height of 0.5 m above the wastewater surface), they recorded from $1.3 \cdot 10^4$ to $2.4 \cdot 10^4$ of mesophilic bacteria. The given ratio of mesophilic bacteria present in bioaerosol to the amount of these microorganisms in wastewater (1 m^3) averaged $1.7 \cdot 10^{-10}$. Mesophilic fungi reached in the wastewater (1 m^3) the amount of $2.8 \cdot 10^{10}$, in the air (1 m^3) from $1.4 \cdot 10^3$ to $2.4 \cdot 10^3$, and the ratio of airborne fungi to their amount in the wastewater was around $7.5 \cdot 10^{-6}$.

In the tested wastewater plant in Srem, quantitative proportions of microorganisms at the heights of 20, 50 and 100 cm were very similar and higher concentrations of bacteria

than fungi in bioaerosols were also noticed. At the same time the first measurement was made at the height of 20 cm and it can be assumed that some of the microorganisms that were raised into the air to the height of 15 cm were not captured by the microbial air sampler MAS-100 Eco. It can also be assumed that they were microorganisms of the greatest biomass, which quickly fell to the wastewater layer. While the forms that were detected at the heights of 20, 50 and 100 cm could have been lighter or smaller as a result of an intensive evaporation of aerosol droplets, and with wind they were raised in the form of bioaerosol much higher above the surface of the treated wastewater in the denitrification and nitrification chambers. Among the captured bioaerosol particles there could have been secondary, very fine aerosol droplets of the wastewater that were produced as a result of splashing of the wastewater surface layer by falling droplets originally produced as a result of aeration and mixing of the wastewater. The emission of microorganisms from wastewater into the air is also influenced by the type and nature of a treatment, the number of microorganisms in wastewater, operating conditions of wastewater plant equipment and environmental conditions [30, 31].

In the tested wastewater treatment plant (Srem - Poland) a fine air-bubble aeration method in the nitrification chamber was used (V_{max} = 1708 m^3), and above that chamber lower emission of the microorganisms was reported in comparison to the denitrification chamber (V_{max} = 1046 m^3), in which a mechanical mixing a and strong turbulence of the wastewater took place. At the same time, municipal wastewater was supplied to that wastewater treatment plant, which were mechanically and biologically treated, and the number of psychrophilic bacteria was higher than the mesophilic ones and microscopic fungi in the activated sludge chambers. It can be assumed that in the sewage influent apart from bacteria associated with people (mesophilic) there was also other microflora (psychrophilic) coming from the water used in everyday life, e.g. from dishwashing, preparing meals, washing, laundry, etc. In addition, many types of psychrophilic bacteria are responsible for biological wastewater treatment process, which takes place right in the denitrification and nitrification chambers.

The structure, size and concentration of microorganisms in the bioaerosols vary depending on the source of emission and environmental factors affecting their spread in the air. Such environmental factors are mainly the temperature, wind velocity and humidity. When the relative humidity is greater than 35 % or the wind speed is stronger, then the amount of the raised air-borne microorganisms is higher [17]. Too high or low temperatures can kill or inhibit the growth of the microorganisms suspended in the air. Increasing survival of the microorganisms is also determined by the relative humidity of the air, and the intensity of the winds is responsible for the spread of bacteria and microscopic fungi both on and off-site treatment plant. Malakootian et al. [17] also reported indicator microorganisms from wastewater emission into the air, which was the ratio of the concentration of microorganisms contained in bioaerosol concentrations in the air to the bacteria present in the wastewater.

Our findings suggest that the relative humidity of the air over the denitrification and nitrification chambers was mostly less than 35 %, and the wind velocity was relatively low, ranging from 0.2 to 1.6 $m \cdot s^{-1}$. It can be concluded that the environmental conditions were not conducive to raising and spreading of microorganisms. Only the air temperature in the summer and spring reached values from 19.4 to 26.4 °C and it was the only positive factor influencing evaporation of aerosol droplets and raising them to the atmosphere. In winter, however, the temperature could impede the emission and propagation of microorganisms.

Conclusions

1. The emission of microorganisms in the atmosphere is influenced by the type of a chamber in which sewage is located.
2. In all test dates a greater number of psychrophilic than mesophilic bacteria and microscopic fungi was found in bioaerosol coming from the denitrification and nitrification chambers.
3. The calculated percentage emission factor (*ER*) ranged from $1.13 \cdot 10^{-8}$ % (microscopic fungi) to $24.53 \cdot 10^{-9}$ % (psychrophilic bacteria).
4. It was found that the process of mixing, aeration of wastewater, have an effect on the emission of microorganisms.

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