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NUMBERS OF AMMONIFYING, NITRIFYING AND DENITRIFYING BACTERIA IN SEWAGE TREATED IN A SYSTEM OF BIOLOGICAL STABILISATION PONDS[1]

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Summary. The paper presents an analysis of the numbers of bacteria participating in the transformation of nitrogen forms in sewage treated in a wastewater treatment plant based on the utilisation of a system of 3 wastewater ponds (anaerobic and aerated). Seasonal changes were observed in bacterial populations in the sewage in 2009. Thermal conditions prevailing in August and November were conducive to the growth of ammonifying, nitrifying and denitrifying bacteria. In February and May notably lower numbers of those bacterial groups were observed. Changes in the numbers of bacteria were also observed at the particular stages of purification of wastewater. The highest numbers of ammonifying and nitrifying bacteria were recorded in raw sewage, and the lowest in the treated sewage. The greatest numbers of phase I and II nitrifying bacteria, as well as an increase in the concentration of $N\text{-}NO_3^-$ and $N\text{-}NO_2^-$, were noted in wastewater flowing out of pond No. 2 (aerated), though the efficiency of nitrification observed in that pond was not too high. In the analysed system of biological stabilisation ponds, and especially in pond No. 3, there were no truly favourable conditions for the process of nitrification, primarily due to the excessively high concentration of oxygen dissolved in wastewaters in that reservoir. This situation was probably the cause for the low efficiency of total nitrogen removal in the object under study, that amounted to only 22%.

Key words: wastewater treatment, biological stabilisation ponds, bacteria, nitrogen, ammonification, nitrification, denitrification

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INTRODUCTION

In wastewater treatment plants three groups of bacteria participate in nitrogen elimination with the biological method: ammonifying, nitrifying and denitrifying bacteria. Each of those bacterial groups requires a different environment for optimum growth [Podedworna and Żubrowska-Sudoł 2004].

Ammonifying bacteria, responsible for the initial stage of degradation of nitrogen compounds occurring in sewage, are the most diversified in terms of their physiology (aerobic and anaerobic, capable of growth and activity at highly varied pH of the environment). In the next stage of nitrogen elimination, the sewage treatment system should be populated by nitrifying bacteria, such as *Nitrosomonas*, *Nitrosococcus* or *Nitrosovibrio*, that oxidise $N\text{-}NH_4^+$ to $N\text{-}NO_2^-$ utilising ammonium ions produced by ammonifying bacteria, and mainly by *Nitrobacter*, that oxidise $N\text{-}NO_2^-$ to $N\text{-}NO_3^-$. In turn, denitrification is a process of dissimilation of nitrate and nitrite nitrogen as a result of the activity of facultative heterotrophic bacteria (*Achromobacter, Aerobacter, Bacillus*) [Bernacka *et al*. 1995].

The processes of ammonification and nitrification lead primarily to a change in the forms of nitrogen compounds, while denitrification permits the elimination of $N\text{-}NO_3^-$ through their reduction to gaseous nitrogen, and thus causes a decrease in the content of total nitrogen in wastewaters. High efficiency of nitrogen compounds removal in sewage treatment plants depends primarily on the conditions under which the above processes take place.

The objective of the study presented here was an analysis of the numbers of bacteria participating in the transformation of nitrogen forms in wastewater treated in a communal sewage treatment plant based on the utilisation of a system of 3 biological stabilisation ponds (anaerobic and aerated).

MATERIAL AND METHODS

The study was conducted on an object situated in the locality of Ludwin in the Lublin Province. The sewage treatment plant has been operating since 1992 and receives around 90 $m^3 \cdot d^{-1}$ of wastewater from the sewerage system of the commune of Ludwin and additionally ca. 50 $m^3 \cdot d^{-1}$ of sewage brought in by cesspool emptier trucks from the recreational resorts in the area of the Łęczna-Włodawa Lakeland.

The technological scheme of the presented sewage treatment plant comprises a system of 4 ponds: 1 – anaerobic pond, 2A and 2B – aerated pond with submerged biological bed, 3 – aerobic-anaerobic pond with recirculation of wastewater (Fig. 1). Pond No. 1 performs the accumulation function and retains mechanical contaminants (screenings, sand and organic suspensions) and ensures anaerobic purification of sewage in the process of fermentation, Ponds No. 2A and 2B with submerged biological bed, with volumes of 32 m^3 (pond 2A) and 16.2 m^3 (pond 2B) are aerated by means of a small-bubble grating and their function is biological purification of wastewaters. Thanks to the biofilm accumulated on the bed surface aerobic degradation of contaminants is possible. Next, excess of the biofilm undergoes sedimentation in pond No. 2B, followed by its mineralisation and condensation on the bottom of the reservoir. Final purification of the wastewaters takes place in pond No. 3, where under aerobic conditions (close to the surface) and anaerobic conditions (near the bottom) the remaining contaminants are neutralised and biogenic compounds – nitrogen and phosphorus – are eliminated [Jóźwiakowski and Kotulska 2006].

Table 1 presents the technological parameters of the object under discussion.

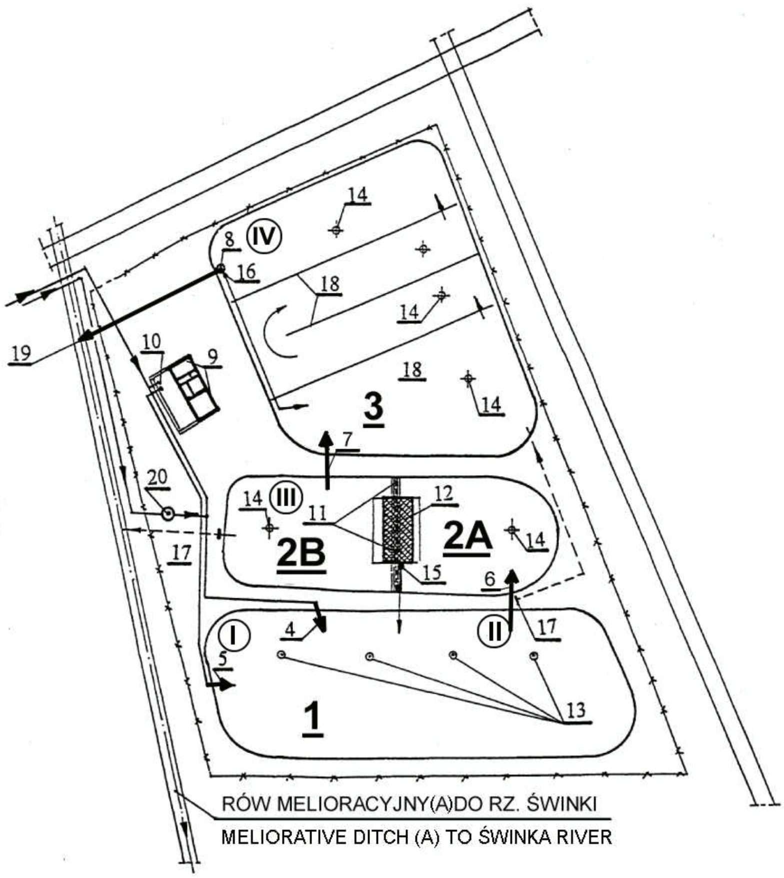

Fig. 1. Technological scheme of wastewater treatment plant in Ludwin: 1 – anaerobic pond, 2A and 2B – aerated pond with submerged biological bed, 3 – sedimentation pond, 4 – inflow of sewage from pumping station, 5 – inflow of brought sewage, 6 – sewage inflow to pond No. 2A, 7 – sewage inflow to pond No. 3, 8 – outflow of treated sewage, 9 – building of service of wastewater treatment plant, 10 – dumping point of brought sewage, 11 – linear aerator, 12 – biological submerged bed, 13 – aerial mixer in pond No. 1, 14 – stirring oxygenator, 15 – excess sludge mammoth pump, 16 – recirculation mammoth pump, 17 – bolt on bypass of pond 2A and 2B, 18 – labyrinth baffle, 19 – outflow of sewage to ditch A, 20 – sewerage vacuum pump, I, II, III, IV – sampling points [Jóźwiakowski and Kotulska 2006]

Table 1. Technological parameters of wastewater treatment plant in Ludwin
[Jóźwiakowski and Kotulska 2006]

Parameters	Pond No. 1	Pond No. 2A	Pond No. 2B	Pond No. 3
Useful reservoir capacity, m^3	6089	2000	2000	4700
Operating depth, m	3.0	3.0	3.0	1.8
Sewage retention time, days:				
In summer period	20	13		16
In winter period	39	27		32

Wastewater samples for analyses were taken in February, May, August and November, 2009, from four points of the sewage treatment plant: I – inflow to pond No. 1 (raw sewage), II – outflow from pond Nr. 1 (sewage after mechanical purification), III – outflow from pond No. 2, IV – outflow from pond No. 3 (treated sewage). In the samples of wastewaters the numbers of ammonifying bacteria, phase I nitrifying bacteria (oxidising $N\text{-}NH_4^+$ to $N\text{-}NO_2^-$) and phase II nitrifying bacteria (oxidising $N\text{-}NO_2^-$ to $N\text{-}NO_3^-$) and denitrifying bacteria (reducing $N\text{-}NO_3^-$ to $N\text{-}NO_2^-$) were determined with the index method, the most probable number MPN) of those bacteria was read from Mc Crady tables, on the basis of calculus of probability. The numbers of ammonifying bacteria were determined with the test tube method acc. to standard PN-75 C-04615/18, of autotrophic nitrifying bacteria – with the method of culturing in liquid media acc. to standard PN-77 C-04615/20, and of denitrifying bacteria – with the test tube method acc. to standard PN-75 C-04615/19. Other parameters determined in the sewage samples included also pH, concentration of O_2, total suspended solids, levels of BOD_5 and COD, as well as concentrations of total nitrogen and phosphorus, $N\text{-}NH_4^+$, $N\text{-}NO_3^-$, $N\text{-}NO_2^-$. Those analyses were made following the commonly applied methods [Hermanowicz *et al.* 1999]. The microbiological analyses of the sewage samples were made at the Faculty of Agricultural Microbiology, and the physicochemical ones at the Water and Sewages Analytics Laboratory, Department of Melioration and Agricultural Construction, University of Life Sciences in Lublin.

RESULTS AND DISCUSSION

Air temperature is one of the main factors determining the efficiency of processes of pollution removal in wastewater treatment plants. As the temperature decreases there is a decrease in the intensity of nitrification and denitrification, and below 5°C those processes crease due to the inhibition of bacterial growth [Bernacka *et al.* 1995]. Fig. 2 presents mean monthly air temperatures for the period of 1996–2008 in Lublin, situated 30 km from the object under study [GUS 1997–2009]. As can be seen from the graph, the most favourable conditions for sewage treatment and bacterial growth in the plant under study prevail in the period from April till October.

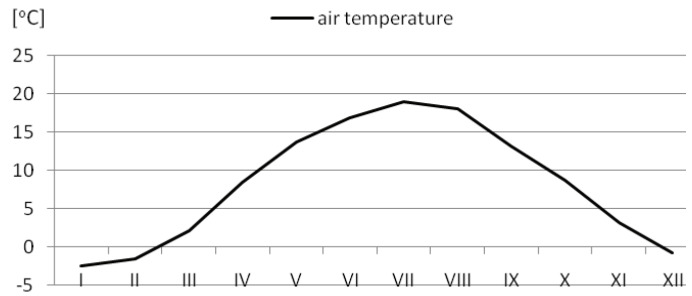

Fig. 2. Mean monthly air temperatures in Lublin for the period of 1996–2008 [GUS 1997–2009]

Figure 3 presents the mean values of indices and components of pollution in the sewage at various stages of treatment in February, May, August and November 2009, as a background for analysis of the results of determination of the numbers of ammonifying, nitrifying and denitrifying bacteria.

In raw sewage flowing into the anaerobic pond I the reaction oscillated at the level of 7.57 to 7.65 pH, and the concentration of dissolved oxygen varied from 0.24 to 0.80 mg $O_2 \cdot dm^{-3}$. The wastewater under analysis contained from 143 to 200 $mg \cdot dm^{-3}$ of total suspended solids, the level of BOD_5 was 195–365 mg $O_2 \cdot dm^{-3}$, and COD 230–450 mg $O_2 \cdot dm^{-3}$. Raw sewage flowing into the object studied was characterised by a very high content of total nitrogen (81.0–110 $mg \cdot dm^{-3}$) and phosphorus (29.3–30.4 $mg \cdot dm^{-3}$) (Fig. 3), i.e. values much higher than those observed in typical municipal sewage [Bernacka *et al.* 1995, Butler *et al.* 1995].

Ammonifying bacteria. The results obtained (Tab. 2) indicate that the highest mean number of ammonifying bacteria was recorded for the raw sewage – 149375 NPL/1 ml. This was probably due to the conditions prevailing in the anaerobic pond I, where the concentration of dissolved oxygen did not exceed 0.8 mg $O_2 \cdot dm^{-3}$, and that of $N\text{-}NH_4^+$ varied from 43.9 to 98.0 $mg \cdot dm^{-3}$ (Fig. 3). According to Abeliovich and Azov [1976], domestic sewage containing high levels of ammonia may be toxic to the process of photosynthesis and to microorganisms, and may also lead to the development of anaerobic conditions in a sewage treatment plant [Abeliovich 1987].

Table 2. Most probable number of ammonifying bacteria in 1 ml of wastewater

Sample No.	Kind of sewage	Period of analyses				
		II	V	VIII	XI	Mean
1	Raw sewage	2500	95000	250000	250000	149375
2	After pond 1	2500	9500	4500	45000	15375
3	After pond 2	2500	2500	45000	9500	14875
4	After pond 3	950	950	950	7500	2588

In wastewater samples from the successive ponds Nos 1, 2, 3 a decrease was observed in the numbers of ammonifying bacteria, being a result of increase

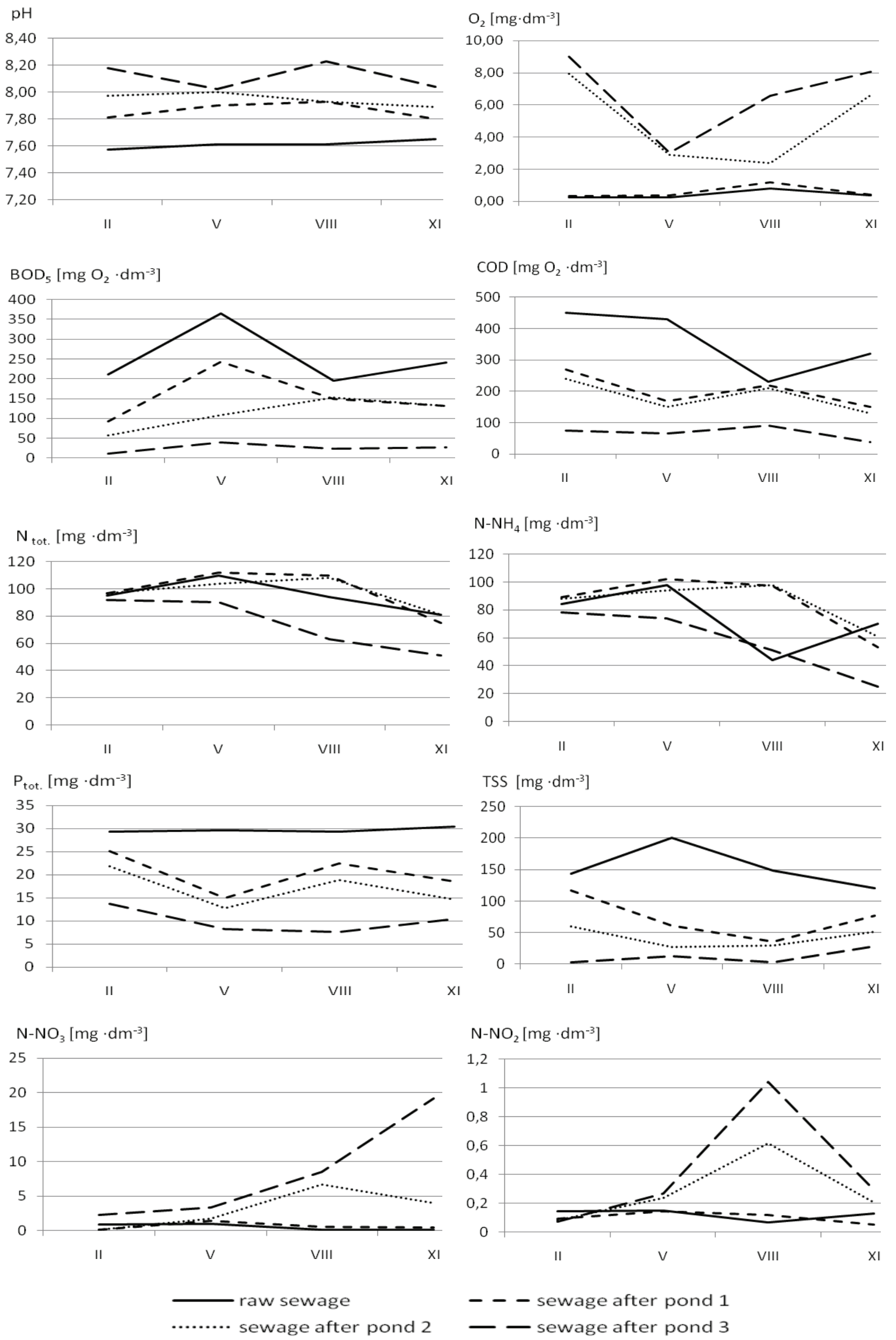

Fig. 3. Values of indices and pollution components in treated sewage in the system of biological stabilisation ponds in 2009

of dissolved oxygen concentration in those reservoirs (Fig. 3). The lowest numbers of those bacteria were found in treated sewage flowing out from pond 3 – mean of 2588 MPN/1 ml (Tab. 2).

Seasonal variation in the numbers of ammonifying bacteria was observed, especially in raw sewage. The lowest number of those bacteria was recorded in February, and their numbers increased with increasing air temperature (Fig. 2) in subsequent months of the experiment. In treated sewage the highest numbers of ammonifying bacteria were recorded in November – 7500 MPN/1 ml, and in other periods of analysis the numbers were constant at 950 MPN/1 ml of wastewater (Tab. 2).

Nitrifying bacteria are among the most sensitive of the microbial populations that take part in sewage treatment, their numbers being affected by such factors as temperature, pH, oxygen concentration, load of organic pollutants, presence and concentration of toxic substances, and nitrogen concentration in the inflow to the treatment plant. The intensity of nitrification decreases with decreasing temperature, and below 5°C the processes ceases due to inhibition of the growth of nitrifying bacteria [Bernacka *et al.* 1995].

Unfavourable oxygen conditions prevailing in pond I (Fig. 3) caused that the numbers of phase I and II nitrifying bacteria in wastewater in that pond were low, and 27 and 26 MPN/1ml, respectively (Tab. 3). In the raw sewage in pond I also the concentrations of $N\text{-}NO_3^-$ and $N\text{-}NO_2^-$ were low – they varied in the range of 0.07–0.97 and 0.068–0.151 $mg\cdot dm^{-3}$, respectively (Fig. 3). These results support the observation of Bernacka *et al.* [1995] that in raw domestic sewage $N\text{-}NO_3^-$ and $N\text{-}NO_2^-$ usually occur in small amounts – their total level is typically below 0.5 $mg\cdot dm^{-3}$.

Table 3. Most probable number of phase I and II nitrifying bacteria in 1 ml of wastewater

Sample No.	Kind of sewage	Period of analysis									
		II		V		VIII		XI		Mean	
		Phase I	Phase II	Phase I	Phase II	Phase I	Phase II	Phase I	Phase II	Phase I	Phase II
1	Raw sewage	9	25	9,5	9,5	45	45	45	25	27	26
2	After pond 1	95	45	45	95	2500	2500	450	950	772	897
3	After pond 2	9	9	150	150	2500	2500	950	950	902	902
4	After pond 3	250	25	45	45	450	450	450	450	299	242

The highest mean number of phase I and II nitrifying bacteria – average of 902 MPN/1ml (Tab. 3), and increased concentrations of $N\text{-}NO_3^-$ and $N\text{-}NO_2^-$ (Fig. 3) were noted in wastewater flowing out of pond 2 (aerated), which indicates that the process of nitrification did take place in that reservoir, though at a low intensity. Therefore, the system of biological stabilisation ponds under study did not ensure effective elimination of $N\text{-}NH_4^+$, as evidenced by the high concentrations of that nitrogen form in wastewaters flowing out of ponds 2 and 3 throughout the year of the study (Fig. 3), and by the low reduction of N-NH_4^+ that amounted in 2009 to an average of 22%.

Seasonal variation in the numbers of phase I and II nitrifying bacteria was observed in the ponds under study. The thermal conditions prevailing in August – mean monthly air temperature of 18°C (Fig. 2), stimulated the growth of those bacteria. The lowest numbers of nitrifying bacteria in sewage at all stages of treatment were recorded in February and May (Tab. 3). The low number of those bacteria in February can be attributed to low air temperature – below 0°C, while in May the time of wastewater passage through the whole pond system, amounting to 98 days (over 3 months) (Tab. 1) was the probable cause of the low numbers of nitrifying bacteria, especially in treated wastewaters flowing out of pond No. 3 (Tab. 3).

Denitrifying bacteria. Seppäneni and Hooli [1974] found that the largest amounts of nitrogen are eliminated in biological stabilisation ponds through the process of denitrification. Also in the opinion of Machnicka *et al*. [2004] only denitrification can contribute to the elimination of nitrogen from ecosystems. Denitrification may also lead to the production of another gas – N_2O [Kester *et al*. 1997, Sliekers *et al*. 2002].

The results presented in Table 4 indicate that the greatest numbers of denitrifying bacteria reducing N-NO_3^- to N-NO_2^- were noted in raw sewage (mean of 6500 MPN/1ml), and over 6-fold lower in treated sewage flowing out of pond 3 (mean of 930 MPN/1ml).

Table 4. Most probable number of denitrifying bacteria reducing N-NO_3 to N-NO_2 in 1 ml of wastewater

Sample No.	Kind of sewage	Period of analysis				
		II	V	VIII	XI	Mean
1	Raw sewage	2500	4500	9500	9500	6500
2	After pond 1	450	250	4500	9500	3675
3	After pond 2	950	250	4500	2500	2050
4	After pond 3	20	250	950	2500	930

Seasonal changes were observed in the numbers of denitrifying bacteria. The lowest seasonal numbers of bacteria reducing N-NO_3^- to N-NO_2^- were noted in February (from 2500 MPN/1ml in raw sewage to 20 MPN/1ml in wastewater after pond 3), which was probably due to low air temperature. In subsequent months the numbers of denitrifying bacteria increased successively, up to the maximum values in November (Tab. 4). It was in November and August that the highest effects of total nitrogen removal in the analysed system of biological ponds were observed, at 37 and 33%, respectively.

The results obtained indicate a highly unfavourable effect of low temperature on the processes of nitrogen elimination taking place in the ponds, which is also confirmed by other authors [Horan *et al.* 2006]. In the system under analysis, in February, at air temperatures below 0°C, the efficiency of total nitrogen elimination was as low as 3.2%.

According to Bernacka *et al*. [1995], the process of denitrification proceeds the best under conditions of sufficient supply of $N\text{-}NO_3^-$ and easily available carbon substrates, and when the concentration of oxygen does not exceed 0.5 mg $O_2\cdot dm^{-3}$. Higher oxygen concentration inhibits the process, as under such conditions the source of oxygen for the bacteria is dissolved oxygen and not oxygen contained in $N\text{-}NO_3^-$. In the system of biological stabilisation ponds under analysis, and especially in pond No. 3, the conditions were unfavourable for the process of denitrification – the concentration of dissolved oxygen in wastewaters in that pond varied during the whole year of the study from 3.03 to 9.01 mg $O_2\cdot dm^{-3}$. That situation was probably the cause of the low efficiency of total nitrogen elimination that in 2009 amounted to a mean of 22%.

According to Seppäneni and Hooli [1974], a method that can be employed to regulate the functioning of biological stabilisation ponds is to maintain the concentration of pollution in wastewaters at a level that is optimal for the activity of the particular bacterial groups. The efficiency of nitrogen reduction in biological ponds can also be enhanced through the creation of suitable oxygen conditions for the correct run of the processes of ammonification, nitrification and denitrification.

According to Rheinheimer [1985], in the aquatic environment only the most active bacteria are capable of complete denitrification. However, to achieve complete denitrification in a given wastewater treatment plant, more detailed research is needed that may lead to the optimisation of the effects of nitrogen elimination. In the opinion of Lim *et al*. [2005], analysis of the numbers of micro-organisms involved in nitrogen transformations is the necessary condition for full understanding of the processes of removal of that biogen in wastewater treatment plants.

CONCLUSIONS

1. Seasonal variations were observed in the numbers of bacteria participating in nitrogen transformations in wastewater in the system of biological stabilisation ponds. Thermal conditions prevailing in August and November stimulated the growth of ammonifying, nitrifying and denitrifying bacteria. In February and May notably lower numbers of the bacterial groups under study were observed.

2. Changes in the numbers of the bacteria under study were also noted at the particular stages of treatment. The highest numbers of ammonifying and denitrifying bacteria were noted in raw sewage, and the lowest in the treated wastewater. The highest numbers of phase I and II nitrifying bacteria and increased concentrations of $N\text{-}NO_3^-$ and $N\text{-}NO_2^-$ were observed in wastewater flowing out of pond 2 (aerated), yet nitrification in that pond was of low intensity.

3. In the system of biological stabilisation ponds under analysis, and especially in pond No. 3, the conditions were unfavourable for the process of denitri-

fication due to excessively high concentration of dissolved oxygen in wastewaters in that pond. That situation was probably the cause of the low efficiency of total nitrogen removal in the object under study that amounted to 22%.

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LICZEBNOŚĆ BAKTERII AMONIFIKACYJNYCH, NITRYFIKACYJNYCH I DENITRYFIKACYJNYCH W ŚCIEKACH OCZYSZCZANYCH W SYSTEMIE BIOLOGICZNYCH STAWÓW ŚCIEKOWYCH

Streszczenie. W pracy przedstawiono analizę liczebności bakterii uczestniczących w przemianach form azotu w ściekach oczyszczanych w oczyszczalni opartej na wykorzystaniu systemu 3 biologicznych stawów ściekowych (beztlenowych i tlenowych). Zaobserwowano sezonowe zmiany liczebności bakterii w ściekach w 2009 r. Warunki termiczne panujące w sierpniu i listopadzie sprzyjały rozwojowi bakterii amonifikacyjnych, nitryfikacyjnych i denitryfikacyjnych. W lutym i w maju notowano znacznie niższe liczebności badanych grup bakterii. Stwierdzono także zmiany liczebności bakterii w poszczególnych etapach oczyszczania ścieków. Największą liczebność bakterii amonifikacyjnych i denitryfikacyjnych notowano w ściekach surowych, a najmniejszą w ściekach oczyszczonych. Największą liczbę bakterii nitryfikacyjnych I i II fazy oraz wzrost stężenia N-NO_3^- i N-NO_2^- stwierdzono w ściekach odpływających z 2 stawu (napowietrzanego), jednak w stawie tym nie zaobserwowano zbyt wydajnej nitryfikacji. W analizowanym systemie biologicznych stawów ściekowych, a szczególnie w stawie nr 3, brakowało także korzystnych warunków do procesu denitryfikacji, przede wszystkim ze względu na zbyt wysokie stężenie tlenu rozpuszczonego w ściekach w tym zbiorniku. Sytuacja taka była prawdopodobnie przyczyną niewielkiej skuteczności usuwania azotu całkowitego w badanym obiekcie, która wynosiła 22%.

Słowa kluczowe: oczyszczanie ścieków, biologiczne stawy stabilizacyjne, bakterie, azot, amonifikacja, nitryfikacja, denitryfikacja