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DENITRIFICATION RATE IN BOTTOM SEDIMENTS OF SULEJÓW RESERVOIR

The aim of investigation was to compare the denitrification rate in Sulejow reservoir using two methods:

1. The *in situ* chamber method – denitrification rate was calculated from the total N_2 flux out of the sediment, being measured directly by means of gas chromatography.

2. Estimation of the size of denitrifying bacteria population by means of the most probable number method and plate counting method.

Positive correlations between the content of organic matter in sediments and the number of denitrifying bacteria ($r = 0.86$) and between the denitrification rate ranging from 0 to 677 µmol $N_2/m^2/h$ and the content of organic matter ($r = 0.84$) were found.

Number of denitrifying bacteria in the sediment estimated by means of the MPN method and the plate counting method ranged from 0.05% to 15.8% and from 4.6% to 26% of total microflora, respectively. About 50% of bacterial strains being isolated accumulate nitrite during nitrate denitrification.

1. INTRODUCTION

Nitrogen and phosphorus are the most important factors limiting algal growth in natural waters and may contribute to eutrophication processes (MOSELLO et al. [16]). The denitrification process may be applied in removing aromatic and nitrogen compounds from industrial and communal wastewater and eutrophicated reservoirs and lakes. Under denitrifying conditions nitrates, which cause of morbidity on methemoglobinemia, are also removed from drinking water. Denitrification processes may contribute to formation of nitrogen oxides which are responsible for acid rain and reduction in the stratospheric ozone level (BŁASZCZYK [4]). Highly unfavour-

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able phenomena such as toxic nitrite accumulation may also occur due to denitrification process.

If the participation of nitrite-accumulating bacteria in total denitrification activity is essential, the enrichment of soil or surface waters with nitrate compounds may endanger the balance of natural environment, or even restrict its use because of the accumulation of toxic nitrite products (BŁASZCZYK [4]).

2. MATERIAL AND METHODS

The area studied. The investigations were conducted at the Sulejów reservoir situated in central Poland. The Sulejów reservoir (surface area of 22 km^2) is a shallow (mean depth of 3.2 m) polymictic reservoir of a maximum storage capacity of 75×10^6 m^3 and a mean retention time of about 30 days (AMBROŻEWSKI [1]). The Sulejów reservoir supplies drinking water to the city of Łódź (nearly 1 million inhabitants) and is a recreational area for up to 60 000 people. The occurrence of toxic algal blooms and temporal accumulation of nitrite during denitrification processes are highly dangerous and may restrict the reservoir's use. Sampling stations with the highest denitrification rates in the bottom sediments were placed near the islands and bays of the Sulejów reservoir, where the conditions for the organic matter accumulation seemed advantageous (BEDNAREK et al. [3]).

***In situ* denitrification measurements.** The denitrification rate in the sediments of the Sulejów reservoir was measured in summer 1998 at 8 sampling stations (TOMASZEK [18], CZERWIENIEC [7], BEDNAREK et al. [3]). This rate was estimated based on the products of gaseous reaction, calculated from the total N_2 flux out of the sediment, and presented in μmol N_2 m^{-2} h^{-1}. Organic substances in the sediments collected were determined as the products of denitrification reaction according to PIPER [17] and JANUSZKIEWICZ [11].

Microbiological analyses. For preliminary comparison of denitrification rate using the *in situ* chamber method, 7 samples of sediments for bacteriological testing were collected in autumn 2000 at the same sampling stations.

Occurrence of denitrifying bacteria was determined by means of the most probable number (MPN) and plate counting (PC) methods (DĄBROWSKA [8], BEDNAREK et al. [3], GAMBLE et al. [9]). Identification of denitrifying bacteria was made according to the colouring Gram method, production of fluorescent pigment on King's A and B, starch hydrolysis, presence of cytochromium oxidases (BURZYŃSKA [6]), the API 20 NE (bioMerieux), a standardised micro-method combining 8 conventional tests and 12 assimilation tests.

Analytical tests were carried out according to HERMANOWICZ et al. [10]. The optical density of the bacterial culture was measured spectrophotometrically ($\lambda = 580$ mn).

3. RESULTS AND DISCUSSION

Denitrifying bacteria naturally occur in sediments, surface waters, soils and wastes due to their ability to use different compounds as a carbon source (KNOWLES [14]). It has been known that due to the fermentation process bacteria can survive in unfavourable anaerobic conditions, being temporarily deprived of oxidised nitrogen compounds (JORGENSEN and TIEDJE [12]). Depending on the type of environment, different species can predominate or coexist within the denitrifier population (BŁASZCZYK [4]).

Table 1

The number of denitrifying bacteria in relation to the total number of bacteria in the sediments from selected environmental stations determined by the MPN method

Number of bacteria × 10^4	Station No.						
	1	2	3	4	5	6	7
Number of heterotrophic bacteria	16320	24300	675	3688	6260	1.35	40600
Number of denitrifying bacteria	2580	243	0.31	77.8	10.3	0.017	85.5

Table 2

The number of denitrifying bacteria in relation to the total number of bacteria in the sediments determined by the PC method

Station No.	Number of bacterial colonies × 10^6 cell/g d.m.	Number of isolates analysed	% content of denitrifiers	Number of denitrifiers × 10^6
1	3.03	205	16.5	0.50
2	0.21	142	9.8	0.021
3	13.2	175	4.6	0.61
4	1.08	119	26.0	0.28
5	0.12	191	7.8	0.01
6	3.32	120	10.8	0.36
7	3.61	70	10.0	0.36

The data presented in tables 1 and 2 allow comparison of the amount of denitrifying bacteria in the bottom sediments of the Sulejów reservoir with a total number of bacteria estimated by the MPN and PC methods. The number of all bacteria counted by means of the PC method was usually by 1–2 orders of magnitude higher than the number of bacteria determined using the MPN method, excluding stations 3 and 6. These results suggested that growth of some bacteria taken from natural environment could be inhibited when grown on plates, which resulted in the denitrifying microorganism selection (BŁASZCZYK [4]). However, the amount of denitrifying bacteria in the sediment estimated by the MPN method ranged from 0.05% to 15.8% and from 4.6% to 26% of the total microflora if the PC method was used.

In all sampling stations, the predominant denitrification bacteria are the members of *Pseudomonas* genus (table 3). This genus accounts for 66.7% (station 5) to 100% (stations 4 and 7) of the total number of denitrifying bacteria; *P. fluorescens* (stations 2, 4 and 7) was most frequently isolated species. In the sediment at station 6, bacteria from *Pseudomonas* (46.2%) and *Bacillus* (53.8%) genera coexist. The predominance of these species corresponded well with the results obtained by GAMBLE et al. [9] and JORGENSEN and TIEDJE [12]. GAMBLE et al. [9] determined 34% of bacteria representing 146 strains selected from 19 different samples as *P. fluorescens.* JORGENSEN and TIEDJE [12] isolated up to 75% of *P. fluorescens* from sediments taken from the eutrophic Wintergreen Lake and the Saginaw River.

Table 3

Percentage of denitrifying bacteria species in the sediment samples

Station No.	Genus			
	Pseudomonas	*Alcaligenes*	*Bacillus*	Not determined
1	68.6	31.4	0	0
2	92.8	0	7.2	0
3	75.0	0	12.5	12.5
4	100	0	0	0
5	66.7	26.7	6.6	0
6	46.2	0	53.8	0
7	100	0	0	0

The domination of *P. fluorescens* is associated with its potential for using a broad range of different organic compounds present in sediments (in preference to glucose), its short generation time (Kunicki-Goldfinger, 1982), and its ability to produce toxins which protect it from phagocytosis exhibited by protozoans (KNORR [13], BŁASZCZYK [4], [5]).

Due to the denitrification process, highly unfavourable phenomenon such as toxic nitrite accumulation may occur in the bottom sediment of the reservoir (table 4). The participation of nitrite-accumulating bacteria is alarmingly high – close to 50% in the case of the bacteria isolated from natural microflora. All isolated denitrifiers accumulate toxic nitrite during the denitrification process, with the strains of *P. stutzeri* predominating. These results suggest that further enrichment of the soil or surface water with nitrate compounds may endanger the balance of the natural environment or even exclude environment from using because of the accumulation of toxic nitrite products (BŁASZCZYK [4]).

Table 4

Significance (%) of strains with different ability to accumulate nitrites in denitrification process

Accumulation of $N-NO_2$ during nitrate reduction (140 mg NO_3/dm^3)		
Without accumulation	Low accumulation <70 mg $N-NO_2/dm^3$	High accumulation > 70 mg $N-NO_2/dm^3$
39.6	12.5	47.9

Spatial variation in the denitrification rate measured by the *in situ* chamber method was determined mainly through different substrate availability in the sediment structure (CZERWIENIEC [7]). The *in situ* denitrification rate ranged from 0 to 677 μmol $N_2/m^2/h^1$, and the values obtained were characteristic of eutrophic reservoirs. The positive correlation between the denitrification rate and the content of organic carbon in the bottom sediments ($r = 0.84$) (BEDNAREK et al. [3]) was found.

The presented results indicated that the denitrification rate and the amount of denitrifying bacteria depended on the availability of organic substrate. The content of organic carbon in sediments at station 4 was the highest (6425.3 μg C-org./g dry matter), where the highest denitrification rate was also measured (677 μmol $N_2/m^2/h$). At stations 2 and 3 the substrate availability was the lowest (465.8 and 302.8 μg C-org./g dry matter), which was reflected in the lowest denitrification rate (0 and 15 μmol $N_2/m^2/h$).

Table 5

Chemical composition of bottom sediments and average denitrification rate

Station No.	Composition of sediments (% dry weight)			Denitrification rate (μmol $N_2 \cdot m^{-2} \cdot h^{-1}$)
	Organic matter	Organic carbon	Total nitrogen	Average
1	0.89	0.94	0.58	56
2	0.41	0.60	0.06	0
3	0.61	0.54	0.18	15
4	20.30	10.32	9.29	677
5	21.77	9.33	8.25	267
6	17.72	8.51	7.66	317
7	4.23	2.49	0.43	278
8	10.09	5.35	3.15	344

A positive correlation between the content of organic carbon in sediment samples and the percentage of denitrifying bacteria was found ($r = 0.86$). The highest percentage share of denitrifiers (26%) was determined at station 4, where the content of organic carbon approached the highest value (6425.3 μg C-org./g dry matter). The low-

Table 6

Correlation between the amount of organic carbon (μg C-org./g d.m.) and percentage of denitrifying bacteria

Station No.	Organic carbon (μg /g d.m.)	% of denitrifiers	Correlation
1	891.9	17.0	
2	465.8	9.8	
3	302.8	4.6	
4	6425.3	26.0	0.86
5	498.2	7.8	
6	1683.3	10.8	
7	2039.9	10.0	

est share of denitrifiers in the sediments from selected environmental stations was found at station 3 (4.6%), where the content of organic carbon reached its lowest value (302.8 μg C-org./g dry matter).

4. CONCLUSIONS

1. In the sediment, the amount of denitrifying bacteria estimated by the MPN method and the PC method reached, respectively, 0.05%–15.8% and 4.6%–26% of total microflora.

2. The most frequently isolated bacteria were identified as the members of the genera of *Pseudomonas, Alcaligenes* and *Bacillus*, with a predomination of the *Pseudomonas* genus and the *P. fluorescens* and *P. stutzeri* species.

3. The positive correlations between the content of organic matter in sediments and the amount of denitrifying bacteria ($r = 0.86$; $p < 0.05$) and between denitrification rate and the content of organic carbon ($r = 0.84$) were found.

4. An alarmingly high number – about 50% – of the bacteria isolated from natural microflora accumulate toxic nitrite in total denitrification activity.

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REFERENCES

[1] AMBROŻEWSKI Z., *Monografia zbiornika Sulejówskiego*, WKiL, Warsaw, 1980, pp. 1–184.

[2] AZNAR R., ALCAIDE E., GARAY E., *Numerical taxonomy of Pseudomonads isolated from water, sediment and eels*, System. Appl. Microbiol., 1992, 14, 235–264.

[3] BEDNAREK A., ZALEWSKI M., TOMASZEK J., CZERWIENIEC E., *Znaczenie procesu denitryfikacji dla dynamiki azotu w Sulejówskim Zbiorniku Zaporowym*, Post. Inżyn. Środ., I Ogólnopolska Konferen. Naukowo-Techn., Rzeszów-Polańczyk, 1999, 203–211.

[4] BŁASZCZYK M., *Denitrifying sediment bacteria from man-made reservoir of fertilizer nitrogen plant wastewaters (RFNPW)*, Acta Microbiol. Polon., 1997, 46, 313–323.

[5] BŁASZCZYK M., *Denitrification of nitrate by Pseudomonas stutzeri*, Acta Microbiol. Polon., 1995, 44, 149–160.

[6] BURZYŃSKA H., *Metody wykrywania i identyfikacji pałeczek z grupy Pseudomonas–Achromobacter i grup pokrewnych*, Roczniki PZH, 1964, 15, 171–181.

[7] CZERWIENIEC E., *Denitryfikacja i zużycie tlenu w osadach dennych wybranych zbiorników zaporowych*, dysertacja doktorska, 1998.

[8] DĄBROWSKA E., *Bakterie denitryfikacyjne w osadach Sulejowskiego Zbiornika Zaporowego*, praca magisterska. Instytut Mikrobiologii, Uniwersytet Warszawski, 2001.

[9] GAMBLE T.N., BETLACH M.R., TIEDJE J.M., *Numerically dominant denitrifying bacteria from world soils*, Appl. Environ. Microbiol., 1977, 33, 926–939.

[10] HERMANOWICZ W., DOŻAŃSKA W., DOJLIDO J., KOZIOROWSKI B., *Fizyczno-chemiczne badanie wody i ścieków*, Arkady, Warszawa, 1976.

[11] JANUSZKIEWICZ T., *Studia nad metodyką analizy składu współczesnych osadów dennych jezior*, Zeszyty Naukowe ART, Olsztyn, Ochrona Wód i Rybactwo Śródlądowe, 1978, 8, 3–30.

[12] JORGENSEN K.S., TIEDJE J.M., *Survival of denitrifiers in nitrate-free, anaerobic environments*, Appl. Environm. Microbiol., 1993, 59, 3297–3305.

[13] KNORR M., *Studies on the biological barrier to bacteria and viruses during vertical filtration through soil*, Schweiiz. Z. Hydrobiol., 1960, 22, 493–502.

[14] KNOWLES R., *Denitrification*, Microbiol. Rev., 1982, 46, 43–70.

[15] MADIGAN M.T., MARTINKO J.M., PARKER J., *Brock Biology of Microorganisms*. 8th ed., Prentice Hall International, Inc., 1997.

[16] MOSELLO R., BARBIERI A., BRIZZIO M., CALDERONI A., MARCHETTO A., PASSERA S., ROGORA M., TARTARI G., *Nitrogen budget of Lago Maggiore: the relative importance of atmospheric deposition and catchment sources*, J. Limnol., 2001, 60 (1), 27–40.

[17] PIPER C.S., *Soil and plant analysis*, PWN, Warsaw, 1957.

[18] TOMASZEK J., *Biochemical transformation of nitrogen compounds in the bottom sediments of the superficial waters*, Rzeszów Technical University Journal, 1991, 13, 1–155.

[19] XU B., ENFORS S.O., *Influence of nitrate starvation on nitrite accumulation during denitrification by Pseudomonas stutzeri*, Appl. Microbiol. Biotechnol., 1996, 45(1/2), 229–235.

PRZEBIEG PROCESU DENITRYFIKACJI W OSADACH DENNYCH SULEJOWSKIEGO ZBIORNIKA ZAPOROWEGO

Celem badań było określenie znaczenia procesu denitryfikacji w zbiorniku Sulejowskim przez porównanie tempa procesu denitryfikacji mierzonego metodą *in situ* i liczebnością populacji bakterii denitryfikacyjnych.

Oszacowano, że bakterie denitryfikacyjne stanowią od 0,05 do 15,8% wszystkich bakterii heterotroficznych oznaczonych metodą NPL oraz od 0,4 do 26% bakterii oznaczonych metodą płytkową. Wykazano dodatnią korelację między zawartością węgla organicznego a liczbą bakterii denitryfikacyjnych (r = 0,86) oraz tempem denitryfikacji a zasobnością pokarmową (r = 0,84). Prawie 50% izolatów bakterii denitryfikacyjnych w procesie oddychania azotanowego kumuluje przejściowo lub następczo azotyny.

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