

## Denitrification in the sediment of a eutrophic reservoir measured with the isotope pairing technique

Renata Gruca-Rokosz\*, Janusz A. Tomaszek, Piotr Koszelnik

*Rzeszów University of Technology  
Faculty of Civil and Environmental Engineering  
Department of Chemistry and Environmental Engineering  
ul. W. Pola 2, 35-959 Rzeszów, Poland*

**Key words:** denitrification,  $^{15}\text{N}$ , stable isotope, bottom sediment, Rzeszów Reservoir

### Abstract

Denitrification rates in the bottom sediment of the Rzeszów Reservoir (southeastern Poland) were measured using the isotope pairing technique. At the stations studied rates were reported in the range of about 26 to 610  $\mu\text{mol N}_2 \text{ m}^{-2} \text{ h}^{-1}$ , with the figures generally highest in summer. Simple and multiple regression analyses of relationships with selected abiotic factors gave rise to a model that revealed a statistically significant influence on rates of denitrification in the bottom sediment of the Rzeszów Reservoir due to concentrations of nitrate in overlying water, temperature, and the organic matter content in the sediment. The present study confirms that nitrate concentration in the overlying water is the main factor controlling sedimentary denitrification.

---

\* corresponding author: [renatagr@prz.rzeszow.pl](mailto:renatagr@prz.rzeszow.pl)

## INTRODUCTION

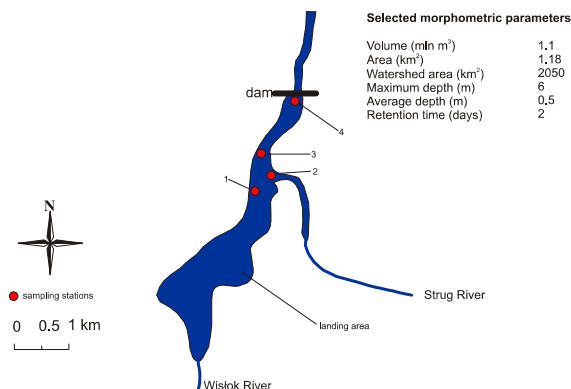
Denitrification is a microbial process that converts nitrate to dinitrogen gas under anoxic conditions (Tiedje 1988). It is of particular interest because it represents N removal from an aquatic ecosystem. In characterizing denitrification, it is essential that an ecosystem's handling of increased N loads be understood. Previous mass-balance studies in the Rzeszów Reservoir point to high nitrogen loading and a rapid acceleration of eutrophication in this water body, while also suggesting that sediment denitrification could be an important retention mechanism (Koszelnik and Tomaszek 2002). The quantification of denitrification rates in its sediment was therefore deemed important.

Thus, the aim of the present study was to measure sediment denitrification rates at different sites in the Rzeszów Reservoir, and to identify factors influencing the process. The factors considered were temperature (T), nitrate concentration in water ( $\text{N-NO}_3^-$ ), and sediment organic matter (OM). Multiple regression analysis was used to determine the simultaneous influence on denitrification rates.

## MATERIALS AND METHODS

Investigations of denitrification process were conducted using samples from Rzeszów Reservoir obtained in 2002 and 2003. During this time, 18 measurement series were carried out – three in spring (April – May), nine in summer (June – August), and six in fall (September–November). Constructed in 1973, the Rzeszów Reservoir is located on the Wisłok River in southeastern Poland. After 20 years of exploitation, its water volume decreased considerably, and the fast growth of aquatic plants encroached on previously open surface water. Despite an attempt at reconstruction in 1996, the reservoir has mostly silted up (Tomaszek 1995). Both of the reservoir's tributaries, the Wisłok and Strug rivers, are highly polluted with nutrients. The drainage basin of the reservoir is mainly agricultural with a few industrial centers.

Sediment and overlying water samples were collected at four stations (Fig. 1). Undisturbed sediment cores were collected with Plexiglass tubes from 0.5 m depth to determine denitrification rates. These were measured using the Isotope Pairing Technique (IPT) (Nielsen 1992). For each core,  $^{15}\text{NO}_3^-$  was added to the overlying water to obtain final concentrations of labeled nitrate less than 10% of the in situ concentration. The cores with the overlying water were then closed and incubated for 1.5 hours. The temperature that occurs in the reservoir was maintained in the cores during that time. Water above the sediment was stirred gently by a small Teflon-coated magnet suspended 5 cm above the sediment to ensure the homogeneous mixing of the water column (without disturbing the



**Fig. 1.** Locations of sampling stations and some of the physical features of the Rzeszów Reservoir.

sediment cores). When the incubation was stopped, samples for  $^{15}\text{N}$  analysis of  $\text{N}_2$  were collected after carefully mixing the sediment (porewater) with the overlying water. Bacterial activity was stopped after the addition of 250  $\mu\text{l}$  of 7 M  $\text{ZnCl}_2$  (volume of sediment-water slurry was 7 ml). The  $^{15}\text{N}$ -labeled  $\text{N}_2$  was extracted from the water in glass gastight vials by replacing 2 ml of the water with a He headspace and shaking vigorously for 5 minutes. The gas phase was then analyzed for the concentrations and isotopic distribution of  $^{29}\text{N}_2$  and  $^{30}\text{N}_2$  on a combined gas chromatograph-isotope ratio mass spectrometer (IRMS DELTA<sup>+</sup> Finnigan on line with GC/CIII). The isotope pairing approach was used to calculate the rate of denitrification (Nielsen 1992). Details of incubation and calculations can be found in Risgaard-Petersen and Rysgaard (1995).

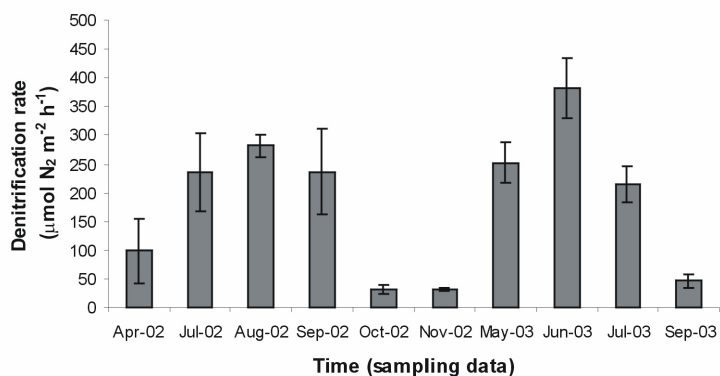
Nitrate concentrations in overlying water were determined using colorimetric analysis (reaction with 2,6-dimethylphenol, photometer WTW PhotoLab S12). Loss on ignition was determined as the weight loss after heating the dried sediment in 550°C for 4 h. The *Statistica PL* program (StatSoft) was used for statistical calculations (ANOVA, simple and multiple regression analyses).

## RESULTS AND DISCUSSION

The denitrification rates noted for the Rzeszów Reservoir during the study were about 26 to 610  $\mu\text{mol N}_2 \text{ m}^{-2} \text{ h}^{-1}$  (at reported temperatures in the range of 7-23°C). The values obtained are characteristic for eutrophic ecosystems (Tomaszek 1991). As differences in denitrification rates between stations proved non-significant (ANOVA,  $F = 0.7$ ,  $p = 0.553$ ), the influence of the

selected abiotic factors on denitrification rates were examined for each date (with no separation into individual stations). The mean temporal changes in sedimentary denitrification rates are as shown in Figure 2.

Table 1 presents mean seasonal changes of the denitrification rate and the characteristics of water and sediment. The seasonal diversification of denitrification rate was confirmed. The highest average denitrification activity (about  $383 \mu\text{mol N}_2 \text{ m}^{-2} \text{ h}^{-1}$ ) and the lowest average rate of the process (about  $30 \mu\text{mol N}_2 \text{ m}^{-2} \text{ h}^{-1}$ ) were noted in June 2003 and October 2002, respectively. This was probably because of the temperature differences during the study. Temperature and denitrification activity were correlated ( $R^2 = 0.61$ ,  $p < 0.0001$ ), which agree with previous studies attesting to the influence of seasonal temperature patterns (Zimmerman and Benner 1994, Pind et al. 1997).



**Fig. 2.** Temporal changes in denitrification rates ( $\mu\text{mol N}_2 \text{ m}^{-2} \text{ h}^{-1}$ ) for the Rzeszów Reservoir in the years 2002 and 2003.

**Table 1**

Mean seasonal changes in the denitrification rate, and characteristic of water and sediment for the Rzeszów Reservoir in the years 2002 and 2003.

	Denitrification rate [ $\mu\text{mol N}_2 \text{ m}^{-2} \text{ h}^{-1}$ ]			Temperature of overlying water [°C]			N-NO <sub>3</sub> <sup>-</sup> in overlying water [mg dm <sup>-3</sup> ]			Sediment organic matter [%]		
	Mean	SD	n	Mean	SD	n	Mean	SD	n	Mean	SD	n
Spring	210.45	124.88	11	16.18	4.00	11	1.30	0.28	11	9.00	1.18	11
Summer	280.22	101.95	36	19.94	1.11	36	1.46	0.31	36	7.95	1.40	36
Fall	136.49	157.55	24	14.17	5.79	24	1.17	0.39	24	7.32	1.37	24

Livingstone et al. (2000) concluded that this temperature dependence probably reflected the role of denitrification as a process following on from the decomposition of organic matter to ammonium and its subsequent nitrification.

A previous study by the present authors (Gruca-Rokosz and Tomaszek 2006) revealed an inverse relationship for temperature coupled denitrification–nitrification. Throughout the measurement period, and especially at higher temperatures, nitrate diffusing into the reduced zone of bottom sediment from the overlying water were the main source of nitrate for the denitrification process. The positive and linear correlation between nitrate concentrations in overlying water and denitrification rates ( $R^2 = 0.67$ ,  $p < 0.0001$ ) attests to this importance of the water column environment in controlling the denitrification process in the bottom sediments of the Rzeszów Reservoir. According to Newell et al. (2002), where the nitrate concentrations in overlying water are low to moderate, the rates of denitrification may be limited by the reduced diffusion of nitrate into the anaerobic sediment zone.

While the great majority of scientists report a positive dependent relationship between nitrate concentration in water and denitrification (e.g., Nielsen et al. 1995, Wall et al. 2005), failures to confirm this relationship have also been noted (e.g., Pfenning and McMahon 1996).

Denitrification usually requires a supply of readily-biodegradable organic C (Knowels 1982), and the present work confirms that an increase in sedimentary organic matter accelerates denitrification activity ( $R^2 = 0.19$ ,  $p < 0.001$ ). Other studies have also indicated there is such a relationship with sediment OM (e.g., Tomaszek 1991, Rysgaard et al. 1993). These findings together confirm that the presence of electron donors, i.e., carbon compounds, is an essential factor controlling dissimilatory nitrate-reduction processes.

Determinations of the simultaneous influences of the aforementioned variables using multiple regression analysis (Table 2) showed that  $\text{N-NO}_3^-$  concentration in overlying water at the Rzeszów Reservoir sites is a significant variable and accounts for only 11% of the variation in denitrification potential.

**Table 2**

Results of multiple regression analysis of selected parameters which influence the denitrification rate in the Rzeszów Reservoir.

$R = 0.88$ , $R^2 = 0.77$ , Standard error of estimation: 68.6, $F(3.67) = 75.96$ , $p < 0.001$ , $n = 71$				
	Coefficient B	Standard error of B	Significance level $p$	Semi-partial correlation
Intercept	-364.7	49.5	< 0.001	
Temperature	12.0	2.6	< 0.001	0.27
Nitrate	193.1	33.8	< 0.001	0.33
Sediment organic matter	15.0	6.1	0.016	0.14

Temperature and sediment organic matter are in turn responsible for about 7.5% and 2%, respectively, of the variation. However, the multiple regression model obtained is capable of describing about 77% of the overall variation in rates of the denitrification process ( $R^2 = 0.77$ ). The model was obtained for the data from all stations throughout the measurement period.

The present study confirming nitrate concentration as the main factor controlling sedimentary denitrification contrasts a similar study by Barnes and Owens (1998), who observed nothing more than a marginal influence of water-column concentrations of nitrate on denitrification, and therefore concluded that the organic status of sediments might be the major controlling factor. Although in the current study trends were noted in sediment denitrification associated with temperature and sedimentary organic matter, these variables explained only a small part of the variability in sediment denitrification compared to previous works in other aquatic ecosystems (e.g., Livingstone et al. 2000).

#### ACKNOWLEDGEMENTS

This work was supported by grant 6 P04G 127 21 from the Poland's State Committee for Scientific Research.

#### REFERENCES

- Barnes J., Owens N.J.P., 1998, *Denitrification and nitrous oxide concentrations in the Humber estuary, UK, and adjacent coastal zones*, Mar. Pollut. Bull., 37: 3-7
- Gruca-Rokosz R., Tomaszek J., 2006, *Quantification of denitrification rates in sediments of Solina Reservoir*, Proceedings of Conference: Progress in Environmental Engineering, Rzeszów – Bystre: 403-12, (in Polish)
- Knowles R., 1982., *Denitrification*, Microbiol. Rev., 46: 43-70
- Koszelnik P., Tomaszek J.A., 2002, *Loading of the Rzeszów reservoir with biogenic elements – mass balance*, Environment Protection Engineering, 28(1): 99-105
- Livingstone M.W., Smith R.V., Laughlin R.J., 2000, *A spatial study of denitrification potential of sediments in Belfast and Strangford Loughs and its significance*, The Science of Total Environment 251/252: 369-80
- Newell R.I.E., Cornwell J.C., Owens M.S., 2002, *Influence of simulated bivalve biodeposition and microphytobenthos on sediment nitrogen dynamics: A laboratory study*, Limnol. Oceanogr., 47(5): 1367-79
- Nielsen K., Nielsen L.P., Rasmussen P., 1995, *Estuarine nitrogen retention independently estimated by the denitrification rate mass balance methods: a study of Norsminde Fjord, Denmark*, Mar. Ecol. Prog. Ser., 119: 275-83
- Nielsen L.P., 1992, *Denitrification in sediment determined from nitrogen isotope pairing*, FEMS Microbiol. Ecol., 86: 357-62
- Pfenning K.S., McMahon P.B., 1996, *Effect of nitrate, organic carbon, and temperature on potential denitrification rates in nitrate-rich riverbed sediments*, Journal of Hydrology, 187: 283-95

- Pind A., Risgaard-Petersen N., Revsbech N.P., 1997, *Denitrification and microphytobenthic  $\text{NO}_3^-$  consumption in Danish lowland stream: diurnal and seasonal variation*, *Aquat. Microb. Ecol.*, 12: 275-84
- Risgaard-Petersen N., Rysgaard S., 1995, *Nitrate reduction in sediments and waterlogged soil measured by  $^{15}\text{N}$  techniques* [in:] *Methods in applied soil microbiology and biochemistry*, Eds. Alef K., Nannipieri P., London, Academic Press, pp. 279-88
- Rysgaard S., Risgaard-Petersen N., Nielsen L.P., Revsbech N.P., 1993, *Nitrification and denitrification in lake and estuarine sediments measured by the  $^{15}\text{N}$  dilution technique and isotope pairing*, *Appl. Environ. Microbiol.*, 59(7): 2093-98
- Tiedje J.M., 1988, *Ecology of denitrification and dissimilatory nitrate reduction to ammonium*. [in:] *Biology of anaerobic microorganisms*, Ed. Zehnder A.J.B., New York, John Wiley & Sons, pp.179-244
- Tomaszek J., 1991, *Biochemical transformation of nitrogen compounds in the sediment of surface water*, *Oficyna Wydawnicza PRz*, 84 (13):1-155, (in Polish)
- Tomaszek J.A., 1995, *Protection and reclamation of Rzeszów Reservoir*, *Proceedings of XVI Symposium of Polish Committee IAWQ, Zabrze*, pp.133-50 (in Polish)
- Wall L.G., Tank J.J., Royer T.V., Bernot M.J., 2005, *Spatial and temporal variability in sediment denitrification with an agriculturally influenced reservoir*, *Biogeochemistry*, 76: 85-111
- Zimmerman A.R., Benner R., 1994, *Denitrification, nutrient regeneration and carbon mineralization in sediments of Galveston Bay, Texas, USA*, *Mar. Ecol. Prog. Ser.*, 114: 275-88