Presence of nitrogen cycle bacteria in the water of Strezevo reservoir

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Abstract

Strezevo reservoir is important source of water supply for public utilities, agriculture and industry. An excessive inflow of biogenic elements caused by agricultural and domestic effluents is the main cause of deterioration of water quality of the reservoir, which reflects on the composition and the number of bacterial communities. Aim of this investigation was to determine seasonal and vertical changes in the number of nitrogen cycle bacteria (ammonifying, nitrifying, and denitrifying bacteria) in the water of Strezevo reservoir during the period of one year. Denitrifying bacteria dominated in 55,55% of water samples. Nitrogen cycle bacteria were found more numerous in depth of 5m of the reservoir, in different investigative periods. This stays in relationship with existing physicochemical conditions of the water, for example: temperature, pH value, oxygen contents, organic and mineral compound of nitrogen contents. Season changes showed the highest rates in early summer and late autumn, when concentrations of organic matter were higher.

Key words: Strezevo reservoir, nitrogen cycle bacteria

Introduction

Strezevo reservoir (Fig. 1) is located in the south western part of the Republic of Macedonia and it provides water for public utilities, for irrigation, for the industry, etc. It has surface area of 450 ha, maximum depth of 70m and average depth about 45m. This reservoir is fed by Semnica River and Alimentation Canal.

Figure 1. Strezevo reservoir

The aim of present paper was to determine season and vertical changes in the number of ammonifying, nitrifying, and denitrifying bacteria, which are responsible for the nitrogen cycling process in the water of Strezevo reservoir.

For normal development all living organisms require between 30-40 elements and their availability depend on different biogeochemical cycles, which make possible to use them again and again, transforming them and re-circulating them through the different living environments (www.biology.qmul.ac.uk/research/staff/s-araya/Hand-Outs.html).

Among those cycles, nitrogen cycle is most complex and very important for the life on earth. These bacteria which carried out nitrogen cycling process play a vital role in water quality control (Jun *et al*, 2000). Changes in their number are usually a reflection of the current state of the water in the reservoir.

Materials and Methods

Microbiological researches were carried out at 7 research sites, 3 from surface waters at sites P_1, P_2, and P_3 and 4 from different depths (5m, 10m, 15m, and 20m) at site P_2. Samples were collected at monthly intervals in all seasons.

Microbiological researches were completed with measurements of some physic-chemical parameters, such as: water temperature, pH, dissolved oxygen, BOD_5, permanganate value and nutrients. Water samples were collected in Ruttner bottle and taken to the laboratory. Time from the moment of taking the samples to conducting the analyses did not exceed 3 hours.

Physic-chemical parameters were determined according to Standard Methods (1992). Ammonifying bacteria (Fig. 2) were cultivated on nutrient broth for 5-7 days at a temperature of 22°C, by the method proposed by Rodina (1965).

Figure 2. Ammonifying bacteria cultivated on nutrienth broth

The plate method was used for the determination of nitrifying (Fig. 3) and denitrifying bacteria (Fig. 4). Their number was determined using mineral agars as the medium, prepared in our laboratory. The bacterial colonies that grew up were counted after 5-7 days of incubation at a temperature of 22°C. The results were calculated per 1ml of water.

Figure 3. Nitrifying bacteria cultivated on mineral agar

Figure 4. Denitrifying bacteria cultivated on mineral agar

Results

It follows from the data presented in Tab.1 concerning the presence of ammonifying bacteria, Fig. 5 concerning the number of nitrifying bacteria and Fig.6 concerning the number of denitrifying bacteria, that these data depend on the season and depth at which the researches were conducted.

Ammonifying bacteria were found in almost all examined waters, but mostly in autumn and winter.

Nitrifying bacteria fluctuated between 255-6300 ind/1ml, and their maximum number was found in early summer and minimum in autumn. At the depth of 5 m a distinctly greater number of nitrifying bacteria was observed in summer, than in the other depths.

Denitrifying bacteria fluctuated between 195-14400 ind/1ml and their maximum number was found in early summer and minimum in autumn. As nitrifying bacteria, denitrifying bacteria also had maximum number at 5m depth. Denitrifying bacteria in ratio with nitrifying bacteria were dominant in 55.55% of examined waters.

	M	J	J	A	S	O	N	D	J
P1V2-s.	+	+	+	+	+-	+	+	+	+
P2V2-s.	+	+	+	+	+-	+	+	+	+
P2V2-5m	+	+	+-	-	-	+	+	+	+
P2V2-10m	+	+	+	+	-	+	+	+	+
P2V2-15m	+	+	+-	+	-	+	+	+	+
P2V2-20m	+	-	+	-	-	+	+	+	+
P3V2-s.	+	+	+	+	-	+	+	+	+

Table 1. Estimation of ammonifying bacteria with titar method in nutrient broth in reservoir in the period between May–January

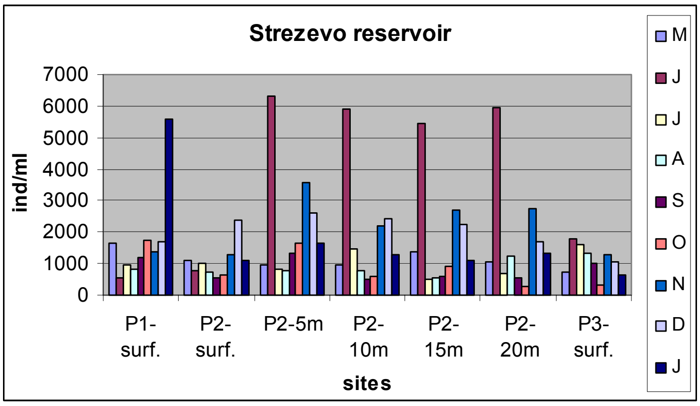

Figure 5. Comparative presentation of the total number of nitrifying bacteria in reservoir in the period between May – January

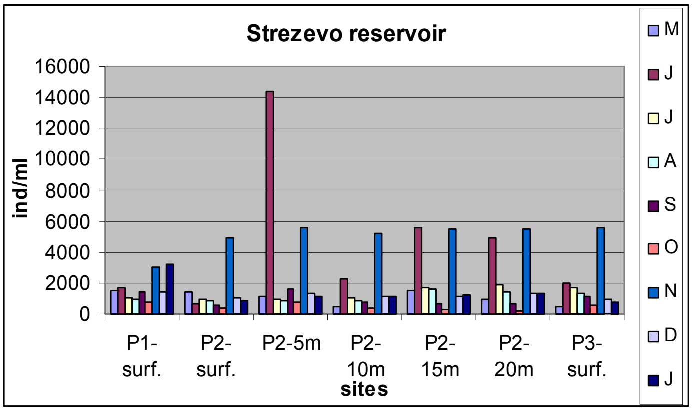

Figure 6. Comparative presentation of the total number of denitrifying bacteria in reservoir in the period between May – January

Discussion

Process called ammonification is carried out by aerobic and anaerobic bacteria, such as: *Pseudomonas, Vibrio, Proteus, Serratia, Bacillus* and *Clostridium* (Herbert, 1999). Ammonifying bacteria during the decomposition of nitrogenous organic matters from the dead organisms, or from their waste, converted them to ammonia, which is assimilated by phytoplankton, or heterotrophic bacteria.

Ammonifying bacteria in Strezevo reservoir were found in all examined waters, except in June at 20m depth, in August at 5m and 20m depth, in September at 5m, 10m, 15m, and 20m depth and also in the surface water at site P3. The examined waters without ammonifying bacteria were also without ammonia.

Ammonifying bacteria were mostly found in the middle of the autumn and in the early winter. This suggests that in that seasons complex nitrogenous matters were present in the waters. Increased presence of the ammonifying bacteria was the result of increased inflow of water with allochtonic organic matter in the reservoir.

Increased presence of the ammonifying bacteria correlated with increased amount of ammonia in the water, which is consistent with the observations of Lokoska (2000). In the opinion of Lokoska (2000) increased amount of ammonia may be result of absence of phytoplankton, so ammonia was unused. In the summer, ammonifying bacteria were less numerous, similar to examinations of Donderski and Kalwasińska (2003).

There are many studies about the relationship between ammonifying bacteria and physic-chemical factors.

Jørgensen (1989), showed that the number of ammonifying bacteria was higher when the number of denitrifying bacteria was lower. Mc Cready *et al* (1983) reported that ammonifying bacteria have a more versatile metabolism and hence may grow under nitrate free conditions. Jun *et al* (2000) found that ammonifying bacteria have high correlation with dissolved oxygen content, which accelerates the decomposition process of organic matter.

Another, very important group of bacteria engaged in nitrogen cycle is nitrifying bacteria which oxidised reduced nitrogenous matter to nitrite and then to nitrate (Hesselsøe, 2001), and they make link between reduced and oxidized nitrogen compounds (Prinčič *et al*., 1998).

First step of nitrification is carried out by ammonia oxidizing bacteria such as: *Nitrosomonas, Nitrosococcus, Nitrosospira, Nitrosolobus and Nitrosovibrio* (Herbert, 1999), which oxidize ammonia into nitrite. Second step of nitrification is carried out by nitrite oxidizing bacteria, such as: *Nitrobacter, Nitrosococcus, Nitrospina* and *Nitrospira* (Herbert, 1999), which oxidize nitrite into nitrate.

Nitrifying bacteria in Strezevo reservoir were found with maximal values in the early summer, when phytoplankton growth was maximal. Also, maximal values of these bacteria were found in the raining periods because of the increased inflow of allochtonic organic matter.

Cèbron *et al*. (2003) reported that large biomass, number and nitrifying activity into the Seine River occurred in summer, at a high temperature, when amounts of dissolved oxygen and ammonia were lower, and amounts of nitrate were higher.

In this study, lower number of nitrifying bacteria was found in the late summer and early autumn. Absence of ammonia and upper amounts of nitrate in the water of Strezevo reservoir in September were the result of oxidation by the nitrifying bacteria.

The relationship between different physic-chemical factors and the number of nitrifying bacteria has received attention of many researches.

Prinčič *et al* (1998) reported that nitrification depended on these factors: water temperature, pH, amount of dissolved oxygen and substrate concentration.

Nitrification of ammonia was higher, when oxygen concentration was higher, because nitrifying bacteria would use dissolved oxygen for the nitrification process.

Cèbron *et al*. (2003) reported that some nitrifying bacteria can survive in low oxygen concentrations. In Hamilton Harbour Roy *et al* (1996) showed that nitrifying bacteria may have great importance for summer hypolimnetic O_2 depletion.

The number of nitrifying bacteria is linked with the presence of nitrogenous organic and inorganic compounds, so in olygotrophic waters with low amounts of that compounds the number of nitrifying bacteria would be lower, but in eutrophic waters, their number would be higher.

Denitrification is biological reduction of nitrate to gaseous N_2 and this process has been recognized as a major sequence in the nitrogen cycle (Gamble *et al*, 1977). Denitrifying bacteria, such as: *Pseudomonas, Bacillus* and *Alcaligenes* (Hargreaves,1998), are facultative aerobic bacteria, which in anoxic environments mediated the reduction of nitrate to N_2 and to $NH4+$ (Sørensen,1978).

Denitrifying bacteria in Strezevo reservoir were found with maximal values in the early summer and in the late autumn because of the increased inflow of allochtonic organic matter.

Donderski and Kalwasińska (2003) showed that denitrifying bacteria were most numerous in the summer period which was probably connected with influx into the Chelmzyńskie lake of waters rich in nitrogen substances washed off from the fields and the rise in temperature that stimulates bacterial activity, and also with the oxygen deficit that occurs in connection with the development of aerobic heterotrophic bacteria conducting mineralising processes. The same thing happened in Strezevo reservoir, where the higher number of denitrifying bacteria was in connection with the higher number of aerobic heterotrophic bacteria.

In this study, lower number of denitrifying bacteria was found in October.

According to standards proposed by Kuznecov (1970) water from Strezevo reservoir was eutrophic in 76,2% of cases, and mesotrophic in 23,8% of cases.

Denitrifying bacteria in ratio with nitrifying bacteria dominated in 55, 55% of examined waters.

Many authors found that the number of denitrifying bacteria have high correlation with nitrate content in water (Oremland *et al*, 1984, Sørensen *et al*, 1979, Donderski and Kalwasińska, 2003, Samuelsson,1985).

But, the other authors (Sørensen , 1978, Jørgensen and Tiedje, 1993, Francis *et* al, 1983), showed that denitrifying bacteria existed in nitrate free environments, where they are in latent condition. Some authors found that the number of denitrifying bacteria depend on phosphate content in water. Lokoska (2000) showed that large amounts of phosphates have high correlation with the number of denitrifying bacteria. Jun *et al* (2000) showed that increase of phosphorus content inhibits the activity of denitrifying bacteria, in the pond.

Denitrification can benefit aquatic environments faced with eutrophication, by removing nitrate from shallow waters.

Conclusions

1. Ammonifying bacteria in Strezevo reservoir were found in almost all examined waters. They were mostly found in the middle of the autumn and in the early winter. These suggest that in that seasons complex nitrogenous matters were present in the waters. Increased presence of the ammonifying bacteria was the result of increased inflow of water with allochtonic organic matter in the reservoir.

2. Nitrifying bacteria in Strezevo reservoir were found with maximal values in the early summer, when phytoplankton growth was maximal. Also, maximal values of these bacteria were found in the raining periods because of the increased inflow of allochtonic organic matter.

3. Denitrifying bacteria in Strezevo reservoir were found with maximal values in the early summer and in the late autumn because of the increased inflow of allochtonic organic matter. According to standards proposed by Kuznecov (1970) water from Strezevo reservoir was eutrophic in 76, 2% of cases, and mesotrophic in 23,8% of cases. Denitrifying bacteria in ratio with nitrifying bacteria dominated in 55, 55% of examined waters.

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