IMPACT OF NITRATE IONS CONCENTRATION ON THE DENITRIFICATION PROCESS

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Abstract

Underground and surface water are contaminated by nitrate in several ways. Nitrate originated from agriculture is increasingly growing all over the world due to the extreme use of fertilizers. Nitrate salts reach the groundwater as they percolate through the soil. Some other sources of nitrate in ground and surface water are from uncontrolled discharges of treated or un-treated wastewater from domestic and industrial sources, landfills and animal waste particularly from animal farms.

Various methods for treatment of water from nitrates are known, but the majority of them generate secondary pollutants. An exception is the biological denitrification, at which nitrates are reduced to harmless nitrogen gas, and side waste products practically do not occur.

In this paper the influence of nitrite ions on the process of denitrification was investigated. It is established that the denitrification process depends on the ratio between nitrate and nitrite concentration. The process is inhibited even at low nitrite concentration.

Keywords: *denitrification, Pseudominas denitrificans, nitrate ions, nitrites ions*

INTRODUCTION

The nitrogen-containing compounds are identified as one of the dangerous pollutants in drinking water. Their throwing out into the surrounding environment leads to the eutrophication of the rivers [1] and it presents a serious risk to human's and animal's health.

Nitrates are reduced to nitroamines in result of biochemical processes in the body, which are a precondition for gastritis [2, 3, 4].

The water sources can be contaminated with nitrate in several ways. The increasing use of chemical fertilizers in agriculture in the world is cause of the nitrate pollution. Some studies have shown that groundwater pollution with nitrate is due to agricultural sources [5]. Nitrate ions penetrate the soil and thus they fall into the groundwater. Another way to contaminate surface waters with nitrates is household wastewater, industry, animal farms [6, 7].

The concentration of nitrogen compounds in some industrial wastewater is higher than in surface and groundwater. The biggest problem with nitrogen compounds is the formation of ammonia and nitrates as well as their separation from wastewater. Ammonia in industrial waste water is usually removed by nitrification, which is expressed in the total oxidation of ammonia to nitrate [1]. This method is an unavoidable step in industrial waste water treatment. It was reported in the literature that different industrial wastewaters contain much higher concentrations than 200 mg/l NO_3^- ions. For example, in the production of explosives, chemical fertilizers [8], pectin, cellophane and metals, the nitrates ions concentration in wastewater is higher than 1000 mg/l [3]. Moreover, in the nuclear industry wastes with very high nitrate content are thrown out. At the nuclear weapons production from radioactive materials and also at the radioactive purification, the research laboratories have found nitrate concentrations of more than 50 000 mg/l, mainly nitric acid used for metals purification [9].

Various methods of water purification are known, with which almost complete nitrate removal is achieved. An exception is made in biological denitrification at which nitrates are reduced to nitrogen only [10]. The traditional methods are not able to eliminate the nitrates, since only nitrate separation into new concentrated streams takes place. Compared with traditional methods the biological methods are promising. They provide complete elimination of the pollutant by full nitrate reduction. Although aerobic denitrification, which is reported [11, 12, 13], most denitrification studies are conducted with

facultative anaerobic organisms in oxygen absence [14]. At biological denitrification in the absence of oxygen, denitrifying bacteria use nitrates as the ultimate electron acceptor in their breathing process. The potential of this method is great. Its flexibility makes it applicable for the purification of domestic, agricultural and industrial waters of nitrates of varying concentrations.

Likewise, in contrary to some pollutants whose processing requires the introduction of a certain type of bacteria, the denitrifying bacteria are found everywhere in nature [15]. Furthermore, microbiological nitrate elimination can be a very cost-effective strategy for the treatment of contaminated and waste water [16]. As a result, there is a great interest to biological denitrification, because it is a cheap method, although its rate is very slow and required time to proceed completely, especially when treating waste water with very high concentrations of nitrate [2].

The denitrification process is most often inhibited by the accumulation of nitrite ions. The aim of our study is to determine of the ratio between initial concentrations of nitrate and nitrite ions, in which the denitrification process is inhibited.

MATERIALS AND METHOD

Strain bacteria and culture medium

The strain Pseudomonas denitrificans (NBIMCC 1625), obtained from the Bulgarian National Bank for Industrial Microorganisms and Cell Cultures, was inoculated into a medium containing 10 g/l peptone, 1 g/l yeast, 10 g/l NaCl. The inoculum was grown for 24 hours at 30°C on a shaker at 50 rpm to avoid undesirable aeration.

Crude cell-free extracts

The cell-free extracts were obtained by supersonic disintegration of cells from the cultured strain. Disintegration was performed with a UD-20 disintegrator (manufactured in Poland) at 14 kHz for 30 minutes. During the disintegration, the culture was placed in an ice bath to avoid thermal denaturation of the enzyme.

Concentration of nitrite

Nitrite is determined photometrically by a standard method based on a Griess reaction involving the formation of a raspberry-red azo dye by treating the nitrite-containing sample with sulphanilic acid and naphthyl-1-amine (Grant and Payne, 1981) in acid medium. The standard error of this analysis is ±4%.

Reagents. To analyze the samples by this method it is necessary to prepare the following two reagents: Reagent Griess 1 consists of 0.5 g of sulphanilic acid dissolved in 150 ml of 12% aqueous acetic acid while heating. Reagent Griess 2 contains 0.25 g of α-naphthylamine dissolved in 20 ml of distilled water. This solution boils for 5 min, then is filtered through a hot filter. The filtrate is mixed with 150 ml of a 12% aqueous acetic acid solution.

Procedure: One milliliter of the sample is made up to 50 ml with distilled water in a volumetric flask. Mix 1 ml each of Griess 1 and Griess 2 reagents. After a 40 minute residence time, measure the light absorption of the treated sample by photometrating on the spectrophotometer at wavelength λ = 543 nm. The nitrite concentration is calculated by a calibration curve obtained by the same method at known concentrations of 0.05 to 1 g / l.

RESULTS AND DISCUSSION

The studies were carried out under batch conditions in a thermostated cell at 30°C and, respectively, at different ratios of the initial concentrations of the two substrates (nitrate and nitrite ions). Experiments with two crude enzyme preparations (Fig. 1) were performed to verify the reproducibility of the experimental results obtained. It can be seen from Figure 1 that the reproducibility obtained is satisfactory.

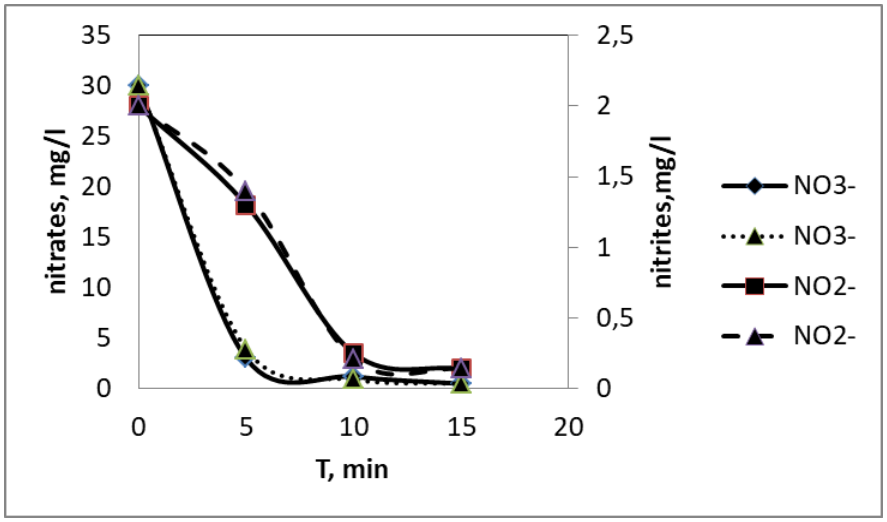

Fig. 1. Reproducibility of experimental data in disintegrated cells with initial concentrations of substrates: nitrate ions - 30 mg / l, nitrite ions - 2 mg / l.

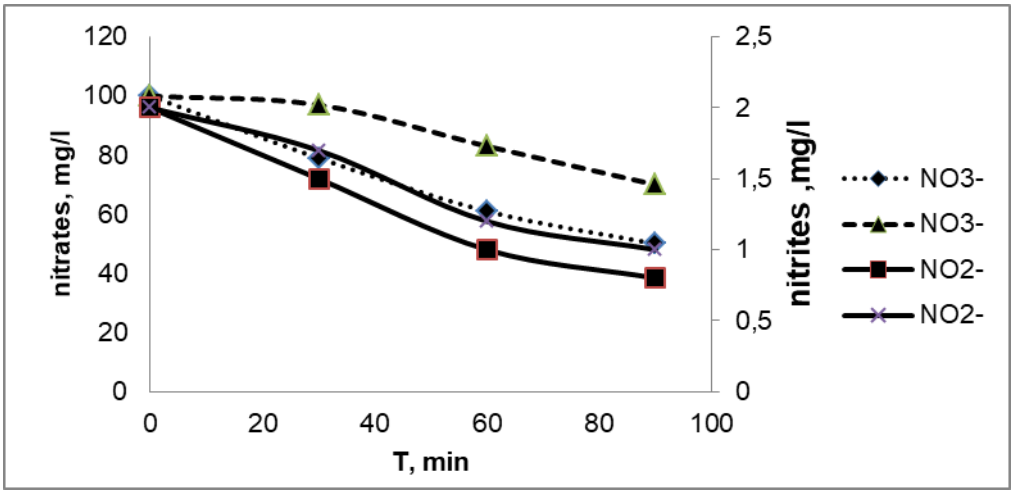

Fig. 2. Comparison of nitrate and nitrite reduction potential between whole and disintegrated Pseudomonas denitrificans cells at an initial concentration of nitrates - 100 mg / l and nitrite - 2 mg / l where: ■- nitrite ions by disintegrated cells, ♦- nitrate ions by disintegrated cells, ※ - nitrite ions by intact cells, ▲ - nitrate ions by intact cells.

The results of the experiments with whole and correspondingly disintegrated cells are presented in Fig.2. It can be seen from the graph that the denitrification rate is higher at a reduction performed with the untreated cell-free extracts. Denitrification process in whole cells is associated with accumulation of cellular biomass, which passes through a longer period of adaptation and respectively a slow process of reduction of nitrate and nitrite ions. Experiments performed by disintegrated cells indicate that substrate inhibition is observed at the lower ratios of the initial concentrations of nitrate and nitrite ions. These results are illustrated in graphs (Figure 3 - Figure 6).

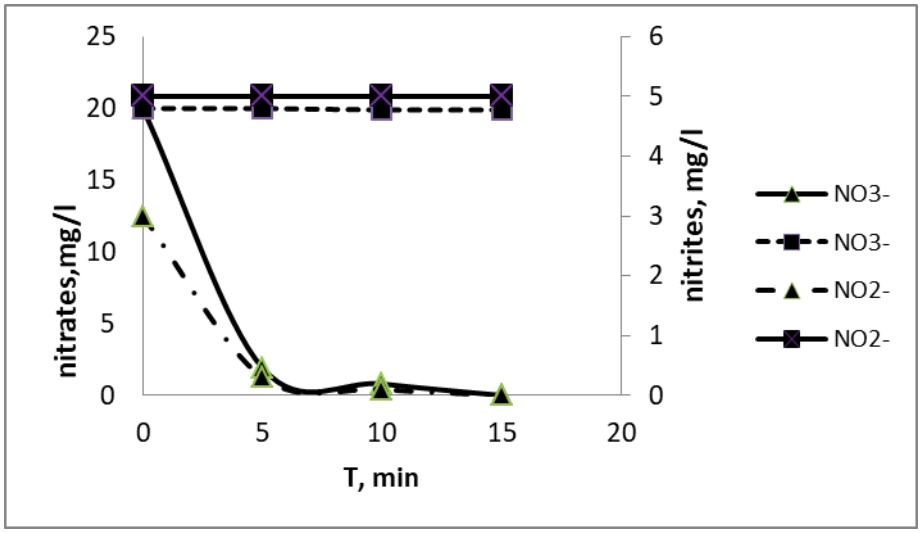

Fig. 3. Comparison of the rate of reduction of nitrate and nitrite ions at different initial ratios of substrate concentrations (NO3- / NO2-); 20/3, 20/5.

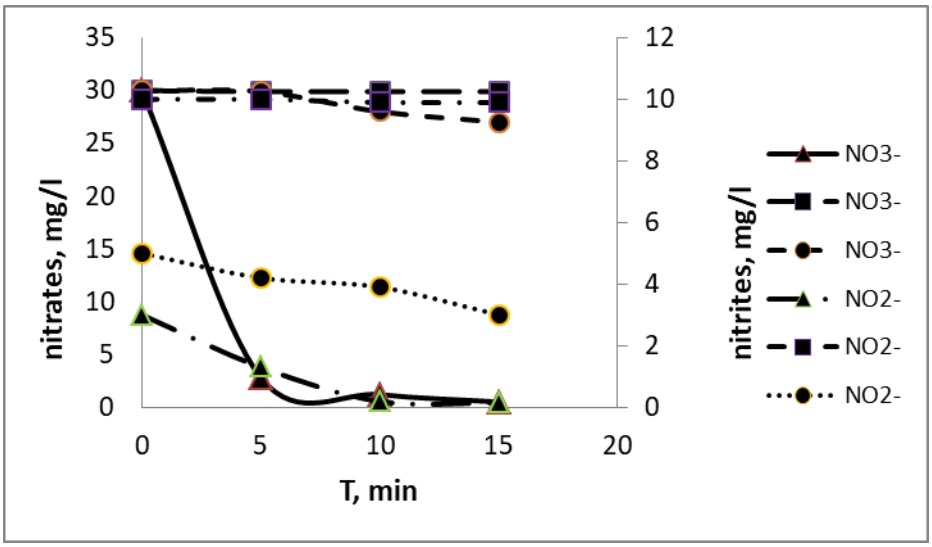

Fig. 4. Comparison of the rate of reduction of nitrate and nitrite ions at different initial ratios of substrate concentrations (NO3- / NO2-); 30/3, 30/5, 30/10.

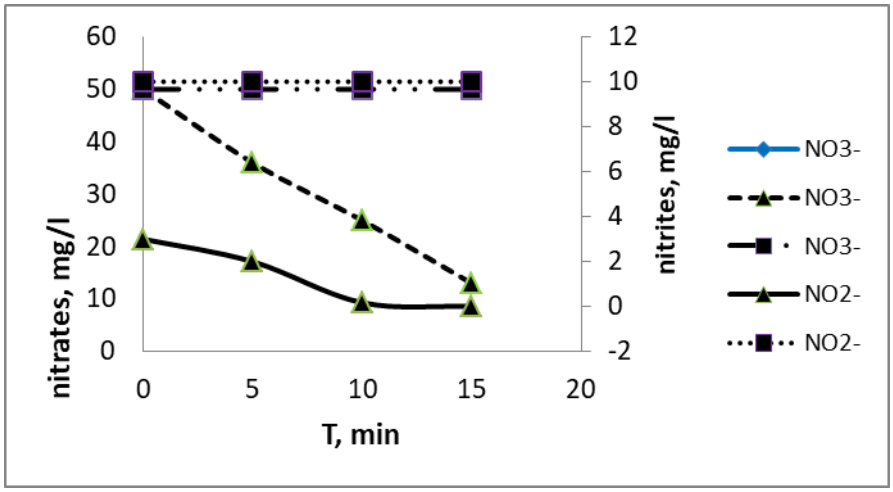

Fig. 5. Comparison of the rate of reduction of nitrate and nitrite ions at different initial ratios of substrate concentrations (NO3- / NO2-); 50/3, 50/10.

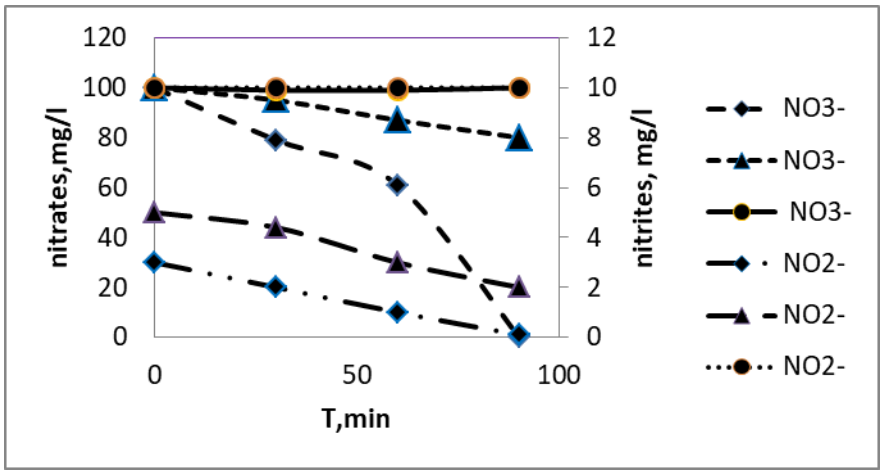

Fig. 6. Comparison of the rate of reduction of nitrate and nitrite ions at different initial ratios of substrate concentrations (NO3- / NO2-); 100/3, 100/5, 100/10.

CONCLUSION

On the based results we can make the following conclusions:

1. The rate of reduction is higher in denitrification with disintegrated cells than that performed with live cells. We can assume that the reason is in the diffusion constraints arising from the passage of substrates through the walls of living cells, as well as in unexplained physiological causes.
2. Lower ratios of initial substrate concentrations lead to strong substrate inhibition.

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