AUTOTROPHIC DENITRIFICATION OF BACTERIA THIOBACILLUS DENITRIFICANS IN THE PRESENCE OF PHOSPHORUS AND MOLYBDENUM

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Abstract

Bacteria *Thiobacillus (Th.) denitrificans* belongs to gram-negative, obligate chemolithoautotrophic and facultative anaerobic microorganisms. *Th. denitrificans* uses the oxidation of reduced inorganic sulfuric compounds for the respiratory reduction of nitrates or nitrites under anaerobic conditions. Denitrification can be affected by many factors. The aim of the work was to describe the positive influence of different concentrations of phosphorus and molybdenum to autotrophic denitrification by *Th. denitrificans,* and to determine optimal amount phosphorus and molybdenum for specific conditions. The best added concentrations of phosphorus was from 1,0 to 1,5 mg / l and molybdenum was 0,1 mg/l for autotrophic denitrification of bacteria *Th. denitrificans* in tested batch reactors.

Key words:

Denitrification, molybdenum, phosphorus, *Thiobacillus denitrificans*.

Introduction

Nitrate and nitrite ions in water cause common and dangerous problems. There are many methods of their removing from contaminated water. One of them is autotrophic denitrification by bacteria *Th. denitrificans* (Oh et al., 2000). It belongs to group of biological methods because it is environmentally friendly, decomposes pollutants and completely removes them from the environment without the formation of undesirable secondary waste (Moon et al., 2004).

Bacteria *Th. denitrificans* are gram-negative, obligate chemolithoautotrophic and facultative anaerobic microorganisms, which can be mainly found in water and soil.

The metabolism of *Th. denitrificans* is based on the obtaining of energy from oxidation of elemental sulfur or certain inorganic sulfuric compounds (hydrogen sulfide, pyrite etc.). Electrons are released during the processes and under anoxic conditions, nitrate and nitrite ions are acceptors of the electrons and they are reduced to gaseous nitrogen, which is released into the environment (Kelly, Wood, 2000; Beller et al., 2006).

The optimal function of these processes is influenced by a number of agents and factors (Liu, Koenig, 2002; Manconi, Carucci, Lens, 2007; Shao, Zhang, Fang, 2010). It also includes the correct dosage of nutrients such as phosphorus and molybdenum. The aim of the work was to monitor the autotrophic denitrification of bacteria *Th. denitrificans* in the presence of different concentrations of phosphorus and molybdenum.

Materials and methods

Nine batch reactors (1 l) were prepared for testing the effect of phosphorus and molybdenum at the rate and course of the denitrification processes of bacteria *Th. denitrificans*. All reactors contained crushed sulfur (size fraction 2,5–5,0 mm), limestone for the pH control, solution of $NaNO_3$ with initial concentration of 100 mg / l NO_3^- and a suspension of bacterial cells *Th. denitrificans* (optical density of the suspension was OD = 0,17). One reactor was left as a control sample; in all other was added phosphorus in the form of NaH_2PO_4, or molybdenum in the form of $Na_2MoO_4 \cdot 2H_2O$ in various concentration ranges, as shown in Tab. 1.

The denitrification process in the reactors was monitored for 40 days and the values of concentrations of nitrate, nitrite and phosphate, pH, temperature, redox potential and dissolved oxygen concentration were measured. During the experiments, it was also verified the presence of bacteria by microscopic techniques.

Tab. 1: Summary of batch experiments and their contents

Label	S (g)	$CaCO_3$ (g)	NO_3^- (mg/l)	Suspension of *Th. denitrificans*, OD = 0,17 (ml)	P (mg/l)	Mo (mg/l)
K					-	-
P1					0,5	-
P2					1,0	-
P3					1,5	-
P4	50	50	100	1	2,0	-
P5					3,0	-
Mo1					-	0,1
Mo2					-	0,5
Mo3					-	1,0

Results and discussion

On the Fig. 1 and Fig. 2, there are results of studies the influence of different concentrations of phosphorus added to the denitrification processes *Th. denitrificans*, i.e. to the rate of degradation of nitrate and nitrite ions.

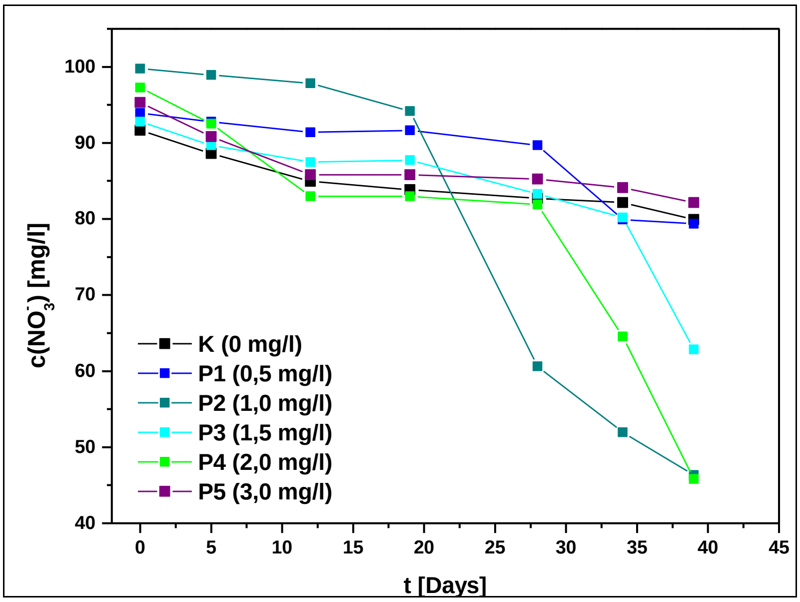

Fig. 1: The rate of removal of nitrate ions in testing the effect of different concentrations of phosphorus to autotrophic denitrification of bacteria *Th. denitrificans*

From both Figs., there can be seen the adaptation phase of microorganisms to environment (lag phase) in approximately the first 10 to 15 days. There is also seen minimal loss of nitrate ions, and formation of a corresponding amount of nitrite ions. There exists another reason for the low reduction of nitrate to nitrite ions - the system still contents oxygen, which has precedence over nitrate and nitrite ions as a terminal electron acceptor in energy metabolism of bacteria *Th. denitrificans*.

The lag phase ends when oxygen is consumed and the bacterial cells are adapted to the environment and there is a massive reduction of nitrate to nitrite ions. Then the nitrite ions are also degraded. After lag phase, there are seen the most distinct differences in the rate of reduction of nitrate and nitrite due to the addition of various concentrations of phosphorus. From the tested concentration range from 0,5 to 3,0 mg / l phosphorus to autotrophic denitrification in batch experiments, nitrate reduction occurs most rapidly at 1,0 to 2,0 mg / l added phosphorus.

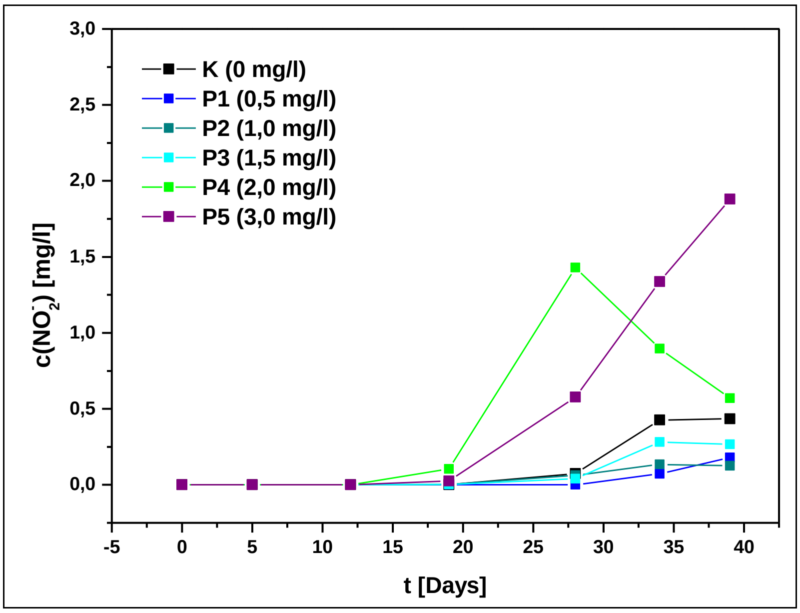

Fig. 2: The rate of removal of nitrite ions in testing the effect of different concentrations of phosphorus to autotrophic denitrification of bacteria *Th. denitrificans*

From the viewpoint of the rate of nitrite reduction in batch reactors, the best initial concentration of added phosphorus was up to 1,5 mg/l. At higher addition of phosphorus, there was seen increased growth of nitrite concentration. This is undesirable, because in the case of using denitrification processes *Th. denitrificans* to remove nitrates from contaminated water, there would be removing nitrates from purified water, but also the production of nitrites as secondary waste.

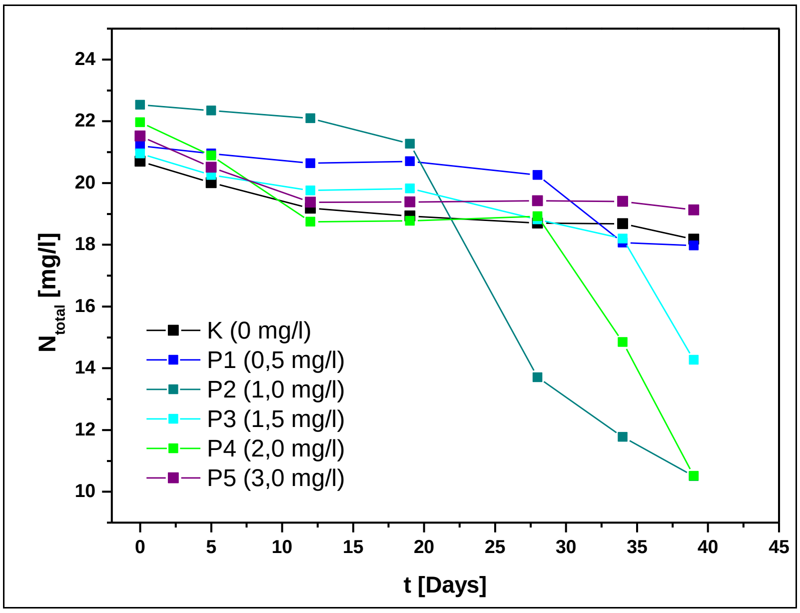

Fig. 3: The rate of removal of total nitrogen in testing the effect of different concentration of phosphorus to autotrophic denitrification of bacteria *Th. denitrificans*

The Fig. 3 shows the concentration of total nitrogen in time in batch reactors. The values of total nitrogen were obtained as the sum of nitrate and nitrite nitrogen and they showed the effect of different concentrations of added phosphorus to denitrification in the reduction of nitrate and nitrite ions together. The obtained results from experiments indicated, that the best initial concentration of added phosphorus was from 1,0 to 1,5 mg/l phosphorus from the viewpoint of the rate denitrification in batch reactors.

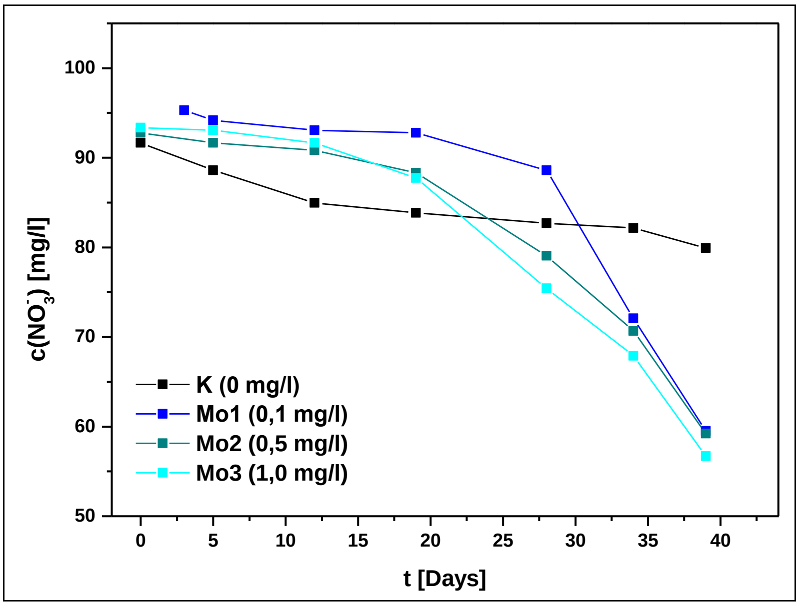

Fig. 4: The rate of removal of nitrate ions in testing the effect of different concentration of molybdenum to autotrophic denitrification of bacteria *Th. denitrificans*

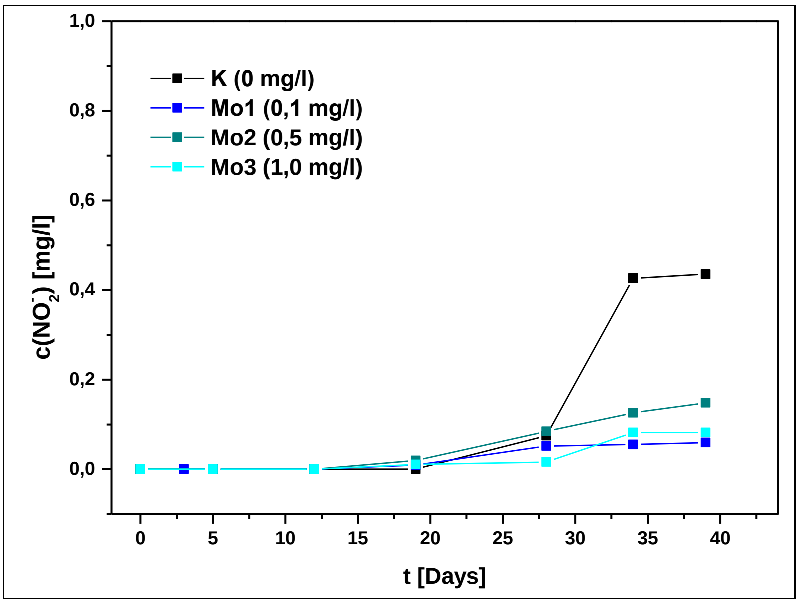

Fig. 5: The rate of removal of nitrite ions in testing the effect of different concentration of molybdenum to autotrophic denitrification of bacteria *Th. denitrificans*

Other experiments were focused on testing the effect of different concentrations of molybdenum to autotrophic denitrification of bacteria *Th. denitrificans*. The course of the denitrification in batch reactors is shown in Fig. 4 and Fig. 5. There are time dependencies of the concentration of nitrate and nitrite ions, on the Figs.

In the first 10 to 15 days, there is again seen the lag phase. After its termination, there can be seen the significant positive effect of three different additions of molybdenum, from the viewpoint of the rate of nitrate and nitrite reduction in batch reactors. Reduction of nitrate ions is accelerated against the same experiment in the control sample without molybdenum.

The same results were obtained in the case of reduction of nitrites, which were reduced faster in the presence of molybdenum than in the control sample without molybdenum; this is shown on the Fig. 5.

This was also confirmed by monitoring of the decrease of total nitrogen in time, as is evident from the Fig. 6. It is evident, that the total nitrogen was degraded out of the system more rapidly with addition of molybdenum than without it. Experiments with different concentrations of molybdenum did not show a statistically significant difference in the rate or course of denitrification in batch reactors, therefore 0,1 mg/l of molybdenum is sufficient addition to denitrification.

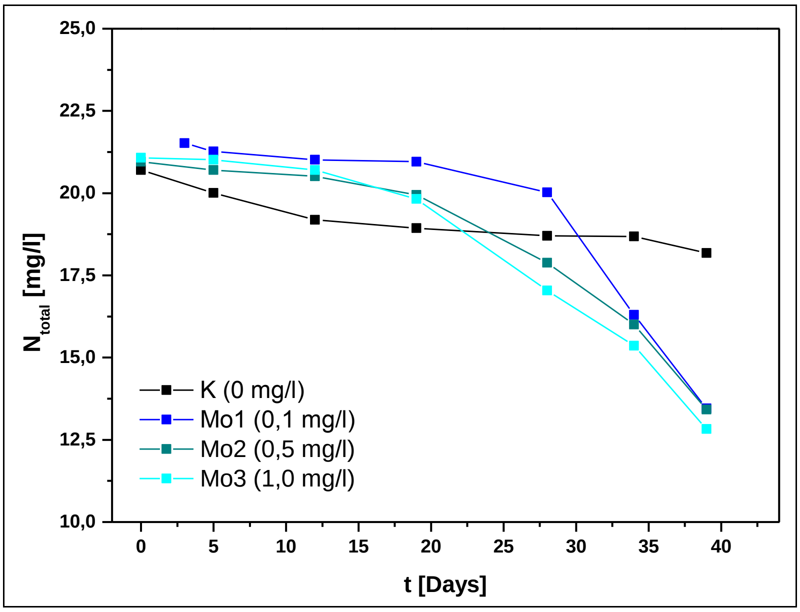

Fig. 6: The rate of removal of total nitrogen in testing the effect of different concentration of molybdenum to autotrophic denitrification of bacteria *Th. denitrificans*

Conclusions

The experiments have shown the correct dosing of phosphorus and molybdenum during denitrification of bacteria *Th. denitrificans* for significantly speed-up these processes. It was found that the addition of phosphorus or molybdenum does not affect to the initial lag phase, lasting 10 to 15 days, but the effect is only after this adaptation period. The best initial concentration was from 1,0 to 1,5 mg/l of phosphorus and 0,1 mg/l of molybdenum for autotrophic denitrification od bacteria *Th. denitrificans* in batch reactors.

Acknowledgement

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