

NITRITE IONS REDUCTION BY IMMOBILIZED MICROBIAL CELLS ON GRANULATED ACTIVATED CARBON

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Abstract

Nowadays pollution of natural waters, rivers and lakes by nitrate is one of the main problems related to the environment. Nitrate originated from agriculture is increasingly growing all over the world due to the extreme use of fertilizers. Nitrate salts reach the underground water as they percolate through the soil. There are various methods for treatment of water for nitrates, but the majority of them yield 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. The potential of this method is high.

The process of nitrate reduction is inhibited by nitrite accumulation being intermediate of microbial denitrification. In the present work the influence of nitrite ions on the process of microbial denitrification was investigated. Both free and immobilized microbial cells were tested. Granulated activated carbon was chosen as support for microbial cell immobilization.

It was found out that granulated activated carbon enables microbial denitrification at very high concentrations of nitrite ions reaching 100 mg /l.

Key words: wastewater, denitrification, nitrate/nitrite reduction, granulated activated carbon

INTRODUCTION

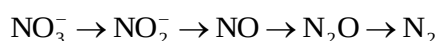
Water is of essential importance for life on earth. The mechanism of metabolism, in the cells of living organisms are closely related to the specific characteristics of water. The water resources are limited and only 2.66% of the total global water resources comprising groundwater, lakes and rivers, polar ice and glaciers are fresh water. Furthermore, only a small fraction, about 0.6%, is usable as drinking water. For this reason, water resources must be necessarily treated properly and wastewater treatment must be done efficiently (Ghafari et al., 2008) (Shimali et al., 2001).

Nitrate and nitrite are ubiquitous within environmental, food, industrial and physiological systems, and although our understanding of their role within such matrices has increased, a substantial degree of uncertainty and speculation remains (Matthew et al. 2001)

Nitrate and nitrite have become intertwined with domestic life, and it is effectively impossible to proceed in everyday activities without encountering these ions or the products of their use (Matthew et al. 2001).

In recent years, nitrate pollution of water resources has become a serious environmental problem due to discharge of domestic and industrial wastewater and widespread use of nitrogen fertilizers (Darvishi, 2016).

The traditional methods (coagulation, ion exchange, reverse osmosis and electro-dialysis UV, filtration, chlorination, ozonation) for waste water treatment are very expensive and not sufficiently useful for the elimination of nitrate ion from the water. Therefore, to remove nitrate ion, a supplementary method is necessary (Sumino et al., 2006). For this reason, biological denitrification is an important approach which actually removes nitrate from the drinking water sources. In biological denitrification nitrate successively reduced to nitrogen gas passing through nitrite, nitrous oxide and nitric oxide, according to the following scheme (Payne, 1973):



Reduction of NO_2^- to NO , N_2O and N_2 , so-called nitrifier denitrification, is very important, because nitrous oxide (N_2O) emission from soils is a major contributor to the atmospheric loading of this potent greenhouse gas (Shaw, 2006).

The goal of the present work is test the nitrite reduction by bacterial strain *Pseudomonas denitrificans* immobilized on granulated activated carbon (GAC).

MATERIALS AND METHODS

Strain

A strain of *Pseudomonas denitrificans* (NBIMCC 1625), provided from the Bulgarian National Bank of industrial Microorganisms and Cell Cultures, was used. The inoculum was cultivated in a medium containing: peptone, 10 g/l; yeast extract, 1 g/l; NaCl, 10 g/l, and was incubated at 30°C for 24 h in a rotary shaker at low stirring speed (50 rpm). The culture medium comprised two solutions, each of which was sterilized separately:

- Phosphate buffer at pH= 7, containing also: $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.2 g/l; $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, 0.2 g/l; NaCl, 5 g/l.
- Potassium nitrate stock solution – 10 g/l.

The latter was added to the phosphate buffer after sterilization in a calculated amount to reach your initial concentrations of nitrate in the culture medium, ranging from 50 to 300 mg / L.

Immobilization procedure

The immobilization of *Pseudomonas denitrificans* cells was accomplished on granulated activated carbon (GAC). The preliminary cultivated bacterial suspension was mixed with the washed and dried to constant weight of GAC. Then the mixture was transferred to shaking flasks and they were incubated at 30°C for least 48 hours.

The *Pseudomonas denitrificans* cells are capable to produce exo-polysaccharides in the biofilm layer, which serve as binding agents between carbon particles and microbial cells (Beschkov, V.).

GAC

Granulated activated carbon, product of Fujikasui, Japan, with specific active area of 680 m^2/g), was used. Two very important properties thereof are used: first, the *Pseudomonas denitrificans* cells are easily attached to the carbon surface; second, the toxic compounds are adsorbed by the activated carbon. As a result, the high concentrations of the substrate are reduced to not dangerous values for the microbial cells and therefore avoiding inhibition caused by high concentrations of the pollutant. (Beschkov)

Analyses

During the experiments, the pH value was measured periodically.

Biomass concentration

The biomass concentration was controlled by optical density readings of the broth at $\lambda=660$ nm using a Spekol spectrophotometer.

Nitrite concentration

The concentration of nitrite in the pre-centrifuged samples was determined spectrophotometrically by the azo-dye formed by the diazonium salt of sulphanilic acid (formed with the nitrites are present) coupled with α -naphthylamine at a pH of 2.0-2.5. Analyses of nitrites are held according to the following procedure. It includes two reagents:

- Griess reagent 1 consists of 0.5 g of sulfanilic acid dissolved in 150 ml of 12% solution of acetic acid.

- Griess reagent 2 consisting of 0.25 g α -naphthylamine dissolved in 20 ml of distilled water. This solution was boiled for 5 minutes under a hood, and then hot filtered through a filter. The filtrate was mixed with 150 ml of 12% acetic acid. After that, one ml of the centrifuged sample is mixed of 1 and 2 Griess reagents, 1 ml each. The mixture was diluted to 50 ml in a volumetric flask. The light absorption of this solution was measured after 40 min using a spectrophotometer at Spekol at 543 nm against distilled water. If necessary, samples were diluted prior to the addition of reagents 1 and 2 Griess. Nitrite concentrations were calculated using a standard curve composed by the same method, in the concentration range of 0.05 to 1 mg / L.

RESULTS AND DISCUSSION

The experiments with free and immobilized cells were carried out in flasks of 250 ml in a rotary shaker at 100 rpm and at 30 °C and initial concentrations of nitrite ions: 2, 3, 10 and 100mg/l.

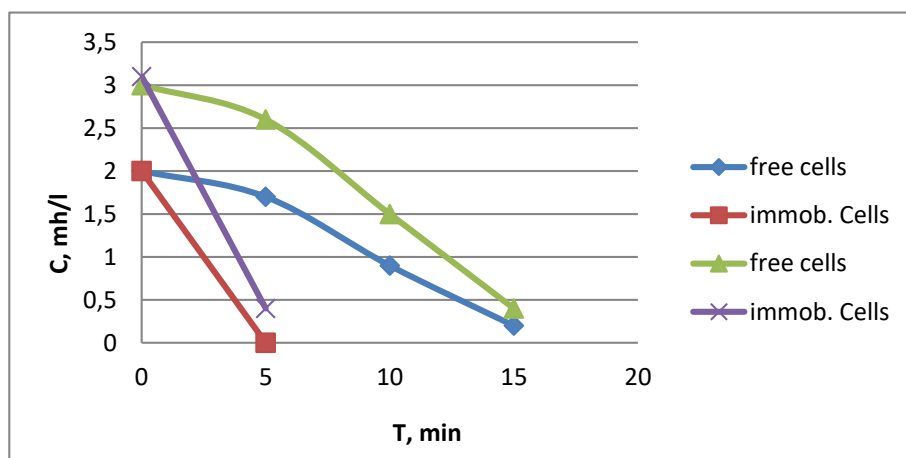


Fig. 1. Comparison of nitrite reduction by free and by immobilized cell, at different initial nitrate concentrations: ■ and ◆ - 2mg/l, ※ and ▲ - 3mg/l.

In the experiments with free culture sterile working solution (phosphate buffer at pH = 7, containing: $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.2 g/l; $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, 0.2 g/l; NaCl, 5 g/l) was inoculated with 10% vol. inoculum and then the desired amount of solution of potassium nitrate was added.

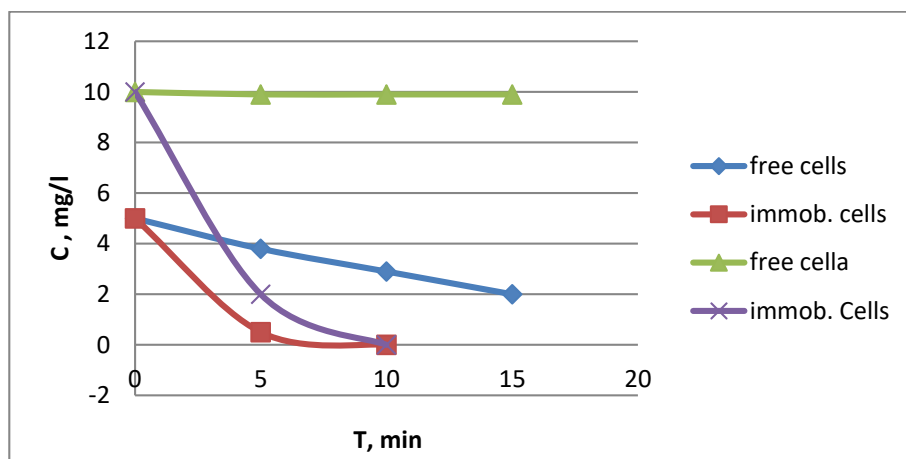


Fig. 2. Comparison of nitrite reduction by free and by immobilized cell, at different initial nitrate concentrations: ■ and ◆ - 5mg/l, ※ and ▲ - 10mg/l.

The experimental results for biodegradation of nitrite ions by free and immobilized cells of *Pseudomonas denitrificans* is shown in Figs 1-3. There is in all cases nitrite reduction at concentrations 2mg /l and 3mg/l, but with different reduction rates. The nitrite reduction rate by immobilized cells is significantly higher than by free cells. This effect is better pronounced at higher initial concentrations of nitrite, up to 100 mg/l, cf. Fig. 3.

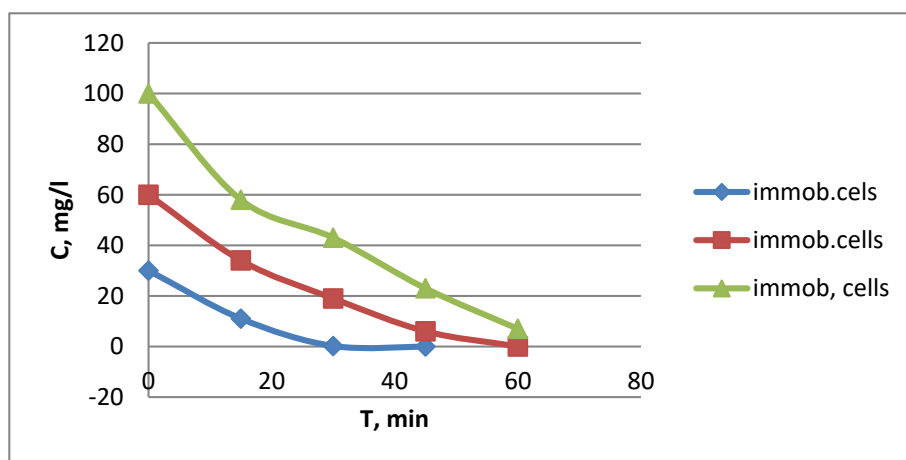


Fig. 3. Comparison of nitrite reduction by free and immobilized cell, at different initial nitrate concentrations: ◆ - 30mg/l, ■ - 60mg/l, ▲ - 100mg/l.

Images of the granulated activated carbon carrier with and without immobilized *Pseudomonas denitrificans* cells were made using scanning electron microscopy. They are shown in Fig. 4a-c.

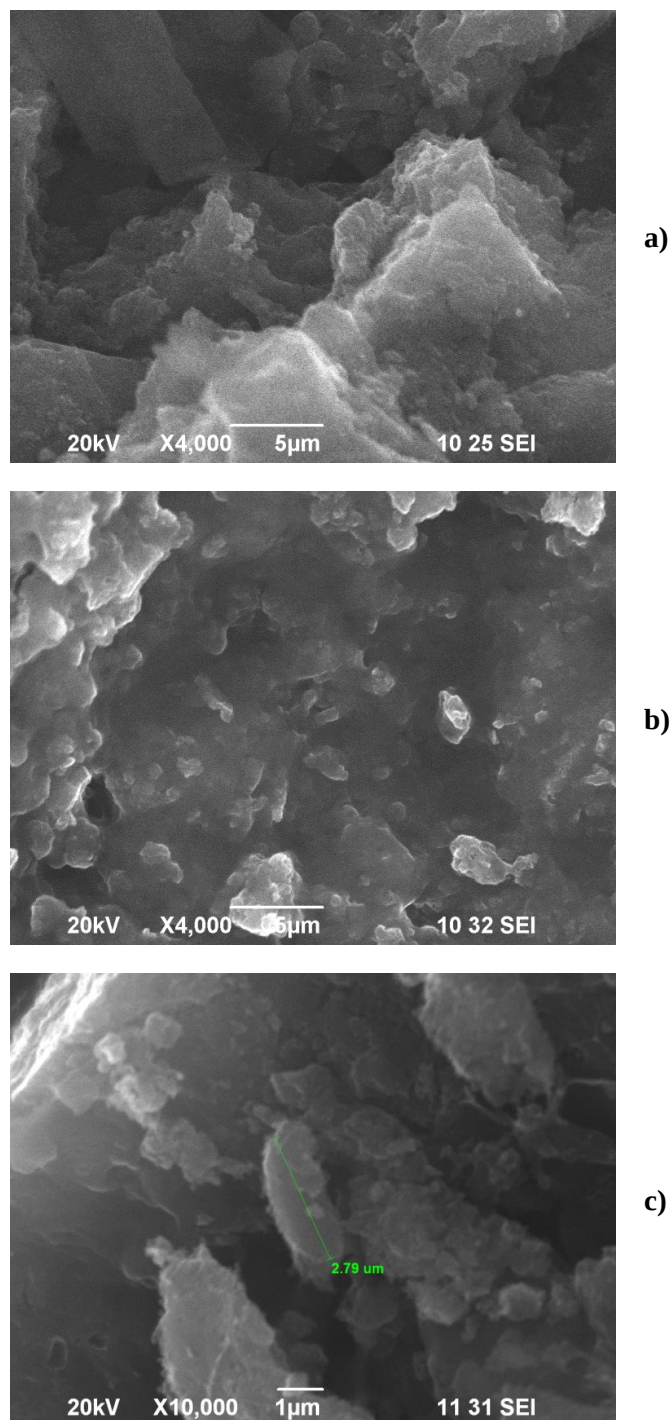


Fig. 4. Images of the surface of the activity granulated carbon (AGC) with scanning electron microscopy. **4a.** GAC surface without bacteria (4 000 times magnification); **4b.** GAC surface with bacteria (4 000 times magnification); **4c.** Surface AGC with bacteria (10 000 times magnification).

CONCLUSION

In conclusion we can say that nitrate reduction by bacterial cells immobilized on granular activated carbon as a carrier is appropriate because it allows us to:

- To avoid substrate inhibition.
- To operate at high initial concentrations of nitrite ions.
- To increase considerably the rate of nitrite reduction.

ACKNOWLEDGEMENTS

This work is supported under project E02/16. The authors would like to thank the National Science Fund, Ministry of Education and Science of the Republic of Bulgaria.

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