APPLIED BIOCHEMISTRY AND BIOTECHNOLOGY 7, 39–41 (1982)

Immobilized Cells in Microbial Nitrate Reduction

INGE NILSSON*,[1] AND STEN OHLSON[2]

Divisions of Technical Microbiology[1], and Pure and Applied Biochemistry,[2] Chemical Center, University of Lund, PO Box 740, S-220 07 Lund 7, Sweden

Accepted February 18, 1981

Abstract

The microorganism *Pseudomonas denitrificans* was immobilized in alginate. These immobilized cells were capable of reducing 0.8 mg NO_3^-/min/g wet weight of cells.

Index Entries: *Pseudomonas denitrificans*, in nitrate reduction; immobilized cells, of *Pseudomonas denitrificans;* cells, immobilized, of *Pseudomonas denitrificans;* nitrate reduction, by immobilized *Pseudomonas denitrificans;* reduction, of nitrate by immobilized *Pseudomonas denitrificans nitrate.*

Introduction

The increasing content of nitrate in different types of water has drawn attention to methods for nitrate reduction. The possible deterioration of groundwater supplies by the extensive use of nitrogen fertilizers emphasizes the need for efficient nitrate reduction processes. Several methods have been suggested for this kind of treatment, including algae ponds, bacterial assimilation, microbial denitrification, and ion exchange (*1*, *2*).

Here we describe the use of alginate-immobilized *Pseudomonas denitrificans* in the purification of high-nitrate drinking water. Microbial denitrification is an anaerobic process in which the oxidation of organic carbon sources is accompanied by a reduction of nitrate, via nitrite, to gaseous compounds, mainly nitrogen.

0273–2289/82/0912–0039$02.00

Results

Pure cultures of *Pseudomonas denitrificans* (ATCC 13867) were grown in flasks using a complex medium (*3*). Experiments showed an increasing specific nitrate reduction capacity when growing cells in batch culture. The cells should preferably be harvested in the late logarithmic phase. The maximum capacity was 0.8 mg NO_3^-/min/g wet weight of cells. The need for an appropriate C/N ratio in water denitrification was also investigated using free cells. It was shown that a C/N ratio of 2.0 in the substrate would assure a complete reduction.

Immobilization was performed as follows: 1 g cell suspension (4×10^{11} living cells/g wet weight) was added to 9 g of 2% sodium alginate solution. This mixture was pumped into 0.1*M* $CaCl_2$. The gel particles were cured for about 2 h at room temperature.

When using gel particles with a diameter of 3 mm, the nitrate reduction rate was about 50% that of the free cells. This decrease mainly results from diffusional limitations within the gel matrix. These effects could also be seen when increasing the cell concentration in the gel (Table 1). In a mixture containing more than 20% cell suspension, the reduction capacity does not increase and there are great difficulties in producing small particles.

In a continuously operating four-column system, the nitrate and nitrite concentrations were determined when introducing a substrate containing nitrate (100 mg/L) and ethanol (Table 2). The results show the typical intermediate production of nitrite in the first part of the column system. A 100-mL column containing 50 g

TABLE 1
Effects of Different *Pseudomonas dentrificans* Cell Concentrations in Alginate Gel

	Cell concentration in gel, %					
	1.7	3.2	6.8	9.7	12.7	15.6
Activity, mg NO_3^-/min/g gel $\times 10^3$	4.5	6.6	9.1	11.2	11.8	12.6

TABLE 2
Nitrate Reduction in a Four-Column System Using Alginate Immobilized *Pseudomonas dentrifficans* Cells[a]

		Column No.			
	Inlet	1	2	3	4
Conc. NO_3^-,	104	39	14	0.1	0.1
Conc. NO_2^-, mg/L	0.0	8.2	7.2	2.4	0.3

[a]Column volume, 40 mL; amount of gel, 20 g; flow rate, 3.2 mL/min.

of alginate gel fibers can produce about 10 L of nitrate- and nitrite-free water per day, if the water contains 100 mg NO_3^-/L.

Conclusions

This study shows that pure cultures of *Ps. denitrificans* entrapped in alginate gel can be used for continuous denitrification of water. We consider this to be a useful alternative to other nitrate reduction methods already established.

References

1. Christensen, M. H., and Harremoes, P. (1977), *Prog. Water Tech.***8** (4/5), 509.
2. Sutton, P. M., Murphy, K. L., and Dawson, R. N. (1975), *J. Water Pollut. Control Fed.* **47,** 122.
3. Nilsson, I., Ohlson, S., Häggström, L., Molin, N., and Mosbach, K. (1980), *Eur. J. Appl. Microbiol. Biotechnol. 10,* 261.