Appl Biochem Biotechnol (2008) 151:193–200
DOI 10.1007/s12010-008-8170-y

Biotreatment of High Strength Nitrate Waste Using Immobilized Preadapted Sludge

Rashmi R. Nair · Pradip B. Dhamole · S. S. Lele · Stanislaus F. D'Souza

Received: 18 December 2007 / Accepted: 1 February 2008 /
Published online: 8 March 2008

Abstract One of the major wastes generated by fertilizer, explosive, and nuclear industries are nitrate (as high as 1,000 ppm NO_3N) whose removal before disposal has become a growing concern. In this study, an active denitrifying sludge was immobilized onto support materials like cloth and polyurethane foam and their denitrification efficiency on high nitrate wastes [1,000 ppm NO_3 (225 ppm NO_3N), 5,000 ppm NO_3 (1,129 ppm NO_3N), 7,500 ppm NO_3 (1,693 ppm NO_3 N)] was studied. Results showed complete degradation of the nitrate wastes (225 ppm NO_3N, 1,129 ppm NO_3N, and 1,693 ppm NO_3N) without any accumulation of nitrite in a period of only 1, 4, and 10 h, respectively. Based on adhering and entrapment principle, an immobilization unit was developed using a combination of cloth and foam as well as both individually. This system used for treating such high nitrate wastes was found to be quite effective in waste water treatment, particularly in problems associated with solid–liquid separation. The batch column reactor was run in about 45 batches without any loss in activity or reactor stability.

Keywords Denitrification · Immobilization · Cloth · Polyurethane foam · Activated sludge

Introduction

Due to increasing environmental concern and regulatory requirements, industries will no longer be allowed to directly discharge their waste. Therefore, appropriate methods for

R. R. Nair · S. F. D'Souza (✉)
Nuclear Agriculture and Biotechnology Division, Bhabha Atomic Research Centre,
Mumbai 400 085, India
e-mail: sfdsouza@barc.gov.in

P. B. Dhamole
Department of Biotechnology, Sinhgad College of Engineering, Wadgaon (Bk), Pune 411041, India

S. S. Lele
Food and Fermentation Technology Department, Institute of Chemical Technology,
University of Mumbai, Matunga, Mumbai 400 019, India

handling industrial waste are necessary before they are discharged. The consumption of drinking water contaminated with nitrate causes serious health risks because of the secondary and tertiary effects, i.e., the reduction to nitrite and the formation of nitrosamines [1]. Nitrate is limited in drinking water to 10 ppm NO_3N in the USA and to 12 ppm NO_3N in Europe to avoid adverse health effects. A potentially cost-effective means for nitrate removal is biological denitrification. Various biological denitrification technologies have been established to solve the problem of nitrate in drinking water and groundwater. Denitrification is used at municipal treatment plants to treat wastewater containing 10–200 ppm NO_3–N. However, research focused on the denitrification of industrial wastewater containing high concentrations of nitrate has not been performed extensively.

Anaerobic process have been proven to be effective in treating various wastewaters owing to the inherent advantages like less consumption of energy for operation and minimum sludge production. However, the slow generation rate of organisms makes the process a major drawback in reactors based on the anaerobic processes. By effective biomass retention, this problem is sought to be overcome in present day advanced reactors [2, 3].

A variety of support materials have been used as matrices for the formation of biofilms. While some researchers have looked into low-cost commercial supports [4, 5], others have carried out studies on the adhesion phenomenon in several materials, with emphasis on the fundamental aspects governing the formation of biofilm [6–8]. Polyurethane foam has been widely studied as a support matrix to immobilize anaerobic biomass [9–12]. Because of the chemical inertness, mechanical stability, capacity for oxygen transfer, and porosity of polyurethane foam, its application to the immobilization of microorganisms has been found to be effective [13]. The greatest advantage of immobilization in polyurethane foam is the stable maintenance of the quantity of microorganisms required in a reactor. The ease of control of pore size and large-scale application at low cost are its other advantages. It has been mainly used in fixed-bed reactors and provides adequate environmental condition for biomass growth and retention [14].

Studies from our lab and others have shown the use of cloth in a frame reactor [15] or a rectangular film bioreactor [16] or in a column reactor [17]. Cloth or other matrices have also shown potentials in the fabrication of spiral wound annular reactors containing immobilized cells for fermentation [18, 19]. The major advantage of cloth is its availability in a fibrous matrix form with different thickness and surface areas. Based on these reports, it is evident that the characteristics of flannel cloth and polyurethane foam play an important role and possibly influence the biomass immobilization process.

The present paper discusses the potential advantage of using flannel cloth and foam as a useful support for the immobilization of sludge. It also investigates its use for the complete degradation of high nitrate waste in batch process when used individually and also in combination.

Materials and Methods

Materials

Flannel cloth was obtained from Bombay Dyeing Textile Mills, Bombay, India. Polyurethane foam was obtained from a local market. All other chemicals of analytical grade were obtained from standard sources.

Microorganisms and Media

The sludge used for immobilization was obtained from the denitrification plant of a fertilizer industry. This sludge was acclimated to nitrate ions as high as 1,693 ppm NO_3N in the process described in our previous work [20]. All the studies were carried out in synthetic waste of the following composition (g l^{-1}): Na_2HPO_4 7, K_2HPO_4 1.5, $MgSO_4$ 0.1, NaCl 0.3, and trace element solution 2 ml l^{-1}. Trace element solution consisted (g l^{-1}) of $CaCl_2$ 5.54, $FeSO_4 \cdot 7H_2O$ 5.0, $MnCl_2 \cdot H_2O$ 5.06, $ZnSO_4 \cdot 7H_2O$ 2.2, $CuSO_4 \cdot 5H_2O$ 1.51, $CoCl_2 \cdot H_2O$ 1.61, ethylenediaminetetraacetic acid 50, $(NH_4)_6MO_7O_{24} \cdot H_2O$ 1.1. The nitrate source used for the study was sodium nitrate. The rate of denitrification is significantly dependent on the availability of a sufficient quantity of a suitable organic carbon source. As nitrate effluents are generally low in organic carbon, the addition of a carbon source may be necessary. In this study, sodium acetate was used as the carbon source. A C/N of 2.25:1 was used for the present study. The denitrifying conditions for all the studies were provided as optimized in our previous work [21].

Adhesion of Sludge on Flannel Cloth

Flannel cloth (104×7 cm^2) was washed and dried. This cloth piece was mounted on an acrylic frame of the cloth bioreactor designed by D'Souza and Kamath [22] and immersed in the reactor containing the activated sludge. This reactor was used only for the formation of biofilm on the cloth. The frame of the reactor has parallel sheets (on which the cloth is wound) which assure the formation of biofilm on both sides of the cloth. The sludge was circulated in the reactor using a peristaltic pump so that the consortium is in constant motion and does not settle at the bottom of the reactor. This setup was kept for a period of 30 days to form a thick biofilm on both sides of the cloth. This cloth with the immobilized sludge was used in the column bioreactor.

Adsorption and Entrapment of Sludge in Polyurethane Foam

Polyurethane foam cubes [30 No ($2 \times 2 \times 2$ cm^3)] were washed and dried. This was placed in a column (h/d=3.2, h=16 cm) filled with activated sludge. To avoid settling of the sludge at the bottom of the column, it was circulated in the column using a peristaltic pump for a period of 30 days. The foam cubes with the immobilized sludge were then used for denitrification of 225 ppm NO_3N and 1,129 ppm NO_3N synthetic nitrate waste.

Denitrification of Nitrate Waste Using Sludge Immobilized on Cloth

Washed and dried cloth pieces [27 No (3×3 cm^2)] were placed in activated sludge for 30 days until a biofilm was formed on the cloth. These cloth pieces were then inoculated in a 150-ml flask containing 100 ml synthetic nitrate waste. The flask was fitted with a cork having two glass pipes. Denitrifying conditions was provided by flushing the flask and purging the waste with N_2 gas through one of the pipes. The mouth of the flask and the pipes were then sealed using a parafilm. The flasks were placed on a shaker (100 rpm) for gentle shaking. Denitrification of 225 ppm NO_3N and 1,129 ppm NO_3N synthetic nitrate waste were studied in this system, and the nitrate and nitrite concentration was assayed at regular time intervals. The reactor was run as a batch with decantation and refilling of fresh synthetic medium after complete removal of nitrate.

Denitrification of Nitrate Waste Using Sludge Immobilized in Polyurethane Foam

Polyurethane foam cubes (10 No) with the immobilized sludge were packed into a column (h/d=3.2, h=16 cm), and 100 ml of synthetic nitrate waste was circulated in the column using a peristaltic pump. Denitrifying conditions were maintained because of the packed foam cubes. The reactor which was run as a batch process was used for the denitrification of 225 ppm NO_3N and 1,129 ppm NO_3N synthetic nitrate waste. The nitrate and nitrite concentration of the waste was analyzed at regular time intervals.

Bioreactor Setup

The flannel cloth (104×7 cm^2) and the polyurethane foam cubes [30 No ($2\times2\times2$ cm^3)] with the immobilized sludge were used in combination by forming an immobilized sludge unit. The bioreactor consisted of a glass column (h/d=6, h=42 cm) with an immobilized unit. The unit comprised of a stainless steel wire mesh rolled to have a dimension (h/d=6, h=30.5 cm). The long hollow gap of the rolled wire mesh was packed with the polyurethane foam cubes with the immobilized sludge. The rolled wire mesh was then wound with the (104×7 cm^2) flannel cloth having the thick biofilm of sludge. To make the whole unit stable and compact, a polypropylene mesh (104×7 cm^2) was wound over the flannel cloth and sewed together. This whole immobilized unit was immersed into the column reactor. The designing of the reactor (immobilized unit) ensured that anaerobic conditions were maintained, which is required for denitrification. The studies of nitrate removal were carried out in batch for different concentrations of synthetic nitrate waste (225, 1,129, and 1,693 ppm NO_3N). The waste was kept in circulation using a magnetic stirrer (400 rpm).

Analysis

Nitrate and nitrite was analyzed using DIONEX Ion chromatograph, AS11, 2-mm column. NaOH (12 mM) was used as eluent. Samples were centrifuged and filtered before analysis. Deionized water was used for dilution. The biomass of immobilized cells was assessed by dry weight method. For the dry weight estimation, the weight of the foam cube/cloth piece with the entrapped/attached cells was determined after drying at 60 °C for 24 h.

Results and Discussion

Nitrate Removal by Sludge Immobilized on Flannel Cloth

Activated sludge immobilized onto flannel cloth pieces were used for studying their denitrification activity by monitoring their nitrate and nitrite profile. Twenty-seven cloth pieces having an attached biomass of 1 mg cm^{-2} dry weight were inoculated into 100 ml of 225 ppm NO_3N synthetic waste. The nitrate and nitrite profile for this set is shown in Fig. 1, which shows that there was complete degradation of nitrate in a period of 2 h with a buildup of nitrite which degrades eventually, thereby showing complete degradation in a period of 2 h. Figure 2 shows the nitrate and nitrite profile during the denitrification of 1,129 ppm NO_3N which was degraded in a period of 6 h.

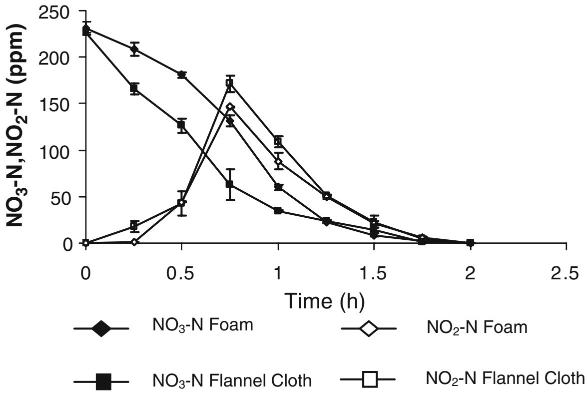

Fig. 1 Nitrate and nitrite profile during denitrification of 225 ppm NO_3N influent synthetic nitrate waste by sludge immobilized in foam cubes and flannel cloth pieces. Attached biomass (foam)=47 mg/cube (10 No), attached biomass (flannel cloth)= 1 mg cm^{-2} (27 No). Error bars indicate standard deviation of triplicate samples

Nitrate Removal by Sludge Immobilized in Foam

Foam cubes with the entrapped biomass (47 mg/cube) were used to denitrify 225 and 1,129 ppm NO_3N synthetic nitrate waste. The nitrate and nitrite profile during the denitrification of 225 ppm and 1,129 ppm NO_3N synthetic waste is shown in Figs. 1 and 2, respectively. The time taken to degrade 225 ppm NO_3N was also 2 h. The total amount of attached biomass in the two reactors (cloth reactor and foam reactor) was found to be 2.7 and 2.3 g l^{-1}, respectively. As the amount of attached biomass was approximately similar for both the reactors, the time taken to degrade 225 ppm NO_3N and 1,129 ppm NO_3N was also similar, i.e., 2 and 6 h, respectively. The reactor was run successfully for 15 days, and no change in the denitrification activity was observed. No significant change was observed even in the amount of attached biomass, and this could be because of the anaerobic conditions provided for the denitrification process. The biomass used in these studies was initially acclimatized to high nitrate wastes before immobilization. Our earlier work [20, 21] on denitrification of high strength nitrate waste showed that acclimatization of sludge to high nitrate wastes increases the specific rate of denitrification, i.e., it results into rapid removal of nitrate. This was attributed to enrichment of sludge and induction of enzymes [21]. Denitrifiers which usually have a slow growth under anaerobic conditions can be

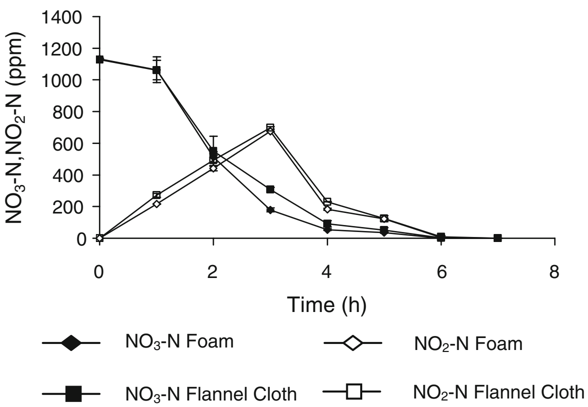

Fig. 2 Nitrate and nitrite profile during denitrification of 1,129 ppm NO_3N influent synthetic nitrate waste by sludge immobilized in foam cubes and flannel cloth pieces. Attached biomass (foam)=47 mg/cube (10 No), attached biomass (flannel cloth)=1 mg cm^{-2} (27 No). Error bars indicate standard deviation of triplicate samples

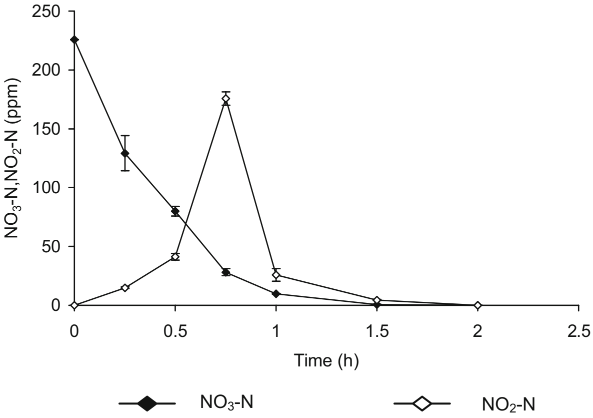

Fig. 3 Nitrate and nitrite profile during denitrification of 225 ppm NO_3N influent synthetic nitrate waste by sludge immobilized in foam cubes and flannel cloth (immobilized unit). Attached biomass (foam)=47 mg/cube (30 No), attached biomass (flannel cloth)=1 mg cm^{-2} (104×7 cm^2 cloth piece). Error bars indicate standard deviation of triplicate samples

retained at a higher concentration in film bioreactors as compared to free cell reactors and therefore was found to be advantageous in increasing the denitrification rate. Thus, cloth and foam were found to be good materials for immobilization of whole cells. These materials were used in combination in a separate bioreactor system for denitrification of high nitrate waste (1,693 ppm NO_3N).

Denitrification of Nitrate Waste in a Batch Column Reactor

The immobilized sludge unit was immersed into the column reactor having a working volume of 700 ml. Synthetic NO_3N (225 ppm) waste was subjected to degradation, and nitrate and nitrite were analyzed at regular time intervals. As can be seen in Fig. 3, the nitrate and nitrite degraded within 1.5 h. Figure 4 shows the nitrate and nitrite profiles during denitrification of 1,129 ppm NO_3N and 1,693 ppm NO_3N. While it took only 4.5 h to degrade 1,129 ppm synthetic NO_3N waste, it took 10 h to degrade 1,693 ppm NO_3N synthetic waste completely. These studies were carried out for 15 days for each nitrate concentration, and the values reported here depict an average of the daily analysis data. The bioreactor was run for 45 days with 100% activity. After each batch test, the synthetic waste

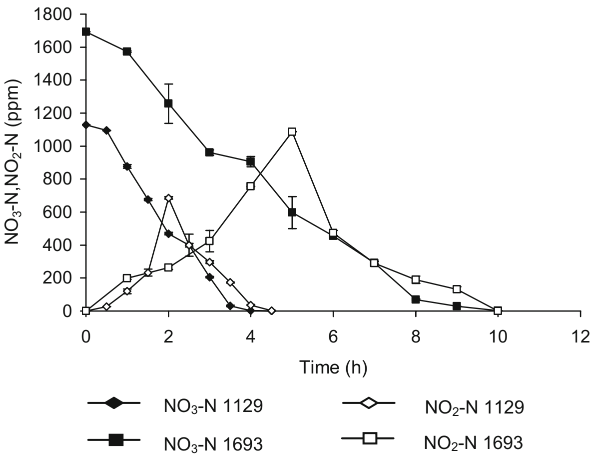

Fig. 4 Nitrate and nitrite profile during denitrification of 1,129 ppm NO_3N and 1,693 ppm NO_3N influent synthetic nitrate waste by sludge immobilized in foam cubes and flannel cloth (immobilized unit). Attached biomass (foam)=47 mg/cube (30 No), attached biomass (flannel cloth)=1 mg cm^{-2} (104×7 cm^2 cloth piece). Error bars indicate standard deviation of triplicate samples

was replaced with fresh synthetic waste. The treated waste did not show any turbidity, unlike batch tests with free cells which had to be kept for settling for 2 h to separate out the cells from the treated waste [20]. Thus, the two materials (cloth and polyurethane foam) used in combination were not only found to be good support materials for immobilization of whole cells in waste treatment but also effective and time-saving.

Conclusions

The processes used to treat nitrate wastes include the use of immobilized enzymes, cells, and other biomaterials in batch/continuous flow reactors. And for treatment of wastes using immobilized cells, materials like cloth and foam have been found to be good supports for immobilization of whole cells. This process of immobilization by passive adsorption of cells on surfaces is one of the most common approaches/techniques for cell adhesion and has played an important role in many biotechnological applications such as waste water treatments and fermentation like vinegar [23]. In this study, sludge from an ETP plant adapted to high nitrate waste was immobilized on cloth and foam pieces, and their use for denitrification of synthetic nitrate waste as high as 1,693 ppm NO_3N was analyzed. Sludge immobilized onto cloth and foam pieces were found to be capable of degrading 225 and 1,129 ppm NO_3N synthetic nitrate waste completely in a period of 2 and 6 h, respectively, without any loss in activity over a period of 15 days.

The immobilized sludge unit comprising of both flannel cloth and polyurethane foam were found to be capable of degrading 225, 1,129, and 1,693 ppm NO_3N synthetic nitrate waste completely in a period of 1.5, 4.5, and 10 h, respectively.

The system remained stable without any loss in activity over a period of 45 days, and the immobilized cells were found to easily adapt to the increase in nitrate load. There was no significant increase in the biomass density in the foam and cloth pieces, and at no point was detachment of the cells from the support observed.

This method of immobilization of whole cells was found to be quite economical and efficient in degrading high nitrate waste. Also, the non-toxicity of the support materials makes the process of discarding treated waste into the environment convenient without any post-treatment. Thus, this method not only increased the efficiency of the process but also facilitated separation of solid (biomass) and liquid (waste).

Acknowledgments The authors thank Department of Atomic Energy (India) for funding this work and Rashtriya Chemicals and Fertilizers, Mumbai (India) for providing the sludge.

References

1. Bouchard, D. C., Williams, M. K., & Surampalli, R. Y. (1992). *Journal of the American Water Works Association*, *84*, 85–90.
2. Young, J. C., & Yang, B. S. (1989). *Pollution Control Federation*, *61*, 1576–1587.
3. Albagnae, G. (1990). *Water Science and Technology*, *22*, 17–24.
4. Cheng, S. S., Huang, S. Y., Lay, J. J., Tsai, P. S., & Cho, L. T. (1992). *Water Science and Technology*, *26*, 503–510.
5. Camargo, S. A. R., & Nour, E. A. A. (2001). *Water Science and Technology*, *44*, 63–70.
6. Verrier, D., Mortier, B., & Albagnae, G. (1987). *Biotechnology Letters*, *10*, 735–740.
7. Vandevivere, P., & Kirchman, D. L. (1993). *Applied Environmental and Microbiology*, *59*, 3280–3286.
8. Picanco, A. P., Vallero, M. V. G., Gianotti, E. P., Zaiat, M., & Blundi, C. E. (2001). *Water Science and Technology*, *44*, 197–204.

9. Huysman, P., van Meneen, P., van Assche, P., & Verstraete, W. (1983). *Biotechnology Letters*, *5*, 643–648.
10. Calzada, J. F., Arriola, M. C., Castañeda, J. E., Godoy, J. E., & Rolz, C. (1984). *Biotechnology Letters*, *6*, 385–388.
11. Gizgen, H. J., Schoenmakers, T. J. M., Caerteling, C. G. M., & Vogels, G. D. (1988). *Biotechnology Letters*, *10*, 61–66.
12. Zaiat, M., Vieira, L. G. T., & Foresti, E. (1997). *Water Research*, *31*, 1760–1766.
13. Havens, P. L., & Howard, F. R. (1993). *Industrial & Engineering Chemistry Research*, *32*, 2254–2258.
14. Varesche, M. B., Zaiat, M., Vieira, L. G. T., Vazoller, R. F., & Foresti, E. (1997). *Applied Microbiology and Biotechnology*, *48*, 534–538.
15. D'Souza, S. F., & Kamath, N. (1988). *Applied Microbiology and Biotechnology*, *29*, 136–140.
16. Joshi, S., & Yamazaki, H. (1984). *Biotechnology Letters*, *6*, 797–802.
17. D'Souza, S. F., & Kubal, B. S. (2002). *Journal of Biochemical and Biophysical Methods*, *51*, 151–159.
18. Lewis, V. P., & Yang, S. T. (1992). *Biotechnology and Bioengineering*, *40*, 465–474.
19. D'Souza, S. F., & Melo, J. S. (2001). *Process Biochemistry*, *36*, 677–681.
20. Dhamole, P. B., Nair, R. R., D'Souza, S. F., & Lele, S. S. (2007). *Bioresource Technology*, *98*, 247–252.
21. Nair, R. R., Dhamole, P. B., Lele, S. S., & D'Souza, S. F. (2007). *Chemosphere*, *67*, 1612–1617.
22. D'Souza, S. F., & Kamath, N. (1991). *Enzyme and Microbial Technology*, *13*, 935–938.
23. Marshall, R. C. (1984). In *Microbial adhesion and aggregation*. New York: Springer.