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Denitrification of high nitrate wastewater in a cloth strip bioreactor with immobilized sludge

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Abstract Denitrification of synthetic high nitrate wastewater containing 40,000 ppm NO_3 (9,032 ppm NO_3-N) was achieved using immobilized activated sludge in a column reactor. Active anoxic sludge adsorbed onto Terry cloth was used in the denitrification of high nitrate wastewater. The operational stability of the immobilized sludge system was studied both in a batch reactor and in a continuous reactor. The immobilized sludge showed complete degradation of different concentrations of NO_3-N (1,129, 1,693, 3,387, 6,774, and 9,032 ppm) in a batch process. The reactors were successfully run for 90 days without any loss in activity. The immobilized cell process has yielded promising results in attaining high denitrifying efficiency.

Keywords Denitrification · Terry cloth · Immobilization · High strength nitrate · Wastewater

Introduction

Nitrogen-containing compounds released into the environment are a growing concern because they create serious problems, such as eutrophication of rivers, deterioration of water quality, and are potential hazards to human health. In the gastrointestinal tract, nitrate can be reduced to nitrite ions, which have the potential to form carcinogenic N-nitrous compounds [14]. According to the US Environmental Protection Agency [26], the permissible level for nitrate in drinking water is 10 ppm. The most commonly used process for the treatment of such nitrate wastewater is denitrification, wherein nitrate is converted to nitrite and then to nitrous oxide and consequently to nitrogen gas. Biological denitrification is widely used for treating nitrate wastewater but as it is usually slow, a high rate denitrification process is needed. Adapted mixed bacterial cultures from various industrial wastewater treatment plants are very useful in treating such high nitrate wastewater [6–8, 15, 25].

Immobilized cells have a major advantage in the development of wastewater treatment because the use of free cells in a continuous reactor leads to a decrease in biomass with each washout of the column reactor. An immobilized cell system improves the reactor capacity by increasing the biomass retention time rather than the liquid retention time. It also allows for an easier solid–liquid separation thereby eliminating problems associated with the occurrence of bulking. Large amounts of biomass can be retained on a support in the reactor, resulting in a high wastewater processing speed. Sludge immobilization is a technology developed to enhance sludge separation. Because sludge with high bioactivity is physically adsorbed onto a support matrix, effluent can be discharged from the reactor without much sludge washout [16, 17].

Different systems are used for the denitrification process, including the sequencing batch reactor [5, 15, 23], packed bed reactor [19], and rotating biological contactor [4, 16]. Recent developments in cell immobilization techniques have enhanced the efficiency of such reactors. Whole cells have been immobilized using a variety of supports and techniques [2, 11, 24, 27], in which adsorbed cells and biofilm play an important role in many natural and biotechnological applications [13, 21]. Surface immobilization of the cells by adhesion helps the direct contact of the cells with the liquid phase containing the substrates, though they are distinctly separated. This

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reduces mass transfer problems which are generally found in gel entrapment techniques. Moreover a high cell concentration can be retained. Unlike the particulate or beaded polymers, a support matrix in the form of fabric in general and cotton cloth as a specific example are amenable for use in various novel reactor systems. The major advantage of cotton cloth is that it is available in a fibrous matrix form with different thickness and surface areas. The immobilization technique is usually followed by colonization of the microbes, by recycling cell biomass along with nutrients such that a biofilm of the cells is formed gradually [20]. The studies from our laboratory and others have demonstrated the use of cloth in a frame reactor [12], rectangular film bioreactor [17], or column reactor [9, 24]. Cloth and other matrixes have also shown potential in the fabrication of spiral wound annular reactors containing immobilized cells for fermentation [10, 20]. Novel techniques for immobilized viable and nonviable cells through adhesion on a variety of polymeric surfaces including glass, cotton fabric, and synthetic polymeric membranes using polyethyleneimine (PEI) have also been developed in our laboratory [10, 12, 13, 18, 22, 24].

Studies on the treatment of high nitrate wastewater using adapted sludge have been carried out successfully on a laboratory scale [6–8, 24, 25] as well as on a pilot scale [3]. Though biological treatment of nitrate wastewater using immobilized systems has been suggested, none of them claim the treatment of wastewater containing as high as 40,000 ppm NO_3 (9,032 ppm NO_3-N) using a simple yet durable support material in a continuous reactor. The present work deals with the denitrification of synthetic nitrate wastewater using active adapted sludge immobilized on Terry cloth strips and their use in the designing of a continuous bioreactor system for the treatment of high nitrate wastewater.

Materials and methods

Microorganisms

The sludge used for immobilization was obtained from the denitrification plant of a fertilizer factory. This sludge was acclimated to nitrate ions as high as 9,032 ppm NO_3-N, in the process described in our previous work [8, 25].

Experimental setup

A Terry cloth was cut into strips (no.15) of 3 cm breadth and 60 cm in length. These strips were placed in sludge for biofilm formation. The sludge was kept activated by the daily replacement of used up synthetic nitrate wastewater with fresh synthetic nitrate wastewater [25]. These sludge-immobilized cloth strips were stitched together with polypropylene mesh strips in such a way that each polypropylene mesh strip (3 cm × 60 cm) was flanked by two cloth strips. This allows the cloth strips to hang freely without adhering to each another. Each one of the polypropylene mesh strips thus held two cloth strips. The polypropylene mesh strips (no. 16) containing the sludge-immobilized cloth strips were held together and hung with the help of an aluminum wire frame. The frame containing the cloth strips was then loaded inside a 1-m temperature-controlled column with an inner diameter of 5 cm (Fig. 1a). The temperature of the column was maintained by pumping water into the outer jacket using a thermoregulated water bath.

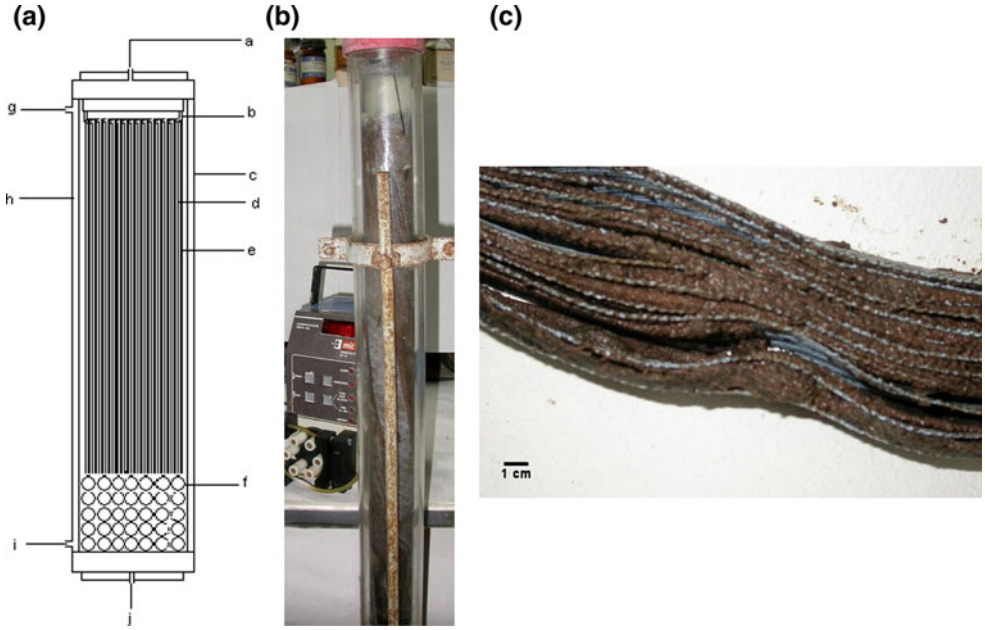

Fig. 1 **a** Schematic diagram of cloth strip bioreactor. *a* Feed inlet, *b* aluminum wire, *c* glass column, *d* cloth strip, *e* polypropylene mesh strip, *f* glass marbles, *g* water outlet, *h* water jacket, *i* water inlet, *j* product outlet. **b** Photograph of the working bioreactor. **c** Photograph of cloth strips with the immobilized biomass flanking polypropylene mesh strips (*scale bar* 1 cm)

The synthetic nitrate waste was pumped into the bioreactor from the top using a peristaltic pump.

Composition of synthetic wastewater

The synthetic wastewater was composed of (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, EDTA 50, and $(NH_4)_6MO_7O_{24}{\cdot}H_2O$ 1.1 [8, 25]. Sodium acetate was used as a carbon source and sodium nitrate was used as nitrogen source. A C/N ratio of 2.25:1, as optimized in our previous experiments [25], was used in this study.

Batch experiment

Batch tests were carried out in the bioreactor with a working volume of 1 l (Fig. 1b). The denitrification process using the immobilized sludge was optimized as described in our previous work [25] and the present reactor was run in the same conditions. The pH of synthetic wastewater was adjusted to 7.2 and the temperature of the column was maintained at 37 °C by pumping water into the outer jacket using a thermoregulated water bath. The studies were carried out initially at a lower concentration of 1,129 ppm NO_3-N. Concentrations of the synthetic nitrate wastewater were then subsequently increased to 1,693, 3,387, 6,774, and 9,032 ppm NO_3-N. Each concentration was studied for a period of 15 days. The synthetic wastewater was circulated in the reactor using a peristaltic pump. The nitrate and the nitrite profile were studied for each concentration of nitrate wastewater.

Continuous experiment

The continuous experiments were also carried out in the reactor of same working volume as the batch tests. Every test began as a batch test of 15 days. When nitrate was completely degraded, a continuous flow of feed was started. For the continuous process, synthetic wastewater was pumped with a peristaltic pump at different flow rates into the reactor to give different hydraulic retention times (HRTs). An HRT of 8, 3, 2, and 1.6 h was used in this study, for three different concentrations of synthetic nitrate wastewater (1,129, 1,693, and 3,387 ppm). The effluent wastewater was collected and tested for NO_3, NO_2, and chemical oxygen demand (COD).

Analysis

The nitrate and nitrite concentrations in the samples were analyzed using a DIONEX ion chromatograph (AS11, 2 mm column) with 12 mM NaOH as the eluent. COD was estimated using the reflux method [1]. All the samples were centrifuged and filtered before analysis. Dilution was carried out using deionized water.

Results and discussion

Adhesion of sludge to Terry cloth

The strips of Terry cloth were placed in sludge to allow the formation of biofilm. The sludge was kept in an activated condition by the regular addition of fresh synthetic wastewater. This system was maintained for a period of 2 weeks. As seen in Fig. 1c, the sludge formed a very thick biofilm on the cloth strips after 2 weeks of contact with the activated sludge.

Batch experiment

Adapted sludge after immobilization onto Terry cloth was used for denitrification studies using a column reactor. All the conditions required for the denitrification process and optimized in our laboratory were provided for the biotreatment of high nitrate wastewater using the immobilized sludge [6–8, 24, 25]. In the first trial, 1,129 ppm NO_3-N synthetic wastewater was subjected to degradation. The nitrate and nitrite profile for this set is shown in Fig. 2. It was seen that in a period of 0.75 h there was total degradation of nitrate with a buildup of nitrite, which was degraded completely thereby showing complete denitrification in a period of 1.25 h. The system was run as a batch process at this concentration for a period of 15 days. The nitrate concentration was sequentially increased to 1,693 ppm NO_3-N. Figure 2 also shows the nitrate and nitrite profiles during the denitrification of 1,693 ppm of

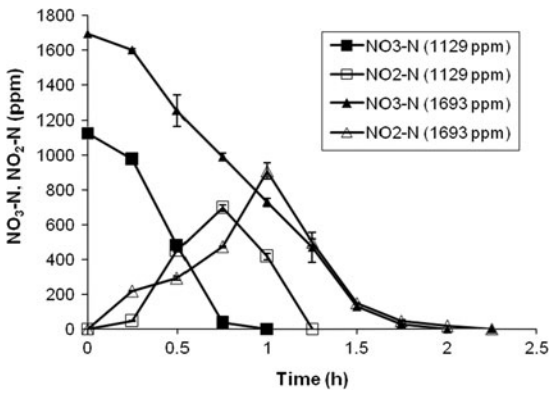

Fig. 2 Nitrate and nitrite profile during denitrification of 1,129 ppm NO_3-N influent synthetic nitrate waste and 1,693 ppm NO_3-N influent synthetic nitrate waste. *Error bars* are/ indicate standard deviation of triplicate samples

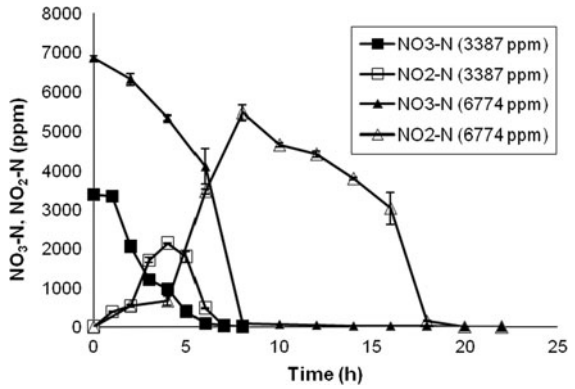

Fig. 3 Nitrate and nitrite profile during denitrification of 3,387 ppm NO_3-N influent synthetic nitrate waste and 6,774 ppm NO_3-N influent synthetic nitrate waste. *Error bars* are/ indicate standard deviation of triplicate samples

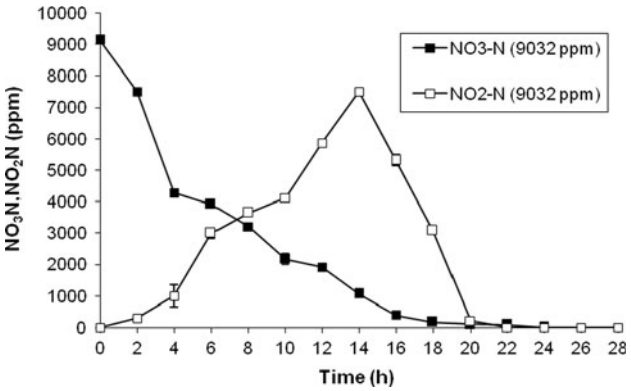

Fig. 4 Nitrate and nitrite profile during denitrification of 9,032 ppm NO_3-N influent synthetic nitrate waste. *Error bars* are/ indicate standard deviation of triplicate samples

NO_3-N. It was observed that nitrate was reduced completely to nitrite in less than 2 h with a buildup of nitrite that subsequently degraded. Thus complete degradation was observed in 2.25 h. Input nitrate concentration was doubled to 3,387 ppm of NO_3-N. This set was continued for another 15 days. Figure 3 shows the nitrate and nitrite profiles during the degradation of 3,387 ppm of NO_3-N. Complete degradation of nitrate was observed here in a period of 7 h, with nitrate degrading completely to nitrite in 6 h.

Input nitrate concentration was again doubled and denitrification in this setup was also studied for 15 days. The nitrate and nitrite profiles during denitrification of 6,774 ppm NO_3-N are shown in Fig. 3. Though the nitrate was degraded completely in a period of 8 h, the nitrite that formed degraded only within 18 h. Denitrification studies at a higher concentration (9,032 ppm NO_3-N) of nitrate were also carried out for 15 days. Input nitrate concentration was increased to 9,032 ppm NO_3-N. The nitrate and nitrite (after 15 days of adaptation to 6,774 ppm NO_3-N) profiles for the degradation of 9,032 ppm NO_3-N is shown in Fig. 4. It can be seen from the figure that nitrate is reduced to nitrite in just 18 h and there is a buildup of nitrite that subsequently degrades within 22 h. At a concentration of 6,774 and 9,032 ppm NO_3-N it can be seen that the nitrite buildup has increased subsequently. When all of these five different concentrations of influent are compared, an increase in nitrite buildup was found with an increase in influent nitrate concentration. The nitrite buildup is explained in the following reaction:

$$NO_3 \rightarrow NO_2 \rightarrow NO \rightarrow N_2O \rightarrow N_2$$

The first step of biological denitrification starts with nitrate first being converted to nitrite and then further reduced to N_2O or N_2 gas. When the rate of reduction of nitrate to nitrite is higher than the rate of degradation of nitrite, a buildup of nitrite is observed.

The bioreactor was run for 2 weeks at each nitrate concentration and the column was found to be stable throughout the different concentrations. In our previous work [8, 25], denitrification of such high nitrate wastes was studied in a sequence batch reactor. In such reactors, management of biomass after every run was tedious. It required settling and separation of the biomass from the treated wastewater. Also there was a need to remove the bulk biomass produced during the treatment process. Immobilizing the sludge can reduce and eradicate these problems as the biomass is adsorbed onto the cloth support and the treated effluent could be discharged easily from the reactor without any post sludge removal treatment. Unlike other immobilization supports like polyurethane foam, polyvinyl alcohol gels, or cellulosic materials like DEAE cellulose, cloth not only represents a more compact structure but also acts as a completely retrievable system in a batch reactor.

Denitrification process in a continuous packed bed reactor

Each test started as a batch and when nitrate was completely reduced (1.25, 2.25, and 7 h for 1,129, 1,693, and 3,387 ppm, respectively), continuous flow of feed solution was started. Synthetic nitrate wastewater used as feed solution was prepared daily and checked for NO_3-N, NO_2-N concentrations, COD, and pH. The performance of denitrification in the bioreactor at different feed NO_3-N concentrations and HRTs is shown in Figs. 5, 6, and 7. Figure 5 indicates the nitrate, nitrite, and COD profile during denitrification of 1,129 ppm NO_3-N at different hydraulic retention times (8, 3, 2, and 1.6 h). Nitrate and nitrite were completely degraded in all four HRTs. The column was run at an HRT of 8 h with no nitrate, nitrite,

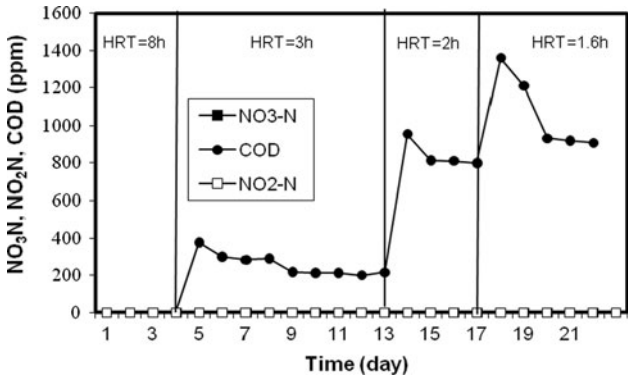

Fig. 5 Nitrate, nitrite, and COD profile during denitrification of 1,129 ppm NO_3-N at different HRTs using sludge immobilized onto Terry cloth in a continuous cloth strip bioreactor

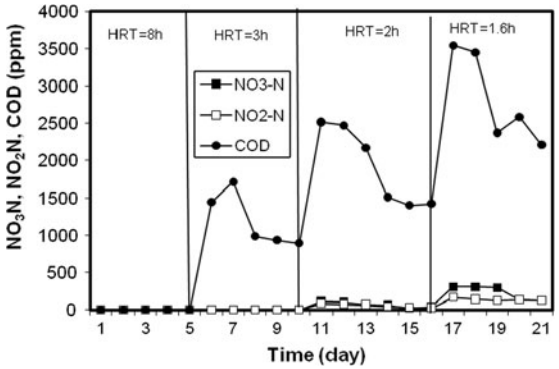

Fig. 6 Nitrate, nitrite, and COD profile during denitrification of 1,693 ppm NO_3-N at different HRTs using sludge immobilized onto Terry cloth in a continuous cloth strip bioreactor

and COD in the outlet. But with a decrease in HRT, there was an initial increase in COD which decreased eventually. The HRT was changed at a stable COD.

The nitrate, nitrite, and COD profile during the continuous denitrification of 1,693 ppm NO_3-N synthetic nitrate wastewater is shown in Fig. 6. At an HRT of 8 h there were no NO_3, NO_2, and COD in the effluent. But when the HRT was reduced to 3 h, though there were no NO_3 and NO_2 in the outlet, there was an increase in the COD. This increase was due to a buildup of unused carbon which was used partially in the course of time, thereby showing a decrease. When the COD was stable, the HRT was further reduced to 2 h. At an HRT of 2 h, NO_3 and NO_2 could be detected in the outlet. The COD increased to 2,520 ppm, which decreased eventually to 1,421 ppm. On further decrease in HRT to 1.6 h, NO_3 and NO_2 ions were found in the outlet, which showed an increase initially and later a decrease, with its consumption.

With the complete degradation of 3,387 ppm NO_3-N synthetic nitrate wastewater, the reactor was made continuous, and the NO_3, NO_2, and COD profile was studied

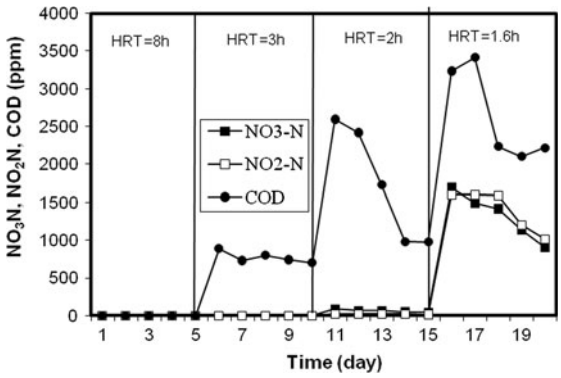

Fig. 7 Nitrate, nitrite, and COD profile during denitrification of 3,387 ppm NO_3-N at different HRTs using sludge immobilized onto Terry cloth in a continuous cloth strip bioreactor

Table 1 Nitrate, nitrite, and COD levels in the outlet during the denitrification of 1,129, 1,693, and 3,387 ppm NO_3-N at different HRTs (8, 3, 2, and 1.6 h) in a continuous cloth strip bioreactor

Influent NO_3-N concentration (ppm)	Hydraulic retention time (h)	NO_3-N (ppm)	NO_2-N (ppm)	COD (ppm)
1,129	8	–	–	–
	3	–	–	215
	2	–	–	800
	1.6	–	–	910
1,693	8	–	–	–
	3	–	–	890
	2	24	29	1,400
	1.6	121	127	2,210
3,387	8	–	–	–
	3	–	–	700
	2	9	4	976
	1.6	203	308	2,214

for a period of 20 days at different HRTs. In Fig. 7, it can be seen that, at an HRT of 8 h, there were no NO_3, NO_2, and COD in the outlet. But with further decrease to 3 h, there was an increase in COD in the outlet. A total of 19 ppm NO_3-N and 5 ppm NO_2-N were also found in the outlet, which decreased to 9 ppm NO_3-N and 4 ppm NO_2-N. Such increases in NO_3-N and NO_2-N were also found at an HRT of 2 and 1.6 h. A detailed list of the nitrate, nitrite, and COD values during the continuous denitrification of different concentrations of synthetic nitrate wastewater at different HRTs is given in Table 1. At no point was the column clogged or any change in the flow rates observed. Table 1 depicts that at an HRT of 8 h, the immobilized cells were able to completely denitrify with zero COD in the outlet for all three concentrations of NO_3-N studied (1,129, 1,693, and 3,387 ppm). But with a decrease in HRT, the COD values increased, because of the

accumulation of unused carbon. In our previous studies [25], the denitrification process was optimized in a batch scale reactor and we had shown how C/N ratio was an important factor in controlling the denitrification activity by influencing the denitrification rate. A C/N ratio of 2.25:1 was found to be optimum for the process, though a residual carbon of 143 ppm (below the permissible disposable limit of 250 ppm) was also detected. Such COD levels are appropriate for a batch process, but in a continuous process they lead to accumulation of unused carbon, thereby creating disposal problems. Thus to avoid carbon accumulation in a continuous reaction process, it could be proposed to either maintain an HRT of 8 h for 1,129, 1,693, and 3,387 ppm NO_3-N, where no carbon accumulations occur, or decrease the C/N ratio below 2.25:1 such that the outlet COD value is below permissible disposable limits. But this can slightly reduce the denitrification efficiency.

The above experiments show that immobilization of the adapted sludge on Terry cloth was quite effective in denitrifying high nitrate wastes. These wastewaters with high nitrate concentration usually make adhering of the cells to immobilization supports difficult as they cannot withstand such high ionic concentrations, pH, and flow rates. Cells entrapped in gels or immobilized onto a plain surface like the RBC biofilm [16, 27] usually tend to be washed off due to high ionic wastewaters and are good only for low strength nitrate wastewaters. The experiments discussed above showed that such conditions of wastewater which normally disrupt ionic interactions fail to desorb cells immobilized on cloth by adhesion, as the adhesion is very strong. Moreover, in the denitrification process, N_2 gas is the end product formed by degradation of nitrate and removal of the gas is important for the smooth running of the reactor. This gas generally gets trapped in the reactor in case of cells immobilized in alginate or polyvinyl alcohol beads. It not only leads to attrition but also the destruction of the beads. In such cases, Terry cloth is advantageous as problems of gas entrapment are not encountered. The gas bubbles easily find their way out through the polypropylene mesh to the top of the column reactor. Treatment of high nitrate wastewaters also requires a high cell to substrate ratio. This is achieved in Terry cloth owing to its fibrous matrix, thickness, and the surface area, which allow more cells to adhere to the surface as compared to simple cotton cloth (flannel) [17, 18, 22], gel beads [27], or RBC biofilm [16].

Conclusions

To evaluate the prospects of Terry cloth as a support for whole cell immobilization and its use in long-term denitrification, a cloth strip column bioreactor was operated. High concentrations of synthetic nitrate wastewater could be degraded efficiently when the reactor was run as a batch. In the continuous reactor system, although high nitrate removal was achieved at an HRT as short as 3 h, a higher HRT had to be provided for significant nitrate and COD removal. Thus this immobilization method which is based on entrapment and adhesion of microorganisms was found to be quite economical and efficient in degrading high nitrate wastewater. Also the nontoxicity of the support and the immobilization process makes the disposal of the wastewater convenient by discarding it directly into the environment without any post sludge removal treatment. On the basis of these experiments, we could operate the reactor for more than 90 days without any loss of activity or reactor stability. Terry cloth thereby holds potential for use in large-scale applications owing to its simplicity, strong binding, and good operational stability.

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