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Denitrification of high sodium nitrate bearing effluents using flow-through bioreactor

U. Sandhya*, D. Banerjee, I.J. Singh, P.K. Wattal

Process Development Division, Bhabha Atomic Research Centre, Trombay, Mumbai, India
Tel. +91 22 25594106; Fax: +91 22 25505150; email: sandhyau@barc.gov.in

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ABSTRACT

Denitrification of sodium nitrate solution has been studied by isolating biomass, identification of suitable growth medium and finally its use in batch bioreactor. The progress of denitrification of 730 mg/l nitrate as $NaNO_3$ solution has been evaluated for 10 consecutive cycles. The use of harvested cells with gradual increase of nitrate concentration in the batch bioreactor resulted in better acclimatized biomass. Complete denitrification of different concentrations of $NaNO_3$ solution up to 8800 mg/l NO_3^- was reached in batch studies. A flow through bioreactor was assembled by growing biomass on to interstices of stainless steel modules and this system was used to study the effects of process variables like C/N ratio, addition of trace elements, etc., on denitrification of high nitrate laden solutions. Based on the gradual acclimatization of biomass to high nitrate bearing solutions, complete denitrification of 12,400 mg/l of nitrate was achieved. Successful demonstration of the process by continuous operation of the column over a period of six months shows the viability of the process in practical application, for denitrification of effluents generated during back end of the nuclear fuel cycle.

Keywords: Biodenitrification; Pseudomonas; Radioactive effluent; Nitrate; Bioreactor; Sodium nitrate; Biomass

1. Introduction

Increasing levels of nitrate in ground water as well as in surface water are of concern as it finally enters into human body through drinking water and causes serious health hazards. Consumption of nitrate leads to problems like blue baby syndrome (methemoglobinemia) in infants and conversion of nitrates to nitrites in saliva causes formation of nitrosamines, which are known carcinogens. In addition, waste waters with excess nitrate when discharged into surface waters are toxic to certain fishes, lead to algal blooms and also cause various ill effects when used as water for livestock. In order to protect our environment and to avoid the adverse effects associated with nitrates, WHO and US environment protection agency has regulated limits for nitrate in drinking water as 50 mg/l NO_3^- (11.1 mg/l NO_3–N) and 10 mg/l NO_3–N, respectively [1,2]. In India, limits for NO_3^- in drinking water and discharge to environment are set at below 45 mg/l [3,4]. The elevated nitrate levels in water are primarily due to overgrowing use of fertilizers, discharge of waste water from various domestic and industrial sources and animal waste. Nitrate salts are highly soluble in water and its high mobility accelerates its transport to ground water and surface water by percolation through soil [5]. Removal of nitrate from such effluents before discharge is thus considered necessary for reduction of nitrate load to environment.

While the nitrate level in effluents generated from various industrial processes, like production of fertilizers,

*Corresponding author.

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explosives, pectin, metal finishing, NO_x absorption in air washing devices are in the range of a few thousand mg/l, the NO_3^- concentrations in the effluents regenerated from various stages of nuclear fuel cycle, mainly from reprocessing plants are significantly higher, notably more than 50,000 mg/l [6,7]. Three types of wastes viz., high level, intermediate level and low level, are generated from reprocessing of spent fuel. In India, high level waste is acidic in nature and contains molar level of nitrates in the form of nitric acid which is used for dissolution of the spent fuel. The intermediate level waste, generated during volume reduction of high level waste by evaporation, is also acidic in nature and thereafter it is made alkaline and stored in carbon steel tanks. The NO_3^- concentration in this waste is also more than 50,000 mg/l and it is in the form of $NaNO_3$ [8]. The low level wastes are near neutral and have much lower nitrate levels of a few thousand mg/l.

Various methods of denitrification have been studied including chemical and electrochemical methods, ion exchange, reverse osmosis, thermal methods, biological denitrification etc., [9–11]. Among these, biodenitrification has recently emerged as the most effective, both with regard to secondary waste generation and technological status. In this process, nitrates are reduced to N_2 gas by the denitrifying microorganisms [12]. Commonly known denitrifies in wastewater system belong to *Achromobacter, Bacillus, Halobacterium, Pseudomonas, Thiobacillus* species etc. These microorganisms utilize nitrate as an energy source in the presence of a carbon source like ethanol, methanol, acetate, glucose, formate etc. Careful selection of biomass and medium constituents are important to obtain maximum denitrification efficiency.

Industrial scale application of the biological based denitrification processes has so far been limited to the treatment of low nitrate bearing effluents only [13–16]. Batch bioreactors and several fixed bed columns packed with activated carbon, sand, gravel, lava stones, plastics, ceramics etc. as support for microbial growth have been extensively used for denitrification of drinking water [13,14]. Some of the processes including fluidized-bed, packed bed and immobilized biomass have been used in treatment of low nitrate bearing effluents too [15,16]. In situ biological denitrification processes have also recently attracted interest especially in ground water studies where substrates and nutrients are injected into aquifers to enhance denitrification rate [12]. With regard to high nitrate bearing effluents, one of the first successful studies is reported by Francis and Mankin who used a column packed with fine particles of anthracite coal in suspension for denitrification of about 20,000 mg/l of NO_3^- wastes [17]. Glass and Silverstein from ORNL laboratory accomplished denitrification of 36,000 mg/l of NO_3^- as $Ca(NO_3)_2$ in a tank as well as pond bioreactors [18]. A successful attempt of a similar concentration level of $NaNO_3$ from BARC, India has also been reported recently [19]. This is also a batch process in which a stirred tank is used as reactor. These results indicate that gradual acclimatization of sludge to the wastewater nitrate concentration helped to develop a suitable consortium for treatment of high nitrate bearing effluent.

Present communication reports denitrification of 12,400 mg/l of nitrate using a novel flow through bioreactor system. Isolation of the biomass and its use in batch bioreactor for denitrification of up to 8800 mg/l of nitrate as $NaNO_3$ has been carried out initially. The biomass was then allowed to grow on stainless steel wire gauge modules which were then used to build a flow through bioreactor. Optimization of process variables like C/N ratio, concentration of trace elements, etc., has been carried out at various stages during gradual increase of NO_3^- concentration. This study helped to develop a process for complete denitrification of 12,400 mg/l of NO_3^- bearing solution. It is noteworthy to mention that growth of microbial culture in a minimal medium is maintained throughout the investigation in order to keep COD of treated effluent minimum. Excellent performance of the column during continuous operation over a period of six months shows the usefulness of the process for treatment of high nitrate bearing nuclear effluents generated from reprocessing plants.

2. Experimental

2.1. Materials

All the chemicals used in feed preparation are of AR grade, S.D. Fine, India make. Feed solution was prepared in tap water (pH 7.2) and stored in poly propylene carboy. Nitrate concentration in tap waste was checked before feed preparation and found to be less than 2 mg/l. The feed solution used in column study was prepared twice a week to minimize the loss of methanol by evaporation at ambient conditions. The wire gauge modules used in column as biomass support was made of stainless steel 304 l. Sludge collected from an Indian industrial plant, wherein it was being used for denitrification of low levels of fertilizer waste streams, was inoculated into a 1 l solution containing about 700 mg NO_3^- as $NaNO_3$, 1 ml methanol and 10 mg phosphate as Na_2HPO_4. The solution was magnetically stirred continuously for a week and then the grown biomass was used in our studies as described below.

2.2. Batch study

2.2.1. Batch study in replenished feed

A batch bioreactor was set up using a high neck 3 l round bottom flask loaded with 3 l solution containing

about 6.0 g of wet biomass and 730 mg/l of NO_3^- as $NaNO_3$ along with 3 ml methanol and 10 mg/l phosphate as Na_2HPO_4. The reactor was stirred continuously with the help of magnetic stirrer bar. About 10 ml of sample was withdrawn periodically and analyzed for both NO_3^- and NO_2^- spectrophotometrically using method of Kamphake et al. [20]. After complete denitrification (NO_3^- concentration in effluent below 20 mg/l), the solution was replenished by adding requisite quantity of $NaNO_3$ (in solid form), methanol and the total volume made up to 3 l with water. Likewise, the extent of denitrification was monitored in 10 consecutive cycles.

2.2.2. Batch study using harvested biomass

This study was also carried out in the 3 l batch reactor. The biomass obtained at the end of earlier study was harvested and used here. The procedure followed for harvesting biomass includes separation of the biomass by centrifugation, washing thrice in distilled water and then storage in freezer. In order to compare the denitrification performance of harvested biomass with earlier study, the feed solution containing 730 mg/l of NO_3^- as $NaNO_3$, 3 ml methanol and Na_2HPO_4 (10 mg/l as PO_4) was used in the first three cycles. In each cycle, freshly prepared feed and 6.0 g (wet) of biomass was used. Likewise, two more sets of experiments, each set consisting of three cycles, were carried out using NO_3^- concentration 2200 and 4400 mg/l, respectively. In addition, one run was carried out with 8800 mg/l NO_3^- bearing feed. It is to be noted that a constant ratio of C/N and 10 mg/l of phosphate was maintained in all above experiments.

2.3. Column study

Stainless steel wire gauge packing modules (length–16 cm, diameter–10 cm), as shown in Fig. 1a, was chosen as support for biomass growth. Initially, for the growth of biomass into these modules, the modules were immersed in solution containing 730 mg/l as NO_3^-, methanol, PO_4^{3-} and inoculated with biomass from batch study. The feed was replenished weekly and after about a month, substantial growth of biomass was seen. Then four such modules were loaded into a 1 m long glass column (Fig. 1), which gave the operating volume of the bed of about 4.8 l. The feed solution was passed through the column, in the up flow mode using a peristaltic pump. The column was run continuously and effluent samples were collected daily and analyzed for NO_3^- and NO_2^-. After complete denitrification of 730 mg/l NO_3^-, stepwise acclimatization of the column was done by passing the feed solution (i) 2200 mg/l NO_3^-, methanol (C:N = 1.78) and 10 mg/l PO_4^{3-} and (ii) 4400 mg/l NO_3^-, methanol (C:N = 1.78) and 10 mg/l PO_4^{3-}. After ensuring complete denitrification of the 4400 mg/l feed, through the column was used for optimization

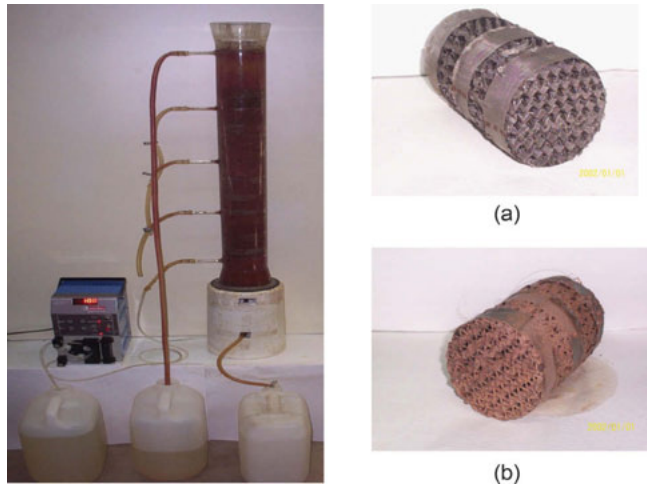

Fig. 1. Flow through bioreactor with biomass on stainless steel wire gauge packing modules, a) module before biomass growth and b) module after biomass growth.

of process variables as described below. It is to be noted that though the experiments were carried out at ambient conditions, room temperature varied from 28–34°C due to change of seasons.

2.3.1. Optimization of C/N ratio

For optimization of C/N, the column was first stabilized with a 4400 mg/l NO_3^- feed and methanol as the carbon source in the C:N ratio of 1.78 and 10 mg/l PO_4^{3-}, at the flow rate of 480 ml/h (HRT= 10 h). Then each denitrification cycle with varying C/N ratio ranging from 1.78 to 0.94 was monitored for a week keeping other parameters constant.

2.3.2. Trace element addition

The effect of trace element addition on denitrification performance was examined with the column stabilized for complete denitrification of 4400 mg/l NO_3^- feed, with methanol (C/N = 0.94) and 10 mg/l PO_4^{3-} at the flow rate of 480 ml/h (HRT = 10 h). In the feed, 1 ml/l of trace element solution of composition shown in Table 1 was added and denitrification performance was monitored.

2.3.3. Column acclimatization for higher NO_3^-

The column stabilized for complete denitrification of 4400 mg/l NO_3^- feed, with methanol (C/N = 0.94), 10 mg/l PO_4^{3-} and 1 ml/l trace solution, at the flow rate of 480 ml/h (HRT = 10 h), was used for gradual adaptation of biomass to higher nitrate solution. The nitrate concentration in feed was raised stepwise upto 12400 mg/l at increments of 1000 mg/l keeping other constituents constant. Every time, change in feed nitrate to higher

Table 1
Trace element composition

Element	Concentration (mg/l)
Co^{2+}	0.020
Ni^{2+}	0.042
Cu^{2+}	0.064
Mn^{2+}	0.358
Zn^{2+}	0.341
Mg^{2+}	1.725
Ca^{2+}	0.801
K^{+}	2.939
SO_4^{2-}	8.111
PO_4^{3-}	6.978
NO_3^{-}	0.043
Cl^{-}	4.0

concentration was done only after ensuring stable performance for a week. At a concentration of 12400 mg/l NO_3^- the run was continued under the same operating conditions for six months to ensure its performance.

2.4. Identification of the biomass

The acclimatized biomass from column studies was streaked on sterile nutrient agar plates and the predominant colonies were isolated, inoculated into slants and sent for identification at the Institute of Microbial Technology, Chandigarh, India.

3. Results and discussion

3.1. Pathway of denitrification

Bio-denitrification is a natural phenomenon and can be described as a respiratory process where nitrate oxygen is used as energy source. A large number of micro organisms are known to be capable of denitrification where the nitrate is reduced to nitrogen gas through a sequence of enzymatic reactions [21]. Biotechnology makes use of these microorganisms by providing optimum conditions for increased denitrification. The basic mechanism for nitrate reduction is known to follow the pathway as shown below [12]:

$$NO_3^- \rightarrow NO_2^- \rightarrow NO \uparrow \rightarrow N_2O \uparrow \rightarrow N_2 \uparrow$$

In the present study, a detailed analysis of effluents was performed with the samples withdrawn in batch studies using inoculated biomass and a representative profile is shown in Fig. 2. It can be seen that the NO_3^- concentration in effluent decreases systematically while NO_3^- concentration increases initially and then decreases slowly. Further the evolution of nitrogen gas from column was seen. From the disappearance of NO_2^- and evolution of N_2 gas, it can be inferred that the above mentioned breakdown pathway for denitrification of $NaNO_3$ is followed in the present study.

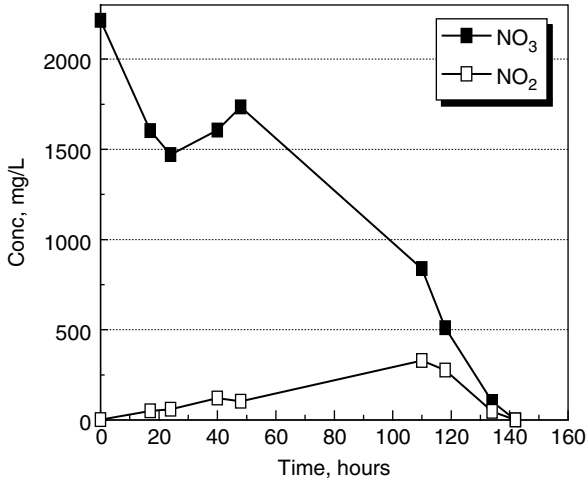

Fig. 2. Typical effluent profile during denitrification in batch bioreactor.

3.2. Batch study in replenished feed

Fig. 3 shows the denitrification performance of the biomass in batch bioreactor for ten consecutive cycles. It can be seen that the duration for complete denitrification of a batch reduced substantially from cycle to cycle. For example, the time taken for complete denitrification of the effluent in first cycle was 144 h and it then decreases to 40 h in 10th cycle. This is attributed to the fact that the biomass is well acclimatized to the medium leading to increase in the denitrification rate.

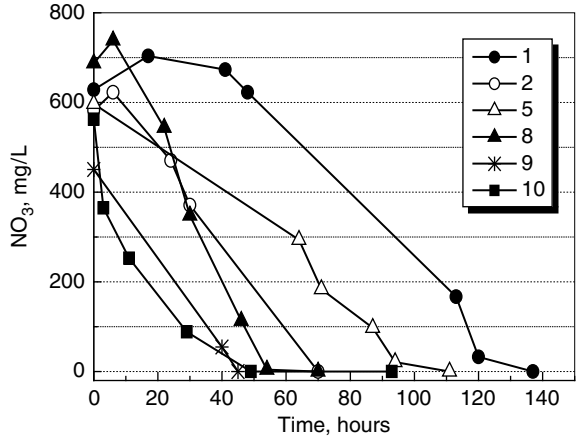

Fig. 3. Denitrification profile of $NaNO_3$ (730 mg/l NO_3) solution in batch bioreactor in 10 consecutive cycles.

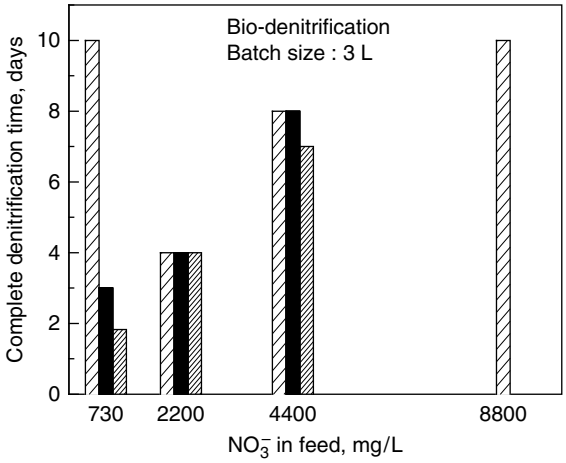

Fig. 4. Denitrification of $NaNO_3$ solution in batch bioreactor using harvested biomass.

3.3. Batch study using harvested biomass

Fig. 4 shows the denitrification performance using harvested cells. It is clearly evident that the rate of denitrification improved significantly with the use of harvested biomass in every cycle. For example, denitrification of 730 mg/l NO_3^- bearing feed was complete in 44 h in 3rd cycles itself, instead of earlier 10th cycle. In the case of 2200 mg/l and 4400 mg/l NO_3^-, denitrification was completed in 4 d and 7 d, respectively. This faster rate of denitrification is due to the use of harvested cells in fresh medium whereby ill effects due to build up of waste products was avoided. When biomass was further exposed to a higher nitrate level of 8800 mg/l, complete denitrification of NO_3^- was achieved indicating that exposure of the biomass to higher NO_3 bearing feed helped in selection of better adapted biomass.

From these results, it can be inferred that the biomass isolated in the present study is suitable for denitrification of the higher $NaNO_3$ bearing effluents. However, the observed rate of denitrification is slower than that reported by other investigators [19]. This may due to the use of minimal growth conditions and lower biomass loading in the present study. The use of minimal conditions can be considered advantageous, inspite of the lower rate of denitrification, as a minimum COD level in effluents can be maintained, thereby further treatment before discharge to environment can be avoided.

3.4. Column experiments

For column study, stainless steel wire gauge packing module was selected as support for bacterial growth because these modules have a very high internal surface area, where bacterial growth can take place within its pores. Further, these modules are physically sturdy to keep biomass attached for long periods of time and will not be affected by release of gases formed in the reactor. As expected, biomass growth was observed on the interstices of the metal sheets as can be seen from Fig. 1b. The geometrical volume of four modules loaded on the glass column was 4.8 l (before biomass growth). After biomass growth on modules, the volume was found to decrease to less than 2 l. This confirmed that extensive growth took place inside the module and it is very difficult to dislodge it physically.

3.4.1. Optimization of C/N ratio

Fig. 5 shows the results of optimization of C/N ratio for denitrification of the $NaNO_3$ bearing effluent. Optimization of carbon requirement is important as any excess will remain in the effluent leading to increase in effluent COD. Methanol was chosen as it is one of the simplest carbon sources for utilization by the organisms and will lead to low bacterial cell yield [22]. For optimization of C/N, the study was begun at C:N ratio of 1.78, and subsequently the ratio was decreased stepwise to 0.94. The lowest values of 0.94 used for the study was arrived at theoretically considering 30% for cell growth and stoichiometric requirement as per the equation below [12]:

$$6NO_3^- + 5CH_3OH \rightarrow 3N_2 + 5CO_2 + 7H_2O + OH^-$$

In all cycles, complete denitrification of 4400 mg/l NO_3^- was achieved during the course of study. The use of high carbon is preferable for rapid biomass growth and higher denitrification rate, while lower C/N ratio will lead to decreased efficiency [22]. In our study, denitrification efficiency was unaffected at a C/N ratio of 0.94 and was therefore adopted for optimization of

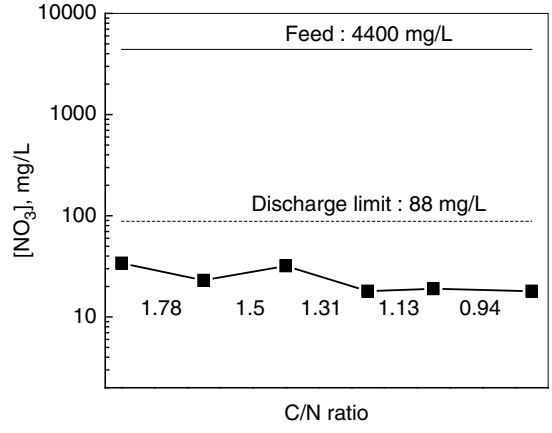

Fig. 5. Effect of C/N ratio on denitrification of $NaNO_3$ solution in flow through bioreactor.

other process variables. Woodbury and Dahab has also reported efficient denitrification using a C/N ratio of as low as 0.92 (with methanol as carbon source) [23].

3.4.2. Addition of micronutrients

Although complete denitrification of 4400 mg/l NO_3^- was achieved without additional micronutrients, it is well known that microbial growth requires various heavy elements in trace quantities to maintain good microbial growth. The addition of trace elements was adopted in the continuous system.

It was seen that the use of micronutrients resulted in better biomass growth on column as well faster rate of denitrification and was therefore used as a component of feed for all further experiments.

3.4.3. Column acclimatization for higher NO_3^-

Based on the results of optimization of process variables, the optimized feed composition of C/N=0.94, 10 mg/l PO_4^{3-} 1 ml/l trace element solution was selected for acclimatization of the biomass to higher nitrate levels. For acclimatization, gradual increase of NO_3^- concentration at increments of 1000 mg/l was adopted. It was observed that the introduction of a higher nitrate containing feed initially leads to a rise in effluent nitrate concentration which then decreases with time. A typical denitrification performance of the column during gradual acclimatization of biomass is shown in Fig. 6. Every time, the change in feed nitrate to higher concentration was done only after ensuring stable performance for a week. With the gradual increase of NO_3^- concentration to 12,400 mg/l NO_3 better adapted biomass was obtained as can be inferred from the efficient denitrification of this high nitrate bearing solution.

The extended column operation at 12400 mg/l NO_3^- bearing feed over a period of six months shows that effluent nitrate and nitrite levels remained below discharge levels throughout the study. The efficient and trouble free operation of the bioreactor shows promise for practical application in continuous, long term treatment of nitrate laden waste streams.

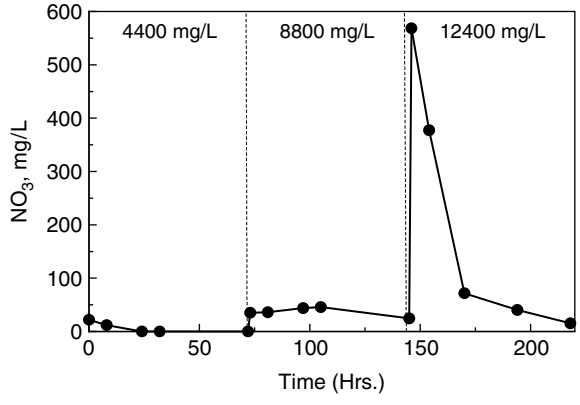

Fig. 6. Performance of flow through bioreactor for denitrification of higher $NaNO_3$ bearing solutions.

3.5. Identification of the biomass

From the microbial tests, it is seen that the predominant microorganisms are gram negative, short, motile rods. Results of biochemical tests show that the organisms were found to be catalase and cytochrome oxidase positive and were identified as *Pseudomonas aeruginosa.*

4. Conclusions

Microbial culture isolated and used in batch bioreactor for denitrification of $NaNO_3$ solution under minimal growth conditions has shown good growth as well as good denitrification ability. It is confirmed that *Pseudomonas aeruginosa* is the principal biomass species responsible for denitrification. The use of harvested biomass in batch bioreactor showed improved denitrification performance. The growth of biomass on the interstices of the metal sheets of wire gauge packing modules was found to be conducive to building the flow through bioreactor. The optimization of C/N ratio of 0.94, use of trace elements (1 ml/l) and gradual acclimatization of biomass to higher concentrations of nitrates, helped obtain suitably adapted biomass for denitrification of as high as 12400 mg/l NO_3 bearing effluent. From excellent performance of the process during continuous operation over a period of six months, it can be concluded that the process is promising for treatment of high nitrate bearing nuclear effluents generated from back end of nuclear fuel cycle.

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