**Biological Process for Denitrification of High Sodium Nitrate Bearing Waste Effluents of Reprocessing Origin**

Process Development Division

**Abstract**

A process for treatment of high sodium nitrate loaded reprocessing waste has been developed and demonstrated in a flow through bioreactor. Denitrification of as high as 28000 mg/L of NO\(_3^-\) (~0.45 M NaNO\(_3\)) bearing waste to below 20 mg/L was accomplished at a residence time of 50 hours by passing the waste solution spiked with methanol (a carbon source) and traces of micronutrients. The process was successfully tested for treatment of actual intermediate level waste solution of reprocessing origin. Traces of radionuclides did not have any deleterious effect on denitrification performance of the biomass. Steady performance on continuous operation of the column over six months has assured the viability of the process for practical applications.

**Keywords:** Biodenitrification, Pseudomonas, radioactive effluent, sodium nitrate, bioreactor

**Introduction**

Growing use of nitrate bearing products in our daily life (fertilizers, food preservatives, pectin, etc.) coupled with the increasing discharge of nitrate bearing waste from various domestic and industrial sources have led to radical changes in nitrate and nitrite proportions in the environment leading to ecological imbalance. Increasing levels of nitrate in ground water as well as in surface water are also of concern as it finally enters the human body through drinking water and can cause serious health hazards. Stringent norms for nitrate discharge and limits are in force everywhere and WHO and EEC has set 50 mg/L NO\(_3^-\) (11.1 mg/l NO\(_3^-\)N) as the limit in groundwater, while regulatory standards in India specify that concentration of nitrates in treated effluents should be below 45 mg/L NO\(_3^-\), before such effluents can be discharged to the environment.

Internationally, various methods including chemical, ion exchange, reverse osmosis, thermal degradation, biological denitrification, etc., are being considered as feasible options, yet it is accepted that all processes, except biodenitrification, partition the waste effluent into two streams viz., a small volume fraction containing the entire nitrate and a nitrate lean large volume fraction suitable for direct discharge. In contrast, biodenitrification fulfills the ultimate objective of converting nitrate to nitrogen gas. However, industrial scale application of the process has so far been limited for purification of drinking water, and to some extent for treatment of low nitrate bearing waste-water from fertilizer industry, nuclear industry, etc. [1-2]. A biodenitrification plant is functional in WMD, BARC for treatment of up to 2000 mg/L nitrates in low level waste [3]. For treatment of high nitrate bearing effluents, Francis and Mankin accomplished denitrification of about 20,000 mg/L of NO\(_3^-\) wastes using a column packed with fine particles of anthracite coal in suspension [4]. Glass and Silverstein achieved success in denitrification of 36,000 mg/L of NO\(_3^-\) as Ca(NO\(_3\))\(_2\) in a tank as well as in pond bioreactors [5] and Dhamole et. al. from BARC, India successfully denitrified a 40,000 mg/L of NO\(_3^-\) as NaNO\(_3\) using a stirred tank reactor [6].
Investigations carried out in our laboratory over the last several years have helped to develop a flow through bioreactor capable of denitrification of higher NaNO₃ bearing effluent. An overview of the process development work is presented here.

**Origin and characteristics of waste effluents generated in nuclear industry**

Processing of uranium from barren liquor at the front end and reprocessing of spent fuel at the back end of nuclear fuel cycle results in various types of nitrate bearing waste effluent. Nitrate in the form of ammonium nitrate and magnesium nitrate are generated at the front end, while three types of waste including NaNO₃ loaded declad waste, neutralized evaporator condensate and acidic (HNO₃) HLW are generated from reprocessing operations. From radioactivity point of view, these NaNO₃ bearing effluents, as intermediate level waste, are stored in underground carbon steel tanks. The NaNO₃ concentration in such waste varies from 50,000 - 150,000 mg/L. As the process of nitrate removal is applicable after decontamination of waste, a dilution of about 2-5 times is expected during treatment as well as transfer of the waste.

**Biodenitrification process**

Bio-denitrification is a natural phenomenon accomplished by the microorganisms as a part of their respiratory process where nitrate serves as an electron acceptor and the carbon source as an electron donor. A large number of microorganisms are known to be capable of denitrification where the nitrate is reduced to nitrogen gas through a sequence of enzymatic reactions, viz.,

\[
\text{NO}_3^- \rightarrow \text{NO}_2^- \rightarrow \text{NO} \uparrow \rightarrow \text{N}_2 \text{O} \uparrow \rightarrow \text{N}_2 \uparrow
\]

The overall reaction can be represented as follows:

\[
\text{NO}_3^- + \text{carbon source} \rightarrow \text{N}_2 + \text{CO}_2 + \text{H}_2\text{O} + \text{OH}^-
\]

Biotechnology makes use of these microorganisms by providing suitable conditions, essentially an external carbon source and micronutrients for microbial respiration and growth leading to denitrification. In general, the rate of denitrification by microorganisms is quite slow by its very nature and high nitrate environments are usually toxic to most microbes. Identification of a suitable microbial strain or consortia of organisms, which can grow in high nitrate concentrations and with minimal growth requirements, is the most important step for a practically viable process.

**Biomass and bioreactor of present study**

Biomass from an industrial fertilizer unit for treatment of nitrate at low concentrations (~2000 mg/L) was used as inoculum and adapted during batch studies to a ‘minimal medium’ containing methanol as carbon source. The inoculated biomass was then allowed to grow in stainless steel wire gauge modules (Fig.1) stacked in a glass column. These sturdy modules although new for this application, were selected with the expectation that its high internal surface area would be colonized by bacterial growth which would remain attached to it and that the release of gases like CO₂ and N₂ would have no effect on functioning of the column during prolonged use. To initiate
biomass growth, a nitrate bearing feed containing 730 mg/L NO$_3^-$ as NaNO$_3$, minimal growth medium including methanol as carbon source and traces of micronutrients was fed through the column in the up flow mode using a peristaltic pump. Denitrification performance was monitored by analyzing effluent samples for NO$_3^-$ and NO$_2^-$. After achieving complete denitrification and stable column performance over a period of two months, conditions were optimized wherein a C/N ratio of 0.94 and the minimal micronutrient composition required in feed was determined [7]. Using these optimized feed conditions the column was then run with gradual increase of nitrate concentration. This approach was adopted to gradually select acclimatized biomass for denitrification of high nitrate bearing effluent.

**Identification of the biomass**

After about 2 years of operation of the column, biomass sample from column was sent for identification at the Institute of Microbial Technology, Chandigarh, India. The biomass was identified as aerobic bacilli bearing Gram negative, short, motile rods which were oxidase positive, indicating *Pseudomonas aeruginosa*. The features showing short motile rods, with length ranging from 1.5-3.0 μm, are seen in SEM micrographs (Fig.2). The selective growth of *Pseudomonas* species are expected, as these are known to be ubiquitous organisms with the ability to colonize diverse niches due to its range of metabolic capabilities and ability to overcome environmental challenges.

**Treatment of actual effluent**

The actual ILW received after necessary pretreatment for removal of $^{137}$Cs, $^{90}$Sr, $^{99}$Tc and $^{106}$Ru was found to contain 17,500 mg/L nitrate and 1x10$^{-3}$ mCi/L of gross β activity. For denitrification, the column was acclimatized by passing a NaNO$_3$ solution of similar concentration level followed by actual feeding of radioactive waste. The typical profile of column acclimatization followed by treatment of actual waste is shown in Fig.3 [8]. Exposure of the biomass to high nitrate conditions leads to an increase in effluent nitrate concentration initially which then decreases over a period of time. This shows that acclimatization of the biomass is a gradual process. Interestingly, once the biomass acclimatization phase is through, steady operation is obtained over an extended period of time. Though only about 50 L of the waste (20 column volumes) was treated due to limited waste availability, the run was continued with the same denitrification performance for six months with simulated waste. The criteria used for selection of modules as support for biomass growth were vindicated as can be seen from the file photograph of the internal part of the modules (Fig 1). Under stable operating conditions, about 50% of module volume was found to be occupied by the biomass (wet weight of the module...
increased from 400 g to about to about 1500 g due to biomass growth). Results of the study suggest that the bioreactor is effective for treatment of NaNO$_3$ bearing reprocessing waste.

**Effect of gamma radiation**

Because of the low volumes of actual waste used in treatment, the effect of gamma radiation on denitrification performance of the column could not be ascertained in the earlier study. However, exposure of the biomass to radiation environment from the low level streams over a prolonged time period during actual operation is inevitable. To study the effect of such radiation, $^{137}$Cs radiotracer was added into feed solution (activity level: 0.05 mCi/L) and passed through the bioreactor (2.5 L) already stabilized with 20,000 mg/L of NO$_3^-$, at the flow rate of 50 mL/h (residence time: 50 h) over a period of one month. Based on the analysis of $^{137}$Cs, NO$_3^-$ and NO$_2^-$ activity, it was found that the waste was completely denitrified throughout the course of the study and almost no activity was absorbed by the biomass. Biomass had therefore received a constant radiation dose similar to exposure to a column filled with 0.05 mCi/L of $^{137}$Cs solution for a period of one month. This corresponds to exposure of the biomass in column to low level waste having 1x10$^{-3}$ mCi/L $^{137}$Cs activity for a period of 50 months. It can therefore be concluded that the biomass used in present study is suitable for treatment of low level effluent.

**Effect of flow rate and process performance under optimized conditions**

Studies on flow rate variation are integral to establishing optimum conditions for good plant throughput. It was practically observed that as in case of change of nitrate concentration in feed, flow rate variations also required time for biomass acclimatization. For example, a flow rate variation study from 50 to 70 mL/h, after ensuring stable denitrification performance of a column at 17,500 mg/L of NO$_3^-$ with a residence time of 50 hours (flow rate: 50 mL/h, column volume 2.5 L), was found to show satisfactory results. However further increase in flow rate to 80 mL/h led to a sharp rise in effluent nitrate level. It can therefore be stated that denitrification of 17,500 mg/L NO$_3^-$ can be carried out at an average residence time of 35 hours. This was confirmed at several concentrations during the process of gradual adaptation of biomass in another (4.8 L) column. The performance of the column under optimized conditions was monitored over several months and the results are shown in Fig.4. Complete denitrification of 4400 mg/L nitrate required only 2 hours while it takes about 50 hours for denitrification of 28,000 mg/L NO$_3^-$ bearing effluent. Results of this study are useful in scale up of the process to meet desired plant throughput.

**Conclusions**

Operations of the column under minimal growth conditions and gradual acclimatization of the biomass to higher nitrate bearing environment by the *Pseudomonas sp* has been established. 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 biomass has demonstrated capability for denitrification of as high as 28,000 mg/L of nitrate bearing solution with only methanol as carbon source (C/N ratio of 0.94) and traces of...
micronutrients. It is established that denitrification performance of the biomass will not be affected even in presence of traces of $^{137}$Cs activity, and hence the process is promising for treatment of high nitrate bearing low level nuclear effluents generated from back end of nuclear fuel cycle. Efforts are being made to demonstrate the process on a larger scale.

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References


