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Denitrification of high strength nitrate bearing acidic waters in granular sludge sequencing batch reactors

Y.V. Nancharaiah [a, b, *], T.V. Krishna Mohan [a, b], P.M. Satya Sai [b, c], V.P. Venugopalan [a, b]

[a] Water and Steam Chemistry Division, Bhabha Atomic Research Centre, Kalpakkam 603102, Tamil Nadu, India
[b] Homi Bhabha National Institute, Anushakti Nagar, Mumbai 400 094, India
[c] Waste Immobilization Plant, NRB, Bhabha Atomic Research Centre, Kalpakkam 603102, India

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ABSTRACT

Nitrate containing acidic wastewaters are generated in nuclear fuel cycle operations. These waters are often neutralized prior to either storage or biological treatment. In this study, treatment of high strength nitrate bearing acidic waters was investigated in a sequencing batch reactor. A six liter reactor was inoculated with activated sludge and operated in sequencing batch reactor mode by feeding simulated nitrate wastewater at pH 7.5. After establishing denitrifying microbial consortia in the form of compact granules, the pH of the feed was gradually decreased to 5.0 and then to 4.0. The *in situ* neutralization of the feed in the reactor by the denitrification-driven alkalinity and the acclimatization strategy helped to achieve complete and stable denitrification during long term operation. The denitrifying microbial community developed in the sequencing batch reactor predominantly consisted of rod- and cocci-shaped microorganisms. This study showed that nitrate bearing acidic effluents can be directly denitrified in granular sludge sequencing batch reactors without a prior treatment. Thus, *in situ* neutralization and acclimatization strategy can be a potential approach to be considered for treating nitrate contaminated acidic waters.

1. Introduction

Nitrate (NO_3^-) is one of the widespread oxidised contaminants in natural water resources worldwide. In spite of NO_3^- being an essential nutrient in plants, it has become a common pollutant of groundwater and surface water creating problems for drinking water supply. Anthropogenic activities such as agricultural runoff, wastewater from feedstocks, high strength nitrate effluents from industries, and discharging improperly treated wastewaters have greatly contributed to increased nitrate concentrations in the surface and ground waters (Camargo et al., 2005; Smith, 2003). Nitrate pollution of natural waters is a concern because it affects ecosystem well-being and public health (Ward et al., 2005). For example, nitrate contamination causes eutrophication of water bodies and blue-baby syndrome in infants (Camargo et al., 2005; Smith and Schindler, 2009). Thus, nitrate is a priority pollutant identified by the US Environmental Protection Agency (Morris et al., 2009). Both the European Union and World Health Organization have set the maximum contaminant level (MCL) for NO_3^- in drinking water supplies at 11.3 mg NO_3^--N l^{-1} (50 mg NO_3^- L^{-1}). While the MCL set by both the US EPA and Bureau of Indian Standards in water supplies is more stringent at 10 mg NO_3^--N l^{-1} (45 mg NO_3^- l^{-1}) (Suthar, 2011). The regulatory standards in India specify a limit of ≤45 mg/l NO_3^- in treated effluents prior to environmental discharge. Nitrate contamination of water bodies is caused from the release of nitrate from point and non-point sources that includes nitrogen fertilizers, sewage and industrial effluents. High strength nitrate effluents are generated in industries such as fertilizer, ammunition, pharmaceutical, metal finishing and nuclear (Glass and Silverstein, 1998, 1999; Nancharaiah and Venugopalan, 2011). In nuclear industry, use of nitric acid in various phases of nuclear fuel fabrication and reprocessing of spent nuclear fuel result in generation of nitrate containing effluents. Majority of the high strength nitrate effluents are acidic at their source of generation. These acidic nitrate effluents are neutralized and stored for subsequent treatment.

Biological denitrification is the sequential microbial reduction of nitrate to dinitrogen gas, through intermediates such as nitrite, nitric oxide and nitrous oxide. It is an energy yielding process in

* Corresponding author. Water and Steam Chemistry Division, Bhabha Atomic Research Centre, Kalpakkam 603102, Tamil Nadu, India.
E-mail addresses: yvn@igcar.gov.in, venkatany@gmail.com (Y.V. Nancharaiah).

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microorganisms, wherein nitrate reduction is catalyzed sequentially by four specific reductases i.e. nitrate reductase, nitrite reductase, nitric oxide reductase and nitrous oxide reductase (Chen and Strous, 2013). Heterotrophic denitrification has been employed in engineered wastewater treatment systems for removing low strength nitrates worldwide (Park and Yoo, 2009; Nancharaiah et al., 2016). Microbial denitrification has attracted interest as a potential treatment method for removing nitrate from high strength wastes (Biradar et al., 2008; Dhamole et al., 2007, 2015; Foglar et al., 2005; Glass and Silverstein, 1998, 1999; Krishna Mohan et al., 2016a,b; Nancharaiah et al., 2016). An organic carbon source such as methanol, ethanol or acetate is needed for the heterotrophic denitrification of nitrate (Park and Yoo, 2009). High strength nitrate wastes of nuclear origin are typically devoid of organic carbon, thus addition of sufficient amounts of electron donor is needed for achieving complete conversion of nitrate to molecular nitrogen. Previous studies have reported that acetate is a preferred electron donor for high strength nitrate denitrification because of ease of metabolism and faster denitrification rates (Dhamole et al., 2007, 2015; Foglar et al., 2005; Glass and Silverstein, 1998; Krishna Mohan et al., 2016a,b).

Dissimilatory reduction of nitrate or nitrate is performed by various phylogenetically distinct microorganisms belonging to different genera and species (Zumft, 1997). Factors like oxygen availability, substrate availability (nitrate or nitrite), pH, temperature and the availability and abundance of denitrifiers can influence the process of denitrification (Saleh-Lakha et al., 2009). Among these, substrate availability, the absence of oxygen, and the presence of active denitrifying microorganisms are considered the main controlling factors (Saleh-Lakha et al., 2009), whereas, pH and temperature play a role in denitrification by influencing growth of the denitrifying microorganisms and expression and activity of denitrifying enzymes. Previous studies have shown that the denitrification rates decrease in soils with low pH values (Šimek et al., 2002). For treatment of acidic effluents, a pre-treatment (i.e. alkali addition) is essential to increase the pH to a level, where biological denitrification will proceed without any inhibition. In most of the studies on biological denitrification, the feed pH was 7 and above (Pan et al., 2012; Papirio et al., 2013, 2014; Saleh-Lakha et al., 2009; Watanabe et al., 2001; Krishna Mohan et al., 2016a,b). Papirio et al. (2014) have operated fluidized-bed reactors (FBR) for treating acidic wastewater containing 186 mg/l nitrate. Although denitrification was inhibited in batch essays at pH 4.8, FBR permitted denitrification of feed having pH 2.5 due to pH neutralization in the reactor. However, denitrification by activated sludge was inhibited in sequencing batch reactors (SBRs) when fed with simulated nitrate wastewater at low pH $\leq$ 7.0, due to accumulation of nitrite (Glass and Silverstein, 1998).

SBRs can efficiently retain biomass in the form of granular sludge allowing high rate biological conversions (Nancharaiah et al., 2015; Kiran Kumar Reddy et al., 2016). Krishna Mohan et al. (2016a, b) have demonstrated cultivation of denitrifying granular sludge for rapid and stable denitrification of high strength nitrate bearing waters in sequencing batch reactors. However, the pH of the nitrate bearing waters was adjusted to 7.5 before addition to the reactor. This study evaluated the feasibility of nitrate denitrification in granular sludge SBRs by directly feeding acidic (pH 5.0 and 4.0) simulated nitrate wastewater. Therefore, the aims of this work were as follows: (1) cultivation of denitrifying granular sludge by feeding simulated nitrate wastewater with pH 7.5, (2) evaluating nitrate denitrification by directly feeding simulated nitrate wastewater with low pH values of 5.0 and 4.0 and (3) characterization of denitrifying granular sludge. Operational strategy comprising of *in situ* neutralization by utilizing the alkalinity produced consequent to biological denitrification and adaptation was applied for treatment of nitrate bearing waters having pH 5.0 or 4.0.

2. Materials and methods

2.1. Composition of nitrate bearing acidic water

Simulated nitrate waste was prepared in deionized water based on typical nitrate bearing effluents of nuclear industry. Sodium acetate was added as the electron donor for denitrification studies. The simulated nitrate wastewater contained the following (in g l^{-1}): sodium acetate 3.54, sodium nitrate 4.16, $MgSO_4 \cdot 7H_2O$ 0.08, KCl 0.035, K_2HPO_4 0.06, KH_2PO_4, 0.028, and trace elements mix 0.1 ml l^{-1} (Nancharaiah et al., 2008). The pH of simulated nitrate wastewater was observed to be 7.5 without adjustment. The acetate- carbon to nitrate-nitrogen mass ratio was fixed at 1.5 based on previous studies (Krishna Mohan et al., 2016b).

2.2. Denitrification at different initial pH in serum bottles

In order to determine the optimum pH for denitrification, batch experiments were performed in 125 ml volume serum bottles. Simulated nitrate wastewater was prepared as described above. The pH of the simulated nitrate waste was adjusted to 4.0, 5.0, 6.0 or 7.5 using 0.1 N HCl or NaOH. The simulated nitrate wastewater and serum bottles were autoclaved separately. Aliquots of 100 ml medium was dispensed into 125 ml serum bottles containing 4 g (wet weight) of acetate-fed anoxic granular biomass (equivalent to 0.094 $\pm$ 0.002 g dry biomass) (Nancharaiah and Venugopalan, 2011). The serum bottles were sealed with butyl rubber stoppers, and purged with ultra high purity nitrogen gas for 5 min. The serum bottles were incubated at 30 °C on an orbital shaker set at 100 rpm. All the experiments were performed in duplicates. Liquids samples were collected at regular time intervals for measuring pH, nitrate and nitrite.

2.3. Denitrification of simulated nitrate wastewater with different initial pH in sequencing batch reactors

Sequencing batch reactors were chosen for denitrification because they allow cultivation of granular biomass, ease of scale up and their proven ability for treatment of high strength nitrate wastewaters (Glass and Silverstein, 1998). Two bench scale column-type reactors were fabricated with polycarbonate material (total height: 0.86 m; 0.74 m with 5.4 cm ID; expanded region with 16 cm ID) and operated in sequencing batch reactor mode (Fig. 1). The reactors were inoculated with activated sludge collected from the aeration tank outlet of an operating sewage treatment plant, Kalpakkam, India (Nancharaiah et al., 2006). The reactors were operated with 24 h cycle (10 min fill, 23 h reaction, 5 min settle, 15 min decant and 30 min idle period) and 50% volumetric exchange ratio. During reaction period, mixing was provided by recirculation of liquid by collecting at the top and pumping at bottom of the reactor using a peristaltic pump at an up-flow velocity of 3 m h^{-1}. The sequencing batch reactors were operated for 2 weeks for establishment of stable denitrification and cultivation of granular sludge. For the initial 2 weeks, both the reactors were supplied with feed at pH 7.5. For one of the SBRs (R1), the feed pH was decreased in steps from 7.5, to 5.0 and 4.0. After attaining steady-state denitrification with feed pH 7.5, R1 was fed with simulated nitrate wastewater whose pH was adjusted to 5.0. After attaining stable denitrification with feed pH 5.0, R1 was supplied with feed having pH 4.0. The second reactor (R2) functioned as a control, was fed with a feed of pH 7.5 throughout the experimental duration. Liquid samples were collected during cycle time and analysed for nitrate, nitrite and acetate. Granular sludge collected from the reactors supplied with

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