Denitrification of Highly Alkaline Nitrate Waste Using Adapted Sludge

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

Abstract Uranium extraction and regeneration of ion exchange resin generates concentrated nitrate effluents (typically 500 to 10,000 ppm NO_3–N) that are highly alkaline in nature (pH 9.0 to 11.0). It is difficult to remove nitrate from such solutions using standard physiochemical and biological methods. This paper reports denitrification of such wastes using preadapted sludge (biomass), which was acclimatized to different influent pH (7.5 to 11.5) in a sequencing batch reactor (4 l) for 2 months. Performance of the developed consortia was studied under different pH (7.5 to 12). Biomass denitrified the synthetic wastewater containing 1,694 ppm NO_3–N at a pH of 10.5. Decrease in nitrite build up was observed at higher pH, which differs from the reported results. Kinetic analysis of the data showed that specific rate of nitrate reduction was highest (78 mg NO_3–N/g MLSS/h) at higher pH (10.5). This was attributed to the acclimatization process. Thus, high-strength nitrate wastewater, which was highly alkaline, was successfully treated using preadapted sludge.

Keywords Denitrification · pH · Acclimatization · Kinetics

Nomenclature

K_N	Zero-order rate constant for nitrite–N loss, mg N/l/h
K_{NO_2}	Zero-order rate constant for nitrite reduction, mg NO_2–N/l/h

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

R. R. Nair · S. F. D'Souza
Nuclear Agriculture and Biotechnology Division, Bhabha Atomic Research Centre,
Mumbai 400085, India

S. S. Lele (✉)
Food Engineering and Technology Department, Institute of Chemical Technology, Matunga,
Mumbai 400019, India
e-mail: sslele@vsnl.net

K_{NO_3}	Zero-order rate constant for nitrate reduction, mg NO_3–N/l/h
k_{NO_2}	Zero-order rate constant for actual nitrite formation, mg NO_2–N/l/h
K'_{N}	Specific rate of nitrite–N loss, mg N/g MLSS/h
K'_{NO_3}	Specific rate of nitrate reduction, mg NO_3–N/g MLSS/h
K'_{NO_2}	Specific rate of nitrite reduction, mg NO_2–N/g MLSS/h
k'_{NO_2}	Specific rate of nitrite formation, mg NO_2–N/g MLSS/h
RR	Relative rate of nitrate reduction to nitrite reduction in presence of nitrate
X	Biomass concentration (MLSS), g/l

Introduction

Deterioration of water sources, eutrophication of receiving water bodies, and rising health problems (mainly blue baby syndrome) by nitrate-bearing wastes have made nitrate removal mandatory to the industries. Besides excessive application of fertilizers, the industrial effluents, particularly from explosive, fertilizer, metal finishing industries, regeneration of ion exchange resins, and nuclear fuel processing, are the major sources of nitrate pollution [1–3]. Wastewater generated in these industries is highly alkaline (pH>9.0) in nature, and treatment of such wastes has been a major concern for these industries. The most widely used method of nitrate removal is biological denitrification [4]. However, very few studies have been reported on denitrification of alkaline nitrate wastes [1, 5–8]. Furthermore, the biomass is sensitive to alkaline pH (>9.0). Optimum pH for denitrification is reported to be in the range of 7.0–7.5 [5]. The performance of biomass decreases with increase in pH and alkaline pH results in accumulation of nitrite, which inhibits the denitrification [1, 5, 9]. Thus, denitrification of alkaline nitrate wastes requires a robust consortium that can sustain the extreme conditions and results into no or low accumulation of nitrite. Such a consortium can be developed by acclimatization of the sludge. In our earlier work, it was reported that sequential adaptation of sludge develops a consortia that can sustain extreme conditions [10, 11]. It was found that acclimatization of biomass to high strength nitrate waste leads to enrichment of sludge with respect to denitrifying bacteria.

The sludge consists of different types of microbes that can be broadly divided as pH tolerant and pH intolerant. Sequential adaptation of sludge to high pH would balance the growth of these microbes. It would limit the growth of pH intolerant bacteria, and only those capable of withstanding high pH would survive and multiply. Based on this theory, the present work was undertaken to develop the consortia and test its performance for degradation of highly alkaline nitrate waste. The present work was divided into three stages: development of suitable consortia, denitrification studies at high pH (7.5, 9.0, 10.5, and 12.0) and kinetic analysis of the data.

Materials and Methods

Reactor Details

All the studies were conducted in a 5-l sequential batch reactor (working volume=4 l and headspace=1 l). Biomass was maintained in a suspension condition by a four-bladed stainless steel impeller driven by 1/2 hp overhead motor.

Composition of Synthetic Waste

Synthetic waste consisted of $NaNO_3$=10.28 g/l, CH_3COONa=9.84 g/l, Na_2HPO_4=7 g/l, K_2HPO_4=1.5 g/l, $MgSO_4$=0.1 g/l, and 2 ml of trace element solution. The composition of the trace element solution was as follows: $ZnSO_4 \cdot H_2O$=2.2 g/l, $CaCl_2 \cdot 2H_2O$=5.5 g/l, $MnCl_2 \cdot H_2O$=5.06 g/l, $FeSO_4 \cdot H_2O$=5.0 g/l, $(NH_4)_6MO_7O_{24} \cdot H_2O$=1.1 g/l, $CuSO_4 \cdot H_2O$= 1.51 g/l, $COCl_2 \cdot H_2O$=1.61 g/l, EDTA=50 g/l, distilled water=1000 ml.

Acclimatization

The reactor was seeded with fresh sludge (i.e., biomass expressed as mixed liquor suspended solids) from a full-scale effluent treatment plant of a fertilizer industry. Acclimatization was carried out in four stages in a sequential manner. Influent pH (pH of the synthetic waste) was varied from 7.5, 9.0, 10.5, and 11.5 and is called stage I through IV. Operating pH (pH in the reactor) was measured but not controlled during the adaptation. Each stage was continued for a period of 15 days. Initially, the sludge was acclimatized to an influent of pH 7.5 for 15 days (stage I). The same biomass was then adapted to influent of pH 9.0 for the next 15 days (stage II) followed by 15 days acclimatization to each pH of 10.5 (stage III) and 11.5 (stage IV). Reactor was operated in a cycle of 24 h which was divided into 22 h for denitrification and 2 h for settling, decantation, and refilling. After every 24 h, half of the reactor volume (2 l) was replaced by fresh synthetic waste, which resulted into dilution of the feed. Thus, 2 l feed containing 1,694 ppm NO_3–N was diluted to ∼ 850-ppm NO_3–N (approximately) on addition to the reactor. NaOH solutions, 5 N, and 5 N H_2SO_4 were used to adjust the pH of the feed.

Denitrification Studies

The performance of the adapted biomass was tested for the denitrification of 1,694 ppm NO_3–N at three-controlled operating pH (7.5, 9.0, and 10.5). In these experiments, the operating pH was controlled using NaOH and H_2SO_4.
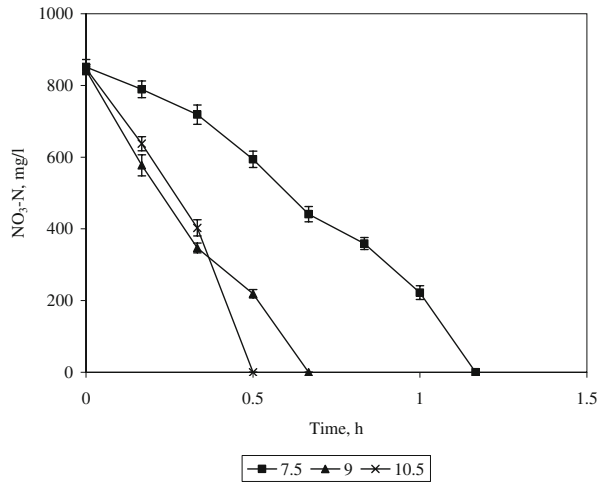
Analysis

Nitrate and nitrite were analyzed using DIONEX Ion chromatograph, AS11 (2 mm) column and guard column. NaOH, 12 mM, prepared using deionized water was used as eluent. pH was measured using ELICO pH meter. Biomass, i.e., sludge expressed as mixed liquor suspended solids (MLSS) was determined by following standard methods [12]

Results and Discussion

Acclimatization of Biomass to Higher pH

Biomass was acclimatized in four stages in a sequential manner to increasing influent pH (7.5, 9.0, 10.5, and 11.5). Irrespective of the influent pH, the operating pH stabilized in between 9.2 to 10.5 (±0.3). Initially, the reactor was inoculated with 12 g/l of sludge, which increased to 23 g/l during the first 15 days and remained constant thereafter (data not reported here). For the first 3 days, nitrate and nitrite was detected in the treated waste.

Absence of nitrate and nitrite in the treated waste and steady MLSS concentration indicated that the system was stabilized for the particular influent pH. This process was continued for a period of 2 months.

Denitrification of Alkaline Nitrate Waste

The biomass developed by the above method was used to study the denitrification of 1,694 ppm NO$_3$–N at higher pH (7.5, 9.0, and 10.5). Fig. 1 and 2 shows the corresponding nitrate and nitrite profiles obtained during the denitrification of 1,694 ppm NO$_3$–N. Complete denitrification was observed at all the pH (Fig. 1–2). It can be seen from the nitrate and nitrite profile that the denitrification time decreased from 2.5 to 1.33 h when the operating pH was increased from 7.5 to 10.5. Maximum amount of nitrite build up at pH of 7.5, 9.0, and 10.5 was, respectively, 774, 624, and 464 ppm NO$_2$–N. A 40% reduction in peak nitrite value was observed at a pH of 10.5.
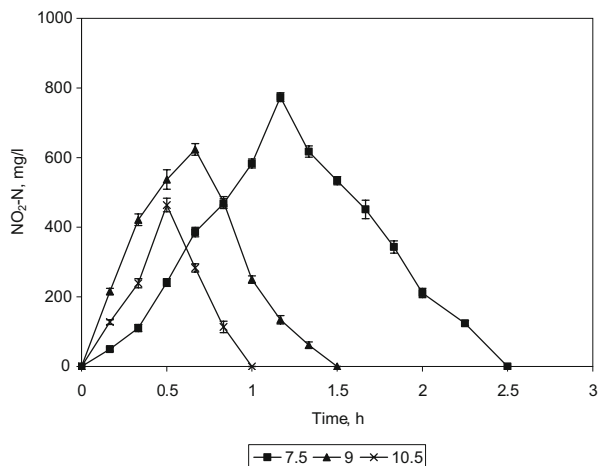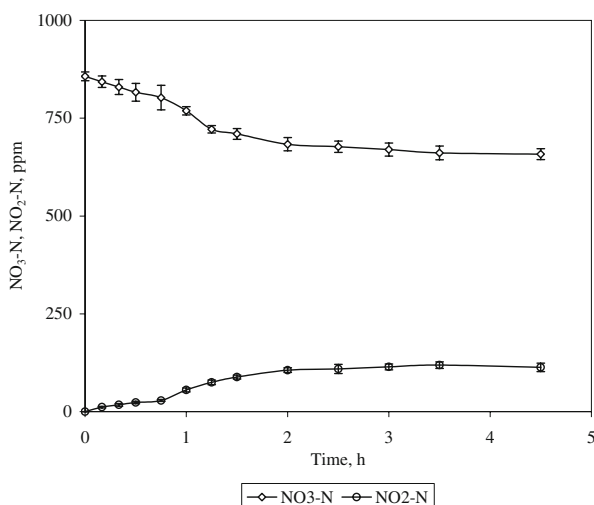
Fig. 3 Denitrification of 1694 ppm NO_3–N (actual concentration in the reactor~850 ppm) at a pH of 12.0. MLSS=20 g/l (*Y error bar is less than 5 %*)


The relationship between pH and denitrification is well documented in the literature [1, 5, 8]. It is reported that high pH (>7.0) results in accumulation of nitrite. It was explained by the action of nitrite reductase and proton availability [8]. In the case of nitrite reduction, the protons are often taken from the periplasmic membrane. At high pH, protons may be relatively scarce in the periplasmic space, reducing the rate of nitrite reduction and accumulation of nitrite. However, in the present work, the reverse trend was observed. At a pH of 10.5, the nitrite build up was less compared to a pH of 7.5. This could be attributed to the existing microflora. Acclimatization might have developed the consortia that perform better at higher pH. It would result into decay of pH-intolerant microbes and growth of pH tolerant ones. Constant biomass value suggests that the decay rate of pH intolerant microbes and growth rate of pH tolerant microbes is same. This would result in a consortium rich in pH tolerant microbes to which it is acclimatized and a better performance at that pH. However, pH higher than that will act as a shock. This theory was verified by conducting an experiment at a higher pH (12.0) using the same biomass which was used in earlier studies. It was seen that higher operating pH (12.0) resulted in incomplete denitrification (Fig. 3), as the biomass was acclimatized to an influent of pH 11.5. It shows that acclimatization of the biomass is necessary for treatment of nitrate waste under extreme conditions. It develops a consortium capable of working under extreme environment.

Table 1 Rate of nitrate and nitrite reduction at different pH and specific rate of nitrate (K'_{NO_3}) and nitrite reduction (in the presence of nitrate, i.e., K'_N and in the absence of nitrate, i.e., K'_{NO_2}) at different operating pH during the denitrification of 1,694 ppm NO_3–N.

Operating pH	K_{NO_3} mg NO_3–N/ l/h	k_{NO_2} mg NO_2–N/ l/h	K_N (K_{NO_3}– k_{NO_2}), mg N/l/h	RR	K_{NO_2} mg NO_2– N/l/h	K'_{NO_3} (K_{NO_3}/X) (mg NO_3– N/g MLSS/h)	k'_{NO_2} (k_{NO_2}/X), (mg NO_2– N/g MLSS/h)	K'_N (K_N/X) (mg N/g MLSS/h)	K'_{NO_2} (K_{NO_2}/X) (mg NO_2– N/g MLSS/h)
7.5	640	590	50	13	615	32	30	2.5	31
9.0	1,300	1,030	270	5	845	65	51	14	42
10.5	1,550	855	695	2	975	78	43	35	49

MLSS = 20 g/l

Fig. 4 Specific rate of nitrate, nitrite reduction, and relative rate at alkaline pH (7.5, 9.0, and 10.5)
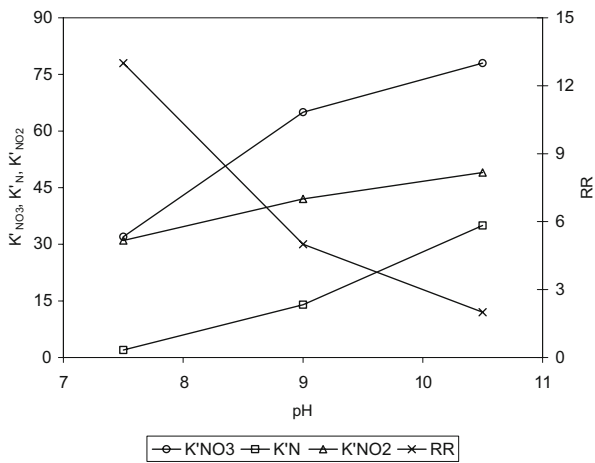
Kinetics of Denitrification

Moore and Schroeder [13] demonstrated that as long as there is sufficient amount of carbon available, denitrification is fast and has constant rate until nitrate concentration becomes 0.3 ppm. In other words, at high nitrate concentration, denitrification follows zero-order kinetics. The mechanism of biological denitrification is as follows:

$$NO_3 \xrightarrow{K_{NO_3}} NO_2 \xrightarrow{K_{NO_2}} N_2$$

The rate equations are:

$$\frac{d\,NO_3}{d\,t} = -K_{NO3} \tag{1}$$

(rate of nitrate reduction)

$$\frac{d\,NO_2}{d\,t} = k_{NO_2} \tag{2}$$

(actual rate of nitrite formation in the presence of nitrate)

$$\frac{d\,NO_2}{d\,t} = K_{NO_3} - k_{NO_2} \tag{3}$$

Table 2 Relationship between various specific rate of nitrate reduction (K'_{NO_3}), specific rate of nitrite reduction in the presence of nitrate (K'_N), specific rate of nitrite reduction in the absence of nitrate (K'_{NO_2}) and relative rate (RR) with pH.

Data fitting equation	Regression
$K'_{NO_3} = 138.03\ Ln\ (pH) - 243.69$	0.9614
$K'_N = 97.103\ Ln\ (pH) - 195.45$	0.9588
$K'_{NO_2} = 53.703\ Ln\ (pH) - 76.826$	0.9937
$RR = -33.03\ Ln(pH) + 78.93$	0.9572

(rate of nitrite reduction in the presence of nitrate)

$$\frac{d\,NO_2}{d\,t} = -K_{NO_2} \qquad (4)$$

(rate of nitrite reduction in the absence of nitrate) where, k_{NO_3}, k_{NO_2} and K_{NO_2} are zero-order rate constants. Eqs. 1–4 were used to fit the experimental data for estimating rate of nitrate and nitrite reduction.

Relative rate of nitrate reduction and nitrite reduction is given by

$$RR = \frac{K_{NO_3}}{K_{NO_3} - k_{NO_2}} \qquad (5)$$

The nitrite build up can be explained by rate of reduction of nitrate, nitrite, and relative rate. Theoretically, rate of nitrate reduction (K_{NO_3}) and rate of nitrite formation (k_{NO_2}) are equal, i.e., $K_{NO_3} = k_{NO_2}$. However, reduction of nitrite also takes place simultaneously; therefore, nitrite formation rate (k_{NO_2}) calculated from experimental data would be less than theoretical (K_{NO_3}). The difference between theoretical rate of nitrite formation (K_{NO_3}) and actual rate of nitrite formation (k_{NO_2}) will give the actual rate of nitrite reduction in the presence of nitrate. In the case of no nitrite build up, RR would be equal to 1, and $k_{NO_2} = 0$. RR ≥ 1 signifies build up of nitrite.

Eqs. 1-5 were used to fit the experimental data. Zero-order rate constants were estimated by fitting a straight line passing through the data. Nitrite profile was divided into two parts, as nitrite reduction in the presence of nitrate and nitrite reduction in the absence of nitrate. The slope of a straight line will yield the rate of nitrate reduction (K_{NO_3}), actual rate of nitrite formation (k_{NO_2}), and rate of nitrite reduction in the absence of nitrate (K_{NO_2}). The difference between the rate of nitrate reduction and actual rate of nitrite formation will give the rate of nitrite reduction in the presence of nitrate (i.e., $K_{NO_3} - k_{NO_2}$). The above calculations are included in Table 1.

The relationship between specific rate, relative rate, and pH is obtained by fitting the data (Fig. 4). The data-fitting equation and regression values are included in Table 2. It was observed that specific rate increases exponentially with increase in pH, and relative rate decreases exponentially with increase in pH. Decrease in RR values (from 13 to 2.5) signifies that accumulation of nitrite is low. It other words, as pH increases, preference to nitrite as an electron acceptor increases resulting into low nitrite build up. It is supported by the specific rate of nitrite reduction in the presence of nitrate (K'_N) and in the absence of nitrate (K'_{NO_2}). Specific rate of nitrite reduction is higher in the absence of nitrate than in the presence of nitrate, which clearly shows that in the presence of nitrate and nitrite, nitrate is preferred over nitrite (Table 1 and Fig. 4).

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