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# Effect of Arsenic (III) on the Denitrification Process

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## Abstract

A sequencing batch reactor (SBR) was operated to develop denitrifying bacteria that had a mean specific denitrification rate of 0.11 g $NO_3^-$-N/gVSS/day. Another system (an anaerobic digester) was operated to generate volatile fatty acids (VFAs) with the effluent concentration being measured to be 5655 ± 876 mg/L as acetic acid. Using the denitrifying biomass developed in the SBR and VFAs generated in the digester as an external carbon source, a series of denitrification batch tests were conducted. The denitrification batch reactors were spiked with $NO_3^-$-N (to get a C:N ratio of 3.0) and different arsenite concentrations to quantify the effect of arsenite on the denitrification rate. A steady deterioration in the ability of the biomass to denitrify under increasing arsenite concentrations was observed, with the mean specific denitrification rate dropping from 0.183 g $NO_3^-$-N/gVSS/day at an arsenite concentration of 5 mg/L, to 0.047 g $NO_3^-$-N/gVSS/day at a concentration of 25 mg/L.

**Keywords:** Denitrification; arsenic; volatile fatty acids, sequencing batch reactor

## 1. Introduction

Arsenic contamination of groundwater drinking supplies is a global health problem, [1], [2], [3]; being most severe in India, [4], [5], and Bangladesh [6], [7]. Arsenic exists in natural waters in both the organic and inorganic forms. Depending upon the redox potential and pH, inorganic arsenic present in natural waters is mainly in two oxidation states; trivalent and pentavalent arsenic. Trivalent arsenic, commonly known as arsenite or As (III) is predominant under a reducing condition, while pentavalent arsenic, commonly known as arsenate or As (V) is predominant in an oxidizing environment. At pH > 2.3 arsenate is present mainly in ionic forms ($H_2AsO_4^-$ or $HAsO_4^{2-}$) whereas arsenite is present in a non-ionic form as ($H_3AsO_3$) up to pH 9, [8], [9].

Several technologies have been proposed to remove arsenic from waters. Some conventional physical – chemical methods include coagulation, precipitation, coprecipitation, filtration, ion exchange, adsorption on a suitable media and reverse osmosis, [10], [11], [12], [13]. Most of these technologies are not efficient at removing the non-ionic form of arsenite; therefore, a pre-oxidation step is usually required to transform the arsenite to arsenate. The oxidants most frequently used in such processes include potassium permanganate, chlorine, ozone, hydrogen peroxide or manganese oxides [10]. Although these chemicals are effective in oxidizing arsenite, they may cause several problems such as the formation of by-products, large volumes of residue and significantly high operational costs [14], [15], [16]. Only a few studies have investigated the interaction of arsenic with microbial communities, [17], [18], [19]. One study, [20] found that autotrophic microorganisms were able to derive energy from the oxidation of arsenite to arsenate under aerobic conditions. Another study, [21], found that *Bacillus sp. strain SF-1*, rapidly reduced arsenate to arsenite under anoxic conditions. Bradley and Chapelle, [17] investigated the effect of arsenate on denitrification in nitrate-contaminated sediments and found that the denitrification rate was considerably inhibited.

Although low concentrations of arsenite may have little impact on microbial respiration, it is also reasonable to suspect that microbial communities will encounter severe problems when subjected to substantial loads of arsenite. In particular, no study to date has quantified the effect of any form of arsenic on the denitrification process, particularly in the bulk liquid environment. Hence, the research described in this paper had conducted a series of denitrification batch tests under increasing contamination levels of arsenite in water by using naturally-produced volatile fatty acids (VFAs) generated from an anaerobic digester as a carbon source. The main objective of the research was to report kinetic information with respect to denitrification as well as the VFAs consumption rate.

## 2. Material and Methods
### 2.1 Experimental Set-Up
The experimental apparatus used in this research required the construction of three physical systems: a sequencing batch reactor (SBR) fed domestic wastewater and containing a denitrifying biomass; an anaerobic digester that generated VFAs from a soy flour influent feed solution; and a series of batch reactor used to study the effects of arsenic on the denitrification process. These latter reactors received incremental loadings of arsenite as well as a nitrate

($NO_3^-$-N) concentrations to match specific carbon-to-nitrogen (C:N) ratios.

**1st System: SBR**
A 21 L SBR was seeded with activated sludge and fed raw domestic wastewater (composition shown in Table 1) both from the Christchurch City Wastewater Treatment Plant (CCWTP) located in Christchurch, New Zealand. Each cycle of the SBR consisted of a 1-h 30-min anoxic, 5-h 30-min aerobic, 30-min settling, 5-min decanting and 5-min filling period. Track studies were performed to measure the SBR performance in terms of COD, $NO_3^-$-N, and $NH_4^+$-N. The SBR had an hydraulic retention time (HRT) of 15 h 20 min, a targeted MLSS concentration of approximately 3000 mg/L; and a solids retention time (SRT) of $20 \pm 2.5$ d. Wasted biomass was stored at $4°C$ for subsequent use in the denitrification batch tests; however, first the biomass was washed in order to remove residual COD and different forms of nitrogen.

Table 1. Mean values of some major constituents of influent wastewater of the SBR

| Constituent of Wastewater | Mean Value |
|---|---|
| CODTotal | $600 \pm 118$ mg/L |
| CODSoluble | $300 \pm 45$ mg/L |
| TOC | $78 \pm 14$ mg/L |
| TSS | $150 \pm 120$ mg/L |
| Ammonia Nitrogen ($NH_4^+$-N) | $32.5 \pm 3.5$ mg/L |
| Nitrate Nitrogen ($NO_3^-$-N) | $4.8 \pm 0.3$ mg/L |
| C:N | 2:1 |
| pH | 6.5 to 7.0 |
| Alkalinity (as $CaCO_3$) | $200 \pm 11$ mg/L |

**2nd System: Anaerobic digester**
A 20 L anaerobic digester was used to generate VFAs. Two litres of 40 g/L soy solution ($COD_{total}$ = 56200 mg/L, $COD_{soluble}$ = 9450 mg/L, VFAs = 0 mg/L) were fed into the digester each day. The same volume was wasted daily to maintain a 10 d SRT. To observe the performance of the digester, pH, MLSS, VFAs, and COD of the effluent were monitored regularly. The VFAs generated were used to the denitrification batch reactors as an external carbon source.

**3rd System: Denitrification batch tests**
Four batch tests using 5-L Erlenmeyer flasks were operated at $20 \pm 2°C$. Concentrated waste activated sludge from the SBR was put into each reactor along with 100 mL of filtered VFA-rich effluent from the digester. The flasks were then diluted with tap water to the 5 L mark achieving a MLSS concentration of $1200 \pm 50$ mg/L. One flask had no arsenic (i.e. a control) while the remaining three flasks were spiked with concentrated arsenic tri-oxide ($As_2O_3$) to yield

concentrations of arsenite of 5, 18 and 25 mg-As/L. The lower bound was from preliminary tests that revealed no significant effect on the ability of the biomass to denitrify up to 5 mg/L. The upper bound was due to handling and toxicity concerns. The test flasks were then spiked with potassium nitrate ($KNO_3$) solution to achieve an initial $NO_3^-$-N concentration of approximately 25 mg/L. The combination of carbon (from the VFAs) and nitrogen (from the spikes) resulted in a C:N ratio of approximately 3.0. A C:N ratio of $\geq 2.0$ is sufficient to ensure carbon-limiting conditions are not experienced during denitrification, [22]. Samples for MLVSS, $NO_3^-$-N and COD were taken every 30 min until the reaction was complete (usually within 4 h).

**2.2 Analytical Methods**
All samples were first filtered through 0.45 μm micromillipore filters to measure soluble parameters ($NH_4^+$-N, $NO_3^-$-N, VFAs, TOC and soluble COD). These parameters, in addition to total COD, alkalinity, D.O., pH, MLSS and MLVSS were analysed according to Standard Methods, [23]. Analyses of VFAs and TOC were carried out on an HP 6890 Series Gas Chromatograph and an Apollo 9000 TOC Combustion Analyzer respectively.

## 3. Results and Discussion
### 3.1 Denitrification in the SBR
Figure 1 shows a representative plot depicting COD, $NO_3^-$-N and $NH_4^+$-N from the SBR track studies. The initial values associated with the influent (Table 1) were diluted by the bulk liquid concentrations carried over from the previous cycle. During the aerobic phase, the $NH_4^+$-N was reduced to 0 mg/L with all the nitrogen showing up as $NO_3^-$-N (i.e. 100% oxidation). Similarly, the $NO_3^-$-N was completely reduced to gaseous nitrogen during the anoxic period (i.e. complete denitrification).
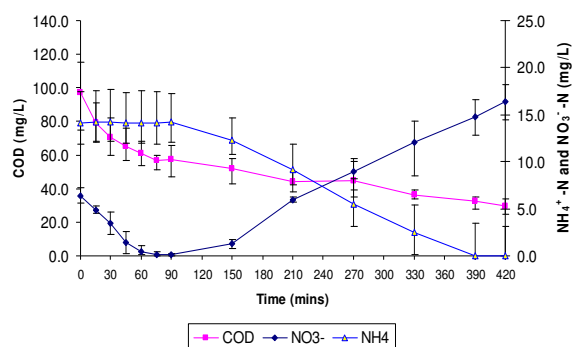


Fig.1. Typical track study of COD, $NO_3^-$-N, and $NH_4^+$-N in the SBR

Figure 1 also shows the COD consumption during the anoxic phase, as the carbon is used to support denitrification. The mean MLVSS concentration in the reactor was 2400 mg/L which was used to calculate the specific denitrification rate (0.11 g $NO_3^-$

N/gVSS·d) This rate is comparable to other research, [24], [25]; and, clearly indicates that a healthy denitrifying biomass was developed in the SBR. Thus, the C:N ratio in the SBR (2:1) was sufficient to ensure both complete removal of nitrates and the majority of influent carbon.

## 3.2 Generation of VFAs from the Anaerobic Digester

Table 2 indicates that the effluent from the digester experienced an 11 % increase in the soluble fraction of the total COD as compared to the influent value. The particulate organic matter in the influent was converted to predominantly VFAs since the influent VFA concentration was zero. It is noted however that the total COD reduced between the influent and effluent; thus, some carbon was lost as gas. This was not of great concern, since the main purpose of the digester was merely to produce VFAs. The net measured VFA individual concentrations were 2423 mg/L (HAc), 2071 mg/L (HPr), 1554 mg/L (HBu), 491 mg/L (iso-HVa) and 762 mg/L (n-HVa) respectively leading to a mean total value of 5655 ± 876 mg/L (expressed as HAc). It is noted that acetic (34% of total) and propionic (28 % of total) acids were the major acids generated which was fortuitous since they are the most preferred VFAs for denitrification [22].

Table 2. Major parameters in the effluent of the anaerobic digester.

| Parameters | Mean Value | |
| --- | --- | --- |
| | Influent | Effluent |
| $COD_{Total}$ | 56200 ± 2500 mg/L | 53000 ± 3000 mg/L |
| $COD_{Soluble}$ | 9450 ± 150 mg/L | 14800 ± 450 mg/L |
| COD soluble fraction | 16.8 % | 27.9 % |
| TOC | n/m | 3200 ± 520 mg/L |
| pH | 4.2 to 4.5 | 4.7 to 4.9 |
| MLSS | n/a | 26292 ± 1828 mg/L |
| MLVSS | n/a | 23372 ± 1752 mg/L |
| Total VFAs (as HAc) | 0 | 5655 ± 876 mg/L |
| Alkalinity (as $CaCO_3$) | n/a | 1120 ± 115 mg/L |
| Ammonia Nitrogen ($NH_4^+$-N) | n/a | 348 ± 4.5 mg/L |
| Nitrate Nitrogen ($NO_3^-$-N) | n/a | 5.4 ± 1.5 mg/L |

n/m – not measured
n/a - not applicable

## 3.3 Effect of Arsenite on Denitrification Rates

As mentioned, four denitrification batch tests were carried out at different arsenite concentrations (0, 5, 18 and 25 mg/L). In the control reactor (i.e. no arsenite) the concentration of $NO_3^-$-N dropped from 23 to 0 mg/L in 120 min, while at 5 mg/L arsenite, the $NO_3^-$-N concentration dropped from 30 to 0 mg/L in 240 min (Figure 2). Note that the difference between the initial $NO_3^-$-N concentrations of 23 and 30 mg/L for the two cases of 0 and 5 mg/L arsenite is explained by the fact that arsenite reacts with nitrate, [26]. Thus, it was necessary to estimate the amount of $NO_3^-$ added (as a function of the added arsenate concentration) in order to get an initial value of $NO_3^-$-N close to 25 mg/L.

The results clearly show that the biomass was able to completely denitrify the initial $NO_3^-$-N concentration; however, the arsenite made a distinct impact on the length of time it took the biomass to completely denitrify. This impact was further confirmed by the results from the subsequent two batch tests. That is, when the arsenite concentration was increased to 18 mg/L and then to 25 mg/L, in this instance the reactor failed to completely denitrify in the 4 hours allotted to the test (i.e. 47.6 % and 28.7 % denitrification respectively).
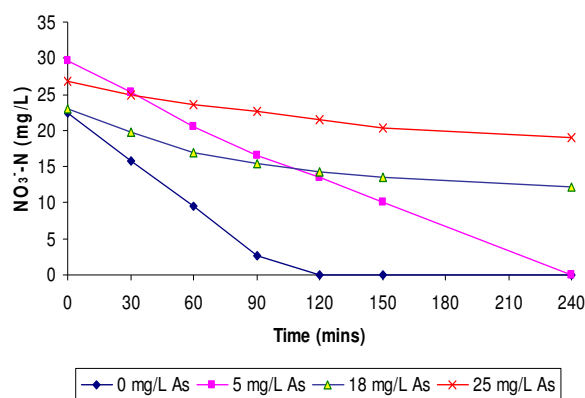


Fig. 2. Track study of $NO_3^-$-N during denitrification batch tests.

Additional evidence of denitrification can be inferred by examining the COD removal patterns (Figure 3). It is reasonable to suppose that much of the carbon was being used to support denitrification, with the rate of consumption clearly slowing as the arsenite concentration increased. Since the total amount of carbon removed was fairly similar in all 4 batch reactors, it is suspected that some carbon was also being removed by non-denitrifying heterotrophic activity.
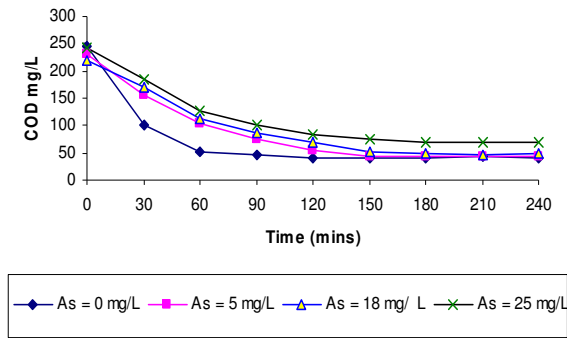
Figure 3. Consumption of COD during denitrification tests.

The rates of denitrification obtained through the batch tests were plotted against the concentrations of arsenic (Figure 4). A considerable decrease in the denitrification rate occurred as the concentration of arsenite increased. In particular, the specific denitrification rate decreased from a high of 0.34 g $NO_3^-$-N/gVSS/day in the control reactor to a low of 0.047 g $NO_3^-$-N/gVSS/day at 25 mg/L arsenite. It is noted that the denitrification control reactor experienced a higher mean specific denitrification rate than the SBR reactor. Since both systems were operated with favourable C:N ratios, the higher rate experience in the denitrification reactor is attributed to VFAs being more amenable as a carbon source than the domestic sewage fed to the SBR.
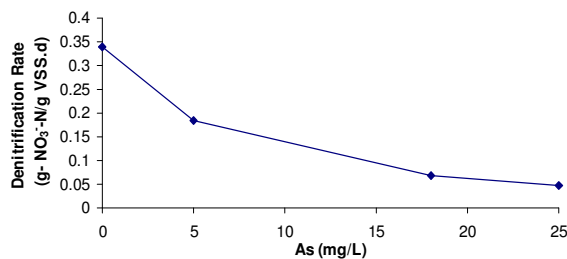


Fig. 4. Decreasing denitrification rate with increasing concentration of arsenite.

## 4. Conclusions

This research has shown that the denitrification process can continue under reasonably high arsenic concentrations (up to 25 mg/L). The ability of the biomass to denitrify however was affected at the higher concentrations studied, as the 25 mg/L arsenic concentration recorded the lowest specific rate (0.047 $NO_3^-$-N/gVSS/day) and the lowest total amount of nitrate removed (28.7 %).

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