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Effect of arsenic on nitrification of simulated mining water

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HIGHLIGHTS

- Arsenic effects on nitrification were revealed using FBR enriched cultures.
- Nitrification was not affected by 100 mg As_{TOT}/L at neutral pH.
- 150–200 mg As_{TOT}/L inhibited ammonium oxidation by 25%.
- As(III) oxidation to As(V) decreased arsenic toxicity to nitrification.
- FBR enrichment consisted of *C. Nitrospira defluvii* and other nitrifying species.

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ABSTRACT

Mining and mineral processing of gold-bearing ores often release arsenic to the environment. Ammonium is released when N-based explosives or cyanide are used. Nitrification of simulated As-rich mining waters was investigated in batch bioassays using nitrifying cultures enriched in a fluidized-bed reactor (FBR). Nitrification was maintained at 100 mg As_{TOT}/L. In batch assays, ammonium was totally oxidized by the FBR enrichment in 48 h. As(III) oxidation to As(V) occurred during the first 3 h attenuating arsenic toxicity to nitrification. At 150 and 200 mg As_{TOT}/L, nitrification was inhibited by 25%. *Candidatus Nitrospira defluvii* and other nitrifying species mainly colonized the FBR. In conclusion, the FBR enriched cultures of municipal activated sludge origins tolerated high As concentrations making nitrification a potent process for mining water treatment.

1. Introduction

Mining is responsible for worldwide environmental contamination by arsenic of surface water streams and groundwater. 20% of total As is released into the environment by the mining industry (Nriagu and Pacyna, 1988). Arsenic co-occurs with ammonium especially in gold mining where gold is associated with arseno-sulfidic minerals such as arsenopyrite (Dave et al., 2008). The use of nitrogenous blasting agents, cyanide and ammonia as lixiviant for copper and nickel recovery are among the main sources of ammonium in mining effluents (EPA, 2003). Ammonium and free ammonia cause eutrophication, reduction of oxygen content and toxicity to living organisms in aquatic ecosystems (Paredes et al., 2007). Municipal and industrial wastewater treatment commonly uses nitrification to control oxygen consumption in the recipient water. The sum nitrification reaction is as follows (Eq. (1)):

$$NH_4^+ + 2O_2 \rightarrow NO_3^- + H_2O + 2H^+ \quad (1)$$

Alkalinity is required in the water to balance the acid produced by nitrification. Nitrate in mine waters can be reduced to nitrogen gas through biological denitrification (Papirio et al., 2014; Zou et al., 2014).

In a combined nitrification/denitrification treatment system, nitrification is generally rate limiting. Slow growth rates of nitrifying bacteria and sensitivity to pH, temperature, oxygen and toxic compounds may result in low nitrification efficiencies (Antoniou et al., 1990; Dangcong et al., 2000; Hu et al., 2004). The influence of heavy metals on nitrification in conventional activated sludge systems has been widely investigated (Hu et al., 2004; You et al., 2009). Beg et al. (1982) studied the inhibition kinetics of arsenic towards nitrification but, however, the arsenic effects on nitrifying cultures remain unclear. The toxic effects of arsenic are related to its oxidation state (Jain and Ali, 2000). Different oxidation states

Abbreviations: HPLC, high pressure liquid chromatography; HG, hydride generation; AFS, atomic fluorescence spectrometry.

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affect arsenic bioavailability and toxicity to microorganisms (Lyubun et al., 2013).

The aim of this work was to reveal the effects of arsenic on nitrification of simulated mining water. Nitrifiers were enriched in a fluidized-bed reactor (FBR) and then this enrichment culture was used in batch assays. Arsenic speciation was monitored during nitrification. The FBR enrichment cultures were profiled using a DNA extraction polymerase chain reaction (PCR) – denaturing gradient gel electrophoresis (DGGE) sequencing approach.

2. Methods

2.1. Reactor operation

A 600 mL glass FBR was operated for the enrichment of nitrifying cultures from activated sludge (1.84 g VSS/L) collected from a municipal wastewater treatment plant in Tampere, Finland. The FBR used was as reported by Kinnunen and Puhakka (2004). An aeration unit was placed in the recirculation line and an aquarium pump was used for system aeration. The FBR was operated at room temperature (21–22 °C) for 420 days. Filtrasorb®200 granular activated carbon (Calgon Carbon, USA) was used as the biofilm carrier and fluidized at a recirculation flow rate of 650 mL/min. Carrier fluidization was maintained at 25%. The FBR feed solution was prepared every 10 days and composed of 77.8 mg/L N-NH_4^+ (added as NH_4Cl), nutrients and 595 mg/L $CaCO_3$ of alkalinity (added as 1000 mg/L $NaHCO_3$). Alkalinity was added in excess. Nutrient stock solution was prepared in mQ-water. Nutrient concentrations were as reported by Papirio et al. (2014). The feed pH ranged between 7.5 and 8.3. During the first 15 days, the FBR was operated in batch mode in order to allow bacterial colonization onto activated carbon. Subsequently, FBR feed was continuous by using first a 12 h (days 15–84) and then a 7 h HRT (days 85–420). The FBR was sampled twice a week for ammonium, pH, DO, alkalinity, nitrate and nitrite analyses. Ammonium and pH were monitored in the feed with the same temporal frequency.

2.2. Batch bioassays for arsenic effects

Batch bioassays were used to investigate the effect of arsenic on nitrification at initial As(III) concentrations of 0, 5, 20, 50, 100, 150 and 200 mg/L at pH 7.5. The tests were performed in 100-mL flasks on a gyratory shaker (200 rpm) at room temperature (22 °C). Each flask contained 50 mL of solution and 5 mL of biofilm-coated activated carbon (0.49 g TSS/mL, 0.24 g VSS/mL) taken from the nitrifying FBR after 370 days of operation. Ammonium and nutrients were added at same concentrations as present in FBR feed solution. A phosphate buffer (50 mM HPO_4^{2-} and 50 mM $H_2PO_4^-$) was used to buffer the pH. Control tests without ammonium were used in order to estimate the release of nitrate from activated carbon/biofilm. During 48 h batch assays, the bottles were monitored for arsenic (total As, As(III) and As(V)), ammonium, nitrate, nitrite, pH and dissolved oxygen (DO).

2.3. Analytical methods

Non-filtrated samples were used for DO, pH, and alkalinity measurements. In batch bioassays, DO and pH were measured directly from the flasks. DO and pH analyses were performed with a HQ 40d multi-meter equipped with LDO101 and pHC101 electrodes (Hach-Lange, Germany). In the FBR samples, total alkalinity was analyzed according to the SFS-EN ISO 9963-1 standard potentiometric method by using a TITRONIC titration system (Schott, Germany) and hydrochloric acid (0.1 M) as titrating solution. Samples for ammonium, nitrate, nitrite, total As, As(III) and As(V) were filtered through 0.2 μm Chromafil Xtra PET-20125 membranes (Macherey–Nagel, Germany). Ammonium concentration in the FBR was analyzed with Kjeldahl method according to SFS-EN 25663 standard method by using a Ordior Kjeltec™ 2100 distillation unit (FOSS, Denmark). In batch bioassays, ammonium was determined photometrically using a DR2800 photometer and cuvette tests (Hach-Lange, Germany). TSS, VSS, nitrate and nitrite were measured as reported by Papirio et al. (2014). Atomic absorption spectrophotometry was used for total As analysis as described by Zou et al. (2014). As(III) and As(V) concentrations were determined by using an isocratic LC system HPLC–HG–AFS 1200 (Agilent, USA) equipped with an atomic fluorescence PSA Millenium Excalibur 10.825 detector (PS Analytical, UK) and a column C18 15 cm × 4.6 mm (Discovery, USA). A 10 mM NaH_2PO_4 + 1.5 M tetrabutylammonium hydroxide solution was used as mobile phase at a flow rate of 1.7 mL/min.

2.4. Microbial community analysis

The microbial communities were studied three times during FBR operation and once in batch tests. Microorganisms were identified with 16s rRNA based profiling technique. The protocol according to Kolehmainen et al. (2007) was used for DNA extraction and PCR. A 30 mL of sample, containing both solution and carrier material, were filtered with Cyclopore track etched 0.2 μm membranes (Whatman, USA) for DNA analysis. DreamTaq DNA Polymerase and Ba357F-GC primer (5′-CGCCCGCCGCGCGCG GCGGGCGGGGCGGGGGC ACGGGGGGCCTACGGGAGGCAGCAG-3′) were used for PCR. Safe Imager Blue Light Transilluminator (Invitrogen), PowerShot A640 camera (Canon) and 1% (w/v) agarose gel with Sybr® Safe DNA gel stain were used with gel electrophoresis to visualize DNA yield. DGGE was accomplished according to the protocol developed by Koskinen et al. (2007). Denaturing gradient in polyacrylamide gel was from 30% to 70%. Gels were run at 60 °C and 100 V for 20 h. Forward primer Ba357F (5′-CCTACGGGAGGC AGCAG-3′) was used in PCR after DGGE. Sequencing was performed by MacroGen (Seoul, Korea). Sequence data were analyzed with Ridom TraceEdit software (version 1.0, Ridom, Germany) and compared to the database of the National Center for Biotechnology Information.

3. Results and discussion

3.1. Nitrification performances in the FBR

A nitrifying FBR was operated for 420 days for enrichment and maintenance of nitrifying microbial cultures using 65.0–77.8 mg/L N-NH_4^+. Ammonium, nitrate, nitrite and DO profiles were as reported in Fig. 1A. Ammonium was completely oxidized throughout the operation. DO was in average 7.0 mg/L during days 15–84. Nitrite remained below the detection limit, except at the beginning when 16.8 mg/L N-NO_2^- (22% of the feed N-NH_4^+) was detected. N-NO_3^- rapidly increased to 76.7 mg/L (340 mg/L as NO_3^-) in the first days and nitrate yields averaged 89% during the first 84 days. From day 85 on, ammonium concentration slightly varied in the feed with the mean value of 73.4 mg/L N-NH_4^+ (Fig. 1A). HRT decrease from 12 to 7 h and subsequent DO variation between 4.8 and 7.8 mg/L did not affect ammonium oxidation but the mean ammonium oxidation to nitrate decreased to 71% during the second experimental phase (days 85–420), indicating a larger discrepancy in the nitrogen mass balance (Fig. 2). Nitrite remained below the detection limit. N-NO_3^- concentration strongly fluctuated with the mean value of 52 mg/L (230 mg/L as NO_3^-). This may have resulted from the varying DO conditions and simultaneous nitrification/denitrification in the FBR. DGGE results indicate the presence of