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Fluidized-bed denitrification of mining water tolerates high nickel concentrations

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HIGHLIGHTS

- Ni impact on denitrification was revealed in two continuous FBRs.
- Denitrification tolerated soluble Ni concentrations as high as 500 mg/L.
- Ni speciation was investigated by using both modeling and XRD analysis.
- $Ni_3(PO_4)_2$ precipitates were detected by XRD on activated carbon at FBR termination.
- The high tolerance of *Dechloromonas* to Ni successfully maintained denitrification.

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ABSTRACT

This study revealed that fluidized-bed denitrifying cultures tolerated soluble Ni concentrations up to 500 mg/L at 7–8 and 22 °C. From 10 to 40 mg/L of feed Ni, denitrification resulted in complete nitrate and nitrite removal. The concomitant reduction of 30 mg/L of sulfate produced 10 mg/L of sulfide that precipitated nickel, resulting in soluble effluent Ni below 22 mg/L. At this stage, *Dechloromonas* species were the dominant denitrifying bacteria. From 60 to 500 mg/L of feed Ni, nickel remained in solution due to the inhibition of sulfate reduction. At soluble 60 mg/L of Ni, denitrification was partially inhibited prior to recover after 34 days of enrichment by other Ni-tolerant species (including *Delftia*, *Zoogloea* and *Azospira*) that supported *Dechloromonas*. Subsequently, the FBR cultures completely removed nitrate even at 500 mg/L of Ni. Visual Minteq speciation model predicted the formation of NiS, $NiCO_3$ and $Ni_3(PO_4)_2$, whilst only $Ni_3(PO_4)_2$ was detected by XRD.

1. Introduction

Nitrogenous compounds and heavy metals are commonly present in mining and industrial wastewaters (Papirio et al., 2014b). Contamination of water bodies by nitrogen and heavy metals poses environmental challenges due to eutrophication and toxicity to aquatic species. Ammonium and nitrate can be removed from metal-containing mining waters by using nitrification and denitrification (Papirio et al., 2014a,b; Zou et al., 2014). Denitrification is primarily performed by heterotrophic bacteria that use organic carbon as source of energy and electrons (Park and Yoo, 2009). In the presence of heavy metals, the activity of microorganisms could be repressed according to the environmental conditions and chemical behavior of the metal species (Bartacek et al., 2008).

Nickel is a common metal in mining environments that can occur in soluble, sulfidic (i.e. NiS, NiS_2), elemental (Ni) and oxidic (i.e. NiO, $Ni(OH)_2$, $NiCO_3$) forms (Schaumlöffel, 2012). Nickel has been reported to exhibit both stimulatory and inhibitory effects on microbial processes at low and elevated concentrations, respectively (Gikas, 2008; Zandvoort et al., 2006). Ni speciation determines its bioavailability and toxicity to microorganisms (Yebra-Biurrun and Castro-Romero, 2011). However, Ni toxicity can be mitigated by the formation of Ni complexes with dissolved organic matter (DOM) or soluble microbial products (SMP) (Doig and Liber, 2007; Kuo and Parkin, 1996) and bio-sorption of Ni on microorganisms (Fomina and Gadd, 2014).

Abbreviations: DGGE, denaturant gradient gel electrophoresis; DOM, dissolved organic matter; ESEM, environmental scanning electron microscopy; EPS, extracellular polymeric substances; FBR, fluidized-bed reactor; FID, flame ionization detector; GC, gas chromatograph; HRT, hydraulic retention time; ICP–OES, inductively coupled plasma–optical emission spectrometry; JCPDS, joint committee on powder diffraction standards; SMP, soluble microbial products; XRD, X-ray diffraction.

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Denitrifying microorganisms have been found in a variety of taxonomic groups (Zumft, 1997). Bioprocess operating conditions influence the composition of denitrifying microbial communities and denitrification potential (Papirio et al., 2014a; Rasool et al., 2014). Modern molecular techniques have allowed revealing the effects of environmental conditions (Cao et al., 2008) and heavy metals (Magalhães et al., 2011) on denitrifying microorganisms in soil and sediments. However, the understanding of the evolution of denitrifying communities in biofilm reactors remains limited.

The present work investigated the effects of increasing Ni concentrations on heterotrophic denitrification and microbial population in two fluidized-bed reactors (FBRs), operated at 7–8 and 22 °C under neutral conditions. Denaturing gradient gel electrophoresis (DGGE) was used to reveal the evolution of the FBR denitrifying microbial communities under gradual feed Ni increases. Ni speciation and solid phase characterization were studied in both FBRs.

2. Methods

2.1. FBR operation

Two FBRs (FBR1 and FBR2) were used to study denitrification in presence of soluble Ni under neutral conditions. The FBR scheme and characteristics were as reported by Papirio et al. (2014a). Prior to this, steady denitrification with ethanol as organic electron donor was initially maintained for 415 days in FBR1 and FBR2, operated at 7–8 and 22 °C, respectively, with the aim of investigating the effects of feed pH, HRT, ethanol/nitrate ratio and temperature on the process (Papirio et al., 2014a). Subsequently, Ni was injected twice to FBR1 and FBR2 between days 416 and 567, in order to study Ni impacts at feed pH of 2.5 (Zou et al., 2014). Finally, from day 568 to 632, feed pH was increased from 2.5 to 5.5 and Ni was not supplemented to FBRs, in order to recover the denitrifying process in both reactors.

In this work, the feed Ni concentration was gradually increased from 10 to 500 mg/L in both FBRs between days 633 and 887. Nitrate and ethanol concentrations were maintained at 200 and 123 mg/L, respectively. Feed pH was kept at 5.5 and HRT was 5.4 h. The composition of the mineral medium was as reported by Papirio et al. (2014a). From day 864 to 887, feed nitrate was increased from 200 to 300 mg/L in order to investigate FBR denitrifying potential at feed Ni 500 mg/L. Ethanol concentration was accordingly increased to 185 mg/L to maintain the ethanol/nitrate ratio constant (0.84 mol/mol). Both FBR1 and FBR2 were terminated on day 887. Effluent samples were taken every three days for pH, nitrate, nitrite, ethanol and Ni measurements.

2.2. Sample analysis

Samples of FBR effluents were filtered through 0.2 μm Chromafil Xtra PET-20125 membranes (Macherey–Nagel, Germany). pH, nitrate, nitrite and Ni concentrations were measured in both FBRs as reported by Zou et al. (2014). Ethanol was analyzed with a gas chromatograph (GC-2010 Plus, Shimadzu, Kyoto, Japan) equipped with a ZB-WAX plus column (Phenomenex, USA) and a flame ionization detector (FID). The alkalinity in the effluent was measured as reported by Papirio et al. (2014b).

The microbial communities were studied both in absence and in presence of nickel at different feed Ni concentrations. Samples of biofilm/activated carbon were taken four times during FBRs operation (on days 607, 704, 788 and 887). PCR-DGGE analysis was performed as reported by Papirio et al. (2014b). The unambiguous bands in the DGGE gel were excised and the DNA was sequenced by MacroGen (Seoul, Korea). Sequencing data were compared to the database of the National Center for Biotechnology Information.

At FBR termination, the precipitates on the biofilm-coated activated carbon were characterized by ESEM, XRD and ICP–OES analyses. Environmental scanning electron microscopy analysis (ESEM) was performed on an Electroscan (Wilmington, USA) Type II LaB6 gun microscope. X-ray diffraction (XRD) analysis was performed on a Bruker D8 Advance diffractometer equipped with an energy dispersion Sol-X detector with copper radiation (CuKα, $\lambda = 0.15406$ nm). The acquisition was recorded between 10° and 80°, with a 0.02° scan step and 1 s step time. The precipitates were dissolved in 1 M HNO_3 and the solubilized elements were analyzed by inductively coupled plasma–optical emission spectrometer (ICP–OES) (Perkin-Elmer Optima 8300). Phosphorus concentration was determined according to the standard colorimetric method (Rodier et al., 2009). The analysis of the elemental composition of the precipitates was performed in triplicate.

2.3. Thermodynamic modeling by Visual MINTEQ

The chemical equilibrium code Visual MINTEQ v3.1 (Visual MINTEQ is a freeware chemical equilibrium model that can be downloaded from http://vminteq.lwr.kth.se/- accessed on December 4th, 2014) was used to identify the possible phases controlling Ni solubility in the bioreactor. The concentrations of the elements used in the feed were input into the model. Besides, the temperature was kept constant at 8 and 22 °C, the pH was set to 7.0, the oversaturated solids were allowed to precipitate, and the alkalinity measured in the effluent was specified.

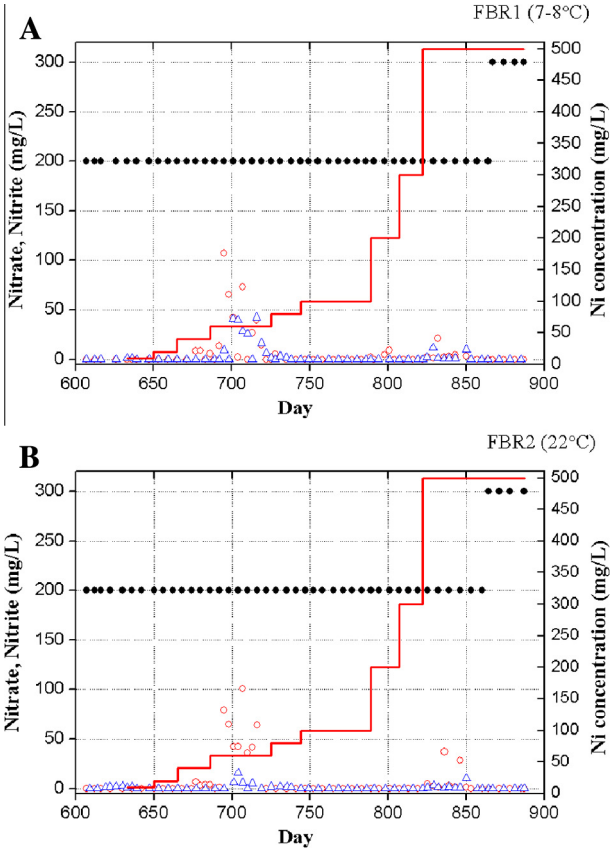

Fig. 1. Feed Ni and nitrate and nitrite profiles in FBR1 (A) and FBR2 (B). Explanation of symbols: influent nitrate (closed circles); effluent nitrate (open circles); effluent nitrite (open triangles); feed Ni (solid line).