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Short Communication

Distribution and genetic diversity of the microorganisms in the biofilter for the simultaneous removal of arsenic, iron and manganese from simulated groundwater

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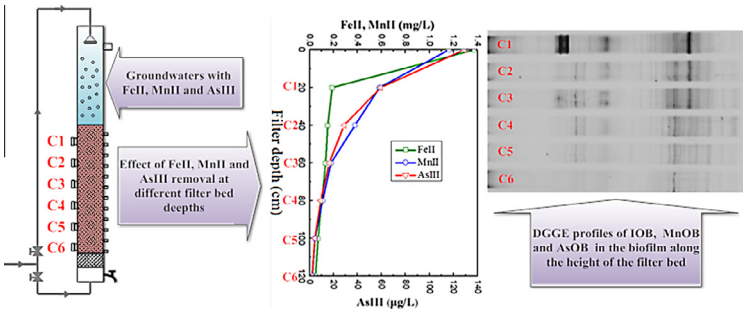
HIGHLIGHTS

- Simultaneous removal of FeII, MnII and AsIII was achieved in a biofilter.
- The removal of FeII, MnII and AsIII was different along the height of the filter.
- IOB, MnOB and AsOB co-existed in the bio-films at different filter bed depth.
- Bacteria distribution could be a main factor to affect biofilter performance.

GRAPHICAL ABSTRACT



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ABSTRACT

A biofilter was developed in this study, which showed an excellent performance with the simultaneous removal of AsIII from 150 to 10 mg L^{-1} during biological iron and manganese oxidation. The distribution and genetic diversity of the microorganisms along the depth of the biofilter have been investigated using DGGE. Results suggested that Iron oxidizing bacteria (IOB, such as *Gallionella*, *Leptothrix*), Manganese oxidizing bacteria (MnOB, such as *Leptothrix*, *Pseudomonas*, *Hyphomicrobium*, *Arthrobacter*) and AsIII-oxidizing bacteria (AsOB, such as *Alcaligenes*, *Pseudomonas*) are dominant in the biofilter. The spatial distribution of IOB, MnOB and AsOB at different depths of the biofilter determined the removal zone of FeII, MnII and AsIII, which site at the depths of 20, 60 and 60 cm, respectively, and the corresponding removal efficiencies were 86%, 84% and 87%, respectively. This process shows great potential to the treatment of groundwater contaminated with iron, manganese and arsenic due to its stable performance and significant cost-savings.

1. Introduction

Arsenic is a contaminant of well-known toxicity and commonly occurs in groundwater. Several studies have reported that arsenic is a carcinogen and its effects are primarily due to consumption of arsenic contaminated drinking water at concentrations around 100 mg L^{-1}. The WHO provisional guideline of 10 μg L^{-1} has been adopted as the drinking water standard in many countries. Arsenic exists mainly in groundwater with two main oxidation states, AsIII and AsV. The distribution of arsenic species (AsIII, AsV) in natural waters is mainly dependent on redox potential and pH conditions. AsV is the thermodynamically stable form of inorganic species and it generally predominates in surface waters. AsIII is favored under reducing conditions, such as in anaerobic groundwater. AsIII species are more toxic and exist as the undissociated molecule of arsenious acid (H_3AsO_3), at the pH values usually encountered in most natural water sources (Smedley and Kinniburgh, 2002). So

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they are more difficult to be removed by the conventional physicochemical treatment methods than AsV (Gupta and Sankararamakrishnan, 2010; Zhang et al., 2013).

The biological oxidation of iron and manganese as a treatment method for arsenic removal is a relatively new method which is based upon the fact that groundwater contaminated with arsenic usually occurs with iron and manganese (Lehimas et al., 2001; Streat et al., 2008). Biological iron or manganese oxidation results in the formation of insoluble products (iron or manganese oxides), which are subsequently removed from water by filtration. If arsenic is simultaneously present in the water, it can be removed by oxidation and sorption onto the iron and manganese oxides.

In this study, a lab-scale biofilter was established for the simultaneous removal of FeII, MnII and AsIII from simulated groundwater. Approximately180 d long-term operation was evaluated with respect to AsIII, FeII and MnII removal. The distribution and genetic diversity of the microorganisms in the biofilter were further analyzed by DGGE to gain a deeper insight into the mechanism of Fe, Mn and As removal. This study may further lead to the application of the biofilter for simultaneous removal of iron, manganese and arsenic in groundwater.

2. Methods

2.1. Biofilter configuration and groundwater simulation

The removal of arsenic was examined simultaneously with biological iron and manganese oxidation. The treatment process was based on a biofilter unit, as shown in Fig. 1. The apparatus consisted of a Plexiglas column, which was filled with quartz sand. The mature filtration media (containing large amounts of IOB and MnOB) from a full-scale plant for the simultaneous removal of iron and manganese in groundwater was put on the upper 10 cm of the filtration bed as inoculum.

The feed solution was the result of mixing a concentrated solution of arsenic, iron and manganese with tap water from the water supply network (containing negligible arsenic, iron and manganese amounts) in the influent sampling vessel. Table S1 presented the physicochemical characteristics of the supply network water. AsIII stock solutions were prepared by dissolving sodium arsenite ($NaAsO_2$) in deionized water. Iron stock solution containing 2000 mg L^{-1} FeII was prepared from $FeSO_4{\cdot}7H_2O$ by dissolving in deionized water. Manganese stock solution containing 1000 mg L^{-1} MnII was prepared from $MnSO_4{\cdot}H_2O$ by dissolving in deionized water. The iron and manganese stock solutions were not kept more than three weeks.

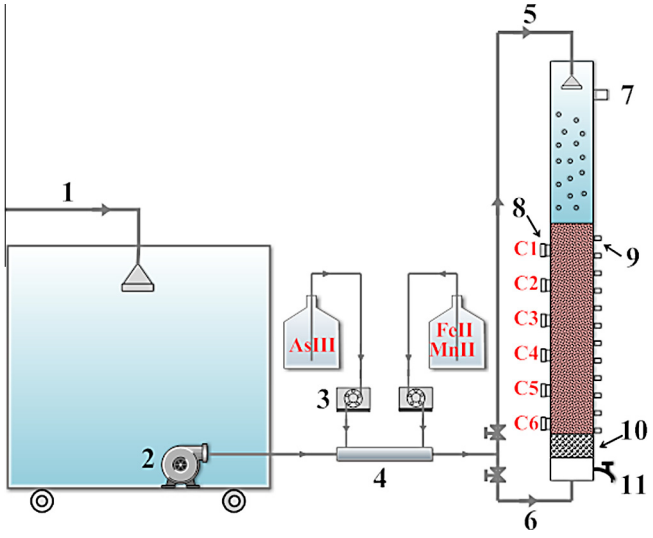

Fig. 1. Schematic drawing of the biofilter arrangement. (1) Water supply network, (2) water pump, (3) peristaltic pump, (4) influent sampling vessel, (5) influent, (6) backwashing water, (7) excess water outlet, (8) sand sampling valve, (9) water sampling valve, (10) support gravel media and (11) effluent. Column characteristics: active height: 2.5 m, inner diameter: 100 mm, bed height: 1.2 m, bed diameter: 1 ~ 1.2 mm. Sand sampling valve: C1 ~ C6, interval: 20 cm. Water sampling valve: 1 ~ 12, interval: 10 cm.

2.2. Sampling and chemical analysis

Water sampling from the inlet and outlet was performed every day and water sampling along the height of the filter bed was performed twice a week during the continuous operation of the biofilter. The determination of Iron, manganese and total arsenic were performed by ICP-OES (Perkin Elmer Optima 5300DV). The temperature, pH and Oxidation reduction potential (ORP) measurements were made using a pH meter (WTW, pH7310). Dissolved oxygen content was measured by the Dissolved Oxygen Meter (WTW, oxi315i). SEM analysis (FEI Quanta 200) was used for observing the microorganisms (Hashimoto et al., 2007).

2.3. DNA extraction, PCR amplification and DGGE analysis

Sand samples from different biofilter bed depths were collected and stored in 50 mL sterile plastic test tubes at −20 °C until DNA extraction performed using the PowerSoil DNA Isolation kit (MoBio Laboratories Inc, USA) according to the manufacturer's instructions. The concentration and quality of DNA were examined by agarose gel (1%) electrophoresis and UV spectrophotometry (NanoDrop200, USA).

The V3 and V4 region of 16S rRNA genes were amplified using primers of 341F-GC: 5′-CGC CCG CCG CGC CCC GCG CCC GTC CCG CCG CCC CCG CCC GCC TAC GGG AGG CAG CAG-3′ (Muyzer et al., 1993) and 907R: 5′-CCG TCA ATT CMT TTGAGT TT-3′ (Muyzer et al., 1995). PCR products were detected by agarose gel (1.5%) electrophoresis.

For DGGE analysis, PCR products were separated on polyacrylamide gels (8%) with a 30–70% linear gradient of denaturant (100% denaturant = 7 M urea plus 40% formamide). The gel was conducted at 60 °C in 1 × TAE buffer (40 mM Tris–acetate, 20 mM sodium acetate, 1 mM Na_2EDTA, pH 7.4) at 90 V for 10 h on a Dcode Universal Mutation Detection System (Bio-Rad). After electrophoresis, the gel was stained using silver-staining method and visualized on the scanner (UMAXPowerLook1000).

2.4. Cloning, sequencing and phylogenetic analysis

Specific gel bands were excised and dissolved in 50 μL 1 × TE buffer (10 mM Tris–HCl, 1 mM EDTA, pH 8.0) at 4 °C overnight. 1 μL 1 × TE solution as DNA template was re-amplified and checked on a DGGE gel for purity and migration to the same gradient position as in the original sample. The target DNA fragments were then excised and re-amplified. The DNA fragments were ligated into pMD19-T plasmid vector (TaKaRa, Japan) and transferred into competent Escherichia coli DH5a (TaKaRa, Japan). The positive colonies of each sample were randomly chosen and validated by colony PCR, and sequencing was carried out on an ABI 3730 DNA sequencer by a commercial service (Sangon, China).

Homology searches were carried out by basic local alignment search tool (BLAST) (Morgulis et al., 2008). Phylogenetic trees were constructed using the neighbor-joining (NJ) algorithm with a bootstrap value of 1000 by Kimura 2-parameter model (MEGA software, version 5.05) (Tamura et al., 2011).