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Treating organic cyanide-containing groundwater by immobilization of a nitrile-degrading bacterium with a biofilm-forming bacterium using fluidized bed reactors☆

Xuejiao An [a], Yi Cheng [b], Mingyan Huang [a], Yueling Sun [a], Hailan Wang [a], Xi Chen [a], Jinming Wang [a], Dapeng Li [a], Chunyan Li [a], *

[a] College of Resources and Environment, Northeast Agricultural University, Harbin 150030, Heilongjiang, PR China
[b] College of Science, China Agricultural University, Beijing 100083, PR China

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ABSTRACT

Organic cyanide are widely used as an ingredient in the production of plastics, synthetic rubbers, polymers, pharmaceuticals and pesticides or used in laboratories and industries as solvents. Although nitrile-containing wastewater is subjected to primary and secondary treatments, residual nitriles may slowly seep and further migrate through groundwater, resulting in the micropollution of groundwater by organic pollutants. In this study, water samples were collected from different study areas in North China during a period of 3y (from 2013 to 2015) and analyzed to evaluate organic cyanide (CN^-) contamination in groundwater. Three parallel lab-scale fluidized bed reactors (FBRs) were tested for their ability to remove organic cyanide from groundwater. The organic cyanide concentration in groundwater increased significantly ($P < 0.05$) from 2013 to 2015. With an optimal hydraulic residence time (HRT) of 54 min, reactor R3 (inoculated with a nitrile-degrading bacterium, BX2, and a biofilm-forming bacterium, M1) effectively removed 99.8% of CN^- under steady operation, which was better than that of other reactors. Short-term shutdowns of FBRs had no serious effects on the efficiency of treating organic cyanide. This work demonstrated that the biofilm-forming bacterium could facilitate the fixation of nitrile-degrading bacterium and enhance the efficiency of removing organic cyanide from groundwater.

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1. Introduction

Nitriles are a class of organic cyanide compounds (e.g., acetonitrile, acrylonitrile and crotononitrile) that are widely used as an ingredient in the production of plastics, synthetic rubbers, polymers, pharmaceuticals and pesticides or used in laboratories and industries as solvents and to for extractions (Li et al., 2007). Many studies have demonstrated that nitriles are highly toxic, carcinogenic and mutagenic, and nitrile-containing wastes are often present in effluents from its production and application (Ramteke et al., 2013; Li et al., 2008). Although nitrile-containing wastewater is subjected to primary and secondary treatments, residual nitriles may slowly seep and further migrate through groundwater, resulting in the micropollution of groundwater by organic pollutants. Organic cyanide-containing groundwater posed a significant long-term environmental threat to water quality and ecosystem and public health (Lovecka et al., 2015). Thus, some countries and organizations set limits on the concentration of cyanide in groundwater. For example, the European Union's administrative agency sets the standard limit for a single nitrile in groundwater and drinking water at 0.1 μg L^{-1}. The Quality Standard for the Groundwater of China (SEPA, 2002) sets the limit for cyanide at 0.05 mg L^{-1}. Few surveys have reported the status of organic cyanide pollution in groundwater near acrylic fiber plants, and the long-term monitoring of groundwater contamination by organic cyanide has particularly not been conducted.

Strategies to remove contaminants from groundwater mainly include physicochemical and biological methods. However, these physicochemical processes may be cost-prohibitive and complex to operate. The biodegradation of contaminants in groundwater has received much attention in recent years. Several previous studies have shown that specific microorganisms could be applied to

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* Corresponding author.
E-mail address: chunyanli@neau.edu.cn (C. Li).

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degrade toxic organic pollutants in groundwater. For example, the use of species such as *Rhodococcus ruber* ENV425, *Acinetobacter* sp. WSD, *Aminobacter* sp. MSH1 and *Desulfitobacterium* spp. has effectively treated contaminated groundwater (Webster et al., 2013; Shao et al., 2015; Careghini et al., 2015; Albers et al., 2015). Unfortunately, the high efficiencies of removing pollutants by groundwater treatment systems rarely persist because the degrading microorganisms are washed out of the system with the repeated replacement of groundwater. Immobilizing degrading microorganisms in groundwater treatment systems has been suggested as a strategy to maintain high pollutant removal efficiency. Different approaches of immobilizing the degrading microbes to promote the effectiveness of groundwater treatment have been reported. Zhou et al. (2009) found that the immobilization of denitrifying bacteria on the surface of activated carbon fiber (ACF) cathodes enhanced the biodegradation of nitrates and organic pollutants in a three-dimensional bio-electrochemical reactor. Cesar and Ros (2013) found that when they investigated the bioaugmentation of nitrite, nitrate and pesticide in a two-stage continuous system, the removal efficiency increased because microorganisms were immobilized within ceramic carriers. Nevertheless, because the degrading microorganisms that are used in groundwater treatment systems were poorly immobilized, the efficiency of pollutant removal was not ideal.

A biofilm is generally defined as a population of bacteria surrounded by a matrix of extracellular polymeric substances (EPSs) and is adherent to the surface of abiotic or biotic substances (Han et al., 2016). Biofilm formation is conducive to immobilizing degrading bacteria in groundwater treatment systems because biofilms can form protected patterns that shield bacteria from damage by harmful substances, resist harsh environments, adsorb organic pollutants in groundwater and enhance the removal of the pollutants by the resident microorganisms (Eberl et al., 2017). Inoculating specific contaminant-degrading and biofilm-forming bacteria into a biological treatment system is therefore a useful strategy for removing pollutants from groundwater.

FBRs are fixed-film bioreactors composed of a vessel that contains a carrier (usually sand, polyvinyl chloride (PVC) or granular activated carbon (GAC)) that exhibits a large surface area to foster the growth of microorganisms (Sutton, 2006). Large amounts of biomass can be fixed to this carrier, leading to the removal of contaminants in shorter HRTs (Tisa et al., 2014). Solid and liquid phases formed excellent contacts, and high microbial activity was achieved. The FBR has advantages similar to previously reported biological reactors, such as packed bed bioreactors (PBRs), sequencing biofilm batch reactors (SBBRs) and rotating biological contactors (RBCs) (Campos et al., 2006; Kumar et al., 2015; Singh and Balomajumder, 2017; White and Schnabel, 1998; Sirianuntapiboon and Chuamkaew, 2007; Singare and Dhabarde, 2017; Acheampong et al., 2010). The FBR also has other benefits, such as stable operation, an ability to handle a high loading rate, better removal of trace substances and scalable application (Moussavi et al., 2014; Mowla and Ahmadi, 2007; Webster et al., 2013).

In this study, we assessed the quality of groundwater near an acrylic fiber plant in North China in terms of organic cyanide contamination from 2013 to 2015. A nitrile-degrading bacterium (*Rhodococcus rhodochrous* BX2) and a biofilm-forming bacterium (*Bacillus mojavensis* M1) isolated by our laboratory were used together as inocula for the FBR start-up and operation. The removal of organic cyanide from groundwater by three parallel lab-scale FBRs was studied. The effects of short-term shutdowns on the treatment of micropolluted groundwater in the FBRs was investigated. To the best of our knowledge, this paper is the first survey of organic cyanide in groundwater and organic cyanide removal from groundwater by the coaggregation of highly effective nitrile-degrading bacteria and biofilm-forming bacteria in FBRs. This study could draw people's attention to the problem of organic cyanide contamination in groundwater and support administrations to develop more reasonable policies for the prevention of groundwater pollution by organic cyanide based on joint efforts by local governments and enterprises. In addition, the method presented here will contribute valuable information for the treatment of organic cyanide and other organic contaminants in groundwater.

2. Materials and methods

2.1. Chemicals, bacterial strains and culture medium

Acetonitrile (CAS no. 75-05-8) was supplied by Fisher Scientific Co. (Shanghai, China). Acrylonitrile (CAS no. 107-13-1) and crotononitrile (CAS no. 4786-20-3) were obtained from the Tokyo Chemical Industry (TCI) Co., Ltd. (Tokyo, Japan). All other chemicals were of analytical grade and purchased from the Chengdu Aike Chemical Reagent Co., Ltd. (Chengdu, China).

Rhodococcus rhodochrous BX2 was isolated from soil and found to efficiently degrade aliphatic nitriles (Fang et al., 2015). *Bacillus mojavensis* M1 is a biofilm-forming bacterium with a high biofilm-forming capacity (see Supplementary data for a detailed description). All of the above mentioned strains were stored in our laboratory.

The composition of the mineral salt (MS) medium used for CN^- utilization experiments was as follows: KH_2PO_4 1700 mg L^{-1}, Na_2HPO_4 9800 mg L^{-1}, $MgSO_4 \cdot 7H_2O$ 0.1 mg L^{-1}, MgO 10 mg L^{-1}, $ZnSO_4 \cdot 7H_2O$ 1.44 mg L^{-1}, $FeSO_4 \cdot 7H_2O$ 0.9 mg L^{-1}, $CuSO_4 \cdot 5H_2O$ 0.25 mg L^{-1}, and H_3BO_3 0.06 mg L^{-1}, at an initial pH of 7.0. Luria-Bertani (LB) medium contained tryptone 10 g L^{-1}, yeast extract 5 g L^{-1}, and NaCl 10 g L^{-1}.

2.2. Water source, water sampling and sample analysis

2.2.1. Water source

In this study, three sampling campaigns were conducted during the period from 2013 to 2015 in Heilongjiang province, northern China (Fig. S1). Water was collected from the following seven different sampling sites: 1) The primary effluent was sampled from the drainage outlet of an acrylic fiber plant (S1), 2) the secondary effluent was obtained from an oxidation pond gate (S2), 3) a groundwater sample was collected from the village near the oxidation pond gate (S3), 4) a river water sample was obtained from upstream of the oxidation pond gate (S4), 5) a groundwater sample was obtained from the village located upstream of the oxidation pond gate (S5), 6) a river water sample was collected from downstream of the oxidation pond gate (S6), and 7) a groundwater sample was obtained from the village located downstream of the oxidation pond gate (S7). The GPS locations of the sampling sites are presented in Table S1.

2.2.2. Water sampling

Duplicate samples were collected at each site in precleaned and sterilized PVC plastic flasks. All samples were rapidly transported to the laboratory under low temperature conditions and immediately stored at 4 °C for further analysis. All analyses were completed within a week.

2.2.3. Sample analysis

All the water samples were analyzed according to the standard methods for water and wastewater quality provided by the China State Environmental Protection Administration (SEPA, 2002). CN^- concentrations were measured using GC-MS equipment from

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