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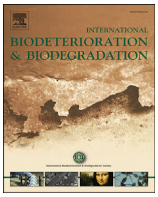

Application of sulfate reduction mechanisms for the simultaneous bioremediation of toluene and copper contaminated groundwater

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ABSTRACT

Groundwater at many industrial polluted sites is usually contaminated by complex contaminants including petroleum hydrocarbons and heavy metals. In this study, the effectiveness of sulfate-reducing mechanisms on toluene and copper contaminated groundwater cleanup was evaluated in microcosm experiments. Sulfate was supplied into microcosms containing toluene (17.5 mg/L) and copper (12 mg/L) contaminated groundwater to activate the sulfate reducing process. The inocula used in the microcosms contained petroleum-hydrocarbon contaminated soils and sludge collected from an anaerobic basin of a wastewater treatment facility. Approximately 99% of toluene and copper could be removed during the 40-day operational period with the decay rates of 0.19 and 0.12 1/d, respectively. Under sulfate-reducing mechanisms, toluene could be biodegraded and a consumption of 0.105 g/L of sulfate was observed. Copper removal (dropped to below 0.1 mg/L) was due to the bioprecipitation mechanisms, which could be confirmed by the occurrence of sulfate reduction mechanisms (sulfide increased from 8.6 to 686 μg/L) and copper sulfide formation. Increased hydrogen sulfide concentrations also resulted in the inhibition of microbial growth. Dominant bacterial species and microbial communities were characterized by molecular biological technologies. A total of 12 dominant sulfate-reducing bacteria and petroleum-hydrocarbon degraders were detected. Results show that simultaneous toluene and copper removal could be achieved via the sulfate reduction and heavy metal bioprecipitation mechanisms. Sulfate reduction became the predominant biodegradation mechanism after sulfate supplement under anaerobic conditions. Results from this study can be applied to develop a cost-effective bioremedial system to cleanup heavy-metal and petroleum-hydrocarbon polluted groundwater.

1. Introduction

Groundwater at many industrial sites is polluted by petroleum hydrocarbons and heavy metals. In situ bioremediation (ISB) is a commonly used site cleanup technology for contaminated site treatment. ISB contains natural bioremediation (NB) and enhanced bioremediation (EB). NB is an approach that uses intrinsic mechanisms to remove pollutants in groundwater. To minimize the remediation time of the NB, EB can be applied at polluted sites to accelerate the remediation processes (Xiong et al., 2012). Electron acceptors are usually provided to enhance the EB rates of groundwater contaminated by petroleum hydrocarbons and heavy metals.

Under aerobic conditions, dissolved oxygen (DO) is supplied as an electron acceptor to enhance the aerobic biodegradation rate. Currently, biosparging and oxygen releasing compounds have been applied for oxygen delivery to the subsurface to enhance the EB rates under aerobic conditions (Farhadian et al., 2008). Under anaerobic conditions, microorganisms can use other electron acceptors (e.g., NO_3^-, Mn^{4+}, Fe^{3+}, SO_4^{2-}, CO_2) to enhance the rates of EB (Xiong et al., 2012). However, groundwater in most petroleum-hydrocarbon polluted sites is usually under reduced conditions (Wilson and Kolhatkar, 2002; Kao et al., 2010), and sulfate is a more water-soluble electron acceptor than oxygen (Cuthbertson and Schumacher, 2010). Application of sulfate-reducing processes to remediate petroleum-hydrocarbon polluted groundwater becomes

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a more practical and cost-effective remedial option.

Sulfate-reducing bacteria (SRB) use organic compound (e.g., petroleum hydrocarbon) as the electron donor and sulfate as the electron acceptor (Mbadinga et al., 2012). The main advantages for using sulfate as the electron acceptor are as follows: (1) sulfate is a relatively cheap chemical with high water soluble [Depending on the cation in the sulfate compound, the solubility of sulfate salts varied from 2 g/L (CaSO4) to 500 g/L (ZnSO4)], (2) SRB can grow in wider pH conditions (pH 5 to 9.5), and (3) Sulfate reduction usually became the dominant mechanism at petroleum-hydrocarbon contaminated sites because oxygen and nitrate are rapidly consumed once the contamination occurs (Cuthbertson and Schumacher, 2010; Zhang et al., 2016).

Sulfate reduction process will produce sulfide, which can react with cations (e.g., metals ions) and form insoluble precipitates. Bioprecipitation is a process to result in metal precipitates with the supplement of carbon sources as electron donors for metal bioprecipitation (e.g., formation of metal sulfide mediated by SRB) (Diels et al., 2002; Wolicka and Borkowski, 2007; Miao et al., 2014). The total mass of the metals cannot be changed via the bioprecipitation mechanism, which only stabilizes the metals in the solid phase (Luptakova and Kusnierova, 2005; Satyawali et al., 2010; Kiran et al., 2017).

Conventional methods for heavy-metal contaminated groundwater treatment include the following methods: ion-exchange, chemical assisted precipitation, and adsorption (Azabou et al., 2007; Xiong et al., 2012). The bioprecipitation of heavy metals by SRB via the formation of heavy metal sulfide has become an alternative method to remediate heavy-metal contaminated groundwater (Azabou et al., 2007; Xiong et al., 2012). SRB can oxidize petroleum hydrocarbons and transform the sulfate to sulfide via sulfate-reducing reactions (Kiran et al., 2017). The stoichiometric equation for the sulfate reduction of petroleum hydrocarbons (using toluene as the target compound) is as follows (Equation (1)) (Azabou et al., 2007; Hoa et al., 2007):

$$C_7H_8 + 4.5\ SO_4^{2-} + 9\ H^+ \rightarrow 2.25\ H_2S + 2.25\ HS^- + 7\ CO_2 + 4\ H_2O \tag{1}$$

Hydrogen sulfide will then react with metal ions and produce precipitates of metal sulfide (Cabrera et al., 2006) (Equation (2)).

$$M + H_2S \rightarrow MS_{(S)} + 2H^+ \tag{2}$$

Compared to conventional chemical precipitation, in situ bioprecipitation has attracted people's attention due to the following advantages: applicable for the remediation of low heavy metal concentrations, lower operational cost and less sludge production, and treatment in situ (Cabrera et al., 2006; Biswas et al., 2009; Mal et al., 2016). Thus, biological heavy metal precipitation can be used to remediate heavy-metal contaminated groundwater via sulfate-reducing reactions (Azabou et al., 2007; Hsu et al., 2010; Zhang and Wang, 2016).

Researchers have applied molecular biological methods [e.g., polymerase-chain reaction (PCR), nucleotide sequence, denaturing-gradient gel electrophoresis (DGGE)] to evaluate variations in bacterial diversity during the bioremediation processes (Li et al., 2012; Chimutsa et al., 2015). Molecular biological techniques are useful methods to evaluate the biodegradation pathways and dominant bacteria during the contaminant biodegradation process (Corseuil et al., 2011). Results from the molecular biological analyses could be used as a reference for the occurrence of bioremediation and changes of microbial diversity during the bioremediation process (Chiu et al., 2013; Chimutsa et al., 2015).

In most heavy-metal contaminated groundwater sites, heavy metals usually coexist with other organic contaminants (e.g., petroleum hydrocarbons). Therefore, it is possible to simultaneously remove petroleum hydrocarbons and heavy metals from groundwater under sulfate-reducing conditions if the site is contaminated by both petroleum hydrocarbons and heavy metals. Although sulfate reduction process can be applied to bioremediate petroleum-hydrocarbon and heavy-metal contaminated sites, the mechanisms and effectiveness of simultaneous hydrocarbon and heavy metal removal are not clear, which need to be confirmed and clarified.

Both petroleum hydrocarbon (toluene) and heavy metal (copper) were selected as the target compounds in the microcosm study. Soils collected from a petroleum-hydrocarbon contaminated site and anaerobic activated sludge collected from the anaerobic biological treatment unit of an industrial wastewater treatment plant were used as inocula in the microcosms. The goals of this research included the following (1) assessment the feasibility of using enhanced sulfate reduction to remediate toluene and copper contaminated groundwater, (2) determine the dominant bacterial species, which were able to remove toluene and copper from contaminated groundwater under the sulfate reduction processes, and (3) determine the major mechanisms resulted in toluene and copper removal via the sulfate reduction and bioprecipitation processes, respectively.

2. Materials and methods

2.1. Batch study

Groundwater at an industrial site located inside an industrial park in southern Taiwan was contaminated by toluene and copper. In this research, a batch microcosm study was conducted to evaluate the mechanisms and effectiveness of simultaneous removal of toluene and copper from contaminated groundwater via the anaerobic sulfate-reducing reactions.

Inocula applied in this research contained soils from the contaminated site and activated sludge collected from an anaerobic treatment unit of an industrial wastewater treatment plant containing petroleum hydrocarbons in the influent. The treatment efficiency of the simultaneous toluene and copper removal from collected groundwater was evaluated in this study during 40 days of operation. The components of three groups of microcosms [kill control microcosm (KC), live control microcosm (LC), and sulfate reduction microcosm (SR)] are presented in Table 1.

The anaerobic microcosm was constructed with 35 mL of contaminated groundwater (containing toluene and copper), 20 g of site soils, and 5 mL of sludge (inocula) (or 5 mL of mineral medium in control microcosms) in a glass serum bottle (70 mL). The components of mineral medium used in microcosms are described in Huang and Kao (2015). Sodium sulfate was added into the sulfate reduction and kill control microcosms (Groups SR and KC) for sulfate supplement, and the initial sulfate concentration was 236 mg/L in microcosms. The initial toluene and copper concentrations in all microcosms were 17.5 and 12 mg/L, respectively. Sulfate in site groundwater ranged from 12 to 23 mg/L.

Microcosms were prepared under anaerobic conditions in an anaerobic glovebox and the mineral medium solution (pH = 7.5) was autoclaved before use. Nitrogen gas was used to purge the collected site soils before moving into the glovebox for the anaerobic microcosm preparation. Live control bottles (Group LC) contained soils and activated sludge, but sulfate was not supplied in microcosms. NaN_3 (500 mg/L) and $HgCl_2$ (250 mg/L) were supplied in the kill control (KC) microcosms, and sludge was not added into the bottles.

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