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Kinetics of nitrobenzene degradation coupled to indigenous microorganism dissimilatory iron reduction stimulated by emulsified vegetable oil

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ABSTRACT

Widespread contamination by nitrobenzene (NB) in sediments and groundwater requires better understanding of the biogeochemical removal process of the pollutant. NB degradation, coupled with dissimilatory iron reduction, is one of the most efficient pollutant removal methods. However, research on NB degradation coupled to indigenous microorganism dissimilatory iron reduction stimulated by electron donors is still experimental. A model for remediation in an actual polluted site does not currently exist. Therefore, in this study, the dynamics was derived from the Michaelis–Menten model (when the mass ratio of emulsified vegetable oil and NB reached the critical value 91:1). The effect of SO_4^{2-}, NO_3^-, Ca^{2+}/Mg^{2+}, and the grain size of aquifer media on the dynamics were studied, and the NB degradation dynamic model was then modified based on the most significant factors. Utilizing the model, the remediation time could be calculated in a contaminated site.

Introduction

Nitrobenzene (NB) is a toxic organic chemical material that is commonly utilized in pharmaceuticals, dyes and other applications. Due to inappropriate disposal during its usage, storage, and transportation processes, NB is already responsible for quite serious pollution in many groundwater systems (Yin et al., 2015). NB does not readily biodegrade and is highly toxic to humans and the environment, so it is listed as a priority pollutant by the US EPA (Dong et al., 2013, 2015). In subsurface environments, the presence of dissimilatory metal-reducing bacteria and organic matter provides a remarkably effective technology for the remediation of contaminated soils and groundwater (Lovley et al., 1989; Lovley and Lonergan, 1990). Geochemical evidence has indicated that organic pollutants, such as nitroaromatic compounds, can be degraded in anaerobic subsurface environments in which iron reduction is an intermediate electron-accepting process (Luan et al., 2009). Fe(III) is one of the most abundant potential electron acceptors in underground environments (Lovley, 1991). Microbial dissimilatory iron reduction coupled to NB degradation has already shown promise in the remediation of NB-polluted aquifers (Heijman et al., 1995).

Under anaerobic subsurface conditions, Fe(III)-reducing microorganisms obtain energy for growth by oxidizing fermentable organic compounds to transform Fe(III) to Fe(II). The microbially generated Fe(II) species can then reabsorb to

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iron-bearing minerals and catalyze the degradation of various classes of contaminants, including nitroaromatic compounds, which are degraded to aniline (AN) through a series of intermediate products (Tobler et al., 2007). Organic substrate and NB contents, active ions in the groundwater, and the characteristics of aquifer media are the most important factors in the iron reduction-coupled NB degradation process.

The addition of fermentable organic substrates to an aquifer is one of the most common methods of enhancing *in situ* anaerobic bioremediation (Ellis et al., 2000). Emulsified vegetable oil (EVO) is favored due to its long chain molecular structure and low cost (US Air Force, 2007; Lindow, 2004; Tang et al., 2013a). EVO consists of food-grade soybean oil, surfactants, dissolved organic matter, and water blended to form a stable microemulsion with small and uniformly sized oil droplets (Ma, 2013; Tang et al., 2013b). Once injected into the subsurface, EVO can maintain long-term reducing conditions and provide a long-lasting carbon source and electron donor for bioremediation (Sheu et al., 2015). Recent laboratory and field studies have shown that injecting EVO into a subsurface can effectively enhance anaerobic bioremediation of chlorate, perchlorate (Borden et al., 2007), nitrate, chromate, AMD (acidminedrainage) (Lindow, 2004), chlorinated solvents (Sheu et al., 2015), and U(VI) (Tang et al., 2013b) in contaminated groundwater and sediment. Addition of edible oil can rapidly reduce contaminant concentrations in the aqueous phase by partitioning off a portion of the solvent mass into the edible oil (US Air Force, 2007).

Many studies have been conducted on NB degradation using zero valent iron (ZVI) or nano-scale zero-valent iron (NZVI) because of their fast and efficient action (Fu et al., 2014; Mueller et al., 2012); however, the poor mobility of NZVI, and especially ZVI, can cause the clogging of aquifers, and also might be toxic to indigenous bacteria and hinder their participation in the cleanup process (Chen et al., 2011). Overall, the addition of ZVI improves the remediation cost and increases the probability of secondary contamination. By comparison, the addition of EVO is economical, safe and can provide a long-lasting carbon source and electron donor for bioremediation (Dong et al., 2014). Biodegradation of the oil then stimulates anaerobic conditions and multiplies the indigenous microorganisms, including dissimilatory iron-reducing bacteria, which are ubiquitous in aquifers, and couple the oxidation of EVO with the reduction of Fe(III) in natural minerals in the aquifer media to generate Fe(II) species.

Relatively few studies as of now have investigated the dynamics of NB degradation coupled to microbial iron reduction in underground environments, especially regarding adding the electron donor EVO to stimulate the process. Many studies have, though, investigated iron reduction dynamics, which can be described by the Michaelis–Menten model, Monod model, or first-order kinetic equation. The kinetic parameters have proven to differ significantly between different experimental environments (Bonneville et al., 2004, 2006; Jaisi et al., 2007; Liu et al., 2001; McCormick et al., 2002; Ross et al., 2009), in which most kinetic models have used either pure culture microbes or synthetic iron mineral. Prior to now, no research project has estimated the EVO electron donor dose or remediation period in any actual contaminated site. An NB degradation kinetic model that closely reflects real-world conditions is urgently required in order to guide engineers at work remediating actual contaminated sites.

The present study investigated the kinetics of NB degradation using EVO as an electron donor to stimulate indigenous microorganism dissimilatory iron reduction by simulating an *in situ* underground environment. The research consisted of two parts: (1) developing the NB degradation dynamics model in the presence of excess electron donor (EVO) in the simulated underground system; and (2) investigating the major factors that affect NB degradation in order to refine the NB degradation model accordingly.

1. Materials and methods

1.1. Materials

The preparation of EVO: A homogenizer (HJ-6A, Jiangsu Jinyi Instruments Technology Co., China) was used to make an emulsion with commercial soybean oil, Tween-80 (analysis grade), yeast extract (Sinopharm Chemical Reagent Co., Ltd., China) and deionized water. The mass concentration of oil used was 10%. EVO was prepared by homogenizing for 24 hr at 3000 r/min.

NB solution was prepared by dissolving analytically pure nitrobenzene (Sinopharm Chemical Reagent Co., Ltd., China) in deionized water and mixing until uniform. The final concentration of NB was 40 mg/L.

Five types of sand with D_{50} of 0.04 mm, 0.23 mm, 0.42 mm, 0.61 mm, and 0.8 mm were used as aquifer media and were prepared by sieving and grading from commercial river sand.

The inorganic salts used were sodium sulfate (Na_2SO_4), sodium nitrate ($NaNO_3$), magnesium chloride ($MgCl_2$) and calcium chloride ($CaCl_2$). All the inorganic salts were obtained from Sinopharm Chemical Reagent Co., Ltd., China.

1.2. Experimental design

1.2.1. Verification of NB biodegradation coupled with dissimilatory iron reduction

Four gas-tight flasks (with 250 mL capacity) fitted with rubber stoppers were used as microcosms for the NB degradation simulations. Two-hundred grams sand (D_{50} = 0.23 mm) was placed in each of the four flasks as aquifer media, and the four flasks were filled with deionized water, NB (41.7 mg/L), EVO (18.18 g/L), EVO (18.18 g/L) and NB (40 mg/L), respectively. All of the flasks were incubated anaerobically in a light–tight constant temperature incubator (Shanghai Boxun Medical Biological Instrument Corp., China) at 30°C. Samples were taken once a day, and concentrations of NB, AN, total Fe, and Fe(III) were analyzed. Each experiment was replicated three times.

1.2.2. EVO addition stimulating NB biodegradation coupled with dissimilatory iron reduction

Four flasks with 200 g sand (D_{50} = 0.23 mm) as aquifer media, 220 mL liquid phase composed of NB 40.2 mg/L, Na_2SO_4 150 mg/L, $NaNO_3$ 5 mg/L, $MgCl_2$ + $CaCl_2$ 100 mg/L (molar ratio of $MgCl_2$:$CaCl_2$ = 1:1) and variable concentrations of EVO (0.18, 0.36, 3.63, 18.18 g/L) were incubated anaerobically in a light–tight constant temperature incubator at 30°C. Samples were taken once a day, and concentrations of NB,

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