# Mechanism on emulsified vegetable oil stimulating nitrobenzene degradation coupled with dissimilatory iron reduction in aquifer media

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GRAPHICAL ABSTRACT
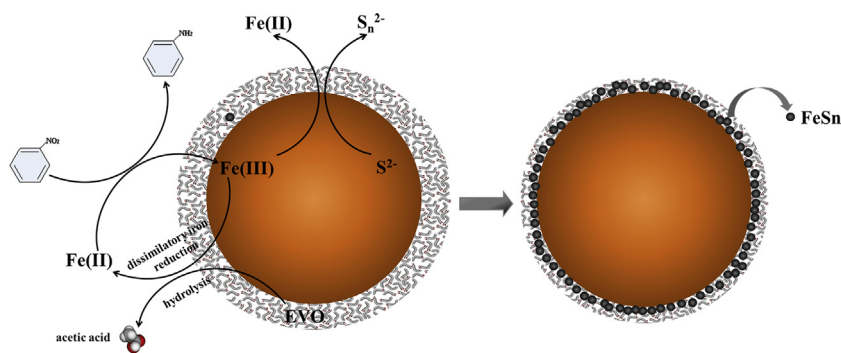
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ABSTRACT

Microbial dissimilatory iron reduction could remediate reducible pollutants in groundwater, such as nitrobenzene (NB). But the natural attenuation rate in aquifer is limited. To stimulate this process, emulsified vegetable oil (EVO) was injected as a remediation agent. The mechanism of this process was studied. Results showed that the addition of EVO made iron easier used by microorganisms and thus promoted dissimilatory iron reduction. The readily used $Fe(III)$ served as electron acceptor and was reduced to $Fe(II)$. $Fe(II)$ supplied electrons to NB, with NB reduced to aniline. Sulphide in the aquifer media also donated electrons and oxidized to polysulfide, then forming precipitates with $Fe(II)$ to the surface of aquifer media, and thus slowing down the electron supplying of EVO and forming permanent efficiency for NB remediation. The work helps to complete a systematic understanding of NB remediation process under stimulation of EVO.

1. Introduction

Nitrobenzene (NB) is one kind of highly toxic compounds and is widely used for the production of insecticides, dyes, feed stock, aniline, and other applications(Zhang et al., 2017). NB has been found as a common groundwater contaminant, and is listed as a priority pollutant by the US EPA for it is difficult to biodegrade and is harmful to natural environment and human health (Zhu et al., 2014). Many studies have shown that contaminant degradation coupled with dissimilatory iron reduction under the stimulation of some fermentable organic substrates is an effective remediation technology for contaminated groundwater and soil (Huang et al., 2015). Dissimilatory iron reduction is the

reduction of Fe(III), which is the most common form in aquifer (Schwertmann et al., 2000), using fermentable organic substrates as electron donors by indigenous microorganisms. In the process, electron donors (eg. glucose, acetate and ethanol, etc.) transfer electron to Fe (III) through microbial metabolism, and then the microbially generated Fe(II) was absorbed to the surface of aquifer medium to reduce contaminant, through a series of intermediate products (Lu et al., 2017), such as NB to aniline (AN) which is more readily to biodegrade. Some studies used acetate and lactate to stimulate the process, and results indicated the rapid depletion of the contaminant, but the resolubilization of the contaminant would reappear caused by depletion of acetate and lactate (Lloyd and Renshaw, 2005; Molins et al., 2015). Consequently, emulsified vegetable oil (EVO), a long-lasting fermentable organic substrates, having long chain molecular structure, was used as electron donor in our previous study to stimulate NB degradation coupled with dissimilatory iron reduction in aquifer media, and got a high remediation efficiency of 100% for NB contaminated aquifer in 12 days without resolubilization (Dong et al., 2017) .

In the process of NB degradation coupled with dissimilatory iron reduction, the actual electron donor is supposed to be microbially generated Fe(II) species (Lu et al., 2017), which were generated from indigenous Fe(III)-reducing microorganisms (such as *Shewanella, Geobacteraceae, Rhodopseudomonas*) using acetic acid, H^+ and hydrocarbons as substrates and transferring electron to the extracellular insoluble Fe(III) by the ferric-chelate reductase under anaerobic conditions (Ghorbanzadeh et al., 2014; Pierre et al., 2002). While not all the speciations of iron in aquifer could be used by indigenous microorganisms (Tessier et al., 1979). In fact, iron is divided into six fractions by sequential extraction technique: water soluble, exchangeable, bound to carbonates, bound to Fe-Mn oxides, bound to organic matter, and residual (Cai et al., 2018). Among of them, residual and bound to organic matter are known as unavailable forms, which exist in the crystal lattice of minerals in aquifers, and can hardly be used by microorganisms (Tessier et al., 1979). Water soluble and exchangeable fractions are easy to utilize, but the contents are very limited. Only bound to carbonates and bound to Fe-Mn oxides species of iron in minerals are effective fractions for the process of dissimilatory iron reduction (Tessier et al., 1979).

So far, few researches have focused on the iron speciation distribution in the contaminant remediation process coupled with dissimilatory iron reduction. While how to increase the content of bioavailable iron is the basic mechanism of NB degradation coupled with dissimilatory iron reduction stimulating by EVO. Therefore, the objective of the study was to study the process and give a systematic understanding of NB remediation process with EVO addition in aquifer.

2. Materials and methods

2.1. Materials

EVO was prepared with commercial soybean oil, tween-80 and yeast extract with a homogenizer at 3000 rpm for 24 h, with the mass concentrations of 10%, 2% and 0.1% respectively. NB solution was prepared by dissolving nitrobenzene in water and mixing until uniform, and the theoretical concentration was 100 mg/L. All the chemicals used in this study were of analytical grade, and all aqueous solutions were prepared with deionized water. The fine sand with particle sizes of 0.1 ~ 0.25 mm were used as aquifer media and were obtained by sieving and grading from commercial river sand.

2.2. Experimental design

Seven head space bottles (with 500 mL capacity) were used as microcosms, and were placed with 600 g fine sand as aquifer media, 350 mL NB solution (100 mg/L) as polluted groundwater. The sand and solution were mixed thoroughly for 24 h in a light-tight constant

temperature incubator at 30 °C to reach absorption equilibrium as other studies (Long and Borden, 2006; Wen et al., 2017; Zhao et al., 2016). After 24 h, 7 mL EVO were added to each bottle, to reach a concentration of 20 g/L in the system. The bottles were incubated anaerobically in the incubator at 30 °C. The concentrations of NB, AN, total Fe, Fe(III), pH, oxidation-reduction potential (ORP) in the solution, and the speciation distribution of iron in the aquifer media were analyzed at regular time (1 d, 3 d, 5 d, 12 d, 22 d, 32 d, 42 d, 52 d and 62 d). Each bottle was used once to keep the anaerobic reduction environment. Each experiment was replicated for three times.

2.3. Analytical methods

The liquid samples were extracted with injector and filtered using 0.22 μm filter before analyzing concentrations of Fe(III), Fe(II), NB and AN. The solid samples were extracted by HCl (V/V = 1:1) for 24 h, and the extract liquid was analyzed for the concentrations of Fe(III) and Fe (II).

Fe(III) and total iron concentrations were determined by the thiocyanate colorimetric assay method at 480 nm (Dong et al., 2017), and the concentration of Fe(II) was obtained by subtracting the concentration of Fe(III) from the total iron. NB and AN were measured by high-performance liquid chromatography (HPLC) (Agilent 1260, USA) using C18 column (150 mm × 4.6 mm, Agilent) maintained at 25 °C. The mobile phase consisted of acetonitrile and acetic acid-ammonium acetate buffer solution (65:35%) at a flow rate of 1.0 mL/min. NB and AN were detected at 262 nm and 280/340 nm (λex/λem) respectively. The composition and chemical states of iron and sulfur on the aquifer media surface were characterized by X-ray photoelectron spectroscopy (XPS), using a Thermo ESCALAB 250 X-ray photoelectron spectrometer employing a monochromated Al KαX-ray source (hm = 1486.6 eV), and the charge shifted spectra were corrected using the adventitious C 1 s photoelectron signal at 285 eV.

The speciation distribution of iron in the aquifer media were analyzed based on Tessier sequential chemical extraction procedure (Tessier et al., 1979). The extracting solutions for irons of water soluble, exchangeable, bound to carbonates, bound to Fe-Mn oxides, bound to organic matter, and residual were distilled water, magnesium chloride solution (1 mol L^{-1}), sodium acetate solution (1 mol L^{-1}, pH = 5), hydroxylamine hydrochloride-hydrochloric acid solution (1:1, mol/ mol) (0.25 mol L^{-1},), ammonium acetate-nitric acid solution (1:1, mol/ mol) (3.2 mol L^{-1}), and nitric acid-hydrochloric acid-hydrofluoric acid (9:3:1, v/v), respectively. The extracting solutions were separated from the solid phase after each stage by centrifugation at 3000 rpm for 20 min, and the supernatant was stored for later analysis.

3. Results and discussion

3.1. Effect of EVO addition on the physicochemical property and iron speciation distribution in aquifer media

The pH value in the system has a decreasing trend during the experiment as shown in Fig. 1(a), because of the linear long-chain aliphatic compounds in the EVO degraded to low-molecular-weight organic acids (Watson et al., 2013). But pH drops less than 1, which indicated that aquifer media in the system has large buffer capacity and can maintain a mild pH level. The addition of EVO would not cause seriously pH levels change in the subsurface environment and thus would not affect the in situ reactions. ORP in the system dropped to below −100 mV within 12 days and maintained during the experimental period as shown in Fig. 1(b). The result shows that EVO can quickly deplete the oxidizing substances in the underground environment and can provide a steady anaerobic reduction environment after injecting (Borden et al., 2006), which is benefit for NB reduction.

Fe (II) in the system began to accumulate at the 22nd day and the maximum concentration reached 54.3 mg/L within 52 days (Fig. 1(c)).