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Kinetics of BTEX biodegradation coupled with Fe(III) reduction by indigenous microorganisms in simulated underground environment

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ABSTRACT

Pressure on widespread contamination of BTEX (benzene, toluene, ethylbenzene, and xylene) in sediments and groundwater requires better understanding of biogeochemical removal process of the pollutant. Oxidation of BTEX coupled with reduction of Fe(III) is one of the most efficient ways. BTEX degradation dynamics was investigated by observing BTEX oxidation rates at different concentrations (from 50 to 200 mg/L) at the presence of Fe (III) (from 106 to 212 mg/L). The dual-substrate Michaelis–Menten model was derived ($V_{max} = 10.14$ mg/L d, $K_{m,Fe(III)} = 20.02$ mg/L, $K_{m,BTEX} = 128.75$ mg/L). Experiment data agreed well with the model when the initial BTEX concentration was under 100 mg/L, which indicated that microorganisms in the system could keep balance. With the increasing of initial BTEX concentration, oxidation rates did not fit the model, indicating that high BTEX concentration would be toxic to the function microorganisms and would dampened the BTEX oxidation reaction. The dynamics of BTEX with different compositions (the B:T:E:X ratios) coupled to the dissimilatory Fe(III) reduction in contaminated underground environment was also modified. The proposal is to remedy contaminated groundwater by using Fe(III) in aquifer material instead of other substances, so that the operating conditions do not destruct ecological environment of groundwater. These findings provide important parameters for the remediation of BTEX in sediments and groundwater coupled with Fe(III) reduction.

Keywords: Underground; BTEX degradation; Dual-substrate Michaelis–Menten model; Fe(III) reduction

1. Introduction

Benzene, toluene, ethylbenzene, and xylene (BTEX) which are a group of monoaromatic petroleum-derived pollutants are widespread contaminations occurred in sediments and groundwater due to the leaking of underground fuel storage tanks and petroleum products spills [1,2]. Thus, BTEX pollutant in underground environment especially in groundwater requires urgent remediation. Biogeochemical removal of organic pollutants including aromatic compounds has been investigated in different redox zones of aquifers, and attenuation was observed in the iron-reducing zone [3–5]. Fe(III) was thus proved to be an important electron acceptor for BTEX oxidation.

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Bioremediation of BTEX coupled with Fe(III) reduction is an important process for the removal of BTEX in groundwater and sediments.

Function microorganisms for iron reduction process in iron-reducing zone obtain energy for growth from oxidizing organic compounds such as ammonia compounds, aromatic hydrocarbon, heavy metal, and BTEX to transfer Fe(III) to Fe(II) [4,6–13]. An important factor influencing function microorganisms activity is the content of BTEX and Fe(III). Many studies investigated iron reduction dynamics, which can be described by Michaelis–Menten model or first-order kinetics equation, but the kinetics parameters were found significantly different with varying experiment environments [13–18]. And dynamics of BTEX in underground environment was not reported.

Considering bioremediation of BTEX coupled with Fe(III) reduction in iron-reducing zone of underground environment, the sole substrate BTEX or Fe (III) oxidation or reduction kinetics do not fit for the real groundwater condition. Therefore, the current study aims to investigate the substrate kinetics of BTEX oxidation at different BTEX and Fe(III) concentrations by simulating *in situ* underground environment. A dual-substrate model for BTEX bioremediation dynamics in the simulated underground system is developed, and the major factors that affect BTEX oxidation are investigated. Critical concentration of BTEX that could be biodegraded is inferred.

2. Materials and methods

2.1. Preparation of substrates

Goethite (α-FeOOH) and akaganeite (β-FeOOH) were synthesized by hydrolyzing $FeCl_3{\cdot}6H_2O$ according to procedures introduced by Schwertmann and Cornell [19]. Following preparation, the goethite and akaganeite were dialyzed against deionized H_2O to remove soluble salts.

The function microorganisms were isolated from BTEX-contaminated aquifer previously under anaerobic condition. The details of culturing procedures and function microorganisms harvest were described in detail in reference [20]. The composition of culture solution for indigenous function microorganisms is NaCl 0.1 g/L, $CaCl_2{\cdot}2H_2O$ 0.2 g/L, $NaHCO_3$ 2.5 g/L, NH_4Cl 1.5 g/L, and KH_2PO_4 1 g/L. Citric acid was added to the culture solution (20.82 mmol/L) serving as electron donor. After incubation, the indigenous function microorganisms were added into each microcosm.

2.2. Experimental design

Gas-tight flasks (with 120 mL capacity) fitted with rubber septa were used as microcosms for the BTEX degradation simulations. Function microorganisms mixing with variable concentrations of iron hydroxides (106 and 212 mg/L) and BTEX (50, 100, 150, and 200 mg/L) (molar ratio of benzene: toluene: ethylbenzene: xylene = 1:1:1:1) were placed in separated flasks and incubated anaerobically with continuous shaking, and the experimental design is shown in Table 1. Samples were taken at selected time point, and concentrations of BTEX, total Fe, and Fe(III) were analyzed.

2.3. Analytical methods

BTEX headspace concentrations were quantified by comparing with headspace concentrations of anaerobic aqueous solutions of single BTEX compounds prepared and stored under the same conditions as the microcosms [11]. Fe(III) and Fe(II) concentrations were determined according to water quality determination of iron-phenanthroline spectrophotometry described in Environmental Protection Standard of China [21]. Total microbial activity was measured by fluorescein diacetate (FDA) hydrolysis. FDA can be hydrolyzed by both free and membrane-bound enzymes releasing a colored end product, fluorescein which absorbs strongly in the visible wavelength (490 nm) and can be measured by spectrophotometry [22,23].

3. Results and discussion

3.1. BTEX-Fe(III) dual-substrate model for BTEX degradation

The biochemical reaction rate is usually expressed by the Michaelis–Menten equation:

$$V = \frac{V_{\max}[S]}{[S] + K_m} \tag{1}$$

where V is the substrate degradation rate (mg/L d), $V_{\max}$ is the maximum substrate degradation rate (mg/

Table 1
Experimental design (microcosms)

	BTEX (mg/L)			
Fe(III) (mg/L)	50	100	150	200
106	M1	M2	M3	M4
212	N1	N2	N3	N4

L d), [S] is the substrate concentration (mg/L), and K_m is the apparent half-saturation constant (mg/L).

BTEX degradation has both substrates of BTEX and Fe(III). Thus, in principle, both substrates could be the limiting factors. A BTEX-Fe(III) dual-substrate equation deduced from the Michaelis–Menten equation is therefore proposed.

$$V = V_{\max} \frac{[S_{\mathrm{Fe(III)}}]}{[S_{\mathrm{Fe(III)}}] + K_{m,\mathrm{Fe(III)}}} \times \frac{[S_{\mathrm{BTEX}}]}{[S_{\mathrm{BTEX}}] + K_{m,\mathrm{BTEX}}} \tag{2}$$

where $[S_{\mathrm{BTEX}}]$ is the BTEX concentration (mg/L), $[S_{\mathrm{Fe(III)}}]$ is the Fe(III) concentration (mg/L), and $K_{m,\mathrm{BTEX}}$, and $K_{m,\mathrm{Fe(III)}}$ are the apparent half-saturation constants (mg/L) of BTEX and Fe(III), respectively.

For each given Fe(III) concentration, where $[S_{\mathrm{Fe(III)}}]$ is set as a constant, the above equation can be converted into the following:

$$\frac{1}{V} = \frac{[S_{\mathrm{Fe(III)}}] + K_{m,\mathrm{Fe(III)}}}{[S_{\mathrm{Fe(III)}}] V_{\max}} \cdot K_{m,\mathrm{BTEX}} \times \frac{1}{[S_{\mathrm{BTEX}}]} + \frac{[S_{\mathrm{Fe(III)}}] + K_{m,\mathrm{Fe(III)}}}{[S_{\mathrm{Fe(III)}}] V_{\max}} \tag{3}$$

Therefore, the relationship between $1/V$ and $1/[S_{\mathrm{BTEX}}]$ can be simplified as:

$$y = ax + b \tag{4}$$

Here, $y = 1/V$ denotes the reverse of the BTEX degradation rate, which can also be expressed as $1/V_{\mathrm{BTEX}}$, $x = 1/[S_{\mathrm{BTEX}}]$ denotes the reverse of the BTEX concentration at reaction time t, $a = \frac{[S_{\mathrm{Fe(III)}}] + K_{m,\mathrm{Fe(III)}}}{[S_{\mathrm{Fe(III)}}] V_{\max}} \cdot K_{m,\mathrm{BTEX}}$, which stands for the slope of the y–x line graph, and $b = \frac{[S_{\mathrm{Fe(III)}}] + K_{m,\mathrm{Fe(III)}}}{[S_{\mathrm{Fe(III)}}] V_{\max}}$, which stands for the intercept of the graph.

According to the slope and intercept on the line $1/V_{\mathrm{BTEX}} - 1/[S_{\mathrm{BTEX}}]$ graph based on the experimental data at the initial Fe(III) concentration 106 mg/L, as shown in Fig. 1, $K_{m,\mathrm{Fe(III)}}$, $K_{m,\mathrm{BTEX}}$, $V_{\max}$ were calculated. The experimental data showed a good agreement with Eq. (4) with correlation coefficients $R^2 >$ 0.98. Then Eq. (2) could be converted into a BTEX-Fe (III) dual substrates equation as shown below:

$$V = 10.14\,\mathrm{mg/L\ d} \times \frac{[S_{\mathrm{Fe(III)}}]}{[S_{\mathrm{Fe(III)}}] + 20.02\,\mathrm{mg/L}} \times \frac{[S_{\mathrm{BTEX}}]}{[S_{\mathrm{BTEX}}] + 128.75\,\mathrm{mg/L}} \tag{5}$$

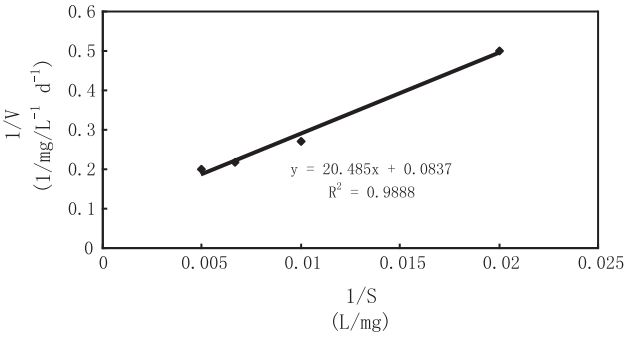

Fig. 1. Correlation of $1/V_{\mathrm{BTEX}}$ (reversed BTEX oxidation rate) and $1/[S_{\mathrm{BTEX}}]$ (reversed BTEX concentration) at Fe(III) concentration of 106 mg/L.

Eq. (5) could be simplified under certain conditions. For instant, under a low BTEX concentration (BTEX < 128.75 mg/L) and a high Fe(III) concentration (Fe(III) > 20.02 mg/L) (Fe(III) addition was used as electron-transfer agents at light BTEX polluted underground site), the equation could be converted into:

$$V = 10.14\,\mathrm{mg/L\ d} \times \frac{[S_{\mathrm{BTEX}}]}{128.75\,\mathrm{mg/L}} = 0.079 \times [S_{\mathrm{BTEX}}]\,\mathrm{mg/L\ d} \tag{6}$$

This equation indicates that the BTEX degradation process follows the first-order kinetic law under this condition and is in accordance with those of McCormick, Liu, and Roden's studies [13,14,18].

Whereas at high BTEX concentrations as in a BTEX-contaminated underground environment, and no additional Fe(III), that is low Fe(III) concentration, the equation could be expressed as:

$$V = 0.51\,\mathrm{mg/L}[S_{\mathrm{Fe(III)}}] \times \frac{[S_{\mathrm{BTEX}}]}{[S_{\mathrm{BTEX}}] + 128.75\,\mathrm{mg/L}} \tag{7}$$

This equation confirmed the result of Bonneville who implied the process following Michaelis–Menten kinetics [16,17].

Nanh Lovanh et al. investigated the effect of ethanol on BTEX biodegradation kinetics, and dual-substrate utilization (e.g. benzene and ethanol) was modeled satisfactorily under carbon-limiting conditions [24]. Jun Dong et al. investigated oxidation of aromatic hydrocarbons (e.g. BTEX) coupled with bacterial reduction of iron and indicated that the process followed first-order kinetics [25]. Ivana Ribeiro de Nardi et al. investigated kinetics of BTEX degradation, and found that a first-order kinetic model fitted

the experimental data well, showing correlation coefficients higher than 0.994 [26]. Although most researchers modeled the process with first-order kinetics, it is undeniable that Fe(III) and additional carbon sources have important effect on BTEX degradation.

In underground system with high concentration BTEX pollution of bioremediation using additional Fe (III) as electron acceptor, both BTEX and Fe(III) are high and vary over time. Thus, none of the simplified models could be used. Therefore, the dynamics of BTEX-Fe(III) dual-substrates model would be more fitting.

3.2. BTEX-Fe(III) dual-substrate model verification

The above BTEX-Fe(III) dual-substrate model was verified in the experiment.

Fig. 2 show the variations of substrates concentrations in simulated microcosms with a fixed Fe(III) concentration of 106 mg/L at different BTEX concentrations ranging from 50 to 200 mg/L. The experiment results showed that, the BTEX and Fe(III) concentrations decreased with time and product of Fe(II) increased, which confirmed that function microorganisms oxidized BTEX in the presence of Fe(III) as the electron acceptor. With the initial BTEX concentration increased from 50 to 100 mg/L, as shown in Fig. 2(A) and (B), the final concentrations of Fe(II) increased from 36 to 52 mg/L, but with the BTEX concentration increased from 100 to 150 and then to 200 mg/L, as shown in Fig. 2(C) and (D), the final concentrations of Fe(II) decreased from 52 to 43 mg/L and then 16 mg/L, which indicated function microorganisms dying out because of enhanced BTEX toxicity.

Fig. 3 shows the comparison of the measured and simulated BTEX concentrations at different initial BTEX concentrations from 50 to 200 mg/L at the initial Fe(III) concentration of 106 mg/L. The dual Michaelis–Menten model was found to agree well with the experimental data when the initial BTEX concentrations were 50 and 100 mg/L, as shown in Fig. 3(A) and (B). The phenomenon showed that function microorganisms in the system could adapt concentration of BTEX below 100 mg/L. However, when the initial BTEX concentrations were raised to 150 and 200 mg/L, as shown in Fig. 3(C) and (D), the dual Michaelis–Menten model did not fit for the experimental data. The phenomenon showed that function microorganisms would not be tolerant when the BTEX concentrations were higher than 100 mg/L, which was in accordance with Fig. 2(C) and (D).

The other microcosms had the same trend (data not shown). The results confirm that critical concentration of BTEX in underground environment of bioremediation is 100 mg/L.

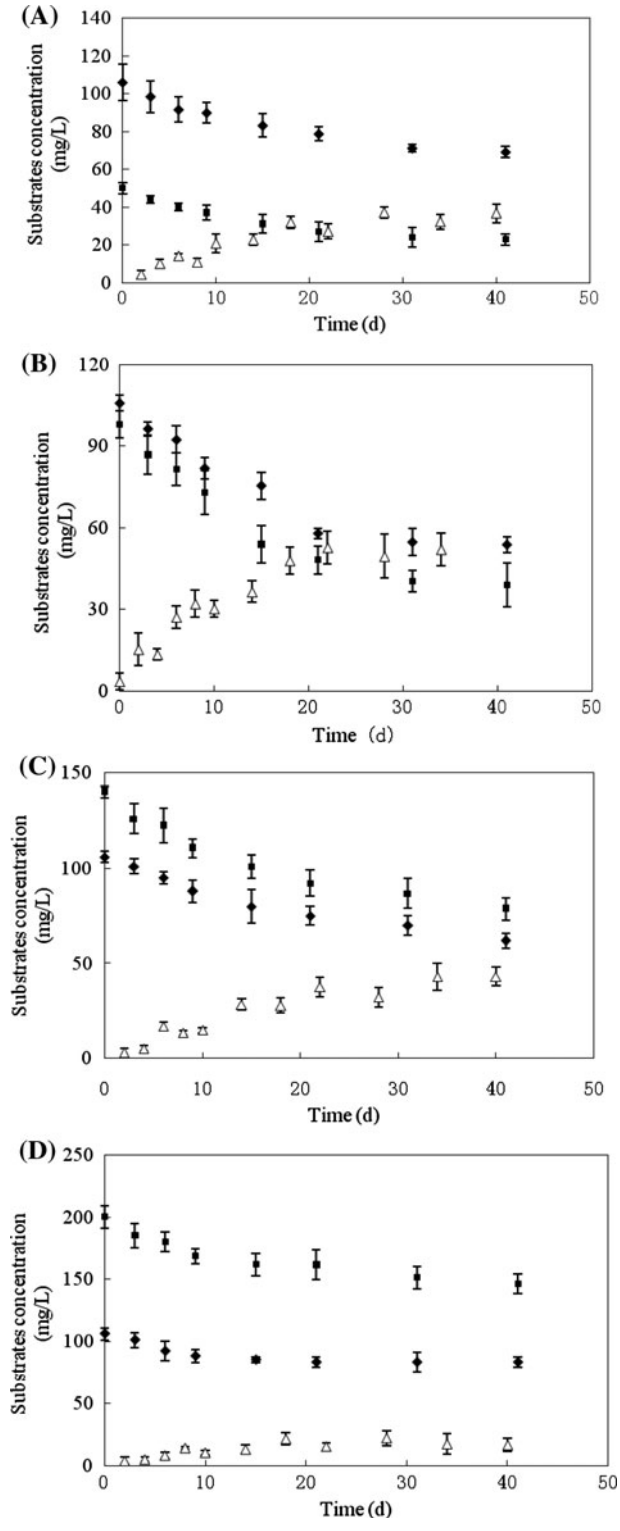

Fig. 2. Variations of substrates concentrations in microcosms at initial BTEX concentrations of 50, 100, 150, and 200 mg/L with an initial Fe(III) concentration of 106 mg/L. (A) Microcosm M1, (B) microcosm M2, (C) microcosm M3, and (D) microcosm M4. (■) BTEX, (♦) Fe(III), (Δ) Fe(II).

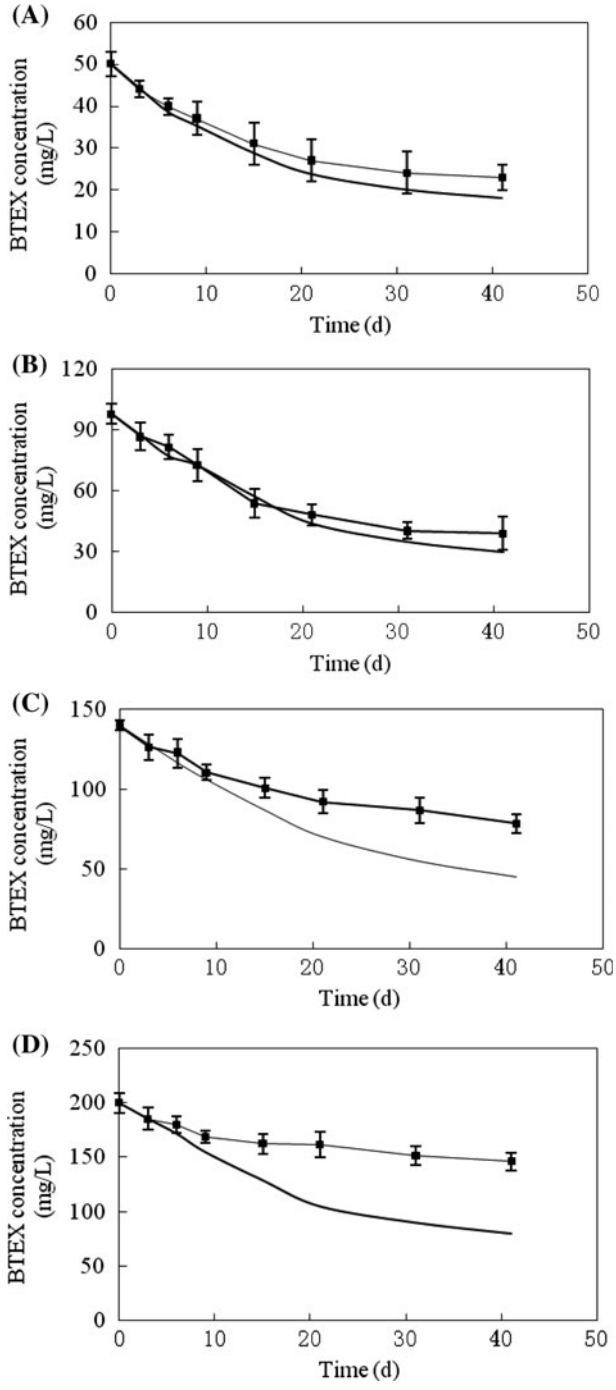

Fig. 3. Comparison of the experimental data and model simulation (initial Fe(III) concentrations of 106 mg/L). BTEX concentrations of 50 mg/L (A), 100 mg/L (B), 150 mg/L (C), and 200 mg/L (D). (■) experimental data (–) simulation data.

Several studies have reported that high BTEX concentrations may inhibit bioremediation [27,28], which is confirmed in this study. Lin reported that BTEX could be degraded when applied BTEX concentrations <114 mg/L in PVA-immobilized degrader [29] and <120 mg/L in immobilized cell beads [30]. While in our study, the tolerance level of BTEX that could be biodegraded in underground environment is about 100 mg/L. The critical concentration is little lower, which is mainly because the immobilized substances contained a high biomass density of immobilized BTEX degraders than in underground environment of our study.

3.3. Effect of aromatic compounds ratios on BTEX oxidation

The stoichiometric reaction for the complete oxidation of monoaromatic hydrocarbons coupled to Fe(III) oxides reduction can be described as the following: Reactions (8)–(10) [1,11,19,31]:

$$C_6H_6 + 30Fe(III) + 12H_2O \rightarrow 30Fe(II) + 6CO_2 + 30H^+ \quad (8)$$

$$C_7H_8 + 36Fe(III) + 21H_2O \rightarrow 7HCO_3^- + 36Fe(II) + 43H^+ \quad (9)$$

$$C_8H_{10} + 42Fe(III) + 16H_2O \rightarrow 42Fe(II) + 8CO_2 + 42H^+ \quad (10)$$

From stoichiometric reactions (8)–(10), degradation of BTEX has a relationship with C number in the molecule. Benzene, toluene, ethylbenzene, and xylene degradation graphs are shown in Fig. 4. The changing trends of the four curves were similar. Removal efficiencies of benzene, toluene, ethylbenzene, and xylene were 28, 36, 45, and 46%, respectively, at total BTEX concentration of 50 mg/L, and were 46, 54, 59, and 60%, respectively, at total BTEX concentration of 100 mg/L. It confirmed that, in the same condition, BTEX degradation ratio has a positive correlation with number of C in BTEX molecule.

BTEX degration concentrations via average C number in BTEX are shown in Fig. 5. Both the slopes of the two lines (total BTEX concentration of 50 and 100 mg/L, respectively) were 1.01 mg/L. That is to say, degradation of BTEX would increase 1.01 mg/L when the average C in BTEX increases by 1. In the study, ratio of benzene, toluene, ethylbenzene and xylene was 1:1:1:1, so the average number of C in BTEX is 7.25. On the assumption that the number of C in BTEX in the contaminated underground environment is m, then based on the lines slope in Fig. 5, Eq. (11) was derived.

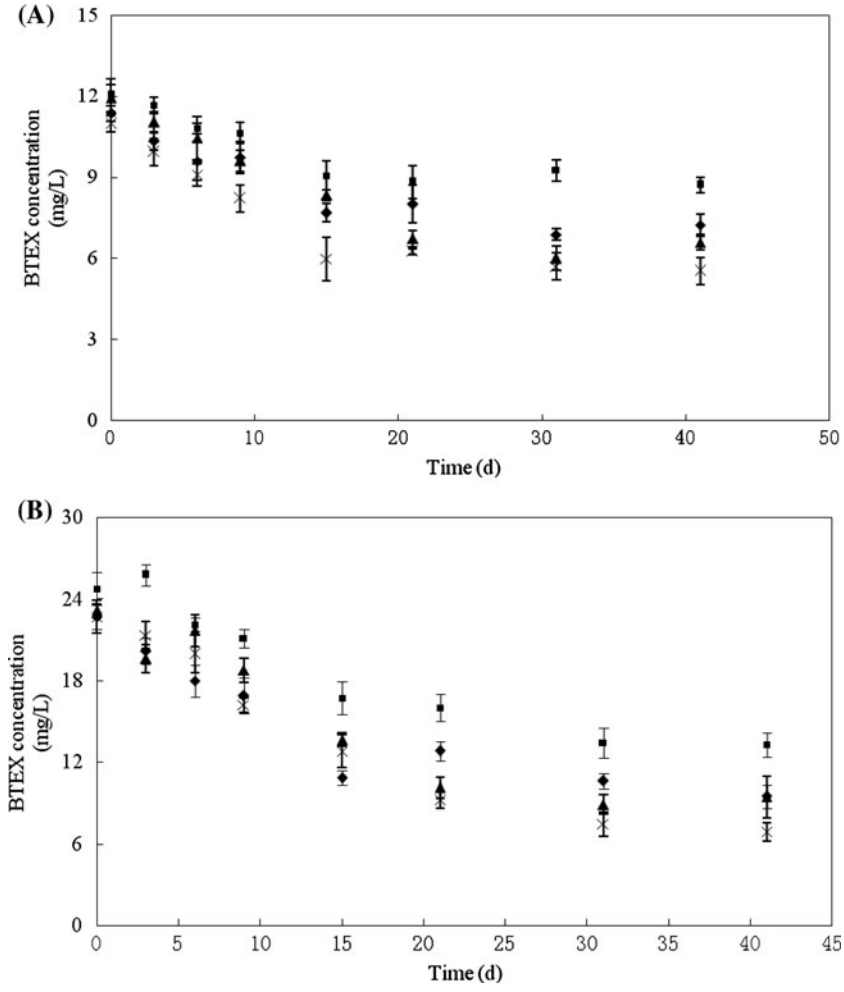

Fig. 4. Benzene, toluene, ethylbenzene, and xylene degradation graphs in the microcosms. (A) Total BTEX concentration of 50 mg/L. (B) Total BTEX concentration of 100 mg/L. (■) Benzene, (♦) toluene, (Δ) ethylbenzene, (×) xylene.

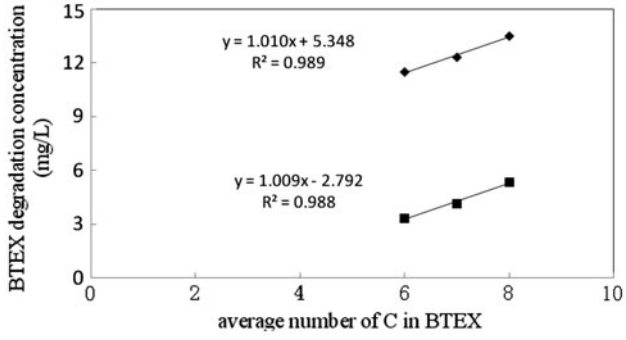

Fig. 5. Experimental data of BTEX degration concentrations via average C number in BTEX. (■) total BTEX concentation of 50 mg/L. (♦) total BTEX concentration of 100 mg/L.

$$\frac{V_m - V_{7.25}}{m - 7.25} = 1.01\ \text{mg/L d} \tag{11}$$

That is,

$$V_m = 10.14\ \text{mg/L d} \times \frac{[S_{\text{Fe(III)}}]}{[S_{\text{Fe(III)}}] + 20.02\ \text{mg/L}} \times \frac{[S_{\text{BTEX}}]}{[S_{\text{BTEX}}] + 128.75\ \text{mg/L}} + 1.01\ \text{mg/L d} \times (m - 7.25) \tag{12}$$

From the above results, a modified model of BTEX dynamics coupled to the dissimilatory Fe(III) reduction in contaminated underground environment was derived.

Though there are some researches on BTEX biodegradation, most of which on fixed BTEX compositions (e.g. B:T:E:X = 1:1:1:1) [29,30,32,33]. While in contaminated sediments and groundwater systems, BTEX compositions (the B:T:E:X ratios) vary greatly, e.g. the relative proportion of BTEX compounds [B:T:E:X] was found to vary systematically in groundwater samples collected at a gasoline-contaminated site [34]. Therefore, the modified model as shown in Eq. (12) could be used more widely.

4. Conclusion

This study verified the BTEX biodegradation coupled with Fe(III) reduction by indigenous microorganisms. A BTEX-Fe(III) dual-substrate equation was built. The study also showed that if the concentration of BTEX in subsurface environment was less than100 mg/L, BTEX biodegradation coupled with Fe(III) reduction could be used. Thus, from the study, it can be concluded that Fe(III) is apt in the remediation of contaminated groundwater in aquifer material, instead of introducing other substances which may also be contaminants of groundwater, so that the operating conditions do not destruct ecological environment of groundwater. Hence, this would be very economically feasible.

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