

## Interactions Between BTEX, TPH, and TCE During Their bio-removal from the Artificially Contaminated Water

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**Abstract**— Many environmental sites are becoming contaminated by mixed wastes, including such organic compounds as BTEX (benzene, toluene, ethylbenzene, and three isomers of xylene), TPH (total petroleum hydrocarbons), and CAHs (chlorinated aliphatic hydrocarbons) including TCE (trichloroethylene). TCE, as a representative CAH, has been widely used in various industrial processes and is among the most prevalent hazardous organic compounds present in the environment. In this study, a microbial pure culture enriched and isolated from a heavily oil-contaminated site in Xiamen, China, was used to remove mixtures of BTEX, TPH, and TCE under different pHs (5, 7, and 9) at 25°C, from the artificially contaminated water and to assess the interactions between those compounds during their bio-removal. The mixtures of BTEX (BTE<sub>o</sub>X, BTE<sub>m</sub>X, and BTE<sub>p</sub>X) and TPH showed different trends when TCE added to the mixture. TCE showed an inhibitory effect on bio-removal of *o*-xylene in BTE<sub>o</sub>X mixture, while it showed a stimulatory effect on the overall removal efficiencies for BTE<sub>m</sub>X and BTE<sub>p</sub>X mixtures. On the other hand, TCE was the most efficiently removed when mixed with BTE<sub>o</sub>X and TPH, except at pH 5, while less removed in other mixtures at almost similar amounts, regardless of pH. The highest removal of BTEX and TPH occurred at pH 7 or 9, while the highest removal of TCE occurred at pH 7, regardless of mixtures.

**Keywords** – BTEX; interaction; mixture; pH; TCE; TPH

### I. INTRODUCTION

Due to the industrialization, petroleum production is not only the primary source of fuel but also the contamination source of soil and groundwater [1]. Besides of basic usage of these productions, lots of them are lost by leakage. Major causes of crude oil-contaminated soil include leaking storage tanks and pipelines, land disposal of petroleum waste, and accidental or intentional spills [2]. There are many compounds in petroleum. Benzene, toluene, ethylbenzene, and three isomers (*ortho*-, *meta*-, and *para*-) of xylene, collectively known as BTEX, are among these compounds, which are also widely used as industrial solvents for organic synthesis and equipment cleansing [3]. Trichloroethylene (TCE) is widely used in various industrial processes, such as industrial dry cleaning, textile manufacturing, etc. It is among the most prevalent hazardous organic compounds

present in the environment [4]. TCE is also carcinogenic and would cause other serious health effects on humans [5].

There have been lots of studies focused on removing these contaminants. Among all the current technologies, biological treatment is regarded as the most economical and environmentally sound approach [6]. However, no microorganism capable of growing on TCE as a sole carbon or energy source has yet been isolated [7]. Instead, several anaerobic and aerobic bacteria are known to degrade TCE by the co-metabolic transformation. Hence, a non-growth-supporting substrate is transformed through the catalysis of non-specific enzymes synthesized by bacteria in the presence of a growth substrate [8].

Many studies have proved that TCE has an inhibitory effect to other compounds during the bio-removal process. TCE significantly inhibited phenol degradation during the degradation of phenol and trichloroethylene by *Pseudomonas cepacia* G4 [9]. TCE was regarded an inhibitor to 1,1-dichloroethylene bio-removal [10] and also showed an inhibitory effect to isopropyl alcohol [11]. On the other hand, the efficacy of TCE biodegradation varies with different compounds under different conditions. The combination of BTEX, TPH, and TCE can be frequently found in the environment.

The efficacy of biodegradation is also influenced by a number of factors including bioavailability, quality and quantity of contaminants, temperature, pH, and oxygen. These factors are essential for formulating successful bioremediation strategies [8]. In this study, one indigenous microorganism isolated from a heavily oil-contaminated site in Xiamen, China was used to remove mixtures of BTE<sub>o</sub>m/pX (350 mg/L), TPH (1,000 mg/L), and TCE (15 mg/L) under three different pH values (5, 7, and 9) at 25°C, from the synthetic contaminated water. Indigenous microorganisms were first enriched and isolated from the contaminated soil. The interaction between BTEX, TPH, and TCE during their bio-removal from the artificially contaminated water under different pH conditions was evaluated. Results would help find out the interactions and the most appropriate combination of contaminants and environmental conditions when the bioremediation technology applied to the mixed wastes contaminated sites.

## II. MATERIALS AND METHODS

### A. Chemicals

Benzene (purity, 99.7%), toluene (purity, 99%), ethylbenzene (purity, 99%), *ortho*-xylene (purity, 99%), *meta*-xylene (purity, 99%), and *para*-xylene (purity, 99%) were purchased from the International Laboratory (USA). TCE (purity, 99%) was purchased from Da Mao Chemical Manufacture in Tianjin, China. TPH were purchased from the Caltex Company in Macau Special Administrative Region (SAR), China, and the TPH stock solution was prepared using the 1:1 ratio (v/v) of unleaded gasoline and diesel mixed with [dimethylformamide](#) (DMF).

### B. Soil sample collection

Indigenous microorganisms were isolated from the soil samples, potentially contaminated with petroleum compounds and obtained from a site near a gas station in Xiamen, China. Soil samples were stored in a freezer until use.

### C. Enrichment and isolation

The microbial pure cultures were enriched and isolated from the soil sample. Soil sample (5%, w/w) was first added into the nutrient broth (contained 3.0 g/L beef extract and 5.0 g/L peptone) in a serum bottle, and then 150 mg/L of toluene was added as a substrate for the microbial growth. After the serum bottles were covered with stoppers (90% teflon/10% silicone) and aluminum crimp sealed, they were inverted and placed on an orbital shaker (IKA) at 150 rpm and 25°C. Then, 10% (v/v) inocula from these bottles were aseptically inoculated into the mineral salts medium (MSM contained  $\text{KH}_2\text{PO}_4$  1.0 g/L;  $\text{K}_2\text{HPO}_4$  1.0 g/L;  $\text{NH}_4\text{NO}_3$  1.0 g/L;  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$  0.2 g/L;  $\text{Fe}_2(\text{SO}_4)_3$  0.5 g/L; and  $\text{CaCO}_3$  0.02 g/L) containing 150 mg/L of toluene as a sole substrate. After several weeks of subculturing, pure cultures were isolated from the bottles by using nutrient agar (NA) plates. The pH of medium was adjusted by adding  $\text{HNO}_3$  (1.0 mol/L) or  $\text{NaOH}$  (1.0 mol/L) solution. All the apparatus and media were autoclaved (HIRAYAMA) for 20 min at 121°C, under 15 psi (103.5 kPa) in advance.

Several microbial colonies with different morphologies were chosen from the NA plates and aseptically transferred to test tubes containing MSM with 150 mg/L of toluene. After the tubes were incubated on the shaker at 150 rpm and 25°C, one microbial pure culture from the soil sample, which showed higher turbidities by measuring optical density (OD) at 600 nm and higher toluene removal efficiencies, was chosen for further experiments. The selected pure culture was further transferred, 10% (v/v), into the newly prepared MSM containing mixtures of BTEX and TPH.

### D. Analytical methods

The concentrations of BTEX, TPH, and TCE were measured using a gas chromatograph (Agilent, 6890N, Agilent Technologies Co., Ltd, China) equipped with a flame ionization detector (FID) and a capillary column (HP-5; 30 m  $\times$  0.53  $\mu\text{m}$  I.D. with a stationary-phase film thickness of

0.88  $\mu\text{m}$ ). One microliter of liquid samples was injected by the autosampler injector (7638 Series, Agilent Technologies Co., Ltd, China) equipped with a tapered microsyringe (5181-1267, Hamilton Company, USA). Nitrogen was used as a carrier gas. The inlet and detector temperatures were 280°C and 300°C, respectively. The column temperature was programmed as initial temperature, 40°C (hold for 2 min), then incrementally increased at 12°C/min to 300°C (hold for 10 min).

### E. Bio-removal of mixtures

After several weeks of subculturing, the pure cultures (10%, v/v) were added into the fresh MSM which contained different concentrations of BTEo/m/pX/TPH/TCE mixtures at different pH values (5, 7, and 9) at 25°C.

After the serum bottles were sealed with stoppers and aluminum crimps, they were inverted to minimize the volatilization of these compounds, then incubated on the shaker at 150 rpm. Bottles containing MSM with these compounds but without microorganisms served as controls. Sample aliquots of 2 mL were periodically withdrawn from the bottles and analyzed for the concentrations of BTEX, TPH, and TCE.

## III. RESULTS AND DISCUSSION

The experimental results for the interactions between BTEX (350 mg/L for BTEo/m/pX), TPH (1,000 mg/L), and TCE (15 mg/L) in the artificially contaminated water at different pH values at 25°C are shown below.

### A. Removal of BTEoX and TPH

The removal efficiencies for benzene, toluene, ethylbenzene, *ortho*-xylene, and TPH in BTEoX/TPH mixture without and with TCE after 144 hours of incubation are shown in Figure 1 and 2, respectively. For the mixture without TCE, the highest total removal efficiencies for BTEoX mixture and TPH were 84.7% at pH 7 and 63.7% at pH 9, respectively, and the lowest total removal efficiencies were 29.1% at pH 5 and 34.5% at pH 5, respectively (data not shown). On the other hand, for the mixture with TCE, the highest removal efficiencies for BTEoX mixture and TPH were 74.5% at pH 9 and 61.5% at pH 7, respectively, and the lowest total removal efficiencies were 28.9% at pH 5 and 35.8% at pH 5, respectively (data not shown). These results suggested the inhibitory effect of TCE on the removal of BTEoX mixture and TPH. The removal efficiency for *o*-xylene in BTEoX/TPH mixture was most affected by the presence of TCE, regardless of environmental conditions. After TCE was added into the mixture of BTEoX (350 mg/L) and TPH (1,000 mg/L) at 25°C, the removal efficiency for *o*-xylene dropped rapidly from 17.6% to 9.9%, from 82.3% to 13.8%, from 83.2% to 10.3% at pH 5, 7, and 9, respectively.

### B. Removal of BTEmX and TPH

The removal efficiencies of benzene, toluene,

ethylbenzene, *meta*-xylene, and TPH in BTE<sub>m</sub>X/TPH mixture without and with TCE after 144 hours of incubation are shown in Figure 3 and 4, respectively. For the mixture of BTE<sub>m</sub>X and TPH without TCE, the highest removal efficiencies for BTE<sub>m</sub>X mixture and TPH were 82.4% at pH 7 and 70.4% at pH 7, respectively, while the lowest removal efficiencies were 39.2% at pH 5 and 36.7% at pH 5, respectively (data not shown). On the other hand, for the mixture with TCE, the highest removal efficiencies for BTE<sub>m</sub>X mixture and TPH were 85.5% at pH 9 and 64.0% at pH 7, respectively, while the lowest removal efficiencies were 49.4% at pH 5 and 28.1% at pH 5, respectively (data not shown). Overall, these results indicated a slightly stimulatory effect of TCE towards the removal of BTE<sub>m</sub>X mixture. However, as shown in Figures 3 and 4, the removal efficiencies for individual compound in BTE<sub>m</sub>X mixture and TPH were not significantly affected by the presence of TCE, regardless of pH.

C. Removal of BTE<sub>p</sub>X and TPH

The removal efficiencies for benzene, toluene, ethylbenzene, *para*-xylene, and TPH in mixture of BTE<sub>p</sub>X and TPH without and with TCE after 144 hours of incubation are shown in Figure 5 and 6, respectively. For the mixture without TCE, the highest removal efficiencies for BTE<sub>p</sub>X mixture and TPH were 82.6% at pH 9 and 60.7% at pH 7, respectively, while the lowest removal efficiencies 42.0% at pH 5 and 30.5% at pH 5, respectively (data not shown). On the other hand, for the mixture with TCE, the highest removal efficiencies for BTE<sub>p</sub>X mixture and TPH were 85.0% at pH 9 and 61.9% at pH 9, respectively, while the lowest removal efficiencies were 49.0% at pH 5 and 26.1% at pH 5, respectively (data not shown). As for the BTE<sub>m</sub>X/TPH mixture, TCE showed a slightly stimulatory effect on the removal of BTE<sub>p</sub>X mixture and TPH. The removal efficiencies for BTE<sub>p</sub>X mixture with TCE compared to without TCE were 49.0% vs. 42.0% at pH 5, 85.5% vs. 82.6% at pH 9, while there was about 0.5% difference at pH 7. As shown, the removal efficiency for TPH in the mixture was most significantly stimulated by the presence of TCE at pH 9.

D. Removal of TCE

Table 1 shows the removal efficiencies for TCE (15 mg/L) at different pH values at 25°C when mixed with BTE<sub>o</sub>X (350 mg/L)/TPH (1,000 mg/L), BTE<sub>m</sub>X (350 mg/L)/TPH (1,000 mg/L), or BTE<sub>p</sub>X (350 mg/L)/TPH (1,000 mg/L) mixtures. TCE was the most efficiently removed when mixed with BTE<sub>o</sub>X and TPH at pH 7 or 9, while less removed in other mixtures at almost similar amounts regardless of pH. The highest removal of TCE occurred at pH 7, regardless of mixtures. The amounts of chloride generated from the co-metabolism/mineralization of TCE at each pH are stoichiometrically equivalent to the bio-removal efficiencies except when mixed with the BTE<sub>m</sub>X/TPH mixture at pH 7 and 9.

IV. CONCLUSION

This study evaluated those interactions among the most frequently found organic environmental contaminants (BTEX, TPH, and TCE) when they existed in mixtures at different pH values at 25°C. The highest removal efficiencies for the mixtures of BTEX and TPH were at pH 7 or 9, while the lowest were at pH 5, regardless of the presence of TCE, implying the isolate (indigenous microorganism) preferring neutral or slightly alkaline condition. When the removal efficiencies for BTE<sub>o</sub>X, BTE<sub>m</sub>X, or BTE<sub>p</sub>X mixed with TPH were compared with or without TCE, TCE showed an inhibitory effect on the *o*-xylene removal in BTE<sub>o</sub>X mixture, while it showed a slightly stimulatory effect on the overall removal efficiencies for BTE<sub>m</sub>X and BTE<sub>p</sub>X mixtures. On the other hand, TCE was the most efficiently removed when mixed with BTE<sub>o</sub>X and TPH at pH 7 or 9, while less removed in other mixtures regardless of pH, and the highest TCE removal occurred at pH 7 regardless of mixtures. Even though this study warrants more works, such as effects of different temperatures and dissolved oxygen levels and contaminants concentrations, to further evaluate the contaminants' interactions in mixtures, these preliminary results would still help improve the applicability of bioremediation technology to the mixed wastes contaminated sites.

TABLE I. BIO-REMOVAL EFFICIENCIES (%) FOR TCE, MIXED WITH BTEX/TPH, UNDER DIFFERENT PH CONDITIONS (MEAN ± SD FOR DUPLICATES) (CHLORIDE IN MG/L)

pH	Removal Efficiency	BTE <sub>o</sub> X/TPH TCE	BTE <sub>m</sub> X/TPH TCE	BTE <sub>p</sub> X/TPH TCE
5	Biotic	12.8	17.2	14.1
	Abiotic	6.1	7.5	10.2
	Total	18.9±7.2	24.7±10.2	24.3±12.7
	Chloride	2.1±1.0	3.8±0.4	2.0±0.3
7	Biotic	31.6	19	20.8
	Abiotic	8.5	10.9	5.3
	Total	40.1±6.2	29.9±8.4	26.1±8.3
	Chloride	4.0±1.2	2.2±0.9	3.1±1.2
9	Biotic	25.8	14.1	12.2
	Abiotic	10.3	7.1	8.6
	Total	36.1±7.0	21.2±13.4	20.8±8.5
	Chloride	2.9±0.5	2.6±1.1	1.9±0.3

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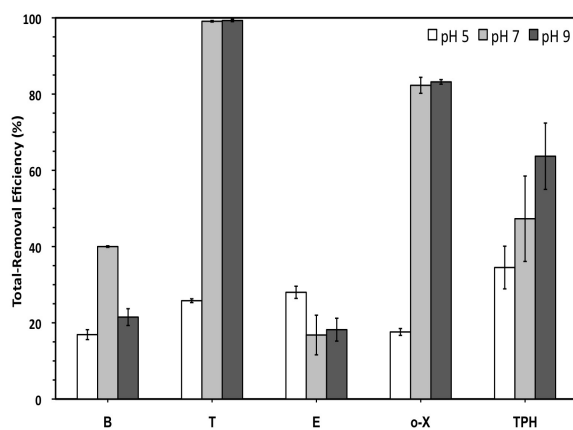


Figure 1. Removal efficiencies (%; average of replicates) for benzene, toluene, ethylbenzene, ortho-xylene, and TPH in BTEoX/TPH mixture after 144 hours of incubation in MSM at different pHs at 25°C.

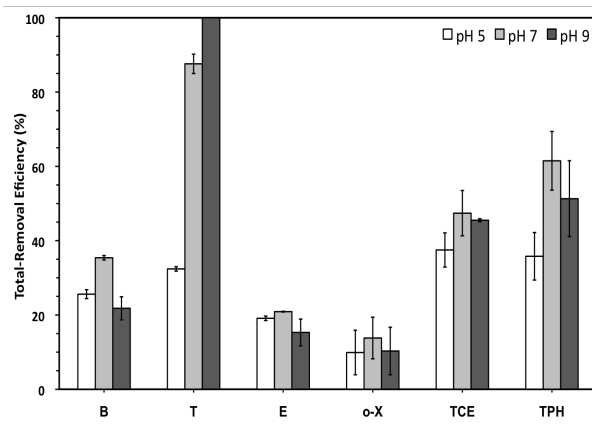


Figure 2. Removal efficiencies (%; average of replicates) for benzene, toluene, ethylbenzene, ortho-xylene, TPH, and TCE in BTEoX/TPH/TCE mixture after 144 hours of incubation in MSM at different pHs at 25°C.

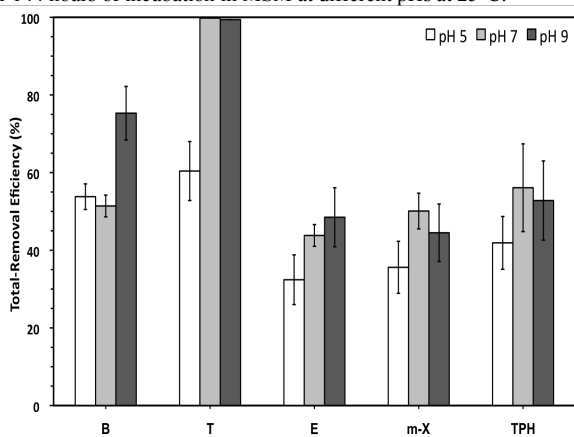


Figure 3. Removal efficiencies (%; average of replicates) for benzene, toluene, ethylbenzene, meta-xylene, and TPH in BTEmX/TPH mixture after 144 hours of incubation in MSM at different pHs at 25°C.

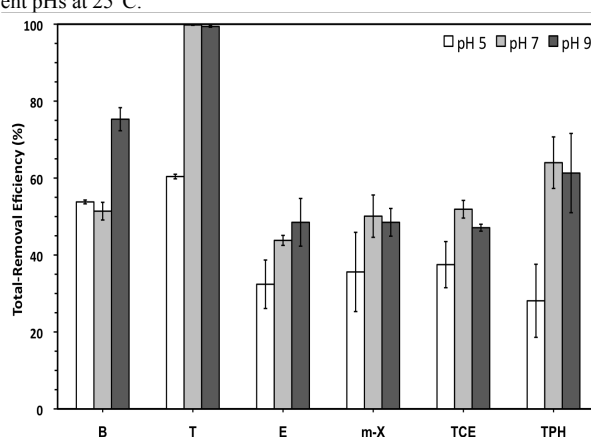


Figure 4. Removal efficiencies (%; average of replicates) for benzene, toluene, ethylbenzene, meta-xylene, and TPH in BTEmX/TPH/TCE mixture after 144 hours of incubation in MSM at different pHs at 25°C.

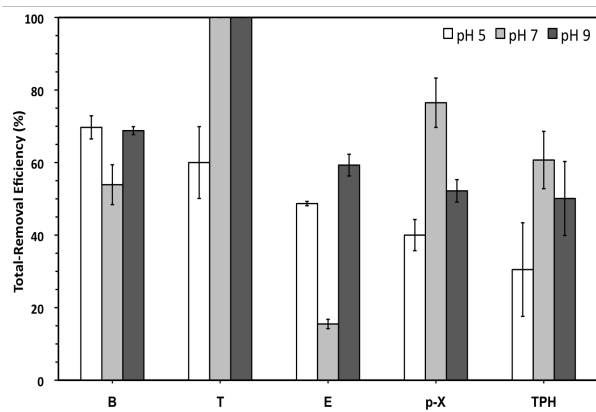


Figure 5. Removal efficiencies (%; average of replicates) for benzene, toluene, ethylbenzene, *para*-xylene, and TPH in BTEpX/TPH mixture after 144 hours of incubation in MSM at different pHs at 25°C.

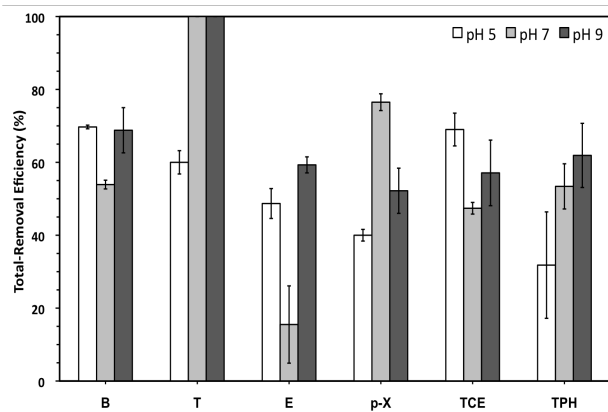


Figure 6. Removal efficiencies (%; average of replicates) for benzene, toluene, ethylbenzene, *para*-xylene, and TPH in BTEpX/TPH/TCE mixture after 144 hours of incubation in MSM at different pHs at 25°C.