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Bio-removal of mixture of benzene, toluene, ethylbenzene, and xylenes/total petroleum hydrocarbons/trichloroethylene from contaminated water

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Abstract

Four pure cultures were isolated from soil samples potentially contaminated with gasoline compounds either at a construction site near a gas station in Fai Chi Kei, Macau SAR or in the northern parts of China (Beijing, and Hebei and Shandong). The effects of different concentrations of benzene, toluene, ethylbenzene, and three isomers (*ortho*-, *meta*-, and *para*-) of xylene (BTEX), total petroleum hydrocarbons (TPH), and trichloroethylene (TCE), when they were present in mixtures, on the bio-removal efficiencies of microbial isolates were investigated, together with their interactions during the bio-removal process. When the isolates were tested for the BTEX (50–350 mg/L)/TPH (2000 mg/L) mixture, BTE*o*X in BTE*o*X/TPH mixture was shown with higher bio-removal efficiencies, while BTE*m*X in BTE*m*X/TPH mixture was shown with the lowest, regardless of isolates. The TPH in BTE*m*X/TPH mixture, on the other hand, were generally shown with higher bio-removal efficiencies compared to when TPH mixed with BTE*o*X and BTE*p*X. When these BTEX mixtures (at 350 mg/L) were present with TCE (5–50 mg/L), the stimulatory effect of TCE toward BTE*o*X bio-removal was observed for BTE*o*X/TCE mixture, while the inhibitory effect of TCE toward BTE*m*X for BTE*m*X/TCE mixture. The bio-removal efficiency for TPH was shown lower in TPH (2000 mg/L)/TCE (5–50 mg/L) mixtures compared to TPH present alone, implying the inhibitory effect of TCE toward TPH bio-removal. For the mixture of BTEX (417 mg/L), TPH (2000 mg/L) along with TCE (5–50 mg/L), TCE was shown co-metabolically removed more efficiently at 15 mg/L, probably utilizing BTEX and/or TPH as primary substrates.

Key words: bio-removal; benzene, toluene, ethylbenzene, and xylenes (BTEX); co-metabolism; trichloroethylene; total petroleum hydrocarbons

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Introduction

Volatile organic compounds generally arise from a wide variety of industrial sources, especially fine chemical and petrochemical industries (Jo *et al*., 2008). An unfortunate side effect of large scale petroleum production and processing is the likelihood of accidental spillage. It has been reported that recently more and more oil spill accidents occur and 74% of accidents occurred in petroleum refineries, oil terminals, or storage facilities (Chang and Lin, 2006). Whatever the origin of contamination, some petroleum or decomposition products may reach groundwater reserves, lakes, or water courses providing water for domestic and industrial use (Sunday *et al*., 2007). Apart from possible hazards to health such as liver damage and skin problems, such contamination is objectionable because of the very low concentration at which petroleum hydrocarbons and associated materials can be detected by their smell and taste (Anyaegbu, 1987; Nwankwo and Irrechukwu, 1987).

Benzene, toluene, ethylbenzene, and three isomers (*ortho*-, *meta*-, and *para*-) of xylenes, collectively known as BTEX, are widely used as industrial solvents for organic synthesis and equipment cleansing (Shim *et al*., 2005). These BTEX compounds can rapidly be percolated through the vadose zone, and upon reaching the water table, quickly migrate downgradient as a plume of hazardous material. BTEX compounds can also persist as an immiscible coating on soil particles for a long period. Trichloroethylene (TCE), on the other hand, is widely used in various industrial processes (e.g., degreasing of fabricated metal parts, industrial dry-cleaning, textile manufacturing). It is among the most prevalent hazardous organic compounds present in groundwater and soil. Its widespread occurrence in soil and subsurface media is an important environmental issue due to its carcinogenicity and other serious health effects on humans (ATSDR, 1997), and it is therefore strictly regulated (Kocamemi and Cecen, 2007). TCE is regulated to the level of 5 μg/L under the Safe Drinking Water Act.

Because of the ecological risk posed by BTEX, total petroleum hydrocarbons (TPH), and TCE contaminated

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soil and water, various alternative treatment technologies have been developed. Among all the currently existing technologies, biological treatment is regarded as the most economical and environmentally sound approach. No microorganism has been isolated, which can grow using TCE as a sole carbon or energy source (Hyman *et al*., 1995). 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 susbstrate (Kocamemi and Cecen, 2007). In the environment, TCE is degraded anaerobically via reductive dechlorination to the less chlorinated ethenes, *trans*-1,2-dichloroethylene (*trans*-DCE), *cis*-1,2-dichloroethylene (*cis*-DCE), 1,1-dichloroethylene (1,1-DCE), vinyl chloride (VC), ethene and ethane (Sharma and McCarty, 1996). In comparison, TCE can also be oxidized to form TCE epoxide, which is rapidly converted to glyoxylic acid, and finally to CO_2 and chloride, under the aerobic conditions.

This study was to utilize already existing indigenous microorganisms to remove these contaminants (BTEX, TPH, and TCE present at different concentrations) from the artificially contaminated water through the lab-scale batch experiments. Indigenous microorganisms were first enriched and isolated from the soils potentially contaminated with gasoline compounds, regionally and at the northern parts of China. These microbial isolates were investigated for their bio-removal efficiencies toward mixtures of BTEX/TPH as substrates, using serum bottles. To assess the possibility for these isolates to co-metabolize TCE, the bio-removal efficiencies for the mixtures of BTEX/TCE, TPH/TCE, and BTEX/TPH/TCE were evaluated, and the results were compared for their interactions during the aerobic bio-removal process.

1 Materials and methods

1.1 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). Gasoline (purity, 99%) and diesel (purity, 99%) were purchased from the Shell Company in Macau, China. All the other chemicals involved in the experiments were also purchased from the International Laboratory (USA). All the materials used were of the highest purities available and were used without further purification.

1.2 Soil sample collection

Soil samples were collected either from a construction site nearby a gas station in Fai Chi Kei, Macau Special Administrative Region, China (the collection depth of soil sample was 5.0 m and the soil sample appeared like marine clay with a grayish black color) or from the potential contaminated sites in northern parts of China (Beijing, Hebei and Shandong), and the microbial pure cultures were enriched and isolated from the soil samples.

1.3 Enrichment and isolation

Two kinds of microbial culture media were used. One was the defined mineral salts medium (MSM) which contained (in g/L): KH_2PO_4 1.0; K_2HPO_4 1.0; NH_4NO_3 1.0; $MgSO_4{\cdot}7H_2O$ 0.2; $Fe_2(SO_4)_3$ 0.05; and $CaCO_3$ 0.02. The other was nutrient broth (NB) consisted of 3.0 g beef extract and 5.0 g peptone per liter of water. The desired amounts of BTEX, TPH (1:1 ratio of gasoline and diesel), and TCE were aseptically added into the media using micropipettes, directly from the stock solutions. The pH of the medium was adjusted to around 7.0 by adding either HCl or NaOH. All the apparatus and media were autoclaved in advance.

The soil sample (5%, *W/W*) was first added into the NB in a serum bottle, followed by the addition of 150 mg/L toluene provided as co-substrate. After the serum bottle was covered with stopper (90% teflon/10% silicone) and sealed with aluminum crimp, it was inverted and placed on an orbital shaker at 150 r/min and 20°C. Ten percent (*V/V*) inoculum from this bottle was then aseptically inoculated into the MSM containing 150 mg/L toluene added as a sole substrate before the bottle was placed on the shaker. The subsequent sub-culturing was followed with toluene supplied as substrate every week.

Different pure cultures were isolated from the nutrient agar (NA) plates inoculated from the bottle containing toluene as substrate. Different bacterial colonies could be seen after the incubation period of 1–2 d, and some representatives were chosen according to the bacterial morphology and isolated as pure cultures. Each pure culture was further inoculated aseptically into MSM, containing 150 mg/L toluene as substrate, in a test tube, and the tube was placed on the orbital shaker at 150 r/min for one week. The tubes with higher turbidities were selected and incubated further. The chosen bacterial strains from tubes were inoculated in serum bottles containing MSM with toluene (150 mg/L) as substrate and the bottles were placed on the orbital shaker at 150 r/min. The sample aliquot was withdrawn from the bottle after one week for the measurement of optical density (OD) as well as the toluene concentration. Then, the pure cultures with the high toluene removal efficiencies were further sub-cultured by aseptically transferring 10% (*V/V*) inoculum into the newly prepared MSM containing BTEX in mixture, as substrates at appropriate concentrations.

1.4 Bio-removal of mixtures

For the bio-removal of BTEX/TPH mixtures, as substrates, 10% (*V/V*) inoculum from the pure cultures (one from Macau and three from the northern parts of China) grown in serum bottles containing MSM and 350 mg/L BTEX as substrates was inoculated into the bottles containing MSM and BTEX (BTE*o*X, BTE*m*X, and BTE*p*X at 350 mg/L)/TPH (2000 mg/L) mixtures as substrates. The bottles containing MSM but without microorganisms

served as controls. After stoppered and aluminum crimp sealed, the bottles were inverted to minimize the volatilization of substrates and incubated on the orbital shaker at 150 r/min and 20°C. At specific intervals, sample aliquots were withdrawn from the bottles and analyzed for the microbial growth measured as OD at 600 nm and the substrate concentrations.

For the bio-removal of BTEX/TCE mixtures, one microbial isolate from the northern part of China was inoculated into the MSM containing BTEX (BTE*o*X, BTE*m*X, and BTE*p*X at 350 mg/L)/TCE (5–50 mg/L) mixtures for its co-metabolic capability toward TCE. At specific intervals after incubation, sample aliquots were withdrawn from the bottles and analyzed for the microbial growth and the removal efficiencies for BTEX and TCE.

Similarly, the microbial isolate from the northern part of China was inoculated into the modified MSM containing TPH (2000 mg/L)/TCE (5–50 mg/L) mixtures or BTEX (including all six individual compounds according to the gasoline composition, at 417 mg/L)/TPH (2000 mg/L)/TCE (5–50 mg/L) mixtures. At specific intervals after incubation, sample aliquots withdrawn from the bottles were analyzed for the microbial growth and the removal efficiencies for BTEX, TPH, and TCE.

1.5 Analytical methods

The magnitude of OD was measured at 600 nm using a spectrophotometer (DR 2800, Hach Company, USA). 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 × 0.53 μm I.D. with a stationary-phase film thickness of 0.88 μm). One microliter of liquid sample was injected by the autosampler injector (7638 Series, Agilent Technologies Co., Ltd., China) equipped with a tapered microsyringe (Hamilton 5181-1267, Hamilton Company, USA). The concentration of chloride generated from the TCE mineralization was measured using an ion chromatograph (Dionex ICS 2500, Dionex Corporation, USA) equipped with separator column (IonPac AS11-HC), guard column (IonPac AG11-HC), GP50 gradient pump, suppressor (ASRS-ULTRA II 4-mm), AS50 autosampler, and ED50 electrochemical detector.

2 Results and discussion

2.1 Bio-removal of BTEX/TPH mixture

Table 1 shows the removal efficiencies of four isolates (one from Macau and three from the northern parts of China) toward the mixture of 350 mg/L BTEX mixtures and 2000 mg/L TPH. BTE*o*X in BTE*o*X/TPH mixture was shown with higher bio-removal efficiencies (53%–79%) than BTE*m*X in BTE*m*X/TPH mixture (34%–49%) and BTE*p*X in BTE*p*X/TPH mixture (41%–52%), regardless of isolates. On the other hand, TPH in BTE*m*X/TPH mixture were generally shown with higher bio-removal efficiencies (62%–76%) than TPH in BTE*o*X or BTE*p*X/TPH mixture. Compared to the same BTEX mixtures present alone at 350 mg/L, the bio-removal efficiencies for BTEX were lower when the BTEX mixtures were present with TPH at 2000 mg/L, implying the inhibitory effect of TPH toward BTEX bio-removal. In comparison, compared to TPH present alone at 2000 mg/L, the bio-removal efficiencies for TPH in BTEX/TPH mixtures were higher, implying the stimulatory effect of BTEX toward TPH bio-removal (data not shown).

Table 1 Removal efficiencies (%, average of replicates) for BTEX/TPH mixtures

Isolate	Removal	BTE*o*X/TPH	BTE*m*X/TPH	BTE*p*X/TPH
1	Biotic	79/67	34/76	43/60
(Macau)	Abiotic	11/19	24/19	12/28
	Total	90±12/86±16	58±15/95±8	55±13/88±7
2	Biotic	62/58	49/65	52/63
(China)	Abiotic	11/19	24/19	12/28
	Total	73±18/77±11	73±13/84±9	64±8/91±8
3	Biotic	59/54	36/70	41/63
(China)	Abiotic	11/19	24/19	12/28
	Total	70±15/73±13	60±12/89±9	53±11/91±8
4	Biotic	53/70	35/62	47/65
(China)	Abiotic	11/19	24/19	12/28
	Total	64±15/89±12	59±13/81±7	59±9/93±9

The first value is removal efficiency for BTEX and the second for TPH.

2.2 Bio-removal of BTEX/TCE mixture

Table 2 shows the bio-removal efficiencies of one isolate (from the northern part of China) toward the mixture of BTEX mixtures (BTE*o*X, BTE*m*X, and BTE*p*X at 350 mg/L) as substrates and TCE (at 5–50 mg/L) along with the concentration of chloride generated from the TCE mineralization.

When the BTE*o*X mixture was present alone without TCE, the bio-removal efficiency for the mixture was about 65%, while the removal efficiency for benzene, toluene, and mixture of ethylbenzene and *o*-xylene was 80%, 95%, and 98%, respectively (data not shown). In comparison, when the same mixture was present with 5 mg/L TCE, the bio-removal efficiency for TCE and BTE*o*X was 63% and 82%, respectively, while 2.5 mg/L chloride was generated (Table 2) and the removal efficiency for benzene, toluene, and mixture of ethylbenzene and o-xylene was 91%, 99%, and 91%, respectively. When the mixture was present with 15 mg/L TCE, the bio-removal efficiency for TCE and BTE*o*X was 90% and 87%, respectively, while 7.0 mg/L chloride was generated and the removal efficiency for benzene, toluene, and mixture of ethylbenzene and o-xylene was 98%, 100%, and 97%, respectively. Finally, when the mixture was present with 50 mg/L TCE, the bio-removal efficiency for TCE and BTE*o*X was 51% and 55%, respectively, while 3.1 mg/L chloride was generated and the removal efficiency for benzene, toluene, and mixture of ethylbenzene and *o*-xylene was 70%, 87%, and 50%, respectively. Therefore, the bio-removal efficiency for the BTE*o*X mixture increased with the presence of TCE up to 15 mg/L, implying the stimulatory effect of TCE toward BTE*o*X bio-removal when they present in mixture, while the removal efficiency for individual compounds in BTE*o*X mixture significantly decreased at 50 mg/L TCE

Table 2 Removal efficiencies (average of replicates) for BTEX/TCE mixtures and amount of chloride generated

BTEX	Removal	TCE			
		0 mg/L	5 mg/L	15 mg/L	50 mg/L
BTE*o*X	Biotic (%)	64.8	81.9/62.6[a]	87.1/89.8	54.5/50.7
	Abiotic (%)	26.3	13.4/5.0	11.4/7.3	14.7/13.1
	Total (%)	91.1	95.3/67.6	98.5/97.1	69.2/63.8
	Chloride generated (mg/L)	–	2.5	7.0	3.1
BTE*m*X	Biotic (%)	74.4	83.6/54.2	82.1/50.2	72.4/37.3
	Abiotic (%)	15.3	8.5/16.3	14.1/11.9	19.0/10.7
	Total (%)	89.7	92.1/70.5	96.2/62.1	91.4/48.0
	Chloride generated (mg/L)	–	4.2	7.5	15.8
BTE*p*X	Biotic (%)	ND[b]	82.7/57.8	51.9/85.4	55.5/73.6
	Abiotic (%)	ND	15.9/4.6	14.9/4.8	11.5/3.8
	Total (%)	ND	98.6/62.4	66.8/90.2	67.0/77.4
	Chloride generated (mg/L)	–	7.8	11.5	12.9

[a] The first value is the removal efficiency for BTEX mixture and the second for TCE. [b] Not determined.

present. On the other hand, the bio-removal efficiency for TCE in BTE*o*X/TCE mixture increased up to 15 mg/L before significantly decreased at 50 mg/L, and the cell growth indicated by OD was also observed decreased with the increased TCE concentration (data not shown). At 5 mg/L TCE, the stoichiometric amount of chloride was generated, corresponding to the amount of TCE removed biologically, while at higher TCE concentrations, up to 65% of the respective chloride generated.

When the BTE*m*X mixture was present alone without TCE, the bio-removal efficiency for the mixture was 74%, while the removal efficiency for benzene, toluene, and mixture of ethylbenzene and *m*-xylene was 84%, 91%, and 96%, respectively (data not shown). When this mixture was present with 5 mg/L TCE, the bio-removal efficiency for TCE and BTE*m*X was 54% and 84%, respectively, while 4.2 mg/L chloride was generated and the removal efficiency for benzene, toluene, and mixture of ethylbenzene and *m*-xylene was 88%, 95%, and 85%, respectively. When the mixture was present with 15 mg/L TCE, the bio-removal efficiency for TCE and BTE*m*X was 50% and 82%, respectively, while 7.5 mg/L chloride was generated and the removal efficiency for benzene, toluene, and mixture of ethylbenzene and *m*-xylene was 96%, 97%, and 96%, respectively. When the mixture was present with 50 mg/L TCE, the bio-removal efficiency for TCE and BTE*m*X was 37% and 72%, respectively, while 15.8 mg/L chloride was generated and the removal efficiency for benzene, toluene, and mixture of ethylbenzene and *m*-xylene was 91%, 92%, and 91%, respectively. In general, the bio-removal efficiency for BTE*m*X mixture decreased with the increased TCE concentration, showing the inhibitory effect of TCE toward BTE*m*X bio-removal when they present in mixture, and the bio-removal efficiency for TCE in BTE*m*X/TCE mixture and the cell growth indicated by OD also decreased with the increased TCE concentration. On the other hand, the stoichiometric amount of chloride was generated, corresponding to the amount of TCE removed co-metabolically, regardless of TCE concentrations.

When the BTE*p*X mixture was present with 5 mg/L TCE, the bio-removal efficiency for TCE and BTE*p*X was 58% and 83%, respectively, while 7.8 mg/L chloride was generated and the removal efficiency for benzene, toluene, and mixture of ethylbenzene and *p*-xylene was 99%, 100%, and 96%, respectively (data not shown). When the mixture was present with 15 mg/L TCE, the bio-removal efficiency for TCE and BTE*p*X was 85% and 52%, respectively, while 11.5 mg/L chloride was generated and the removal efficiency for benzene, toluene, and mixture of ethylbenzene and *p*-xylene was 94%, 56%, and 56%, respectively. When the mixture was present with 50 mg/L TCE, the bio-removal efficiency for TCE and BTE*p*X was 74% and 56%, respectively, while 12.9 mg/L chloride was generated and the removal efficiency for benzene, toluene, and mixture of ethylbenzene and *p*-xylene was 94%, 50%, and 66%, respectively. Therefore, the bio-removal efficiency for BTE*p*X mixture generally decreased with the increased TCE concentration, showing the inhibitory effect of TCE toward BTE*p*X bio-removal when they present in mixture, while the bio-removal efficiency for TCE in BTE*p*X/TCE mixture was the highest at 15 mg/L TCE followed by 50 and 5 mg/L TCE. The cell growth indicated by OD, on the other hand, was not significantly affected by the increased TCE concentration (data not shown). Up to 15 mg/L TCE, the stoichiometric amount of chloride was generated, corresponding to the amount of TCE removed co-metabolically, while up to 45% of the respective amount generated at 50 mg/L.

2.3 Bio-removal of TPH/TCE mixture

Table 3 shows the bio-removal efficiencies of the isolate from the northern part of China toward TPH (2000 mg/L)/TCE (5–50 mg/L) mixtures along with the amount of chloride generated. When TPH was present along with TCE at 15 mg/L, the bio-removal efficiencies were higher

Table 3 Removal efficiencies (average of replicates) for TPH/TCE mixtures and amount of chloride generated

Removal	TCE			
	0 mg/L	5 mg/L	15 mg/L	50 mg/L
Biotic (%)	75.9	61.5/36.4[a]	69.1/57.6	52.7/34.7
Abiotic (%)	16.3	18.1/5.0	12.2/5.6	14.6/15.8
Total (%)	92.2	79.6/41.4	81.3/63.2	67.3/50.5
Generated chloride (mg/L)	–	5.6	3.4	3.9

[a] The first value is the removal efficiency for TPH and the second for TCE.

Table 4 Removal efficiencies (average of replicates) for BTEX/TPH/TCE mixtures and amount of chloride generated

Removal	BTEX (417 mg/L)/TPH (2000 mg/L)/TCE (0–50 mg/L)[a]			
	0 mg/L	5 mg/L	15 mg/L	50 mg/L
Biotic (%)	53.6/60.5	58.9/49.6/51.7[b]	50.2/54.4/65.3	59.0/56.5/46
Abiotic (%)	5.9/19.3	5.5/27.0/16.3	7.7/13.8/12.4	7.4/19.5/13.8
Total (%)	59.5/ 79.8	64.4/76.6/68.0	57.9/68.2/77.7	66.4/76.0/59.8
Chloride generated (mg/L)	–	5.0	6.4	8.0

[a] Concentrations of BTEX, TPH, and TCE were 417, 2000, and 0 to 50 mg/L, respectively.
[b] Values show the biotic removal efficiency for BTEX, TPH, and TCE, respectively.

for both TPH (69.1%) and TCE (57.6%) with 3.4 mg/L chloride generated, compared to TCE at 5 or 50 mg/L. The bio-removal efficiencies for both TPH and TCE increased with the increased TCE concentration up to 15 mg/L, but decreased with further increasing TCE concentration. On the other hand, in terms of chloride generated from the TCE mineralization, the stoichiometric amount of chloride was generated at 5 mg/L TCE, corresponding to the amount of TCE removed co-metabolically, while up to 45% of the respective chloride generated at higher TCE concentrations.

2.4 Bio-removal of BTEX/TPH/TCE mixture

Table 4 shows the bio-removal efficiencies of the isolate from the northern part of China toward BTEX (417 mg/L)/TPH (2000 mg/L)/TCE (5–50 mg/L) mixtures along with the amount of chloride generated. The BTEX concentration in this case, 417 mg/L, was based on all six individual compounds considered following the composition of unleaded gasoline. When the TCE concentration was 15 mg/L, the bio-removal efficiency for BTEX was lower (50.2%), while the bio-removal efficiencies for TPH and TCE were higher compared to other two mixtures with TCE at 5 and 50 mg/L. In terms of chloride generated from the TCE mineralization, the stiochiometric amount of chloride was generated at 5 mg/L TCE, corresponding to the amount of TCE removed co-metabolically, while up to 80% of the respective chloride generated at higher TCE concentrations.

Even though lots of studies have been done with the removal of BTEX (singly and in mixtures), TPH, or TCE (singly and in mixtures with other chlorinated aliphatic compounds; using such substrates as toluene for the enzymes TOM and TOD, *ortho*-xylene for ToMO, and methanol for MMO) individually, unfortunately almost no study seems dealing with the interaction (stimulatory or inhibitory to each other's removal) among BTEX, TPH, and TCE when they exist in mixtures of BTEX/TPH, BTEX/TCE, TPH/TCE, and BTEX/TPH/TCE, as tested in this study. The results from this study further show some contradictory interaction results among BTEX, TPH, and TCE when they existed in mixtures, similar to the interaction results previously shown when BTEX were present in mixtures (e.g., benzene biodegradation was shown stimulated by the presence of toluene or *ortho*-xylene in one study but shown inhibited by the presence of toluene in others, under the same aerobic condition but for the different microorganisms). Since this study is just preliminary toward the interactions among these very commonly found organic environmental contaminants present in mixtures, further studies are definitely warranted in order to find out more exact interactions among them, due to their co-existence at many actual contaminated sites.

3 Conclusions

Four pure cultures isolated from the regional soil samples potentially contaminated with gasoline compounds were shown with various responses in terms of bio-removal efficiencies toward mixtures of BTEX, TPH, and TCE at different concentrations. When BTEX (BTE*o*X, BTE*m*X, or BTE*p*X at 350 mg/L) and TPH (at 2000 mg/L) were mixed together, BTE*o*X was shown with the highest bio-removal efficiencies, followed by BTE*p*X and BTE*m*X, regardless of isolates. TPH in BTE*m*X/TPH mixture, on the other hand, were generally shown with higher bio-removal efficiencies compared to TPH mixed with BTE*o*X or BTE*p*X. When these BTEX mixtures were present with TCE (at 5–50 mg/L), the stimulatory effect of TCE toward BTE*o*X bio-removal was observed, compared to its inhibitory effect toward BTE*m*X. The bio-removal efficiency for TPH was shown lower in TPH (at 2000 mg/L)/TCE (at 5–50 mg/L) mixtures compared to TPH present alone, implying the inhibitory effect of TCE toward TPH bio-removal. For the mixture of BTEX (at 417 mg/L), TPH (at 2000 mg/L) along with TCE (at 5–50 mg/L), TCE was shown better co-metabolically removed at 15 mg/L. Even though the preliminary experimental results from this study still warrant further detailed studies, especially in terms of more detailed interactions among BTEX, TPH, and TCE during their bio-removal process, they still imply that the development of this kind of innovative biotechnology would be potentially applicable, especially for the environment contaminated with petroleum hydrocarbons together with chlorinated hydrocarbons.

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