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RESEARCH ARTICLE

# Combined removal of a BTEX, TCE, and *cis*-DCE mixture using *Pseudomonas* sp. immobilized on scrap tyres

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**Abstract** The simultaneous aerobic removal of a mixture of benzene, toluene, ethylbenzene, and *o*,*m*,*p*-xylene (BTEX); *cis*-dichloroethylene (*cis*-DCE); and trichloroethylene (TCE) from the artificially contaminated water using an indigenous bacterial isolate identified as *Pseudomonas plecoglossicida* immobilized on waste scrap tyres was investigated. Suspended and immobilized conditions were compared for the removal of these volatile organic compounds. For the immobilized system, toluene, benzene, and ethylbenzene were completely removed, while the highest removal efficiencies of 99.0±0.1, 96.8±0.3, 73.6±2.5, and 61.6±0.9 % were obtained for *o*-xylene, *m*,*p*-xylene, TCE, and *cis*-DCE, respectively. The sorption kinetics of contaminants towards tyre surface was also evaluated, and the sorption capacity generally followed the order of toluene>benzene>*m*,*p*-xylene>*o*-xylene>ethylbenzene>TCE>*cis*-DCE. Scrap tyres showed a good capability for the simultaneous sorption and bioremoval of BTEX/*cis*-DCE/TCE mixture, implying a promising waste material for the removal of contaminant mixture from industrial wastewater or contaminated groundwater.

**Keywords** Bioremoval · BTEX · *Cis*-DCE · Immobilization · Mixture · Scrap tyres · Sorption

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## Introduction

Industrialization has generated large quantities of hazardous wastes and more sites have become contaminated with mixed wastes of organics. Chlorinated aliphatic hydrocarbons (CAHs) and petroleum hydrocarbons are among the most widespread contaminants in subsurface, due to their increased use and accidental spillage (Li et al. 2014). Trichloroethylene (TCE), as a representative CAH, has historically been used as a cleaning and degreasing solvent. It is among the most prevalent hazardous organic compounds present in the environment and has been considered as a suspected carcinogen and hazardous to humans (Collins et al. 2002; Tobiszewski and Namiesnik 2012). TCE can be degraded via a reductive dechlorination process, mainly resulting in the formation of *cis*-1,2-dichloroethylene (*cis*-DCE) and vinyl chloride (McCarty 1997), which are more toxic than the parent compound.

Benzene, toluene, ethylbenzene, and three isomers (*ortho*-, *meta*-, and *para*-) of xylene, collectively known as BTEX, are the major aromatic and most toxic components in many petroleum products and widely used as industrial solvents for organic synthesis and equipment cleansing. As CAHs are frequently found together with BTEX at many contaminated sites, it would be beneficial if they can be removed simultaneously (Shim et al. 2009). This co-contamination would allow the occurrence of aerobic cometabolism of CAHs utilizing BTEX as primary substrates to achieve the simultaneous mineralization of both types of contaminants.

Over 3 billion car and truck tyres have been reported discarded worldwide each year, with over 10 billion tyres stockpiled in landfills (Association 2006). Therefore, the proper management of waste tyres for disposal and recycling has been a major environmental concern (Williams 2013). The tyre crumb rubber consists of a complex mixture of elastomers including polyisoprene, polybutadiene, and styrene-butadiene. Additionally, since stearic acid, zinc oxide, extender oil, and carbon black are important components of tyres (Amari et al. 1999), waste tyres could exhibit remarkable adsorption properties as activated charcoal, a well-known adsorbent used to remove organic and inorganic compounds from air, soil, and liquids (Alamo-Nole et al. 2011). Waste tyres were utilized as the sorption phase in a two-phase partitioning bioreactor (TPPB) for the biodegradation of a binary mixture of 2, 4-dichlorophenol (DCP) and 4-nitrophenol (4NP). A significant reduction in the DCP toxicity and an improvement in the substrate removal efficiency (up to 83 % for DCP and almost 100 % for 4NP) were obtained for the TPPB operated with 10 and 15 % (*v*/*v*) tyres (Tomeia et al. 2014).

To our best knowledge, there has been no study that investigated the utilization of waste tyres as a matrix for the immobilization of microorganisms to remove contaminant mixtures. *Pseudomonas plecoglossicida*, previously isolated from a heavily oil-contaminated site, was the strain chosen for the bioremoval studies due to its excellent performance on the removal of BTEX and chlorinated aliphatic compounds (Li et al. 2014). Microbial immobilization has been known offering many advantages compared to the suspended microbial growth technology, achieving high biomass, high metabolic activity, and strong resistance to toxic chemicals (Barreto et al. 2010).

Although the occurrence of co-contamination sites is being found more often, there have been only a few data available on the simultaneous bioremoval of BTEX/CAH mixtures in environmental samples (Johnston et al. 1996; Li et al. 2011, 2014). Despite the information already available on the microbial and physical removal of BTEX compounds from liquids using activated carbon (Yakout and Daifullah 2013), the development of low-cost adsorbent materials is further necessary (Ali et al. 2012). In this study, the utilization of waste scrap tyres for the simultaneous sorption and bioremoval of a mixture of BTEX, *cis*-DCE, and TCE was investigated.

## Materials and methods

### Chemicals and scrap tyres

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). *Cis*-DCE (purity, 99 %) was purchased from the Tokyo Chemical Industry Co., Ltd (Japan), and TCE (purity, 99 %) was purchased from Damao Chemical Manufacture (China). The stock standard solution of BTEX mixture was prepared at 10,000 mg/L in *N*,*N*-dimethylformamide and stored at 4 °C until used.

The waste scrap tyre used was from Bridgestone (Battlax BT-39 tubeless). Scrap tyres were cut into small pieces (0.2 cm×0.2 cm×0.2 cm), washed in deionized water using an ultrasonic cleaner for 1 h, and dried in an oven (60 °C), and the amounts needed for the sorption and bioremoval experiments were carefully weighed and autoclaved for 1 h (121 °C, 103.5 kPa) before use.

### Microbial culture

The microbial pure culture used in this study, identified as *P. plecoglossicida*, was isolated from a heavily petroleum-contaminated soil near a gas station in Xiamen, China. This strain already showed the ability to simultaneously remove two representative CAH (*cis*-DCE and TCE) and BTEX mixtures under aerobic conditions (Li et al. 2014). The respective enrichment and isolation process can be found elsewhere (Li et al. 2011). Toluene (at 150 mg/L, unless otherwise stated) was first added as the sole carbon source in a serum bottle containing mineral salt medium (MSM), and the bottle, after sealed with Teflon/silicon rubber and aluminum crimp, was inverted and shaken at 150 rpm and 25 °C. The culture was subcultured (10 % *v*/*v* inoculum size) every week in a bottle containing toluene (150 mg/L) as a growth substrate in a fresh MSM containing (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 pH of the medium was adjusted to 7.0 by adding either NaOH (1.0 mol/L) or $HNO_3$ (1.0 mol/L) solution. All the apparatus and liquid media were autoclaved for 20 min at 121 °C under 103.5 kPa in advance.

### Analytical methods

The concentrations of *cis*-DCE, TCE, and BTEX were measured on a gas chromatograph (Agilent, 6890N, Agilent Technologies Co., Ltd, China) equipped with a flame ionization detector and a capillary column (Agilent HP-5; 30 m× 0.53 μm I.D. with a stationary-phase film thickness of 0.88 μm). The temperatures of injector and detector were 120 and 260 °C, respectively. The initial temperature programme for column was 40 °C and incrementally increased (6 °C/min) to 110 °C.

The microbial growth was measured as the optical density (OD) at 600 nm using a spectrophotometer (DR 2800, Hach Company, USA). To evaluate the OD in microcosms with scrap tyres as immobilization matrices, the following protocol was used. The OD of freely suspended cells was measured directly from the serum bottle samples, and the immobilized cells were first suspended in sterile deionized water through the tyre sonication for 5 min before the respective OD was measured.

On the other hand, the colony-forming unit (CFU) assays were performed in duplicates to estimate the number of viable bacteria, freely suspended in solution as well as attached on tyre surfaces. For this assay, the samples were first diluted in the range of $10^{-1}$ to $10^{-8}$ with sterile distilled water, and then the diluted samples were aseptically poured onto the nutrient broth (NB) agar plates. After the plates were incubated at 30 °C for 2 days, the CFUs were counted.

### Experimental setup

The microcosm studies were conducted in 160-mL serum bottles covered with stopper (90 % Teflon/10 % silicone; Ohio Valley Specialty, USA), sealed with aluminum crimp, inverted to minimize the volatilization of contaminants (BTEX, *cis*-DCE, and TCE), and shaken at 150 rpm. The BTEX mixture (300 mg/L, unless otherwise stated) was prepared following the ratio of benzene/toluene/ethylbenzene/*o*-xylene/*m*-xylene/*p*-xylene at 22.7:48.3:4.6:6.3:6.9:11.1 %, based on the mass fractions in crude oil (U.S. Environmental Protection Agency 2011) and mixed together with TCE (10 mg/L) and *cis*-DCE (5 mg/L) (Lu et al. 1998; Li et al. 2014). For the suspended microbial system, each contaminant was added into serum bottles containing 45 mL of MSM solution, and the bottles were sealed and shaken for 6 h until the equilibrium between liquid and gas phases was reached. Then, 5 mL of early stationary-phase inocula from the subculture grown on toluene (150 mg/L) were transferred to each serum bottle. In case of waste scrap tyres used, different amounts of scrap tyres were added to the bottles after the addition of contaminants. The abiotic loss of volatile organic compounds (VOCs; BTEX, *cis*-DCE, and TCE) was measured through the controls without inoculum. All the treatments were incubated in the dark at 25 °C and pH 7.0, in duplicates, and each bottle was measured twice for the liquid phase concentrations of VOCs.

To evaluate the sorption kinetics of VOCs on tyre surface, BTEX mixture (300 mg/L) was added at the same concentrations used for the bioremoval experiments (benzene 68.1 mg/L; toluene 145.0 mg/L; ethylbenzene 13.8 mg/L; *o*-xylene 20.7 mg/L; *m*-xylene 18.9 mg/L; *p*-xylene 33.3 mg/L), together with TCE (10 mg/L) and *cis*-DCE (5 mg/L). Then, 1.5 g of tyre pieces was added and the bottles were sealed and shaken. The concentrations of individual VOCs were measured at intervals (after 0.25, 0.5, 1, 1.5, 24, 48, and 72 h). The sorption kinetics of BTEX only was also evaluated with the equal concentration (100 mg/L) of each BTEX compound.

The sorption capacity of adsorbent ($q_e$) was calculated by the following equation.

$$q_e = V(C_0 - C_e)\Big/m$$

Where $C_0$ and $C_e$ are the initial and equilibrium concentration (mg/L), respectively, $m$ is the mass of adsorbent (g), and $V$ is the solution volume (L).

### Statistical analysis

The freely suspended and immobilized microbial system data are shown in means±standard deviations (SD). The statistical analysis was performed by one-way analysis of variance (ANOVA) using the SPSS statistic software, and the values of $P<0.05$ were considered to indicate a statistical significance.

## Results and discussion

### Sorption of BTEX/*cis*-DCE/TCE mixture on tyre surface

Figure 1 shows the sorption kinetics of BTEX, *cis*-DCE, and TCE on scrap tyres over time at 25 °C and pH 7.0. The amounts of all VOCs sorbed on scrap tyres were greatly affected by the contact time, reaching the maximum capacity in about 1 day, and then leveled off. Toluene was the most sorbed, followed by benzene, xylenes, ethylbenzene, TCE, and *cis*-DCE. This order also followed the concentration of each VOC used in a mixture, with toluene at 145 mg/L and *cis*-DCE at 5 mg/L, further suggesting that the concentration of contaminant (in mixture) played an important role in the sorption capacity on tyre.

The sorption capacity of individual BTEX compounds was further evaluated with each compound present at the same concentration (100 mg/L), individually and in a mixture. Figure 2a shows the sorption kinetics of each BTEX compound on tyre, and Fig. 2b shows the comparison of the removal (due to sorption) efficiencies for BTEX between when present singly and when present in mixture after 72 h of contact time. It should be noted that both *m*-xylene and *p*-xylene showed one combined peak on a gas chromatogram. As shown, when BTEX compounds were present individually at the same concentration, their sorption capacities were different compared to when they were present at different concentrations in mixture, corresponding to their mass fractions in

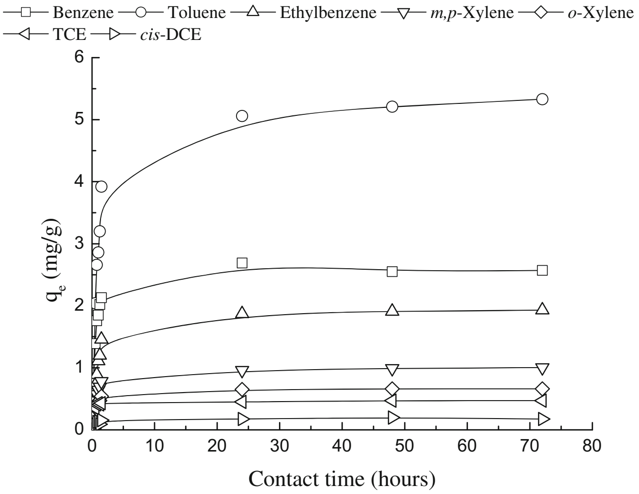

**Fig. 1** Sorption kinetics of each BTEX compound (total concentration at 300 mg/L), TCE (10 mg/L), and *cis*-DCE (5 mg/L). Weight of tyre is 1.5 g

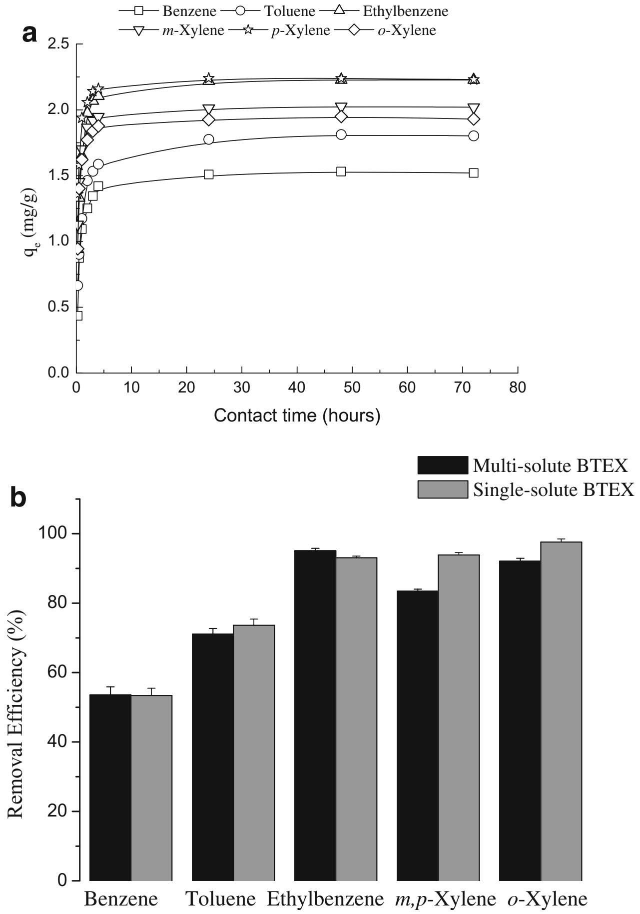

**Fig. 2** **a** Sorption kinetics of individual BTEX compounds (each at 100 mg/L) in mixture (multi-solution). Weight of tyre is 1.5 g. **b** Removal efficiencies for individual BTEX compounds in multi- and single-solution

crude oil (Fig. 1). The sorption capacity of individual BTEX compounds when present singly was in the order of *p*-xylene>ethylbenzene>*m*-xylene>*o*-xylene>toluene>benzene. This favourable sorption of xylenes and ethylbenzene compared to toluene and benzene may be attributed to the higher octanol-water partition coefficient ($K_{ow}$), 3.15 for xylenes and ethylbenzene, compared to 2.73 for toluene and 2.13 for benzene (Ranck et al. 2005). This result was also in accordance with another study (Zhang et al. 2013) which attributed the higher adsorption capacity to the lower water solubility and the higher hydrophobic nature. Yakout and Daifullah (2013) further reported that the adsorption capacity of BTEX on activated carbon prepared from rice husk followed the same order as in the current study and suggested that the adsorption rate would increase as the number of methyl groups attached to the aromatic ring increases (one methyl group for toluene to two for xylenes). As the side chain length increases, the compound tends to be more hydrophobic and consequently the adsorption increases. The adsorption capacity of BTEX compounds was also similar to the study of Silva Almeida el al. (2012), which used *Moringa oleifera* seed cake (MOSC) as adsorbent. The adsorption capacity was mainly attributable by the inductive effect of the substituents (–$CH_3$ and –$CH_2CH_3$) and also by the interactions between the electronically dense aromatic rings of BTEX and charged functional groups on the MOSC surface. According to Li et al. (2010) and Villacañas et al. (2006), the interactions of aromatic compounds with activated carbons are mainly with dispersive, electrostatic, and water cluster blocking nature. Since BTEX compounds are present in molecular forms in an aqueous solution, the dispersive interaction referred to as the interaction of the $\pi$ orbit on the carbon basal planes with the electron density of the aromatic rings is expected to play a significant role in the adsorption process. Considering that carbon black and styrene-butadiene rubber are the main components of tyres (Kaminsky and Mennerich 2001) and carbon black is known to present low content of acidic groups (carboxylic, lactonic, and phenolic groups) (Boehm 1994), the dispersive interaction is considered the main interaction between BTEX and scrap tyres in the current study. On the other hand, the removal efficiencies (due to sorption) for each BTEX compound, as shown in Fig. 2b, did not differ significantly between multi- and single-solute solutions, further suggesting that there was no competitive adsorption among BTEX compounds in mixture for the available active sites on tyre surfaces.

## Simultaneous sorption and bioremoval of BTEX/*cis*-DCE/TCE mixture

Figure 3 shows the growth of *P. plecoglossicida*, previously isolated from a heavily oil-contaminated site, on toluene with or without the presence of tyres (0.1, 0.5, and 1.0 g). According to Mao et al. (2013), *P. plecoglossicida* is a bacterium, physically and genetically closely related to *Pseudomonas*

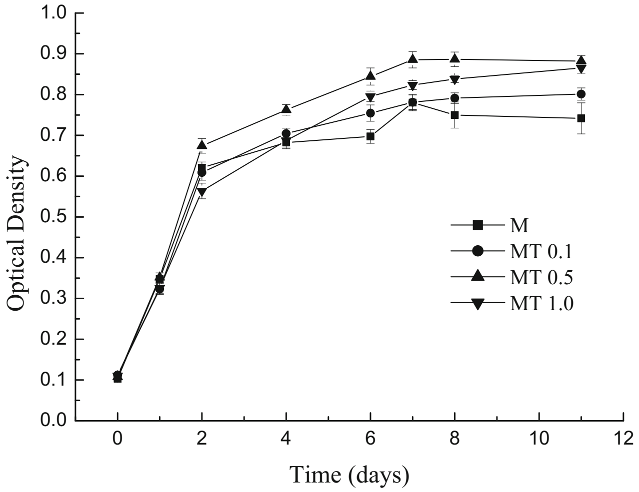

**Fig. 3** Microbial growth after 11 days of incubation using BTEX as a growth substrate (300 mg/L). *M* represents microorganisms only; *MT* 0.1, 0.5, and 1.0 represent microorganisms present together with different weights (g) of scrap tyres (0.1, 0.5, and 1.0 g)

*putida. P. putida* is known utilizing toluene as the sole source of carbon and energy for growth, and the catabolic pathway is carried out by a multicomponent enzyme system, toluene dioxygenase (Zylstra and Gibson 1989).

When the scrap tyre was present as a matrix for the microbial immobilization, the microorganism grew better showing a higher OD, compared to the suspended microbial system, and the more tyres were provided, the better the microbial growth. The statistical analysis (one-way ANOVA) indicated that the difference between freely suspended and immobilized systems (both with 0.1, 0.5, and 1.0 g of tyres) was statistically significant ($P<0.05$).

Table 1 shows the removal efficiencies for individual compounds under different conditions after 5 days of incubation. The abiotic losses of BTEX, *cis*-DCE, and TCE in a mixture, mainly due to volatilization and/or sorption to the surface of serum bottle or stopper, were almost negligible (data not shown). Scrap tyres were shown with the higher sorption capacities towards BTEX compounds, compared to *cis*-DCE and TCE. The adsorption of toluene, ethylbenzene, xylenes, and TCE did not increase apparently with the increased tyre weight, probably due to their adsorption to the tyre surface followed by the establishment of equilibrium between adsorbed contaminants and those that remained freely in the solution. A similar behaviour was also observed by Yao et al. (2014) during the adsorption of arsenic from aqueous solutions onto iron oxide/activated carbon magnetic composite. In comparison, benzene and *cis*-DCE were shown with the highest increase of adsorption (around 17 %) with the increased tyre weight.

Tyre is generally considered with a non-wetting surface with predominantly hydrophobic nature but also contains some hydrophilic functional groups according to the results from the thermos gravimetric analysis of tyres (data not shown). The presence of hydrophilic groups on the tyre surface may thus be attributed to the higher adsorption of benzene and *cis*-DCE.

When microorganisms (*P. plecoglossicida*) were present together with 4.0 g of tyre pieces (19.2 cm$^2$ of surface area provided), benzene, toluene, and ethylbenzene were completely removed. In comparison, Li et al. (2014) previously reported that this microbial isolate was not able to utilize xylenes as substrates that much. Atlas (1981) reported that the microbial utilization of hydrocarbons is remarkably dependent on the contaminant's chemical structure and an extensive methyl substitution can inhibit the initial oxidation of contaminant to some extent, and this further justifies the lower bioremoval efficiencies for xylenes obtained in this study (Table 1). In case of two chloroethenes, when *P. plecoglossicida* was present without tyres, while utilizing BTEX as growth substrates, 20.4 % of *cis*-DCE and 36.3 % of TCE were cometabolized. One possible reason for these lower bioremoval efficiencies for these two chloroethenes would be due to the lack of primary substrate (toluene in BTEX mixture) and insufficient oxygenases induced for their cometabolic removal (Lu et al. 1998). The presence of other primary substrates in the BTEX mixture could act as inhibitors for the microbial cometabolism of CAHs (Hopkins and McCarty 1995).

On the other hand, regardless of the amount of tyre pieces (surface area) used, xylenes were sorbed more to the tyre surface, followed by benzene and toluene, and two chloroethenes, in a mixture after 5 days (Table 1). In cases of immobilized (tyres+microorganisms) treatments, the highest combined (sorption+biological) removal efficiencies for each compound were achieved when 4.0 g of tyre pieces (19.2 cm$^2$ surface area) was provided, except for *cis*-DCE and TCE (the highest combined removal efficiencies for each, at 61.9 and 73.6 %, with 1.5 and 3.0 g of tyre pieces provided). Compared to the treatments with tyres only, the bioremoval efficiencies for toluene accounted for about 17 % more (in addition to about 83 % removal by sorption), followed by 11 % more bioremoval for benzene (89 % removal by sorption) (Table 1).

The addition of different amounts of tyres did not enhance the bioremoval of *cis*-DCE and TCE (Table 1). Substrate limitation is a common concern due to the fact that BTEX, *cis*-DCE, and TCE in mixture must compete for the same enzyme

**Table 1** Removal efficiencies (%) for each compound in mixture under different conditions after 5 days of incubation

| Surface area (cm$^2$) | Benzene | Toluene | Ethylbenzene | *m,p*-Xylene | *o*-Xylene | *cis*-DCE | TCE |
|---|---|---|---|---|---|---|---|
| $T^{1.5}$7.2 | 70.6±3.2 | 75.4±2.3 | 92.8±1.4 | 92.0±1.1 | 92.1±0.9 | 42.6±3.4 | 54.1±3.4 |
| $T^{3.0}$14.4 | 84.2±2.1 | 82.4±1.2 | 95.6±1.8 | 96.8±0.4 | 97.2±0.3 | 53.7±3.1 | 64.0±2.8 |
| $T^{4.0}$19.2 | 88.6±1.3 | 82.8±2.2 | 98.3±0.3 | 97.6±0.2 | 97.9±0.4 | 64.6±2.2 | 62.5±2.5 |
| $MT^{1.5}$7.2 | 99.6±0.2 | 100 | 95.5±0.7 | 96.5±0.3 | 98.2±0.2 | 61.9±1.7 | 70.3±1.7 |
| $MT^{3.0}$14.4 | 100 | 100 | 97.8±0.4 | 96.3±0.4 | 98.5±0.3 | 60.8±2.4 | 73.6±2.5 |
| $MT^{4.0}$19.2 | 100 | 100 | 100 | 96.8±0.3 | 99.0±0.1 | 61.6±0.9 | 71.0±2.8 |
| M | 99.5±0.2 | 97.6±0.1 | 88.0±0.4 | 64.5±3.3 | 56.0±2.7 | 20.4±1.2 | 36.3±2.0 |

Removal efficiencies are shown in mean value±standard deviation; 1.5, 3.0, and 4.0 represent different weights of tyres used (1.5, 3.0, and 4.0 g)

*T* tyres only, without microorganisms; *MT* tyres together with microorganisms; *M* microorganisms only, without tyres site. The substrate (BTEX) is almost removed by *P. plecoglossicida* within 1 day of incubation, and the rate and extent of the cometabolic transformation of both chloroethenes may be limited by the absence of growth substrate, which can act as an inducer for enzyme production. The removal of *cis*-DCE by adsorption only with 4 g of tyre pieces was higher (64.6 %) compared to the combined adsorption/biological process (61.6 %). The decrease of *cis*-DCE removal efficiency might be due to the generation of by-products during the bioremoval of BTEX, *cis*-DCE, and TCE, with the high binding affinities that can directly compete for the available adsorption sites and consequently displace *cis*-DCE from the tyre surface.

In general, the biological process played a major role in the removal of benzene, toluene, and ethylbenzene, while xylenes, *cis*-DCE, and TCE were mainly removed by the adsorption on tyre surfaces. Fletcher and Loeb (1979) used *Pseudomonas* NCIMB 2021 in laboratory experiments and found out that the bacterial adhesion occurred at hydrophobic surfaces more rapidly than at hydrophilic surfaces. According to Christensen et al. (1985), the extracellular polymers in *Pseudomonas* were changed with the stage of the growth cycle. During the exponential growth, cells attached poorly to hydrophobic surfaces. However, there was a 25-fold increase in the number of adhered cells when the bacteria entered the stationary phase. This behaviour coincided with the production of a hydrophobic carbohydrate polymer quite different in structure from the one produced earlier in the growth cycle. Tyre is predominantly hydrophobic and consequently can be used as a favourable matrix for the adhesion of *P. plecoglossicida* on surface. When the colony-forming unit (CFU) was used to estimate the number of viable bacteria, freely suspended in solution as well as attached on tyre surfaces, results showed that 20–25 % of microorganisms adhered to the tyre surface, suggesting tyres as a promising matrix for the development of hybrid technologies employing physical (adsorption) and biological (immobilization) methods to clean up the mixture-contaminated sites.

Figure 4 further shows the removal trends for TCE and *cis*-DCE at different treatments after 5 days of incubation. As shown, when tyres (1.5 g) were present with or without the presence of microorganisms, the concentrations of both chloroethenes sharply decreased within a day (around 50 % of TCE and 35 % of *cis*-DCE removed) after the injection, and then leveled off in case of tyres only. In comparison, however, with microorganisms immobilized on tyre surface, the two chloroethenes were further removed, resulting in the additional removal efficiencies of 13 and 16 % for *cis*-DCE and TCE, respectively, after 5 days (Table 1). For the immobilized treatments (tyres+microorganisms), the concentrations of both chloroethenes showed continuous smooth declines, caused by the mineralization of growth substrates (BTEX) in the first 3 days, then followed by the cometabolism of TCE and *cis*-DCE.

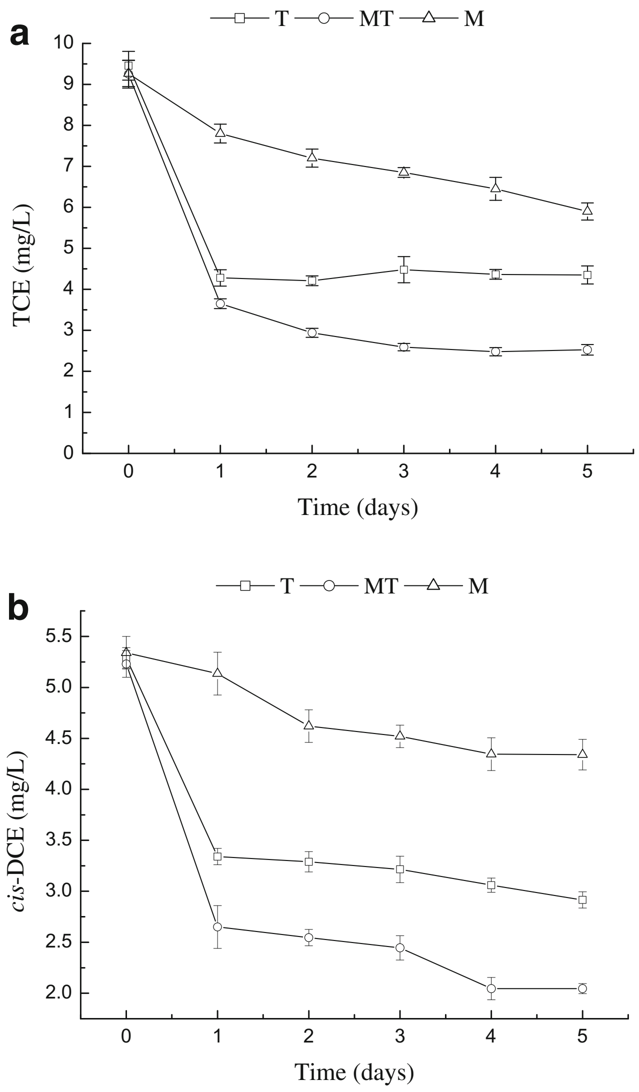

**Fig. 4** Removal trends for TCE (**a**) and *cis*-DCE (**b**) after 5 days of incubation using BTEX (300 mg/L) as a growth substrate and 1.5 g of tyres

The concomitant use of scrap tyres and microorganisms as a hybrid physical/biological remediation technology in this study showed the removal of BTEX and two chloroethenes from mixture can be carried out to some extent and can be further optimized to achieve higher removal efficiencies especially for chloroethenes. The utilization of tyres as sorbents has some advantages compared to the carbon-based sorbents previously used (Villacañas et al. 2006; Li et al. 2010; Yakout and Daifullah 2013; Zhang et al. 2013), mainly due to no need for pre-chemical or thermal treatment and easy acquisition without any cost as waste material. In addition, scrap tyres can be used as a matrix for the microbial growth/immobilization, providing a more favourable environment for the efficient substrate use (Hamid et al. 2014). Future study would include the bioregeneration of tyres (as a biologically activated carbon) for the long-term treatment performance of this hybrid remediation technology.

## Conclusion

The current study demonstrates that scrap tyre is a good candidate to remove such volatile organic compounds (VOCs) as BTEX, *cis*-DCE, and TCE, due to its remarkable sorption property as well as the capability to immobilize microorganisms. Scrap tyres showed high sorption capacities towards BTEX compounds. When microorganisms and tyres were used together, the removal efficiencies for each VOC in a mixture were higher compared to the treatments where microorganisms and tyres are separately used, suggesting a promising reutilization of waste tyres as sorbents and immobilization matrices for the contaminant removal from mixed waste-contaminated environments. Further studies are in progress to evaluate whether the immobilized microorganisms possess the same initial removal efficiency after the reutilization cycles, including whether higher concentrations of contaminants can also be applied.

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