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Response of a solid–liquid two-phase partitioning bioreactor to transient BTEX loadings

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ABSTRACT

A two-phase partitioning bioreactor (TPPB) consisting of an aqueous phase containing a bacterial consortium and a polymeric phase of silicone rubber pellets (solid volume fraction 0.1) was used to treat a gaseous waste stream containing benzene, toluene, ethylbenzene and *o*-xylene (BTEX). The function of the solid polymer phase was to absorb/desorb the gaseous volatile organic compounds providing a buffering effect to protect the cells from high transient loadings and to sequester the BTEX for subsequent degradation. The TPPB was subjected to high and fluctuating inlet loadings of BTEX in the form of 4 h step changes of 2, 4, 6 and 10 times the nominal inlet loading of 60 g m^{-3} h^{-1} total BTEX in approximately equal amounts, and removal efficiencies and elimination capacities were determined. It was found that overall removal efficiencies of greater than 95% can be achieved while obtaining overall elimination capacities of up to 282 g m^{-3} h^{-1} during transient operation and TPPB operation succumbs to toxic substrate levels between step changes of 6 and 10 times the nominal loading value (360–600 g m^{-3} h^{-1}). BTEX concentrations in the aqueous phase and the polymer phase of the TPPB were monitored throughout the imposed step changes to determine the extent to which the sequestering phase can buffer the aqueous phase from BTEX. With the polymer phase comprising only 10% of the total working volume of the reactor, the polymer beads accounted for up to 93%, 91% and 70% of the total BTEX present in the working volume for step changes of 2, 4 and 6 times the nominal loading, respectively.

1. Introduction

Benzene, toluene, ethylbenzene and *o*-xylene, collectively known as BTEX, are volatile compounds that are commonly used in many sectors of the petroleum industry and are often emitted in gaseous waste streams. Traditionally, physical or chemical treatment methods have been used in order to stay within regulation emission guidelines (Khan and Ghoshal, 2000), however, biofilters, which provide a low-cost, energy efficient and effective alternative to the destruction of toxic volatile chemicals (Datta and Allen, 2005), have been shown to be successful in treating low concentrations (less than 5 g m^{-3}) of BTEX components in gaseous waste streams (Oh and Bartha, 1997; Torkian et al., 2003). Although biofilters are effective for the treatment of low concentrations of contaminants, there has been recent interest in biological BTEX treatment beyond the current abilities of biofilters; that is, the biological treatment of high and fluctuating loadings of BTEX (Jutras et al., 1997; Stewart et al., 2001).

Two-phase partitioning bioreactors (TPPBs) have recently been shown to be extremely effective for the treatment of high and fluctuating loadings of contaminants in waste gas streams (Van Groenestijn and Lake, 1999; Djeribi et al., 2005; Nielsen et al., 2005a; Boudreau and Daugulis, 2006; Muñoz et al., 2006). TPPBs consist of an aqueous, cell containing phase and a nontoxic, nonbioavailable second phase that traditionally consists of an immiscible organic solvent. These systems are effective for the treatment of toxic contaminants with relatively low water solubility due to the ability of the target compound to partition into the second phase in much higher concentrations than the cell containing aqueous phase, thus buffering the cells from elevated concentrations. As the target compounds are metabolized in the aqueous phase, they are continuously delivered from the sequestering phase based on the metabolic demand of the organisms and the maintenance of thermodynamic equilibrium between the two phases. Due to the difficulty in identifying an organic liquid solvent that would be nonbioavailable to a wide array of bacteria, the application of TPPBs with an organic solvent second phase has been limited to an individual, or very limited mixtures of, volatile organic compounds (VOC) and pure strains of microorganisms. However, to effectively degrade mixtures of BTEX components a bacterial consortium is needed (Bielefeldt and Stensel, 1999). Bacterial consortia have been used in TPPBs with a silicone oil second phase (Van Groenestijn and Lake, 1999; Djeribi et al., 2005), as silicone oil is nonbioavailable to a wide range of bacteria. However, organic solvent second phases can be selected based on their

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affinity to the target contaminant, whereas silicone oil cannot, as its properties are fixed.

A more effective second phase that provides the ability to use a bacterial consortium in a TPPB includes replacing the organic liquid phase with a solid, polymeric phase, as polymers are generally nonbioavailable to a wide range of bacteria. Recent research using solid–liquid TPPBs has shown the effective uptake, release and subsequent degradation of high concentrations of phenol using a bacterial consortium (Prpich and Daugulis, 2005). The use of polymers as a second phase has additional advantages over organic liquids and silicone oil, as they are generally very inexpensive, non-volatile, can be formed into many shapes and sizes, and can be tailored to a particular target molecule through monomer selection, cross-linking, and polymer processing.

In the present work, a solid–liquid TPPB has been used to treat high and fluctuating loadings of a mixture of BTEX simultaneously in a continuous waste gas stream using a bacterial consortium. This is the first report of the use of a solid–liquid TPPB wherein a bacterial consortium aids in providing efficient treatment, as it provides more effective and complete degradation of BTEX mixtures in comparison to pure strains (Alvarez and Vogel, 1991; Attaway and Schmidt, 2002). Bacterial consortia also provide a robustness that is more practical in industrial settings. Moreover, a TPPB has not been used for such a mixture of contaminants to date. Concentrations of BTEX components in the gaseous, aqueous and polymer phases were monitored during imposed inlet concentration step changes in order to quantify the degree to which the polymer phase buffered toxicity in the aqueous phase. Although the ability of TPPBs to outperform bioreactors without a second phase has been previously attributed to the ability of the polymer phase to rapidly sequester large concentrations of target compounds (Boudreau and Daugulis, 2006), it has not been demonstrated to be the case prior to the current study. Finally, this paper also provides results that prompt discussion of current methods for reporting elimination capacity.

2. Materials and methods

2.1. Chemicals

All chemicals used in the fermentation medium were obtained from either Sigma Aldrich (Canada) or Fisher Scientific (Canada). Benzene (99% assay) and *o*-xylene (98%, HPLC grade) were obtained from Sigma Aldrich, and toluene and ethylbenzene were obtained from Fisher Scientific. Tryptic Soy Broth (TSB) for inoculum preparation was obtained from DIFCO (Canada).

2.2. Polymer selection

In order to select an appropriate polymer for the uptake and release of BTEX in a TPPB, a variety of polymers with different properties was tested for BTEX diffusivity, BTEX polymer/aqueous partitioning and bioavailability. The methods for these tests are described elsewhere (Amsden et al., 2003; Prpich and Daugulis, 2004). The polymers tested include; nylon 6,6 (Zytel 42-A®, Dupont), ethylene vinyl acetate (ELVAX 360®, Dupont), styrene–butadiene copolymer (Scientific Polymer Products Inc.), silicone rubber (GE-Mastercraft), poly(butylene terephthalate) (Hytrel 8206®, Dupont) and polyurethane elastomer (Desmopan 9370A®, Bayer Materials Science). On the basis of the aforementioned criteria, it was found that silicone rubber was the most favourable polymer for use in the TPPB.

2.3. Bacterial consortium enrichment

A bacterial consortium to metabolize BTEX components was enriched from petroleum contaminated soil (Sarnia, Ontario) and a commercial mixture of petroleum hydrocarbon metabolizing bacteria composed of strains of *Pseudomonas* (Petrox-1, CL Solutions). The enrichment was completed by adding 0.5 g of both the contaminated soil and the commercial mixture to a 1.5 L bioreactor with a 1 L working volume consisting of medium with the following composition ($g\,L^{-1}$); 7 $(NH_4)_2SO_4$, 0.75 $MgSO_4 \cdot 7H_2O$, 6.6 K_2HPO_4, 8.42 KH_2PO_4, and 1 mL L^{-1} trace elements. Trace element solution was prepared as follows ($g\,L^{-1}$): 16.2 $FeCl_3 \cdot 6H_2O$, 9.44 $CaHPO_4$, 0.15 $CuSO_4 \cdot 5H_2O$, and 40 citric acid (Davidson and Daugulis, 2003). This medium formulation was used for all experiments in this study.

The bioreactor was maintained at a temperature of 30 °C, pH of 6.9 and agitation of 800 rpm. Gaseous BTEX was continuously fed into the reactor as the sole carbon source at a rate of 50 g m^{-3} h^{-1} with approximately equal amounts of each component along with air at a flow rate of 1 L min^{-1}. The continuous addition and removal of medium was undertaken to achieve an aqueous dilution rate of 0.1 h^{-1} in order to effectively washout the bacteria that were not metabolizing the BTEX. This enrichment was conducted for 12 d, and the resulting culture was stored for long-term maintenance in 12% dimethyl-sulfoxide at −70 °C in 1.5 mL vials.

A denatured gradient gel electrophoresis was completed by Microbial Insights (TN, USA) using 16S rRNA to determine the dominant members of the microbial population. It was found that the bacterial consortium used in this study was composed of seven unique species of *Pseudomonas*, 5 with an excellent similarity index and 2 with a good similarity index.

2.4. Experimental setup

The TPPB setup for experimentation performed in this study is shown in Fig. 1. The bioreactor used was a 6.5 L New Brunswick BioFlo III with a working volume of 3 L. 10% of this working volume consisted of silicone rubber polymer beads with a diameter of 2.2 mm and density of 1150 g L^{-1} and the other 90% consisted of aqueous medium. This polymer volume was found to be the largest fraction possible while avoiding operational difficulties such as clogging in the bioreactor. The bioreactor was automatically maintained throughout all experimentation at a temperature of 30 °C, an agitation of 800 rpm to disperse the polymer beads in the aqueous phase and a pH of 6.9 (by the addition of 6 M KOH). Dissolved oxygen was continuously monitored using a polarographic-membrane electrode (Broadley and James Corp.) and automatically recorded. The nominal operating point from which step changes were imposed was a loading of approximately 60 g m^{-3} h^{-1} which consisted of a total gas flow rate of 0.55 L min^{-1} (enriched BTEX gas flow and makeup aeration) and an approximate average concentration of 5.5 g m^{-3} total BTEX in approximately equal parts. Empty bed residence time (EBRT) for all experimentation was 5.5 min. The inoculum for the TPPB was prepared by adding 1 mL of the culture stored for long-term maintenance to 100 mL of sterile TSB medium and incubating the mixture for 24 h at 180 rpm and 30 °C. The resulting culture was then centrifuged, resuspended in medium and added in an appropriate volume to the TPPB to obtain an initial cell concentration of 0.5 g L^{-1}.

2.5. Step changes

After approximately 200 h of TPPB operation at the nominal loading, the system reached a characteristic quasi-steady state biomass concentration that arises in response to the carbon source being directed towards maintenance energy as described by Nielsen et al. (2005b). At this point, 4 h step changes were imposed on the system in order to simulate fluctuations of high BTEX loadings in the inlet gas stream. Step changes were performed at approximately 2, 4, 6 and 10 times the nominal loading (2×, 4×,

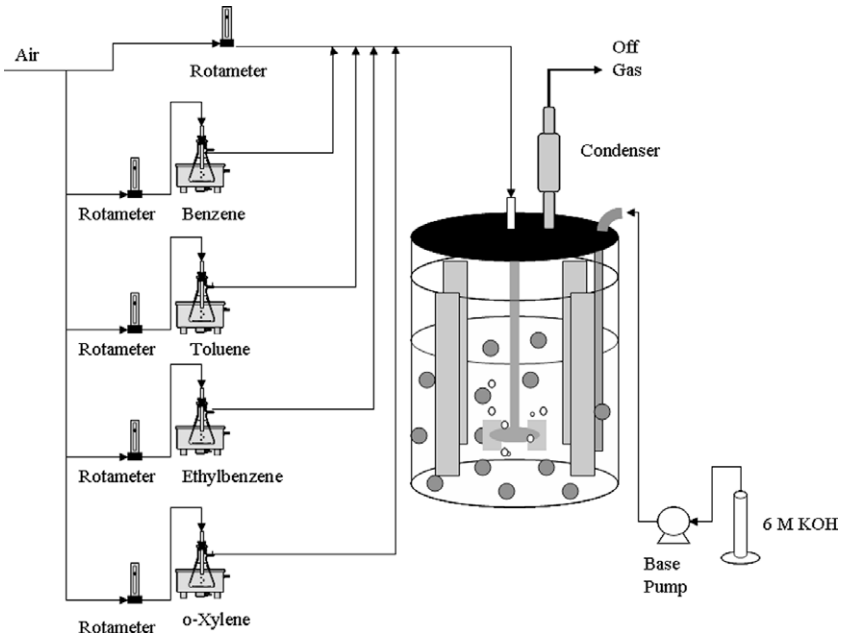

Fig. 1. TPPB setup for BTEX treatment.

6× and 10×, respectively). During step changes, the total gas flow rate was kept constant at approximately 0.55 L min^{-1} and only the BTEX concentration was increased in order to increase loading. Prior to the system reaching quasi-steady state and during all step changes, BTEX components in the inlet gas stream were maintained at approximately equal concentrations.

2.6. Sampling procedure

Prior to achieving quasi-steady state operation, inlet gas samples were taken periodically (12–24 h) and analyzed using GC/FID using a method described elsewhere (Littlejohns and Daugulis, 2008). Aqueous phase samples from the reactor were also taken periodically in order to determine biomass concentration and identify when the system had reached quasi-steady state. Biomass concentration was determined by optical density measurements with a Biochrom Ultrospec 3000 UV/vis spectrophotometer (Biochrom, Ltd., UK) at 600 nm. During the imposed step changes, inlet and outlet BTEX concentrations and biomass measurements were taken approximately every 13 min. In addition, polymer phase BTEX concentrations for step changes of 2×, 4× and 6× and liquid phase BTEX concentrations for step changes of 2×, 4×, 6× and 10× were determined approximately every 30–50 min. Polymer phase concentrations throughout the step change of 10× were regrettably not measured as the system was clearly failing and the information was deemed unnecessary as the biological component of the TPPB was not functioning. Only a polymer concentration for the 10× loading at t = 182 min is reported in this study.

To determine aqueous phase and polymer phase BTEX concentrations, two samples were taken simultaneously from the bioreactor; an aqueous sample consisting of 10 mL of the aqueous phase and an aqueous/polymer sample consisting of 10 mL of combined polymer and aqueous phases. Aqueous phase samples and aqueous/polymer samples were withdrawn from a port in the bioreactor using a 10 mL pipette with a narrow inlet hole and a 10 mL pipette with an enlarged inlet hole, respectively, in order to disallow/allow entry of the polymers into the sample. After the sample was withdrawn from the bioreactor it was rapidly delivered into an airtight 125 mL amber bottle containing approximately 5 drops of phosphoric acid to immediately kill all biomass and capped with a Teflon lined, silicone rubber septum. Both the aqueous samples and polymer/aqueous samples in the amber bottles were permitted to equilibrate for 24 h at 30 °C and the headspace from each bottle was sampled and measured using GC/FID. Concentrations of BTEX in the aqueous phase of the bioreactor were determined using Eq. (1).

$$S_{i,\mathrm{R,aq}} = \frac{\left(S_{i,\mathrm{g,aq}} V_{\mathrm{g,aq}}\right) + \left(\frac{S_{i,\mathrm{g,aq}}}{H_i} V_{\mathrm{L,aq}}\right)}{V_{\mathrm{L,aq}}} \tag{1}$$

where $S_{i,\mathrm{R,aq}}$ is the concentration of species i (one of benzene, toluene, ethylbenzene or o-xylene) in the aqueous phase of the bioreactor (g m^{-3}), $S_{i,\mathrm{g,aq}}$ is the concentration of species i in the gas phase at equilibrium in the glass bottle containing the aqueous phase sample (g m^{-3}), H_i is the Henry's constant for species i (mg L^{-1} gas phase L mg^{-1} liquid phase), $V_{\mathrm{g,aq}}$ is the volume of headspace in the glass bottle containing the aqueous phase sample (m^3), and $V_{\mathrm{L,aq}}$ is the volume of the aqueous phase sample extracted from the bioreactor (m^3).

Concentrations of BTEX in the polymer phase were determined using Eq. (2).

$$S_{i,\mathrm{R,poly}} = \frac{\left(S_{i,\mathrm{g,poly}}\ V_{\mathrm{g,poly}}\right) + \left(\frac{S_{i,\mathrm{g,poly}}}{H_i}\ V_{\mathrm{L,poly}}\right) + \left(\frac{S_{i,\mathrm{g,poly}}}{H_i}\ K_i\ V_{\mathrm{P,poly}}\right) - \left(S_{i,\mathrm{R,aq}}\ V_{\mathrm{L,poly}}\right)}{V_{\mathrm{P,poly}}} \tag{2}$$

where $S_{i,\mathrm{R,poly}}$ is the concentration of species i in the polymer phase of the bioreactor (g m^{-3}), $S_{i,\mathrm{g,poly}}$ is the concentration of species i in

the gas phase at equilibrium in the glass bottle containing the aqueous/polymer phase sample (g m^{-3}), K_i is the partition coefficient for species i (mg L^{-1} gas phase L mg^{-1} liquid phase), $V_{g,poly}$ is the volume of headspace in the glass bottle containing the aqueous/polymer phase sample (m^3), $V_{L,poly}$ is the volume of the aqueous phase in the glass bottle containing the aqueous/polymer phase sample and $V_{P,poly}$ is the volume of polymer in the aqueous/polymer phase sample (m^3).

Henry's coefficients used in Eqs. (1) and (2) were determined by adding a known volume of each BTEX compound into an airtight 125 mL septa bottle containing 50 mL of water. The bottle was then left for 24 h at 30 °C to equilibrate and the headspace concentration was measured allowing for the determination of the aqueous phase concentration. The Henry's constants for B, T, E and X were found to be 0.26, 0.35, 0.43 and 0.25 mg L^{-1} gas phase L mg^{-1} liquid phase, respectively. Partition coefficients used in Eq. (2) were determined during the polymer selection experiments, and the polymer volume used in Eq. (2) was estimated by multiplying the number of polymer beads taken in a particular sample by an average bead volume calculated using an average polymer bead diameter (n = 100).

2.7. Performance quantification

The performance of VOC treatment systems is often quantified using two instantaneous measurements; removal efficiency (RE) (Eq. (3)) and elimination capacity (EC) (Eq. (4)).

$$\mathrm{RE} = \frac{Q_g\left(S_g^{in} - S_g^{out}\right)}{Q_g S_g^{in}} \times 100\% \quad (3)$$

$$\mathrm{EC} = \frac{Q_g(S_g^{in} - S_g^{out})}{V_{total}} \quad (4)$$

where Q_g is the gas flow rate (L h^{-1}), S_g^{in} is the instantaneous concentration of BTEX in the inlet gas (g m^{-3}), S_g^{out} is the instantaneous concentration of BTEX in the outlet gas (g m^{-3}) and V_{total} is the working volume of the TPPB (L). The instantaneous REs and ECs were determined prior to and during each step change. Overall REs and ECs (calculated from the initiation of the transient until the system returning to quasi-steady state) will be discussed in a later section.

3. Results and discussion

3.1. TPPB performance

During quasi-steady state operation of the TPPB prior to the application of step changes, an instantaneous maximum RE of 99% and instantaneous minimum RE of 95% were obtained during all four experimental runs. During BTEX concentrations step changes of 2× and 4×, maximum ECs of 157 and 285 g m^{-3} h^{-1} were achieved with the RE remaining above 95% (Fig. 2a and b). However, when a step change of 6× was imposed on the system, the RE ranged from a maximum value of 95% to a minimum value of 68% (Fig. 2c). Furthermore, following the step change, the RE fell

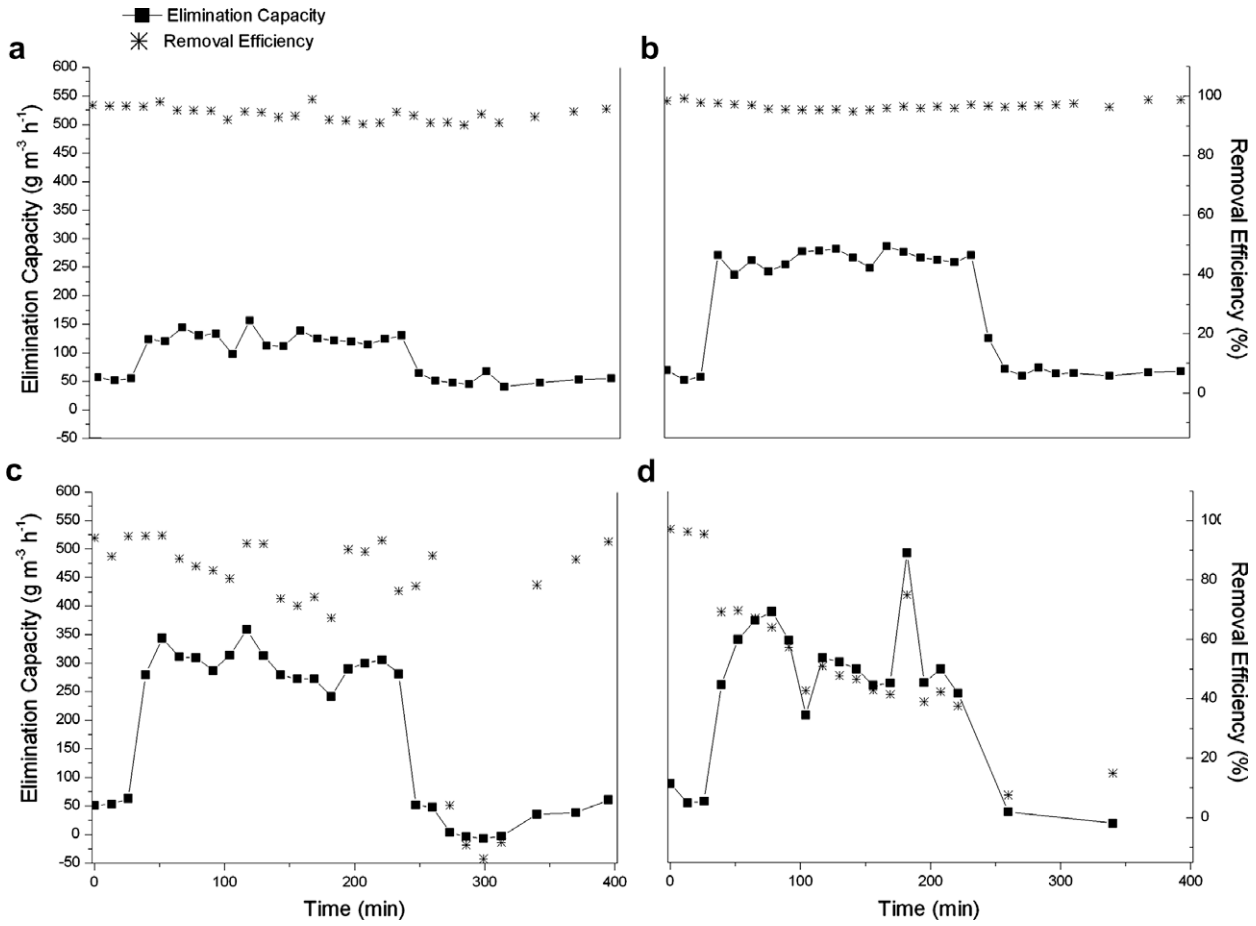

Fig. 2. Total BTEX elimination capacities and removal efficiencies for (a) 2× step change, (b) 4× step change, (c) 6× step change and (d) 10× step change. Vertical dashed lines indicate step change time period.

to a minimum of −10% but recovered to original levels in less than 60 min after the loading was returned to nominal levels. Although the performance decreased relative to the step changes of 2× and 4×, the TPPB system was able to eventually recover to performance levels that were obtained before the introduction of the step change. Shown in Fig. 2d are the instantaneous ECs and REs for a step change of 10×. The RE and EC drop rapidly after the imposition of the step change and do not recover after step change completion. Samples taken 24 h after the completion of the step change (data not shown) confirm that the system did not recover as the EC was <10 g m^{-3} h^{-1} for nominal loadings. Fig. 2 reveals that the maximum step change loading after which the TPPB could subsequently recover to original RE levels lies between 360 and 600 g m^{-3} h^{-1}. The step change of 6× does not appear to be mass transfer limited (gas to liquid), as large removal efficiencies are shown at the beginning of the step change; however, the step change of 10× does appear to be mass transfer limited (gas to liquid) as removal efficiencies drop immediately after step change initiation.

During all nominal loadings and step change conditions, the RE for individual BTEX compounds was in the order benzene > toluene > ethylbenzene > *o*-xylene (data not shown). This was an expected result, as the degradation kinetics for this particular bacterial consortium have been previously characterized (Littlejohns and Daugulis, 2008) and degradation rates were found to be in an identical order to those found in the current study for benzene, toluene and ethylbenzene. It was also previously determined that *o*-xylene is cometabolized in the presence of both benzene and toluene and, therefore, *o*-xylene degradation rates are expectedly lower than those for benzene and toluene. Dissolved oxygen was measured and was found to remain above 80% during each step change of 2×, 4×, 6× and 10× (data not shown). This reveals that oxygen was not limited during step change experimentation, which may be partially due to the addition of silicone rubber pellets as Littlejohns and Daugulis (2007) showed that the addition of silicone rubber pellets increases the oxygen transfer rate into the working volume of a TPPB system during dynamic operation.

3.2. Overall elimination capacities and removal efficiencies

Fig. 2c shows that subsequent to the 6 times step change, a negative EC was calculated for a short period of time (<60 min). However, the capacity of a bioreactor to eliminate compounds entering the inlet stream should intuitively be greater than or equal to 0. The operating conditions reflected in the data of Fig. 2c reveal a situation in which current reporting methods for EC are somewhat ambiguous. For dynamic situations during which a bioreactor system sequesters high concentrations of target compounds, as is particularly the case for TPPBs, reporting ECs on an instantaneous basis may not be reflecting the true permanent removal of compounds from the waste gas stream. During a step change imposed on a solid–liquid TPPB system a concentration driving force exists in the direction of the polymer. Subsequent to the step change, elevated concentrations can remain in the TPPB working volume and the same compounds that were removed earlier from the waste gas stream can be stripped and detected in the outlet gas stream, reducing instantaneous ECs. In order to account for this situation, we propose that an overall EC be reported during periods of operation starting from the beginning of the imposed dynamic until the system has returned to original quasi-steady state values. In order to approximate the overall elimination capacity ($EC_{overall}$), Eq. (5) can be used.

$$EC_{overall} = \frac{Q_g}{V_{total}\, t_{overall}} \sum_{i=1}^{n} \left(S_{g,i}^{in} - S_{g,i}^{out}\right) t_{i,i+1} \quad (5)$$

Table 1
Overall performance of TPPB

Step change	Time period of dynamic operation (min)	Average biomass (g L^{-1})	$EC_{overall}$ (g m^{-3} h^{-1})	$RE_{overall}$ (%)	Normalized $EC_{overall}$ (g m^{-3} h^{-1} g^{-1} biomass)
2×	39–249	5.9	134	94	23
4×	39–249	8.2	282	96	34
6×	39–313	6.8	230	81	34

where i is the sample number, n is the total number of samples taken during the overall period, $t_{overall}$ is the time of the overall period (min) and $t_{i,i+1}$ is the time between sample i and the next proceeding sample (min). $EC_{overall}$ for step changes of 2×, 4× and 6× can be seen in Table 1.

In addition to the current study, several authors have observed lower than expected REs subsequent to step changes in inlet loading (Marek et al., 2000; Boudreau and Daugulis, 2006). An overall RE ($RE_{overall}$) can be approximated using Eq. (6).

$$RE_{overall} = \frac{Q_g \sum_{i=1}^{n} \left(S_{g,i}^{in} - S_{g,i}^{out}\right)}{Q_g \sum_{i=1}^{n} \left(S_{g,i}^{in}\right)} \times 100\% \quad (6)$$

$RE_{overall}$ values for the present system can also be seen in Table 1.

Slightly different average quasi-steady state values for biomass concentration were obtained for each run which is shown in Table 1. Therefore, $EC_{overall}$ values were normalized with respect to biomass concentration and are also reported in Table 1. These normalized $EC_{overall}$ values consistently reveal that during the imposition of larger step changes (4× and 6×) each gram of cells is able to eliminate approximately 34 g L^{-1} h^{-1}, which suggests that this is the maximum utilization rate for the bacterial consortium in this TPPB system.

3.3. Aqueous phase and polymer phase concentrations

Aqueous phase concentrations for individual BTEX components for all step change conditions can be seen in Fig. 3. Data from the smallest step change of 2× (approximately 120 g m^{-3} h^{-1}), seen in Fig. 3a, show that during the step change (time 39–249 min), concentrations of all BTEX components increase slightly relative to concentrations before the step change, however, remain at <2 mg L^{-1} in all cases. Following the step change, aqueous concentrations gradually drop back to levels similar to those before the imposition of the step change due to a combination of physical desorption of BTEX from the polymer to the aqueous phase and microbial degradation. Fig. 3a also reveals that benzene and toluene are present in much lower concentrations in the aqueous phase than ethylbenzene and *o*-xylene.

Fig. 3b shows aqueous phase BTEX concentrations during a step change of 4× (approximately 240 g m^{-3} h^{-1}). Again, concentrations of BTEX components increase during the step change period relative to before the step change is applied, but stay below 3.5 mg L^{-1}. Also, concentrations gradually decrease to original levels after the step change is completed.

Aqueous phase BTEX concentrations during a step change of 6× are shown in Fig. 3c. Again, concentrations of all BTEX components in the aqueous phase increase during the step change, but remain below 10.5 mg L^{-1}, and decrease after the completion of the step change. However, the order of individual BTEX concentrations in the aqueous phase in Fig. 3c does not follow the expected trend that was observed during 2× and 4× step changes wherein the magnitude of individual BTEX concentrations was inversely proportional to individual degradation rates found for this particular consortium (Littlejohns and Daugulis, 2008). In contrast, the order

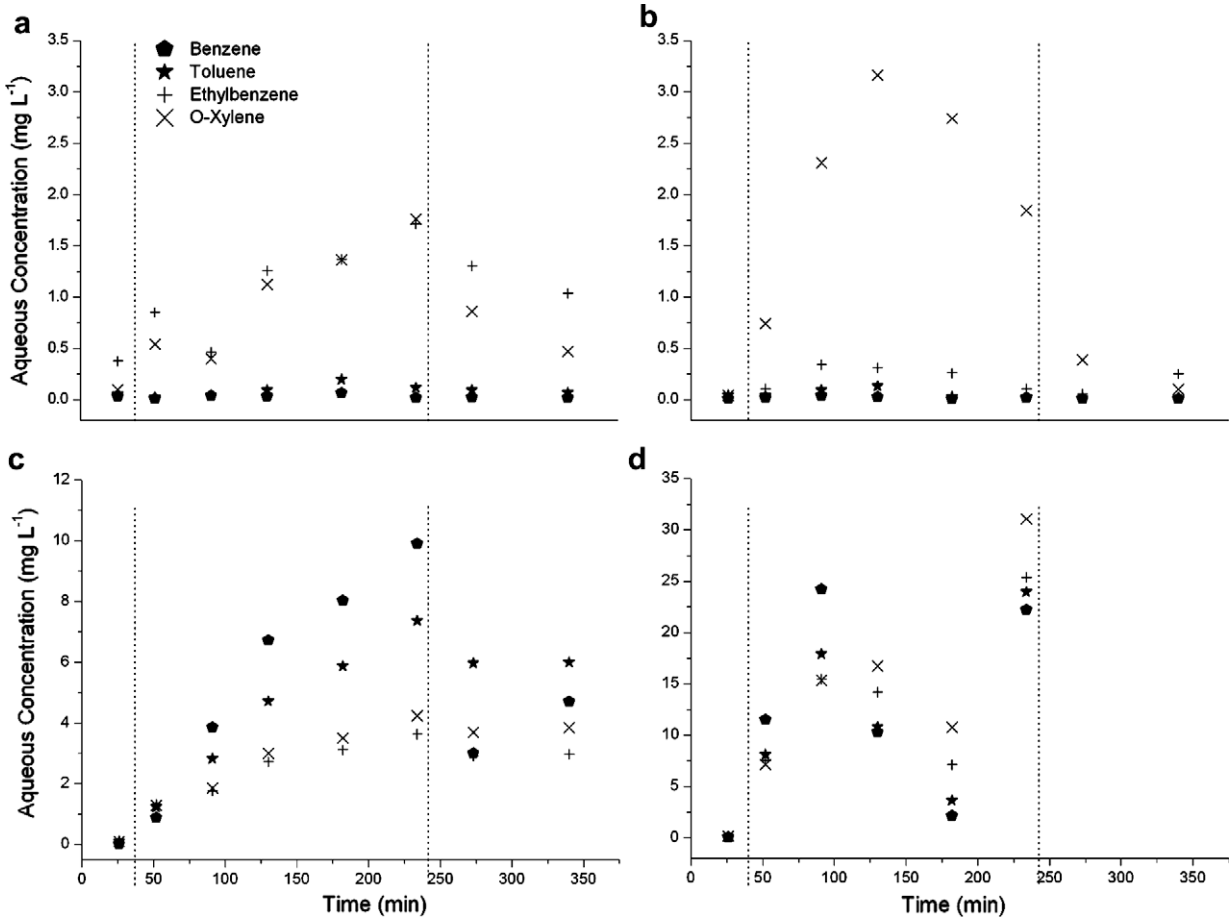

Fig. 3. Aqueous phase BTEX concentrations for (a) 2× step change, (b) 4× step change, (c) 6× step change, (d) 10× step change. Vertical dashed lines indicate step change time period.

of aqueous phase individual BTEX concentrations observed during the step change of 6× is benzene > toluene > *o*-xylene > ethylbenzene. It should be noted that this order follows what would be expected for an abiotic system, as the compounds with the highest aqueous phase concentration would be those with the highest water solubility; benzene (1780 mg L^{-1}) > toluene (515 mg L^{-1}) > *o*-xylene (198 mg L^{-1}) > ethylbenzene (152 mg L^{-1}) (Kuo, 1999). This observation can be attributed to the bacterial consortium not effectively degrading the influx of BTEX compounds caused by the step change, which is confirmed in Fig. 2c as the RE decreases significantly during the step change period. Fig. 3d shows BTEX concentrations in the aqueous phase during a step change of 10×. It can be seen that BTEX concentrations accumulate to high levels even after the completion of the step change when the BTEX loading is reduced back to nominal levels (total BTEX is 162.5 mg L^{-1} at t = 340 min), indicating that biological activity has ceased, possibly due to inhibitory aqueous phase concentrations. Although different bacterial strains have different inhibitory aqueous phase concentrations, it has been found that for *Pseudomonas putida* F1, substrate inhibition (decline of specific growth rate) occurs for benzene concentrations >20 mg L^{-1} and toluene concentrations >30 mg L^{-1} (Abuhamed et al., 2004).

BTEX concentrations in the polymer phase for step changes of 2×, 4× and 6× are shown in Fig. 4. An entire set of data for BTEX concentrations in the polymer phase for the step change of 10× was not obtained, as explained previously, but polymer concentrations at t = 182 min were determined to be 5367, 5154, 4854 and 5581 mg L^{-1} for benzene, toluene, ethylbenzene and *o*-xylene, respectively. Fig. 4 shows that concentrations in the polymer phase are much higher than the aqueous phase concentrations, as expected. For all step changes, the trends for individual BTEX components in the polymer phase are similar to those observed in the aqueous phase. An exception to this can be seen for the step change of 6×, wherein benzene has the lowest polymer phase concentration (Fig. 4c), but the highest aqueous phase concentration (Fig. 3c). This, however, is expected, as partition coefficients that were measured during the polymer phase selection process for silicone rubber revealed that BTEX components have partition coefficients (mg L^{-1} gas phase L mg^{-1} liquid phase) in the following order: ethylbenzene (593 ± 5) > *o*-xylene (414 ± 2) > toluene (200 ± 4) > benzene (62 ± 2) and thus, as benzene concentrations in the aqueous phase are not exceptionally higher than the other BTEX compounds, benzene concentrations in the polymer phase are expected to be lower than the other BTEX compounds.

An important point shown by Fig. 4 is that polymers appear to effectively buffer large concentrations of BTEX compounds in a solid–liquid TPPB. This is the first study to monitor polymer phase concentrations during transient loadings applied to a TPPB to reveal conclusively that uptake by polymers is responsible for the increased performance of a solid–liquid TPPB relative to a single phase reactor. With the polymer phase composing only 10% of the total working volume of the reactor, the polymer beads accounted for 93%, 91% and 70% of the total BTEX present in the working volume for step changes of 2×, 4× and 6×, respectively (at t = 237 min).

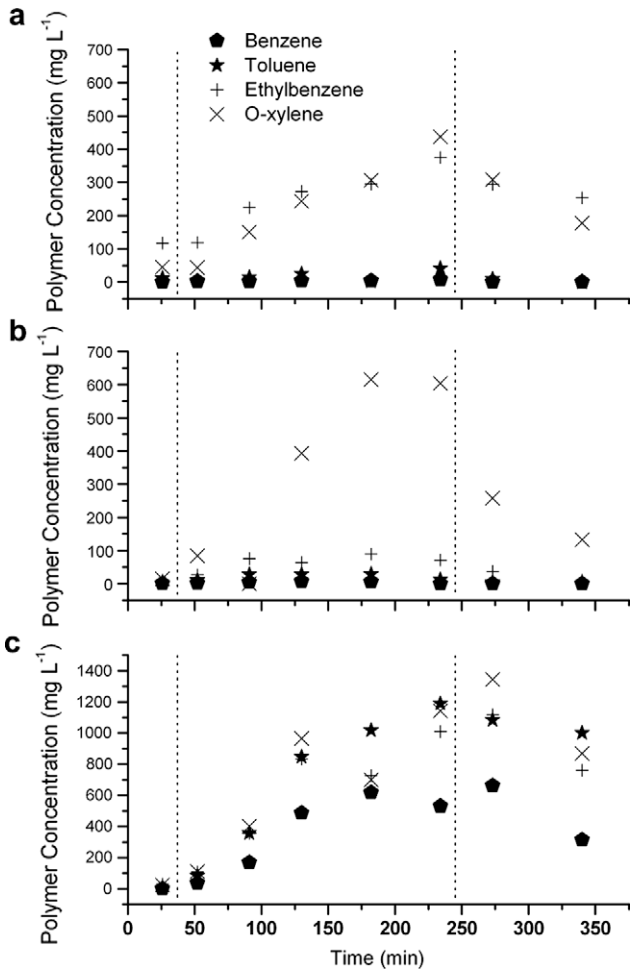

Fig. 4. Polymer phase BTEX concentrations for (a) 2× step change, (b) 4× step change, (c) 6× step change. Vertical dashed lines indicate step change time period.

3.4. System comparison and limitations

There is difficulty in comparing the system in the present work (well mixed) to biofiltration methods (plug flow) as the responsiveness of these systems are dissimilar and the step changes tested on biofilters are typically longer. However, there have been several studies that investigated the performance of biofilters during step changes in inlet concentrations of less than 5 h (Deshusses et al., 1996; Marek et al., 2000), although step changes typically tested for biofilters generally used much higher gas flow rates with lower concentrations than the system in question and render system comparisons difficult.

Technologies that can be compared to the solid–liquid TPPB include liquid–liquid TPPBs, single liquid phase bioreactors and extractive membrane bioreactors. Nielsen et al. (2005a) examined the treatment of high and fluctuating loadings of benzene in a waste gas stream using a liquid–liquid TPPB containing 33% *n*-hexadecane and a pure strain of *Achromobacter xylosoxidans* Y234 (Nielsen et al., 2005a). During 4 h loading step changes of up to 650 g m^{-3} h^{-1}, REs of greater than 99% were maintained (EBRT of 3 min). The REs during these step changes are higher than those obtained in the current study, possibly due to the combined effect of organic solvents being more rapid in the uptake of contaminants in comparison to polymer beads and the difficulty in treating a multi-component waste gas (this study) as opposed to treating a single contaminant (the work by Nielsen et al., 2005a).

Jianping et al. (2006) investigated step changes of toluene using a gas–liquid–solid airlift loop bioreactor wherein the intension of the solid (activated charcoal) was to immobilize cells, not to sequester contaminants. During 24 step changes of up to 3 times the nominal concentrations (1.5 g m^{-3}) in the inlet gas stream, an RE of greater than 80% was maintained with a maximum instantaneous EC of approximately 168 g m^{-3} h^{-1} (EBRT of 0.66 min). This performance is comparable to our system, however, the gas–liquid–solid airlift treated a single contaminant, toluene, which is notably less difficult than treating all four BTEX compounds, particularly *o*-xylene which is notoriously more recalcitrant than the other three compounds (Attaway and Schmidt, 2002). Neal and Loehr (2000) compared single liquid phase bioreactors to biofilters for the degradation of toluene containing waste gas streams for different loadings at steady state operation. The single liquid phase bioreactor obtained extremely high REs (96–99.7%) for mass loadings ranging from 5 to 30 g m^{-3} h^{-1} at low gas flow rates from 0.071 to 0.13 L min^{-1} (EBRT of 13.3 min). The RE achieved was similar for the biofilter that was operated at similar loadings with higher gas flow rates (EBRT of 1–2 min). These REs achieved are higher than the current study, but at much lower ECs.

Little investigation has been made on the response of membrane bioreactors to transient loadings in waste gas streams, and much more research is necessary to understand these systems better (Kumar et al., 2008). It is known that membrane bioreactors are limited by the surface area of the membrane (Wilderer, 1995) and are extremely sensitive to biofilm thickness (Dubus, 1995). These limitations are addressed by the use of a solid–liquid TPPBs as polymer beads provide a much larger surface area for absorption and biofilms do not form on the polymer surface (Amsden et al., 2003).

Experimental testing in this study has utilized lower waste gas flow rates than is typically used for conventional biotreatment systems in order to be able to simulate a high concentration waste gas stream while maintaining a reasonable loading for the bioreactor. Therefore, the TPPB in the current study may be limited by size constraints in the treatment of higher gas flow rates. In addition, the energy intensive stirred tank configuration used in this study may not be practical upon scale up and research entailing replacement of the stirred tank with an airlift bioreactor is currently underway in our laboratory. Also, the dynamic periods investigated were of relatively short duration and longer perturbations may lead to different system responses. Moreover, it is important to note that the effect of cycling step changes will likely affect microbial culture evolution and future work characterizing the solid–liquid TPPB during long-term operation would be beneficial. From the results of this study, it is also of intent to recognize that many of the VOC treatment devices mentioned here, such as biofilters, may also benefit from the addition of solid polymer beads into the system as a suspended or packing medium, and further work in this area is suggested.

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