

# Biodegradation of VOC mixtures of different hydrophobicities in two-phase partitioning bioreactors containing tailored polymer mixtures

María Hernández,<sup>a,b</sup> Raúl Muñoz<sup>b\*</sup> and Andrew J Daugulis<sup>a</sup>

## Abstract

**BACKGROUND:** There is a lack of systematic studies of biodegradation of mixtures of VOCs with different hydrophobicities in two-phase partitioning bioreactors (TPPBs). The role of tailored mixtures of solid polymers on the biodegradation of MEK (low hydrophobicity), toluene (moderate hydrophobicity) and hexane (high hydrophobicity) was evaluated under steady state operation and transient loading (2- and 3.6-fold 4 h step increase) in TPPBs. Two mixtures of polymer beads (A and B) were selected based on their 2 h partition coefficients for the target VOCs tested, biocompatibility and resistance to microbial attack.

**RESULTS:** The addition of polymer mixture A (20%) into the bioreactor resulted in a severe microbial inhibition, likely due to the leaching of a polymer component, however, the presence of polymer mixture B (20%) supported removal efficiencies (REs) comparable with those recorded in the absence of polymers during steady state operation (hexane, toluene and MEK REs of 7%, 76% and 98%). However, the presence of polymer mixture B supported enhanced MEK REs during the 2-fold step increase, and increased toluene removal during the 3.6-fold step increase compared with the system without polymers.

**CONCLUSIONS:** TPPBs with tailored polymer mixtures can improve process performance during VOC transient loadings, however, the interactions between the target VOCs and the solid polymers used should be a key selection criterion in order to avoid microbial inhibition during TPPB operation.

© 2010 Society of Chemical Industry

**Keywords:** volatile organic compounds; gas treatment; pollutant hydrophobicity; solid polymer mixtures; TPPBs; transient performance

## INTRODUCTION

Volatile organic compounds (VOCs) are key atmospheric pollutants due to their ozone depletion and global warming potential, toxicity and carcinogenicity.<sup>1,2</sup> VOCs are emitted from the chemical and petrochemical industries, printing and textile facilities, pulp and paper industries, etc.<sup>3</sup> The large number of solvents used in these industries results in off-gas emissions comprised of mixtures of VOCs with very diverse hydrophobicities, toxicities and biodegradabilities. Despite the fact that conventional physical/chemical technologies are being displaced by biological gas treatment methods, the performance of these processes is often constrained by the hydrophobicity of some specific VOCs (such as alkanes and terpenes), which limits pollutant transfer from the gas to the aqueous phase.<sup>4,5</sup> In addition, biological processes are also challenged by surges in the loading rate of emissions containing moderately soluble toxic VOCs. These surges, caused by variations in concentrations or flow rates, are common in industry due to process failures and operational fluctuations.<sup>6</sup>

Two-phase partitioning bioreactors (TPPBs), which are based on the addition to the biological process of solid or liquid non-aqueous phases (NAPs), can enhance both the transfer of hydrophobic VOCs and process robustness against surges in pollutant concentration. The presence of a NAP with high

affinity for the target VOC can provide a higher driving force for mass transfer and induce an increase in the gas interfacial area, which ultimately improves the transfer of hydrophobic VOCs to the aqueous phase.<sup>7,8</sup> Therefore, this technology has supported unprecedentedly high elimination capacities (ECs). For instance, steady state  $\alpha$ -pinene ECs 10 and 12 times higher than those recorded in a similar system without a liquid NAP were observed by Muñoz *et al.*, (2008)<sup>9</sup> and Montes *et al.* (2009),<sup>10</sup> respectively. On the other hand, NAPs can act as a buffer, absorbing excesses of moderately soluble toxic VOCs entering the system and delivering them back to the aqueous phase at subinhibitory concentrations. For example, Boudreau and Daugulis (2006)<sup>11</sup> reported toluene overall removal efficiencies (REs) of 90% in a stirred tank TPPB during short 10-fold step increases in toluene loading rates

\* Correspondence to: Raúl Muñoz, Department of Chemical Engineering and Environmental Technology, Valladolid University, Dr. Mergelina, s/n, 47011, Valladolid, Spain. E-mail: mutora@iq.uva.es

a Department of Chemical Engineering, Queen's University, K7L 3N6, Kingston, ON, Canada

b Department of Chemical Engineering and Environmental Technology, Valladolid University, Dr. Mergelina, s/n, 47011, Valladolid, Spain

**Table 1.** Polymer properties

Commercial name	Chemical name	Density (g cm <sup>-3</sup> )	Glass transition temperature (T <sub>g</sub> , °C)	Melt point (°C)	Hardness (shore A)	Particle size distribution (mm)
<b>Hytrel 8206</b>	thermoplastic polyester elastomer	1.17	-59	150	35–40 (shore D)	3.3 ± 0.2 × 3.8 ± 0.1
<b>Zytel 42 A</b>	polyamide 6,6	1.13	70	262	40 (Rockwell M)	2.3 ± 0.2 × 4.3 ± 0.2
<b>Zytel RSLC1000</b>	polyamide 1010	1.05	n/A	203	n/A	2.6 ± 0.1 × 4.3 ± 0.1
<b>Engage 8100</b>	ethyleneoctene copolymer	0.87	-52	60	75	4.2 ± 0.1 × 5.1 ± 0.1
<b>Engage 8842</b>	ethyleneoctene copolymer	0.86	-58	35	50	4.0 ± 0.0 × 5.3 ± 0.1
<b>Recycled rubber tires</b>	n/A	1.00	n/A	n/A	n/A	7.4 ± 0.5 × 5.4 ± 0.5 × 3.6 ± 0.5
<b>Silicone Rubber</b>	n/A	1.19	-127	150–165 (processing temperature)	18–87	2.4 ± 0.2 × 2.1 ± 0.2
<b>Kraton G 1657</b>	copolymer based on styrene and butadiene	0.90	-55	190–260 (processing temperature)	47	3.4 ± 0.2 × 3.8 ± 0.2

compared with REs of 69% in a similar system without a NAP. However, despite these promising results (achieved in TPPBs treating single VOC streams or simple mixtures of VOCs with very similar properties such as BTEX), there is a lack of systematic studies with mixtures of VOCs of different hydrophobicities.

This study was therefore conducted to assess the performance of TPPBs containing tailored mixtures of solid polymers for the biodegradation of MEK (low hydrophobicity), toluene (moderate hydrophobicity) and hexane (high hydrophobicity) under steady state and transient (surges in pollutant concentration) conditions. Hytrel 8206, Zytel 42, Zytel RSLC1000, Engage 8842, Engage 8100, silicone rubber, Kraton G1657, and recycled rubber tires were selected as model NAPs.

## MATERIALS AND METHODS

### Chemicals

All chemicals used were purchased from either Sigma-Aldrich (Canada) or Fisher Scientific (Canada). Hexane (>99%) was obtained from Fluka (Canada), toluene (99%) from Acros Organics (USA) and MEK (99%) from Sigma-Aldrich. Tryptic Soy Broth (TSB) for inoculum preparation was also obtained from Fluka. Different solid polymers were tested due to the different hydrophobicities of the target VOCs. Thus, Hytrel 8206 (thermoplastic polyester elastomer), Zytel 42 (polyamide 6, 6) and Zytel RSLC1000 (polyamide 1010) were selected based on their high affinity for polar compounds. Recycled rubber tires (RRT), polydimethylsiloxane (silicone rubber) and Engage (8842 and 8100) (polyolefin elastomers) were selected due to their high affinity for hydrophobic compounds. The main properties of these solid polymers are shown in Table 1.

### Microorganisms and culture conditions

The bacterial consortium was obtained from contaminated soil from the north site of the Sidney Tar Ponds (Nova Scotia, Canada) and a commercial mixture of petroleum hydrocarbon metabolizing bacteria composed of *Pseudomonas* strains (Petrox-1, CL Solutions). This inoculum was acclimated to hexane, toluene and MEK according to Littlejohns and Daugulis (2008).<sup>12</sup> To

furnish fresh inoculum, 1000 mL E-flasks containing 500 mL of TSB solution were inoculated with acclimated bacterial consortium and incubated for 18 h in an orbital shaker at 300 rpm and 30 °C.

### Experimental

#### Partition tests at 2 h

Polymer selection was based on 2 h partition coefficients. For each solid polymer, three sets of 162 mL serum bottles containing 3, 6 and 10 g of polymer beads and 10 mL of mineral salt medium (MSM) prepared according to Littlejohns and Daugulis (2008)<sup>12</sup>, were closed with butyl septa and sealed with aluminium caps. Each set was supplied with 4, 4 and 10 µL of hexane, toluene and MEK, respectively, and incubated under orbital agitation at 300 rpm and 30 °C for a period of 2 h. These tests were conducted in duplicate. The headspace composition of the flasks was monitored for the following 2 h by GC-FID. Aqueous and polymer concentrations of the three VOCs were calculated using Henry's Law and mass balances based on the experimental gaseous VOCs concentrations at 2 h. Gas-polymer and polymer-water partition coefficients were defined by Equations (1) and (2), respectively:

$$K_{gp} = \frac{C_{gas}}{C_{pol}} \quad (1)$$

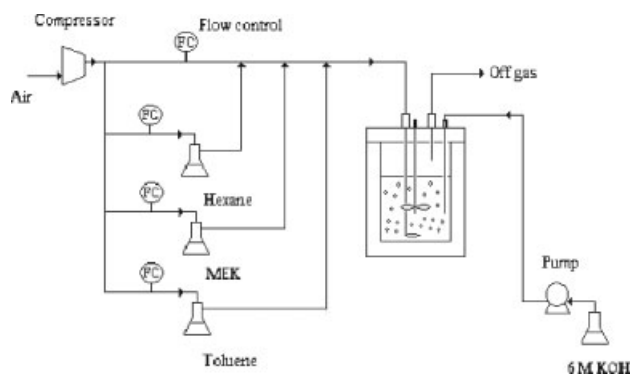
where  $C_{gas}$  and  $C_{pol}$  represent the target VOC concentration in the flask headspace and polymer phase, respectively.

$$K_{pw} = \frac{C_{pol}}{C_{water}} \quad (2)$$

where  $C_{water}$  represents the aqueous VOC concentration.

#### Polymer mixtures toxicity tests

Toxicity tests were conducted in duplicate in 162 mL serum bottles supplied with 38 mL of sterile TSB medium as a carbon source, 10 mL of a sterile polymer mixture A (50% Hytrel 8206 and 50% RRT) or B (33.3% Engage 8100, 33.3% Engage 8842 and 33.3% Hytrel 8206) and 2 mL of fresh inoculum. The flasks were closed with



**Figure 1.** Experimental set-up for hexane, toluene and MEK biodegradation.

butyl septa, sealed with aluminium crimp caps and incubated under orbital agitation at 300 rpm and 30 °C. Control flasks were prepared and incubated under similar conditions without NAPs. No replicates of the polymer mixtures toxicity experiments were conducted. Culture absorbance ( $OD_{600}$ ) was periodically monitored by spectrophotometry under sterile conditions for 24 h. A polymer mixture was considered toxic if the  $OD_{600}$  in the flasks supplied with polymer mixtures was significantly lower than the  $OD_{600}$  in the controls.

#### Performance of polymer mixtures in a stirred tank bioreactor

A 7 L SGS Chemap Series 3000 Fermentor (Mannedorf, Switzerland) was used to evaluate the performance of polymer mixtures A and B during the biodegradation of hexane, toluene and MEK under continuous gas supply (steady state and transient operation). The bioreactor was initially filled with 3500 mL of sterile MSM and 500 mL of fresh bacterial inoculum (previously centrifuged and resuspended in 500 mL of fresh MSM) in order to obtain an initial cell concentration ranging from 0.5 to 1 g l<sup>-1</sup>. The bioreactor operated at 500 rpm, 30 °C and pH 6 (by automatic addition of 6 mol L<sup>-1</sup> KOH). The culture was supplied with hexane, toluene and MEK at individual concentrations of 0.5 g m<sup>-3</sup> by mixing a VOC-free air stream and three air streams containing each VOC (Fig. 1). The nominal operating loading rates were  $88 \pm 3$  g m<sup>-3</sup> h<sup>-1</sup> (4 L min<sup>-1</sup>). Once this NAP-free system (control system) achieved constant ECs (steady state), 800 mL of cultivation broth was replaced with 800 mL of polymer mixture A or B (20%) and the reactor operated under similar experimental conditions until a new steady state was reached. Sigma Antifoam 204 was initially added in order to avoid biomass losses by foam formation. Gas samples of 50 µL were taken twice a day to monitor the inlet and outlet hexane, toluene and MEK concentrations. Additionally, 25 mL of MSM was added daily to minimize water losses by evaporation. The intense floc formation and cell adhesion into bioreactor walls occurring at the high biomass densities present in the bioreactor hindered the accurate determination of biomass concentration by  $OD_{600}$  measurements.<sup>13</sup> Therefore, theoretical biomass concentrations (based on a 50% carbon conversion into biomass) were used in this study.

In addition, process robustness towards 4 h step changes of 2 and 3.6 times the nominal loading rate was assessed in the absence and presence of polymer mixture B according to Littlejohns and Daugulis (2008).<sup>12</sup> During these step changes, the total gas flow rate was maintained constant at 4 L min<sup>-1</sup> while the individual VOC concentrations were increased by 2 and 3.6. Gas samples of

50 µL were taken every 15 min to monitor the inlet and outlet hexane, toluene and MEK concentrations.

No replicates of the experiments with bioreactor were conducted.

#### Cultivation broth toxicity assay

The inhibition of toluene biodegradation by a potentially toxic metabolite excreted during the treatment of the target VOCs was assessed in 162 mL serum bottles filled with 28 mL of cultivation medium consisting of different ratios of centrifuged cultivation broth from the bioreactor and fresh MSM (0 : 28, 14 : 14 and 28 : 0) and inoculated with 2 mL of fresh inoculum. The systems were supplied with 5 µL of toluene, closed with butyl septa, sealed with aluminium caps and incubated under orbital agitation at 300 rpm and 30 °C. Toluene headspace concentration was periodically monitored by GC-FID for 5 h. No replicates of the cultivation broth toxicity assays were conducted.

#### Analytical procedure

Hexane, toluene and MEK concentrations in the gas phase were determined using a Varian 450 gas chromatograph (Palo Alto, CA, USA) equipped with a flame ionization detector and a Varian VF-WAXms capillary column (30 m × 0.53 mm × 1 µm). Injector and detector temperatures were set at 250 °C while oven temperature was increased from 110 °C to 140 °C at 50 °C min<sup>-1</sup>. Helium was used as carrier gas at 2.5 mL min<sup>-1</sup>. Biomass concentration was determined using optical density measurements at 600 nm using a Biochrom Ultraspec 3000 UV/Visible Spectrophotometer (Biochrom, Ltd, UK).

## RESULTS AND DISCUSSION

### Polymer selection

The solid polymers tested exhibited wide and different ranges of  $K_{gp}$  and  $K_{pw}$  (VOC partition coefficients after 2 h), which were correlated with VOC hydrophobicity (Table 2). Low values of  $K_{gp}$  and high values of  $K_{pw}$  have been traditionally considered as parameters determining the high affinity of the tested polymer for the target VOC. The polymers tested presented a low affinity for MEK compared with water ( $K_{pw}$  from 0.070 to 0.67). This affinity was higher for toluene ( $1 < K_{pw} < 180$ ) and even higher for hexane ( $99 < K_{pw} < 13\ 813$ ). Despite this wide range of affinities, very low  $K_{gp}$  values were recorded for most VOCs, which suggest that polymer selection must not be based only on  $K_{gp}$  as has often been the case in the previous literature. In addition, it must be noted that neither Zytel 42A nor Zytel RSLC1000 presented a high affinity for any of the target VOCs.

Partition coefficients determined at equilibrium have been considered as the main NAP selection criterion in TPPBs. However, in this study, the partition coefficient at two hours was chosen as the criterion since it provides a more realistic estimation of the VOC transfer rates into the solid polymers. In this context, Quijano *et al.* (2010)<sup>14</sup> demonstrated that the transfer of gaseous substrates in TPPBs are more influenced by polymer hydrodynamics than by their partitioning coefficients at equilibrium.

Silicone rubber exhibited the highest affinity for the three VOCs tested. However, due to its very high affinity for hexane, which could result in hexane sequestration and hinder its delivery to the microbial community in the aqueous phase, it was decided to work with tailored mixtures of polymers with a moderately high affinity for each of the three target VOCs. Therefore, two polymer

**Table 2.** Partition coefficients at two h

	HEXANE		TOLUENE		MEK	
	$K_{pw}$ ( $C_{pol}C_{water}^{-1}$ )	$K_{gp}$ ( $C_{gas}C_{pol}^{-1}$ )	$K_{pw}$ ( $C_{pol}C_{water}^{-1}$ )	$K_{gp}$ ( $C_{gas}C_{pol}^{-1}$ )	$K_{pw}$ ( $C_{pol}C_{water}^{-1}$ )	$K_{gp}$ ( $C_{gas}C_{pol}^{-1}$ )
Hytrel 8206	99 ± 14	0.76 ± 0.10	51 ± 3	0.0036 ± 0.0002	0.67 ± 0.058	0.0057 ± 0.0006
Zytel 42 A*	-	-	-	-	-	-
Zytel RSLC1000*	-	-	1 ± 0	0.158 ± 0.0135	0.081 ± 0.013	0.066 ± 0.011
Engage 8100	3885 ± 999	0.020 ± 0.004	100 ± 3	0.0018 ± 0.0001	0.088 ± 0.059	0.058 ± 0.041
Engage 8842	1442 ± 571	0.059 ± 0.026	150 ± 8	0.0012 ± 0.0001	0.65 ± 0.30	0.0070 ± 0.0044
Recycled rubber tires	3284 ± 559	0.023 ± 0.004	151 ± 13	0.0012 ± 0.0001	0.26 ± 0.19	0.012 ± 0.013
Silicone Rubber	13 813 ± 667	0.0054 ± 0.0003	180 ± 7	0.0010 ± 0.0000	0.44 ± 0.18	0.0091 ± 0.0035
Kraton G 1657	2764 ± 2246	0.041 ± 0.028	130 ± 6	0.0014 ± 0.0001	0.074 ± 0.061	0.109 ± 0.118

\* There is no VOC absorption into Zytel 42A nor hexane partition into Zytel RSLC1000.

mixtures (A and B) were selected. Polymer mixture A consisted of 50% Hytrel 8206 (affinity for MEK) and 50% RRT (affinity for hexane and toluene). Recycled rubber tires were preferred over Kraton G1657 and Engage 8100, despite their similar  $K_{pw}$  values for being a low cost NAP due to their origin as waste materials. Polymer mixture B consisted of 33.3% Hytrel 8206 (affinity for MEK), 33.3% Engage 8842 (affinity for toluene) and 33.3% Engage 8100 (affinity for hexane).

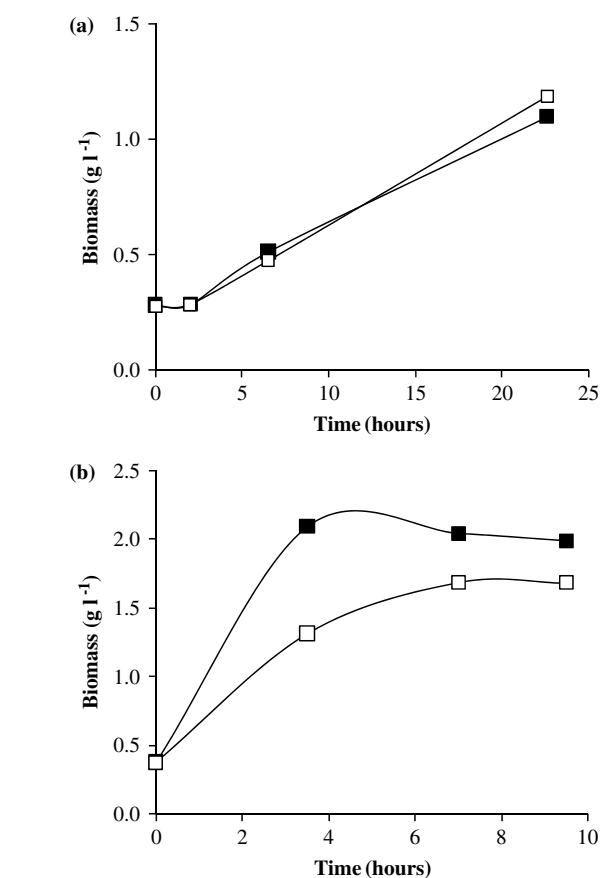
Once polymer mixtures were selected, their toxicity towards the microbial community was evaluated since toxicity constitutes a process-specific NAP selection criterion that must be assessed on a case by case basis. In our particular study, none of the polymer mixtures tested (A and B) was found to be toxic based on the similar biomass concentration recorded in the tests supplied with and without NAP (Fig. 2). Polymer biodegradability, the other selection criterion commonly evaluated in TPPBs, was not assessed based on the study of Boudreau and Daugulis (2006),<sup>11</sup> who reported that polymers beads are non-bioavailable to microorganisms as a result of their large molecular size, poor solubility and resistance to hydrolysis.

### Performance of polymer mixtures in a stirred tank bioreactor

#### Steady state loading

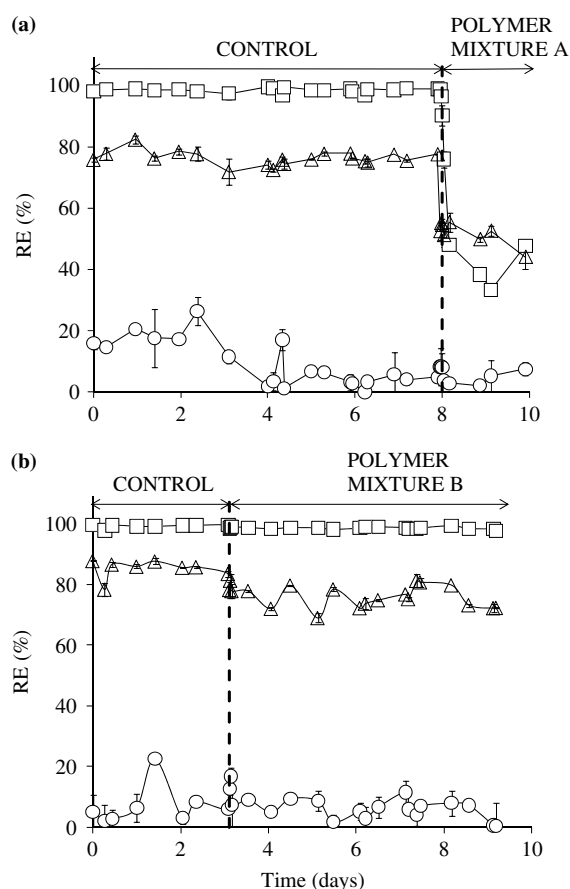
When the system was operated in the absence of polymer mixtures (controls), the process was characterized by steady state REs for hexane, toluene and MEK of  $9 \pm 8 \text{ g m}^{-3} \text{ reactor h}^{-1}$ ,  $76 \pm 3 \text{ g m}^{-3} \text{ reactor h}^{-1}$  and  $98 \pm 1 \text{ g m}^{-3} \text{ reactor h}^{-1}$ , respectively (before the addition of polymer mixture A) and  $7 \pm 7 \text{ g m}^{-3} \text{ reactor h}^{-1}$ ,  $85 \pm 3 \text{ g m}^{-3} \text{ reactor h}^{-1}$  and  $99 \pm 1 \text{ g m}^{-3} \text{ reactor h}^{-1}$ , respectively (before the addition of polymer mixture B) (Fig. 3). These results confirmed that VOC biodegradation was strongly determined by VOC hydrophobicity.<sup>15</sup> Hence, while MEK biodegradation was almost complete as a result of its efficient mass transfer to the aqueous phase, toluene and hexane removal was likely limited by the low concentration gradient available for transfer (due to their high Henry's law values, 74 for hexane and 0.25 for toluene).

The addition of polymer mixture A into the bioreactor prompted a rapid decrease in toluene and MEK REs, which achieved values of 44% and 48%, respectively, within the next 2 days. On the other hand, hexane removal was not substantially affected (REs  $\approx 6 \pm 3\%$ ) (Fig. 3(a)). The pernicious effect induced by the addition of polymer mixture A was likely due to the interaction of the target VOCs with some of the compounds comprising the RRT since no inhibition was recorded in the toxicity tests



**Figure 2.** Time course of biomass concentration in the absence (□) and in the presence (■) of polymer mixtures A (a) and B (b) during the polymers toxicity tests with TSB as carbon source.

carried out with TSB medium. That is, it is possible that the reactivity of the VOCs (such as MEK) used in this study resulted in the leaching of compounds from the RRT, which caused a cytotoxic effect on the cells. This hypothesis was supported by the deterioration of RRT in the presence of MEK (as observed in part of the bioreactor set-up) and by the black color of the cultivation medium after polymer mixture A addition (despite RRT pre-washing for several days). This interaction may be compound (substrate) specific as such a negative interaction was not observed



**Figure 3.** Time course of steady state hexane (○), toluene (Δ), and MEK (□) removal efficiencies in the absence and in the presence of polymer mixtures A (a) and B (b). Vertical bars represent standard deviation.

for the case of sequestration/delivery of diesel fuel from RRT<sup>16</sup> or substituted phenols (unpublished results). Nevertheless, the interactions between the target VOCs and the solid polymers used should now also be a key selection criterion that must be considered in future in order to avoid microbial inhibition during bioreactor operation.

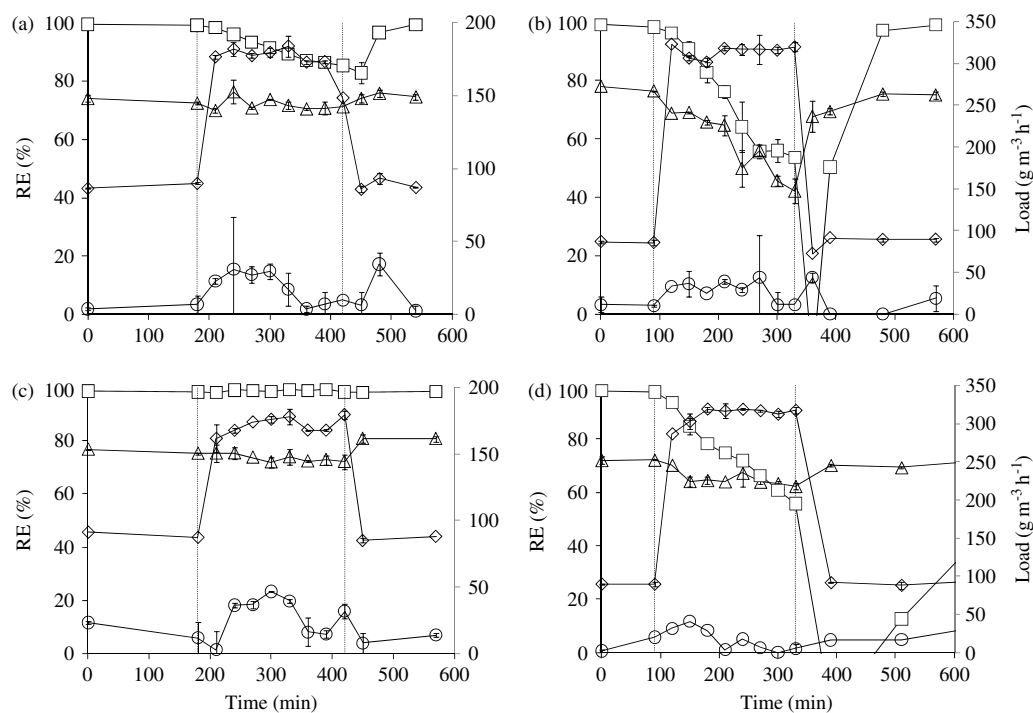
When the system was operated in the presence of polymer mixture B, the process was characterized by a comparable performance with that recorded in the control system. Hence, hexane, toluene and MEK achieved steady state REs of  $7 \pm 4$ ,  $76 \pm 4$  and  $98 \pm 0$ , respectively (Fig. 3(b)). Therefore, this work confirms that the presence of a tailored polymer mixture with high affinity for the target VOCs does not enhance their biodegradation during steady state operation. Likewise, Littlejohns and Daugulis (2009)<sup>17</sup> did not record any improvement during the steady state biodegradation of BTEX (moderately soluble compounds) in a two-phase partitioning airlift bioreactor provided with silicone rubber beads.

#### Transient loadings

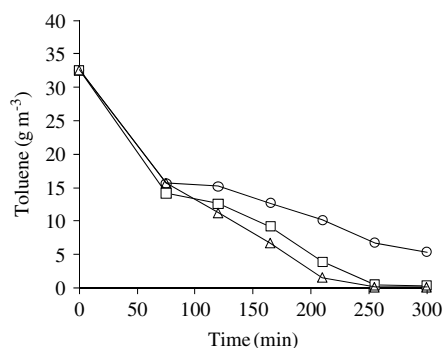
During the 2-fold step increase in the control system toluene REs remained approximately constant ( $72 \pm 2\%$ ), MEK REs decreased gradually from 99% to 85% while hexane REs fluctuated without any clear trend from 3 to 15% (Fig. 4(a)). The system rapidly recovered steady state REs ( $\approx 60$  min) when VOC loading was restored to nominal levels (Fig. 4(a)). The decrease in MEK removal

recorded was likely due to limitations in microbial activity. This limitation can originate from either a limited amount of VOC-degrading biomass or from the occurrence of microbial inhibition in the presence of a toxic metabolite excreted as result of the increase in VOC loading. The occurrence of mass transport limitations in MEK biodegradation was ruled out based on the gradual nature of the decrease recorded and on the high solubility of this particular VOC. In addition, the fact that toluene REs remained constant during the 2-fold step change suggest that the dissolved oxygen concentration also did not limit the biodegradation processes. However, when polymer mixture B was present the REs for MEK remained constant at approx.  $99 \pm 1\%$  (Fig. 4(c)). This enhancement in the biodegradation of MEK suggests that no limitation due to microbial activity occurred during this 2-fold step increase since the biomass concentration in the tests conducted in the presence of the polymer mixture B was lower than in the control test ( $7.4$  and  $13.5 \text{ g L}^{-1}$ , respectively). Therefore, this biodegradation enhancement could be explained by the ability of the solid polymers to absorb any potential toxic metabolite(s) excreted during process overload. Similarly, Morrish and Daugulis (2008)<sup>18</sup> used a solid-liquid two-phase partitioning bioreactor to improve the performance and operability of the biotransformation of carveol to carveone, which suffered from substrate and by-product inhibition. In this process, the presence of a single polymer and a mixture of polymers mediated the absorption of these compounds into the solid polymers, which protected the system against inhibitory carveone aqueous concentrations and therefore, resulted in improved carveone productivities.

During the 3.6-fold step increase in the absence of polymers, hexane REs fluctuated between 3% and 12%, while both toluene and MEK REs decreased gradually from 76% to 42% and from 98% to 54%, respectively. However, the system recovered steady state REs in approximately 180 min after the loading was returned to nominal levels (Fig. 4(b)). Oxygen or VOCs mass transfer limitations would have resulted in a step decrease in both toluene and MEK REs. However, the fact that process performance deterioration was gradual suggests that the process was limited by microbial activity. This limitation could be either due to a low biomass concentration in the system or to the accumulation of a toxic metabolite released from VOCs biodegradation. When polymer mixture B was present MEK REs decreased gradually from 98% to 56%, however, the rate of deterioration of the MEK-RE was lower in the presence of the polymer mixture since the MEK RE decrease took place over 4 h instead of 3 h as was the case in the absence of polymers (Fig. 4(d)). The clearer effect of the 2-fold increase was likely due to the increase in the production rate of toxic metabolites as a result of the higher VOCs input to the bioreactor. The fact that biomass concentration was lower in the presence of the polymer mixture ( $15.0 \text{ g L}^{-1}$  in the control system vs  $10.2 \text{ g L}^{-1}$  in the presence of polymer mixture B) suggests, once again, that the accumulation of a toxic metabolite rather than biomass concentration was probably the reason underlying the deterioration in MEK REs. Toluene REs were constant at  $66 \pm 4\%$ , which rules out potential limitation by low oxygen concentrations. Therefore, the enhancements recorded in MEK and toluene biodegradation could be explained by the absorption into the polymers of a possible toxic metabolite present in the cultivation medium. Surprisingly, the TPPB did not recover its original MEK-REs levels after the loading was returned to nominal levels. This may be due to the high aqueous MEK concentrations during the 3.6-fold step loading, higher than in the control system as a result of the lower aqueous fraction (80%)



**Figure 4.** Time course of VOCs loading rate ( $\diamond$ ) and removal efficiencies for hexane ( $\circ$ ), toluene ( $\Delta$ ), and MEK ( $\square$ ) for a 2-fold step increase in VOC concentrations in the absence (a) and in the presence of polymer mixture B (c), and for a 3.6-fold step increase in the absence (b) and in the presence of polymer mixture B (d). Vertical dashed lines indicate the 4 h step increase in VOC loading and vertical bars represent standard deviation.



**Figure 5.** Time course of toluene headspace concentration in a gas-tight flask supplied with different ratios of centrifuged cultivation broth from the bioreactor and fresh MSM (0:28 ( $\Delta$ ), 14:14 ( $\square$ ) and 28:0 ( $\circ$ )).

in this TPPB (where the  $K_{pw}$  of the polymers for MEK was lower than one) and due to the lower biomass concentration present (Fig. 4(d)).

#### Cultivation broth toxicity assay

Toluene degradation rates decreased at increasing ratios of centrifuged cultivation broth to MSM (Fig. 5). Hence, the rate at a ratio of 0:28 was approximately two times higher than at a ratio 28:0, which suggests that the presence of toxic metabolites excreted during VOCs biodegradation severely hindered toluene biodegradation.

#### CONCLUSION

This innovative study assessed the performance under steady state and transient loadings conditions of TPPBs during the

biodegradation of VOCs of very different hydrophobicities with two tailored mixtures of solid polymers. Process inhibition, which followed the addition of polymer mixture A, was probably due to the complex interactions between the RRT and the target VOCs. The utilization of RRT, or other discarded/recyclable plastics, does, however, remain an interesting opportunity to use waste materials for positive environmental purposes, which has both techno-economic and public acceptance advantages. However, preliminary NAP-VOC interaction tests must be conducted prior to their use. Despite the performance of hexane, toluene and MEK biodegradation did not improve under steady state conditions in the presence of solid polymers with high affinity for these VOCs, tailored polymer mixtures can improve process performance during VOC transient loadings.

#### ACKNOWLEDGEMENTS

This research was supported by the Spanish Ministry of Education and Science (RYC-2007-01667 and BES-2007-15840 contracts, CTQ2009-07601 and CONSOLIDER-CSD 2007-00055) projects. The support of the Natural Sciences and Engineering Research Council of Canada is also gratefully acknowledged.

#### REFERENCES

- 1 BREF – European Commission, Institute for Prospective Technological Studies, 2003. Integrated Pollution Prevention and Control: Reference Document on Best Available Techniques in the Large Volume Organic Chemical Industry. Seville. <http://eippcb.jrc.es/reference/> [Accessed 11 May 2010].
- 2 Knox EG, Childhood cancers and atmospheric carcinogens. *J Epidemiol Community Health* **59**:101–105 (2005).
- 3 Khan FI and Ghoshal AKR, Removal of volatile organic compounds from polluted air. *J Loss Prevent Proc* **13**:527–545 (2000).

- 4 Devinny JS, Deshusses MA and Webster TS, *Biofiltration for Air Pollution Control*. Lewis Publishers, Boca Raton (1999).
- 5 Zhu X, Suidan MT, Pruden A, Yang C, Alonso C, Kim BJ, *et al*, Effect of substrate Henry's constant on biofilters performance. *J Air Waste Manage Assoc* **54**:409–418 (2004).
- 6 Nielsen DR, Daugulis AJ and Mclellan PJ, Transient performance of a two-phase partitioning bioscrubber treating a benzene-contaminated gas stream. *Environ Sci Technol* **39**:8971–8977 (2005).
- 7 Cesário MT, Beverloo WA, Tramper J and Beffink HH, Enhancement of gas-liquid mass transfer rate of apolar pollutants in the biological waste gas treatment by a dispersed organic solvent. *Enzyme Microbiol Technol* **21**:578–588 (1997).
- 8 Quijano G, Rocha-Rios J, Hernandez M, Villaverde S, Revah S, Muñoz R *et al*, Determining the effect of solid and liquid vectors on the gaseous interfacial area in two-phase partitioning bioreactors. *J Hazard Mater* **175**:1085–1089 (2010).
- 9 Muñoz R, Chambaud M, Bordel S and Villaverde S, A systematic selection of the non-aqueous phase in a bacterial two liquid phase bioreactor treating  $\alpha$ -pinene. *Appl Microbiol Biotechnol* **79**:33–41 (2008).
- 10 Montes M, Rene ER, Veiga MC and Kennes C,  $\alpha$ -pinene removal from air in one- and two-liquid-phase thermophilic and mesophilic biotricking filter. *Proceeding of the 3<sup>rd</sup> International Congress on Biotechniques for Air Pollution Control*. Delft, The Netherlands, September 28–30 (2009).
- 11 Boudreau NG and Daugulis AJ, Transient performance of two-phase partitioning bioreactors treating a toluene contaminated gas stream. *Biotechnol Bioeng* **94**:448–457 (2006).
- 12 Littlejohns JV and Daugulis AJ, Response of a solid-liquid two-phase partitioning bioreactor to transient BTEX loadings. *Chemosphere* **73**:1453–1460 (2008).
- 13 Bouchez-Naitali M, Blanchet D, Bardin V and Vandecasteele JP, Evidence for interfacial uptake in hexadecane degradation by *Rhodococcus equi*: the importance of cell flocculation. *Microbiology* **147**:2537–2543 (2001).
- 14 Quijano G, Hernández M, Villaverde S, Thalasso F and Muñoz R, A step-forward in the characterization and potential applications of solid and liquid oxygen transfer vectors. *Appl Microbiol Biotechnol* **85**:543–551 (2010).
- 15 Muñoz R, Villaverde S, Guieysse B and Revah S, Two partitioning bioreactors for treatment of volatile organic compounds. *Biotechnol Adv* **25**:410–422 (2007).
- 16 Prpich GP, Rehmann L and Daugulis AJ, On the use, and re-use, of polymers for the treatment of hydrocarbon contaminated water via a solid-liquid partitioning bioreactor. *Biotechnol Prog* **24**:839–844 (2008).
- 17 Littlejohns JV and Daugulis AJ, A two-phase partitioning airlift bioreactor for the treatment of BTEX contaminates gases. *Biotechnol Bioeng* **103**:1077–1086 (2009).
- 18 Morrish JLE and Daugulis AJ, Improved reactor performance and operability in the biotransformation of carveol to carvone using a solid-liquid two-phase partitioning bioreactor. *Biotechnol Bioeng* **101**:946–956 (2008).