# Transient Performance of Two-Phase Partitioning Bioreactors Treating a Toluene Contaminated Gas Stream

**Neal G. Boudreau, Andrew J. Daugulis**

*Department of Chemical Engineering, Queen's University, Kingston, Ontario, K7L 3N6 Canada; telephone: 613-533-2784; fax: 613-533-6637; e-mail: daugulis@chee.queensu.ca*

*Received 9 September 2005; accepted 22 December 2005*

*Published online 10 April 2006 in Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002/bit.20876*

**Abstract:** Two-phase partitioning bioreactors (TPPBs) consist of a cell-containing aqueous phase and an immiscible organic phase that sequesters and delivers toxic substrates to cells based on equilibrium partitioning. The immiscible organic phase, which acts as a buffer for inhibitory substrate loadings, makes it possible for TPPBs to handle high volatile organic compound (VOC) loadings, and in this study the performance of liquid *n*-hexadecane and solid styrene butadiene (SB) polymer beads used as partitioning phases were compared to a single aqueous phase system while treating transient loadings of a toluene contaminated air stream by *Achromobacter xylosoxidans* Y234. The TPPBs operated as well-mixed stirred tanks, with total working volumes of 3 L (3 L aqueous for the single-phase system, 2 L aqueous and 1 L *n*-hexadecane for the solvent system, and 2.518 L aqueous volume and 500 g of SB beads for the polymer system). Two 60-min step changes (7 and 17 times the nominal loading rates, termed ''small'' and ''large'' steps, respectively) were imposed on the systems and the performance was characterized by the overall removal efficiencies, instantaneous removal efficiency recovery times (above 95% removal), and dissolved oxygen recovery times. For the small steps, with a nominal loading of 343 g/m$^3$/h increasing to 2,400 g/m$^3$/h, the TPPB system using *n*-hexadecane as the second phase performed best, removing 97% of the toluene fed to the system compared with 90% for the polymer beads system and only 69% for the single-phase system. The imposed large transient gave similar results, although the impact of the presence of a second sequestering phase was more pronounced, with the *n*-hexadecane system maintaining much reduced aqueous toluene concentrations leading to significantly improved performance. This investigation also showed that the presence of both *n*-hexadecane and SB beads improved the oxygen transfer within the systems.

**Keywords:** two-phase partitioning bioreactor; VOCs; toluene; transient performance

*Correspondence to:* Dr. Andrew J. Daugulis
Contract grant sponsor: Natural Sciences and Engineering Research Council of Canada

## INTRODUCTION

Biological options for treating contaminated air streams can be effective and economical means for the degradation of low concentrations of contaminants in large quantities of air. The biological treatment of volatile organic compounds (VOC) in air depends on the ability of certain microorganisms to metabolise these VOCs and use them as their sole source of carbon and energy producing carbon dioxide, water vapor, and biomass (Mutafov et al., 2004). Generally, the pollutants are absorbed from the gas phase to an aqueous phase in which the active microbial culture attacks and degrades the target pollutants. The most common types of biological treatment systems are biofilters, biotrickling filters, and bioscrubbers. The basic mechanisms for removal are similar for all reactor types, however, differences are found in the phases in which the microbial population is located (fixed in the case of biofilters and biotrickling filters; suspended in the case of bioscrubbers), and the state of the liquid phase which may be stationary, in the case of biofilters, or flowing in the case of biotrickling filters and bioscrubbers.

Two-phase partitioning bioreactors (TPPBs) are a relatively new method of dealing with VOCs, with the inherent features of these devices allowing them to buffer what could possibly be toxic loadings of VOCs in other types of biological treatment devices. TPPBs contain an aqueous, cell-containing phase, as well as an immiscible organic phase that acts as a reservoir for toxic or inhibitory substrates. The organic phase absorbs the substrate as it enters the reactor and, based on equilibrium partitioning between the two phases, releases it to the cell-containing aqueous phase at low concentrations. Recent work (Amsden et al., 2003; Daugulis et al., 2003; Prpich and Daugulis, 2004, 2005) has shown that the second phase, traditionally an immiscible organic solvent, can be replaced by solid polymer (plastic) beads. One advantage of using polymer beads is that they are non-bioavailable to microorganisms, and thus, a consortium of bacteria, rather than a pure species, can be used for the degradation of pollutants. The ability of solid polymers to

absorb (rather than adsorb) organic molecules has been amply demonstrated starting from the ground-breaking work of Bowen (1970) and has spawned the field of controlled drug delivery (Michaels et al., 1975). Various authors (Schumack and Chow, 1987; David et al., 1989; El-Shahawi, 1994) have shown controlled absorption and release of target molecules from polymers, and have demonstrated (Prpich and Daugulis, 2004) that polymers absorb target molecules characterized by a partition coefficient, analogous to organic solvents. In a somewhat analogous fashion to that employed in TPPBs, some researchers (Aizpuru et al., 2003; Tang et al., 2005; Weber and Hartmans, 1995) have used granular activated carbon (GAC) as part of their biofilter matrix to absorb VOCs, thus mitigating their potentially toxic effects on the microbial community present. Such systems, however, rely on adsorption rather than absorption (which is the VOC uptake mechanism in TPPBs) and are therefore limited by the GAC surface area.

Full-scale industrial air treatment devices are exposed to changes in operating conditions such as variations in waste gas composition and concentration, flow rate, as well as shutdowns in the industrial process. Extreme variations in waste air composition flux also result from upstream process changes and accidental spillages (Deshusses et al., 1996). As a result of this changing environment, it is important to determine how effectively treatment processes will be able to handle these influent fluctuations. Biological treatment options are particularly sensitive to such variations as many pollutants in air streams can be toxic to microorganisms past a certain threshold concentration. Should the concentration of a pollutant surpass the toxicity threshold of the microbes used to degrade it, the bioremediation process could fail. Due to the plug flow nature of biofilters, a large shock can effectively destroy the entire population as biofilters have little ability to buffer such shock loads. Biotrickling filters and bioscrubbers also have no means to buffer a toxic spike in the inlet waste stream and are also prone to decreased effectiveness due to this limitation. Alternatively, the design of TPPBs provides the potential to handle fluctuations in the influent concentration of toxic pollutants. The second, immiscible, non-bioavailable phase acts as a buffer, or a ''sponge,'' and absorbs the high concentrations of pollutants entering the system and, based on equilibrium considerations, delivers subinhibitory levels of the pollutant to the microbial population based on cellular demand.

This work was conducted in order to compare the performance of an organic solvent with polymer beads as second phases in a TPPB treating a continuous air stream contaminated with toluene, while at the same time testing the ability of TPPBs to handle very large VOC loadings. In order to compare the performances of the two systems, small (7–8 times the nominal loading) and large (17–20 times the nominal loading) 1-h step changes were imposed on the systems. The performances of the two systems were compared to one another based primarily on overall removal efficiencies, as well as on instantaneous removal efficiency recovery times, and dissolved oxygen recovery times. The performance of both of these systems was also compared to a bioreactor containing aqueous medium, but no second phase, treating the same toluene contaminated gas stream.

## MATERIALS AND METHODS

### Chemicals

The salts used for this investigation were obtained from Fisher Scientific, Ottawa, ON. The *n*-hexadecane and toluene were obtained from Alfa Aesar of Ward Hill, MA. Yeom and Daugulis (2001) showed that *n*-hexadecane is a suitable solvent for the TPPB treatment of VOCs based on biological factors, such as biocompatibility and non-bioavailability (i.e., it is not used as a carbon source), physical factors, such as low volatility, hydrophobicity, and phase stability, as well as other factors such as cost and safety. The long-term (>1 month) use of hexadecane as a benzene delivery phase of *Achromobacter xylosoxidans* Y234 has been amply demonstrated, although the presence of other organisms in the system (e.g., via contamination, such as in the air stream) may or may not (MacLeod and Daugulis, 2003) result in preferential uptake of the solvent relative to the target molecule. Styrene-butadiene (SB; 28% styrene) ABA co-polymer beads (cylindrical with dimensions of approximately $L = 4.25$ mm, $D = 3.75$ mm) were obtained from Scientific Polymer Products, Inc., (Ontario, New York). SB co-polymer beads (with a density of 0.94 g/mL) were selected as a second phase based on work done with this material and a benzene-contaminated air stream (Daugulis et al., 2003). As has been previously shown (Prpich and Daugulis, 2004), significant differences exist in the ability of polymers to take up target molecules, and rational polymer selection based on polymer chemistry (analogous to rational solvent selection in liquid–liquid TPPBs) is required for effective uptake by the solid phase. SB beads were tested for toluene absorption and were shown to be able to absorb similar amounts of toluene (data not shown).

### Microorganism, Medium, and Culture Conditions

*Achromobacter xylosoxidans* Y23*4* (formerly *Alcaligenes xylosoxidans* Y234), was obtained from Dr. Sung Ho Yeom, Department of Chemical Engineering, Seoul National University. It is known to have the ability to degrade toluene, as well as benzene and *m*-xylene. The growth medium used for *Achromobacter xylosoxidans* Y234 had previously (Davidson and Daugulis, 2003) been developed as: 7 g/L $(NH_4)_2SO_4$, 0.75 g/L $MgSO_4 \cdot 7H_2O$, 6.6 g/L $K_2HPO_4$, 8.42 g/L $KH_2PO_4$, 2 g/L sodium benzoate, and 1 mL/L trace elements. Stock trace element solution was prepared as follows: 16.2 g/L $FeCl_3 \cdot 6H_2O$, 9.44 g/L $CaHPO_4$, 0.15 g/L $CuSO_4 \cdot 5H_2O$, and 40 g/L citric acid. The growth medium has been designed to achieve pH 6.6, determined to be optimal for this bacterium. Eight 125 mL Erlenmeyer shake flasks containing 50 mL of medium composed of the above formulation were inoculated from frozen stock prior to

incubation at 30°C and 150 rpm for 24 h in preparation for their inoculation in the bioreactor. Stock cultures previously grown in the above medium were cryogenically preserved at −86°C with 10% (v/v) dimethyl sulfoxide.

## Reactor Set-up and Operation

A 5 L New Brunswick Scientific BioFlo III reactor was used for all fermentations. The reactor was set to operate at a temperature of 30°C, a pH of 6.6, an agitation speed of 800 rpm and a total working volume of 3 L. The aqueous medium consisted of 14 g/L $(NH_4)_2SO_4$, 1.5 g/L $MgSO_4 \cdot 7H_20$, 13.2 g/L $K_2HPO_4$, 16.84 g/L $KH_2PO_4$, and 0.16 mL/L trace elements. For the fermentations conducted using liquid *n*-hexadecane as the second phase, an organic fraction of 0.33 was used with the remainder being the aqueous, cell-containing phase. The two phases were maintained as a dispersion through agitation. For the fermentations conducted using SB beads as a second phase, 500 g of polymer beads were used with 2.518 L of aqueous medium added for a final total volume of 3 L. This mass of beads was selected based on operational considerations; higher bead fractions were found to result in excessive build up of beads behind baffles and other reactor internals, due to the relatively large size of the beads. The use of smaller beads would allow for higher bead volume fractions in these solid–liquid systems.

The toluene delivery system consisted of an Erlenmeyer flask with approximately 2 L of toluene and a regulated amount (Matheson TriGas Model 603 rotameter) of compressed air being sparged through it that continued into the reactor. The flask was positioned inside a water bath that kept the flask at the 30°C. This air stream was mixed with air for bioreactor aeration and this combined stream was delivered into the reactor through a sparger located at the bottom of the reactor. Two Rushton turbines inside the reactor, as well as metal baffles, were used to help keep the system well mixed. Dissolved oxygen levels were measured with a polarographic-membrane electrode (Broadley and James, Corp., Irvine, CA).

Concentrated nutrient boluses were added periodically (every 2–3 days) to the reactor based on a developed feeding schedule to ensure that the system was not nutrient limited. A small amount of Sigma Antifoam 289 (∼0.5 mL) was added as required.

## Analytics

Liquid samples, periodically taken in order to measure biomass concentration, were transferred to 15 mL centrifuge tubes and centrifuged for 25 min at 4°C. After centrifugation, the liquid supernatant was discarded from the sample tube and the biomass was then re-suspended in deionized water. Appropriate dilutions were then performed to arrive at an absorbance of between 0.2 and 0.8 as measured by a Biochrom Ultrospec 3000 UV/Visible Spectrophotometer at 650 nm and compared to a previously determined calibration curve.

Inlet and outlet gas samples were taken by means of a Hamilton Gas Tight 250 μL syringe. A Perkin Elmer AutoSystem Gas Chromatograph fitted with a flame-ionizing detector and a fused silica capillary column (DB-5, 0.53 mm I.D., 30 m length, 1 μm film thickness, Model 125–503 J, J & W Scientific, Inc., Folsom, CA) was used to analyze toluene concentrations. Helium was used as the carrier gas, flowing at 30 mL/min while injector, column, and detector temperatures were set at 180, 150, and 250°C, respectively. Peak integration was performed using Millennium[32] Version 3.05.01 Software (Waters Corporation) and compared to external standards. The aqueous phase toluene concentration was calculated based on the Henry's Law relationship between air and the aqueous medium previously found to be 0.247 $(mg/L)_{gas}/(mg/L)_{aq}$. Toluene concentrations in *n*-hexadecane and SB beads were determined based on partition coefficients relative to the aqueous medium as determined previously.

## Steady State and Transient Operation

Immediately after inoculation a total flow rate of 1.71 L/min air (0.58 vvm) at a toluene concentration of 10 mg/L was established for a loading rate of 343 $g/m^3/h$. This loading rate was maintained during the biomass growth phase and between dynamic step experiments. The cell growth slowed and reached a steady state in each case within 5–7 days of inoculation at a cell mass in the bioreactor of between 20 and 25 grams (CDW). Achieving a steady state biomass concentration even with continued addition of substrate is due to the use of the consumed substrate for cell maintenance purposes only, rather than cell growth, as will be discussed later. All transient experiments were performed once the biomass levels had stabilized (after the initial 5–7-day growth period).

Inlet toluene steps were introduced to the system for periods of 60 min by varying the proportions of air passing through the toluene flask and the aeration air, after which the toluene loading was reduced to its initial level. The size of the step was determined based on the measured mass of biomass present in the reactor at the time. Normalizing the size of the step with respect to total cell mass present was done to ensure that the performance of each system was not affected by the amount of biomass present. Two-step sizes were conducted for each bioreactor configuration above the steady state feeding condition; one at a rate of approximately 110 $(g_{Toluene}/m^3_{reactor}/h)/(g\text{-cells})$ and one at 280 $(g_{Toluene}/m^3_{reactor}/h)/(g\text{-cells})$. Alternatively, from a stable loading of 343 $g/m^3/h$, steps of approximately 2,400 and 6,000 $g/m^3/h$ were performed. The small step was conducted at an overall aeration rate of 0.57 vvm (same as steady state) while the large step was done at 1.28 vvm. More air was added to the larger step to help avoid oxygen-limiting conditions and was then reduced to nominal conditions (0.57 vvm) immediately after the step was completed. At the end of the step ($t = 60$ min), the flows were readjusted to their original set-points and the inlet and outlet toluene



DOI 10.1002/bit

concentrations were monitored until the instantaneous removal efficiency of the system returned to its original steady state value.

## RESULTS AND DISCUSSION

### Achieving Steady State

Shortly (<2 days) after inoculation of the three systems, the instantaneous removal efficiencies increased to greater than 95% of the inlet toluene fed to the system. At a toluene loading of 343 g/m$^3$/h, the biomass in the systems reached steady state total cell masses in the reactors that ranged between 19.4 and 26.2 g (CDW) within 7 days of inoculation in all three cases (Fig. 1). The instantaneous removal efficiencies of the systems remained greater than 95% for the entirety of the experiments except during transient periods. All transient experiments were performed after the systems had reached steady state conditions (i.e., between days 6 and 16 in Fig. 1), but the transient data are not shown in this figure (transient data are shown on Figures 2–4 and 7–9).

Past work (Daugulis and Boudreau, 2003; Davidson and Daugulis, 2003) on VOC removal performed with continuously-fed TPPBs involved regular medium exchanges to maintain cell concentrations to within a desired "band," as it was believed that if cell growth were allowed to increase unabated, viscosity problems, wall growth, and other operational problems would be likely to occur. However recent work by Nielsen et al. (2005) has shown that a constant cell concentration will eventually be established (as seen in Fig. 1) due to cellular maintenance requirements, which are responsible for all of the substrate consumed. Therefore, in this work medium exchanges were not employed.

### Small Steps

Figures 2–4 show the transient responses of the three systems when the small step change (~110 (g/m$^3$/h)/(g-cells)) was imposed. In this step, the toluene loading was increased from

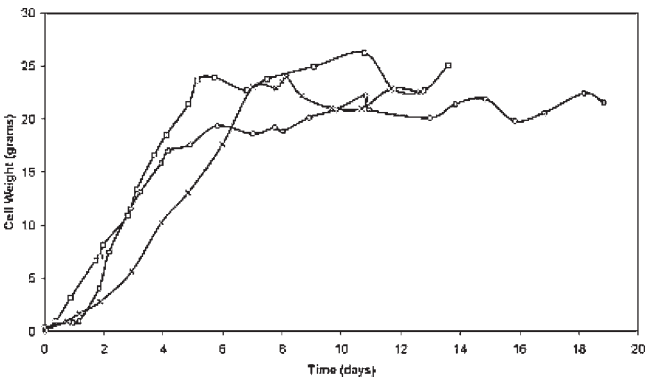


**Figure 1.** Biomass levels in the single phase (○), solvent as a second phase (□), and polymer beads as a second phase (×) systems over the course of the fermentations.

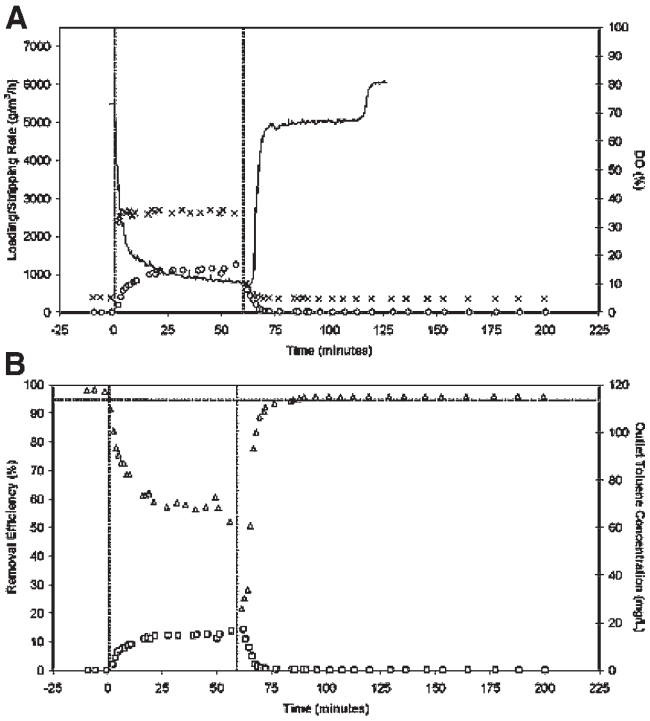


**Figure 2.** **A**: Dynamic response (Inlet (×) and outlet (○)) and dissolved oxygen trace and (**B**) instantaneous removal efficiencies (△) and outlet toluene concentrations (□) of the partitioning bioreactor operated with no second phase when imposed with a 1-h toluene step of 118 (g/m$^3$/h)/(g-cells).

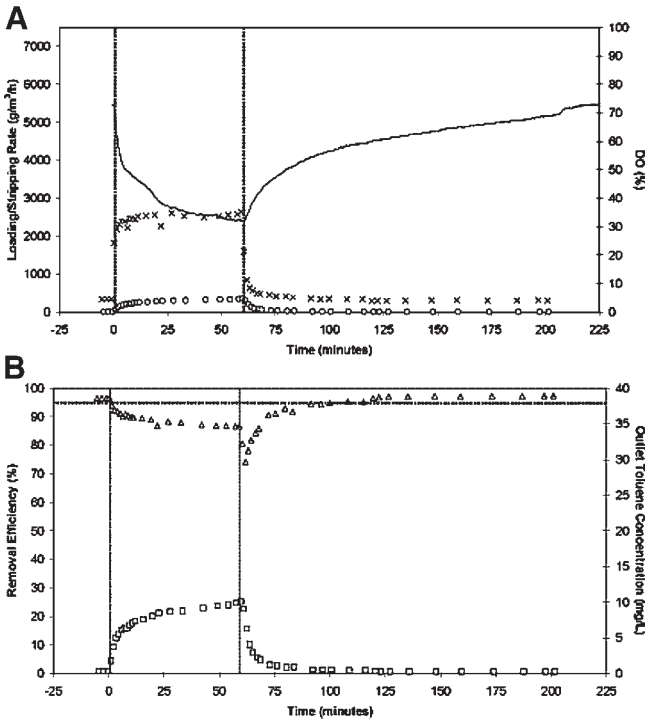


**Figure 3.** **A**: Dynamic response (Inlet (×) and outlet (○)) and dissolved oxygen trace and (**B**) instantaneous removal efficiencies (△) and outlet toluene concentrations (□) of the two-phase partitioning bioreactor operated with styrene-butadiene (SB) co-polymer beads as the second phase when imposed with a 1-h toluene step of 110 (g/m$^3$/h)/(g-cells).

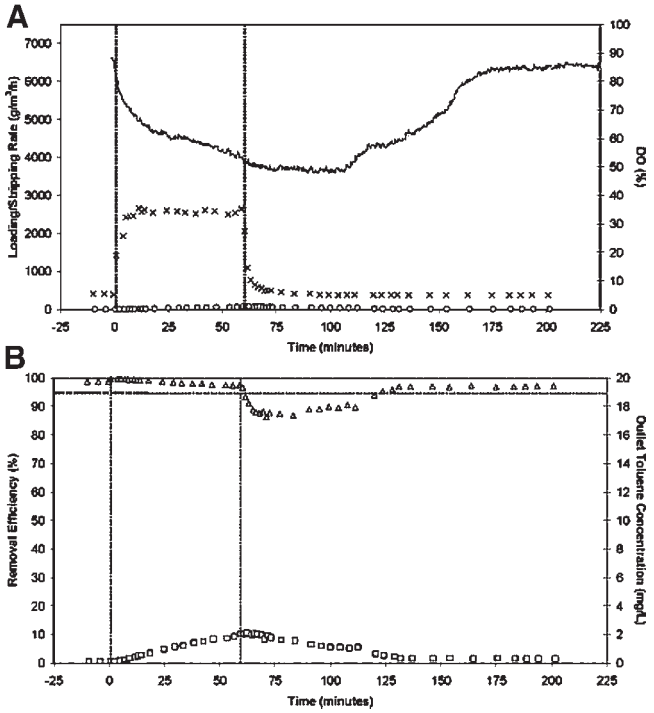


**Figure 4.** **A**: Dynamic response (Inlet (×) and outlet (○)) and dissolved oxygen trace and (**B**) instantaneous removal efficiencies (△) and outlet toluene concentrations (□) of the two-phase partitioning bioreactor operated with *n*-hexadecane as the second phase when imposed with a 1-h toluene step of 113 (g/m$^3$/h)/(g-cells).

its nominal rate of 343 g/m$^3$/h to approximately 2,400 g/m$^3$/h for a period of 60 min. The instantaneous removal efficiencies of the single-phase system and the polymer beads as a second-phase system both dropped immediately upon the onset of the step reaching minimum values of 57% and 87%, respectively before the end of the 60 min step. These reduced efficiencies during the imposed transient are also reflected in the differences between toluene loaded and stripped (Figs. 2A and 3A) as well as in the outlet toluene concentrations (Figs. 2B and 3B), which reached 20 and 10 mg/L, respectively. The instantaneous removal efficiencies of the *n*-hexadecane as a second-phase system remained above 95% for the entirety of the 60-min step, and in fact *increased* at the initial stages of the transient, reflecting absorption of the higher toluene loading. Outlet toluene concentrations remained low for this system, reaching just 2 mg/L, or about one-tenth the concentration of the single-phase system.

Inspection of the overall removal efficiencies (Table I) shows that the solvent as a second-phase system clearly outperformed both the polymers as a second phase and the no second phase cases as may be anticipated. Over the course of the 200 min experiment, the solvent as a second-phase system removed 97% of the toluene fed to the system, with the polymer beads as a second phase being intermediate removing 90% of the toluene, and the no second phase clearly performing the worst of the three systems removing only 69%. The performance comparison between the systems with second phases may not be entirely fair, however, given the different masses of second phases that were used. It can be anticipated that as more polymer phase is used (approaching the mass of *n*-hexadecane) the performance of this system would be closer to the two liquid-phase system. Table I also shows quite clearly that even though the two cases utilizing the second phases take longer to recover due to toluene absorption, the total amount of toluene released is significantly less (i.e., toluene degradation is higher) than for the case with no second phase present.

A direct comparison of the instantaneous removal efficiencies (Fig. 5) of the three systems also clearly shows the enhanced effect that the second phases have on the performance of the systems as measured by the minimum instantaneous removal efficiencies reached. In addition, the 95% instantaneous removal efficiency recovery times (Fig. 5 and Table I) also show a clear trend with the no second-phase system recovering the most rapidly (30 min) as a result of little toluene absorption. The polymer beads as a second-phase system again were intermediate (48 min), while the solvent as a second-phase system took the longest to recover (63 min) as a result of the solvent having the highest degree of toluene absorption.

The DO traces (Figs. 2A, 3A, and 4A) of the three systems (reflecting oxygen transfer to the single or two-phase systems, as well as microbial uptake) varied considerably. The DO in the single-phase system dropped quickly, but also recovered quickly after completion of the step, forming a distinct shoulder shape before fully returning to its original steady state value. The DO traces of the two systems using second phases dropped more gradually and to higher DO levels than the single-phase system, and also recovered much more gradually than the single-phase system. In both cases, with the second phases present, it appears as though there were also small shoulders on the DO traces, however these

**Table I.** Performance summary during imposed step transients.

| | 95% recovery time (min) | DO recovery time (min) | Toluene released during step (mg) | Toluene released after step (mg) | Total toluene released (mg) | Overall removal efficiency (%) |
|---|---|---|---|---|---|---|
| Single-phase (small step) | 30 | 60 | 3002 | 292 | 3294 | 69 |
| Solvent (small step) | 63 | 115 | 116 | 198 | 314 | 97 |
| Polymer beads (small step) | 48 | 162 | 864 | 165 | 1030 | 90 |
| Single-phase (large step) | 87 | 65 | 7918 | 696 | 8614 | 58 |
| Solvent (large step) | 126 | 190 | 641 | 651 | 1292 | 94 |
| Polymer beads (large step) | 118 | 271 | 5894 | 1109 | 7003 | 66 |



DOI 10.1002/bit

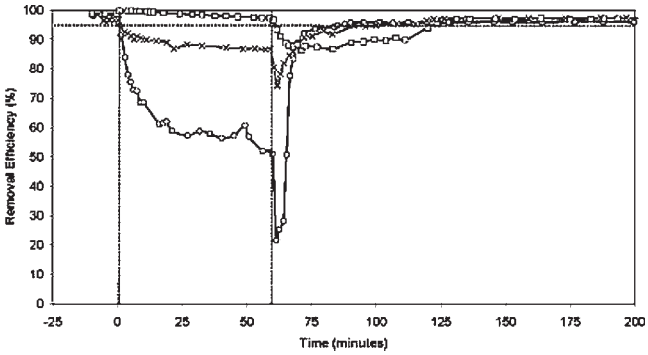


**Figure 5.** Instantaneous removal efficiencies of the single phase (○), solvent as a second phase (□), and the polymer beads as a second phase (×) systems during the small step change.

were much less pronounced than in the case of the single-phase system.

A direct comparison of the DO traces of the three systems (Fig. 6) did, however, show the same trend as seen in the removal efficiencies as the DO level in the system with the solvent as the second phase remained the highest reaching a minimum DO value of 48% of saturation, while that of the polymer bead system was intermediate (33% of saturation), and the DO for the system with no second phase dropped to the lowest level (10% of saturation). The higher level of oxygen in the *n*-hexadecane case may be expected due to the greater capacity for oxygen by this solvent (Nielsen et al., 2003), and it is interesting to see that the SB beads had a similar effect, albeit to a lesser degree with the mass of beads used in this case. Thus the presence of a second phase, originally intended to absorb and sequester toxic VOC substrates, has the added beneficial effect of enhanced oxygen absorption and release. The DO recovery time (Table I) for the system with no second phase was the fastest (60 min). The systems containing the solvent and polymers took longer to recover, likely due to the oxygen requirement associated with degrading the additionally absorbed toluene. In addition, for all cases, the shoulders on the DO traces suggest that toluene-degradation intermediates may have been formed during the step change transient, which were

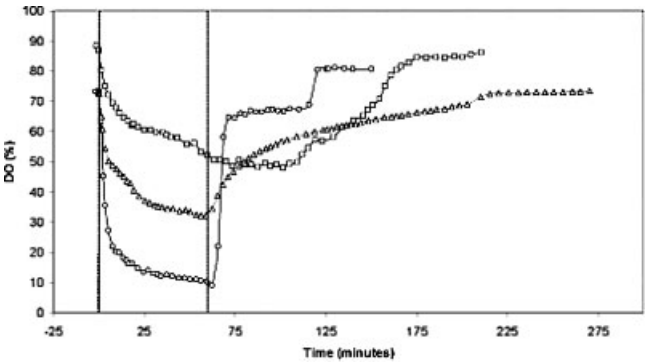


**Figure 6.** Dissolved oxygen traces of the single phase (○), solvent as a second phase (□), and the polymer beads as a second phase (×) systems during the small-step change.

subsequently degraded further (requiring oxygen) during the post-step period.

The small steps clearly showed that having a second phase present provide a significant improvement in the performance of the three systems, with the solvent as a second phase being the most effective.

## Large Steps

Figures 7–9 show the transient responses of the three systems with the large step change (∼280 (g/m$^3$/h)/(g-cells)) imposed. In this larger step, the toluene loading was increased from its nominal rate of 343 g/m$^3$/h to approximately 6,000 g/m$^3$/h for a period of 60 min. The instantaneous removal efficiency of the single-phase system (Fig. 7) dropped immediately upon the onset of the step and appeared to reach a steady state over the course of the step at an instantaneous removal efficiency of 45%. A significant proportion of the toluene loading can be seen to be stripped from the system (Fig. 7A), and outlet toluene concentrations reached 40 mg/L (Fig. 7B). The instantaneous removal efficiencies of the two second-phase systems decreased less dramatically over the course of the step, tracking downwards during the transient, and reached minimum values of 60% for the polymer bead system and 94% for the solvent system. It is again interesting to note the initial *increase* in removal efficiency for the *n*-hexadecane case (Fig. 9) arising from the capacity of the solvent to absorb the imposed higher toluene loading. Lower outlet toluene concentrations were also seen for the

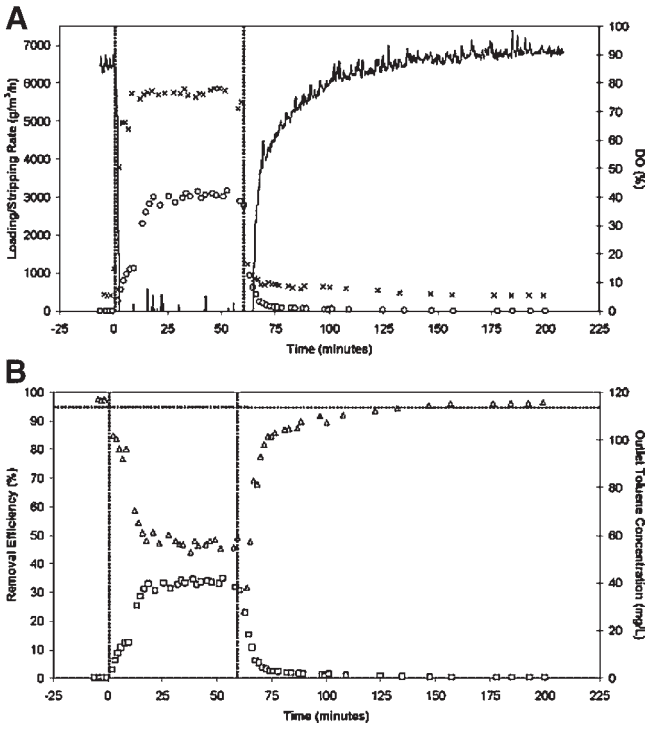


**Figure 7.** **A**: Dynamic response (Inlet (×) and outlet (○)) and dissolved oxygen trace and (**B**) instantaneous removal efficiencies (△) and outlet toluene concentrations (□) of the partitioning bioreactor operated with no second phase when imposed with a 1-h toluene step of 289 (g/m$^3$/h)/(g-cells).



Biotechnology and Bioengineering. DOI 10.1002/bit

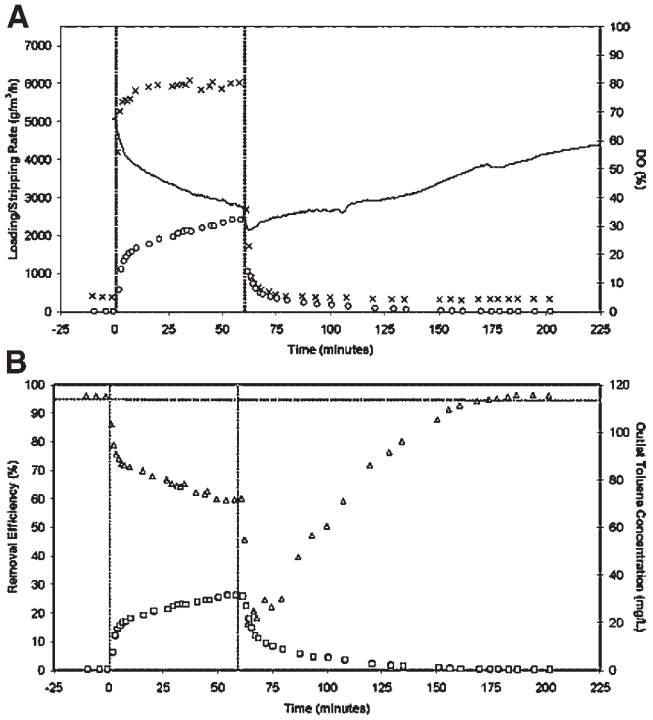


**Figure 8.** **A**: Dynamic response (Inlet (×) and outlet (○)) and dissolved oxygen trace and (**B**) Instantaneous removal efficiencies (△) and outlet toluene concentrations (□) of the two-phase partitioning bioreactor operated with SB co-polymer beads as the second phase when imposed with a 1-h toluene step of 285 (g/m$^3$/h)/(g-cells).

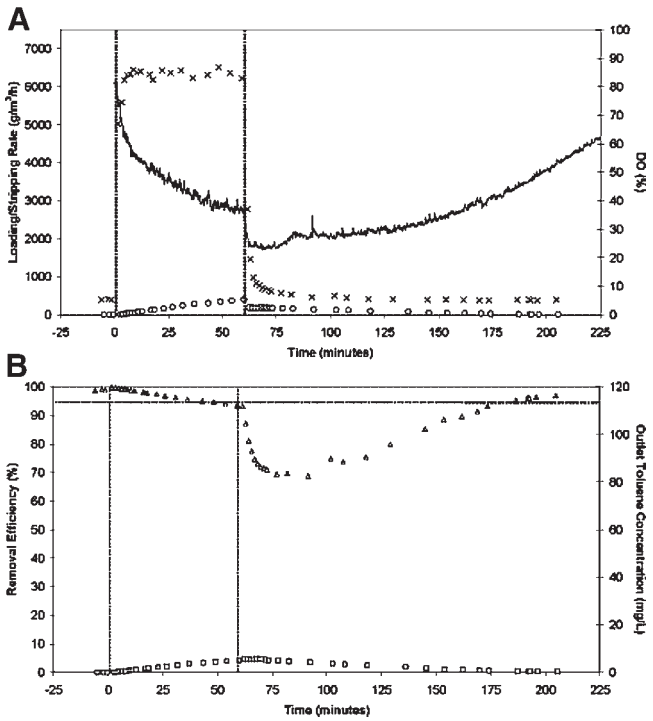


**Figure 9.** **A**: Dynamic response (Inlet (×) and outlet (○)) and dissolved oxygen trace and (**B**) instantaneous removal efficiencies (△) and outlet toluene concentrations (□) of the two-phase partitioning bioreactor operated with *n*-hexadecane as the second phase when imposed with a 1-h toluene step of 280 (g/m$^3$/h)/(g-cells).

two-phase systems, with the *n*-hexadecane system emitting only 6 mg/L at the conclusion of the step (Fig. 9B). As in the case of the small step, immediately upon the completion of the step, the instantaneous removal efficiencies of all three systems decreased sharply, with the systems achieving minimum instantaneous removal efficiencies of 23% for the single-phase system, 16% for the polymer beads as a second-phase system, and 69% for the solvent as a second-phase system. The single-phase, polymer bead and solvent systems eventually recovered and crossed the 95% instantaneous removal line 87, 118, and 126 min after the completion of the step, respectively.

The impact of the second phases in terms of overall removal efficiencies were even more pronounced for the larger step experiments in which aqueous toluene levels had the potential to rise to inhibitory levels. Table I shows that in terms of overall removal efficiency the system with the solvent as the second phase was again superior to the one utilizing polymer beads as a second phase, which surpassed the performance of the single aqueous-phase system. Overall, relative to the total amount of toluene added to the bioreactors, the system using solvent as the second phase released only 1,292 mg of toluene (94% removal), the system using polymer beads as a second phase released 7,003 mg of toluene (66%), while the system with no second phase released 8,614 mg of toluene (58% removal). Comparison of the performances of the two-phase systems again must be done with care, however, given the different masses of second phase present.

Figure 10 shows the estimated (based on known toluene partition coefficient) aqueous phase toluene concentrations over the course of the 200-min experiment. The concentration of toluene at which inhibition occurs (i.e., specific growth rate declines) depends on the organism(s) employed and has been reported as 30 mg/L (Abuhamed et al., 2004), 43 mg/L (Reardon et al., 2000), and 70 mg/L (Elmen et al., 1997). As can be seen, with no second phase present, the aqueous phase toluene concentration remained high (>150 mg/L) throughout the experiment, while the polymer beads as a second phase case surpassed 100 mg/L approximately 20 min into the 60-min step, and the solvent as a second phase system

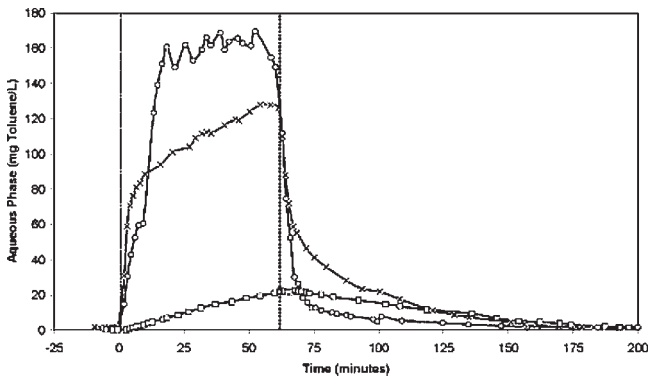


**Figure 10.** Aqueous phase toluene concentrations of the single phase (○), solvent as a second phase (□), and the polymer beads as a second phase (×) systems during the large step change.



DOI 10.1002/bit

never exceeded 20 mg/L of toluene throughout the step, however it also increased throughout the 60 min transient. The fact that the aqueous phase concentration reached such high levels in the system utilizing the polymer beads as a second phase may be the reason why the overall removal efficiency of this system was closer to that of the single-phase system, rather than the solvent case. As can be seen in Figure 8, the instantaneous removal efficiency of the polymer system decreased sharply at the beginning of the step change, then more gradually, throughout the rest of the step change. This is consistent with the initial rapid increase in aqueous toluene concentration at the start of the step (Fig. 10) followed by a more gradual concentration increase during the rest of the step. Inhibitory toluene levels, as may have been the case for the single phase and the polymer system, raise the prospect of process instabilities with a repeating cycle of increasing concentrations causing reduced microbial activity which leads to further increases in toluene concentration and continued deterioration in performance. The DO trace for the polymer system (Fig. 8A), however, continued to steadily decrease over the course of the 60-min step indicating ongoing cellular activity. Ongoing microbial activity throughout the step also appeared to be the case for the single-phase system as the DO remained at zero. In contrast, the solvent as a second-phase system experienced only a very slight drop in performance during the large step (with subsequent decreased removal efficiency as a result of toluene stripping) and abundant DO during the course of the dynamic period. This is likely due to the high affinity (and absorption) by *n*-hexadecane of both toluene and oxygen.

The DO in the single-phase system (Fig. 7A) dropped quickly, to 0% saturation, indicating that the system was oxygen limited, but quickly recovered (65 min after the completion of the step). The DO traces of the two systems using second phases (Figs. 8A and 9A) dropped more gradually than the single-phase system, and also recovered much more gradually than the single-phase system. In both cases with the second phases present, the DO in the system did not appear to become oxygen limiting (remaining above 20% saturation) and both systems recovered at much slower rates than the single-phase system (271 min for the polymer bead system and 190 min for the solvent system).

The 95% instantaneous removal efficiency recovery times (Table I) followed a pattern similar to the small-step experiment, with the solvent, capable of absorbing the most toluene, taking the longest to recover (126 min), while the polymer beads were intermediate (118 min) and the single-phase system recovered the quickest (87 min). It also appears as though none of the systems was permanently affected by the large step change dynamic, as all three systems returned to high performance operation soon after the return of base-case operating conditions.

## Performance Comparison With the Literature

Transient testing of biological remediation systems in the literature ranges widely in the size and duration of spike and step changes imposed. This work is the first investigation that has examined transients under such extreme loadings, as no reports are found in the literature that have been conducted with VOC transients exceeding 1,000 g/m$^3$/h. In this investigation, from a nominal toluene loading of 343 g/m$^3$/h the small and large steps reached toluene loadings as high as 2,701 and 6,423 g/m$^3$/h, respectively. As a result of these high loadings and the comparatively low loadings cited in the literature, it is difficult to compare these results against previously published reports.

Atoche and Moe (2004) imposed toluene and MEK step changes at five times their nominal loading rates in two different types of biofilters. The total toluene removal efficiencies over the course of their steps ranged between 92.1% and 73.0% for their continuous flow biofilter (CFB) and between 98.0% and 75.6% for their sequencing batch biofilter (SBB), and the instantaneous removal efficiencies after the steps were completed also dropped dramatically as was seen in this investigation. The toluene recovery times for these biofilters were under 30 min in every case. These results are very similar to the results seen in this investigation, however, the study by Atoche and Moe dealt with steps that did not exceed 200 g/m$^3$/h of toluene whereas the steps in this investigation had loadings exceeding 10 times that level.

Marek et al. (2000) performed similar 1-h steps with a mixed stream of xylene and toluene as feeds to a biofilter filled with a mixture of peat, bark, and wood inoculated with a mixed microbial population. These steps were at inlet VOC concentrations of 2 mg/L corresponding to a VOC loading of 200 g/m$^3$/h. The biofilters in this investigation had average removal efficiencies of approximately 75% for the step changes and had recovery times on the order of an hour or less. Again, however, the VOC loadings used by Marek et al. were much smaller than the loading used in the present investigation.

Longer step changes were studied by Seignez et al. (2004) who induced 2–3-h step changes in a biotrickling filter treating chlorobenzene-containing waste gas. When the inlet feed stream was increased from 75 to 375 g/m$^3$/h (five times the nominal loading rate) the instantaneous removal efficiency of the system dropped from values above 95% to a minimum of 55% and took almost 6 h to fully recover. Based on the fact that the nominal loading rate for the present investigation was close to that of the largest step in the investigation by Seignez, it is evident that TPPBs have the potential to operate in a competitive fashion to biotrickling filters. Moreover, relatively long term (30 day) operation of TPPBs to treat VOCs using hexadecane as the delivery phase has also been shown (Nielsen et al., 2005).

Tang et al. (1995) investigated the shock loading of toluene on three different biofilters. Each biofilter contained a different filter materials; chaff/compost, D.E. (diatomaceous earth)/compost, and GAC/compost. To achieve transients, the researchers abruptly increased the inlet toluene concentration to each of the biofilters. In each case, the biofilters had been operating at removal efficiencies of over 95% (inlet = 0.051 g/m$^3$), however due to the sudden increase

(inlet = 1.87 g/m$^3$), each of the biofilters' removal efficiencies decreased dramatically (65%–75%). The inlet concentration was then increased once more (inlet = 3.32 g/m$^3$) after a period of approximately 150 h and the removal efficiency once again dropped to 50%–65%. An interesting phenomenon occurred in all three cases when the inlet concentration was dropped from its highest value (3.32 g/m$^3$) back to a lower input value (0.89 g/m$^3$). Under these conditions the instantaneous removal efficiency dipped dramatically (to below 20%) as a result of the toluene that had sorbed to the packing material desorbing, resulting in higher outlet toluene concentrations than were experienced prior to the inlet change. The results from this experiment showed that the GAC/compost biofilter had the highest maximum steady state elimination capacity of 95 g/m$^3$/h.

In the present investigation, the drop in the instantaneous removal efficiencies after the loading to the systems had been returned to their original values does not necessarily reflect a decrease in the intrinsic performance of the system. As can be seen in Figures 2–4 and 7–9, even though the instantaneous removal efficiencies of the systems do decrease, the actual toluene concentration at the outlet does not increase. This delayed decrease in removal efficiency is a result of absorption/desorption of toluene by the aqueous solution, polymer beads and solvent, resulting in higher outlet toluene gas readings than were recorded prior to the transient. Removal efficiency is a function of the concentrations of inlet and outlet toluene gas concentrations and, in these cases, the inlet toluene concentration was decreased, but the outlet toluene concentration does not decrease immediately, due to absorption, resulting in lowered instantaneous removal efficiencies. It may therefore be more useful to consider the overall removal efficiency over the course of the perturbation, rather than drawing conclusions based on performance near a change of conditions. This type of absorption phenomenon has been seen and described previously by Marek et al. (2000) who witnessed negative removal efficiencies during one of their step changes on a biofilter treating xylene and toluene, and has also been observed and discussed by several other researchers (Atoche and Moe, 2004; Campbell and Connor, 1997; Deshusses, 1997; Deshusses et al., 1995; Tang et al., 1995; Zarook et al., 1997). The absorption phenomenon in the case of the single phase system quickly disappeared as a result of the low aqueous solubility of toluene in water and the high Henry's Law value between the two compounds, whereas in the case of the solvent and polymer beads it lasted for much longer periods of time due to high toluene absorption.

## CONCLUSIONS

Based on the results presented in this investigation, it is clear that using *n*-hexadecane as a second phase was superior to using SB copolymer beads in terms of performance based on the overall removal efficiencies over the course of the imposed transients. The presence of the solvent allowed for much lower aqueous phase toluene concentrations resulting in very high overall removal efficiencies and allowed the system to always stay well above oxygen limiting conditions. The use of polymer beads as a second phase also proved to be very beneficial as a second phase and vastly improved the removal of toluene from the contaminated air stream, as well as oxygen delivery, over that of the single phase. It is anticipated that using larger masses of SB beads (approaching the mass of *n*-hexadecane used) may result in comparable performance to the two-liquid system, in terms of both toluene removal and oxygen augmentation. Employing polymer beads in TPPB systems allows the use of microbial consortia (Prpich and Daugulis, 2005), something that is not always possible with the use of immiscible organic solvents; thus contamination would no longer be a concern and sterility precautions would not need to be taken. These potential improvements, along with a systematic selection and specification of polymers for substrate uptake and oxygen delivery are currently under investigation.

The financial support of the Natural Sciences and Engineering Research Council of Canada is gratefully acknowledged.

## References

Abuhamed T, Bayraktar E, Mehmetoglu T. 2004. Kinetics model for growth of *Pseudomonas putida* F1 during benzene, toluene, and phenol biodegradation. Process Biochem 39:983–988.

Aizpuru A, Khammar N, Malhautier L, Fanlo JL. 2003. Biofiltration for the treatment of complex mixtures of VOC influence of the packing material. Acta Biotechnol 23:211–226.

Amsden BG, Bochanysz J, Daugulis AJ. 2003. Degradation of xenobiotics in a partitioning bioreactor in which the partitioning phase is a polymer. Biotechnol Bioeng 84:399–405.

Atoche JC, Moe WM. 2004. Treatment of MEK and toluene mixtures in biofilters: Effect of operating strategy on performance during transient loading. Biotechnol Bioeng 86:468–481.

Bowen HJM. 1970. Absorption by polyurethane foams—new method of separation. J Chem Soc A—Inorganic Physical Theoretical 7:1082–1085.

Campbell HJ, Connor MA. 1997. Practical experience with an industrial biofilter treating solvent vapour loads of varying magnitude and composition. Pure Appl Chem 69:2411–2424.

Daugulis AJ, Boudreau NG. 2003. Removal and destruction of high concentrations of gaseous toluene in a two-phase partitioning bioreactor by *Alcaligenes xylosoxidans*. Biotechnol Letters 25:1421–1424.

Daugulis AJ, Amsden BG, Bochanysz J, Kayssi A. 2003. Delivery of benzene to *Alcaligenes xylosoxidans* by solid polymers in a two-phase partitioning bioreactor. Biotechnol Letters 25:1203–1207.

David H, Bowzon J, Vergnaud JM. 1989. Controlled absorption and release of an active agent by using evac beads—effect of various parameters. Eur Polymer J 25:1007–1011.

Davidson CT, Daugulis AJ. 2003. Addressing biofilter limitations: A two-phase partitioning bioreactor process for the treatment of benzene and toluene contaminated gas streams. Biodegradation 14:415–421.

Deshusses MA. 1997. Biological waste air treatment in biofilters. Curr Opin Biotechnol 8:335–339.

Deshusses MA, Hamer G, Dunn IJ. 1995. Behavior of biofilters for waste air biotreatment. 1. Dynamic model development. Environ Sci Technol 29:1048–1058.



DOI 10.1002/bit

Deshusses MA, Hamer G, Dunn IJ. 1996. Transient-state behavior of a biofilter removing mixtures of vapors of MEK and MIBK from air. Biotechnol Bioeng 49:587–598.

Elmen J, Pan W, Leung SY, Magyarosy A, Keasling JD. 1997. Kinetics of toluene degradation by a nitrate-reducing bacterium isolated from a groundwater aquifer. Biotechnol Bioeng 55:82–90.

El-Shahawi MS. 1994. Retention and separation of some organic water pollutants with unloaded and tri-n-octylamine loaded polyester-based polyurethane foams. Talanta 41:1481–1488.

MacLeod CT, Daugulis AJ. 2003. Biodegradation of polycyclic aromatic hydrocarbons in a two-phase partitioning bioreactor in the presence of a bioavailable solvent. Appl Microbiol Biotechnol 62:291–296.

Marek J, Paca J, Gerrard AM. 2000. Dynamic responses of biofilters to changes in the operating conditions in the process of removing toluene and xylene from air. Acta Biotechnol 20:17–29.

Michaels AS, Wong PSL, Prather P, Gale RM. 1975. A thermodynamic method of predicting the transport of steroids in polymer matrices. AIChE Journal 21:1073–1080.

Mutafov S, Angelova B, Schmauder H-P, Avramova T, Boyadjieva L. 2004. Stoichiometry of microbial continuous-flow purification of toluene contaminated air. Appl Microbiol Biotechnol 65:22–234.

Nielsen DR, Daugulis AJ, McLellan PJ. 2003. A novel method of simulating oxygen mass transfer in two-phase partitioning bioreactors. Biotechnol Bioeng 83:735–742.

Nielsen DR, Daugulis AJ, McLellan PJ. 2005. Quantifying maintenance requirements from the steady-state operation of a two-phase partitioning bioscrubber. Biotechnol Bioeng 90:248–258.

Prpich GP, Daugulis AJ. 2004. Polymer development for enhanced delivery of phenol in a solid-liquid two-phase partitioning bioreactor. Biotechnol Progr 20:1725–1732.

Prpich GP, Daugulis AJ. 2005. Enhanced biodegradation of phenol by a microbial consortium in a solid-liquid two phase partitioning bioreactor. Biodegradation 16:329–339.

Reardon KF, Mosteller DC, Rogers JDB. 2000. Biodegradation kinetics of benzene, toluene, and phenol as single and mixed substrates for *Pseudomonas putida*F1. Biotechnol Bioeng 69:385–400.

Schumack L, Chow A. 1987. Extraction of aromatic organic-compounds by polyurethane foam. Talanta 34:957–996.

Seignez C, Adler N, Thoeni C, Stettler M, Peringer P, Holliger C. 2004. Steady-state and transient-state performance of a biotrickling filter treating chlorobenzene-containing waste gas. Appl Microbiol Biotechnol 65:33–37.

Tang HM, Hwang S-J, Hwang S-C. 1995. Dynamics of toluene degradation in biofilters. Hazard Waste Hazard Mater 12:207–219.

Weber FJ, Hartmans S. 1995. Use of activated carbon as a buffer in biofiltration of waste gases with fluctuating concentrations of toluene. Appl Microbiol Biotechnol 43:365–369.

Yeom SH, Daugulis AJ. 2001. Benzene degradation in a two-phase partitioning bioreactor by *Alcaligenes xylosoxidans* Y234. Process Biochem 36:765–772.

Zarook SM, Shaikh AA, Ansar Z. 1997. Biofiltration of volatile organic compound (VOC) mixtures under transient conditions. Chem Eng Sci 52:4135–4142.