Solid-Liquid two-phase partitioning bioreactors for the treatment of gas-phase VOCs

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ABSTRACT

Two-Phase Partitioning Bioreactors (TPPBs) consist of a cell-containing aqueous phase and a separate, biocompatible and immiscible phase that partitions toxic substrates to the cells based on their metabolic demand and on maintaining the thermodynamic equilibrium of the system. TPPBs have traditionally used immiscible liquid organic solvents as the substrate delivery phase, however, one of the limitations of organic solvents is their potential bioavailability as substrates, and therefore these TPPB systems have generally been limited to the use of pure strains of organisms incapable of metabolizing the solvent. We have replaced the organic solvent phase in TPPBs with inert polymers (plastic beads). A TPPB employing styrene-butadiene beads as the sequestering phase was used to treat high step change loadings of BTEX in a contaminated air stream. The presence of the polymers allowed the system to effectively capture the incoming VOCs, buffer the cells from high VOC levels and release the VOCs to the cells for biodegradation. The polymer TPPB system demonstrated substantially higher performance than an aqueous phase bioscrubber and comparable performance to a solvent-aqueous TPPB. Also of great interest was the increase in oxygen transfer provided to the system by the addition of polymer beads, which have significant affinity for oxygen. The presence of polymer beads, which are biocompatible and non-bioavailable, provides a simple and effective means of enhancing the bioremediation of toxic organics present in gas streams, and potentially other phases.

1 INTRODUCTION

Biological treatment of contaminated air streams can be an effective and economical means for the degradation of volatile contaminants in airstreams. Generally, the pollutants are absorbed from the gas phase to an aqueous phase in which the active

microbial culture degrades the target pollutants. The most common types of biological treatment systems are biofilters, biotrickling filters, and bioscrubbers. The mechanisms for removal are similar for all reactor types, however, differences are found in the phases in which the microbial population is located, and the state of the liquid phase.

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. 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. We have recently shown (Amsden *et al*., 2003; Prpich and Daugulis, 2004, 2005) that the second phase, traditionally an 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. Solid polymers absorb (rather than adsorb) organic molecules, and are characterized by a partition coefficient analogous to organic solvents.

In a somewhat related 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 and 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. The design of TPPBs provides the potential to handle fluctuations with the second immiscible phase acting as a buffer, or a «sponge», and absorbs the high concentrations of pollutants.

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 transient VOC loadings. In addition, the positive effect that the presence of a polymer second phase can have on oxygen transfer was investigated.

2 MATERIALS AND METHODS

2.1 Chemicals

In the two-liquid TPPB arrangement, n-hexadecane was used as the toluene-sequestering phase. n-hexadecane is a suitable solvent for the TPPB treatment of VOCs and the long-term (> 1 month) use of hexadecane as a benzene delivery phase for *A. xylosoxidans* Y234 has been amply demonstrated (Nielsen *et al.*, 2005a). Styrene-butadiene (28% styrene) ABA co-polymer beads (with dimensions of approximately L=4.25 mm, D=3.75 mm and density of 0.94 g/mL) were used as toluene-sequestering phase in the solid-liquid TPPB. For oxygen transfer experiments nylon 6,6, glass beads and silicone rubber beads were used in addition to the styrene-butadiene (SB) beads.

2.2 Microorganism, Medium, and Culture Conditions

Achromobacter xylosoxidans Y234 is known to have the ability to degrade toluene, benzene and m-xylene. The growth medium (Davidson and Daugulis, 2003) was: 7 g/L $(NH_4)_2SO_4$, 0.75 g/L $MgSO_4 \bullet 7H_2O$, 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. Eight 125 mL Erlenmeyer shake flasks containing 50 mL of medium were inoculated from frozen stock prior to incubation at 30°C and 150 rpm for 24 hours in preparation for their inoculation in the bioreactor.

2.3 Reactor set-up and operation

A 5 L New Brunswick Scientific BioFlo III was set to operate at 30°C, a pH of 6.6, an agitation speed of 800 rpm and a total working volume of 3 liters. The aqueous medium consisted of 14 g/L $(NH_4)_2SO_4$, 1.5 g/L $MgSO_4 \times 7H_20$, 13.2 g/L K_2HPO_4, 16.84 g/L KH_2PO_4 and 0.16mL/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, 500g of polymer beads were used with 2.518L of aqueous medium added for a final total volume of 3L. 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 toluene delivery system consisted of an Erlenmeyer flask with 2L of toluene and a regulated amount of compressed air being sparged through it that continued into the reactor. A water bath 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. Dissolved oxygen levels were measured with a polarographic-membrane electrode. Concentrated nutrient boluses were added periodically (every 2-3 days) to

the reactor to ensure that the system was not nutrient limited. A small amount of Sigma Antifoam 289 (~0.5mL) was added as required.

2.4 Analytics

Liquid samples, to measure biomass concentration, were centrifuged, the liquid supernatant was discarded and the biomass was then re-suspended in deionized water. Appropriate dilutions were then performed and measured at 650 nm and compared to a previously determined calibration curve. Inlet and outlet gas samples were taken by means of a 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, 0.53mm I.D., 30m length, 1mm film thickness, Model 125-503J, J & W Scientific, Inc., Folsom, CA) was used to analyze toluene concentrations. The aqueous phase toluene concentration was calculated based on Henry's Law 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.

2.5 Steady state and transient operation

Immediately after inoculation a total flow rate of 1.71L/min air (0.57vvm) 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 reached a steady state in each case within 5 to 7 days 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. All transient experiments were performed once the biomass levels had stabilized after the initial 5 to 7 day growth period.

Inlet toluene steps were introduced for periods of 60 minutes 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 normalized with respect to total cell mass present to ensure that the performance of each system was not affected by the amount of biomass present. The step was imposed for each bioreactor configuration above the steady state feeding condition at a loading of approximately 110 $(g_{Toluene}/m^3_{reactor}.h)/(g\text{-cells})$. Alternatively, from a stable loading of 343 $g/m^3.h$, steps of approximately 2400 $g/m^3.h$ were performed, after which the flows were readjusted to their original set-points and the inlet and outlet toluene levels were monitored until the instantaneous removal efficiency of the system returned to its original steady state value.

2.6 Oxygen transfer experiments

The same New Brunswick Bioflo III operating at 30 °C was used for oxygen transfer experiments. All systems, which consisted of either tap water or 500 g of either glass beads, nylon 6,6, styrene–butadiene copolymer (SB) or silicone rubber in tap water to a total working volume of 3 L, were operated at aeration rates of 0.5 L/min, 0.75 L/min and 1 L/min and agitation rates of 100-800 rpm. Mass transfer measurements were taken in duplicate with the average value being reported, and the effect of probe response was also incorporated in the analysis. All solid particles used were approximately spherical in shape. The unsteady-state method described by Shuler and Kargi (2002) was used to determine mass transfer coefficients that reflected either the presence of inert particles (e.g. glass beads and nylon 6,6) alone, or the presence of particles that also possessed oxygen affinity (e.g styrene–butadiene or silicone rubber). The effect of the presence of inert particles was «separated» from the combined effect of «presence + affinity» using mathematical analysis as previously described (Littlejohns and Daugulis, 2007) to isolate the oxygen transfer enhancement that can be obtained in the presence of polymer beads possessing oxygen affinity.

3 RESULTS AND DISCUSSION

3.1 TPPB operation

Shortly (< two days) after inoculation, the removal efficiencies increased to greater than 95% and at a toluene loading of 343 g/m^3.h, the cells reached steady state masses ranging between 19.4 and 26.2 grams (CDW) within 7 days. The removal efficiencies of the systems remained greater than 95% for the entirety of the experiments except during transients. Recent work by Nielsen *et al*. (2005a) has shown that a constant cell concentration will eventually be established due to cellular maintenance requirements, which are responsible for all of the substrate consumed.

Figures 1-3 show the transient responses when the loading (~110 (g/m^3.h)/(g-cells)) was increased from its nominal rate of 343 g/m^3.h to approximately 2400 g/m^3.h for a period of 60 minutes. The instantaneous removal efficiencies of the single-phase system and the polymer 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 minute step. This is also reflected in the outlet toluene concentrations 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 minute 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 of the single-phase system.

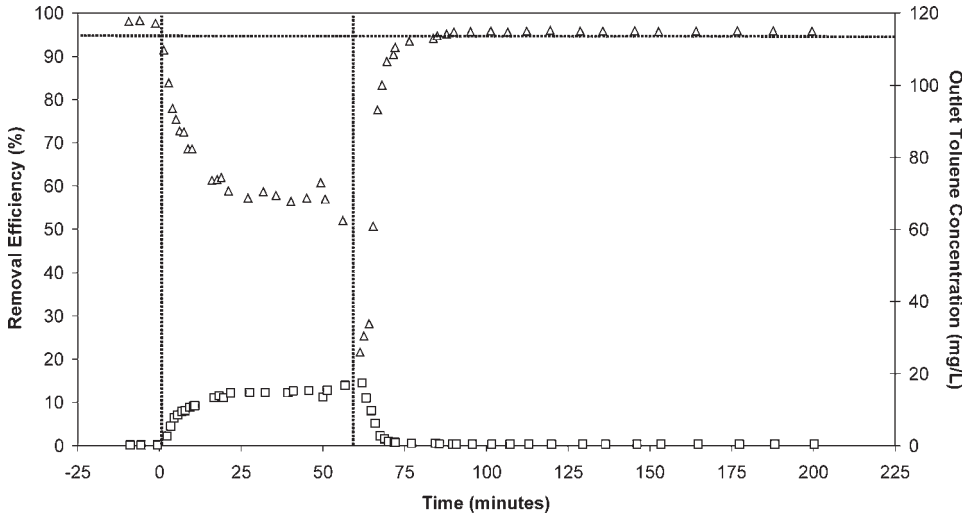

Figure 1. Removal efficiency (triangles) and exit toluene concentration (squares) for single-phase system.

The solvent as a second phase system removed 97% of the toluene fed to the system over the course of the 200 minute experiment (Table 1), with the polymer phase system removing 90% of the toluene, and the single 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.

A comparison of the DO traces of the three systems (Figure 4) shows that DO for the solvent system remained the highest reaching a minimum DO value of 48% of saturation, while that of the polymer 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 also 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.

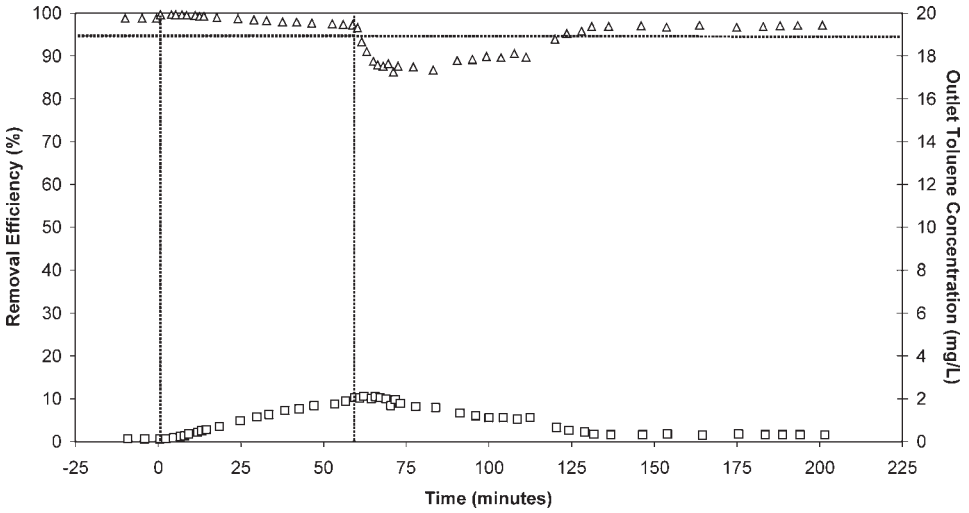

Figure 2. Removal efficiency (triangles) and exit toluene concentration (squares) for solvent-phase system.

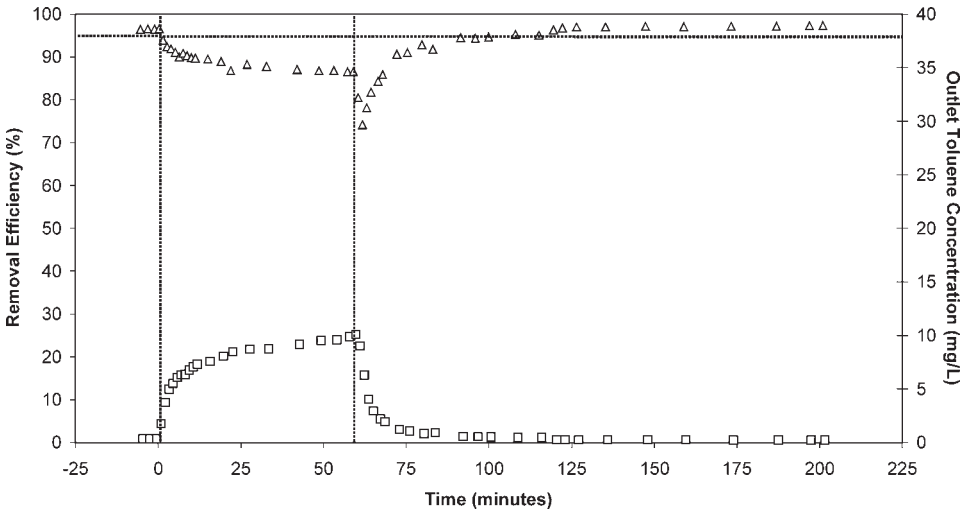

Figure 3. Removal efficiency (triangles) and exit toluene concentration (squares) for polymer-phase system.

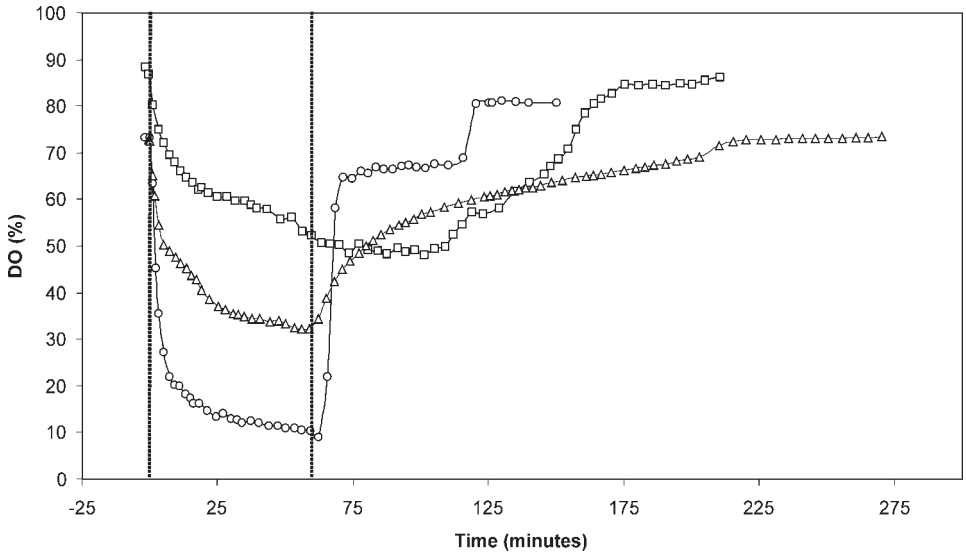

Figure 4. DO profiles for single (circles), polymer (triangles), and solvent (squares) systems.

Table 1.
Performance summary during imposed step transients.

Reactor Type	95% Recovery Time (Minutes)	DO Recovery Time (Minutes)	Toluene Released During Step (mg)	Toluene Released After Step (mg)	Total Toluene Released (mg)	Overall Removal Efficiency (%)
Single -phase	30	60	3002	292	3294	69
Solvent	63	115	116	198	314	97
Polymer	48	162	864	165	1030	90

3.2 Oxygen transfer

In light of the enhanced oxygen transfer seen in the polymer TPPB, the effect of the presence of polymer beads on O_2 transfer was examined in more detail as described elsewhere (Littlejohns and Daugulis, 2007). The presence of the beads could have 2 effects on O_2 uptake: a physical effect arising from their mere presence, and an absorptive effect in which O_2 is actually taken up by the polymer. In order to examine this in detail, polymers with negligible O_2 affinity (e.g. nylon 6,6 with a diffusivity of 1.6×10^{-9} cm^2/s), and polymers with substantial O_2 affinity (SB with a diffusivity of

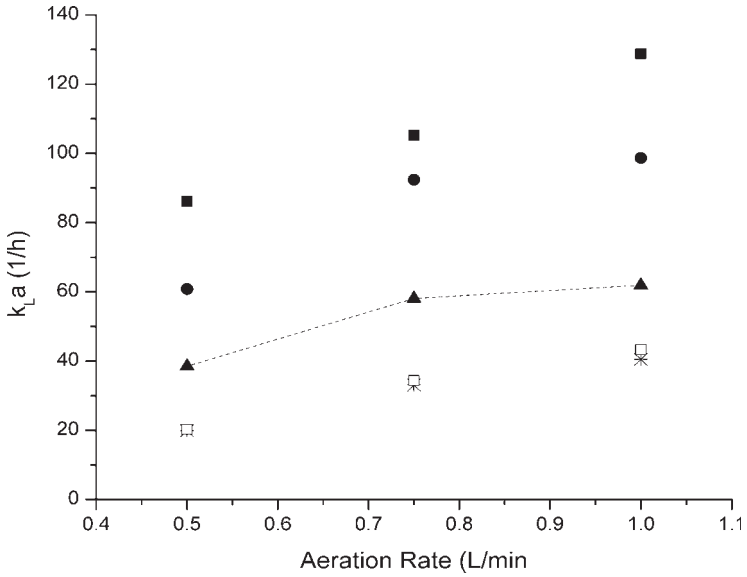

Figure 5. Mass transfer coefficients at 400 rpm for nylon 6,6 (square), glass beads (circle), water (triangle and line), silicone rubber (square) and styrene–butadiene copolymer (star).

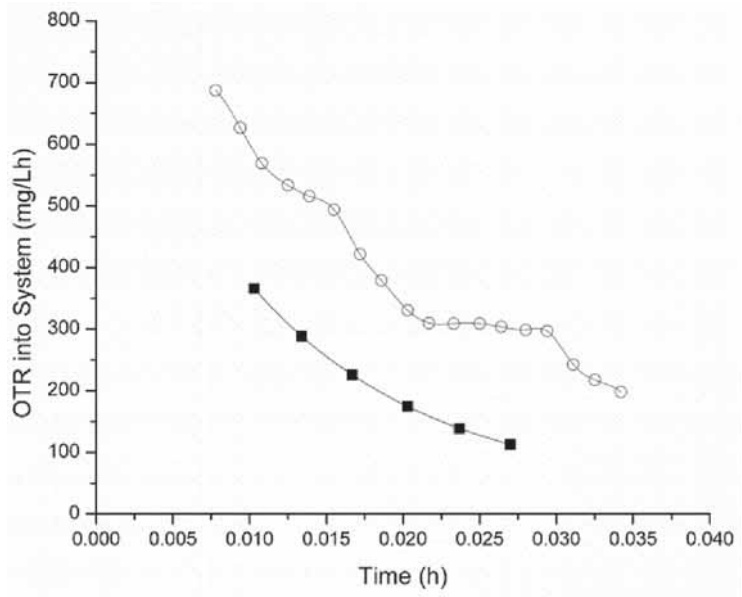

Figure 6. Oxygen transfer rate between 30% and 80% of liquid saturation by a system of water with silicone beads (circles) and water without particles (squares).

1.4 x 10^{-6} cm^2/s, and silicone rubber with a diffusivity of 3.4 x 10^{-5} cm^2/s) were tested along with glass beads with essentially zero O_2 affinity as a control. The presence of beads on both the kLa and on the oxygen transfer rate (OTR) was examined. The volumetric mass transfer coefficients (kLa) for aqueous systems with and without particles are shown in Figure 5 for different aeration rates at 400 rpm. The kLa values are up to 55% *lower* for the system with SB beads relative to the system without particles and up to 63% lower for the system containing silicone rubber beads, which at first seems counter-intuitive, given the earlier TPPB results. For systems containing SB and silicone rubber, the measurement of mass transfer coefficient contains the effect that the solid polymer particles may have on kLa, as well as any effects that they may have on absorbing oxygen. The system containing nylon 6,6 shows up to a 268% *increase* in kLa. Due to the low oxygen diffusion coefficient of nylon 6,6, the effect of the nylon particles on the kLa is isolated, and mass transfer enhancement due to the mere presence of particles alone is clearly observed. In a similar manner to nylon 6,6, glass beads are inert and enhance the kLa up to 159%. Both nylon 6,6 and silicone rubber have very similar dimensions and densities, which have been identified earlier as critical factors for the effect of particles on kLa. Nylon 6,6, can therefore be used to approximate the effect of the presence of silicone rubber beads on kLa, as both the effects of oxygen absorption by the silicone rubber and the effects on the gas–liquid mass transfer are contained within the measured kLa for the silicone rubber system and cannot be separated. In order to demonstrate a *larger* overall uptake of oxygen into a TPPB system relative to a system without a second phase, the instantaneous oxygen transfer rate (OTR) as a function of time at 400 rpm agitation and 1 L/min aeration is shown in Fig. 5. This plot, which was generated by mathematically «separating» the kLa effect from the overall observed measurements, clearly shows that between 30% and 80% of liquid saturation the system containing silicone rubber beads has a much larger OTR during the progression to liquid saturation than the system without a second phase. As well, the system with a second phase reaches 80% liquid saturation much later than the system without a second phase. This is due to the polymers acting as an oxygen sink within the system, in turn causing the liquid oxygen concentration to be lower relative to the system without polymers, at any given time. This decrease in the liquid concentration causes an increased driving force for oxygen between the gas and liquid phases, which causes a larger oxygen transfer rate for an extended period of time. Therefore, although the kLa is measured as lower for the reasons explained above, the overall oxygen transfer rate into the solid–liquid system is *larger* for systems containing particles with oxygen affinity (e.g. silicone rubber or SB). This is due to the oxygen transfer rate not only being proportional to the volumetric mass transfer coefficient, but also to the increased instantaneous concentration driving force. The SB and silicone polymers have a large uptake of oxygen and therefore more oxygen can ultimately be contained within the

system. These results are comparable to those for liquid–liquid systems that have found that oxygen is transferred at a higher rate due to an increased driving force (Nielsen *et al.*, 2005b). However liquid–liquid systems increase the working volume oxygen saturation concentration, whereas the solid–liquid system increases the driving force by decreasing the liquid concentration at any given time, as well as by enhancing gas–liquid mass transfer. Nevertheless, both liquid–liquid and solid–liquid systems can increase the overall amount of oxygen that can be contained within a working volume.

4 CONCLUSION

The presence of a second immiscible phase, whether a liquid, or a carefully selected polymer, has been shown to significantly improve the ability of bioreactors to enhance VOC removal. Although data from the use of a pure strain have confirmed this in the present work, similar results have also been obtained with microbial consortia operating in polymer-based TPPBs. In addition, the presence of these materials can have a significant positive effect on O_2 transfer, which can be critical during dynamic periods of operation treating VOC surges. Adding such polymeric materials to more conventional biotreatment devices (e.g. biofilters) may also provide similar positive benefits on VOC buffering and removal, and on O_2 transfer.

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