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ORIGINAL RESEARCH PAPER

Solid-liquid two-phase partitioning bioreactors for the treatment of gas-phase volatile organic carbons (VOCs) by a microbial consortium

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Abstract A two-phase partitioning bioreactor (TPPB), employing styrene-butadiene co-polymer beads as the sequestering/delivery phase, was used to treat high step change loadings of toluene in a contaminated air stream. The polymers, which are biocompatible and non-bioavailable, allowed the use of a microbial consortium and effectively absorbed and released the toluene vapours for biodegradation, while providing a buffering effect against high toluene transients. Toluene loadings were increased from a base steady state rate of 343–6,000 g/m^3 h for 1 h periods, with the polymer-aqueous system substantially outperforming a single phase system on the basis of improving the toluene removal efficiency and reducing the maximum toluene concentrations emitted during the transients.

Keywords Absorption · Partitioning bioreactors · Polymers · Toluene · VOCs

Introduction

Two-phase partitioning bioreactors (TPPBs) rely on the use of an immiscible organic phase that acts as a reservoir for inhibitory substrates that are delivered to the cell containing aqueous phase at low concentrations determined by thermodynamic equilibrium partitioning (Daugulis 2001). Early TPPB research focused on the use of immiscible organic solvents as the absorption and delivery phase (Déziel et al. 1999; Muñoz et al. 2007) and the use of a single microbial species, as employing several microorganisms increases the chances of the solvent being bioavailable, which could interfere with degradation of the target substrate. Replacing the liquid organic phase in TPPBs with solid polymer (plastic) beads has recently been shown to be feasible (Amsden et al. 2003). It should be emphasized that the use of polymer beads to absorb target molecules is fundamentally different than the use of granulated activated carbon (GAC) to adsorb chemical species (Weber and Hartmans 1995), as GAC uptake of organics is a non-selective process based on available surface area, while absorption by solvents and polymers is specific to the chemical moieties involved requiring rational polymer selection (Prpich and Daugulis 2004) and is driven by partition coefficient-based equilibrium. Polymers are generally very inexpensive, can be formed into many shapes and sizes, can be tailored to a particular target molecule through monomer selection, cross-linking, and polymer processing and, most importantly, are non-biodegradable, thus opening the possibility of using mixed populations of organisms. Using a pure species of *Achromobacter xylosoxidans* Daugulis

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et al. (2003) showed that polymer beads can be used effectively when treating benzene in a TPPB via absorption by poly(ethylene-co-vinyl acetate) (EVA) and styrene-butadiene. Boudreau and Daugulis (2006) also used this pure species and confirmed effective toluene degradation with both liquid and solid delivery phases. To date, the solid-as-delivery phase concept has not been applied to a continuous process for volatile organic carbon (VOC) removal under dynamic conditions when a microbial consortium is employed, made possible by replacing the organic solvent phase with inert polymers. Here the performance of a microbial consortium operating in a TPPB with styrene-butadiene co-polymer beads as the second phase was compared to single-phase operation when large (17–20 times the nominal loading) 60 min step change loadings were imposed on the systems.

Materials and methods

Microorganisms

A selective enrichment was performed to isolate a consortium of bacteria capable of degrading toluene starting with soil samples obtained from a petrochemical refinery and an activated sludge process associated with a pulp mill. After 10 days of chemostat operation with a mineral salts medium and toluene as the sole carbon source, five different morphologies of bacteria were detected. Identification of individual microorganisms was then undertaken using API 20 NE identification strips.

Polymer characterization

Styrene-butadiene (28% styrene) ABA co-polymer beads (cylindrical, with dimensions of approximately $L = 4.25$ mm, $D = 3.75$ mm and density of 0.965 g/cm^3) were obtained from Scientific Polymer Products Inc. (Ontario, New York, USA). Several 125 ml serum bottles with Teflon septa were prepared with 4 g polymer (or no polymer as a control) and 100 ml of mineral salts medium. After toluene additions over the range 50–550 µl and vigorous agitation for 1 h, the concentration of toluene in the headspace was measured and the amount of toluene in the aqueous medium and in the polymer beads was determined by mass balance and Henry's Law. The aqueous phase toluene concentration was then plotted against the polymer bead toluene concentration to obtain the toluene partition coefficient.

Reactor set-up and operation

A 5 l New Brunswick Scientific BioFlo III reactor was prepared and operated as previously described (Boudreau and Daugulis 2006) with an aqueous volume of 3 l for the single phase case, and 500 g polymer beads and 2.518 l aqueous phase for a total working volume of 3 l for the TPPB case. The bioreactors were maintained automatically at pH 6.6, and 800 rpm, this latter value indicating complete mixing of the reactor contents. The toluene delivery system consisted of an Erlenmeyer flask kept at 30°C with 2 l of toluene and a regulated amount of compressed air being sparged through it. This toluene laden air stream was mixed with air for bioreactor aeration and the combined stream was delivered into the reactor through a sparger at the bottom of the reactor. Dissolved oxygen levels were measured with a polarographic-membrane electrode. Concentrated salt boluses were added periodically to the reactor based on a developed feeding schedule to ensure that the system was not nutrient limited.

Analytics

Liquid samples were periodically taken in order to measure biomass concentration as previously described (Boudreau and Daugulis 2006). For toluene, inlet and outlet gas samples were taken by means of a gas tight 250 µl syringe and assayed via gas chromatograph fitted with a flame-ionizing detector and a fused silica capillary column (DB-5 Model 125-503J, J and W Scientific). 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 $(\text{mg/l})_{\text{gas}}/(\text{mg/l})_{\text{aq}}$.

Steady state and transient operation

Immediately after inoculation, a total flow rate of 1.71 l/min air (0.58 vvm) with toluene at 10 mg/l was established for a loading rate of 343 g/m^3 h, which was maintained during the biomass growth phase and between dynamic step experiments. These

conditions provide mean gas-phase residence times of 1.75 and 1.5 min for the single phase and TPPB system, respectively. The cell growth slowed and reached a steady state in each case within 5–7 days of inoculation even with continued addition of substrate, which is due to the use of the consumed substrate for cell maintenance purposes only, rather than cell growth, as discussed previously (Nielsen et al. 2005). Cells were freely suspended in the aqueous phase with no attachment to the polymers, as has been documented previously (Amsden et al. 2003). All transient experiments were performed once the biomass levels had stabilized after the initial 5–7 days growth period. Inlet toluene steps were introduced to the system for periods of 1 h 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 based on the mass of biomass present in the reactors, viz. 280 ($g_{Toluene}/m^3_{reactor}$ h)/(g-cells). Alternatively, from a stable loading of 343 g/m^3 h, steps of approximately 6,000 g/m^3 h were imposed. Comparison of process performance was based on the maximum toluene concentrations observed in the off-gas and the aqueous phases, as well as the overall Removal Efficiencies (RE) during and after the transient, until the system had returned to 95% RE.

Results and discussion

Identification of the microbial consortium

Five individual bacterial isolates were purified with three of the five species being Gram-negative bacteria (identified as *Stenotrophomonas maltiphilia* and *Chromomonas acidivorans* using API 20NE strips, while the identity of the third bacterium, could not be confirmed). The other two were Gram-positive isolates tentatively identified as being members of the genus *Bacillus*.

Polymer partition coefficient

Figure 1 shows the linear relationship between the polymer and aqueous phase concentrations of toluene, the slope providing an estimate of the partition coefficient. The value for toluene, of approximately 465, is of the same order of magnitude as organic solvents that have been used in two-liquid TPPBs to treat VOCs, such as *n*-hexadecane, whose partition coefficient for toluene is 540 (Boudreau and Daugulis 2006).

Achieving steady state

Shortly (<2 days) after inoculation of the aqueous and TPPB systems, the instantaneous removal efficiencies increased to >95% at a toluene loading of 343 g/m^3 h and the biomass reached steady state total cell masses of between 21 and 25 g within 5 days of inoculation. The removal efficiencies of the systems remained >97% for the entirety of the experiments except during transient periods. Recent work by Nielsen et al. (2005) has shown that for a pure strain of bacteria treating VOCs a constant cell concentration will eventually be established due to cellular maintenance requirements, which are responsible for all of the substrate consumed. Here we also show that this will be the case for a mixed population after

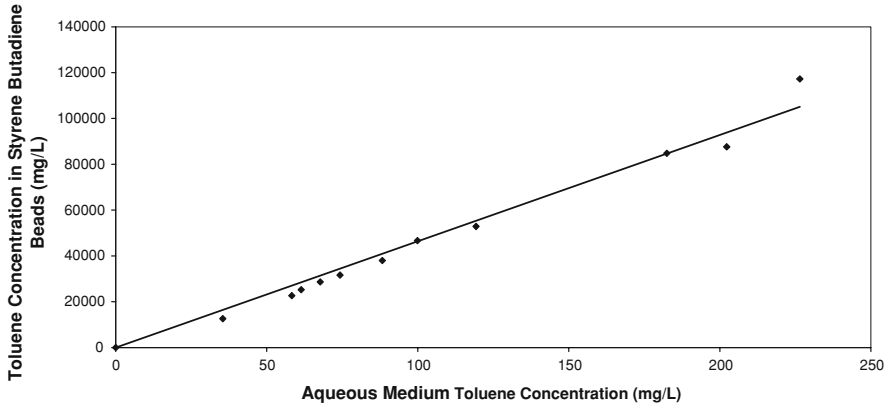

Fig. 1 Partition coefficient of toluene between mineral salts medium and polymer beads

an initial growth period due to the diversion of substrate entirely to maintenance functions.

Transients

The response to the imposed toluene transients for the single-phase system and two-phase system are shown in Figs. 2 and 3, respectively. Significant toluene stripping is seen for the single phase case, along with a rapid drop in RE from >97% to a fairly stable value of about 52%, before a large but brief decrease to 14%, reflecting the increased stripping of accumulated toluene once the step increase had been terminated. As expected, the presence of the polymer beads provided significant buffering of the system to the imposed step change (Fig. 3), as seen in the reduced toluene losses due to stripping as well as the much better RE during and after the imposed transient. The RE stabilized at about 80% during the step, and fell slightly, to 74%, before rapidly returning to its original high value. The DO profile in the TPPB system showed a greater drop due to the increased degradation of toluene, even though it has been shown that the presence of polymers increases the oxygen transfer in two-phase systems (Littlejohns and Daugulis 2007).

A summary of the performance of the two systems in response to the step change is shown in Table 1 which demonstrates the enhancements of performance arising from the presence of the polymer beads as indicated by reduced maximum off-gas concentration, lower maximum aqueous toluene concentrations, and higher REs over the course of, and after, an imposed step increase in toluene. The ability to handle transient loadings is an important consideration when evaluating the potential of a biotreatment system to handle VOCs, since, as noted by Zarook et al. (1997), transient fluctuations can be expected to be encountered more frequently in practice than stable operation. Since the uptake of toluene by polymers is absorptive, rather than adsorptive, the release of toluene to the cells is

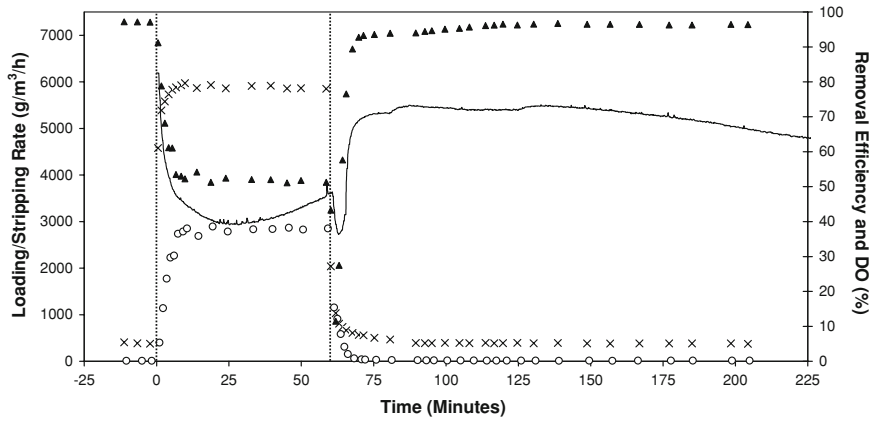

Fig. 2 Dynamic response (Inlet (×) and outlet (◯) toluene rates), dissolved oxygen concentration (–) and instantaneous removal efficiencies (▲) of the single phase partitioning bioreactor with an imposed 1 h toluene step of 283 (g/m^3 h)/(g cells)

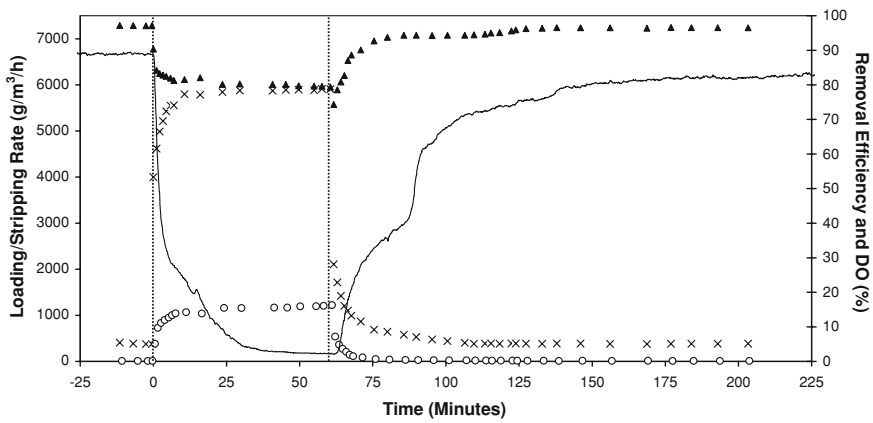

Fig. 3 Dynamic response (Inlet (×) and outlet (◯) toluene rates), dissolved oxygen concentration (–) and instantaneous removal efficiencies (▲) of the two-phase partitioning bioreactor with an imposed 1 h toluene step of 270 (g/m^3 h)/(g cells)

Table 1 Comparison of the performance of a single phase and a polymer two-phase system in treating a toluene-containing gas stream

	Maximum toluene in off-gas (mg/l)	Maximum toluene in aqueous phase (mg/l)	Toluene released during step (mg)	Toluene released after step (mg)	Total toluene released (mg)	Overall removal efficiency (%)
Single phase	37.6	152	8,100	408	8,508	59
Polymer	15.8	64	3,332	329	3,661	83

driven by the system seeking to maintain an equilibrium condition, which is ultimately determined by the instantaneous metabolic demands of the cells in consuming substrate from the aqueous phase. Thus the overall process "runs" on two familiar and fundamental phenomena: thermodynamic equilibrium and cell metabolism. Moreover, the beneficial effects demonstrated by the presence of the polymer beads need not be restricted to this particular polymer shape, as other shapes can be generated as reactor "internals" via the use of thermoplastics (Prpich and Daugulis 2007). Although regulations governing VOC emissions are generally set on the basis of emitting a maximum mass of contaminant per volume of air over a specified time period, removal efficiencies provide good relative estimates of process performance. The 83% obtained for the polymer TPPB system (Table 1) is in the upper end of the range deemed to be typical for VOC biotreatment processes of 60–95% (Khan and Ghossal 2000).

The addition of polymer beads to a VOC-degrading system is an extremely simple and effective way of improving performance, and should also be considered for other types of VOC-remediation devices such as biofilters. The non-biodegradable nature of most polymers makes the use of microbial consortia for VOC removal readily possible, as we have now shown here for the first time, which is an improvement over two-liquid phase systems that have previously used pure cultures for bioremediation applications. It must be stressed, however, that the selection of an appropriate polymer, as is the case in identifying a suitable organic solvent in two-liquid phase systems, must be done with care and consideration of the chemical properties of the materials present.

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