Bioprocess Biosyst Eng (2012) 35:1367–1374
DOI 10.1007/s00449-012-0725-1

ORIGINAL PAPER

Substrate mass transport in two-phase partitioning bioreactors employing liquid and solid non-aqueous phases

Hala Fam · Andrew J. Daugulis

Received: 19 January 2012 / Accepted: 9 March 2012 / Published online: 28 March 2012

Abstract The mass transfer of phenol and butyl acetate to/from water was studied in two-phase partitioning bioreactors using immiscible organic solvents and solid polymer beads as the partitioning phases in a 5-L stirred tank bioreactor. Virtually instantaneous mass transfer was observed with phenol in water/2-undecanone, and with butyl acetate in water/silicone oil systems. The mass transfer of butyl acetate to silicone oil was rapid irrespective of the viscosity of the partitioning phase. When Hytrel® polymer beads were employed as the partitioning phase, substrate transport to the polymer was found not to be externally mass transfer limited, but rather internally by substrate diffusion into the polymer. In contrast to gaseous, poorly soluble substrates studied in other works, mass transfer of soluble substrates such as phenol and butyl acetate to the polymer was unaffected by impeller speed but rather by polymer mass fraction.

Keywords Mass transfer · Partitioning bioreactor · Mass transfer coefficient · Soluble substrates · Organic solvents · Polymers

Introduction

Two-phase partitioning bioreactors (TPPBs) have been shown to be highly effective in overcoming the limitations of substrate toxicity and delivery typically experienced by conventional bioremediation strategies [1]. TPPBs consist of a cell-containing, aqueous phase and a non-biodegradable, immiscible organic phase which acts as a delivery agent that effectively partitions toxic substrates to/from the aqueous phase. Toxic contaminants with low water solubility partition into the organic phase at much higher concentrations than the aqueous phase, thus maintaining their concentration in the cell-containing phase at sub-inhibitory levels. The compounds are continuously delivered to the aqueous phase based on the metabolic demand of the cells and the maintenance of thermodynamic equilibrium between the two phases [2].

Such contaminants can include benzene which is relatively insoluble at 1,600 mg/L, but is toxic to many cells at a concentration of about 100 mg/L. Detoxification by sequestration is one of the primary goals of using TPPBs instead of conventional single-phase biodegradation processes. In studies by Tomei et al. [3], 4-nitrophenol biodegradation was achieved in the TPPB employing polymer beads, while no degradation occurred for the conventional one-phase stirred tank bioreactor. In addition, in studies by Littlejohns and Daugulis [4], polymers were able to buffer large concentrations of benzene, toluene, ethylbenzene and o-xylene (BTEX) in a solid–liquid TPPB and the polymer beads accounted for 93, 91, and 70 % of the total BTEX present in the working volume of the reactor.

In cell processes, if the mass transfer rate between the phases is substantially higher than the microbial consumption rate, the overall process rate is controlled by microbial kinetics [5]. For optimal performance of bioreactors, substrate delivery should be limited by microbial consumption rate rather than by mass transfer. Although several studies have investigated the potential of TPPBs in overcoming the limitations of oxygen mass transfer in biotransformations [6–11], relatively little attention has been paid to investigations involving substrate/contaminant

H. Fam · A. J. Daugulis (✉)
Department of Chemical Engineering,
Queen's University, Dupuis Hall,
19 Division St, Kingston, ON K7L 3N6, Canada
e-mail: andrew.daugulis@chee.queensu.ca

mass transfer. Moreover, most studies on substrate mass transfer have focused on poorly soluble gaseous compounds such as oxygen, hexane, α-pinene, and isopropylbenzene [5, 7, 12–16]. TPPBs have also been successful in the controlled delivery of relatively water-soluble substrates such as phenol [17, 18]. A systematic study of the mass transfer of relatively water-soluble substrates in TPPBs has not been previously undertaken. Cruickshank et al. [19] presented a dynamic mechanistic model describing the mass transport of a water-soluble substrate, phenol, using estimated mass transfer coefficients. In the work presented herein, a systematic study involving operational parameters such as rotational speed and non-aqueous phase fraction are investigated to quantify the effect of these operating variables on the magnitude of the mass transfer coefficient.

Initial research on TPPBs employed low-viscosity organic solvents as partitioning phases [20, 21]. Silicone oil has also been used in TPPBs as the sequestering phase, since it is biocompatible and non-biodegradable [22]. More recently TPPBs have employed solid absorbents such as polymer beads as the partitioning phase [5, 23]. In addition to being biocompatible and non-biodegradable, polymer beads offer operational advantages such as easier separation and recovery [17] and much lower cost [24]. However, the use of solid polymers may present a limitation to mass transport due to low pollutant diffusivity and low interfacial area compared to liquid organic solvents. The purpose of this study was to systematically determine the mass transfer in a TPPB using phenol and butyl acetate as model compounds, which were chosen to represent relatively soluble substrates. The mass transfer coefficient was evaluated as a function of bioreactor operation conditions such as impeller speed and sequestering phase fraction. 2-Undecanone, silicone oil at two different viscosities and solid polymer beads (Hytrel 8206®) were separately employed in the TPPB. 2-Undecanone was chosen as a model for low-viscosity organic solvents, which were initially used as partitioning phases in TPPBs. Silicone oil was also employed to represent a high-viscosity sequestering phase and has been used previously in TPPBs [6, 9, 25]. In addition to these two model solvents, solid polymer beads have also been used as the partitioning phase in the TPPB, and substrate mass transfer was evaluated using these model solvents and the solid polymer.

Materials and methods

Materials

Butyl acetate, phenol, 2-undecanone and silicone oil were obtained from Sigma Aldrich (Oakville, ON, Canada). Butyl acetate at a purity of ≥99 % had a density of 0.88 g/mL at 25 °C and a boiling point of 124–126 °C. 2-Undecanone had a density of 0.825 g/mL at 25 °C and a boiling point of 231–232 °C. Silicone oil (dimethylpolysiloxane) was obtained at two viscosities (5 and 200 cSt at 25 °C). HYTREL® 8206 beads (Dupont, ON, Canada) were used as the solid phase. HYTREL® is a polyether ester thermoplastic (PEEP) consisting of oval-shaped beads, approximately 5.5-mm long and 3.5-mm wide. It has a melting point of 180 °C, a glass transition temperature of −60 °C and a density of 1.17 g cm^{-3}.

Analytical methods

The concentration of phenol was determined with the 4-aminoantipyrene method [26] which is effective at detecting phenol to concentrations of 5 μg/L. Absorbance was measured at 510 nm using an Ultraspec 3000 spectrophotometer and a calibration curve was employed to detect phenol concentrations. The concentration of butyl acetate was determined by high-performance liquid chromatography (HPLC) using UV/Vis at 200 nm and an injection volume of 20 μL. The retention time of butyl acetate was 5.3 min. The concentration was determined by a predetermined area count calibration curve of analytical standards.

Partition coefficients

Partition coefficients were determined for substrate and sequestering phase selection as well as for the calculation of the initial substrate aqueous phase concentration that is required to yield a final sub-inhibitory concentration of 1,000 mg/L in the TPPB. The partition coefficient of phenol in 2-undecanone and of phenol in silicone oil was determined according to the procedure by Collins and Daugulis [27]. The partition coefficient of phenol in silicone oil was found to be nearly 0; silicone oil has virtually no affinity for phenol and appears to be suitable only for highly hydrophobic substrates; therefore, butyl acetate was employed as a model substrate, since it has been shown to partition into silicone oil [28]. The partition coefficient of butyl acetate in silicone oil was also determined according to the procedure by Collins and Daugulis [27]. The partition coefficient of phenol in HYTREL® and of butyl acetate in HYTREL® was determined according to the procedure by Prpich and Daugulis [2].

Model

The diffusion of a solute through a liquid film at a fixed depth δ, into an absorbing matrix (polymer bead) according to the quasi-steady state model used to describe solute transfer to an absorbing domain, is shown in Fig. 1 [29].

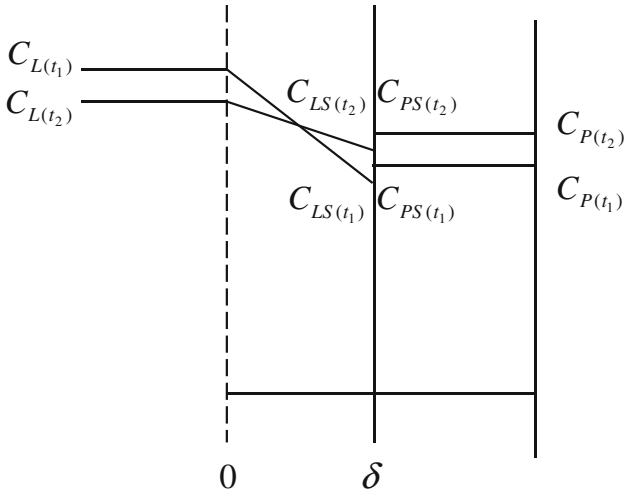

Fig. 1 Substrate transfer model from the aqueous phase to the polymer

The model describes the change in solute concentration with time in the absorbing domain and assumes constant solute concentration within this domain. The assumption is valid for conditions where the solute flux through the diffusion domain adjacent to the absorption domain is large compared to the rate of change in boundary concentrations [29]. The rate of change in solute concentration in the absorbing polymer is given by:

$$\frac{dC}{dt} = \frac{D}{\delta} a(C_L - C_{LS}) \tag{1}$$

where D is the diffusion coefficient, C_L is the substrate concentration in the bulk liquid, C_{LS} is the substrate concentration at liquid surface adjacent to the solid, and a is the liquid–solid interfacial area per unit volume.

At the interface, equilibrium exists between the liquid phase and the absorbing polymer where:

$$Q = \frac{C_{LS}}{C_{PS}} \tag{2}$$

where Q is the partitioning coefficient, and C_{PS} is the substrate concentration at the polymer surface.

Substitution of Eq. 2 into 1 yields:

$$\frac{dC}{dt} = \frac{D}{\delta} a(C_L - QC_{PS}) \tag{3}$$

$$\frac{dC}{dt} = \frac{D}{\delta} a(C_L - QC_P) \tag{4}$$

or

$$\frac{dC}{dt} = Ka(C_L - QC_P) \quad \text{where} \quad K = \frac{D}{\delta} \tag{5}$$

Equation 5 can be expressed using equilibrium concentrations:

$$\frac{dC}{dt} = Ka\left(C_L - C_L^*\right) \tag{6}$$

Assuming Ka is constant with time, Eq. 6 can be integrated between time $t_1$and t_2 to yield:

$$\ln\left(\frac{C_{L2} - C^*}{C_{L1} - C^*}\right) = Ka(t_2 - t_1) \tag{7}$$

Ka can be determined from the slope of the line of $\ln\left(\frac{C_{L2}-C^*}{C_{L1}-C^*}\right)$ versus $(t_2 - t_1)$

Mixing in polymer-loaded systems

To investigate the effect of polymer mass fraction on mixing in the TPPB, and its subsequent effect on the mass transfer coefficient, tracer experiments were conducted using 5-mL pulse injections of 99.7 % pure acetic acid (Fisher Scientific, Nepean, Canada) added to the top of the liquid phase of the TPPB. The working volume was 3 L of tap water and 100, 200 and 300 g polymer beads were employed separately. Triplicate reactor runs were conducted for the three HYTREL® polymer loadings. The change in pH with time was monitored with a data acquisition system before and after the pulse injection of acetic acid until a steady-state value of pH was attained.

The first-order-with-dead-time model is typically used to predict the response of a CSTR to a step change in input [30]:

$$Y'(t) = K(1 - e^{-(t-\theta)/\tau}) \tag{8}$$

where $Y'(t)$ is the output response in deviation variables, τ is the time constant, and θ is the time delay. The time constant is a measure of the time required for complete mixing in the CSTR and hence can be used to compare mixing time in TPPBs employing different polymer mass fraction [30]. Time constant values were obtained for the response in pH to the pulse injection of acetic acid by using the EXCEL® SOLVER function to minimize the square of the residuals between experimental values and those predicted by the model.

Reactor operation

A New Brunswick Bioflo® III bioreactor with an internal diameter of 17 cm, a working volume of 3 L, a single six-blade Rushton turbine impeller of diameter 7.7 cm, four baffles each with a width of 1.5 cm, operating at 30 °C, was used to conduct all mass transfer experiments. The substrate was loaded to the aqueous phase (RO water) and mass transfer was evaluated from the aqueous to the organic solvent/solid phase. The transfer of phenol from RO water to 2-undeconone was achieved by using an organic phase ratio of 25 % (750 mL of solvent) at impeller speeds of 200 and 600 RPM. 10 mL samples were collected from the reactor and allowed to phase separate for

1 min. Aqueous phase samples were aspirated and subsequently analyzed. The transfer of butyl acetate from RO water to silicone oil was determined using silicone oil viscosities of 5 and 200 cSt and 200 g of silicone oil at 400 RPM. Sampling was conducted with the same procedure as that for the phenol in water/2-undecanone system. The rate of substrate release from the polymer was investigated with phenol using RO water and 100 g HYTREL® at 400 RPM, to confirm whether the mass transfer coefficient was equivalent to that obtained when the substrate is absorbed by the polymer. The transfer of phenol from RO water to phenol-free HYTREL® polymer beads was first evaluated to determine the rate of phenol uptake by the polymer. Following the uptake reactor run, phenol-loaded polymer beads were sieved and towel dried. The phenol release from the polymer was conducted by adding the phenol-loaded polymer to the bioreactor and repeating the experiment.

The effect of impeller speed on the mass transfer of phenol between RO and HYTREL® was found at impeller speeds of 200, 400, 600 and 800 RPM and 100 g HYTREL®. In order to determine the effect of polymer mass fraction on substrate mass transfer, 200 g HYTREL® beads were also employed at the same initial phenol concentration as that used when 100 g polymer was used. Additional reactor runs were also conducted to verify the effect of polymer mass fraction on substrate mass transfer using butyl acetate and employing 200 and 300 g HYTREL® separately in the TPPB.

Results and discussion

Partition coefficients

The partition coefficient of phenol in 2-undecanone was found to be 40.4, while it was nearly 0 in silicone oil. The partition coefficient of butyl acetate in silicone oil was found to be 27. The partition coefficient of phenol in HYTREL® was found to be 43 [(mg phenol/g HYTREL®)/(mg phenol/mL solution)] and for butyl acetate in HYTREL® it was found to be 7.4 [(mg butyl acetate/g HYTREL®)/(mg butyl acetate/mL solution)]. Compared to silicone oil, HYTREL® was able to sequester a wider range of substrates and this offers an operational flexibility advantage to solid polymers as opposed to liquid organic solvents such as silicone oil.

Mass transfer of phenol in 2-undecanone

The rate of mass transfer of phenol from the aqueous phase to 2-undecanone occurred rapidly, and the aqueous phase concentration reached equilibrium by the time the first sample was taken at 30 s of reactor operation at 200 and 600 RPM. This demonstrates that in TPPBs operating with dissolved substrates and with non-viscous organic liquids as the partitioning phase, equilibrium is quickly attained. These results also indicate the absence of external mass transfer in the liquid film adjacent to the organic/aqueous phase interface as well as the absence of internal mass transfer within the organic layer. These findings also reveal that for users of TPPB technology with liquid organic solvents and with soluble substrates, mass transfer limitations may be non-existent and optimization of the process can be directed more toward consideration of microbial growth limitations.

Mass transfer of butyl acetate in silicone oil

The mass transfer of butyl acetate from the aqueous phase to silicone oil was also very rapid as the aqueous phase concentration reached equilibrium within the first minute, operating with silicone oil at 5 and 200 cSt. These results indicate that in TPPBs operating with soluble substrates and with silicone oil as the partitioning phase, equilibrium is also reached quickly and the fermentation process would not be limited by mass transfer. As observed with phenol transfer in 2-undecanone, neither external mass transfer in the liquid film adjacent to the organic/aqueous phase interface nor internal mass transfer within the silicone oil droplets (irrespective of viscosity) limits the overall mass transfer at the reactor conditions herein.

In contrast to insoluble substrates [31], operating with higher viscosity silicone oil did not affect mass transfer to the partitioning phase. These findings are also important for TPPB operation where high-viscosity sequestering phases can be employed with soluble substrates with no mass transfer limitation to the overall process rate. The overall process rate may be limited by other biological factors that could be optimized.

Mass transfer of phenol and butyl acetate in polymer beads

Phenol mass transfer to and from polymer beads

Prior to a biodegradation process in a TPPB, either the sequestering phase or the aqueous phase can be loaded with substrate. The transfer of the substrate to/from the aqueous phase occurs until equilibrium is attained. Equilibrium is typically reached before the system is inoculated. In Fig. 2, the rate of substrate uptake by HYTREL® is nearly equivalent to the rate of substrate release. This was also verified by the mass transfer coefficients determined in Table 1. This finding is useful from an operational point of view, where the mass transfer rate between the two phases

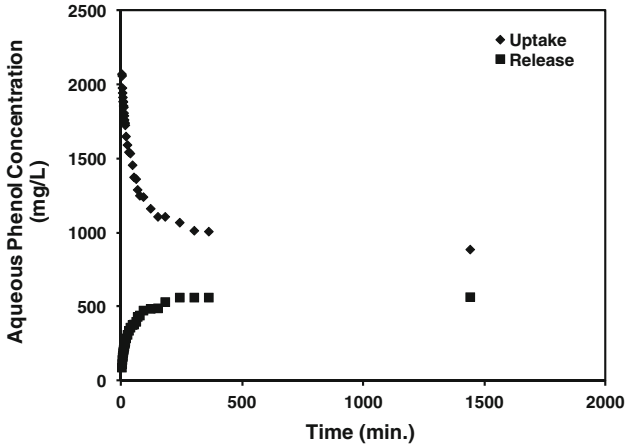

Fig. 2 Phenol uptake and release by HYTREL®

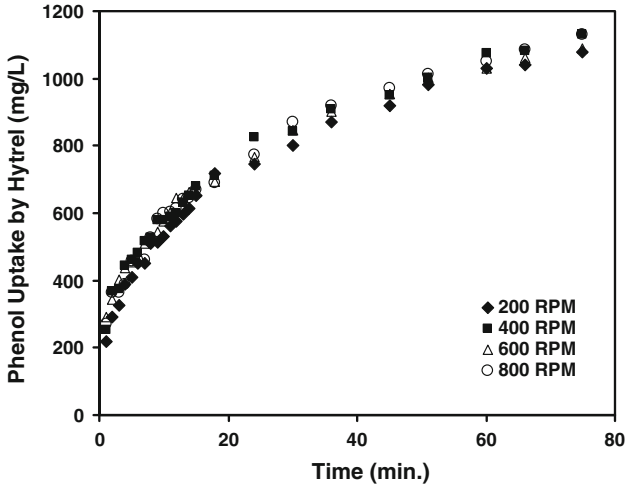

Fig. 3 Phenol uptake by HYTREL® at different impeller speeds

Table 1 Mass transfer coefficients of phenol and butyl acetate in water/HYTREL® TPPBs

Substrate	HYTREL® loading (g)	Impeller speed (RPM)	Ka (min^{-1}) ± 0.0004	a (m^2/m^3)	K ($\times 10^4$ m min^{-1})
Phenol	100	200	0.0158	63	2.51
	100	400	0.0169	63	2.68
	100	600	0.0159	63	2.52
	100	800	0.0169	63	2.68
	200	400	0.0225	126	1.79
	200	800	0.023	126	1.83
Phenol uptake	100	400	0.0165	63	2.62
Phenol release	100	400	0.0156	63	2.48
Butyl acetate	200	400	0.0165	126	1.31
	300	400	0.0192	189	1.02

is independent of which phase is being loaded with the substrate.

Effect of impeller speed

Figure 3 shows the rate of uptake of phenol by HYTREL® at different impeller speeds. Compared to liquid organic solvents [31], mass transfer was not externally controlled by the extent of mixing (i.e., RPM) in the aqueous phase, but by substrate diffusion into the polymer. These findings are significant for users of TPPBs with polymers where mass transfer limitations must be considered for efficient process operation. In slurry systems, the solid/liquid mass transfer coefficient is affected by the degree of suspension of the solids, where solids settling leads to a drop in the mass transfer coefficient [32]. A minimum impeller speed is required for complete suspension, beyond which the solid/liquid mass transfer coefficient is marginally affected by the impeller speed [32]. In Fig. 3, even at the lowest operating impeller speed of 200 RPM, phenol uptake by HYTREL® is not limited by external mass transfer. The impeller speeds employed could be adequate to provide complete suspension that all polymer beads are in direct contact with the aqueous phase and hence no limitation to external mass transfer occurs. As indicated in Table 1, the mass transfer coefficient was unaffected by impeller speed for phenol transfer to HYTREL® at 100 g of polymer loading. Similarly, the mass transfer coefficient for 200 g of HYTREL® loading is almost identical irrespective of impeller speed. These results indicate that phenol mass transfer to HYTREL® is not limited by external transfer, but rather by internal diffusion of substrate within the polymer, which could result in an overall mass transfer limited TPPB. In contrast to insoluble substrates such as anthracene [31], higher impeller speeds did not affect the mass transfer rate of phenol to the polymer beads. These findings have not been reported previously. The significance of these results is that the cost of TPPB operation can be minimized by operating at lower impeller speeds with lower energy requirements.

Effect of polymer beads mass fraction

The effect of polymer mass fraction on the uptake of phenol in HYTREL® is depicted in Fig. 4, which shows that compared to a HYTREL® loading of 100 g, the rate of uptake of phenol by HYTREL® is greater for the 200 g of HYTREL® due to the additional surface area available for mass transfer due to the presence of more polymer beads. This effect was also observed in the increase in *Ka* when 200 g compared with 100 g of HYTREL® beads were employed (Table 1). However, the value of the mass transfer coefficient *K* was lower by 30 % when 200 g HYTREL® beads were used compared to that obtained when 100 g beads were used (Table 1). As the polymer mass fraction increases, the presence of additional beads could affect mixing in the TPPB and hinder the convective transfer of the substrate in the aqueous phase to the surface of the beads. A similar effect of polymer mass fraction on the substrate rate of uptake by HYTREL® and on *Ka* and *K* was obtained when butyl acetate was employed (Fig. 5; Table 1). The value of *Ka* increased due to the additional surface area available for mass transfer, but the value of *K* dropped by 22 % when 300 g of HYTREL® beads were used compared to that obtained when 200 g HYTREL® beads were used. Joosten et al. [33] determined the change in the gas–liquid mass transfer coefficient $k_L a$ as a function of solids (glass, polypropylene and sugar particles) content. At low solids concentrations, $k_L a$ increases to a small extent, however, as more solids are added the value remains constant at first, and then starts declining. According to Mills et al. [34], inert solids can decrease k_L by creating a diffusion blocking effect at higher solids concentration. It is likely that HYTREL® could have contributed to a hindering effect to convective mass transfer similar to that observed by Mills et al. [34] leading to a decrease in *K* with increased mass fraction of polymer beads.

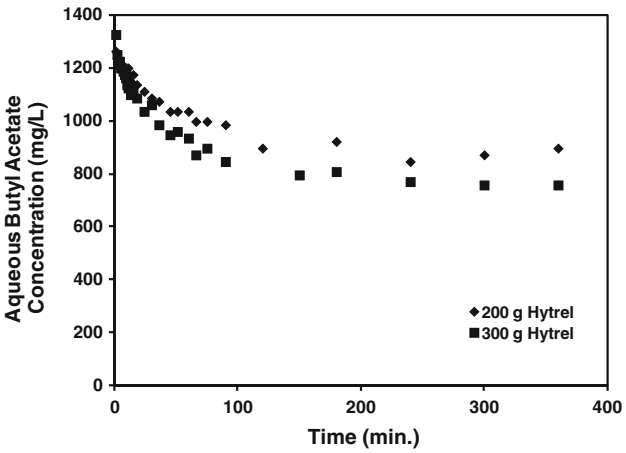

Fig. 5 Effect of polymer loading on the rate of butyl acetate uptake

Mixing in polymer-loaded systems

To investigate further the potential hindering effect to convective mass transfer observed with higher mass fraction of polymer beads, mixing in the TPPB was studied by conducting tracer studies and observing the reactor response. The response of the TPPB to a step change in pH and the model fit of the experimental data to the first-order-with-dead-time model are depicted in Fig. 6. Time constant values were obtained for all reactor runs with 100, 200, and 300 g polymer loadings and are given in Table 2. An unpaired student's *t* test was conducted to determine whether the mean values of the time constants obtained from the model fits for data obtained with different polymer loadings are significantly different. The mean value of the time constant obtained for the 100 g reactor runs was

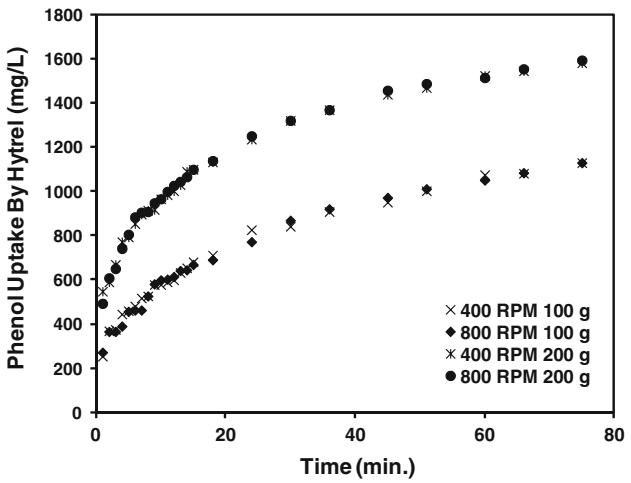

Fig. 4 Effect of polymer loading on the rate of phenol uptake

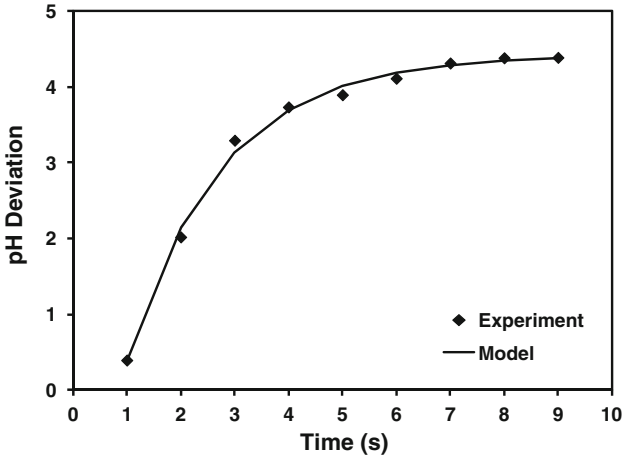

Fig. 6 Response of the TPPB to a step change in pH and the first-order-with-dead-time model fit

Table 2 Time constants τ obtained for TPPBs at different HYTREL® loadings

HYTREL® loading (g)	Test 1 (s)	Test 2 (s)	Test 3 (s)	SD
100	1.45	1.30	1.30	0.085
200	1.56	1.65	1.74	0.091
300	2.10	2.31	2.15	0.111

significantly less than that obtained for the 200 g and the 300 g reactor runs (unpaired t test, $p < 0.05$). Significant difference between the mean values of the time constant was also obtained for experiments employing 200 and 300 g polymer loadings (unpaired t test, $p < 0.05$). The time constant is a measure of how fast the system responds to the step change and is a measure of the degree of mixing in the TPPB [35]. With higher mass fraction of polymer, higher time constants were obtained. The presence of more polymer beads could have altered mixing in the reactor hindering convective mass transfer of the substrate to the bead surface leading to a decrease in K. These findings indicate that when operating with solid polymer beads with high volume fraction in the TPPB, mixing can be reduced and this limitation must be considered in optimizing the overall process.

Future work will focus on the understanding of the limitations to internal mass transfer within the polymer. Polymer beads with different sizes (i.e., diffusional path lengths) and with different diffusivities will be used in the TPPB, and mass transfer of the substrate will be investigated. Such an attempt would provide a deeper understanding of the mechanisms of mass transfer involved and allow for more suitable polymer selection.

Conclusions

The mass transfer of phenol in water/2-undecanone and of butyl acetate in water/silicone oil TPPB system was found to occur rapidly. The significance of these results is that for TPPB process optimization, mass transfer limitations are non-existent when low- and high-viscosity solvents are used as sequestering phases and when soluble substrates are employed. In contrast to insoluble substrates, mass transfer of phenol to the solid polymer beads was unaffected by impeller speed. These findings are important for reducing the operating cost of the TPPB by using lower impeller speeds with lower energy requirements. Substrate transfer of phenol and butyl acetate to the polymer surface was found to be limited by internal diffusion in the polymer and this limitation must be considered in optimizing the overall TPPB process rate. Using higher polymer mass fraction led to an increase in the available area for mass transfer; however, mixing in the reactor was reduced. These results are important in predicting the overall mass transfer rate which is critical in optimizing the overall process rate of the TPPB.

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

References

1. Quijano G, Hernandez M, Thalasso F, Munoz R, Villaverde S (2009) Two-phase partitioning bioreactors in environmental biotechnology. Appl Microbiol Biotechnol 84:829–846
2. Prpich G, Daugulis A (2004) Polymer development for enhanced delivery of phenol in a solid–liquid two-phase partitioning bioreactor. Biotechnol Prog 20:1725–1732
3. Tomei MC, Annesini MC, Rita S, Daugulis AJ (2010) Two-phase partitioning bioreactors operating with polymers applied to the removal of substituted phenols. Environ Sci Technol 44:7254–7259
4. Littlejohns JV, Daugulis AJ (2008) Response of a solid–liquid two-phase partitioning bioreactor to transient BTEX loadings. Chemosphere 73:1453–1460
5. Rehmann L, Daugulis AJ (2007) Biodegradation of biphenyl in a solid–liquid two-phase partitioning bioreactor. Biochem Eng J 36:195–201
6. Quijano G, Revah S, Gutierrez-Rojas M, Flores-Cotera LB, Thalasso F (2009) Oxygen transfer in three-phase airlift and stirred tank reactors using silicone oil as transfer vector. Process Biochem 44:619–624
7. Littlejohns JV, Daugulis AJ (2007) Oxygen transfer in a gas–liquid system containing solids of varying oxygen affinity. Chem Eng J 129:67–74
8. Nielsen D, Daugulis A, McLellan P (2003) A novel method of simulating oxygen mass transfer in two-phase partitioning bioreactors. Biotechnol Bioeng 83:735–742
9. Quijano G, Rocha-Rios J, Hernandez M, Villaverde S, Revah S, Munoz R, Thalasso F (2010) Determining the effect of solid and liquid vectors on the gaseous interfacial area and oxygen transfer rates in two-phase partitioning bioreactors. J Hazard Mater 175:1085–1089
10. Torres-Martinez D, Melgarejo-Torres R, Gutierrez-Rojas M, Aguilera-Vazquez L, Micheletti M, Lye GJ, Huerta-Ochoa S (2009) Hydrodynamic and oxygen mass transfer studies in a three-phase (air–water–ionic liquid) stirred tank bioreactor. Biochem Eng J 45:209–217
11. Bordel S, Hernandez M, Villaverde S, Munoz R (2010) Modelling gas–liquid VOCs transport in two-liquid phase partitioning bioreactors. Int J Heat Mass Transfer 53:1139–1145
12. Dumont E, Delmas H (2003) Mass transfer enhancement of gas absorption in oil-in-water systems: a review. Chem Eng Process 42:419–438
13. Cesario M, Beverloo W, Tramper J, Beeftink H (1997) Enhancement of gas–liquid mass transfer rate of apolar pollutants in the biological waste gas treatment by a dispersed organic solvent. Enzyme Microb Technol 21:578–588
14. Munoz R, Arriaga S, Hernandez S, Guieysse B, Revah S (2006) Enhanced hexane biodegradation in a two phase partitioning bioreactor: overcoming pollutant transport limitations. Process Biochem 41:1614–1619

15. Munoz R, Chambaud M, Bordel S, Villaverde S (2008) A systematic selection of the non-aqueous phase in a bacterial two liquid phase bioreactor treating alpha-pinene. Appl Microbiol Biotechnol 79:33–41
16. Aldric J, Lecomte J, Thonart P (2009) Study on mass transfer of isopropylbenzene and oxygen in a two-phase partitioning bioreactor in the presence of silicone oil. Appl Biochem Biotechnol 153:67–79
17. Prpich G, Daugulis A (2005) Enhanced biodegradation of phenol by a microbial consortium in a solid–liquid two phase partitioning bioreactor. Biodegradation 16:329–339
18. Collins L, Daugulis A (1997) Characterization and optimization of a two-phase partitioning bioreactor for the biodegradation of phenol. Appl Microbiol Biotechnol 48:18–22
19. Cruickshank S, Daugulis A, McLellan P (2000) Dynamic modeling and optimal fed-batch feeding strategies for a two-phase partitioning bioreactor. Biotechnol Bioeng 67:224–233
20. Bruce L, Daugulis A (1991) Solvent selection-strategies for extractive biocatalysis. Biotechnol Prog 7:116–124
21. Deziel E, Comeau Y, Villemur R (1999) Two-liquid-phase bioreactors for enhanced degradation of hydrophobic/toxic compounds. Biodegradation 10:219–233
22. Darracq G, Couvert A, Couriol C, Amrane A, Thomas D, Dumont E, Andres Y, Le Cloirec P (2010) Silicone oil: an effective absorbent for the removal of hydrophobic volatile organic compounds. J Chem Soc Biotechnol 85:309–313
23. Prpich GP, Daugulis AJ (2006) Biodegradation of a phenolic mixture in a solid–liquid two-phase partitioning bioreactor. Appl Microbiol Biotechnol 72:607–615
24. Daugulis AJ, Tomei MC, Guieysse B (2011) Overcoming substrate inhibition during biological treatment of monoaromatics: recent advances in bioprocess design. Appl Microbiol Biotechnol 90:1589–1608
25. Hernandez M, Quijano G, Thalasso F, Dangulis AJ, Villaverde S, Munoz R (2010) A comparative study of solid and liquid non-aqueous phases for the biodegradation of hexane in two-phase partitioning bioreactors. Biotechnol Bioeng 106:731–740
26. Yang RD, Humphrey AE (1975) Dynamic and steady state studies of phenol biodegradation in pure and mixed cultures. Biotechnol Bioeng 17:1211–1235
27. Collins L, Daugulis A (1996) Use of a two phase partitioning bioreactor for the biodegradation of phenol. Biotechnol Tech 10:643–648
28. Gardin H, Lebeault J, Pauss A (1999) Biodegradation of xylene and butyl acetate using an aqueous-silicon oil two-phase system. Biodegradation 10:193–200
29. Weber W, McGinley P, Katz L (1991) Sorption phenomena in subsurface systems—concepts, models and effects on contaminant fate and transport. Water Res 25:499–528
30. Marlin TE (2000) Process Control. Designing processes and control systems for dynamic performance. McGraw-Hill Higher Education, Boston
31. Eibes G, McCann C, Pedezert A, Teresa Moreira M, Feijoo G, Manuel Lema J (2010) Study of mass transfer and biocatalyst stability for the enzymatic degradation of anthracene in a two-phase partitioning bioreactor. Biochem Eng J 51:79–85
32. Pangarkar V, Yawalkar A, Sharma M, Beenackers A (2002) Particle-liquid mass transfer coefficient in two-/three-phase stirred tank reactors. Ind Eng Chem Res 41:4141–4167
33. Joosten G, Schilder J, Janssen J (1977) Influence of suspended solid material on gas–liquid mass-transfer in stirred gas–liquid contactors. Chem Eng Sci 32:563–566
34. Mills D, Bar R, Kirwan D (1987) Effect of solids on oxygen-transfer in agitated 3-phase systems. AIChE J 33:1542–1549
35. Ingham J, Dunn I, Heinzle E, Prenosil J, Snape J (2007) Chemical engineering dynamics: an introduction to modelling and computer simulation. Wiley-VCH Verlag GmbH & Co KGaA, Weinheim