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ORIGINAL RESEARCH PAPER

Passive/aggressive detoxification of continuous flow biotreatment systems using absorptive polymers: partitioning bioreactors treating transient phenol loadings

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Abstract Biotreatment of wastewaters containing toxic compounds can be jeopardized by surges in substrate concentration. Preventative measures include tight monitoring and control to either dilute high concentration transients, or to divert them to surge tanks for later release and treatment. Using phenol as a typical toxic substrate, we have imposed 4 h phenol surges of 3,000, 6,000 and 8,000 mg/l from a steady-state feed of 500 mg/l, demonstrating such reduced treatment efficiency. In contrast, with the addition to the bioreactor of a small amount (3–10 % w/v) of inert polymer beads (Hytrel 8206) possessing a high affinity for phenol (partition coefficient of 39), significant detoxification was achieved, resulting in a 100 % increase in treatment efficiency, and good performance at phenol surges of up to 16,000 mg/l. The addition (passive operation) of polymers to a biotreatment system eliminated the need for intervention during toxic transients while, at the same time, demonstrating that the polymers perform this function automatically and at all times (aggressive operation).

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Introduction

Phenol is a toxic environmental pollutant commonly arising from industrial processes including plastics, pharmaceutical and resin manufacturing, and petroleum processing. Phenol concentrations in effluents from these sources can be as high as 15,000 mg/l, (Ahamad and Kunhi 2011) and, since phenol is recognized by the US EPA as a priority pollutant, effective biotreatment strategies are needed to degrade this xenobiotic to mitigate environmental and human health hazards. Multiple studies have shown that free cell systems can degrade phenol only up to circa 1,000 mg/l due to substrate toxicity (Loh et al. 2000; Ahamad and Kunhi 2011); to address this issue, several phenol degradation strategies have been proposed including pre-adapting pure strains to higher phenol concentrations (Kwon and Yeom 2009), immobilizing pure cultures of organisms in matrices such as agar or calcium alginate beads (Ahamad and Kunhi 2011), cyclodextrin (Safont et al. 2012), or protecting cells within fixed-film systems (Hamoda et al. 1987) or hollow fiber membrane bioreactors (Li and Loh 2007). However, if practical, industrially applicable biotreatment processes for the handling of xenobiotics are to be found, the solution surely must involve the use of mixed populations of organisms,

along with simple and inexpensive processing configurations.

Virtually all modern wastewater treatment plants operate in some form of continuous mode, and the toxicity experienced in the treatment of xenobiotics would therefore arise from transient substrate feeds to such processes. Such variations in feed strength are seldom encountered in domestic wastewater, which has a relatively constant composition, but more commonly in industrial wastewater, which often arises from batch processing, resulting in rapid swings in the types and concentrations of organic pollutants present (Sipma et al. 2010). However, very few studies have been undertaken that examine the impact of transient feeds of inhibitory substrates on the performance of continuous flow biotreatment processes.

Two-phase partitioning bioreactors (TPPBs), in which toxic concentrations are maintained below cytotoxic levels by means of substrate partitioning into an immiscible second phase, have shown to be effective in the treatment of various gaseous and aqueous phase contaminants. In an important sense, such systems are both passive and aggressive: passive, in that they are "in the background", being present during steady feeds to the process, but also at all times operational (aggressive), responding to fluctuating concentrations of target molecules *spontaneously* via equilibrium considerations. TPPBs are therefore operationally simple, requiring minimal monitoring-and-control, or operator intervention, to automatically handle concentration spikes. Although immiscible organic solvents were originally used in TPPBs (Collins and Daugulis 1996), inexpensive, amorphous polymers have also proved to be equally effective in sequestering toxic substrates, and have the advantage of being completely inert, thus allowing the use of mixed populations of organisms. Such solid–liquid TPPBs have been shown to far surpass the performance of single-phase biodegradation systems in the treatment of toxic substrates in batch (Prpich and Daugulis 2006), fed batch (Prpich and Daugulis 2004), and sequencing batch operation (Tomei et al. 2011). The applicability of TPPBs to truly continuous, suspended cell systems in handling surges in feed concentration has not yet been examined, and in this work we demonstrate the resilience and operability of such systems in response to 4 h transients of very high (up to 16,000 mg/l) phenol feeds.

Materials and methods

Materials

Hytrel 8206 polymer beads (approx. the size and shape of rice grains) were donated by DuPont, Canada. All chemicals were obtained from recognized commercial sources.

Microorganisms and medium

A mixed microbial consortium consisting of *Acinetobacter baumannii*, *A. johnsonii*, *Pseudomonas alcaligenes* and *P. putida*, previously isolated via selective enrichment and shown to degrade phenol more efficiently than a pure strain of *P. putida* (Prpich and Daugulis 2005) was used. The medium was (g/l): K_2HPO_4, 2.56; KH_2PO_4, 2.08; NH_4Cl, 1; $MgSO_4 \cdot 7H_2O$, 0.5; yeast extract, 0.1; and phenol, 0.5. Inoculum was prepared in eight 125 ml flasks each containing 50 ml of medium, inoculated with 100 µl microbial consortium and grown for 20 h at 30 °C.

Analytical methods

Phenol concentrations were determined using the 4-aminoantipyrines method, which can detect phenol at 5 µg/l (Yang and Humphrey 1975). Cell dry weights (CDW) were determined from OD_{600} values and converted to CDW. For both cell and phenol concentrations, at each sampling time triplicate measurements were taken and averaged to obtain data points.

Partition coefficient

The partition coefficient of phenol for Hytrel 8206 was determined as described by Fam and Daugulis (2012).

Equipment

Biotreatment of phenol streams was performed using an NBS Bioflo III bioreactor with a 3 l working volume. The operating set points were: 400 rpm, 30 °C, pH 7.0, and air flow at 3 l/min. For CSTR operation, the dilution rate was set to 0.07 h^{-1}, which

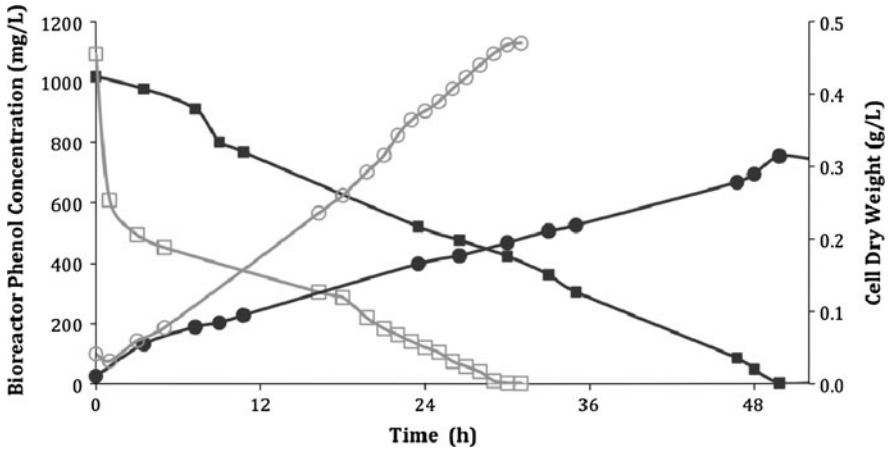

Fig. 1 Batch degradation with and without polymers at an initial phenol concentration of 1,000 mg/l. The *closed circles* and *squares* represent the CDW and phenol concentrations, respectively, with no polymers. The *open circles* and *squares* represent the CDW and phenol concentrations, respectively, with 100 g polymer beads

is in the range of specific growth rates for phenol degradation at concentrations above about 500 mg/l (Pawlowsky and Howell 1973), using a peristaltic pump. The bioreactor was run in batch mode for 24 h and then switched to continuous mode for 12–24 h until steady state conditions were established, as determined by minimal fluctuations in CDW and effluent phenol concentration. Once steady state was reached, a 4 h step change in inlet phenol concentration was implemented by switching to a medium containing the desired phenol concentration. After 4 h, the inlet feed was returned to the original medium, and CDW and phenol were tracked.

Results and discussion

The partition coefficient (PC) of Hytrel for phenol was 39, which is consistent with the value reported earlier (Fam and Daugulis 2012), and is also similar to those reported for immiscible organic solvents (Collins and Daugulis 1996). The most common immiscible solvent used in TPPBs, silicone oil, has virtually no affinity for relatively soluble xenobiotic substrates such as phenol (Fam and Daugulis 2012), and would therefore be of no use in such applications. Uptake of target molecules by amorphous polymers, such as Hytrel, is by *absorption* rather than by *adsorption*, which is the uptake mechanism of activated carbon and "hard" crystalline resins (e.g. poly (styrene-co-divinylbenzene)). This means that substrate uptake by amorphous polymers is selective (Tomei et al. 2011), depending on the polymer chosen (again, in contrast to activated carbon which is relatively non-selective with respect to organic molecules), and that uptake/release by amorphous polymers is governed by maintaining thermodynamic equilibrium (affected by concentration driving forces and cell metabolism) rather than adsorption isotherms.

Figure 1, a preliminary experiment intended to determine the approximate phenol level to which the culture could be exposed, to assess the rate of absorption by Hytrel, and to see the effect of detoxification on cell performance, shows the impact of adding a relatively small amount (3 % w/v) of polymer to a batch system in which approx. 1,000 mg/l had been added. The figure demonstrates that the consortium is quite robust as, in single phase mode, it was able to degrade this level of phenol to completion within about 50 h. In the TPPB case, phenol absorption by Hytrel was very rapid (a 50 % reduction in aqueous phenol concentration within the first hour), and the reduced toxicity allowed the cells to consume all of the phenol in only about 30 h. Release of absorbed substrate by polymers has been confirmed to be essentially to completion (Tomei et al. 2011), and Fig. 1 also shows that higher biomass levels were reached in the TPPB. This may be due to the detoxification provided by the presence of the polymer, which could increase the observed cell yield, since maintenance requirements would be higher (and observed yields lower) at elevated phenol concentrations, as also seen by Ahamad and Kunhi (2011).

Figure 2 shows the response of the single phase system to 4 h step change increases of phenol at 3,000, 6,000 and 8,000 mg/l. Table 1 summarizes some of

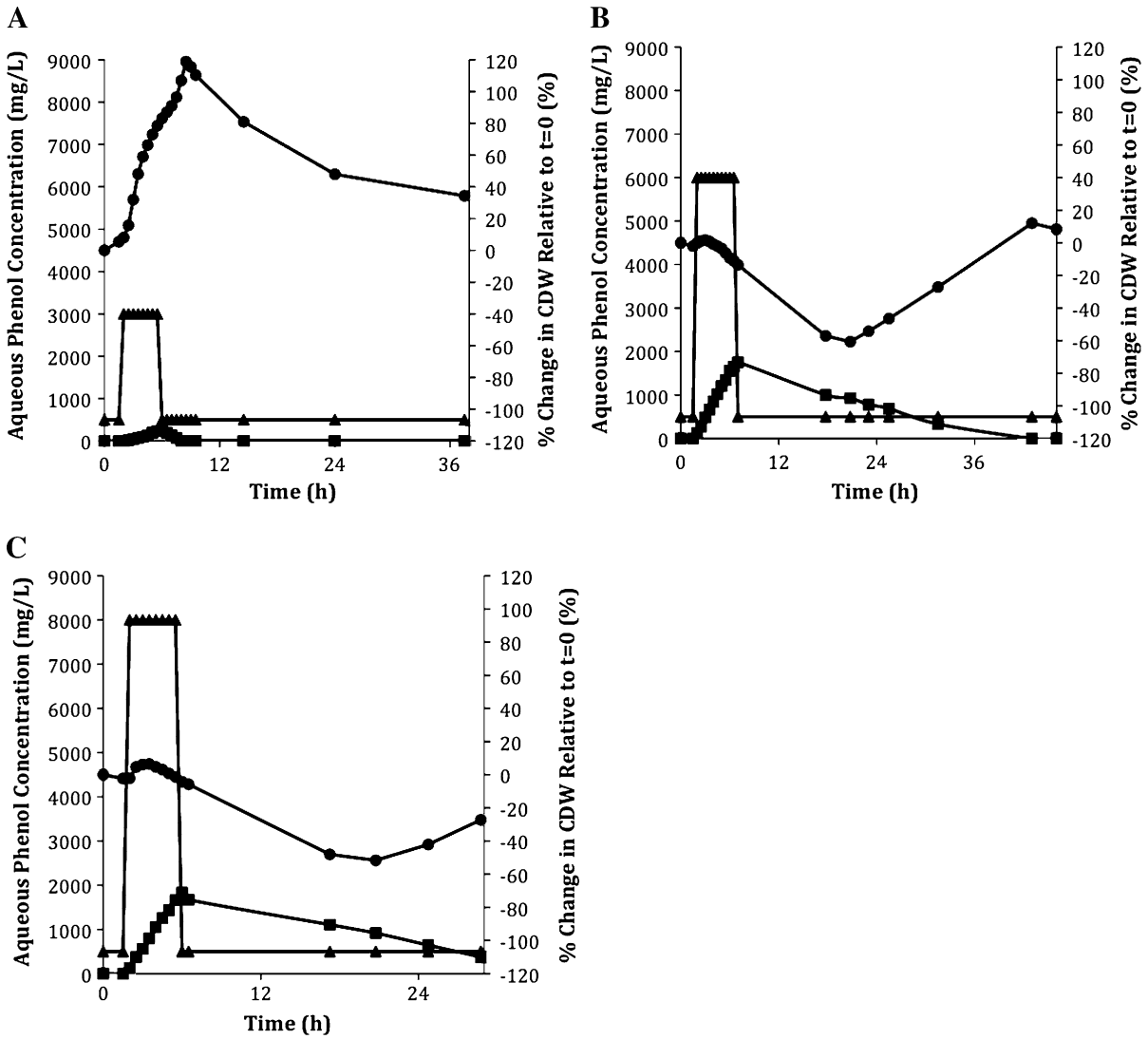

Fig. 2 Single phase continuous operation with 4 h phenol step changes of varying magnitude (**a**: 3,000 mg/l, **b**: 6,000 mg/l, **c**: 8,000 mg/l). *Filled circle* are the percentage change in CDW relative to t = 0, *filled triangle* are the inlet phenol concentration, and *filled square* are aqueous phenol concentration

the key features of these runs. It is clear that for a step change of 3,000 mg/l, the cells were not inhibited; in fact the cell concentration increased during the step, likely reflecting the fact that the aqueous phenol level reached only 243 mg/l during the transient, providing the consortium with extra substrate at a sub-inhibitory level. This result is consistent with the data in Fig. 1, which showed that exposure of the culture to phenol levels of 1,000 mg/l was inhibitory, but that lower concentrations could be readily consumed. The higher concentration step changes shown in Fig. 2 were clearly inhibitory to the cells, as supported by the decrease in biomass levels during and after the steps, and as reflected in the high aqueous phenol levels occurring during the steps (Table 1), which significantly exceeded 1,000 mg/l, albeit at the end of the transient period. The reduced levels of biomass obviously also affected the ability of the system to degrade the residual phenol levels, as seen in the elevated (untreated) aqueous phenol levels leaving the bioreactor in the effluent, after returning the system to a feed of 500 mg/l.

Table 1 Comparison of bioreactor performance for various experimental conditions

Phenol step change (mg/l)	Polymer (%w/v)	Maximum bioreactor phenol concentration (mg/l)	Min/max percent change in CDW relative to t = 0 (%)	Phenol removal efficiency (%)
3,000	–	243	0/119	97
6,000	–	1,753	−61/12	33
8,000	–	1,832	−52/6	35
8,000	5	981	−25/20	65
8,000	10	651	−12/74	74
16,000	10	1,094	−33/0	77

Figure 3 shows TPPB performance at 8,000 mg/l with 5 and 10 % polymer additions, and at 16,000 mg/l with 10 % polymer addition for 4 h phenol transients, with Table 1 also providing summary information. For the 8,000 mg/l step change, the addition of only a small amount (5 %) polymer (Fig. 3a) clearly had a beneficial effect relative to the single phase case (Fig. 2c) in all important aspects: reduced maximum aqueous phenol concentration, reduced negative impact on cell growth, and less emitted (undegraded) phenol leaving the biotreatment system after the transient (Table 1). As anticipated, the higher polymer loading (Fig. 3b) detoxified the system to a greater extent, resulting in a lower maximum phenol concentration (one-third the level found in the single phase case for the same step change), smaller decrease in biomass concentration during the transient, and a faster recovery after the system had been returned to its nominal phenol feed of 500 mg/l. In both TPPB cases, but more pronounced for the 10 % polymer case, the phenol that had been absorbed by the polymer was released by the polymer and generated much higher post-transient biomass levels. Even with a step increase in phenol of 16,000 mg/l (Fig. 3c), the TPPB system sequested large amounts of phenol, keeping aqueous phenol concentrations at levels ($\sim$1,000 mg/l) that did not result in the system washing out.

The magnitude of phenol sequestration is demonstrated in Fig. 4, which shows the calculated amount of phenol in the Hytrel beads during the course of the transient substrate surges. These values were estimated by mass balance from the aqueous phenol concentration, the PC value, the mass of polymers present and the assumption of equilibrium conditions during the transient period; although this last hypothesis may not be entirely valid, the very rapid uptake of phenol by Hytrel (Fig. 1) supports this assumption, and is also borne out by other studies of rapid mass transfer of solute into TPPB polymers (Prpich and Daugulis 2004). A related metric to gauge the role of the polymer in TPPBs is the proportion of the total phenol in the system that is contained within the polymer phase. This was calculated again by mass balance 5.5 h into the step, which was in the last 30 min of the transient and when the bioreactor phenol concentration was near its highest value. At this time for the 16,000 mg/l step change, there was 1,094 mg phenol/l in the aqueous phase and 42,673 mg phenol/l in the polymer phase, which indicates that 77 % of the total phenol present in the system at that time was sequestered within the polymer phase, even though the polymer phase represented only 10 % of the system volume.

Table 1 also shows the impact on biomass concentration excursions arising from the transients. Since there was some run-to-run variability in the steady state biomass levels at the start of the step changes, the biomass concentration changes were set to a normalized value of 0 at the start of each transient, and the changes in biomass levels were then plotted with respect to this starting value. During TPPB operation a smaller biomass decrease and a greater post-transient increase was observed relative to the single phase cases, demonstrating the positive effect that TPPB operation had on enhancing the stability of the system by maintaining biomass levels. In the biological treatment of contaminants there are usually two measures of emissions that are regulated: the maximum concentration of target molecules that is emitted, and the cumulative emission level. As seen in Table 1, the maximum phenol levels emitted are greatly reduced during TPPB operation. The removal efficiencies (cumulative emission levels) for all cases were determined by mass balance, by integrating the area under the inlet phenol concentration curve and effluent bioreactor phenol concentration curve from t = 2 h to t = 26 h (an arbitrary end-point for all runs) and, by multiplying by the flow-rate. Table 1 clearly shows the positive impact that the presence of polymers has on process performance. For single phase operation, from a high of almost 100 % removal

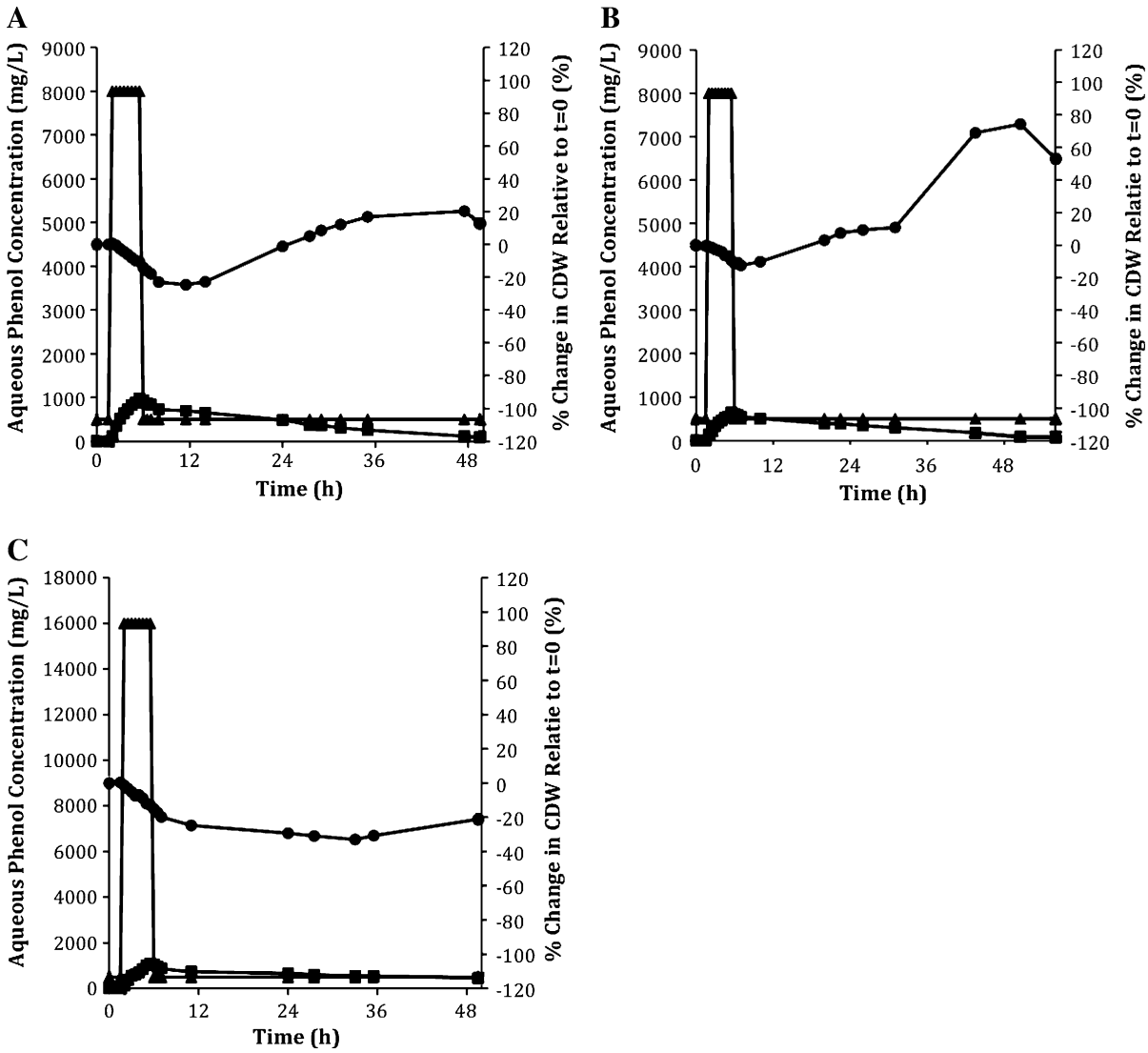

Fig. 3 TPPB operation with 4 h phenol step changes of varying magnitude (**a**: 8,000 mg/l, 5 % (w/v) polymer, **b**: 8,000 mg/l, 10 % (w/v) polymer, **c**: 16,000 mg/l, 10 % (w/v) polymer). *Filled circle* are the percentage change in CDW relative to t = 0, *filled triangle* are the inlet phenol concentration and *filled square* are aqueous phenol concentration

efficiency for the 3,000 mg/l phenol spike, the performance deteriorated to around 33 % removal efficiency at the higher phenol transients. In contrast, the two phase systems' performance was approximately double at the same phenol loadings and, even at the 16,000 mg/l transient, a 77 % removal efficiency was obtained during operation in the presence of polymers.

Many reports in the literature have clearly demonstrated the kinetic advantages conferred upon biological systems that treat cytotoxic substrates (or produce cytotoxic products) using the TPPB platform (Daugulis et al. 2011), but what of the practicality of TPPB systems? The evolution of this technology began with the use of immiscible organic solvents as sequestering phases, culminating in the widespread use of silicone oil for mixed microbial populations in liquid–liquid TPPB systems. More recently, amorphous polymers have been used in TPPBs, and act in exactly the same way as do solvents: absorptive equilibrium partitioning in response to cellular

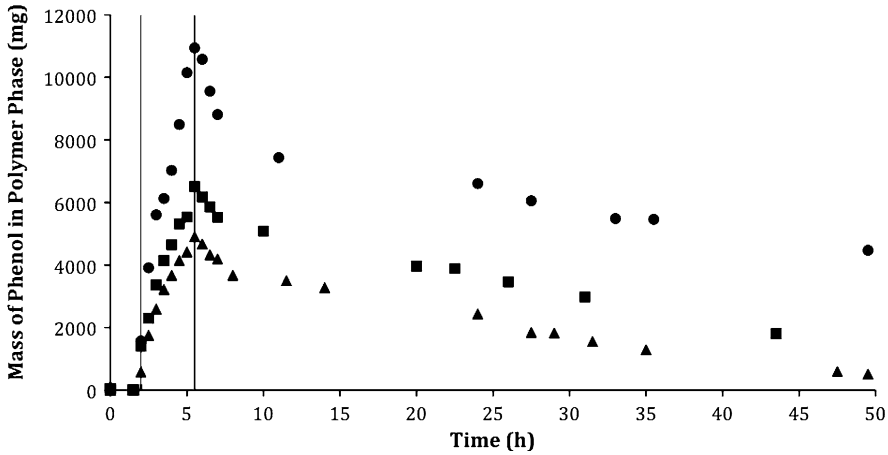

Fig. 4 The mass of phenol in the polymer phase over time. *Vertical line* denotes duration of phenol step change. *Filled triangle* are 5 % (w/v) polymer, 8,000 mg/l phenol step change, *filled square* are 10 % (w/v) polymer, 8,000 mg/l phenol step change and *filled circle* are 10 % (w/v) polymer, 16,000 mg/l phenol step change

metabolic demand. It is our view that in actual wastewater biotreatment applications, the use of organic solvents, including silicone oil, may be impractical given the handling challenges, and potential losses of these materials; certainly from an operational standpoint (adding, removing, retaining polymers within the bioreactor) the handling of solid polymer beads is far easier than dealing with solvent-in-water dispersions. Also as previously noted, silicone oil is restricted to applications involving highly hydrophobic target molecules, while the range of substrates that can be sequestered by commercially-available polymers is extremely broad. On economics alone, polymers also appear to have a very significant advantage, as the cost of Hytrel is approx. €5/kg, compared to circa €150–200/kg for the preferred low viscosity grade of silicone oil (Daugulis et al. 2011).

Thermoplastic polymers such as Hytrel can also be readily manipulated into different shapes and sizes from the as-received form in which they are fabricated (pellets). As an example, bioreactor internals (i.e. baffles) were constructed from Hytrel pellets and used effectively during TPPB operation (Prpich and Daugulis 2007). The use of waste polymers (e.g. automobile tires) has also been shown to be effective in TPPB systems (Prpich et al. 2008), potentially increasing the economic advantage that polymer-based TPPBs already possess. For all of the above reasons, it is our view that the use of targeted polymers, including polymer mixtures, in small proportions can be readily used to protect conventional biotreatment systems (e.g. activated sludge) from transient feeds with virtually no changes in process operation, while minimizing monitoring and control activities. The use of inexpensive, non-volatile, non-flammable, biocompatible and easily-shaped polymers as the sequestering phase in TPPBs is an enormous advance in designing high-efficiency, low-cost biotreatment processes that eliminate cell toxicity and is key to the development of "green", solvent-free processing strategies.

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