

Solid–liquid two-phase partitioning bioreactors (TPPBs) operated with waste polymers. Case study: 2,4-dichlorophenol biodegradation with used automobile tires as the partitioning phase

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Abstract Used automobile tire pieces were tested for their suitability as the sequestering phase in a two-phase partitioning bioreactor to treat 2,4-dichlorophenol (DCP). Abiotic sorption tests and equilibrium partitioning tests confirmed that tire “crumble” possesses very favourable properties for this application with DCP diffusivity ($4.8 \times 10^{-8} \text{ cm}^2/\text{s}$) and partition coefficient (31) values comparable to those of commercially available polymers. Biodegradation tests further validated the effectiveness of using waste tires to detoxify a DCP solution, and allow for enhanced biodegradation compared to conventional single-phase operation. These results establish the potential of using a low-cost waste material to assist in the bioremediation of a toxic aqueous contaminant.

Keywords 2,4-Dichlorophenol · Partitioning bioreactor · Renewable polymers · Used tires

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Introduction

The use of Two-phase Partitioning Bioreactors (TPPBs) is an extremely effective means of biologically destroying xenobiotic substrates. In TPPBs, an organic phase is used to sequester toxic substrates, and to deliver them to the cells in the aqueous phase at sub-cytotoxic levels based on cellular demand and the maintenance of thermodynamic equilibrium between the two phases. In biotreatment systems, in which mixed populations of cells are necessarily used, the sequestering phase would need to be non-bioavailable to cells, however, in the case of immiscible liquid phases, this significantly limits the selection of the partitioning phase, as many/most organic solvents are to some extent biodegradable.

Amorphous (soft) commercial polymers have been shown to function in exactly the same way as immiscible organic solvents in the delivery of toxic substrates to cells in TPPBs; advantageously, however, they are completely inert (non-bioavailable and completely biocompatible), have been shown to be effective for a wide range of toxic substrates of varying hydrophobicity (Daugulis et al. 2011), and are very inexpensive ($\sim \text{€} 5$ per kg). Even with these advantages commercial polymers represent a capital cost in initially supplying a TPPB system with a solid sequestering phase. Waste polymers, in the form of automobile tires, provide an opportunity for not only reducing the initial polymer cost to near-zero, but also for utilizing a waste material for positive environmental purposes. Scrap automobile and truck tires are

generated in very large quantities, on a continuous basis; for example, the estimate of used tires generated in Europe is 3.2×10^6 tonnes per year (European Tyre and Rubber Manufacturers' Association, <http://www.etrma.org/tyres/ELTs>) and in the US 3×10^6 tonnes per year (data of 2003 from US EPA <http://www.epa.gov/osw/conserve/materials/tires/faq.htm>). Although recycling and reuse (e.g. as supplementary fuel in cement kilns, rubber-modified asphalt, surfaces for sports fields) are currently being practiced, significant amounts of used tires are still either landfilled or are discarded in illegal scrap piles. Not only are these materials therefore not reused, but they can even result in environmental hazards, such as fires.

In this work, we have employed used automobile tire pieces as the partitioning phase in a TPPB to biologically degrade 2,4-dichlorophenol (DCP), a highly toxic compound ($EC_{50} = 2.3\text{--}40$ mg/l; Ren and Frymier 2005; Nalbur and Alkan 2007), found in many industrial effluents of pesticide and herbicide production. We have characterized the rate and extent of uptake of DCP by tires, and compared their sorption/desorption characteristics with other commercial polymers successfully employed previously as the partitioning phase in solid–liquid TPPBs for the removal of phenolic compounds. This work has shown that TPPBs using waste tires readily outperform conventional single-phase systems, and that tires have comparable sorption/delivery properties (capacity and diffusivity) to commercial polymers.

Materials and methods

Chemicals

DCP (purity >99 %) was obtained from Sigma Aldrich, and all other chemicals, which were of commercial grade, were purchased from Carlo Erba (Italy). Used tires were obtained from Recovery Technologies Canada Inc., in the form of rubber “crumble”, whose physical appearance is shown in Fig. 1, along with other commercial polymers that have been used in TPPB applications. Table 1 lists properties of various TPPB polymers. The tire crumble is generated by cryogenic pre-treatment of used tires to below their brittleness temperature, followed by grinding, and separation of the rubber from the steel and fibre fractions. The crumble is claimed to have an



Fig. 1 Tires and commercial polymers in the form utilized in experiments with TPPBs (scale bar in cm)

unaltered chemical composition from the original tires, and is currently used for moulded rubber products and sports surfaces. Prior to use tires were pre-treated with a multistep water washing to remove residual contaminants, which could be released during our experiments.

Analytical methods

Biomass concentrations were measured as Volatile Suspended Solids (VSS) according to Standard Methods (APHA 1998). DCP was analysed spectrophotometrically at 280 nm. Its disappearance, along with possible degradation by-products, was also confirmed by periodically measuring the COD of cell-free samples (APHA 1998), which correlated to DCP disappearance as measured spectrophotometrically. In kinetic tests DCP analysis was performed on the supernatant of samples centrifuged for 6 min at 10,000 rpm.

Bacterial culture

The biomass utilized in the kinetic tests originated from a bacterial culture previously utilized in the biodegradation of 4-nitrophenol (Tomei et al. 2009) and was progressively acclimatized to DCP. The culture was grown aerobically in mineral medium on a mixture of DCP and sodium acetate, with the DCP concentration in the feed being progressively increased, and acetate progressively decreased from 30 mg/l to zero, over the course of serial transfers. The

Table 1 Properties and suppliers of used tires and polymers previously utilized in TPPBs for uptake of phenolic compounds (modified from Tomei et al. 2011)

Polymer	Grade	Supplier	Hardness	T _g (°C) ^a	T _m (°C) ^b	Specific gravity	Composition
Hytrel	G3548L	DuPont	40D	−30	170	1.18	Co-polymer of poly(butylene terephthalate) and polyether
	8206		35–40D ^c	−59	189	1.17	
Elvax	770	DuPont	98A 47D	−100 ±	96	0.928	9.50 % vinyl acetate (co-polymer with ethylene)
Tone	P787	Dow Chemical Canada	–	–	60	1.145	Poly-caprolactone polyester
Kraton SBR	D1102	Kraton	66A	−80	150–200 ^d	0.94	Styrene/butadiene linear block copolymer
Fusabond	N416	DuPont	–	17	43	0.869	Chemically modified ethylene elastomer
PEBAX	2322	Arkema	75A	−65	133.5	1.01	Polyether block amide
Used tires		Recovery Technologies Canada	–	–	–	0.8	Copolymer of polyisoprene, styrene-butadiene copolymer and polybutadiene

Hardness: A (Shore A), D (Shore D), R (Rockwell R)

^a Glass Transition Temperature

^b Melting point

^c Personal correspondence with supplier

^d Brittleness temperature

reactor was then fed with 100 mg/l DCP as the sole carbon and energy source.

Test plan

Absorption/desorption tests

To estimate the DCP absorption/desorption rates of tires, duplicate tests were performed at 25 °C, in 300 ml sealed glass flasks with 250 ml medium and a tire fraction of 5 % v/v, with mixing provided by magnetic stirrers. The initial DCP concentration was 75 mg/l, and the DCP concentration in the liquid samples was determined at intervals of 10–15 min using micro-cuvettes (volume 1.5 ml) to minimize liquid volume losses. Parallel control tests were performed under the same conditions without DCP to account for the possible effect of the release of tire contaminants, or particulates. The absorbance readings of the control samples were subtracted from the correspondent test sample readings. The same experimental procedure was used for desorption tests, using the DCP-loaded tires from the absorption tests, desorbing into fresh tap water.

Partition tests

To determine the partitioning coefficient (PC, defined as the ratio of the concentration in the solid and liquid phases) equilibrium tests were performed in duplicate at 25 °C in 100 ml glass flasks (working volume 50 ml) by adding known amounts (1–4 g) of tires to an 80 mg/l solution of DCP in tap water. Mixing was provided by magnetic stirrers, and the flasks were allowed to equilibrate for 24 h, a sufficient time to reach equilibrium. Parallel control tests without DCP were also undertaken as described above.

Kinetic tests

DCP biodegradation tests were performed in parallel in 250 ml flasks with 200 ml of the same minimal growth medium utilized for biomass acclimatization, in single and two-phase systems operated with 5 % (v/v) tires. The tests were run at 25 °C, with DCP and biomass concentrations in the range of 90–130 mg/l and 2,000–3,000 mg VSS/l, respectively. Mixing was by magnetic stirrers and the flasks were continuously aerated. The DCP concentration was measured at time

intervals of ~ 15 min until no appreciable concentration decrease was observed, and an abiotic control test was also performed under the same operating conditions to rule out possible DCP losses via air stripping.

Results and discussion

Absorption/desorption and partition tests

Figure 2a, b show the DCP concentration profiles in the sorption and desorption tests. Although it is clear that 24 h is sufficient to reach equilibrium, it can also be seen that the majority of the uptake takes place within the first 150 min of the test, accounting for about 70 % of the final equilibrium partitioning. Desorption also occurs to the largest extent in the first 150 min of the test. These results can be considered to be an initial validation for the use of tires as the sequestering phase in a TPPB system, since the absorption/desorption rates are similar to those for the uptake of phenolics by commercial polymers such as Hytrel (Prpich and Daugulis 2004; Tomei et al. 2009) and are not anticipated to limit the overall biodegradation process. To estimate the diffusivity of phenol in tires, the sorption kinetics were modelled with a classical Fickian model for diffusion inside the solid phase (Crank 1968) with the simplifying assumption of having particles of spherical form. No resistance to mass transport in the liquid layer external to the particle surface was considered. It is also assumed that the concentration at the particle surface is in equilibrium with the concentration in the bulk liquid phase. From the fitting of the sorption curve data, and the calculated PC value (described below), a diffusion coefficient value of 4.8×10^{-8} cm²/s was calculated.

Figure 2c provides an estimate of the partition coefficient of tires for phenol, with a calculated PC value of 31, and with very good data fit (correlation coefficient $R^2 = 0.99$). This value is similar to previously used commercial polymers such as Hytrel (Fam and Daugulis 2012), as well as immiscible organic solvents (Collins and Daugulis 1996). Again, as a comparison of this value to those of commercial polymers used previously in TPPB applications, Table 2 summarizes the PC and diffusion coefficient values for tires and literature data for sorption of phenolic compounds including DCP. Both PC and

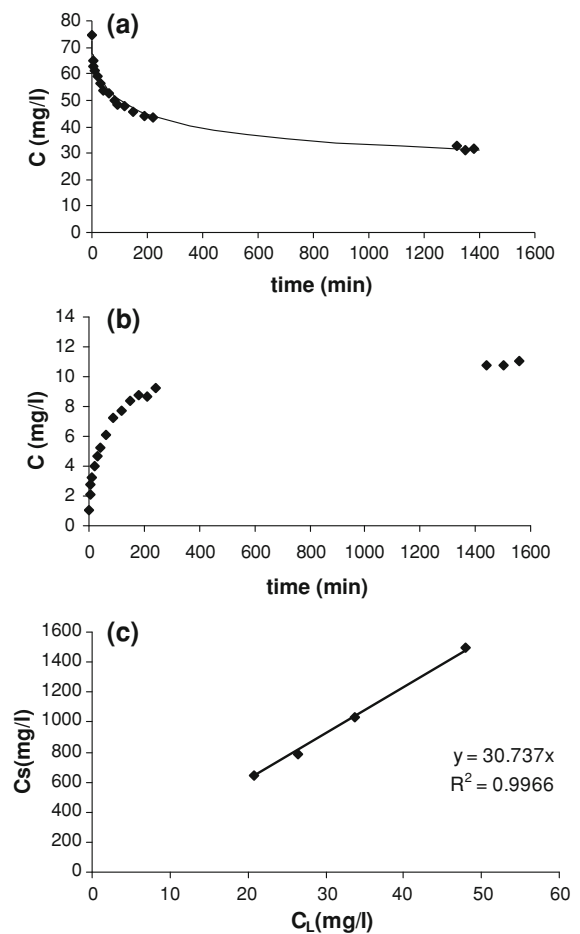


Fig. 2 DCP concentration profiles in absorption (a), and desorption (b) tests. Initial DCP concentration 75 mg/l; tires 5 % v/v. The *solid line* in (a) is from data fitting using the estimated value for diffusivity. Partition test data (c), C_s concentration in the solid phase, C_L concentration in the liquid phase

diffusion data for the tires are intermediate in the range of values reported in the literature, suggesting that this initial validation step for uptake and release of DCP with tires can be considered to be very positive.

Batch kinetic tests

To complete the evaluation of the suitability of using tires as the solid partitioning phase in a two-phase system, DCP biodegradation was investigated through batch biodegradation tests in single and two-phase mode, and the results are summarized in Fig. 3 for initially DCP at 92 and 130 mg/l. In both cases the presence of the partitioning phase was effective in

Table 2 Partition coefficient and diffusion data for commercial polymers and used tires

Compound	Polymer	PC	Diffusivity (cm ² /s)	References
Phenol ^a	Hytrel 8206	43	1.54×10^{-7}	Prpich and Daugulis (2004) Fam and Daugulis (2012)
	Elvax 40W	10.1	3.73×10^{-9}	Prpich and Daugulis (2005) Prpich and Daugulis (2004)
4-Nitrophenol ^b	Hytrel 8206	61	1.45×10^{-6}	Tomei et al. (2010)
	Tone P787	6	1.38×10^{-7}	Tomei et al. (2010)
	Elvax 40W	11.8	–	Tomei et al. (2009)
2,4-Dimethylphenol ^a	Hytrel 8206	201	–	Tomei et al. (2010)
	Kraton-D1102	10.1	–	Tomei et al. (2010)
	Nylon 6,6	38.3	–	Tomei et al. (2010)
2,4-Dichlorophenol ^b	Tone P787	96	6.6×10^{-8}	Unpublished data
	Elvax 40 W	96	6.6×10^{-8}	Unpublished data
	Used tires	31	4.8×10^{-8}	This study

^a Data for distilled water

^b Data for tap water

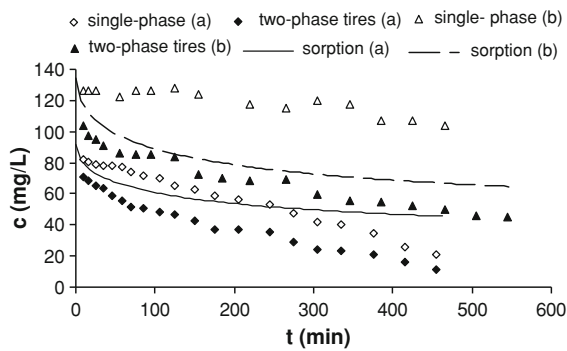


Fig. 3 Experimental DCP concentration profiles of batch biodegradation tests performed in single and two-phase systems. Initial concentrations: DCP = 92 mg/l (test a), 130 mg/l (test b); biomass = 1850 mg VSS/l. The two solid lines represent the simulated concentration profiles obtained by considering only sorption as the removal mechanism

reducing the DCP concentration and, as a consequence, the toxicity to the biomass. Moreover, while at lower initial concentration the conventional system is still able to remove the substrate with reasonable efficiency (74 %), at the higher initial DCP concentration a strong inhibitory effect is observed with very low removal efficiency (18 %). For the two-phase systems superior removal efficiencies of 87 and 66 % for the low and high feed concentrations were obtained.

Another key point to be confirmed in properly working TPPB systems is that the target compound is not simply removed from the liquid phase by

absorption, but that it is also delivered to the cells and biodegraded. To demonstrate this, simulated concentration profiles considering only sorption as the removal mechanism are also shown in Fig. 3, which were generated using the PC and diffusivity values. Comparing the experimental DCP concentration data and the simulated curves demonstrates that the obtained performance is due not just to absorption, but that biodegradation clearly also takes place.

A simple quantification of the differences in removal kinetics in the conventional and two-phase systems is further shown in Fig. 4 where the DCP specific removal rates in the liquid phase are reported versus time. As expected from the concentration profiles in Fig. 3, significantly lower removal rates are observed for the conventional system in both tests with

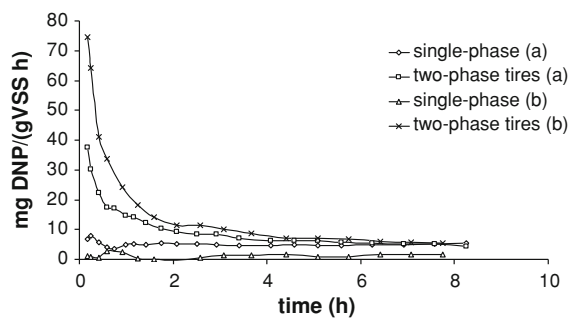


Fig. 4 Specific DCP removal rates for the single-phase (a) and two-phase (b) kinetic tests reported in Fig. 3

stronger evidence of the toxic concentration effect at the higher DCP concentration, which resulted in negligible biodegradation rates during the entire experiment. A marked increase (up to one order of magnitude) is observed for the TPPB system with higher specific rate values in the first part of the test arising from the reduced DCP toxicity attributable to DCP absorption by the tires.

Conclusions

Solid polymers provide comparable performances to organic solvents in the treatment of toxic substrates in TPPBs, while also possessing numerous superior features such as ease of use, applicability to a wide range of target molecules, complete biocompatibility and non-biodegradability, and much lower cost. Nevertheless, the purchase of commercial polymers for TPPBs represents a capital cost. Here we have shown that waste polymers with negligible or negative cost can perform equivalently in the treatment of elevated concentrations of xenobiotic substrates, while significantly outperforming single-phase bio-treatment systems. We believe that this represents a significant incentive for the adoption of the TPPB platform for the biological treatment of recalcitrant substrates.

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