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Simultaneous biodegradation of volatile and toxic contaminant mixtures by solid–liquid two-phase partitioning bioreactors

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HIGHLIGHTS

- We investigate the simultaneous biodegradation of phenol and butyl acetate.
- We identify an effective polymer mixture to selectively absorb each of the substrates and decrease their initial concentration.
- The polymer mixture is used to overcome the high phenol cytotoxicity and reduce the abiotic losses of butyl acetate associated with volatility.
- The solid–liquid Two Phase Partitioning Bioreactor (TPPB) outperforms the liquid–liquid TPPB and the single phase systems.

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ABSTRACT

Microbial inhibition and stripping of volatile compounds are two common problems encountered in the biotreatment of contaminated wastewaters. Both can be addressed by the addition of a hydrophobic auxiliary phase that can absorb and subsequently re-release the substrates, lowering their initial aqueous concentrations. Such systems have been described as Two Phase Partitioning Bioreactors (TPPBs). In the current work the performances of a solid–liquid TPPB, a liquid–liquid TPPB and a single phase reactor for the simultaneous degradation of butyl acetate (the volatile component) and phenol (the toxic component) have been compared. The auxiliary phase used in the solid–liquid TPPB was a 50:50 polymer mixture of styrene–butadiene rubber and Hytrel® 8206, with high affinities for butyl acetate and phenol, respectively. The liquid–liquid TPPB employed silicone oil which has fixed physical properties, and had no capacity to absorb the toxic contaminant (phenol). Butyl acetate degradation was enhanced in both TPPBs relative to the single phase, arising from its sequestration into the auxiliary phase, thereby reducing volatilization losses. The solid–liquid TPPB additionally showed a substantial increase in the phenol degradation rate, relative to the silicone oil system, demonstrating the superiority and versatility of polymer based systems.

1. Introduction

Phenols are common water pollutants found in the effluents of oil refineries, and the plastics and steel industries. They are also found in suburban and agricultural runoff as they are frequently used as pesticides [1]. Phenol concentrations in natural contaminated sources can range from 20 to 2000 mg/L, far exceeding the tolerance limit in surface water of 0.5 mg/l [2]. Accordingly, phenol has been designated by the US EPA as a priority pollutant [3].

Bioremediation has become an effective strategy for the treatment of phenol contaminated sources, although one of the main limitations of bio-treatment processes is the microbial inhibition that can arise even at moderate concentrations of inhibitory substrates such as phenol. In order to overcome this challenge traditional biological treatments have often relied on prior chemical treatment or dilution [4].

Other very different types of organic contaminants, volatile organic compounds (VOC), also present a challenge to conventional biotreatment strategies. In some cases the disappearance of target VOCs is often wrongfully attributed to biodegradation when in reality it may be caused by abiotic stripping. In the case of waste water treatment, air stripping usually takes place in aerated biological treatment processes, such as activated sludge aeration basins [5]. Unfortunately, the transfer of pollutants to the atmosphere is unacceptable despite their apparent "treatment" because even small airborne concentrations of some VOCs have been related to environmental deterioration and public health hazards [6].

Two Phase Partitioning Bioreactors (TPPBs) are an efficient and practical means of biologically treating high concentrations of toxic pollutants while providing sub-inhibitory substrate delivery to microbial populations [4]. TPPBs have also been used to minimize volatility losses during the treatment of xenobiotic

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contaminated soils [7]. TPPBs consist of a cell containing aqueous phase and an immiscible sequestering phase, which can preferentially absorb inhibitory target molecules, and then progressively release them based on the microbial metabolic demand. This dynamic process of absorption and release is governed by thermodynamic equilibrium and the relative affinity between the solutes and the auxiliary phase [8]. TPPBs have been operated as liquid–liquid and solid–liquid configurations, mainly differentiated by the nature of the auxiliary phase; the first type utilizes immiscible organic solvents while the second makes use of amorphous polymers. Solid–liquid TPPBs systems have repeatedly proven to outperform their liquid–liquid counterparts due to the versatility and large variety of existing polymers. In contrast to organic solvents whose properties are fixed, polymers possess a wide variety of functional groups and can often be formed into chemical mixtures, as co-polymers, or as mixtures of individual polymers. These features translate into a greater number of possible interactions with many different solutes, thus broadening the scope of possible applications [9].

TPPBs have been used to treat phenol-containing streams and also streams containing mixtures of substituted phenolics [10]; however, these target contaminants possess very similar chemical structures as well as closely related physical/chemical and toxicological characteristics. In actual wastewaters it can be expected that the contaminant mixtures would vary widely in these properties, thus posing challenges in designing single, effective treatment systems. The main objective of the present work was to assess and compare the performance of solid–liquid TPPBs to single phase and organic-aqueous platforms in the treatment of aqueous mixtures of phenol and butyl acetate, two contaminants of widely different toxicity and volatility. As will be explained later these substrates also pose two different problems during the biotreatment: microbial inhibition at moderate phenol concentrations and volatility losses of butyl acetate. It will be shown how these two relevant issues can be addressed by the use of solid–liquid TPPBs.

2. Materials and methods

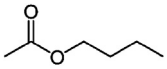
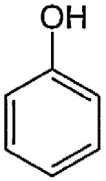
2.1. Chemicals and polymers

All medium components were purchased from Fisher Scientific (Guelph, Canada). Silicone oil (poly(dimethylsiloxane)) with a viscosity of 5 cSt and density of 0.98 g/ml, phenol (99%) and butyl acetate (>99%) were obtained from Sigma–Aldrich. Hytrel® 8206 and the various grades of Nylon were supplied by DuPont (Kingston, Ontario, Canada), Pebax® 1657 was obtained from Arkema (Burlington, Ontario, Canada), and the remaining polymers were purchased from Scientific Polymer (Ontario, New York, U.S.A.). Important butyl acetate and phenol properties are shown in Table 1. For the purposes of this work, the most critical difference are the much higher level of toxicity possessed by phenol, and the much higher volatility of butyl acetate.

2.2. Selective enrichment

A microbial consortium to degrade phenol and butyl acetate was obtained via selective enrichment. Initial seeds included contaminated soil from tar sands deposits in Alberta, Canada and a microbial consortium previously used for phenol degradation [11]. The medium consisted of 2 g/l $(NH_4)_2SO_4$, 0.75 g/l $MgSO_4{\cdot}7H_2O$, 2 g/l K_2HPO_4, 2 g/l KH_2PO_4, 0.1 g/l yeast extract, 1 ml/l trace element solution, prepared as referred in [12], in tap water. Initial seeds were placed in a shake flask containing 50 ml of medium with concentrations of 400 mg/l, increasing to 700 mg/l, of phenol and butyl acetate. The process was repeated for approximately one month until the consortium proved to consistently degrade both substrates. Growth in serum bottles was undertaken toward the end of the enrichment to ensure that the disappearance of butyl acetate was due to microbial activity and not abiotic losses (this was confirmed).

Table 1
Butyl acetate and phenol properties.

Property	Butyl acetate	Phenol
Structure		
Formula	$C_6H_{12}O_2$	C_6H_6O
CAS number	123-86-4	108-95-2
Molecular weight (g/mol)	116.16	94.11
Density (kg/m^3)	880	1070
Octanol–water partition coefficient (Log P)	1.54	1.46
Water solubility at 20 °C (g/l)	10	83
LD_{50} oral, for rats (mg/kg)	10,768	317
Henry's constant (H_{cc}, C_{gas}/C_{aq})	1.14×10^{-2}	3.11×10^{-5}

2.3. Partitioning coefficients

All partitioning coefficient (PC) experiments were performed in quadruplicate as described previously [10]. PCs and relevant polymer properties are shown in Table 2.

2.4. Abiotic volatility tests

Three liters of tap water were added to a 5-L New Brunswick Scientific BioFlo II bioreactor along with phenol or butyl acetate, with aeration and agitation at 1 l/min and 400 rpm respectively. Samples were periodically taken and analyzed. Volatility tests in single phase was performed for both phenol and butyl acetate. Butyl acetate volatility was additionally tested in a system containing 250 g of butadiene–styrene copolymer.

2.5. Toxicity tests

An initial seed was grown for 24 h in a shake flask containing 50 ml of medium with 500 mg/l phenol and butyl acetate. Subsequently, 5 ml was transferred to six other flasks containing 70 ml of media with increasing concentrations of phenol or butyl acetate. Substrate concentrations and cell growth were monitored to evaluate the impact of the initial substrate concentration on biodegradation activity.

2.6. Biodegradation tests

Inoculum was prepared in 50 ml of medium and 500 mg/l phenol and butyl acetate in 125 ml flasks. After incubating at 30 °C and 180 rpm for 24 h, 5 ml was transferred to 6 other similar flasks which were incubated for an additional 24 h, and used as bioreactor inoculum.

Biodegradations tests were undertaken in sequencing batch mode. For cycle 1, 2.67 l of fresh medium was placed in a sterile 5-l New Brunswick Scientic BioFlo III with 500 mg/l of phenol and butyl acetate, followed by inoculation. The pH and temperature were controlled at 6.9 and 30 °C, respectively and agitation and aeration

Table 2
Polymer properties and partitioning coefficients.

Commercial name	Chemical name	Glass transition temperature (T_g, °C)	Density (g/ml)	Butyl acetate PCs ($g/kg_{poly}/g/kg_{aq}$)	Phenol PCs ($g/kg_{poly}/g/kg_{aq}$)
Hytrel 8206	Polyether–ester copolymer	−59	1.17	9.2 ± 0.4	37.40 ± 0.6
Pebax 1657	Polyether–amide copolymer	−40	1.14	3.8 ± 1.8	13.0 ± 0.4
Poly(ethylene succinate)		−1	1.08	1.1 ± 0.7	2.9 ± 0.4
Poly(ethylene adipate)		0	1.18	7.3 ± 0.4	10.9 ± 0.3
Polycaprolactone		−60	1.15	8.2 ± 1.0	9.8 ± 0.1
Poly(1,4-butylene adipate)		−68	1.02	8.1 ± 0.3	12.0 ± 0.40
Poly(ethylene alcohol)		72	1.2	0.0 ± 0.1	0.3 ± 0.1
Nylon-6	Polyamide	47	1.13	0.2 ± 0.2	2.2 ± 0.11
Nylon-12	Polyamide	41	1.02	0.7 ± 0.4	1.7 ± 0.3
Styrene–butadiene rubber		−60	0.91	34.0 ± 1.7	0.8 ± 0.3
Silicone oil	Poly(dimethyl siloxane)	NA	0.98	27[a]	0[a]

[a] Experimental values published in [16].

were maintained at 400 rpm and 1 l/min unless the dissolved oxygen (DO) percentage decreased to a value less than 40%, in which case both were increased to 500 rpm and 4 l/min. At the end of one biodegradation cycle, the system was left operating overnight. Prior to the next cycle broth was pumped from the reactor quickly in order to avoid biomass settling, leaving 600 ml of broth, equivalent to an exchange factor of 80%. The reactor was subsequently filled with 2400 ml of fresh medium with the various phenol and butyl acetate concentrations. The reaction proceeded with the inoculum obtained from the previous cycle. A typical cycle lasted 22 h divided as follows: fill phase 1 min, reaction phase approximately 10 h, idle phase 12 h, decant phase 5 min.

For TPPB operation the sequestering phases were added immediately after removing the broth from the reactor, prior to the addition of fresh medium. In each case 150 g of the selected auxiliary phase was added (silicone oil or polymer). For the polymer experiment a 1:1 mixture of styrene–butadiene rubber and Hytrel® 8206 was utilized.

2.7. Analytical procedures

Reactor samples were centrifuged for 5 min at 10,000 rpm and 5 °C to avoid volatilization losses. Samples were filtered through 2 ml vials using Teflon syringe filters. In the case of silicone oil containing samples, silicone oil supernatant was removed by aspiration to avoid damage to chromatography equipment.

Phenol and butyl acetate concentrations were measured using a Varian Pro Star HPLC with UV/VIS detection, with dual wavelength scans performed to measure the concentration of both analytes simultaneously (butyl acetate: 190 nm, phenol: 260 nm). A mixture of 50:50 water/acetonitrile was used as the mobile phase at 1 ml/min and a total running time of 7 min.

Butyl acetate headspace concentration was measured using a Varian CP 3800 Gas Chromatograph (GC) equipped with a 30 m WCOT fused silica coated capillarity column (Model CP 8771), and FID detector. The carrier gas was helium at 1.5 ml/min. The method was: injector and detector temperatures at 250 °C, initial oven temperature at 30 °C and a temperature program of hold for 2 min followed by a ramp of 30 °C/min until reaching the final temperature of 200 °C, a split ratio of 10 was used. 100 μl samples was taken from the reactor headspace using a Hamilton gas-tight syringe and manually injected. Occasionally gas phase butyl acetate concentrations were determined from aqueous phase concentrations, using the Henry's law constant, and this method was validated in separate experiments (data not shown).

Cell concentration was determined by optical density using a UV/VIS spectrophotometer at 600 nm, and converted to dry cell weight concentrations by means of a calibration curve.

3. Results and discussion

3.1. Polymer selection

Polymer selection was based on the PC values for both substrates, which are shown in Table 2 and are a measure of the polymer absorption capacity for a given solute. Prospective polymers must possess moderate PC values to ensure effective performance in TPPBs; that is, polymers with low PCs do not provide enough uptake to significantly decrease the substrate aqueous concentration, while too high PCs may not re-release sufficient substrate to respond to the microbial demand [10]. PCs in the range of 20–50 have proven to perform well in solid–liquid TPPBs applications [3,13,14]. Table 2 shows that the only two polymers meeting this criterion were Hytrel® 8206 with a phenol PC of 37.4, and styrene–butadiene copolymer with a butyl acetate PC of 34. These polymers possess low T_g values and a relative large portion of soft active segment available for substrate absorption, 95% in the case of styrene–butadiene rubber and more than 50% for Hytrel® 8206, properties that favor higher substrate uptake capacities [15].

A polymer mixture was therefore used in the solid–liquid TPPBs in order to target both substrates simultaneously. Interestingly, the same polymer mixture has been used previously for a significantly different application [14] showing the versatility and adaptability of polymer based systems, particularly as mixtures. Silicone oil (SO) is overwhelmingly the most common organic solvent used in liquid–liquid TPPBs. Previous work has shown that SO provides good affinity for butyl acetate with a PC of 27, nevertheless its phenol partitioning was shown to be literally negligible [16]. Clearly, silicone oil, unlike polymers selected from the many tens of thousands available, will be limited to a small number of TPPB applications. A liquid–liquid TPPB experiment was also carried out using silicone oil as the auxiliary phase in order to compare its performance with its solid–liquid counterpart.

3.2. Substrates volatility

A previous study attempted to overcome volatility problems by performing biodegradation experiments in leak-free sealed systems [17]. This approach, however, has little resemblance to real industrial conditions given that most wastewater treatments processes take place in large aeration tanks completely open to the environment and are subject to high levels of aeration and mixing [5]. In the current work experiments were performed to assess the volatility of each of the substrates via abiotic losses. Fig. 1 shows the time course for butyl acetate and phenol, and in all cases the decrease in the aqueous concentration was attributed to volatility losses and/or polymer absorption.

As seen in Fig. 1 phenol showed virtually no losses due to volatility even at the aggressive conditions of aeration and agitation

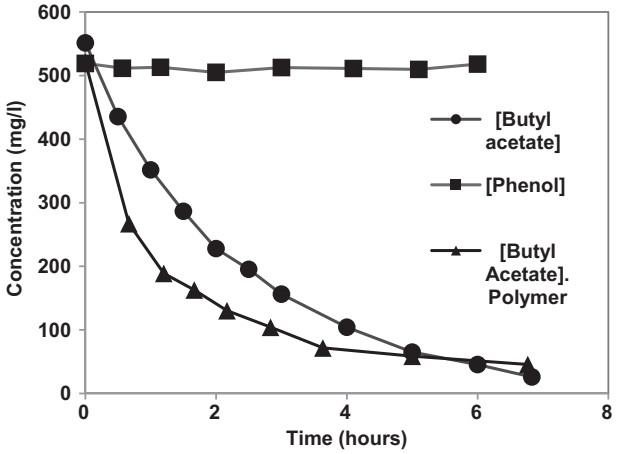


Fig. 1. Time course of phenol and butyl acetate in different volatility experiments.

employed. In contrast, the butyl acetate concentration decreased rapidly in both the single phase and the polymer added systems. The experiment with polymers showed a more pronounced decrease at the beginning of the test compared to the single phase system, due to the combined action of polymer absorption and volatilization. Butyl acetate volatility losses were reduced by 30% with the use of polymers. Based on thermodynamic principles a decrease in the aqueous concentration, in this case due to polymer sorption, will translate into a corresponding reduction in the amount of substrate lost to volatilization [18].

3.3. Substrate toxicity

Previous research has shown that inhibition thresholds are highly variable, and dependent on the biomass used (e.g. pure strains or consortia) and on the type of contact (free cells, immobilized cells, biofilms, etc.) [19,20]. A toxicity test was therefore performed to determine the effect of the initial substrate concentrations on the growth and activity of the microbial consortium employed here. Fig. 2a shows the butyl acetate and cell concentrations at three different initial substrate levels. Butyl acetate disappearance occurred readily, and such disappearance is likely due to simultaneous degradation and volatilization, as noted previously.

At best, inhibition caused by butyl acetate was observed only at the highest initial concentration studied, 2420 mg/l; even at this initial concentration cell growth was reduced only slightly compared to the other two lower concentration cases, suggesting minimal inhibition by butyl acetate. To support this observation initial specific rates for the first 5.5 h of reaction were calculated at the initial concentration of 800, 1640 and 2430 mg/l and were estimated to be 940, 1500, 1440 mg butyl acetate/mg cell h, respectively. Cell growth at the lowest initial butyl acetate concentration continued even after the complete disappearance of the acetate, presumably due to the further degradation of intermediates and metabolites.

Phenol disappearance was significantly slower, as seen in the concentration profiles in Fig. 2b, and is attribute entirely to biodegradation. Cell inhibition was clearer in this case, being present even at moderate concentrations, as seen by the longer degradation times. Initial specific rates for phenol at the initial concentrations of 500, 700, 950 mg/l were estimated as 67.3, 54.7 and 35.0 mg phenol/mg cell h, respectively. In contrast to butyl acetate, phenol initial specific rates presented a monotonic decrease with increasing initial substrate concentrations. Inhibition effects are

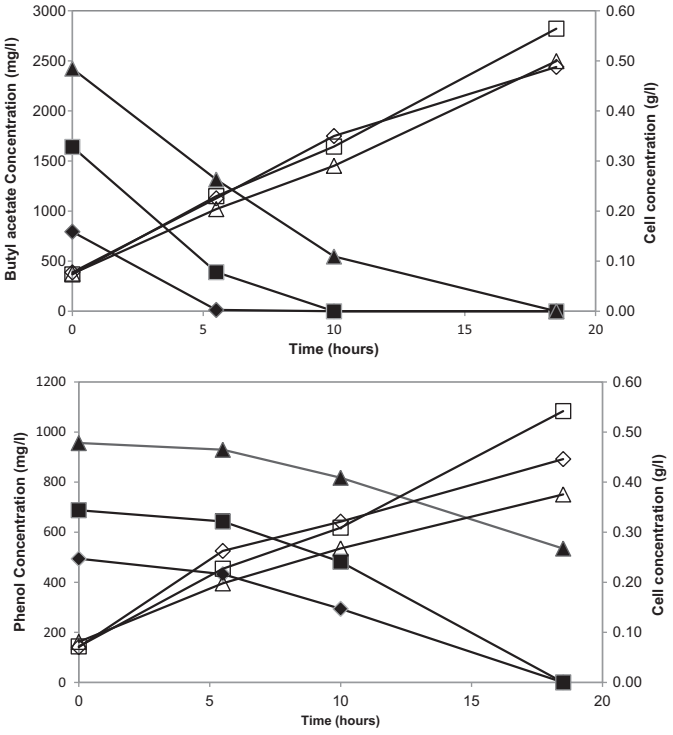


Fig. 2. (a) Butyl acetate time course (solid symbols) and cell concentration profile (open symbols). ▲ – initial concentration 2420 mg/l. ■ – initial concentration 1640 mg/l. ♦ – initial concentration 800 mg/l. (b) Phenol time course (solid symbols) cell concentration profile (open symbols). ▲ – initial concentration 960 mg/l. ■-– initial concentration 690 mg/l. ♦ – initial concentration 500 mg/l.

especially noticeable at 950 mg/l at which concentration the initial specific rate is about half that found for 500 mg/l.

These results reflect the nature of the 2 substrates selected for this work, butyl acetate and phenol, whose principal properties (in terms of this study) were high volatility and minimal toxicity for butyl acetate, and low volatility and high toxicity for phenol.

3.4. Single phase degradation

Sequencing Batch Reactor (SBR) mode was used in this work, as it has been shown to be effective in treating phenolic contaminants [21]. To demonstrate the reproducibility of SBR operation single phase degradation was repeated for four consecutive cycles with an initial concentration of 500 mg/l of both substrates. Cycle 1 was not considered to be characteristic of subsequent cycles because the inoculum conditions were different. Fig. 3 shows the time course, cell concentration profile and dissolved oxygen (DO) trace of a typical single phase cycle. As seen previously butyl acetate disappearance occurs readily at the beginning of the reaction due to cell consumption and volatility; the gradual decrease in the DO profile and the increase in biomass at the beginning of the reaction are evidence of butyl acetate degradation. In contrast, phenol degradation is slower and begins near the end of butyl acetate disappearance. This type of sequential degradation has been observed previously in systems containing multiple substrates such as acetates and phenolic compounds [22,23], and strongly suggests the presence of diauxic growth.

The DO profile shows a steep increase near the last hour of reaction which coincides with the depletion of phenol. Single phase characterization is presented in Table 3. The percentages of butyl acetate degraded and volatilized presented in Table 3 do not add up to 100% because the complete mass balance also considered losses

Table 3
Single phase reactor performance parameters.

Performance comparison	Cycle 2	Cycle 3	Cycle 4	Average values
Total reaction time (h)	10.3	9.0	10.5	9.9 ± 0.7
Butyl acetate rate of disappearance (mg/l h)	101.6	106.8	106.8	105.1 ± 2.5
Phenol rate of degradation (mg/l h)	47.8	56.3	48.2	50.8 ± 3.9
Total mass of butyl acetate volatilized (mg)	787.1	841.2	817.6	815.3 ± 22.2
Total mass of butyl acetate volatilized (%)	52.5	56.1	54.5	54.4 ± 1.5
Total mass of butyl acetate degraded (mg)	673.3	616.8	642.2	644.1 ± 23.1
Total mass of butyl acetate degraded (%)	44.9	41.1	42.8	42.9 ± 1.5

of butyl acetate due to sampling, and such losses were not ascribed to either degradation or volatilization in Table 3.

Single phase experiments were also conducted at 1000 mg/l of butyl acetate and phenol in order to test the system's limitations, and the performance is also presented in Fig. 3. The trends are consistent with the experimental results found at 500 mg/l, and are characterized by an immediate decrease in butyl acetate concentration followed by phenol degradation only after the complete depletion of butyl acetate. Sampling was stopped after 11.5 h, and the phenol profile was therefore not entirely determined. Nevertheless, it is clear from the data obtained at 500 mg/l, that the DO trace is a reliable representation of the microbial activity for the single phase system. The DO trace at 1000 mg/l showed a steep increase in the DO around the twentieth hour of reaction characteristic of the end of microbial activity and the depletion of the substrate.

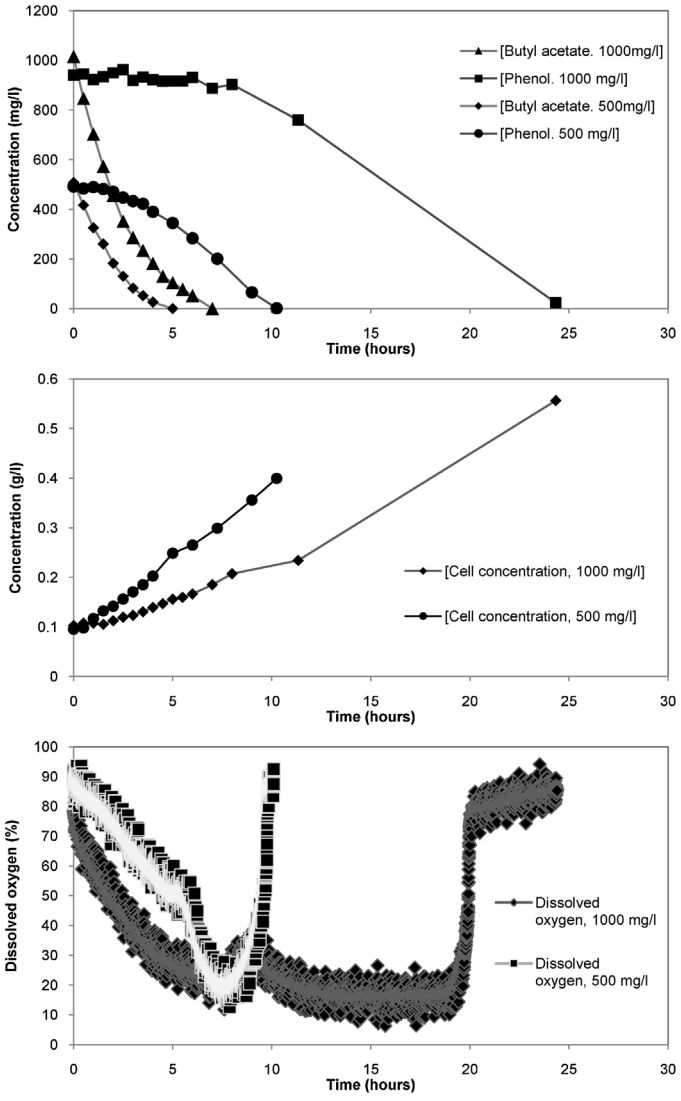


Fig. 3. Single phase biodegradation at 500 and 1000 mg/l. (a) Time course. (b) Cell concentration profile (c) DO trace.

Fig. 3a and b shows the strong inhibitory effect of phenol at higher concentrations. At 1000 mg/l phenol starts being consumed only after the eighth hour of reaction and, additionally, cell growth is clearly hampered compared to the lower concentration experiment.

3.5. Two phase degradation: liquid–liquid

Silicone oil was chosen as the auxiliary phase due to its overwhelming popularity in liquid–liquid TPPB applications. This organic solvent is non-toxic and non-bioavailable for a wide variety of organism, thus making it suitable for many applications including those using microbial consortia. Nevertheless, in contrast to polymers, SO properties are fixed and its thermodynamic affinity to many target molecules is exceedingly low, in addition to possessing a number of practical problems related to its handling [24]. Lastly, SO is considerably more expensive than polymers with prices of up to 386$/l (Sigma–Aldrich catalog price), compared to about $6/kg for polymers [20]. Some authors have claimed to have found lower cost suppliers of SO [25] but until biodegradation experiments are published showing equivalent performance using such low-cost materials, the relative price differences remain as stated above.

Fig. 4 shows biodegradation using SO. Butyl acetate concentration decreased immediately at the beginning of the reaction to a value of 208 mg/l due to its immediate absorption into the SO. This is in agreement with the observations in [16] where it was also shown that the partitioning of butyl acetate into silicone oil occurs instantaneously. A PC of 27.9 was calculated assuming an initial concentration of 500 mg/l based on the amount of butyl acetate added and the reactor volume. On the other hand initial phenol concentrations remained near the original value of 500 mg/l given the poor affinity of SO for this substrate, PC ≈ 0.

The butyl acetate time course had some differences compared to the single phase experiment, showing a straight line trend, with complete degradation being delayed by 1 h compared to the single phase system. These changes in the concentration profile have been ascribed in previous studies to the added system complexity arising from the absorption–desorption phenomenon of the substrate into the auxiliary phase [26,27]. The concentration profile of phenol remained almost unchanged compared to the single phase case, and this was expected given the negligible affinity between phenol and SO. The cell concentration profile could not be successfully tracked, as the presence of the SO promoted emulsion formation which strongly affected the optical density measurements.

The DO profile remained almost constant during the entire reaction, contrary to the single phase experiment. This changed profile can be attributed to an enhanced oxygen transfer capacity provided by the addition of SO. Previous research on the transfer of hydrophobic substrates in TPPBs systems, has shown that the addition of a non-aqueous phase enhances the gas/water transport vector by means of reducing the surface tension and hence air

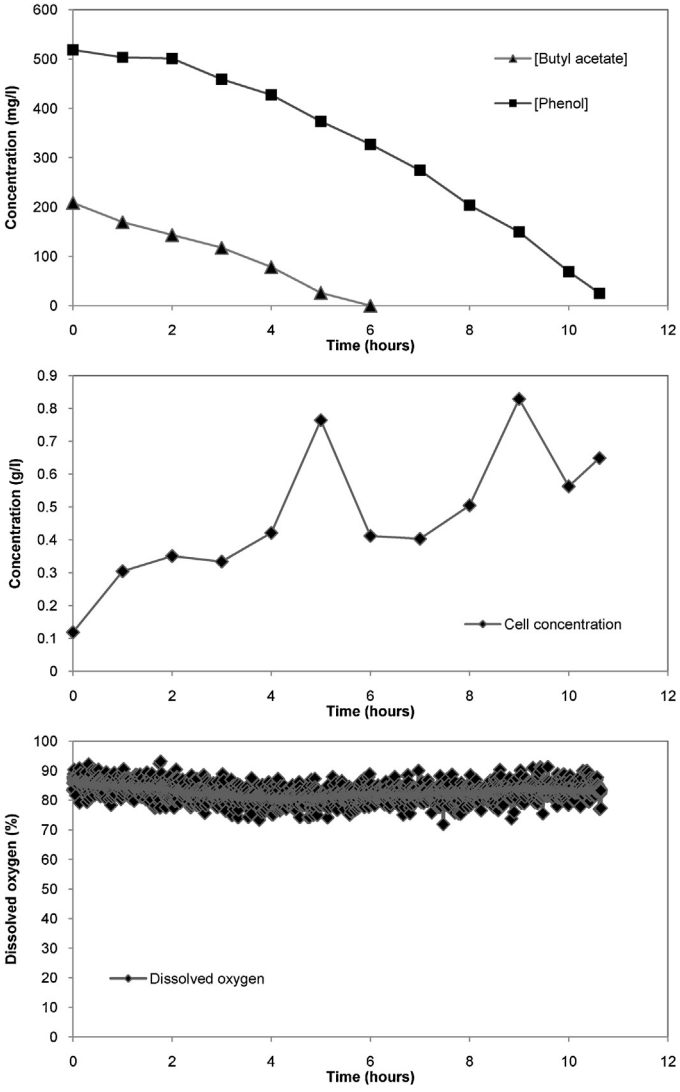


Fig. 4. Liquid–liquid TPPB using silicone oil as the sequestering phase. (a) Time course. (b) Cell concentration profile (c) DO trace.

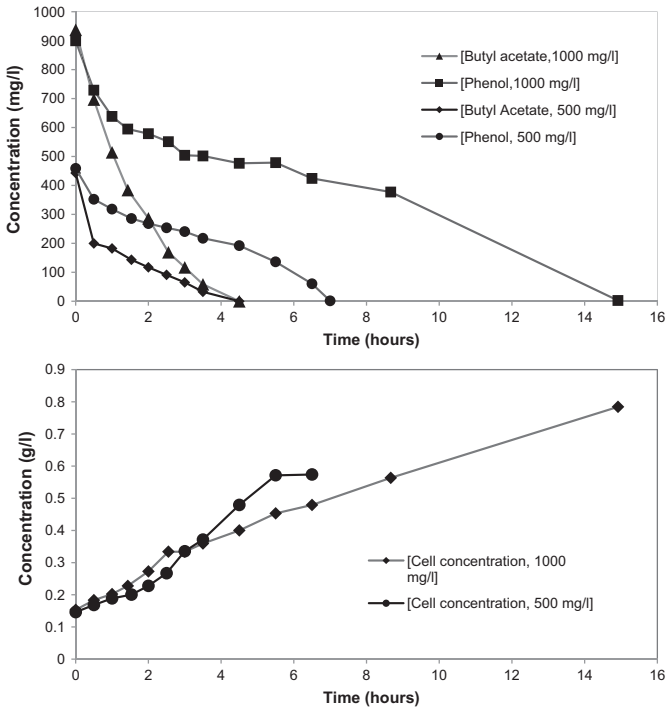


Fig. 5. Solid–liquid TPPB using a polymer mixture of 50:50 SBR and Hytrel® 8206 at 500 and 1000 mg/l. (a) Species time course. (b) Cell concentration profile.

droplets coalescence. The latter allows a greater gas/water interfacial area that translates into an enhanced oxygen transfer capacity. Bubble size distribution and overall gas holdup data presented in some of these studies seem to support this argument, although the interaction between the auxiliary hydrophobic phase and air and the exact transfer mechanism are not yet fully understood [28–30].

3.6. Two phase degradation: solid–liquid TPPB

Fig. 5a and b shows the time course and the cell concentration profiles for the degradation of phenol and butyl acetate at 500 and 1000 mg/l. In the experiment carried out at initial concentrations of 500 mg/l the butyl acetate concentration decreased at the beginning of the reaction due to the combined effect of three phenomena: polymer absorption, volatilization and degradation. In contrast to the SO experiment the absorption of butyl acetate into the polymers did not occur instantaneously, as the concentration decreased sharply during the first 30 min and then adopted a decreasing linear trend similar to the SO experiment. The phenol concentration showed a gradual decrease at the beginning of the reaction attributed to its absorption into the Hytrel® 8206 beads. After the complete disappearance of the acetate at 4.5 h the phenol concentration began to decrease more sharply until complete depletion at 7 h. The total reaction time was considerably less compared to the previous systems confirming that the partial phenol absorption greatly enhanced the microbial activity through reduction in inhibition. The cell concentration profile again suggested the presence of diauxic growth.

In the experiment carried out at initial concentrations of 1000 mg/l the butyl acetate concentration decreased until its complete depletion at 4.5 h, while the phenol time course showed a gradual decrease at the beginning due to polymer absorption and a sharper decrease at 6.5 h until its complete depletion at around 15 h.

3.7. Performance comparison

The biodegradation experiments were characterized in terms of total reaction time, the degradation rates for each substrate and the amount of butyl acetate degraded and volatilized and are summarized in Table 4. The SO system provided a considerable improvement in the amount of butyl acetate degraded relative to the single phase system, due to the reduction in volatility losses. Nevertheless, as expected, there were no significant difference in the total reaction time and in the phenol degradation rate, due to the negligible affinity of SO for phenol.

The polymer system showed faster degradation compared to the other systems, and the reaction time was reduced by 30 and 35% relative to the single phase and the SO systems, respectively. In terms of butyl acetate degraded and volatilized, no significant enhancements were obtained compared to SO, although great improvement was seen relative to the single phase system with an increase of more than 50% in the amount of butyl acetate degraded and a decrease of around 40% in the total amount of butyl acetate volatilized. Butyl acetate rates of disappearance varied, and the highest rate of disappearance was obtained for the polymer system while the lowest was seen in the SO system. An increase in the rate of butyl acetate disappearance is not necessarily associated

Table 4
Performance comparison for all cases studied.

Performance parameter	Single phase	Silicone oil	Polymer mixture	Polymer improvement relative to single phase (%)	Polymer improvement relative to SO (%)
Performance comparison at 500 mg/l					
Total reaction time (h)	9.9	10.9	7.0	29.3	35.8
Butyl acetate rate of disappearance (mg/l h)	105.1	83.3	125.0	–	–
Phenol rate of degradation (mg/l h)	50.8	46.4	71.4	40.6	53.9
Total mass of butyl acetate volatilized (mg)	815.3	576.5	476.4	41.6	17.4
Total mass of butyl acetate degraded (mg)	644.1	901.3	997.8	54.9	10.7
Performance comparison at 1000 mg/l					
Total reaction time (h)	19.8	–	14.9	24.7	–
Butyl acetate rate of disappearance (mg/l h)	145.1	–	222.2	–	–
Phenol rate of degradation (mg/l h)	46.3	–	66.8	44.2	–
Total mass of butyl acetate volatilized (mg)	2014.0	–	1214.6	39.7	–
Total mass of butyl acetate degraded (mg)	886.8	–	1722.1	94.2	–

with an enhancement given the complexity of the system where degradation and volatility occur concurrently.

The relatively high rate of disappearance observed for the single phase system can be mostly attributed to volatilization. For the two phase systems the absorption phenomenon plays an important role in the retention of the butyl acetate within the system, allowing for additional biodegradation. In the case of the SO system the apparently slow butyl acetate disappearance is related to the relatively high affinity between it and the solvent; this causes a slower re-release and ultimately delays the degradation [26]. In the polymer system the apparent higher butyl acetate disappearance is presumably associated with an enhanced microbial activity as a consequence of the lower phenol aqueous concentrations. This is reflected in an increase of the amount of butyl acetate degraded and also in the higher values of cell concentration. By the time butyl acetate is completely depleted in the single phase system, around 5 h, the cell concentration is 0.25 g/l, whereas for the polymer system the cell concentration at butyl acetate depletion time, 4.5 h, is 0.37 g/l. As discussed earlier phenol disappearance unlike butyl acetate, can be mostly attributed to degradation. The polymer system showed a remarkable improvement with respect to the phenol degradation rate with a 40.7 and 53.4% improvement relative to the single phase and the SO systems, respectively.

For the degradation carried out at 1000 mg/l improvements of about 24% were obtained for all the parameters studied. The highest enhancement was seen for the amount of butyl acetate degraded which was increased by almost 100% relative to single phase.

4. Conclusion

The solid–liquid TPPB platform was successfully used to overcome two clearly distinct limitations commonly found in bioremediation applications: stripping losses and microbial inhibition. A polymer mixture of styrene–butadiene rubber and Hytrel® 8206 was chosen for the TPPB experiments due to their high absorption capacity for butyl acetate and phenol, respectively. The degradation patterns for butyl acetate and phenol presented strong evidence of diauxic growth and in the case of the single phase experiment the dissolved oxygen trace provided good insights into the microbial activity variations over the course of the biodegradation. For the liquid–liquid TPPB, SO was found to enhance oxygen transfer, presenting almost no noticeable changes in the DO trace during the whole reaction.

Performance comparisons at two different initial concentrations 500 mg/l and 1000 mg/l were undertaken. At 500 mg/l both TPPB versions clearly outperformed the single phase reactor by decreasing the amount of butyl acetate volatized. The solid–liquid TPPB also outperformed its SO counterpart; in this case the relative improvement in volatility was not as marked but the phenol degradation rate was substantially increased as SO has zero affinity for phenol. Experiments carried out at 1000 mg/l showed a clear superiority of the solid–liquid TPPB relative to the single phase reactor especially in the amount of butyl acetate degraded which was improved by almost 100%.

In the current work polymer selection was carried out based solely on the absorption capacities of the polymer represented by their corresponding PC values. A more systematic protocol founded on fundamental thermodynamic principles is currently being developed with the aim of optimizing the selection of effective polymers for a variety of applications.

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