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Treatment of substituted phenol mixtures in single phase and two-phase solid–liquid partitioning bioreactors

M. Concetta Tomei[a,*], Sara Rita[a], Domenica Mosca Angelucci[a], M. Cristina Annesini[b], Andrew J. Daugulis[c]

[a] Water Research Institute, C.N.R., Via Salaria km 29.300, 00015 Monterotondo Scalo (Rome), Italy
[b] Department of Chemical Engineering Materials & Environment, Sapienza University of Rome, Via Eudossiana 18, 00184 Rome, Italy
[c] Department of Chemical Engineering, Queen's University, Kingston, Ontario, Canada K7L 3N6

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ABSTRACT

The biological treatment of phenolics is constrained by the inherent cytotoxicity of these compounds. One method to alleviate such toxicity is to add a sequestering phase to absorb, and subsequently release, the substrate(s) to the micro-organisms; such a system is termed a Two Phase Partitioning Bioreactor. Here we have compared the performance of a TPPB, relative to single phase operation, in which a small volume (5%, v/v) of beads of the polymer Hytrel 8206 was used to treat aqueous mixtures of 2,4-dimethylphenol and 4-nitrophenol. Hytrel 8206 was selected from a range of polymers that were tested for their partition coefficients (PCs) for the target molecules, with the more hydrophobic compound (2,4-dimethylphenol) having a higher PC value (201) than 4-nitrophenol (143). Significantly increased removal rates for both substrates were demonstrated in TPPB mode relative to single phase operation. Additionally, the differential release of the compounds to the aqueous phase and their distinct PC values changed the kinetic pattern of the biotreatment system, smoothing out the cellular oxygen demand. Release of the substrates by the polymer over 60 operating cycles was virtually complete (>97%) demonstrating the reusability and robustness of the use of polymers in overcoming cytotoxicity of phenolic substrates.

1. Introduction

Phenols, and substituted phenols, are important contaminants in wastewater that are of significant environmental concern because of their toxicity to many life forms; for example the EC_{50} (determined on activated sludge respiration inhibition) of 4-nitrophenol and 2,4-dimethylphenol are 64 and 190 mg/L, respectively [1]. Effluents containing these compounds, present in discharges from a number of industrial activities (e.g. coal conversion processes, coke ovens, petroleum refineries and petrochemical industries, resin and fibreglass manufacturing and herbicide production), must be treated but the ability to eliminate these compounds via biotreatment methods is hampered by their inherent toxicity, which causes inhibition to the microbes responsible for their elimination [2].

An effective means of reducing or eliminating substrate toxicity, while still treating high concentrations of inhibitory compounds, is via the use of Two Phase Partitioning Bioreactors (TPPBs), which consist of a cell-containing aqueous phase, and a sequestering/partitioning phase that reduces aqueous concentrations of toxic substrates via equilibrium partitioning, and releases them to the cells based on metabolic demand. TPPBs have been successfully applied in the removal of xenobiotic compounds from air, water and soil [3–6]. We have demonstrated the enhanced biotreatment of phenol and of 4-nitrophenol (4NP) in TPPBs using immiscible organic solvents [7,8] and commercial polymer beads [9], with the latter approach allowing the use of mixed microbial consortia because of the non-bioavailability and biocompatibility of polymeric materials (e.g. plastics).

Since industrial wastewater is likely to contain mixtures of contaminants it is critical that strategies being developed for commercial application are able to demonstrate effective treatment of such mixtures, and are able to address the kinds of interactions that can occur with multiple toxic substrates. Such substrate interactions can be extremely complex, and can include enhancement, inhibition, and cometabolism as was shown for a mixture of benzene, toluene, ethylbenzene and xylene [10]. In the case of mixtures of substituted phenols, numerous such interactions have also been observed in batch experiments. For example, when cells were grown on a mixture of 4-chlorophenol, 4-nitrophenol and phenol, 4-chlorophenol degradation was delayed until 4-nitrophenol was almost completely depleted [11]. In a dual-substrate system of phenol and 4-chlorophenol, 4-chlorophenol was found to intensely inhibit phenol biodegradation [12]. Alexieva et al. [13] showed

* Corresponding author. Tel.: +39 06 90675800; fax: +39 06 90672787.
E-mail address: tomei@irsa.cnr.it (M.C. Tomei).

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that the presence of p-cresol did not prevent complete phenol degradation but had a significant delaying effect on the phenol degradation dynamics. Also, Wang and Loh [14] found that the addition of a readily utilizable substrate (sodium glutamate) to a ternary substrate system, caused the overall phenol degradation and 4-chlorophenol transformation rates to be greatly enhanced, since glutamate was able to attenuate the toxicity of 4-chlorophenol and therefore increase the cell growth rate.

Although applying TPPBs to mixtures of substituted phenols can reduce toxicity, it adds another degree of complexity since, unlike the above batch experiments, TPPBs also provide for the *re-release* of substrates previously absorbed by the polymer phase on a continuous basis (until substrate depletion) which will vary based on the partition coefficient of each substituted phenol with respect to the partitioning phase, and its demand by the cells. Therefore although TPPBs are extremely effective at detoxifying high initial concentrations of inhibitory substrates, the re-release of these materials to the cells also occurs spontaneously to maintain thermodynamic equilibrium, and can be expected to affect the interactions, kinetics and ultimate performance of a biotreatment system.

In this paper we report the results of an investigation on the performance of conventional and two phase bioreactors applied to the biodegradation of a binary mixture of 2 substituted phenols, 2,4-dimethylphenol (2,4DMP) and 4-nitrophenol (4NP). The objective of the study was to evaluate TPPB technology in terms of the selectivity of the polymer on the partitioning of similar compounds during the biodegradation process, and the effect of the differential partitioning on the biotreatment kinetics.

2. Materials and methods

2.1. Chemicals and polymers

4NP and 2,4DMP (purity > 98%) were obtained from Fluka (Italy). Sodium acetate was obtained from Sigma (Italy). All other chemicals were commercial grade and were supplied by Carlo Erba (Italy). The sources, properties and chemistry of the polymers tested in this work are shown in Table 1.

2.2. Microbial cultures

A mixed culture previously acclimatized to 4NP was available. Details on the culture development are reported elsewhere [15]. An inoculum from this culture was gradually acclimatized to 2,4DMP. In the first phase of the acclimatization procedure sodium acetate was fed in addition to 2,4DMP then, once stable performance was obtained, acetate was progressively reduced stepwise (from 100 mg/L to 0) and 2,4DMP concentration increased from 100 up to 300 mg/L. To ensure the presence of required nutrients and microelements, the feed solution consisted of the MSV mineral medium of Williams and Unz [16]. The mineral medium was formulated to ensure a C:N:P ratio in the influent equal to 100:5:1 with respect to the 4NP and/or 2,4DMP carbon. Once effective performance in biodegradation of the single compounds at a feed concentration of 300 mg/L was verified, two equal aliquots of the microbial cultures degrading the single compounds were mixed and utilized for the kinetic tests on substrate mixtures.

2.3. Reactors

Kinetic tests were carried out in a lab scale Sequencing Batch Reactor (SBR) consisting of a 1-L glass vessel (0.8 L working volume) with a thermostatically controlled water jacket maintaining the temperature at $25 \pm 0.5\,^{\circ}C$. Dissolved oxygen was continuously monitored by a WTW probe (CellOx 325). Feeding, biomass wasting, effluent discharge and acid/base addition for pH control were performed by peristaltic pumps (Cellai, Perinox SF3) through openings located in the reactor cover. Mixing was achieved by a magnetic stirrer that was able to achieve complete mixing conditions even in the presence of the polymer. Air was supplied by a membrane compressor and introduced into the bioreactor through a glass diffuser. Customized software was developed within the Labview-Windows 3.1 environment to manage the sequencing batch working cycle phases, driving of the stirrer, compressors and pumps, and dissolved oxygen (DO) monitoring and control in the range of 3–4 mg/L via an on–off strategy.

A typical SBR operating cycle lasted 12 h distributed as follows: feed 20 min, reaction 580 min, wastage 2 min, settling 90 min, draw 28 min. The feed phase was operated under mixed and aerated conditions. The exchange ratio (added volume/total volume) was 0.5. The SBR was operated as a TPPB by adding the polymer in the ratio of 5% (v/v).

2.4. Analysis

Volatile Suspended Solid (VSS) concentration was determined according to standard methods [17] as an estimate of the biomass concentration. Analysis of 4NP and 2,4DMP was performed on samples after filtering through syringe nylon membrane filters (0.45 μm pore-size) and acidified in order to stop the enzymatic reactions. The filtered samples were then analysed by UV absorbance using a spectrophotometer (Varian, model Cary 1) at 280 and 320 nm for 2,4DMP and 4NP respectively. A double reading of the samples at the two wavelengths was performed for the measurement of the concentration of the two compounds in the binary mixture.

Oxygen Uptake Rate (OUR) was measured from DO data continuously recorded during the reaction phase, and calculated as previously reported [15]. The SOUR (Specific Oxygen Uptake Rate) was evaluated as SOUR = OUR/VSS from OUR data and VSS concentration.

2.5. Partition tests

In the polymer screening tests the partition coefficient measurements were performed on a solution of distilled water containing 4NP and 2,4DMP at a concentration of 1000 mg/L. Two different amounts of polymers (1 and 1.5 g) were added to 12 mL of the solution and allowed to equilibrate on a shaker for 24 h, a time largely overestimated to ensure reaching equilibrium conditions. The final concentration was measured as described above and partition coefficients were determined by mass balance [18].

2.6. Kinetic tests

Kinetic tests were performed in the SBR operated in conventional and two-phase mode. The feed concentration was in the range of 250–450 mg/L 4NP and 2,4DMP, and the polymer/aqueous phase ratio in the reactor was 5% (v/v). The tests were performed by measuring 4NP and 2,4DMP concentrations on samples of the aqueous phase taken from the reactor at predetermined time intervals (5–20 min) during the feed and reaction phases. VSS concentration was also measured but at longer time intervals (hours) due to its very low variation with respect to the typical concentrations in the reactor. In order to verify the reproducibility of the data, biodegradation tests were carried out in at least two replicates under the same operating conditions.

Two series of biodegradation tests were undertaken. In the first one the performance of the SBR operating in a conventional single phase mode was investigated at different concentrations in the feed (in the range of 250–380 mg/L). In the second, the reactor was

Table 1
Properties of polymers tested for uptake of 4NP and 2,4DMP.

Polymer	Grade	Supplier	Hardness	T_g (°C)[b]	T_m (°C)[c]	Specific gravity	Description
Amides							
Pebax	2533	Arkema	75A	−65	133.5	1.01	Polyether block amide
Nylon	6,6	DuPont	90R	50	255	1.24 (crystalline) 1.07 (amorphous)	Polyamide 66
Vinyl acetates							
Elvax	770	DuPont	98A 47D	−100[d]	96	0.928	9.50% vinyl acetate (co-polymer with ethylene)
Other							
Hytrel	G3548L	DuPont	40D	−30	170	1.18	Co-polymer of poly(butylene terephthalate) and polyether
	8206		35–40A[a]	−59	189	1.17	
Kraton SBR	D1102	Kraton	66A	−80	150–200	0.94	Styrene/butadiene linear block copolymer
Fusabond	N416	DuPont		17	43	0.869	Chemically modified ethylene elastomer

Hardness: A (Shore A); D (Shore D); R (Rockwell).
[a] Approximated in personal correspondence with DuPont.
[b] Glass transition temperature.
[c] Melting point.
[d] Brittleness temperature.

operated as TPPB at a polymer/aqueous phase ratio of 5% and feed concentration in the range of 300–450 mg/L.

2.7. Polymer washing

Methanol was utilized as a solvent to extract and quantify the residual amount of 4NP and 2,4DMP in the polymer after the kinetic tests. A multi-step washing procedure with 10 mL of methanol per 0.5 g of polymer was utilized for each washing step until the concentration in the solvent was negligible.

3. Results and discussion

3.1. Polymer screening

The screening of a variety of commercially available polymers was performed by evaluating their partition coefficients in distilled water for the two selected compounds, and the results are reported in Table 2.

In the first instance the value of the partition coefficient for a target molecule is a key feature for assessing the potential effectiveness of a polymer for use as a partitioning phase in a TPPB, and the polymer must possess at least two key characteristics: it should have a high affinity (high partition coefficient) to ensure an efficient uptake of the compounds and a consequent effective reduction in microbial toxicity, and at the same time the affinity should not be so high as to cause too low an equilibrium concentration in the aqueous phase resulting in substrate limitations to the overall degradation process. This latter effect was seen in TPPBs degrading PCBs in which very high affinities for the PCB molecules resulted in kinetically limited operation [19]. Table 2 shows a very broad range of affinities by the polymers tested for the 2 substituted phenols and, with the exception of Hytrel G3548L, 2,4DMP was taken up to a larger extent in all cases. This may be due to the hydrophobicity of 2,4DMP, whose log $K_{O/W}$ (2.3) is higher than that of 4NP (1.91) and is therefore likely attracted more readily by these generally hydrophobic polymers. When selecting TPPB polymers for use with mixtures of toxic substrates the differences in the partition coefficients are also important and must be taken into account when determining polymer phase ratios for a given influent feed concentration, and the desired "detoxified" aqueous phase concentration targets. Of the polymers considered (Table 2), Hytrel 8206 and Pebax 2322 showed substantial affinity for the two compounds, and were also characterized by a high degree of selectivity. In view of the fact that Hytrel 8206 has already been successfully employed with 4-nitrophenol alone [20], it was chosen as the partitioning phase in the TPPB reactor in this work.

Table 2
Partition coefficients of 4NP and 2,4DMP measured in distilled water.

Polymer	4-Nitrophenol	2,4-Dimethylphenol
Elvax 770	1.08	13.60
FUS N416 EPR-g	0.0635	7.99
Hytrel 8206	143	201
Hytrel G3548L	1125	543
Kraton-D1102	0.554	10.1
Nylon 6,6	8.34	38.3
Pebax 2533	107	350

A final significant advantage with the use of polymers as the sequestering phase in TPPBs, in contrast to two-liquid phase systems, is the possibility of using two (or more) different polymers with dissimilar affinities for individual target molecules in a mixture in order to better control aqueous phase concentrations to desired values. The use of polymer mixtures to achieve this additional level of "fine tuning", as has been demonstrated for the uptake of inhibitory fermentation products [21], is an area of current research by us.

3.2. Kinetic tests: single phase reactor

A first series of kinetic tests (S1–S4) with the substituted phenol mixture were performed in a single phase reactor, with the operating conditions of the different tests summarized in Table 3. To investigate the interactive effects of the concentrations of the two compounds on their rate of biodegradation in mixture, tests S1 and S2 were performed at equal influent concentrations of 4NP and 2,4DMP while in tests S3 and S4 different concentrations of the two compounds (in the same ratio) were utilized.

A quantitative evaluation of process performance at these various feed conditions is given by the average specific reaction rates reported in Table 3. These rates were calculated from the start of the SBR cycle until 98% of the substrate had been degraded. The rate values in Table 3 suggest that both compounds are able to sustain metabolic activity with kinetics comparable to those of bacterial populations operating in full scale wastewater treatment plants (i.e. nitrifiers) [22].

Figs. 1–3 show the concentration profiles for 4NP and 2,4DMP and the Specific Oxygen Uptake Rates (SOURs) for tests S2–S4. The reported data are representative of the range of operating conditions; test S1, at the lower concentration for the case of equal feed

Table 3
Operating conditions and average reaction rates for kinetic tests in the single phase reactor. $4NP_{in}$ and $2,4DMP_{in}$ are the influent concentrations and r the reaction rates.

Test	$4NP_{in}$ (mg/L)	$2,4DMP_{in}$ (mg/L)	X^a (mgVSS/L)	r_{4NP} (mg 4NP/(gVSS min))	$r_{2,4DMP}$ (mg 2,4DMP/(gVSS min))
S1	250	250	2870	0.38	0.11
S2	300	300	2580	0.42	0.12
S3	380	250	2740	0.34	0.10
S4	250	380	3050	0.33	0.11

[a] Mean value resulting from multiple measurements during the test.

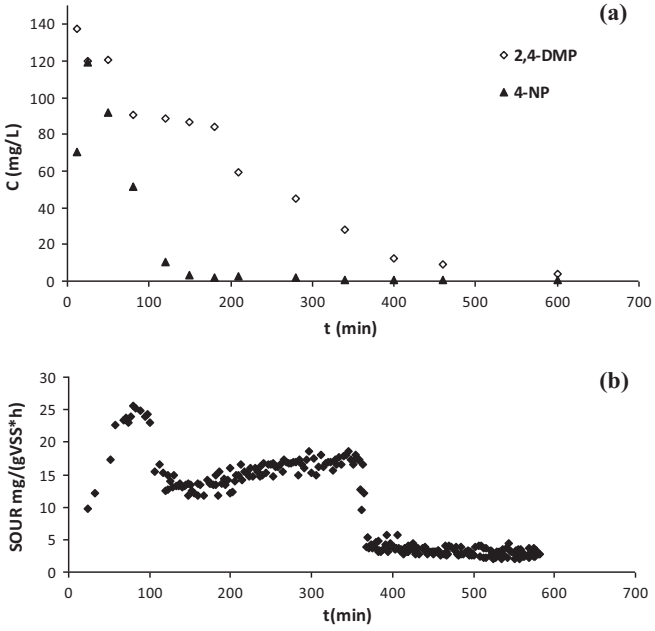

Fig. 1. Concentration profiles (a) and SOUR (b) vs time observed in test S2.

concentrations demonstrated similar SOUR patterns as observed in S2 (and is not shown) and tests S3 and S4 are for the two cases characterized by a higher concentration value of one of the compounds with respect to the other.

In all cases, irrespective of the initial substrate concentration ratio, faster degradation of 4NP is observed, and the corresponding 2,4DMP degradation is characterized by slower kinetics whose rates significantly increase after 4NP is completely depleted. This pattern of slow degradation in the presence of 4NP and faster uptake upon 4NP depletion is more evident at higher 2,4DMP concentrations (i.e. test S4) and is confirmed by the SOUR profiles that are distinguished by a levelling of the rate values corresponding to the transition from growth on 4NP to growth on DMP, as seen by two peaks.

A similar degradation pattern was observed by Unell et al. [11] in the degradation of a phenolic mixture who observed a delayed degradation of 4-chlorophenol that began measurably only when 4NP was almost completely depleted. They suggested that this preferential order of degradation could be attributed to the pK_a values of the two compounds and hypothesized that the dissociated phenolate ions are the substrates. This explanation may also be the case in this work considering that the pK_a values of 4NP and 2,4DMP are 7.15 and 10.6, respectively. An alternative explanation for the delayed degradation kinetics of 2,4DMP in our case may be due to the fact that the biomass used in our study was cultivated for a long period of time (years) on 4NP and only for a shorter period (weeks) on 2,4DMP and therefore an acclimatization effect could play a role in the mixture degradation kinetics. Alternatively, this pattern may be an example of substrate interactions.

The calculated degradation rates confirm faster degradation of 4NP relative to 2,4DMP (Table 3) with a slight reduction in tests S3 and S4 presumably due to the inhibitory effect exerted by the higher substrate concentrations. No significant differences are observed for the 2,4DMP degradation rates.

3.3. Kinetic tests: TPPB reactor

A second series of kinetic tests (T1–T3) were performed in TPPB mode, using a polymer ratio of 5% of the working volume of the

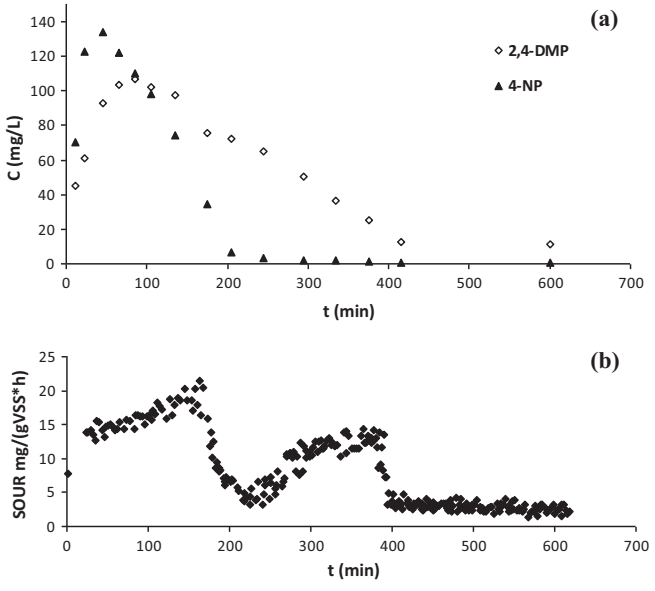

Fig. 2. Concentration profiles (a) and SOUR (b) vs time observed in test S3.

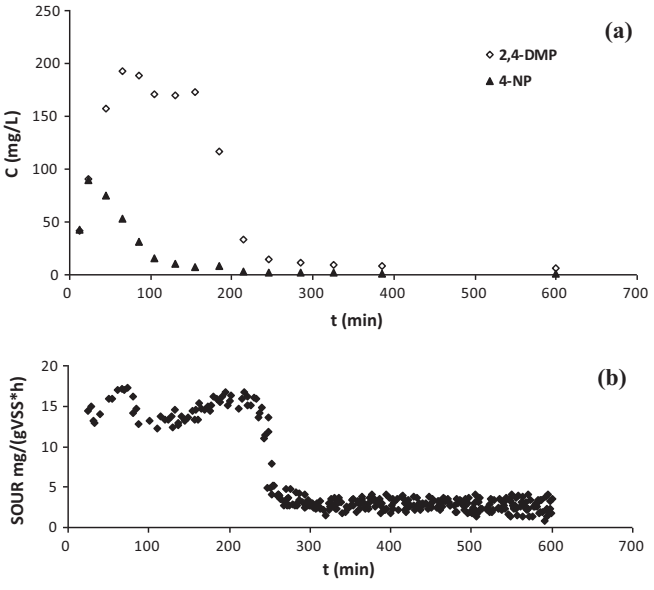

Fig. 3. Concentration profiles (a) and SOUR (b) vs time observed in test S4.

Table 4
Operating conditions and average degradation rates for kinetic tests in the TPPB rector. $4NP_{in}$ and $2,4DMP_{in}$ are the influent concentrations and r is the reaction rates.

Test	$4NP_{in}$ (mg/L)	$2,4DMP_{in}$ (mg/L)	X^a (mgVSS/L)	r_{4NP} (mg 4NP/(gVSS min))	$r_{2,4DMP}$ (mg 2,4DMP/(gVSS min))
T1	300	300	2950	0.61	0.25
T2	350	350	3310	0.57	0.45
T3	450	450	3405	0.56	0.48

[a] Mean value resulting from multiple measurements during the test.

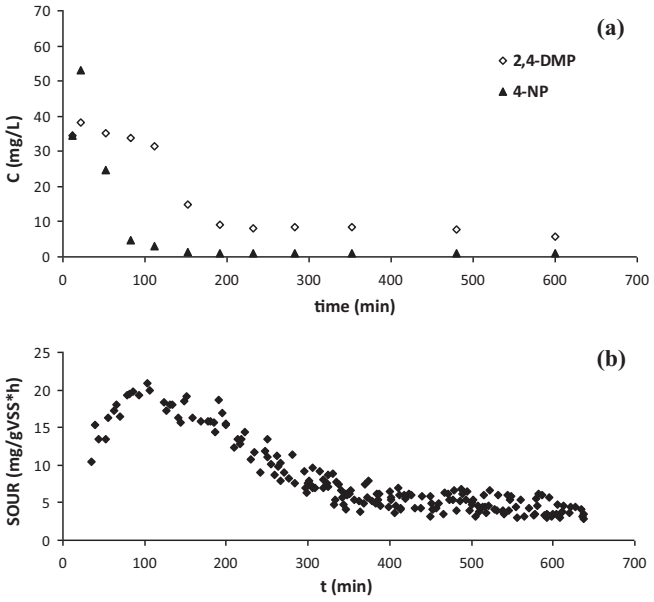

Fig. 4. Concentration profiles (a) and SOUR (b) vs time observed in test T1.

reactor, with the operating conditions and specific reaction rates reported in Table 4. As was done in the single phase case, the reaction rates were calculated based on the time required for 98% substrate removal.

The concentration profiles for 4NP and 2,4DMP and the SOURs for tests T1 and T3 are shown in Figs. 4 and 5. The reported data allow a direct comparison of the performance of the single and two phase reactors operating at the same feed conditions (e.g. T1 with S2) and an evaluation of two phase reactor efficiency at an even higher concentration value (test T3).

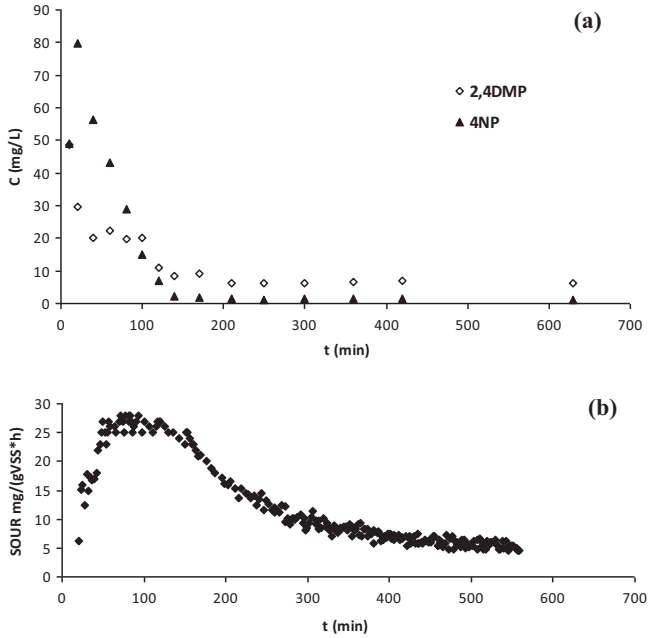

Fig. 5. Concentration profiles (a) and SOUR (b) vs time observed in test T3.

The presence of the polymer had a marked positive effect on the process kinetics, which were significantly improved for both substrates as is shown by the direct comparison of the r values of tests S2 and T1 performed at the same influent substrate concentrations. As expected from the partition coefficient values, the reduction of toxicity (i.e. the decrease in substrate concentration) is much more evident for 2,4DMP, with the 4NP degradation rate being enhanced by 45% with polymer addition, and by more than 100% in the case of 2,4DMP. Moreover, the $r_{2,4DMP}$ values for tests T2 and T3 give a quantitative indication of the enhanced 2,4DMP degradation rate with the use of polymers at even higher influent concentrations. This finding demonstrates the positive effect of selective toxicity reduction arising from the higher partition coefficient for 2,4DMP.

In TPPB mode the concentration profiles of the two compounds also showed delayed 2,4DMP degradation, although this feature was less evident than that seen in the single phase system. However, considering the complexity of the solid–liquid partitioning system, the concentration profiles are in fact not likely representative of the pattern of the intrinsic biodegradation kinetics. This is because of the additional phenomena present in the TPPB system, which include adsorption and desorption of the substrates. An enhanced representation of the kinetic pattern is given by the SOUR profiles, which provide a direct indication of the biological kinetics, because they reflect metabolic activity rather than just aqueous substrate concentrations [23]. From the SOUR profiles it is possible to see the impact of the presence of the polymer partitioning phase on the kinetic pattern. In the two phase system there is only one peak corresponding to the maximum process rate, in contrast to the single phase system that exhibited a double peak followed by a sharp decrease, and a gradual reduction in the SOUR value until the endogenous value of 4–5 mg O_2/(gVSS h) is reached.

This smoothed SOUR profile in the TPPB system is thought to be due to the gradual release of the substrates from the solid phase. A slower release is observed for 2,4DMP attributable to the higher affinity for this compound by the polymer. The observed low residual concentration of 2,4DMP (4–5 mg/L) remaining over a prolonged period can explain the slightly higher final SOUR values; minimal degradation of the released compound is still occurring as the system experiences a condition of slow release (due to the reduced solid–liquid concentration gradient) and slow degradation rate (due to the low concentration in the liquid phase) at a process rate that is comparable to that of endogenous respiration.

The effect of the presence of a solid matrix on the degradation kinetics of phenolic compounds in mixtures was also observed by Unell et al. [11] who found a modified kinetic pattern with respect to aqueous solution kinetics when the phenolic mixture was being degraded in a soil slurry. In their case the soil used was “sandy loam” whose organic content could act as an absorptive phase analogous to the TPPB polymer.

The SOUR values at the end of the reaction phase are indicative of almost complete removal of the compounds from the liquid phase but, to better verify their degree of degradation, the residual amounts in the solid phase were evaluated by a multi-step washing procedure with methanol. The results of the mass balance for the two compounds after 60 operation cycles are reported in Table 5.

Table 5
Residual substrate levels after 60 operation cycles.

Compound	Effluent (%)	Polymer (%)	Removed (%)
2,4DMP	1.54	1.29	97.17
4NP	0.26	0.51	99.23

The data in Table 5 confirm the very high removal efficiency achieved for both the compounds in the TPPB reactor. Moreover, a negligible residual amount was found in the polymer phase even for 2,4DMP in spite of its high partition coefficient.

4. Conclusions

This work has shown that a number of inexpensive and commercially available polymers are effective sequestering phases for detoxifying a mixed feed of substituted phenols in a TPPB. The detoxification of the inhibitory substrates occurs rapidly within the feed cycle of a Sequencing Batch Reactor, and leads to significantly enhanced specific rates of substrate consumption. Individual polymers have varying affinities for different target molecules leading to selective uptake of substrates and, as demonstrated in this study, an overall enhancement arising from the preferred uptake of the more recalcitrant molecule (2,4DMP). This selectivity can be exploited further by the use of mixtures of polymers aimed at reducing the concentration of wider mixtures of substrates to predetermined values. The second function of the partitioning phase, the re-release of the substrate to the cells based on metabolic demand, has resulted in a smoother degradation pattern with a more concurrent uptake of the two substrates and without multiple peaks in oxygen demand. The use of these commercial polymers through many (>60) SBR cycles with minimal accumulation of substrates demonstrates the robustness of these materials in this application. Current work is aimed at the use of even more broadly mixed substrate feeds, the use of inexpensive waste rubber/plastics (e.g. shredded automobile tires) as the sequestering phase, as well as modelling of these systems to predict suitable conditions (polymer selection) and operating regions (SBR cycle) in the treatment of recalcitrant substrates.

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