Two-Phase Partitioning Bioreactors Operating with Polymers Applied to the Removal of Substituted Phenols

M. CONCETTA TOMEI,*,† M. CRISTINA ANNESINI,‡ SARA RITA,† AND ANDREW J. DAUGULIS§

Water Research Institute, C.N.R., Via Salaria km 29.300, CP 10-00015 Monterotondo Stazione (Rome) Italy, Department of Chemical Engineering Materials & Environment, Sapienza University of Rome, Via Eudossiana 18, 00184 Rome, Italy, and Department of Chemical Engineering, Queen's University, Kingston, Ontario, Canada K7L 3N6

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Significant improvement in biodegradation performance has been demonstrated arising from the reduction of cytotoxicity provided by the sequestering of 4-nitrophenol (4NP) within Hytrel polymer beads added to a two-phase partitioning bioreactor (TPPB) operating in sequencing batch reactor (SBR) mode. This reduced toxicity is particularly apparent as the feed substrate concentration is increased; in fact it was shown that at a feed of 1000 mg/L 4NP, the inhibitory effect of the substrate completely prevents degradation from occurring in a single-phase system, whereas at only a 5% polymer loading, rapid and compete biodegradation is achieved. Different polymer/aqueous phase ratios were used to detoxify varying feed concentrations, and degradation rates were enhanced through the use of increased polymer loadings. As demonstrated in oxygen uptake experiments, the addition of polymers also reduces the maximum demand for oxygen, relative to single-phase operation, and smoothes the demand for oxygen throughout the degradation process. Polymer regeneration has also been further characterized by quantifying the number of methanol washes required to achieve satisfactory 4NP residuals, and the addition of a small amount of cosolvent has been shown to dramatically increase the rate of bioregeneration to produce beads ready for reuse.

Introduction

Organic contaminants in wastewater can often be resistant to biotreatment methods due to the cytotoxicity of the target molecules themselves. The resulting challenges in biological treatment systems are prevalent not only under steady-state operation, but may be exacerbated under transient conditions during which surges of toxic organic compounds can severely inhibit, or completely destroy, the microbial populations present. Using phenol as a representative xenobiotic, many researchers have developed biotreatment strategies aimed at protecting cells from such cytotoxic effects, which include immobilizing cells in calcium alginate beads (*1*), acrylamide gels (*2*), membrane protection (*3*), on glass beads as a biofilm in a pulsed plate bioreactor (*4*), and sequestration of the target molecule in a separate immiscible liquid organic phase (*5*).

This last technology platform, termed a two-phase partitioning bioreactor (TPPB), has been successfully used to treat a variety of toxic organic contaminants including phenol (*6*), pentachlorophenol (*7*), biphenyl (*8*), indole (*9*), polyaromatic hydrocarbons (*10*), and PCBs (*11*). One of our recent contributions in this area has been to demonstrate the significantly enhanced degradation of a substituted phenol, 4-nitrophenol (4NP), in a TPPB arising from the reduced cytotoxicity provided by the sequestering second phase (*12*). A major practical limitation of such two-liquid phase systems, however, is identifying an immiscible organic solvent that will be biocompatible with the microbial population present, and also nonbioavailable, as "real world" biotreatment applications will invariably use mixed populations. Silicone oil is the most widely used solvent in these applications, being both biocompatible and nonbioavailable to most cells, however its cost and operating challenges (high viscosity, foaming) are severe limitations.

Recently, we have shown that inexpensive polymer beads can be used to replace immiscible organic solvents to absorb and release organic molecules for degradation by mixed populations in TPPBs (*13*). In such systems the polymers act exactly as does an organic solvent, with uptake/release occurring via absorption, and reduced aqueous xenobiotic levels arising from the maintenance of a thermodynamic equilibrium between the aqueous and polymer phases. The use of inexpensive, nonvolatile, nonflammable, biocompatible, and easily shaped polymers as the sequestering phase in a bioreactor is an enormous advance in designing high-efficiency, low-cost bioprocesses that eliminate cell toxicity and is key to the development of "green", solvent-free biotreatment strategies.

In a recent fundamental study (*14*) we screened for appropriate sequestering polymers for 4NP degradation based on their partition coefficients, demonstrated greatly enhanced kinetic performance relative to a single-phase system, and confirmed the applicability of polymers in TPPBs operating with a microbial consortium. The novelty of the results of the present paper is the demonstration of the advantages of the two-phase polymer−aqueous system in comparison to one-phase reactors in terms of increased process rates with an experimental apparatus closer to a real system, a sequencing batch reactor (SBR). SBR process performance over a range of increasing 4NP concentrations was examined, as was the effect of varying polymer loading, polymer regeneration, and reuse in the combined TPPB-SBR system. Moreover, an alternative bioregeneration method that provides a significant improvement of the process kinetics was also demonstrated.

Material and Methods

Chemicals. 4-Nitrophenol (purity >98%) was obtained from Fluka (Italy). The polyether-ester copolymer, Hytrel 8206, (kindly provided by DuPont Canada) is in the form of oval shaped beads (5 mm length, 1.5 mm diameter) with density 1.17 g/cm^3, melting point 189 °C, and glass transition temperature of −59 °C. All other chemicals were commercial grade and were supplied by Carlo Erba (Italy).

Biomass. A mixed culture previously acclimatized to 4NP as the sole carbon source was used in all biodegradation experiments. The original biomass inoculum was obtained as a mixed liquor sample from a domestic wastewater

* Corresponding author phone: +390690672800; fax: +390690672787; e-mail: tomei@irsa.cnr.it.
† Water Research Institute, C.N.R.
‡ Sapienza University of Rome.
§ Queen's University.

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treatment plant in Rome. The culture was acclimatized by growing on 4NP as the sole carbon and energy source. To ensure the presence of required nutrients and microelements, the feed solution consisted of the MSV mineral medium of Williams and Unz (*15*). 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 carbon. Additional details of the acclimatization procedure have been previously reported (*16*).

Reactors. The SBR reactor consisted of a 5 L glass vessel (4 L working volume) with a thermostatically controlled water jacket maintaining the temperature at 20 ± 0.5 °C. Dissolved oxygen and pH were monitored online by WTW instruments (CellOx 325 and Ino Lab, respectively). Feeding, sludge 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 mechanical stirrer fitted with helicoidal blades, and 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 located below the impellor.

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) and pH monitoring and control via on−off strategies. The DO was maintained in the range of 3−4 mg/L and pH was maintained in the range of 7.5−8.

A typical SBR operating cycle lasted 12 h distributed as follows: fill 30 min, reaction 570 min, wastage 3 min, settling 92 min, draw 25 min. The fill phase 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 predetermined amounts of polymer.

Analysis. Volatile suspended solids (VSS) concentration was determined according to Standard Methods (*17*) as an estimate of the biomass concentration.

4-Nitrophenol analysis was performed on samples after filtering through syringe nylon membrane filters (0.2 μm pore-size) and acidified to stop the enzymatic reactions. The filtered samples were then analyzed by UV absorbance at 320 nm using a spectrophotometer (Varian, model Cary 1). Interference by other compounds in the aqueous solution was excluded by preliminary tests.

Oxygen uptake rate (OUR) was measured from DO data continuously recorded during the reaction phase, and calculated as previously reported (*16*). The specific oxygen uptake rate (SOUR) was evaluated as SOUR = OUR/VSS from OUR data and VSS concentration.

Biodegradation Tests. Biodegradation tests were performed in the SBR operated in conventional and two-phase mode. The feed concentration was in the range of 400−1000 mg/L 4NP, and the polymer/aqueous phase ratio in the reactor was varied from 5 to 10% (v/v). The tests were performed by measuring 4NP concentration on samples of the aqueous phase taken from the reactor at predetermined time intervals (10−20 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 concentration in the reactor. To verify data reproducibility, 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 conventional and TPPB SBRs was investigated at increasing 4NP concentrations in the feed (in the range of 750−1000 mg/L) and with a polymer/aqueous phase ratio of 5% in the TPPB. In the second the TPPB SBR was operated by varying the polymer/aqueous phase ratio in the range of 5−10% v/v. The reactor worked continuously for 8 months (3 with the one-phase configuration and 5 as TPPB) in the first period and for 5 months (3 with the one-phase configuration and 2 as TPPB) in the second period.

Regeneration Tests. Regeneration tests with methanol were performed to extract and quantify the residual amount of 4NP in the polymer after the biodegradation tests. A multistep washing procedure with 10 mL of methanol per g of polymer was utilized for each washing step until the concentration in the solvent was negligible.

A "bioregeneration" procedure was also undertaken on the used polymer beads through prolonged contact with the biomass present in a batch, aerated reactor. The batch reactor was operated at the same polymer/aqueous phase ratio as the main reactor. To measure the residual 4NP in the solid phase, at various time intervals 1 g of polymer was withdrawn and the residual 4NP was extracted by multi step washing with methanol until negligible 4NP values were detected.

An alternative "bioregeneration" method was also preliminarily tested consisting of the addition of a small amount of methanol (0.5% v/v) to the batch reactor. The operating conditions of the reactor and procedures for determining the amount of residual 4NP in the polymer phase were as described above.

Results and Discussion

TPPB vs Conventional SBR: Effect of Feed Concentration. The capability of the TPPB to reduce toxic substrate effects on biomass activity was investigated via biodegradation tests performed at increasing 4NP concentrations in the feed. SBR operation was initiated in conventional one-phase mode then, once a stable operating condition was reached, the 4NP concentration in the influent was increased. When the system showed a significant loss of performance the polymer was added and the reactor was shifted to two-phase operation. The influent concentration values tested in this paper (up to 1000 mg/L) are significantly higher in comparison to those reported in previous papers (*12*, *16*) and are potentially very toxic (for 4NP the EC_{50} = 64 mg/L (*18*) as determined with respect to activated sludge microorganisms).

The polymer Hytrel 8206 was utilized in this study because of its demonstrated superior performance (*14*) in 4NP partition and batch kinetic tests relative to a number of other commercial polymers tested. Tests in the SBR reactor here were performed at a polymer/aqueous phase ratio of 5%.

Figure 1a shows the 4NP concentration profiles observed in the biodegradation tests (two replicates) at a feed concentration of 750 mg/L in conventional single-phase SBR operation. To provide an appropriate comparison of the biodegradation rates the feed and reaction phase concentration curves are plotted vs Xt, a parameter that takes into account the effect of the inevitable slight variations in the biomass concentration among the different tests. With regard to the assumption of a constant biomass concentration value in the single kinetic test, it should be pointed out that the biomass was measured in terms of volatile suspended solids (VSS) a parameter that can be affected by a significant measurement error; at the VSS concentration levels used in the experiments, the variation of the biomass due to net growth was not appreciable.

Good agreement is observed between the replicates. An initial increase in 4NP concentration is seen in the feed phase due to influent addition. A marked inhibition effect can be seen as indicated by the double slope in the concentration curve during the reaction phase. The inhibitory effect markedly decreases below a concentration of about 70 mg/L, which is of the order of the EC_{50} value.

In Figure 1b two replicates of the concentration profiles observed in the TPPB-SBR are shown for the same influent concentration, and in this case good agreement between replicates is also observed. The presence of the polymer

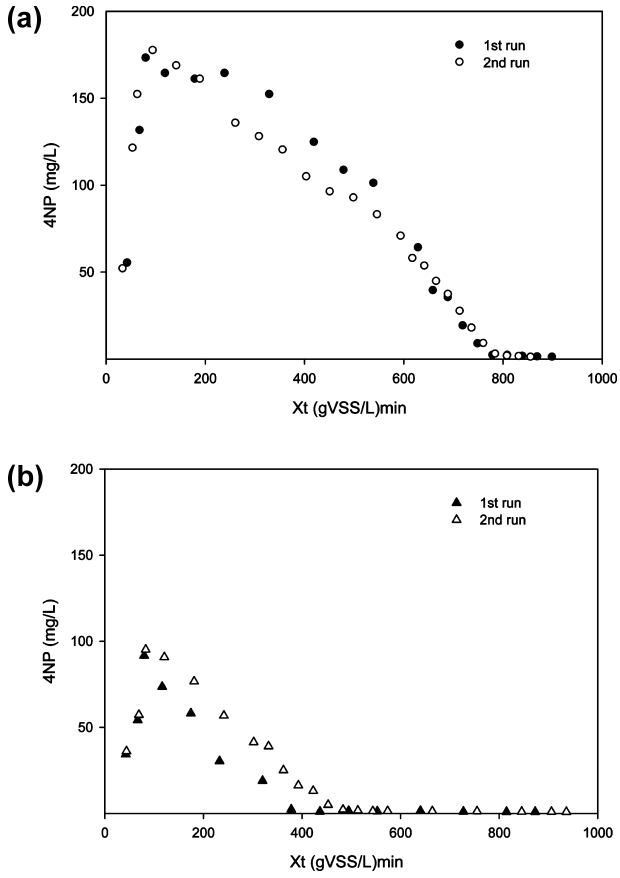

FIGURE 1. (a) Biodegradation tests (with replicate) in the conventional one-phase SBR. Feed concentration 750 mg/L and X = 2700 and 2400 mgVSS/L for the 1st and 2nd runs, respectively (Test T1a). (b) Biodegradation tests (with replicate) in the TPPB-SBR. Feed concentration 750 mg/L and X = 2900 and 3000 mgVSS/L for the 1st and 2nd runs respectively. Polymer/aqueous phase ratio =5% (v/v) (Test T1b).

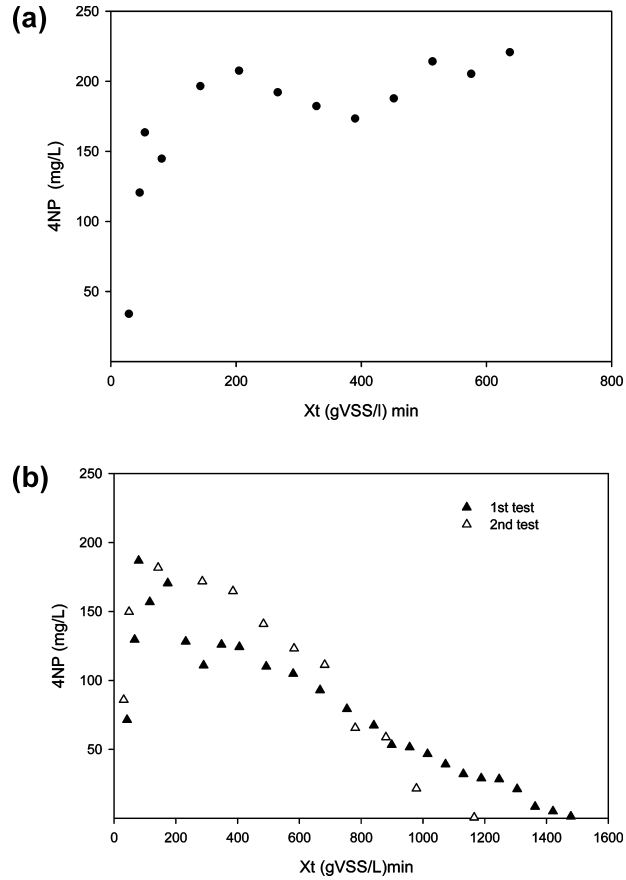

FIGURE 2. (a) Biodegradation test in the conventional one-phase SBR. Feed concentration 1000 mg/L and X = 2100 mgVSS/L (Test T2a). (b) Biodegradation tests performed in the TPPB-SBR. Feed concentration 1000 mg/L and X = 2900 and 2000 mgVSS/L for the 1st (Test T2b) and 2nd test (Test T2c), respectively. Polymer/aqueous phase ratio =5% (v/v). Second test was performed more than 2 months after the first test.

eliminates the inhibitory concentration effect by reducing the 4NP concentration to subinhibitory levels for the most part of the reaction phase. This is also confirmed by the different concentration profiles that do not show the double slope pattern.

The beneficial effect of the polymer was more evident in the tests performed at 1000 mg/L for which results are reported in Figure 2a and b for the one- and two-phase systems, respectively. The conventional SBR is no longer able to remove 4NP at this level, which may have been partially adsorbed on the biomass initially, and progressively released into the liquid phase.

In the case of the TPPB system complete 4NP removal is achieved. It is also important to note that the replicate data in Figure 2b were obtained two months apart, without changing the polymer, and thus the reactor was able to sustain long-term operation without losing efficiency. Moreover the concentration profile of the replicate is seen to be less inhibited than the one performed earlier just after polymer addition. As an alternative means of comparing TPPB reactor performance, average 4NP removal rates were evaluated for the different operating conditions and configurations, and results are summarized in Table 1.

The positive effect of the polymer on the removal rate can be seen for both feed concentrations, as in the test T1b the average reaction rate was increased by about 62% relative to the single-phase case (T1a), with a consequent reduction of the required reactor volume for equivalent treatment performance. In the case of T2 tests the TPPB configuration provides the biological system with significant removal rates just after the polymer addition; furthermore the long-term beneficial effect of the reduction in toxicity is quantitatively demonstrated by the increased rate (47%) in test T2c in comparison with T2b. This could be the result of a positive effect on biomass performance derived from a prolonged exposure, and adaptation, to the reaction environment.

Besides the advantages in terms of biodegradation performance, the solid partitioning phase was demonstrated to be effective in overcoming several operating limitations affecting the liquid−liquid system (*12*). No foaming or emulsion formation were observed during reactor operation, and the polymer beads, after prolonged use, did not show any attached biofilm growing on the surface.

The residual 4NP in the used polymer was quantified by multi-step extraction with methanol. A measured value of 1.70 mg4NP/g polymer was obtained that allows effective long-term operation for the TBBP SBR working at a feed concentration in the range of 750−1000 mg/L.

Effect of the Polymer/Aqueous Phase Ratio. The polymer/aqueous phase ratio is a key parameter in optimizing the operating conditions of the TPPB-SBR reactor. The amount of polymer employed, in fact, determines the pollutant concentration in the liquid phase and the residual amount in the polymer at the end of the work cycle. The first parameter has a direct effect on the process kinetics while the second is critical for long-term repeated utilization of the polymer.

SBR biodegradation tests were performed at polymer/aqueous phase ratios of 5, 7.5, and 10% and compared with

TABLE 1. Comparison of 4NP Specific Removal Rates for Single Phase, and Solid Liquid TPPB Operated with Polymer/Aqueous Phase Ratio 5%

test	configuration	feed concentration (mg/L 4NP)	4NP specific removal rate (mg 4NP/(gVSS-min)
T1a	one-phase	750	0.53
T1b	TPPB	750	0.86
T2a	one-phase	1000	N.D.
T2b	TPPB 1st test	1000	0.34
T2c	TPPB 2nd test	1000	0.50

the test in the conventional one-phase system undertaken at the same operating conditions. The results are reported in Figure 3.

The polymer reduces 4NP inhibition and this positive effect increases with an increased amount of polymer. In terms of reaction time there are no appreciable differences among the three polymer/aqueous phase ratios; this might be explained by considering that the higher polymer amounts result in lower 4NP concentrations in the solid phase with a consequent reduction of the solid−liquid concentration gradient and of the mass transfer rate between the two phases. In other words an increased amount of polymer with a high affinity for a poorly water-soluble target molecule could be the limiting component of the mass transfer step on the overall process rate. On the other hand the use of additional polymer provides a more gradual delivery of the pollutant to the micro-organisms with a more stable operating environment, less prone to process upsets during possible substrate surges. This is confirmed by Figure 4 where the SOUR is reported vs time.

The presence of polymers reduces the maximum oxygen demand relative to the single-phase case, and spreads the oxygen demand out more evenly over the operating cycle. This effect is also seen as the polymer/aqueous phase ratio is increased from 5% to 10%, and both effects (reducing maximum oxygen demand, and providing a more even demand) can be viewed as additional positive effects arising from TPPB operation.

The residual 4NP in the beads was evaluated for the three operating conditions; values equal to 1.19, 0.70, and 0.38 mg4NP/g polymer were obtained for 5%, 7.5%, and 10% polymer/aqueous phase ratio, respectively.

An almost linear decrease in the residual 4NP values was found as the polymer phase ratio was increased. As a consequence, higher polymer amounts are likely more appropriate for long-term repeated utilization without polymer regeneration, as substrate residuals remain lower. Moreover, low substrate levels in the solid phase will also allow the system to better handle substrate transient loadings because of the higher unutilized uptake capacity.

The lower residual value found for the test with 5% Hytrel (1.19) in this second series of tests in comparison with the value detected in the first series (1.70) can be explained by the longer period of operation of the system as a TPPB and by the higher 4NP feed concentration values utilized in the first case.

The residual 4NP in the solid phase was also evaluated in terms of the percent ratio between the total residual amount remaining in the polymer and the total 4NP fed during the period of operation. This calculation was repeated for the three periods, and for all three polymer ratios, and was found to be very low (≤1.5%).

Analysis of the previous results suggests that the best set of operating conditions is determined by a variety of factors that depend on the process characteristics and objectives. If the reactor is treating an influent with constant characteristics in terms of flow rate and concentrations, or if an equalization tank is available, the amount of polymer would primarily be determined by the desired reduction of the toxic effect associated with the influent load to have acceptable process kinetics. In the presence of anticipated load variations higher polymer amounts may be a better strategy. In the event of multiple target substrates, a mixture of rationally selected polymers can be used, as demonstrated for toxic cell products (*19*). Low-cost waste polymers (recycled automobile tires) are also an option for the sequestering phase in TPPBs (*20*).

Polymer Regeneration. On the basis of the results presented here, as well as our experience in the operation of solid−liquid TPPB systems for many weeks, once the optimal polymer fraction is determined, the TPPB reactor is potentially capable of operating indefinitely without polymer regeneration as long as the influent characteristics do not change. In industrial wastewater treatment situations this

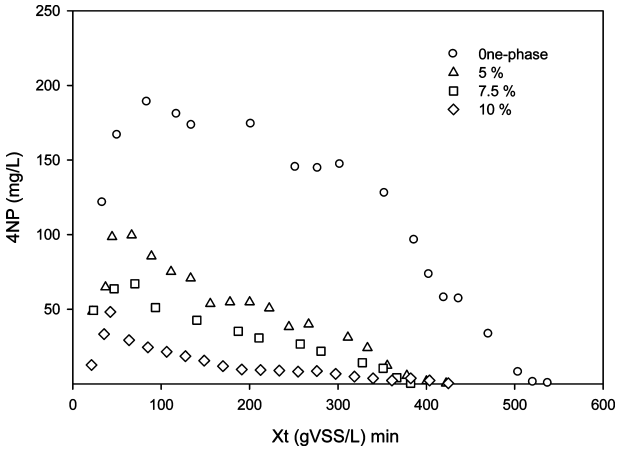

FIGURE 3. Biodegradation tests performed in the conventional and two-phase SBR system at different polymer/aqueous phase ratios. Feed concentration 400 mg/L and $X = 1300-1700$ mgVSS/L.

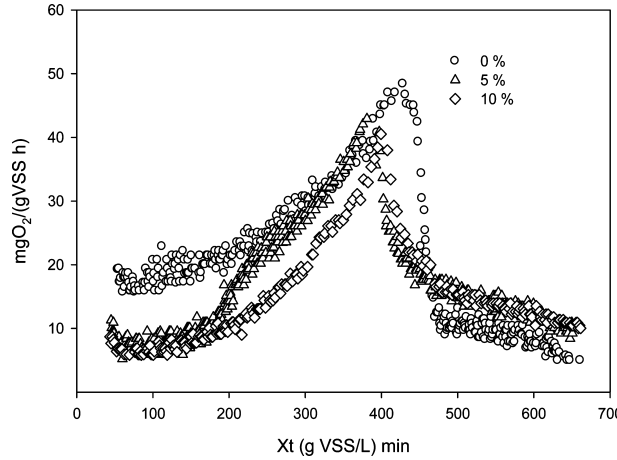

FIGURE 4. Specific oxygen uptake rate (SOUR) profiles observed in tests at different polymer/aqueous phase ratios.

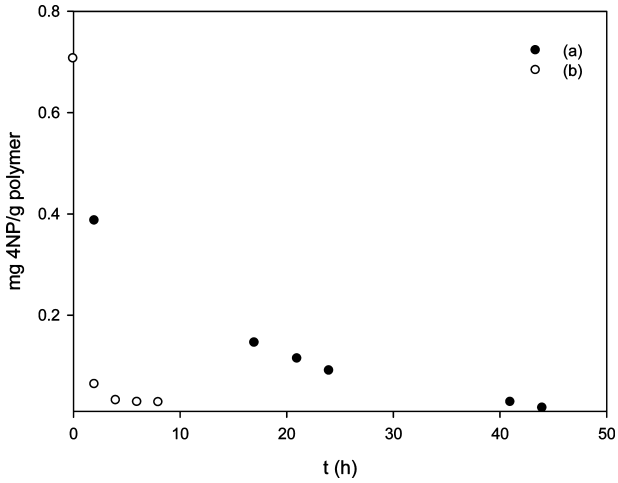

FIGURE 5. Profile of the residual amount of 4NP in the polymer vs contact time for polymer regeneration with biomass ("bioregeneration"). $X = 1700$ mgVSS/L. Polymer taken from the reactor working at 7.5% polymer/aqueous phase ratio; (a) bioregeneration; (b) bioregeneration with methanol cosolvent addition (0.5%v/v).

TABLE 2. Residual Amounts of 4NP in Polymers after Multiple Methanol Washings (mg 4NP/g Polymer)

washing step	Hytrel 5%	Hytrel 7.5%	Hytrel 10%
0	1.1900	0.7000	0.3800
1	0.8416	0.4985	0.2163
2	0.2053	0.1429	0.1114
3	0.0938	0.0439	0.0360
4	0.0274	0.0129	0.0111
5	0.0118	0.0089	0.0067
6	0.0088	0.000	0.000
7	0.0044		

condition is quite common, but in the presence of variable process conditions or modifications of the production cycle, different wastewater characteristics could require polymer regeneration for reuse.

Previously (*14*) we had shown that complete removal of residual 4NP from several different polymers is possible, and here we have refined this procedure by examining the number of methanol washes that are required to reduce 4NP residuals by 1 order of magnitude for the different Hytrel ratios employed in the SBR tests. In addition, although we had shown the potential of bioregeneration for residual 4NP removal (*14*), here we have also examined the effect of adding a small amount of methanol (0.5% v/v) as a cosolvent to improve the 4NP transfer from the polymer beads. Methanol was utilized because it is a readily biodegradable compound, extensively used in conventional wastewater treatment as a carbon source for denitrification (*21*), and was anticipated to be biocompatible with the biomass employed here. The polymer utilized in the bioregeneration test was the Hytrel of the 7.5% test shown in Figure 5.

Table 2 shows the results of the multistep methanol washing method while in Figure 5 the bioregeneration data for the two tested alternatives are reported. The data in Table 2 show that three methanol washing steps are enough to reduce the residual 4NP concentration by 1 order of magnitude to values suitable for polymer reuse.

Bioregeneration (Figure 5) requires 24 h of contact time to reach the same residual values. With methanol addition the process kinetics are significantly improved and only 4 h of contact time are required to completely remove the residual 4NP from the solid phase.

Both processes are suitable for application but bioregeneration, although slower, may be more suitable in wastewater treatment applications and more "environmentally friendly" because it can be performed by the same biomass operating in the process. The process could be sped up by increasing the biomass concentration, by the addition of a suitably inexpensive cosolvent or via physical enhancements such as sonication (*22*).

Having now comprehensively demonstrated the enhanced performance that is provided by the TPPB-SBR system for 4NP biodegradation, our current work is focused on the degradation of multiple substituted phenols, and the rational specification of mixtures of polymers to optimize substrate sequestration and delivery in TPPB-SBRs. In addition we are examining waste plastic/polymeric materials, such as automobile tires, for use in such systems.

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