Biodegradation of 4-Nitrophenol in a Two-Phase System Operating with Polymers as the Partitioning Phase

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The present study has demonstrated the enhanced performance of a two-phase bioreactor, operating with polymers as a partitioning phase, as an alternative to both single phase biotreatment and to the use of an immiscible organic solvent partitioning phase, to deliver a toxic substrate (4-nitrophenol, or 4NP) to a microbial consortium in batch and repeated batch mode. Three commercial polymers were tested, Hytrel, Tone, and Elvax, and were shown to have superior properties related to the use of a consortium, including complete biocompatibility with the biomass and nonbiodegradability. Repeated kinetic tests performed with short reaction times demonstrated the accumulation of 4NP within the polymers in the range of 6−8 mg/g polymer, which reduced polymer performance in subsequent batch operations. Hytrel gave the best performance with residuals of up to 4 mg /g polymer showing no reduction in subsequent use, while for the other polymers a 4NP value lower than 2 mg/g polymer was required to have acceptable performance during repeated polymer use. Polymer reuse without affecting the process efficiency was confirmed with regeneration tests. A conventional methanol extraction method, as well as biological regeneration of the polymers by prolonged contact with the biomass, were assessed for their ability to remove the residual 4NP. Parallel kinetic tests performed with new and regenerated polymers showed a complete overlap of the 4NP concentration profiles indicating that a simple biological regeneration method provides a means of completely restoring polymer performance for repeated batch operation.

Introduction

As an alternative to physical and chemical treatment of organic pollutants, biological processes offer the advantages of operating at ambient temperatures and pressures, with reduced capital and operating costs. A serious limitation of biotreatment systems, however, is the potential toxicity of xenobiotic organic pollutant(s). In practice, this means that great care must be taken in the organic loading of biotreatment systems which, if overly aggressive, could lead to significant lags and even to complete process upsets during transient loadings as the microbes are inhibited or even killed by toxic effects.

A simple means of dealing with high substrate loadings, or transient organic surges, is via the two phase partitioning bioreactor (TPPB) concept. In this process configuration a cell-containing aqueous phase is contacted with an immiscible organic phase in which either high concentrations of substrate can be dissolved (delivering suitably low concentrations to the aqueous phase), or which acts to sequester high substrate loadings from the cells during transient conditions. Cells are therefore protected from toxic substrate levels, and partition more substrate from the immiscible phase based on their metabolic requirements. A TPPB schematic is reported in ref *1*. Although immiscible organic solvents have been demonstrated to function effectively in this manner (*2, 3*), they have generally been restricted to the use of pure microbial strains, or limited microbial consortia, due to the concern that the organic phase would also act as a competing substrate. Even under these conditions, however, effective treatment of contaminants has been achieved (*4, 5*).

Recently, it has been demonstrated that inert and inexpensive polymers can be used, in the same manner as immiscible organic solvents, to partition toxic organic substrates to/away from cells in a TPPB (*6, 7*). The advantages of using polymers, beyond low cost and wide availability, include complete biocompatibility and nonbioavailability, which permit the use of widely mixed consortia. The use of TPPBs in sequencing batch reactors (SBRs) has added another dimension to process operation, as the SBR, characterized by significant flexibility in operating conditions (readily achieved by varying the times of the operating cycle), is an effective means of generating a versatile microbial culture able to develop metabolic pathways for xenobiotic degradation (*8, 9*). Notwithstanding these advantages, and the potential of combining the solid−liquid TPPB concept with SBR operational flexibility, concerns have emerged about the relatively low diffusivity of large organic molecules in polymers, and the constraints that this may pose on the rate/extent of release of target molecules from polymers for biodegradation (*10*).

In this work, we demonstrate, for the first time, the ability of three selected polymers to act as the sequestering/delivery phase for 4-nitrophenol (4NP), in a solid−liquid TPPB operating in discontinuous mode. This organic contaminant was selected because of its environmental risks (*11, 12*) and toxicity to cells when present in concentrations exceeding about 500 mg/L. Earlier (*5*) we had described the enhanced performance of a liquid−liquid TPPB system in which the rate of 4NP removal had been greatly enhanced over single phase operation, but in which there was evidence of solvent uptake by the microbial consortium. In addition, in this work, we have examined the retention by the polymers of increasing amounts of 4NP after numerous repeated cycles that diminished process performance, and have also devised an easy way to regenerate the polymer for extended and multiple reuse.

Material and Methods

Bacterial Culture. The culture utilized in the batch experiments originates from the aeration basin of an urban wastewater treatment plant in Rome and was acclimatized

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to 4NP. It was grown in a conventional 5 L glass SBR at 20 ± 0.5 °C. Dissolved oxygen was controlled in the range of 3−4 mg/L by on -off control. The feed consisted of 4NP and MSV mineral salts medium (*13*), whose composition prepared in deionized water, was, in mg/L: $(NH_4)SO_4$ 500, $MgSO_4 \cdot 7H_2O$ 100, $CaCl_2 \cdot 2H_2O$ 50, K_2HPO_4 110, KH_2PO_4 85, $FeCl_3 \cdot 6H_2O$ 2, NaEDTA 2. The C:N:P ratio in the influent was 100:5:1 with respect to the 4NP carbon. More details on the operating conditions are reported in (*14*).

Chemicals. Fluka (Italy) was the supplier for 4-nitrophenol (purity >98%). The polyether-ester copolymer, Hytrel 8206, (DuPont Canada) is in the form of oval shaped beads (5 mm length, 1.5 mm diameter) with density of 1.17 g/cm^3 and melting point of 189 °C. The polycaprolactone polyester, Tone P787 (Dow Chemical Canada Inc.), is in the form of roughly spherical beads (~4 mm diameter) with density 1.145 g/cm^3 and melting point 60 °C. The Elvax 40W (DuPont Canada) polymer is a polyethylene-vinyl acetate copolymer with density 0.967 g/cm^3 and melting point 47 °C.

Analysis. Volatile suspended solids concentrations were determined according to Standard Methods (*15*). Analysis of 4-nitrophenol in kinetic tests was performed on samples filtered on syringe nylon membrane filters (0.45 μm pore-size) acidified in order to stop 4NP biodegradation by the residual biomass not retained on the filter. They were then analyzed via UV absorbance at 320 nm using a spectrophotometer Varian (model Cary 1). Interference of other compounds in the aqueous matrix was excluded by preliminary tests. The same procedure was followed in the partition tests. For the adsorption curves 4NP was measured with a spectrophotometer (Perkin-Elmer Lambda 20) equipped with continuous flux cuvettes.

Test Plan and Operating Procedures. An overview of the test plan and procedures is reported in the following. All the tests were performed with tap water in order to have a realistic matrix to simulate wastewater systems.

Partition Coefficients. Batch partition coefficients tests were performed at 25 °C ± 0.5 with mixing provided by magnetic stirrers. The working volume was 30 mL and the polymer amount was varied from 0.5 to 4 g. The initial 4NP concentration was 60 mg/L and the final concentration was measured after 60 h, a time sufficiently long to ensure that equilibrium was reached. Partition coefficients were estimated as the ratio of 4NP concentration in the polymer (mg/L), divided by the residual concentration of 4NP in the aqueous phase (mg/L), at equilibrium.

Absorption Tests. Absorption tests, to estimate the rate of 4NP uptake, were carried out in a continuous reactor (volume = 50 mL) connected to a continuous flux cuvette for on line measurement. The reactor was controlled at T = 25 °C ± 0.5, and complete mixing was ensured by the continuous flow. The measurement was stopped when equilibrium conditions were reached, that is, when a constant concentration in the liquid phase was detected.

Batch Kinetic Tests. Batch kinetic tests were conducted using the biomass from the SBR reactor and were performed in parallel in single and two phase systems with the three polymers. The temperature was controlled at 25 ± 0.5 °C, while 4NP and biomass concentrations were in the range of 400−500 mg/L and 2000−3000 mgVSS/L, respectively. The liquid volume was 200 mL. In the tests, the 4NP solution was added to the polymer (10 g, polymer/solution ratio ~5%) and kept in contact for 24 h under mixing conditions. The biomass was then added and 4NP concentration was measured at time intervals of ~5−15 min until a 4NP concentration value ≤1 mg/L was detected.

Repeated Batch Kinetic Tests. To determine the behavior of the polymers subjected to repeated operating cycles a series of repeated kinetic tests was performed as described above but without changing the polymer (i.e., the polymer was reused a number of times). After each test, 1 g of polymer was withdrawn and the residual 4NP absorbed in the polymer was extracted by a five-step washing with methanol. In the subsequent test the liquid volume was proportionally reduced in order to keep a fixed polymer/solution ratio of 5%. This procedure allowed tracking the 4NP accumulation in the polymer phase. The reaction time was the same in all tests and was equal to the time necessary to complete the 4NP removal with the new polymers in the first test.

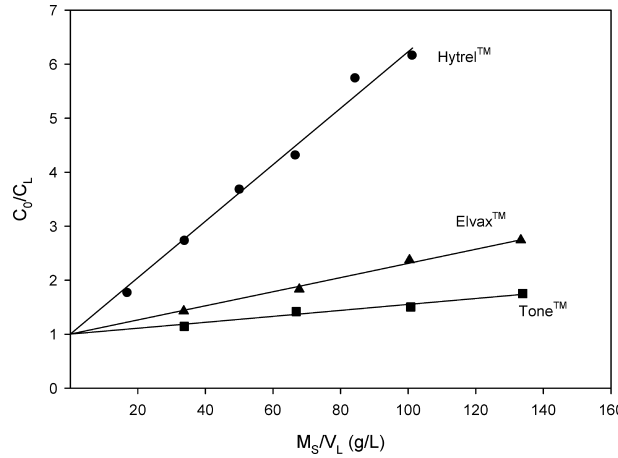

FIGURE 1. Partition coefficients for the three polymers.

TABLE 1. Partition Coefficients for the Investigated Polymers

polymer	P	correlation coefficient	standard error
Hytrel	61.19	0.994	1.37
Tone	6.25	0.982	0.30
Elvax	11.82	0.997	0.23

Bioregeneration Tests. The possibility of polymer regeneration by prolonged contact with the biomass (X ~3000 mg VSS/L) was investigated with the polymers that had undergone the repeated kinetic tests; these were the worst conditions because the beads contained the highest level of residual 4NP. The polymers were contacted with the biomass under aerated conditions, and at prefixed time intervals 0.5 g of polymers were withdrawn and the residual 4NP was extracted by a multi step washing with methanol. The procedure was repeated until a negligible amount of 4NP was detected in the final washing step.

Results and Discussion

Partition Coefficients. The 4NP mass balance in the batch partition coefficient tests is expressed by

$$V_L C_0 = V_L C_L + (M_S/\rho) P C_L \quad (1)$$

where V_L is the liquid volume, C_0 and C_L are the concentrations in the liquid phase at time 0 (before polymer addition) and t (when equilibrium is reached) respectively, M_S is the polymer mass, ρ the polymer density, and P the partition coefficient.

Equation 1 can be expressed in a linear form as

$$\frac{C_0}{C_L} = 1 + \frac{M_S}{V_L}\frac{P}{\rho} \quad (2)$$

To provide a visual representation, partition data, according to eq 2, are reported in Figure 1 as C_0/C_L vs M_S/V_L. The obtained partition coefficients are reported in Table 1.

Absorption Tests. In addition to the partition tests, absorption tests were performed to study the uptake kinetics and determine whether the absorption/desorption rates of

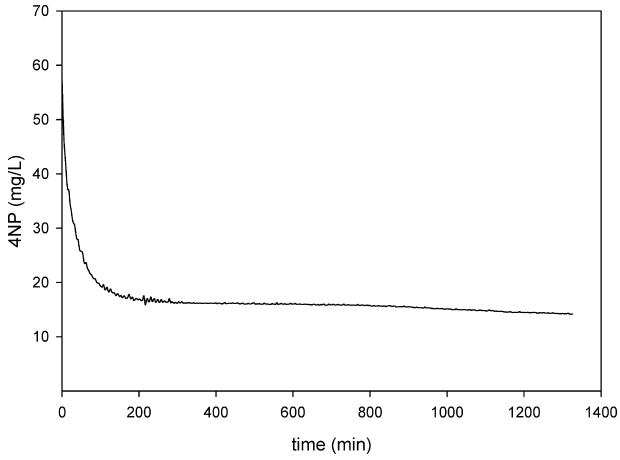

FIGURE 2. 4NP concentration profile in an absorption kinetic test with Hytrel (polymer 10.5 g, 4NPconcentration 58 mg/L).

4NP are high enough to not limit degradation rates. Figure 2 shows the concentration profile determined in the absorption kinetic test with Hytrel; a rapid 4NP uptake was observed in the first 200 min. Similar results have been obtained with Tone and Elvax but, as expected by the partition coefficients, they are characterized by a lower absorption capacity. The rate of uptake/release by the polymers relative to the microbial degradation rate is an important issue that will determine the overall process kinetics, and current work in our group is directed toward modeling the individual mass transfer steps, and overall process dynamics.

The rapid 4NP uptake observed with Hytrel may be advantageously exploited in operating conditions when a rapid detoxification, i.e., transient phases or unexpected variation of influent load, may occur. Under these circumstances, it might be expected that the polymer could act as a "sponge" and rapidly reduce the toxic effects and minimize the decrease of biomass activity. Moreover, correlation of 4NP absorption data on Hytrel, along with the known partition coefficient, gave a diffusivity of 6.5×10^{-6} cm^2/s, close to the value reported in ref *16* for the diffusivity of phenol in Hytrel.

These preliminary tests suggest that the absorption kinetics for the three polymers are appropriate (i.e., rapid enough) for application in the two phase system, however the characteristic absorption/desorption times, depending on the operating conditions, could be of the same order as the reaction times so their effect on overall process kinetics need to be taken into account.

Batch Kinetic Tests. The polymers were then utilized in a series of batch kinetic tests conducted in parallel with a control single phase system, at the same biomass concentration, to compare 4NP removal. Typical concentration profiles are reported in Figures 3 and 4.

In the two phase system, even at initial concentrations up to four times the maximum concentration provided in the conventional single phase reactor, the biomass was exposed to 4NP concentrations that are significantly lower during the entire course of the experiment. As expected from the partition coefficient data, the best results were obtained with Hytrel which, even at the highest initial concentration, rapidly reduced the substrate concentration in the aqueous phase to subinhibitory values (≤60 mg/L). This is certainly an advantage when the system operates with high concentrations of xenobiotics and was obtained with a very small amount of polymer (~5% of the liquid volume). In fact in the first test at 440 mg/L the reaction time required for complete removal in the liquid phase was reduced by 25%, 40% and 65% with Tone, Elvax, and Hytrel, respectively. The positive effect was even more evident at a 4NP concentration of 500 mg/L; in the single phase system the strong inhibitory effect resulting from the higher substrate/biomass ratio caused a low 4NP degradation efficiency (about 50%) while complete 4NP removal from the liquid phase was obtained in both of the two phase systems with reaction times of ~180 min for Hytrel and ~350 min for Elvax and Tone.

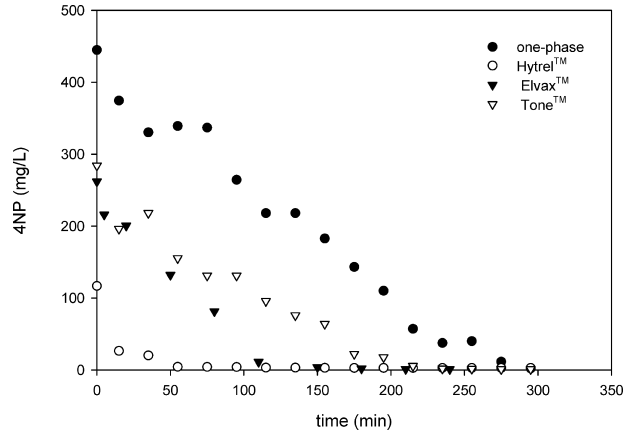

FIGURE 3. 4NP concentration profiles in one and two phase systems vs time in the kinetic test at initial concentration of 440 mg/L, $X = 2300$ mgVSS/L.

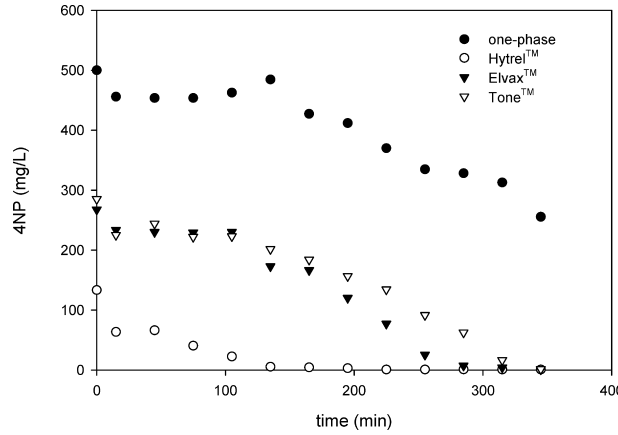

FIGURE 4. 4NP concentration profiles in one and two phase systems vs time in the kinetic test at initial concentration of 500 mg/L, $X = 2700$ mg VSS/L.

TABLE 2. Comparison of 4NP Specific Removal Rates[a] for Single Phase, Liquid−liquid and Solid Liquid[b] TPPB Configurations

configuration	4NP specific removal rate mg 4NP/ (gVSS-min)	references
one-phase	0.60	this study
undecanone	1.01	*5*
Hytrel	1.80	this study
Tone	0.80	this study
Elvax	0.90	this study

[a] Evaluated at 4NP initial concentration of 440 mg/L. [b] For the solid−liquid system, the 4NP fraction retained has already been accounted for in the solid phase at the end of the test.

A comparison of specific removal rates with previous data for 4NP biodegradation performed in a liquid−liquid TPPB with 2-undecanone as the immiscible liquid solvent (*5*) is reported in Table 2. The best results are obtained with Hytrel, whereas the performance of the systems with Tone and Elvax is comparable to the liquid−liquid system which

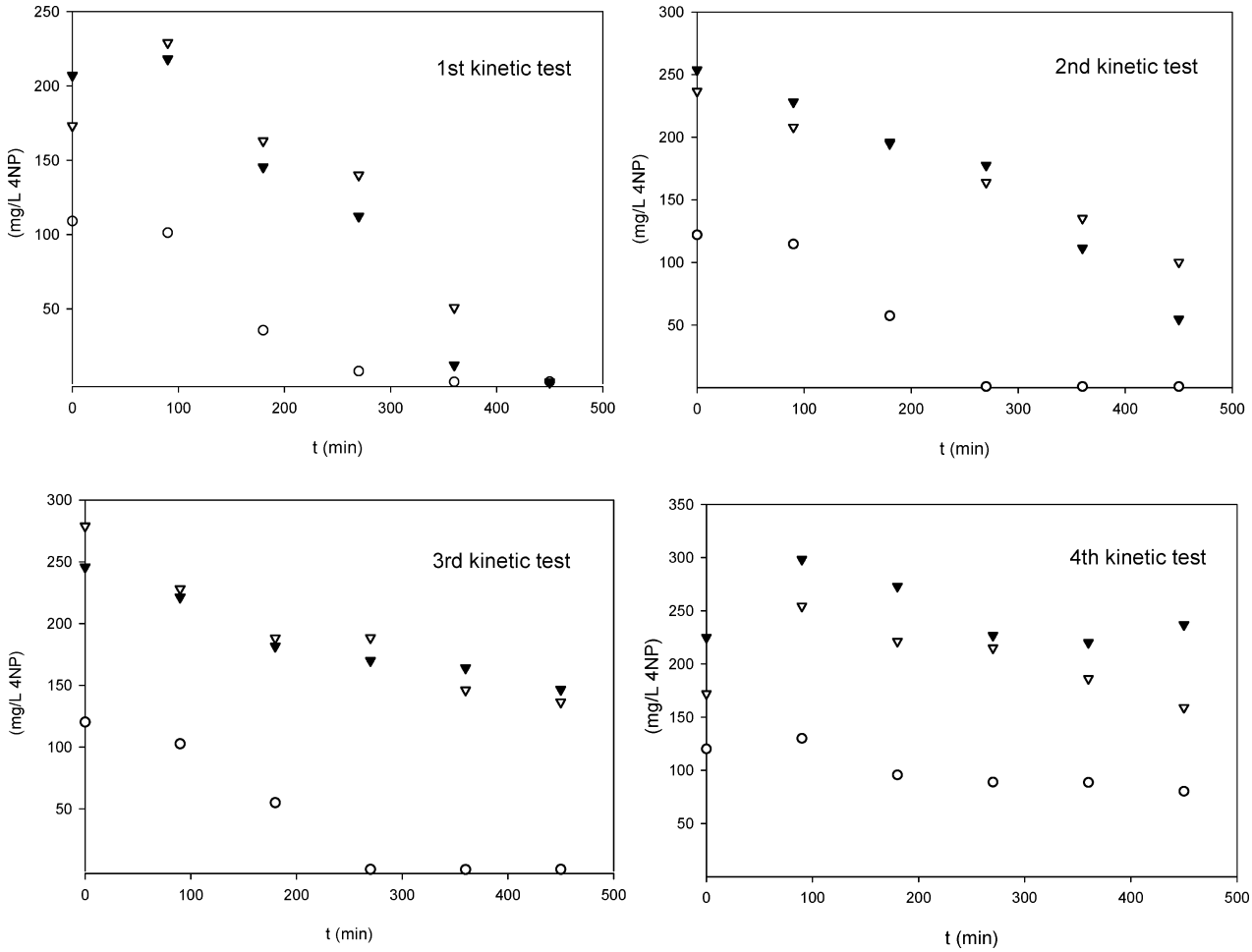

FIGURE 5. 4NPconcentration profiles in the first to fourth repeated kinetic tests, $X = 2500-2800$ mg VSS/L. (○) = Hytrel, (▼) = Elvax, (▽) = Tone.

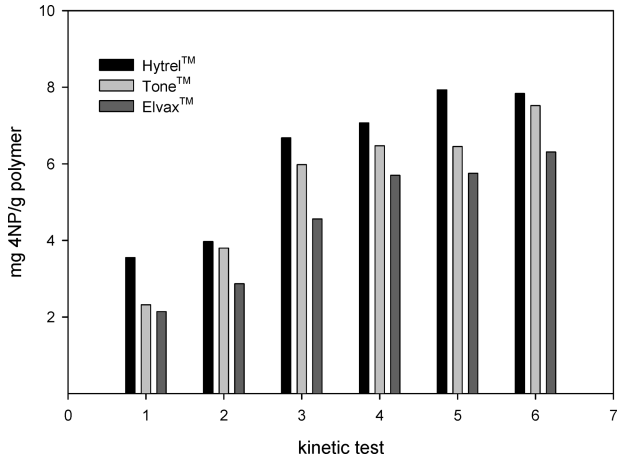

FIGURE 6. 4NP retained in the polymers after the various kinetic tests.

utilized undecanone as the partitioning phase. The results clearly confirm that the two-phase systems are very effective in reducing the inhibitory and/or toxic effects on the biomass thus improving process performance.

Repeated Uptake/Release Tests. To more completely characterize the polymer performance in the uptake and release of 4NP, it is necessary to examine the behavior of the solid phase in terms of residual substrate retained at the end of the kinetic tests. During biodegradation the substrate concentration in the liquid phase is progressively reduced with a consequent reduction of the biodegradation rate. At the same time the absorbed amount also decreases, causing a reduction of the driving force resulting in a lengthy release of residual contaminant. In our experiments we detected a residual fraction of 4NP in the beads depending on the bioreactor operating conditions, in particular on the length of the contact time with the biomass. In order to investigate this aspect and the retention of 4NP by the polymer (affecting transport driving force) repeated uptake tests were conducted.

Figures 5 and 6 show the 4NP concentration profiles during the first four repeated tests and the 4NP amounts retained in the solid phase after each kinetic test. The fifth and sixth kinetic tests are not reported as they were not significantly different from the fourth test. For comparison, the tests were performed with the same contact time as had been used for complete 4NP removal in the liquid phase in the first kinetic test for all the polymers. This is the worst condition because there is no time for the biomass to degrade any nondesorbed 4NP.

TABLE 3. 4NP Residual Amounts in the Polymers As Function of Contact Time

contact time (h)	Hytrel (mg/g pol)	Tone (mg/g pol)	Elvax (mg/g pol)
0	7.84	7.52	6.5
24	1.26	3.94	3.65
72	0.21	3.05	2.24
120	0.17	2.41	1.46
168		1.81	0.69
216		0.29	

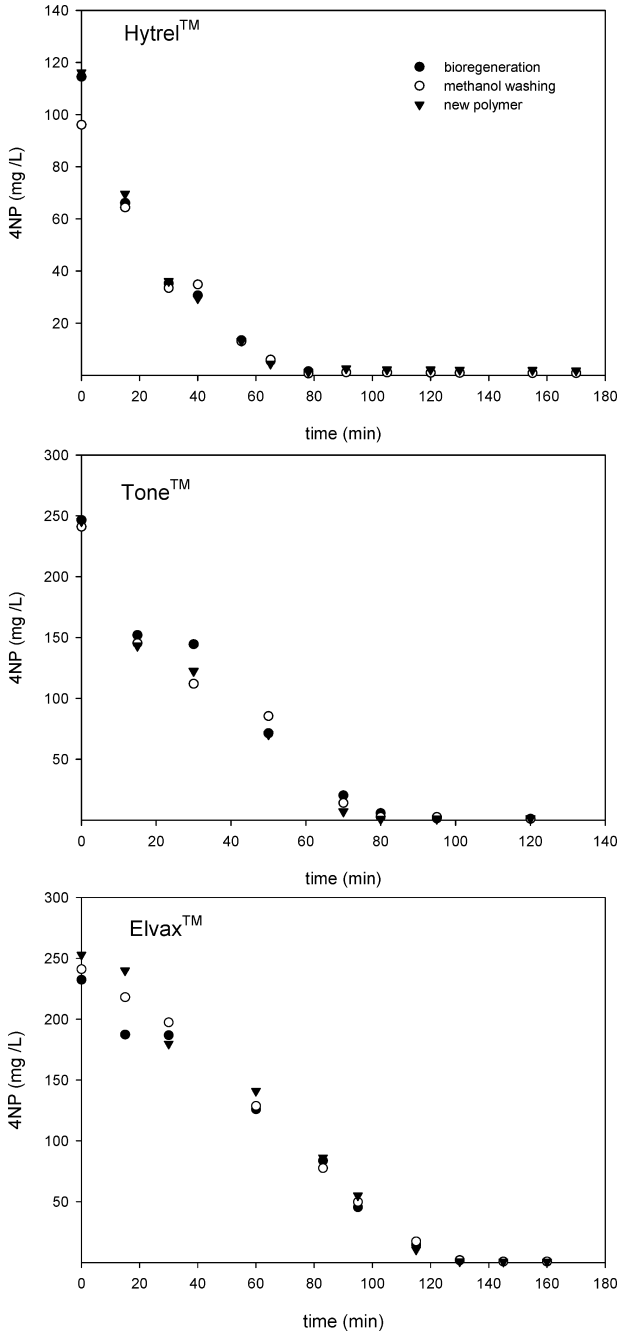

FIGURE 7. Concentration profiles in the kinetic tests with new and regenerated polymer (X = 3000 mgVSS/L, polymer/solution ratio = 5%).

As seen in Figure 5 the best performance is again observed with Hytrel which is able to function efficiently for three repeated kinetics, whereas the other two polymers showed a loss of efficiency beginning in the second kinetic test. With regard to the retained 4NP in the solid phase (Figure 6) a progressive increase is observed, with the saturation value for all the polymers being in the range of 6−8 mg/g polymer. It is important to note that Hytrel is characterized by the highest amount of 4NP absorbed for all the kinetic tests but is also able to provide good performance even up to 4 mg/g polymer of retained 4NP. For the other two polymers, a value lower than 2 mg/g polymer is required to have acceptable performance. These results were confirmed by long-term (∼150 work cycles) utilization of Hytrel in a lab scale TPPB−SBR (data not shown) without loss of efficiency. After this long operation time the residual 4NP in the beads was 1.7 mg4NP/g, significantly lower than the amount evaluated with the current uptake test. In the case of the multiple cycle runs the reaction time (about 8 h) was long enough to obtain 4NP removal in the liquid phase and to maintain a low residual amount of 4NP in the polymer. This result is significant in terms of application because it demonstrates that the sequential system can operate without polymer regeneration for long periods of time but that the reaction phase duration must be optimized to achieve sufficient removal of absorbed 4NP in each cycle to obtain acceptable performance for the next operating cycle.

Bioregeneration Tests. The last important issue to be considered for practical application of these systems is polymer regeneration for reuse. In these tests polymer regeneration with biomass was compared to the solvent extraction method. This latter method was effective but is not as attractive in terms of environmental impact since the solvent must be treated or disposed of. A more sustainable possibility is biological regeneration by prolonged contact time with the biomass, as in this way it would be possible to complete the 4NP degradation process during the regeneration cycle with the same biomass. It is also worth noting that biological regeneration would not subject the polymer to chemical or thermal stress that may alter its structure.

Methanol was used in a multistep extraction procedure, and five washings were required to completely remove the 4NP from the polymer. Bioregeneration was performed in batch tests with the same biomass concentration kept in contact with the polymer for different times. In Table 3 the residual amounts of 4NP in the polymers are reported as a function of contact time.

The main observation from Table 3 is that the biomass can be utilized to regenerate the polymers and, depending on the polymer structure, different times are required: for Hytrel 24 h are sufficient to reach residual values that permit its reuse, whereas higher times are necessary for Elvax and Tone. Moreover, it is important to stress that the contact time data are specific for the adopted experimental conditions and can be optimized by varying operating parameters (i.e., biomass concentration, degree of mixing).

The last validation check for reuse was performed by parallel batch kinetic tests conducted under the same operating conditions with new polymer, polymer regenerated with methanol and the bioregenerated polymer. The results shown in Figure 7 for Hytrel, Tone, and Elvax demonstrate a complete overlap of the three curves for all the polymers, and it can therefore be concluded that the two regeneration methods result in similar polymer performance and confirm the possibility of polymer reuse following bioregeneration.

In terms of environmental impact the "bioregeneration" alternative is certainly advantageous in that it allows complete biodegradation of the residual fraction of the compound retained in the polymers without the production of additional streams to be treated or disposed.

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