

SOLID-LIQUID TWO PHASE PARTITIONING BIOREACTORS AS A TOOL FOR XENOBIOTIC BIODEGRADATION: CASE STUDY OF 4-NITROPHENOL

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In this paper the performance of two phase liquid-solid systems applied to the removal and degradation of xenobiotics was investigated. 4-nitrophenol, a typical representative of substituted phenols, was chosen as the target compound. A polyether-ester copolymer Hytrel 8206, was employed as the partitioning phase in batch tests and in a lab scale sequencing batch reactor. In the two phase reactor even if operated with a very low polymer content (~ 5%), the biomass was exposed to 4-nitrophenol concentrations that are significantly lower if compared to the one-phase aqueous system with consequent drastic reduction of the toxic effect of 4-nitrophenol, and of the reaction times. A process model was also formulated and applied to analyze the performance of the system during different operating conditions. Simulation studies were focused on the fate of the xenobiotic in the solid phase and on the effect of the diffusion time on the entire process kinetics. It was demonstrated the effect of the diffusion time on the overall process kinetics and the potential polymer "bioregeneration" by prolonged contact with the biomass is significant.

1. INTRODUCTION

Biological processes are attractive as "green" remediation strategies for xenobiotic removal from aqueous environments but often possess a major limitation due to substrate toxicity that can significantly reduce process efficiency and the applicable substrate load. In order to overcome this limitation an extremely promising technology based on the use of two phase partitioning bioreactors (TPPBs) has been proposed: the basic principle is to partition the toxic substrate between the aqueous phase containing the micro-organisms and an organic phase which has typically been comprised of an immiscible organic solvent. This configuration allows control of substrate delivery (from the solvent to the water phase) that is determined by the degradation kinetics and the maintenance of thermodynamic equilibrium (Daugulis, 2001, Malinowski, 2001). This approach is suitable for pure cultures (Janikowski *et al.*, 2002; Munoz *et al.*, 2006; Rehmann and Daugulis, 2006). but when mixed cultures are employed (i.e. in industrial wastewater treatment) a reduced efficiency can result arising from the parallel biodegradation of the solvent. Solid polymers beads have recently been proposed as an alternative partitioning phase, having been extensively investigated for the biodegradation of phenol, and have been shown to be an effective alternative to liquid organic solvents in TPPBs when mixed cultures are used (Amsden *et al.*, 2003; Pripch *et al.*, 2006; Tomei *et al.*, 2008). Solid polymer beads have shown partition capabilities similar to those of immiscible liquid solvents but, at the same time, have the significant advantage of being biocompatible with the biomass and non biodegradable, as well as being inexpensive. These characteristics allow operation with mixed cultures without altering the biomass composition. The performance of TPPB bioreactors can be enhanced if the reactor is operated in a sequential mode. In fact, the Sequencing Batch Reactor (SBR), characterised by a large variety of operating conditions (easily obtainable by varying the times of the operating

cycle) and high operating flexibility, is a suitable technological solution in order to obtain a versatile micro-organism culture able to develop metabolic pathways required in the degradation of xenobiotics (Ellis *et al.*, 1996). The advantages of combining the two phase TPPB process with SBR technology is therefore a promising strategy when xenobiotic removal has to be undertaken in particularly critical conditions i.e. very high substrate concentrations.

Substituted phenols are present in industrial effluents originating from many different sources and are major constituents of wastewater from coal conversion processes, coke ovens, petroleum refineries and petrochemical industries, resin and fiberglass manufacturing and herbicide production. Concentrations detected in these effluents are quite high ranging from 10 to $17 \cdot 10^3$ mg/l while the related biodegradable COD fraction varies from 40 to 80% of the total COD (Tsai *et al.*, 1982; Luthy *et al.*, 1983; Suidan *et al.*, 1983;). These numbers provide an idea of the enormous impact of this class of compounds on water pollution. Moreover, because of their toxicity to humans and aquatic life (1mg/l is enough to detect the effects) they are included in the USEPA list of priority pollutants. The objective of this paper, using experimental as well as modeling methods, is to evaluate the potential of the two phase liquid-solid systems applied for the removal of a target compound, 4-nitrophenol (4NP), a typical representative of substituted phenols found in many industrial effluents (manufacture of explosives, drugs, dyes, phosphororganic insecticides, pesticides, leather coloring) (Trapido and Kallas, 2000). The biomass was a mixed culture operating as a conventional Sequencing Batch Reactor (SBR) and acclimatized to 4NP as the sole carbon source. On the base of literature data and previous experiments a polyether-ester copolymer Hytrel 8206 (DuPont) was employed in the liquid-solid system.

2. MATERIAL AND METHODS

2.1 Chemicals

The target compound 4-nitrophenol was in granular form (purity > 98%) and was supplied by Fluka (Italy). 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 1.17 g/cm^3 and melting point $189 \text{ }^\circ\text{C}$.

2.2 Bacterial culture

A mixed culture previously acclimatized to 4NP as the sole carbon source was used in the experiments. The original biomass inoculum was a mixed liquor sample from a full scale urban wastewater treatment plant; the details of the acclimatization procedure are reported in previous papers (Tomei *et al.*, 2003; Tomei and Annesini, 2005). To ensure the presence of required nutrients and microelements, in all cases the aqueous matrix consisted of a 4NP solution with the addition of the mineral medium MSV (Williams and Unz, 1989). The amount of added mineral medium was determined to ensure a C:N:P ratio in the influent equal to 100:5:1 with respect to the 4NP carbon. The culture utilized in the kinetic experiments was grown in a conventional Sequencing Batch Reactor described in the following paragraph.

2.3 Sequencing Batch Reactor

The reactor is a glass vessel of 5 litres equipped with a thermostatically controlled water jacket to maintain the operating temperature at $20 \pm 0.5 \text{ }^\circ\text{C}$. Dissolved oxygen (DO) was controlled in the range of 3-4 mg/l by an on-off control strategy. A typical SBR operating cycle lasted 12 hours distributed as follows: FILL 30 min, REACTION 570 min, WASTAGE 3 min, SETTLE 92 min, DRAW 25 min. The fill phase operated under mixed and aerated conditions. The exchange factor (added volume/total volume) was 0.5. More details on the operating conditions of the SBR are reported in Tomei *et al.* (2008).

2.4 Kinetic tests

In order to compare the performance of the two removal processes, kinetic tests were carried out in single and two phase systems both in batch mode and in the SBR reactor.

Batch kinetic tests were carried out using the biomass from the SBR reactor; Temperature was controlled at $20 \pm 0.5^\circ\text{C}$, while 4NP and biomass concentration were in the range of 400-500 mg/l and 2000-2700 mgVSS/l respectively. The water phase volume was 100 ml. Before the biomass addition the 4NP solution was kept in contact with the polymer (10 g) for 24 hours. In all tests the 4NP concentration during the reaction phase was measured at fixed time intervals of ~10-15 min until a 4NP concentration value ≤ 1 mg/l was detected. VSS were measured at longer time intervals of 20-30 min.

Kinetic tests in the SBR reactor were carried out during the work cycle before and after Hytrel addition. Polymer solution ratio in the TPPB SBR was 5%. Liquid samples were regularly taken and analysed during the fill and reaction phases with the same modalities of the batch tests.

2.5 Analysis

Volatile Suspended Solids concentrations have been determined according to Standard Methods (APHA, 1998). 4-nitrophenol analysis in kinetic tests was performed on samples acidified and filtered on syringe nylon membrane filters (0.45 μm pore-size) by measuring the UV absorbance at 320 nm using a spectrophotometer Varian (model Cary 1).

3. MODELING

The efficiency and applicability of a two phase solid liquid system to xenobiotic biodegradation are strongly dependent on the interaction between the coupled phenomena simultaneously occurring: partitioning and diffusion of the compound inside the solid particles and biodegradation in the liquid phase. The overall process complexity is also increased by the presence of the inhibitory "concentration effect" that characterizes these substrates.

In this context, representative process models could be useful tools to predict the interactions between the physico-chemical and biological processes, to identify the controlling phenomena and to optimize the operating conditions.

In the following a basic model of the two phase solid liquid system is reported.

The first step in modeling is the coupling between the substrate balance in the aqueous and in the polymer phase with the biodegradation reaction rate. The substrate balance equation in the water phase is given by the following equation where the last term on the right hand side accounts for substrate sorption by the polymer beads:

$$V_w \frac{d(C_w)}{dt} = -V_w r_s - S_p D \left. \frac{\partial C_p}{\partial r} \right|_{r=R} \quad (1)$$

where V_w is aqueous phase volume; C_w and C_p are the substrate concentrations in the aqueous and polymer phase, respectively; S_p the polymer surface; R the polymer bead radius; D the substrate diffusivity in the polymer phase, r the radial coordinate and r_s the substrate degradation rate.

For xenobiotic compounds, exerting an inhibitory effect at high concentrations, the substrate degradation rate, r_s , is usually represented by the Haldane equation:

$$r_s = -k^* X \frac{C_w}{C_w + K_s + \frac{C_w^2}{K_I}} \quad (2)$$

where K_s is the saturation constant; K_I is the inhibition constant; k^* a kinetic parameter and X the biomass concentration.

As for the substrate mass balance in the polymer phase, a radial distribution of the substrate concentration and unsteady diffusion inside the polymer beads are considered

$$\frac{\partial C_p}{\partial t} = \frac{D}{r^2} \frac{\partial}{\partial r} \left(r^2 \frac{\partial C_p}{\partial r} \right) \quad (3)$$

with the boundary conditions:

$$r = 0 \quad \frac{\partial C_p}{\partial r} = 0 \quad r = R \quad C_p = P \cdot C_w \quad (4)$$

where P is the partition coefficient. It is worth noting that in (4), mass transfer resistance in the external liquid phase is neglected and equilibrium conditions at the bead surface are assumed.

Finally, obvious initial conditions, at $t=0$ $C_w=0$ and $C_p=0$ are utilized.

4. RESULTS AND DISCUSSION

Kinetic tests were performed with the polymer Hytrel 8206: among the polymer tested in a preliminary screening (Tomei *et al.*, 2009) this polymer was chosen since it showed the higher partition coefficient for 4NP. In Figures 1 and 2 the 4NP concentration profiles of the kinetic tests, performed in a batch reactor at initial concentrations of 430 and 500 mg/l of 4NP, are reported. In the tests with Hytrel the 4NP containing solution was first contacted with the polymer, in order to reduce the substrate concentration. After this sorption phase, the biomass was added to the batch reactor. It was observed that in the two phase batch systems the biomass was exposed to much lower maximum concentrations of substrate during the entire course of the experiments. This is certainly an advantage when the system operates with high concentrations of xenobiotics and the reduced substrate levels were obtained with a low polymer amount (~ 10% of the liquid volume).

In light of the favorable performance observed in batch tests with Hytrel, this polymer was also utilized in preliminary tests in the SBR reactor (figure 3) performed at a lower polymer /solution ratio (~ 5%). The SBR reactor was first operated in conventional one phase mode then the polymer was added to the reactor. Results of figure 3 confirmed the significant potential of polymer use for application to xenobiotic removal. Considering the very low 4NP concentrations obtained in the liquid phase when Hytrel is used, it is anticipated that the system could also utilize more concentrated streams without toxic effects on the biomass thus allowing better performances and reduced reactor volumes in comparison with the conventional one-phase configuration.

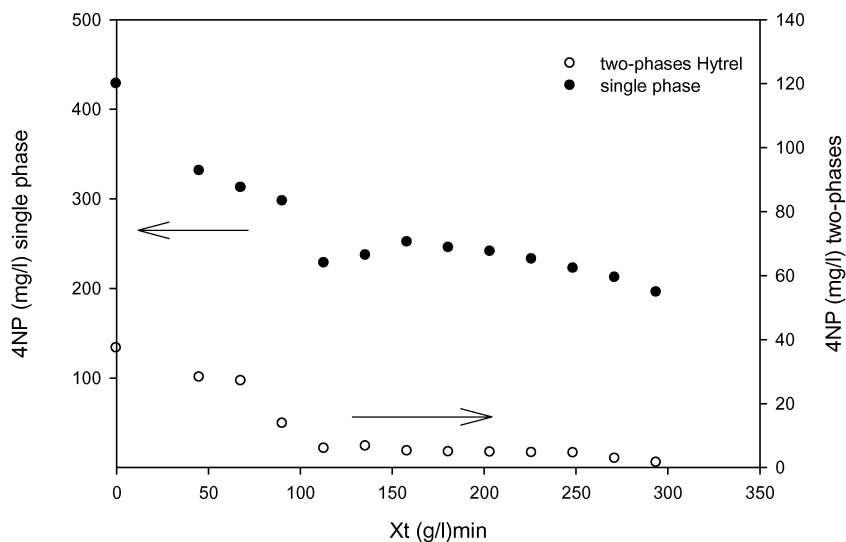


Figure 1. Concentration profiles detected in batch kinetic tests. 4NP initial concentration in the one-phase system 430 mg/l; polymer content 10%, $X = 2260$ mgVSS/l.

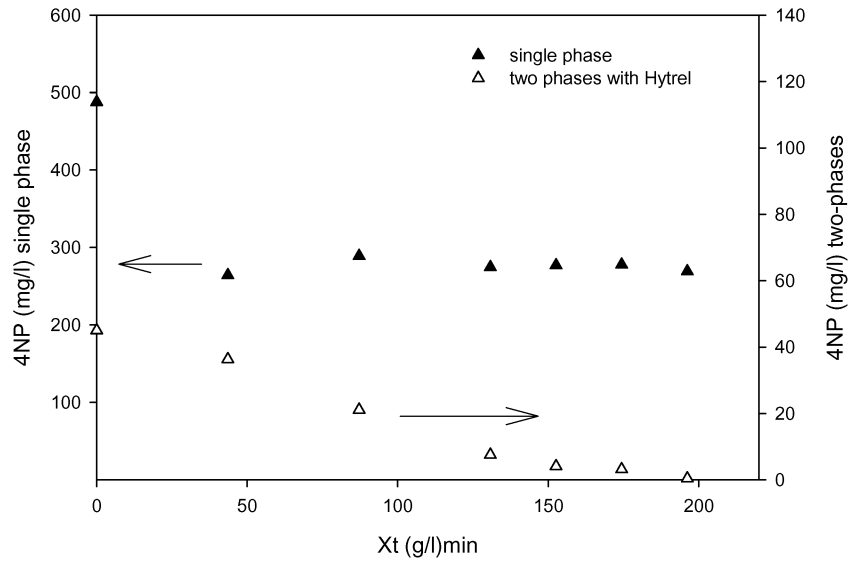


Figure 2. Concentration profiles detected in batch kinetic tests. 4NP initial concentration in the one-phase system 500 mg/l; polymer content 10%, $X = 2180 \text{ mgVSS/l}$.

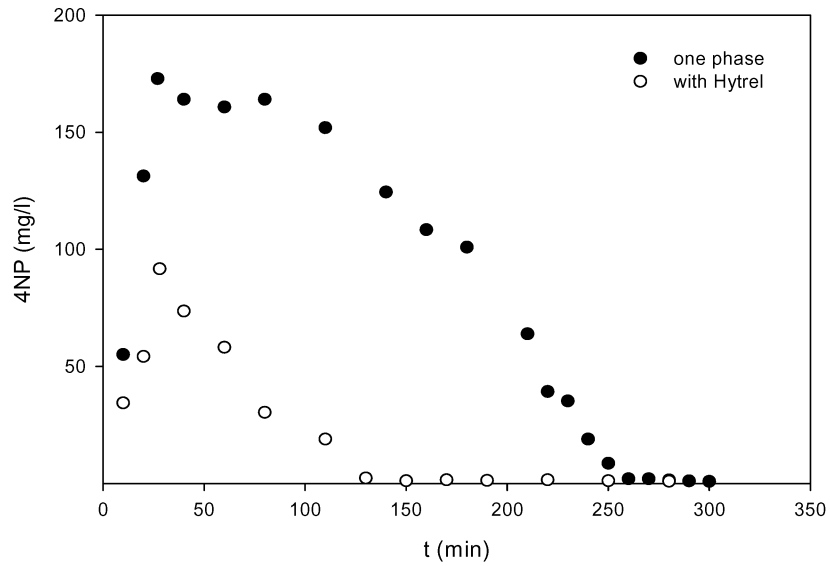


Figure 3. 4NP concentration profiles detected in a reactor kinetic tests. Feed concentration 750 mg/l, feed time 30 min, Hytrel 5%, $X = 2700 \text{ mgVSS/l}$.

In light of the favourable results of the experimental kinetic tests in terms of 4NP removal from the liquid phase, the proposed model was applied to provide a more complete picture of the entire process by simulation studies focused on the fate of the xenobiotic in the solid phase and on the effect of the diffusion time on the overall process kinetics.

Analysis of the model equations suggests the definition of two important characteristic times, a reaction time t_R defined as $t_R = C_w^0 / (r_s(C_w^0) \cdot X)$, where C_w^0 is the 4NP initial concentration (at $t=0$) and an intraparticle diffusion time, t_D defined as d^2/D where d is the particle diameter.

The model was applied to simulate a typical kinetic batch test with an initial sorption phase (contact of polymer/solution), and a subsequent reaction step taking place in the liquid phase after biomass addition. Simulations were performed considering a reaction characteristic time of 4.8 h, as obtained from kinetic parameter values reported in previous work (Tomei *et al.*, 2005) and two different diffusion times, 1 and 6 hours (that are in the range of values of literature data reported for similar systems). In the following discussion, the two simulation cases are referred as low ($t_D/t_R = 0.2$) and high ($t_D/t_R = 1.2$) diffusion times.

Figure 4a shows the substrate concentration profile in the liquid phase and the mean substrate concentration in the solid phases as a function of the dimensionless time t/t_R . In figure 4b the total residual amounts of substrate in a single and two phase systems at the same operating conditions are compared.

Different behaviour is observed depending on the kinetics of substrate diffusion inside the polymer. For low diffusion time (i.e. with lower bead diameter and/or higher substrate diffusivity) polymer beads are practically saturated in the sorption phase. When the biomass is added, a rapid decrease in the substrate concentration both in the liquid and in the polymer phase is observed; the result is a fast overall removal rate and a significant reduction of the reaction time in comparison to that required in a single phase process.

In contrast, in the case of high diffusion time, the polymer loading is slower and a lower amount of 4NP is removed from the liquid phase in the sorption phase. In the reaction phase, a rapid concentration decrease in the liquid phase is observed due to the biodegradation process coupled with slow 4NP release from the polymer. In this case, even when the substrate concentration in the liquid is almost zero, some substrate is still present in the polymer and it is removed by the biomass at a lower rate, controlled by desorption. As a consequence, in the residual amount (see fig. 4b) profile, after a rapid initial decrease, a sharp slope change is observed corresponding to the lower final kinetics. This last phase of desorption from the polymer and biodegradation in the liquid phase, suggests the potential for polymer "bioregeneration" achieved with a prolonged contact time with the biomass. Such a regeneration process is preferable to solvent regeneration (e.g. methanol extraction) method in that provides a complete mineralization of the compound and not concentration in another phase that has to be treated or disposed of.

5. CONCLUSIONS

Utilization of polymers as partitioning phases in TPPB systems as an alternative to a liquid organic solvents provides significant advantages in applications when mixed cultures are employed: complete biocompatibility with the biomass was confirmed, as was the non-biodegradability of the polymers. The formation of emulsions, often associated with immiscible liquid solvents, was eliminated and the polymer beads are easily separated from the biomass, and can be reused. A critical aspect to be further investigated in the use of polymers is the sorption/desorption kinetics that, as shown in the model simulation, could reduce the process rate due to the slow release of the absorbed residual amount of substrate in the solid phase. In any case, the removal of the absorbed xenobiotic by a prolonged contact time with the biomass demonstrated the potential for "bioregeneration" of polymers in a more sustainable way in comparison with the solvent regeneration that has traditionally been applied.

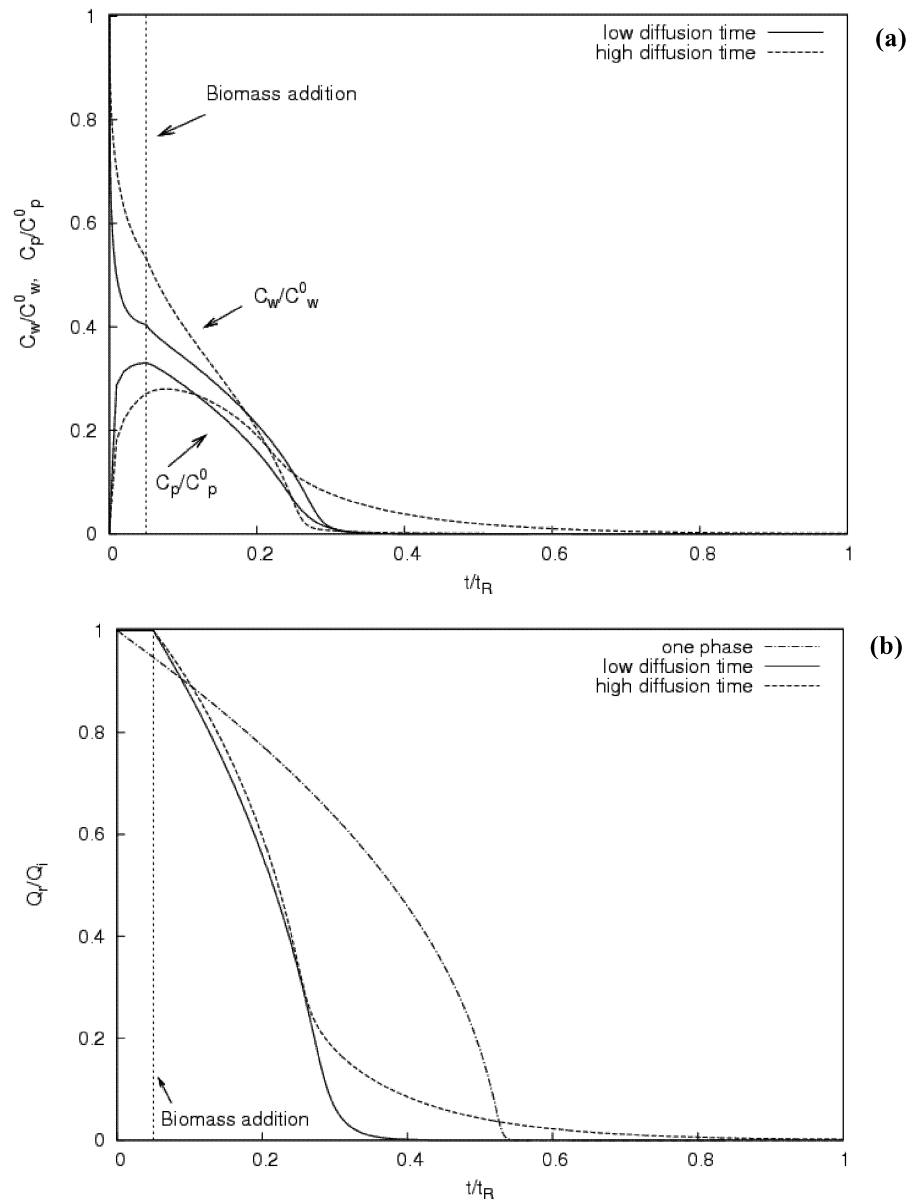


Figure 4. Substrate concentration profile in the liquid and solid phases (a) and total residual amount of substrate in the system (b).

Symbols in figure: $t_R = C_w^0 / [r_s(C_w^0)X]$, C_w^0 is the initial concentration in the liquid phase without polymer, $C_p^0 = PC_w^0$ is the initial concentration in the polymer phase, Q_t = residual amount of substrate in the system, Q_i = amount to be removed.

Values assumed in the simulation: $P = 60$, $R = 1.5 - 4$ mm, $X = 2000$ mgVSS/l, $V_w = 200$ ml, Polymer content $\sim 5\%$, $C_w^0 = 450$ mg/l, $D = 6.5 \cdot 10^{-6}$ cm²/s, $k^* = 0.059$, $K_s = 47.86$ mg/l, $K_I = 17.23$ (mg/l)², $t_R = 4.79$ h.

6. REFERENCES

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