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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 of xenobiotics was investigated. 4-nitrophenol, a typical representative of substituted phenols, was chosen as the target compound. Three polymers, a polyether-ester copolymer Hytrel 8206, a poly-caprolactone polyester Tone P797 and a polyethylene-vinyl acetate copolymer Elvax were utilized in batch kinetic tests. The best performance was obtained with Hytrel, and this polymer was also employed as the partitioning phase in a lab scale sequencing batch reactor. In all cases in the two phase systems, even if operated with a very low polymer content (~ 5%), the biomass was exposed to 4-nitropheol 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 set up and applied to analyze the performance of the system in different operating conditions.

1. Introduction

Biological processes are attractive as "green" remediation strategies for xenobiotic removal from aqueous environments but they have a major limitation due to substrate toxicity that can significantly reduce process efficiency and the applicable substrate load. In order to overcome these limitations 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 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). This approach is suitable for pure cultures but when mixed cultures are employed (i.e. in industrial wastewater treatment) a reduced efficiency can result by the parallel biodegradation of the solvent arising from biomass acclimatization. Solid polymers beads have recently been

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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 (Tomei et al., 2008, Pripch et al., 2006). 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. 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. 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. 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 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 colouring). 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 three polymers, a polyether-ester copolymer Hytrel 8206 (DuPont), a polycaprolactone polyester Tone P797 (Dow Chemical) and a polyethylene-vinyl acetate copolymer Elvax (DuPont). were employed in the liquid-solid system.

2. Material and Methods

2.1 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 and Annesini, 2003, 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.

2.2 Sequencing Batch Reactor

The culture utilized in the batch experiments was grown in a conventional Sequencing Batch Reactor (SBR) that is a glass vessel of 5 litres equipped with a thermostatically controlled water jacket to maintain the operating temperature at 20±0.5°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).

2.3 Kinetic tests

Batch kinetic tests were carried out using the biomass from the SBR reactor; in order to compare the performance of the two removal processes, kinetic tests were carried out in parallel in single and two phase systems. Temperature was controlled at 20+0.5°C, while 4NP and biomass concentration were in the range of 300-500 mg/l and 2000-2700 mgVSS/l respectively. The water phase volume was 200 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 fill and reaction phases with the same sampling modalities of the batch tests.

2.4 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 °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.

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

In Table 1 the mass balance equation referring to substrate, biomass and oxygen are reported. Equations are formulated by taking into account the substrate transfer between the two phases. A radial distribution of the substrate concentration and unsteady diffusion inside the polymer beads are modeled while the resistance in the external liquid phase is neglected.

Table 1. TPPB-SBR fundamentals modeling of a solid-liquid- system

Aqueous phase	
$\frac{dV_w}{dt} = F_{in} - F_{out} - F_{ws}$	
$\frac{d(V_w C_w)}{dt} = F_{in}C_{in} - F_{out}C_{out} - F_{ws}C_w - V_w r_s - S_P D \left.\frac{\partial C_P}{\partial r}\right\|_{r=R}$	***Substrate mass balance***
$r_S = -k^* X \frac{C_w}{C_w + K_s + \frac{C_w^2}{K_I}}$	***Kinetics*** (Haldane Equation)
Partitioning phase	
$\frac{\partial C_p}{\partial t} = \frac{D}{r^2}\frac{\partial}{\partial r}\left(r^2 \frac{\partial C_p}{\partial r}\right)$; $r=0 \ \frac{\partial C_p}{\partial r}=0 \quad r=R \ C_P = P \cdot C_w$	***Boundary conditions***

Symbols in table:
V_W = aqueous phase volume; F_{in} = influent flow rate; F_{out} = effluent flow rate; F_{ws} = wastage flow rate; C = substrate concentration; S_p = polymer surface; R = polymer bead radius; P= partition coefficient; D = substrate diffusivity in the polymer phase; r_S = substrate degradation rate; K_s = saturation constant; K_I = inhibition constant; k* = kinetic parameter; X = biomass concentration.
Subscripts: w = aqueous phase; out = outlet; in = inlet; ws= wastage; P = polymer phase.

In order to analyse the behaviour of polymers in two phase systems, the model was applied to simulate batch tests carried out first with a polymer loading phase then biomass addition and subsequently a reaction phase. In Figure 1 the substrate concentration profile in the liquid phase and the total residual amount of substrate in the system (Qr) are reported vs. dimensionless time and compared to the behaviour of a conventional single phase system (without polymer). Two different diffusion times of 1 and 6 hours (in the range of values of literature data reported for similar systems) are assumed.

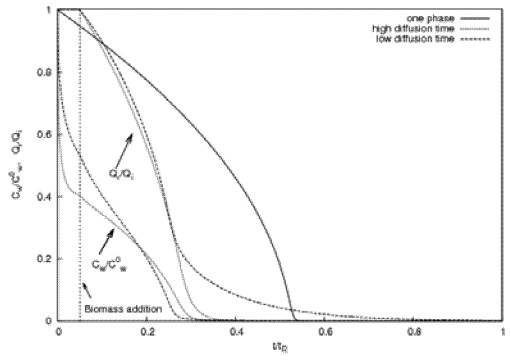

Figure 1. Substrate concentration profile in the liquid phase the total residual amount of substrate in the system.

Symbols in figure: $t_R = C^0_w/[r_S(C^0_w)X]$, C^0_w is the initial concentration in the liquid phase without polymer, Q_r = 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 ~5%, C^0_w = 450 mg/l, D 6.5 10^{-6} cm^2/s.

A significant reduction of the removal time is observed with the polymer particularly in the case of a low diffusion time (i.e. with lower bead diameter and/or higher substrate diffusivity). It is worth noting that if the diffusion time increases, a faster concentration decrease in the liquid phase is observed due to the lower desorption rate from the polymer. As a result, 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. This last phase is important to demonstrate the potential "bioregeneration" of polymers that can be achieved with a prolonged contact time with the biomass, and is a preferred method of polymer regeneration and reuse relative to solvent regeneration (e.g. methanol extraction) that has been used in the past.

4. Results and discussion

In Figures 2 and 3 the 4NP concentration profiles of the kinetic tests performed in batch and in the SBR reactor are reported. It was observed that in the two phase batch systems the biomass is exposed to much lower maximum concentrations of substrate, when compared to the conventional single phase system.

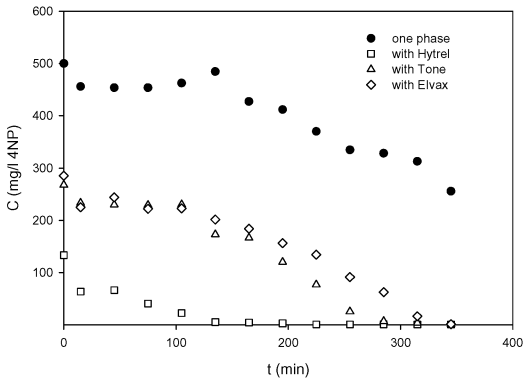

Figure 2. Concentration profiles detected in batch kinetic tests. 4NP initial concentration in the one-phase system 500 mg/l; polymer content5%, X= 2700 mgVSS/l.

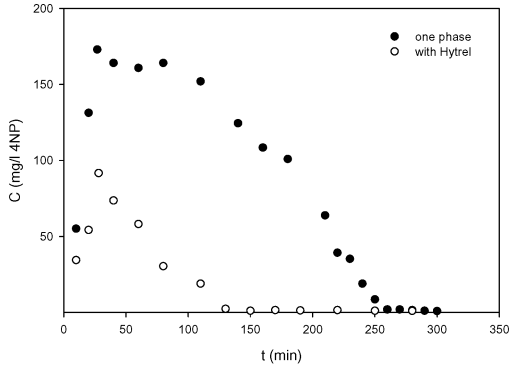

Figure 3. 4NP concentration profiles detected in a reactor kinetic tests. Feed concentration 750 mg/l, feed time 30 min, Hytrel 5%, X= 2700 mgVSS/l.

The best performance was obtained with the polymer Hytrel characterized by the highest partition efficiency resulting in a ~75% reduction of the initial 4NP concentration. In all cases in the two phase systems, the biomass was exposed to 4NP concentrations that are significantly lower if compared to the one-phase system during the entire course of the experiment. This is certainly an advantage when the system operates with high concentrations of xenobiotics and was obtained with a very low polymer amount (~ 5% of the liquid volume). The polymer Hytrel was also utilized in preliminary tests in the SBR reactor, confirming its significant potential for application to xenobiotic removal. Considering the very low 4NP concentrations obtained in the liquid phase when polymers are used, 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.

5. Conclusions

Utilization of polymers as partitioning phases in TPPB systems as an alternative to a liquid organic solvent could give some 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 evaluated from the model application, could slow down 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 “bioregeneration “ of polymers in a more sustainable way in comparison with the solvent regeneration that is usually applied.

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