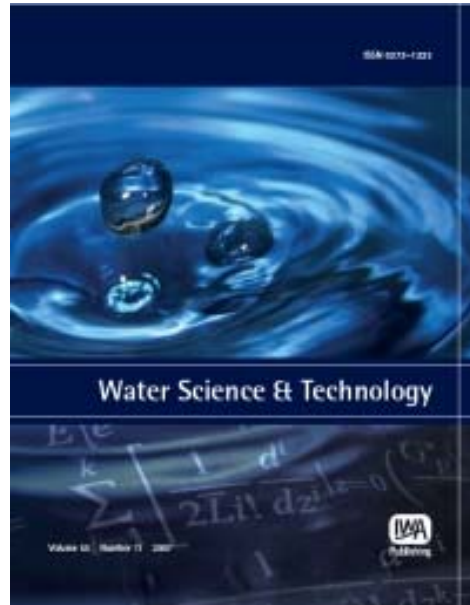


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Two-phase reactors applied to the removal of substituted phenols: comparison between liquid-liquid and liquid-solid systems

M. C. Tomei, M. C. Annesini, V. Piemonte, G. P. Prpich and A. J. Daugulis

ABSTRACT

In this paper, a comparison is provided between liquid-liquid and liquid-solid partitioning systems applied to the removal of high concentrations of 4-nitrophenol. The target compound is a typical representative of substituted phenols found in many industrial effluents while the biomass was a mixed culture operating as a conventional Sequencing Batch Reactor and acclimatized to 4-nitrophenol as the sole carbon source. Both two-phase systems showed enhanced performance relative to the conventional single phase bioreactor and may be suitable for industrial application. The best results were obtained with the polymer Hytrel™ which is characterized by higher partition capability in comparison to the immiscible liquid solvent (2-undecanone) and to the polymer Tone™. A model of the two systems was formulated and applied to evaluate the relative magnitudes of the reaction, mass transfer and diffusion characteristic times. Kinetic parameters for the Haldane equation, diffusivity and mass transfer coefficients have been evaluated by data fitting of batch tests for liquid-liquid and liquid-solid two phase systems. Finally, preliminary results showed the feasibility of polymer regeneration to facilitate polymer reuse by an extended contact time with the biomass.

Key words | bioregeneration, organic solvents, partitioning bioreactors, polymers, substituted phenols

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INTRODUCTION

Substituted phenols in industrial wastewater originate from many different sources such as 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. Moreover, because of their toxicity to humans and aquatic life (1 mg/L is enough to detect the effects) they are included in the USEPA list of priority pollutants. An extremely promising technology for the removal of phenolic compounds and other xenobiotics is based on the use of two phase partitioning bioreactors (TPPBs) whose main function is to reduce substrate toxicity to the biomass

(Deziel *et al.* 1999; Marcoux *et al.* 2000; Daugulis 2001). The operating principle involves the partitioning of the toxic substrate between the aqueous phase containing the micro-organisms and a sequestering phase which is comprised of either an immiscible organic solvent or a solid phase consisting of polymer beads. This configuration allows control of substrate delivery (to the aqueous phase) that is determined by the microbial degradation kinetics and the maintenance of thermodynamic equilibrium within the two-phase system.

Both liquid-liquid and liquid-solid TPPB systems have been successfully investigated for xenobiotic removal and destruction and the optimal choice between the two alternatives has to be evaluated for each specific case

(Prpich & Daugulis 2005; Muñoz *et al.* 2008; Tomei *et al.* 2008, 2009). The use of organic solvents in reactors operating with long biomass retention times and mixed cultures could be affected by a reduced efficiency caused by the parallel biodegradation of the solvent arising from biomass acclimatization. On the other hand, solid polymer beads have the significant advantage of being biocompatible with the biomass and non biodegradable, however a low substrate sorption/desorption rate may significantly reduce the overall process performance.

The objective of this paper is to evaluate the potential of the two liquid-liquid and liquid-solid systems applied to the removal of a target compound, 4-nitrophenol, on the basis of experimental results and theoretical modelling. A complete process model taking into account the interactions between biological processes and mass transfer phenomena occurring in the aqueous and partitioning phases was formulated and applied to analyse the kinetic test data. The dynamic model, shown in this work for the first time, considered complex (i.e. inhibitory) microbial kinetic terms overlain with convective and diffusive mass transfer relationships between the aqueous and sequestering phases, and within the solid polymer phases themselves.

The considered substrate is a typical representative of substituted phenols found in many industrial effluents and the biomass was a mixed culture operating as a conventional Sequencing Batch Reactor (SBR) and acclimatized to the 4NP as the sole carbon source. On the basis of literature data and previous experiments 2-undecanone was chosen as the liquid solvent while a polyether-ester copolymer (Hytrel[™]) and a polycaprolactone polyester (Tone[™]) were employed in the liquid-solid system.

MATERIALS AND METHODS

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).

To ensure the presence of required nutrients and microelements, the aqueous matrix consisted of a 4NP solution with the addition of the mineral medium MSV (Williams & 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 batch experiments was grown in a conventional Sequencing Batch Reactor (SBR) comprised of a 5 L glass vessel controlled at $20 \pm 0.5^\circ\text{C}$ by means of a water jacket. 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 h 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 elsewhere (Tomei *et al.* 2008).

Kinetic tests

Batch kinetic tests were conducted 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 $20 \pm 0.5^\circ\text{C}$, while 4NP and biomass concentration were in the range of 300–500 mg/L and 1,500–2,700 mg VSS/L respectively. The aqueous phase volume was 200 ml. In the liquid-liquid system the solvent/water ratio was 10% (v/v) while in the solid-liquid system the polymer/water ratio was 5% (w/w).

Before the biomass addition the 4NP solution was kept in contact with the partitioning phase under mixing conditions for 20 min and 24 h with the solvent and the polymer, respectively, to ensure equilibrium conditions.

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.

Regeneration tests

To evaluate the capacity of the biomass for polymer bioregeneration fixed aliquots of polymers (1 g) were

withdrawn from the batch reactor at different contact times and washed with methanol. Washing cycles were repeated until a negligible residual concentration in the solvent was detected. The absorbed amount was evaluated by 4NP detection in the washing solvent.

Chemicals

The target compound 4-Nitrophenol (purity >98%) was supplied by Fluka (Italy). The liquid solvent 2-undecanone (purity 99%) was supplied by Sigma Aldrich (Germany).

The polyether-ester copolymer, Hytrel™ 8206, (DuPont Canada, Kingston, Canada) is in the form of oval shape beads (5 mm length, 1.5 mm diameter) with density 1.17 g/cm³ and melting point 189°C. The polycaprolactone polyester, Tone™ P787 (Dow Chemical Canada Inc., Sarnia, Canada), is in the form of roughly spherical beads (~4 mm diameter) with density 1.145 g/cm³ and melting point 60°C.

Analysis

Volatile Suspended Solids concentrations were determined according to Standard Methods (APHA 1998).

4-Nitrophenol analysis in the kinetic tests was performed on samples filtered on syringe nylon membrane filters (0.45 μm pore-size) acidified in order to stop the 4NP biodegradation by the residual biomass not retained in the filter. Samples were then analysed by measuring the UV absorbance at 320 nm using a spectrophotometer Varian (model Cary 1).

MODELLING

The 4NP degradation rate was modelled by the Haldane equation which is commonly utilized for substrate inhibited kinetics:

$$r_s = v \frac{C}{C + K_s + \frac{C^2}{K_I}} = k^* \cdot X \frac{C}{C + K_s + \frac{C^2}{K_I}} \quad (1)$$

where X and C are the biomass and substrate concentration, respectively. In order to have an equation with more representative parameters in relation to the process

kinetics, the Haldane equation was rearranged in a different form:

$$r_s = k_{\max} \cdot X (2 + \beta) \frac{C/C^*}{1 + \beta(C/C^*) + (C/C^*)^2} \quad (2)$$

In Equation (2) $C^* = \sqrt{K_s \cdot K_I}$ is the substrate concentration at which the maximum removal rate occurs, k_{\max} is the maximum removal rate observed at $C = C^*$ and $\beta = \sqrt{K_I/K_s}$ is a parameter that accounts for the extent of the inhibitory effects. The procedure to derive Equation (2) from Equation (1) is reported in Tomei & Annesini (2008).

In modelling the two phase batch systems the substrate mass balance equations in both phases, the kinetic Equation (reported above) and the substrate transfer between the two phases are considered.

In Table 1 the model equations for both solvent and polymer as partitioning phases are listed.

For the liquid-liquid system, a mean substrate concentration in the organic phase is assumed and the substrate transfer rate is described by an overall mass transfer coefficient. For the liquid-solid system a radial distribution of the substrate concentration is considered within the spherical polymer bead and unsteady diffusion inside the

Table 1 | Fundamentals modelling: comparison between liquid-liquid and liquid-solid systems

Substrate mass balance in the aqueous phase	Partitioning phase
$\frac{dC_w}{dt} = aK_{ws} \left(\frac{C_{org}}{P} - C_w \right) - r_s$	Organic solvent
$\frac{dC_w}{dt} = - \frac{1}{1 + \frac{V_{org}}{V_w} P} r_s^{(*)}$	
$\frac{dC_w}{dt} = aK_{ws} \left(\frac{C_p _{r=R}}{P} - C_w \right) - r_s$	Polymer
Substrate mass balance in the partitioning phase	Partitioning phase
$\frac{dC_{org}}{dt} = - \frac{V_w}{V_{org}} aK_{ws} \left(\frac{C_{org}}{P} - C_w \right)$	Organic solvent
$\frac{\partial C_p}{\partial t} = \frac{D}{r^2} \frac{\partial}{\partial r} \left(r^2 \frac{\partial C_p}{\partial r} \right)$	Polymer
<i>Boundary conditions</i>	
$r = 0 \quad \frac{\partial C_p}{\partial r} = 0 \quad r = R \quad -D \frac{\partial C_p}{\partial r} = K_{ws} \left(\frac{C_p _{r=R}}{P} - C_w \right)$	

(*) simplified form assuming equilibrium conditions.

Symbols in table: V_w , aqueous phase volume; V_{org} , organic phase volume; C , substrate concentration; K_{ws} , mass transfer coefficient; a , specific surface area referring to the aqueous phase; r , radial coordinate; R , polymer bead radius; P , partition coefficient; D , substrate diffusion coefficient in the polymer phase; r_s , substrate degradation rate. Subscripts: w, aqueous phase; org, organic phase; P, polymer phase.

polymer as well as resistance in the external liquid phase are also accounted.

Analysis of the model equations suggests the definition of three characteristic times referring to the different fundamental processes involved: the reaction time, t_R , the solvent-water transfer time, t_C , and the time of diffusion inside the polymer beads, t_D .

A t_R value of a few hours for both systems is evaluated based on the kinetic parameters reported in a previous paper (Tomei *et al.* 2003) and average operating conditions adopted in the experiments. With regard to the liquid-liquid system, by assuming the mass transfer coefficient aK_{ws} equal to 250 h^{-1} as reported in Cruickshank *et al.* (2000) for a similar system, a transfer time t_C of less than 1 min is obtained. Since the reaction characteristic time is of the order of magnitude of hours, this result suggests that in the liquid-liquid system the overall kinetics are controlled by the reaction rate, and thermodynamic equilibrium conditions can be assumed as expressed by the simplified equation reported in Table 1.

In contrast, for the solid-liquid system, a diffusion time t_D of the order of magnitude of hours is calculated for 0.4 cm diameter beads with a substrate diffusivity of $1.5 \cdot 10^{-7}\text{ cm}^2/\text{s}$, reported in (Prpich & Daugulis 2004) for phenol. As a consequence, since the intraparticle diffusion time is comparable to the reaction time, diffusion inside the particle has to be taken into account in the evaluation of the overall process kinetics.

RESULTS AND DISCUSSION

Experimental concentration profiles found in batch tests in the presence of undecanone and polymers are shown in Figures 1 and 2, respectively, along with a comparison of the profile obtained in the conventional one-phase system. In the same Figures the fitted curves calculated for the liquid-liquid system (Figure 1) and for the solid-liquid one (Figure 2) are also reported.

In both two phase cases, a significant reduction of substrate concentration to which the biomass is exposed can be observed with a consequent increase of the removal rate. The effect seems more evident in the solid-liquid system where a faster substrate removal is observed. Nevertheless, in the presence of polymers the rapid

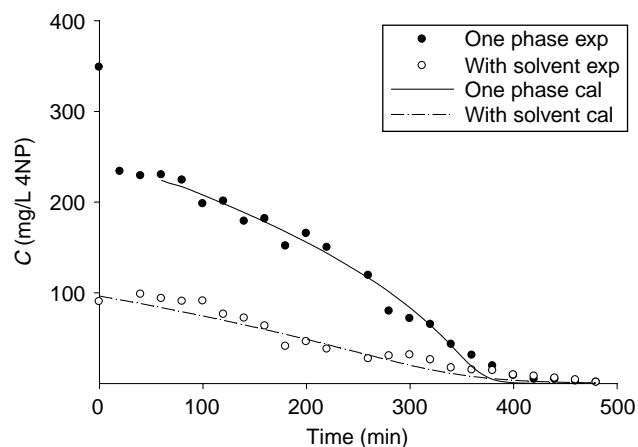


Figure 1 | Concentration profiles detected in batch kinetic tests with 2-undecanone as liquid solvent. 4NP initial concentration in the one-phase system 350 mg/L; solvent/water ratio=10% (v/v); $X=1,800\text{ mg VSS/L}$; exp=experimental; cal=calculated.

concentration decrease in the aqueous phase could be also due to the slow substrate release from the polymer beads, as expected from the comparison of the characteristic times reported above.

The best performance for the solid-liquid systems is observed with Hytrel[™] showing higher partition efficiency in comparison with Tone[™]. This is shown clearly in Figure 2 where the greatest initial concentration decrease is observed for Hytrel[™] (from 500 to 143 and from 500 to 335 mg/L 4NP for Hytrel[™] and Tone[™] respectively) followed by a faster removal from the liquid phase

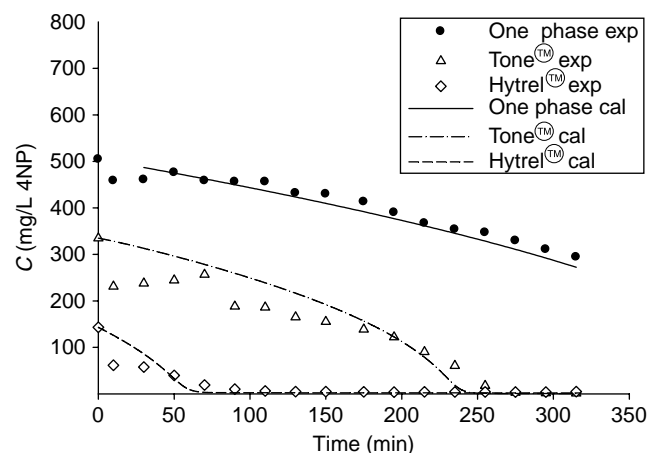


Figure 2 | Concentration profiles detected in batch kinetic tests with polymers. 4NP initial concentration in the one-phase system 500 mg/L; polymer content 5% (w/w); $X=2,300\text{ mg VSS/L}$; exp=experimental; cal=calculated.

(reaction time of 150 and 250 min for Hytrel™ and Tone™ respectively).

In terms of practical aspects, the liquid-liquid system showed the formation of micro-emulsions causing the entrapment and removal of micro-organisms and consequent increase of the effluent turbidity. Occasionally, depending on the mixing conditions, biofilm formation was also observed at the solvent-water interface with consequent reduction of the active fraction of the biomass involved in the biodegradation process. These features were not observed when using polymers as the sequestering phase.

The proposed models were applied to the two phase systems in comparison with the conventional single phase case. A preliminary analysis of the concentration profiles shows that in the one phase systems a rapid decrease of 4NP concentration followed by lag phase were observed as a possible consequence of sorption of 4NP onto the biomass and a storage phenomena due to the rapid exposure of the micro-organisms to high substrate concentrations. In this situation, after an initial period of reduced activity the biomass is able to adapt to the new condition and initiate the biodegradation process. To model the reaction phase (during which the compound biodegradation takes place) this first lag phase of the kinetic tests, that is strongly affected by the above mentioned phenomena, is not relevant and the initial data points of the concentration profiles were not included in the kinetic analysis of the single phase systems.

Another aspect that can justify this approach is that, in the batch systems utilized in the experiments, the substrate-biomass contact is instantaneous being the most favourable condition to induce adsorption and storage. In actual fed batch systems the feed phase time can be properly chosen to ensure gradual substrate-biomass contact thus minimizing the lag phase effect.

With regard to the liquid-liquid system, a detailed analysis is reported in a previous paper (Tomei & Annesini 2008); in the present study we are verifying that the simplified model accounting for thermodynamic equilibrium between aqueous and organic phases, is able to predict the behaviour of a batch liquid-liquid system, once the kinetic parameters are known. Figure 1 shows good agreement between the experimental data and the concentration profiles calculated with the kinetic

parameters obtained from the fitting of single phase data ($\beta = 0.6$, $C^* = 30 \text{ mg/L 4NP}$ and $k_{\max} = 0.05 \text{ mg 4NP mg VSS}^{-1} \text{ h}^{-1}$) and a partition coefficient $P = 30$.

As for the solid-liquid system, the first step in model application is evaluation of the intrinsic kinetic parameters from the one phase batch tests carried out in parallel with the two phase system. With reference to the one phase batch tests in the concentration range of 0–500 mg/L, the best fitting kinetic parameters are $\beta = 0.6$, $C^* = 34.7 \text{ mg/L 4NP}$ and $k_{\max} = 0.09 \text{ mg 4NP mg VSS}^{-1} \text{ h}^{-1}$. It is worth noting that these experiments were performed one year after the previous kinetic tests, and therefore the higher value of the maximum removal rate in this case may be explained by the prolonged contact time of the biomass with the 4NP having a positive effect on mixed culture acclimatization. Once the intrinsic kinetic parameters are known, the model application for the data fitting of the two phase solid-liquid system requires knowledge of the partition coefficient, the diffusivity in the solid phase and the mass transfer coefficient in the liquid phase. From preliminary sorption experiments (data not shown) carried out without biomass in a recirculation system, partition coefficients $P = 61.2$ and $P = 6.2$ have been evaluated for Hytrel™ and Tone™, respectively as well as a diffusion coefficient for Hytrel™ $D = 1.45 \cdot 10^{-6} \text{ cm}^2/\text{s}$. Specific surface areas (a) with respect to the aqueous phase were $6.73 \cdot 10^{-1}$ and $6.91 \cdot 10^{-1} \text{ cm}^{-1}$ for Hytrel™ and Tone™, respectively. Therefore, the 4NP concentration profile for the Hytrel™ test was fitted to obtain the mass transfer coefficient K_{ws} , which was found to be $K_{ws} = 1.2 \cdot 10^{-4} \text{ cm/s}$. Since the experiments were performed in the same fluidodynamic conditions for both polymers, it is reasonable to use the K_{ws} value obtained in the Hytrel™ test also for fitting the Tone™ concentration profile to obtain the diffusion coefficient, which was calculated to be $1.38 \cdot 10^{-7} \text{ cm}^2/\text{s}$. Figure 2 shows reasonable agreement between the model and the experimental data for both polymers.

For both polymers the intraparticle diffusion corresponds to relatively low sorption/desorption rates, and thus at very low substrate concentrations detected in the liquid phase (and high biomass concentrations) significant residual amounts could still be retained in the polymer beads. In order to have an estimation of the residual 4NP retained in the polymers the model was also applied

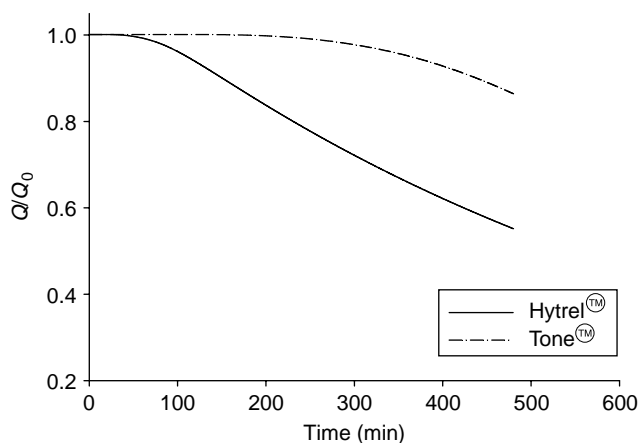


Figure 3 | 4NP retained in the solid phase vs. time. 4NP initial concentration in the one-phase system 500 mg/L; polymer content 5%, $X=2,300$ mg VSS/L. Q =retained amount in the beads, Q_0 =initial amount in the solid phase estimated from the partition data.

to evaluate the 4NP profiles in the solid phase vs. time. The 4NP amount in the solid phase (Q_0) at $t=0$ was evaluated by the partition data and is in agreement with the experimental 4NP mass balance. Simulation curves are reported in Figure 3 and show that about 60% of the initial amount of 4NP is still retained in the Hytrel™ particles at 300 min, when the 4NP removal in the liquid phase is practically completed. Model predictions (5.2 and 2.1 mg 4NP/g for Hytrel™ and Tone™ respectively) are of the same order of magnitude as the experimental values of residual 4NP determined by a multi-step washing with methanol of aliquots of polymers withdrawn from the batch reactor at a contact time of 6 h (see Table 2).

All of the preceding results suggest that the contact time required to remove the xenobiotic in the solid phase is longer than that necessary to reduce the concentration in the aqueous phase to acceptable values. Therefore the contact time is a factor to be closely considered in a TPPBs operating with polymers. As an alternative, in order to avoid excessively long contact times, a periodic polymer regeneration step can be considered. Preliminary bioregeneration

Table 2 | Residual amounts of 4NP in polymers

Contact time (h)	Hytrel™ (mg 4NP/g pol)	Tone™ (mg 4NP/g pol)
6	4.06	2.8
24	1.37	1.62

tests, whose results are reported in Table 2, show that for prolonged contact time the residual amount of 4NP in the polymer significantly decreases, and after 24 h removal efficiencies of 66 and 42% are observed for Hytrel™ and Tone™ respectively. These preliminary results are encouraging in demonstrating that the polymer could be effectively “bioregenerated”.

CONCLUSIONS

Two phase systems have been demonstrated to be an advantageous alternative in comparison to conventional processes for the removal of substituted phenols from concentrated wastewater. Better performance was obtained with solid-liquid systems and the polymer Hytrel™ showed the highest partition coefficient and the best removal efficiency for 4NP.

It is worth noting that in solid-liquid systems the magnitude of the sorption/desorption kinetics could determine the amount of residual xenobiotics remaining in the polymer beads, thus requiring a regeneration process for reuse. Polymers can be easily regenerated by a solvent extraction procedure or better by a “bioregeneration” process utilizing the same biomass operating in the biodegradation process. Preliminary results showed the feasibility of polymer bioregeneration by a prolonged contact time with the biomass that is an environmentally sustainable approach to allow for future polymer reuse, and to provide a means for the ultimate destruction of the contaminants.

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