

Solid-Liquid Partitioning Bioreactors Applied to the Removal of Mixtures of Phenolic Compounds

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In this paper we report the results of an investigation on the performance of conventional and two phase partitioning bioreactors (TPPBs) operating in sequencing batch mode applied to the biodegradation of a binary mixture of 2 substituted phenols, 2,4-dimethylphenol and 4-nitrophenol. The TPPB was operated with the DuPont polymer Hytrel 8206 as the partitioning phase, which was demonstrated to be effective in the uptake and release of a variety of phenolic compounds.

Partition tests were performed on both compounds, and partition coefficients of 201 and 143 were obtained in distilled water for 2,4-dimethylphenol and 4-nitrophenol, respectively. Parallel kinetic tests were carried out in conventional and TPPB bioreactors under the same operating conditions. The TPPB results showed a reduction in the aqueous phase concentration for both substrates to sub-inhibitory levels in all the tests, and the effect was more evident for 2,4-dimethylphenol as expected by the partition coefficient values. Moreover, the presence of the polymer has a marked effect on the process kinetics that are significantly increased for both substrates in comparison to the values of the conventional reactor.

1. Introduction

Phenolic compounds are important contaminants in industrial wastewater and are of significant environmental concern because of their toxicity to many life forms; for example the EC_{50} values for inhibition of biodegradation by 2,4-dinitrophenol and 2,4-dichlorophenol are 31 and 44 mg/L, respectively (Walker, 1989). Effluents containing these compounds, generally in mixtures, arise from a number of industrial activities (e.g. production of pesticides, dyes, explosives, leather colouring etc...). Biotreatment methods are in principle effective for their removal but their performance is hampered by the inherent toxicity of these molecules, which causes inhibition to the microbes catalyzing the biodegradation processes. A suitable technological solution to address this problem is the solid liquid Two Phase Partitioning Bioreactor (TPPB) which is able to reduce the toxic substrate concentration to which the biomass is exposed (Amsden et al., 2003). TPPBs operate with the addition of polymer beads to the liquid phase

containing the biomass. In the same manner as immiscible organic solvents, the polymers are able to partition toxic organic substrates to/away from cells thereby optimizing substrate delivery to the microorganisms. The advantages of using polymers, in addition to their low cost and wide availability, include complete biocompatibility and non-bioavailability, which permit the use of widely mixed consortia as required in industrial wastewater treatment.

TPPBs have been demonstrated to be very effective in the removal of single phenolic compounds (Tomei et al., 2009) but to verify their applicability in industrial wastewater treatment, their performance in treating mixtures has to be confirmed.

In this paper we report the results of an investigation of the performance of conventional and two phase sequencing batch bioreactors applied to the biodegradation of a binary mixture of 2 substituted phenols, 2,4-dimethylphenol and 4-nitrophenol.

2. Material and methods

2.1 Chemicals and polymer

4-Nitrophenol and 2,4-dimethylphenol (purity >98%) were obtained by Fluka (Italy). All other chemicals were commercial grade and were supplied by Carlo Erba (Italy).

Hytrel 8206, a polyether-ester copolymer, was supplied by DuPont (Canada) and is in the form of oval shaped beads (5mm length, 1.5 mm diameter) with density 1.17 g/cm³ and melting point 189 °C.

2.2 Microbial cultures

A mixed culture previously acclimatized to 4NP was utilized for single compound degradation tests. Details on the culture development are reported elsewhere (Tomei et al., 2005). An inoculum from this culture was gradually acclimatized to 2,4 DMP. Once effective performance in biodegradation of the single compounds at a feed concentration of 250 mg/L was verified, two equal aliquots of the microbial cultures degrading the single compounds were mixed and utilized for the kinetic tests on substrate mixtures.

2.3 Reactors

Biomass was grown in a lab scale Sequencing Batch Reactor (SBR) consisting of a 1 L glass vessel (0.8 L working volume) with a thermostatically controlled water jacket maintaining the temperature at 25±0.5°C. Dissolved oxygen was continuously monitored by a WTW probe (CellOx 325). Mixing was achieved by a magnetic stirrer. Air was supplied by a membrane compressor and introduced into the bioreactor through a glass diffuser. Dissolved oxygen (DO) was controlled in the range of 3-4 mg/L via an on-off strategy.

A typical SBR operating cycle lasted 12 hours distributed as follows: Feed 20 min, Reaction 580 min, Wastage 2 min, Settling 90 min, Draw 28 min. The exchange factor (added volume/total volume) was 0.5. The SBR was operated as a TPPB by adding the polymer in the ratio of 5% v/v.

2.4 Analysis

Volatile Suspended Solids (VSS) concentration was determined according to Standard Methods (APHA, 1998) as an estimate of the biomass concentration.

Analysis of 4NP and 2,4DMP in the kinetic tests were performed on samples after filtering through syringe nylon membrane filters (0.45 µm pore-size) and acidified in

order to stop the enzymatic reactions. The filtered samples were then analysed by UV absorbance using a spectrophotometer (Varian, model Cary 1) at 280 and 320 nm for 2,4 DMP and 4NP respectively. A double reading of the samples at the two wavelengths was performed for the measurement of the concentration of the two compounds in the binary mixture.

2.5 Partition tests

Batch Partition tests were performed for each single compound in distilled water. they were performed in a thermostated bath ($T=25\pm 0.5^{\circ}\text{C}$), mixing was ensured by magnetic stirrers, the work volume was 50 ml and the polymer amount was varied in the range 1-3 g. Initial concentration was 100 mg/L and the final concentration was measured after 24 hours to ensure the reaching of the equilibrium conditions.

2.6 Kinetic tests

Kinetic tests were performed in the SBR operated in conventional and two-phase mode. Two series of biodegradation tests were undertaken. In the first one the performance of the SBR operating in a conventional single phase mode was investigated at different concentration in the feed (in the range of 250-380 mg/L). In the second, the reactor was operated as TPPB at a polymer/aqueous phase ratio of 5%v/v and feed concentration in the range of 300-450 mg/L.

The tests were performed by measuring 4NP and 2,4DMP concentrations on samples of the aqueous phase taken from the reactor at predetermined time intervals (5 -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 concentrations in the reactor.

2.7 Polymer washing

Polymer washing with methanol was performed to extract and quantify the residual amount of 4NP and 2,4DMP in the polymer after long term use in the TPPB-SBR. A multi-step washing procedure with 10mL of methanol per 0.5 g of polymer was utilized for each washing step until the concentration in the solvent was negligible.

3. Results and Discussion

3.1 Partition tests

Results of the partition tests are reported in Figure 1 as C/C_0 vs M_S/V_L according to the linearized form of the mass balance equation:

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

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. Evaluated partition coefficients are 143 and 201 for 4NP and 2,4DMP respectively, and both values are sufficiently high as to be considered for use in this application.

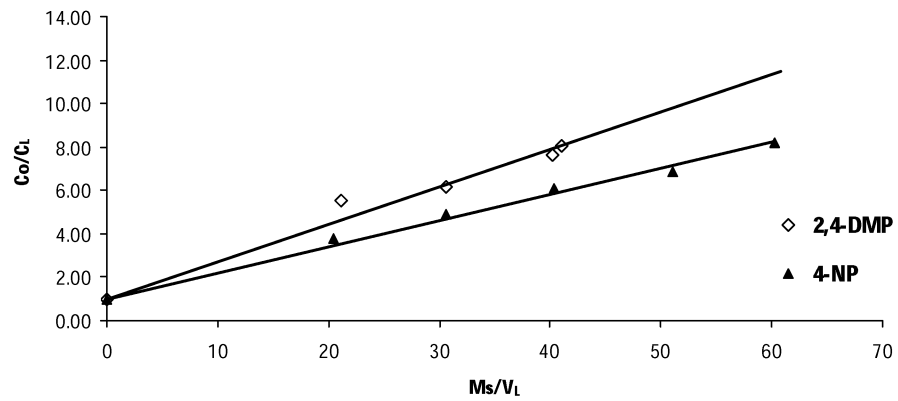


Figure 1: Partition test data in distilled water for 4NP and 2,4DMP.

3.2 Kinetic tests

Conventional single phase system

The first series of kinetic tests was performed in the SBR reactor working with suspended biomass: typical concentration profiles of 4NP and 2,4 DMP are shown in Figure 2.

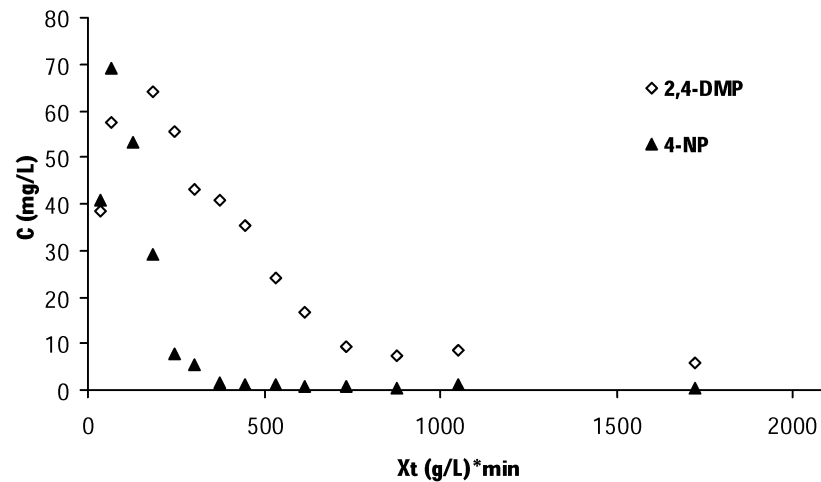


Figure 2: Concentration profiles detected in a kinetic test performed in the single phase SBR. Feed concentration = 250 mg/L for 4NP and 2,4DMP; biomass concentration $X = 2870$ mgVSS/L.

A faster biodegradation of 4NP respect to 2,4 DMP was observed corresponding to removal rates (evaluated on 4 kinetic tests with reference to 98% removal of the influent load) of 0.022 ± 0.002 and 0.00634 ± 0.0006 mg/(mgVSS h) for 4NP and 2,4DMP

respectively. The delayed 2,4 DMP degradation could be caused by a mutual inhibition effect of the mixture due to the presence of 4NP. Mutual interaction of substrates in phenolic mixture biodegradation have also been observed by Monsalvo et al. (2009) in the biodegradation of a mixture of phenol and 4-chlorophenol. They found a marked reduction of the phenol uptake rate (from 2.88 to 1.21 mg phenol/gVSS h) when the 4-chlorophenol concentration in the feed is increased from 1050 to 2100 mg/L. It is also worth noting that the mixed culture used in this study was cultivated for a long period of time (years) on 4NP, and only for a shorter period on 2,4DMP and therefore an acclimatization effect could cause preferential 4NP biodegradation in the mixture.

TPPB -SBR

Typical concentration profiles observed in kinetic tests performed in the TPPB- SBR are reported in Figure 3.

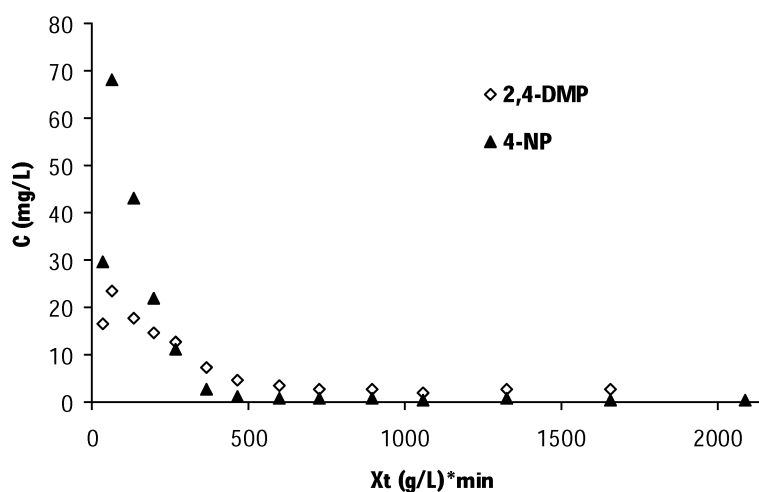


Figure 3: Concentration profiles detected in a kinetic test performed in the TPPB- SBR. Feed concentration = 350 mg/L for 4NP and 2,4DM; biomass concentration $X = 3310$ mgVSS/L, Hytrel 5% v/v.

Concentration profiles for these tests, performed at higher influent concentration of the two substrates, show a marked decrease of the concentration in the liquid phase, more evident for 2,4DMP, as expected, considering the higher value of the partition coefficient.

As a consequence of reduced inhibition, removal rates, evaluated with reference to 98% removal on 3 tests, showed a marked increase with values of 0.035 ± 0.001 and 0.023 ± 0.007 mg/(mgVSS h) for 4NP and 2,4DMP respectively. In both cases a significant improvement of the biodegradation kinetics was found but the effect was more evident for 2,4DMP again demonstrating the importance of the polymer affinity in TPPBs systems. The difference in kinetics also indicates the effect of the presence of

polymer on the biodegradation pattern that shows a reduced delay of the 2,4 DMP degradation with respect to the 4NP in comparison with the single phase system.

Moreover, the TPPB reactor was operated for a prolonged period (about 30 days) with the same polymer with stable performance in terms of removal efficiency of the two compounds.

At the end of the experimental period, to have an estimation of the real degree of mineralization of the two compounds, the residual amount of 4NP and 2,4 DMP remaining in the solid phase was determined by a multistep washing procedure with methanol. For both compounds the fraction retained in the polymer was ~ 1% of the total amount fed during the whole experimental period which is negligible with respect to the mineralized fraction.

4. Conclusions

The solid-liquid TPPB reactor was demonstrated to be very effective in reducing toxicity in the biodegradation process of a binary mixture of substituted phenols (4NP and 2,4 DMP). The polymer Hytrel showed a differential partitioning of the two compounds with a higher partition coefficient for 2,4DMP. This feature was of relevance in the biodegradation process causing a more significant improvement of the removal kinetics for 2,4DMP than for 4NP in the TPPB in comparison to the conventional single phase system. The two compounds were effectively mineralized and not “simply” retained in the polymer since the residual amount in the solid phase was negligible even after a prolonged operating period.

The results of the experiments demonstrated the possibility of utilizing TPPB systems applied to wider substrate mixtures by using mixtures of polymers tailored for the different compounds of interest in the mixture.

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