Removal of xenobiotics in a two phase sequencing batch reactor: kinetics and modelling

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ABSTRACT

The objectives of the paper are to verify the potentialities of a sequential two phase partitioning bioreactor in degrading xenobiotics and to evaluate the kinetic parameters for modelling the system. The target compound investigated was the 4-nitrophenol. Preliminary tests were carried out to define the solvent most appropriate for the compound. Among the three investigated solvents 1-undecanol, 2-undecanone and oleyl alcohol, the 2-undecanone was chosen because of the higher partition coefficient of 30 and the negligible formation of emulsions. Moreover, the tested solvent showed satisfactory “biocompatibility” characteristics for the biomass with minor effects on the intrinsic kinetics. Parallel batch kinetic tests were then performed with the conventional one phase and the two phase systems. In the two phase system the biomass is exposed for all the time to 4NP concentrations that are significantly lower if compared to the conventional system and, for the highest concentration (450 mg/l) in the two phase system a reduction of the reaction time is observed depending on the biomass concentration. Kinetic parameters were also evaluated in both cases by fitting of the experimental data with a modified form of the Haldane equation.

Key words | Haldane kinetics, 4-nitrophenol, sequencing batch reactor, two phase partitioning bioreactor, xenobiotic biodegradation

BACKGROUND AND OBJECTIVES

The main concern in applying biological processes to the removal of xenobiotic compounds is the limit in concentration that the biomass can face without significant kinetics reduction that is often not acceptable in practical applications (i.e. industrial wastewater treatment). So it is required a system able to optimize the “substrate delivery” to the cells in order to keep the substrate concentration at a level high enough to have reaction rates suitable for application but not inhibitory and/or toxic for the biomass.

In response to this challenge a promising technology based on the use of two phase partitioning bioreactors (TPPBs) has been proposed (Collins & Daugulis 1999; Daugulis 2001): it is based on the use of a water-immiscible and biocompatible organic solvent floating on the surface of the aqueous phase containing the micro-organisms. The solvent is able to dissolve large amounts of the compound (due to the hydrophobic nature of the most part of organic contaminants) which then partition in the aqueous phase at a concentration depending on the partition coefficient.

In this way, even if large amounts of the compound are added to the bioreactor, the micro-organism are exposed to low (sub-inhibitory) levels. Moreover, the substrate delivery is completely driven by the cellular processes, in fact when the substrate is consumed in the aqueous phase, the thermodynamic equilibrium has to be restored in the system and this causes the xenobiotic transfer from the solvent to the water phase. The amount of delivered substrate is always the optimal one because the transfer rate is only depending on the biodegradation kinetics.
The concept of the two phase reactors can be practically realized both in continuous (operating in steady state conditions) and discontinuous (operating in dynamic conditions) plants but in the specific case of xenobiotic removal the last solution could be preferable. In fact, the Sequencing Batch Reactor (SBR), characterised by a large variety of operating conditions (easily obtainable on the time scale) and high operation 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 combination of the advantages of the two phase process with the SBR technology seems really promising to be investigated as possible strategy when the xenobiotic removal has to be realized in particularly critical conditions i.e. very high concentration. One typical example is the “remediation of stored xenobiotics” (unfortunately a quite common situation) that is the removal of toxic compounds stored in containers in large amounts as results of industrial closures or of the stockpiling of substances not more usable.

The objectives of the paper are to verify the potentialities of a TPP-SBR in degrading xenobiotics and to evaluate the kinetic parameters to set up a mathematical model of the system. As target compound was chosen the 4-nitrophenol, a typical representative of substituted phenols found in many industrial effluents (manufacture of explosives, drugs, dyes, phosphororganic insecticides, pesticides, leather colouring) and also generated in aqueous matrices during formulation, distribution and field application of pesticides.

**MATERIAL AND METHODS**

**Sequencing batch reactors**

SBR reactors is a glass vessel of 5 litres volume with a thermostated water jacket to control the operating temperature. Dissolved oxygen and pH are on-line monitored and controlled by WTW instruments (CellOx 325 and Ino Lab pH level 2, respectively). Feeding, sludge wasting, effluent discharge and acid/base addition for pH control were performed by peristaltic pumps (Cellai, Perinox SF3) through openings located in the reactor cover. Mixing was ensured by a magnetic stirrer. Air was supplied by variable flow compressors through a glass diffuser.

Time operational sequence and control strategies have been automatically operated by a personal computer interfaced to the reactor. Specialised software has been developed under Labview- Windows to manage time definition of the working cycle phases, dissolved oxygen (DO) and pH monitoring and control by on-off strategies and driving of stirrer, compressors and pumps.

A typical SBR work cycle lasted 8 hours distributed as follows: FILL 30 min, REACTION 340 min, WASTAGE 3 min, SETTLE 92 min, DRAW 15 min. Fill phase was mixed and aerated.

**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 & Annesini 2005).

Fed was constituted by 4NP added of the mineral medium MSV (Williams & Unz 1989) to ensure the required contribution of nutrients and microelements. The composition of the MSV solution, prepared in deionized water, was as follows in mg l⁻¹ units: (NH₄)SO₄ 500, MgSO₄·7H₂O 100, CaCl₂·2H₂O 50, K₂HPO₄ 110, KH₂PO₄ 85, FeCl₃·6H₂O 2, NaEDTA 2. The C:N:P ratio in the influent was 100:5:1 with respect to the 4NP carbon.

**Analysis**

- Volatile Suspended Solids concentrations have been determined according to Standard Methods (APHA 1998).
- 4-Nitrophenol analysis 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. They were then analysed by measuring the 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.
Preliminary tests

Preliminary tests were carried out to define, on the basis of the partition coefficient, the solvent most appropriate for the compound. On the basis of literature data, three solvent were utilized: 1-undecanol, 2-undecanon and oleyl alcohol. The partition coefficients were evaluated in batch tests at \( T = 20 \pm 0.5 ^\circ C \) and different solvent-water ratios (varying from 0.08 to 0.2). Initial 4NP concentration was 100 mg/l.

Kinetic tests

Kinetic tests were performed in batch and in the SBR reactor during the reaction phase of the working cycle. 4NP concentration was measured at time intervals varying from 5 to 10 min, while VSS concentration was controlled at longer time intervals (30–40 min). Furthermore, DO was continuously measured during the test.

A first series of kinetic tests was carried out to evaluate the “biocompatibility” of the solvent, that is its effects on the biomass activity: parallel kinetic batch tests were carried out to evaluate the removal rate of 4NP in water and in a solution saturated with the solvent after a contact time of 24 hours. Operating conditions were \( T = 20 \pm 0.5 ^\circ C \), 4NP concentration equal to 100 mg/l and biomass concentration \( X \sim 4,000 \text{mgVSS/l} \).

A second series batch tests was carried out in parallel with a single and two phase systems in order to compare the performance of the removal processes. Temperature was controlled at 20 \( \pm 0.5 ^\circ C \), while 4NP and biomass concentration were in the range of 300–450 mg/l and 1,400–2,000 mgVSS/l respectively. The solvent/water ratio was 0.1.

Moreover, in order to verify data reproducibility, all kinetic tests have been carried out in at least two replicates under the same operating conditions.

Modelling

To model the inhibited kinetics of 4NP removal the Haldane equation is utilized:

\[
    r_s = \frac{v}{C_{NP} + K_s + \frac{C_{NP}}{K_i}} = \frac{v}{C_{NP} + K_s + \frac{C_{NP}}{K_i}}
\]

where \( X \) and \( C_{NP} \) are the biomass and 4NP concentration, respectively. In this model three fitting parameters, the rate constant \( k^* [\text{MNP} \cdot \text{VSS}^{-1} \cdot \text{t}^{-1}] \) and the saturation and inhibition constants, \( K_s \) and \( K_i \) [ML\(^{-3}\)] are included. In the classical form of the Haldane Equation (1), it is worth noting that the parameters \( k^*, K_s \) and \( K_i \) do not have a precise meaning in terms of process kinetics; in fact no direct information is given on the maximum consumption rate and on the critical substrate concentration (corresponding to the maximum removal rate). 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:

\[
    r_s = \frac{k_{max} X (2 + \beta) C_{NP} C^*}{1 + \beta C_{NP} C^* + (C_{NP} C^*)^2}
\]

In equation (2) \( C^* = \sqrt{K_s K_i} \) is the substrate concentration where 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 smaller \( \beta \) the larger the removal rate reduction at high substrate concentration). The main advantage of this form is to have a direct indication from the kinetic parameters of the possible maximum rate or of the critical substrate concentration value; moreover, working with \( \beta \) and \( C^* \) in the data fitting reduces some numerical problems that are often found with \( K_s \) and \( K_i \) in applying the classical form of Haldane equation. The procedure to derive Equation (2) from Equation (1) is reported in Tomei & Annesini (2007).

The concentration profile is evaluated from the mass balance equation:

\[
    \frac{dC_{NPW}}{dt} = -r_s
\]

for the conventional one-phase system where the subscript \( W \) indicates the aqueous phase. For the two-phase system the Equation (3) is modified as follows:

\[
    \frac{dC_{NPW}}{dt} = -\frac{V_W}{(V_W + PV_O)} r_s
\]

where \( P \) indicates the partition coefficient and the subscript \( O \) the organic phase. In equation is evaluated at the
substrate and biomass concentrations in the aqueous phase where the biochemical reactions occur.

RESULTS AND DISCUSSION

Preliminary tests

A first analysis of the preliminary tests showed for the oleyl alcohol a marked tendency to form emulsions that interfere with the 4NP absorbance measurement, so it was excluded. For the two other solvents 2-undecanone and 1-undecanol the emulsion formation was negligible, so the choice was done on the base of the partition coefficient. The higher partition coefficient, \( P = 30 \), was obtained with 2-undecanone, while the 1-undecanol gave a value of 14, so the 2-undecanone was used in the successive tests. Figure 1 shows the experimental data for the partition of 4NP in the two-phase system 2-undecanone water.

Biocompatibility tests

The biocompatibility tests were performed in order to evaluate the intrinsic kinetics of 4NP biodegradation in presence of the solvent in the most unfavourable conditions that is in a saturated aqueous solution.

This approach is simple to realize and at the same time allows a rapid estimation of the solvent effects on the process rate. Typical profiles detected in biocompatibility tests are reported in Figure 2. It can be observed that in the first part of the test at higher 4NP concentration the degradation rates in the two cases are coincident while a slight decrease is detected in the second part. In any case, the time required to complete the reaction (4NP concentrations \( \leq 1 \text{mg/l} \)) is practically the same. As a consequence, the tested solvent shows good biocompatibility characteristics.

Data analysis was performed by fitting of the experimental data with the model for the one phase system (Equations 2 and 3) and the results are reported in Table 1. According to experimental VSS concentration values it was assumed that the biomass remained practically constant throughout each run. Evaluated best fitting parameters confirm the low difference in kinetics with the same values of \( \beta \) and \( C^* \) and a low decrease of \( k_{\text{max}} \) in the saturated water tests. Moreover, data fitting gives good correlation coefficients (\( \geq 0.98 \)) of the interpolating functions as showed by the calculated concentration profiles reported in Figure 2.

Table 1 | Best fitting parameters for biocompatibility kinetic tests (subscripts: \( \text{TW} = \text{tap water, SW} = \text{saturated water} \). Initial concentration of 4NP = 100 mg/l

<table>
<thead>
<tr>
<th>Test</th>
<th>( X_{\text{mean}} ) (mg/l VSS)</th>
<th>( \beta )</th>
<th>( C^* ) (mg/l 4NP)</th>
<th>( k_{\text{max}} ) (mg4NP/mgVSS·h(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>BC1(_{\text{TW}})</td>
<td>3,990</td>
<td>0.6</td>
<td>30</td>
<td>0.031</td>
</tr>
<tr>
<td>BC1(_{\text{SW}})</td>
<td>3,990</td>
<td>0.6</td>
<td>30</td>
<td>0.025</td>
</tr>
<tr>
<td>BC2(_{\text{TW}})</td>
<td>3,870</td>
<td>0.6</td>
<td>30</td>
<td>0.041</td>
</tr>
<tr>
<td>BC2(_{\text{SW}})</td>
<td>3,870</td>
<td>0.6</td>
<td>30</td>
<td>0.034</td>
</tr>
</tbody>
</table>

Figure 1 | Experimental data of the 4NP partition coefficient in the system 2-undecanone water.

Figure 2 | 4NP typical experimental and calculated concentration profiles detected in biocompatibility tests (Test BC1).
Kinetic tests

The batch kinetic tests were performed in order to have a first comparison of the results obtainable with the conventional SBR and the TPP-SBR. The typical concentration profiles detected in a kinetic test are reported in Figure 3 while in Figure 4 the amount of 4NP removed is reported vs. time. It can be observed that in the two phase system the biomass is exposed for all the time to 4NP concentrations that are significantly lower if compared to the conventional system. This is certainly an advantage when the system operates with high concentrations of xenobiotics as it was also observed in our experiments: in fact in the first two tests at 300 and 350 mg/l the reaction time required for complete removal is almost the same while for the highest concentration of 450 mg/l in the one phase system an increase of the reaction time of about 10% is observed with $X = 1,800$ mg/l VSS. The better operation of the two phase system is also confirmed by the higher amount of 4NP removed (see Figure 4) especially in the first half of the kinetic test where the biomass in the conventional system is inhibited by the higher substrate concentration.

Moreover, the potential advantage obtainable with a two phase system is well demonstrated in Figure 5 and 6 showing the concentration profiles and the removed amount, respectively, detected in kinetic tests performed at 450 mg/l 4NP initial concentration and at lower biomass concentration $X = 1,480$ mg/l. In the one phase system the strong inhibitory effect resulting by the higher ratio...
substrate/biomass causes a low 4NP degradation efficiency (about 40%) while in the same time the complete 4NP removal is attained in the two phase system.

Data analysis was performed by fitting of the experimental data with the model for the two phase system (Equations 2 and 4) and on the base of the previous results for the biocompatibility tests the same $b$ and $C_p$ values were assumed. Results are reported in Table 2. Obtained correlation coefficients are quite satisfactory being in the range of 0.97–0.99.

It is observed that the $k_{max}$ value does not change significantly with the initial 4NP concentration and very close values are observed for the conventional and the two-phase systems. This last finding can be explained considering that in the biocompatibility tests the water phase was saturated (24 hours contact time) while in this series of kinetic tests the solvent was added just before the test.

**CONCLUSIONS**

The TPP-SBR configuration was demonstrated to be effective in degrading 4NP and advantageous for concentration $\geq 450$ mg/l. The process performance is dependent on the solvent employed which has to be tested for the specific case but, considering that most xenobiotics are highly soluble in organic solvents and substantially less in water, the task, as in our case, seems not so difficult to achieve. For the target compound, 4NP, 2-undecanone showed satisfactory results both in terms of partition coefficient ($P = 30$) and biocompatibility. Kinetic data analysis of parallel tests demonstrated that the intrinsic parameters are not significantly affected by presence of the solvent, therefore the two phase system is able to operate with the same kinetics as the conventional one but with the relevant advantage of reducing the toxic effect on the biomass.

**REFERENCES**


### Table 2

<table>
<thead>
<tr>
<th>Test</th>
<th>$C_{NP0}$ (mg/l)</th>
<th>$X$ mean (mg/l VSS)</th>
<th>$k_{max}$ (mg4NP mgVSS $^{-1}h^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T11</td>
<td>300</td>
<td>1,990</td>
<td>0.055</td>
</tr>
<tr>
<td>T12</td>
<td>300</td>
<td>1,990</td>
<td>0.056</td>
</tr>
<tr>
<td>T21</td>
<td>350</td>
<td>1,705</td>
<td>0.050</td>
</tr>
<tr>
<td>T22</td>
<td>350</td>
<td>1,705</td>
<td>0.049</td>
</tr>
<tr>
<td>T31</td>
<td>450</td>
<td>1,800</td>
<td>0.066</td>
</tr>
<tr>
<td>T32</td>
<td>450</td>
<td>1,800</td>
<td>0.065</td>
</tr>
</tbody>
</table>

$C_{NP0}$ = initial concentration of 4NP; $b = 0.6$ and $C_p = 30$ mg/l 4NP.