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Biodegradation of 4-nitrophenol in a two-phase sequencing batch reactor: concept demonstration, kinetics and modelling

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Abstract The objectives of this work were to demonstrate the potential of a two-phase sequencing batch reactor in degrading xenobiotics and to evaluate the kinetic parameters leading to a mathematical model of the system. 4-Nitrophenol (4NP), a typical representative of substituted phenols, was selected as the target xenobiotic; this compound has never been remediated in a two-phase bioreactor before. Partition tests were conducted to determine the most appropriate partitioning solvent, and among the three investigated solvents (1-undecanol, 2-undecanone and oleyl alcohol), 2-undecanone was chosen because of its favourable partition coefficient and its negligible emulsion-forming tendencies. Moreover, the selected solvent showed satisfactory biocompatibility characteristics with respect to the biomass, with only minor effects on the intrinsic microbial kinetics. Kinetic tests were then performed in a sequencing batch reactor (2-l volume) operated in both conventional one- and two-phase configurations, with the two-phase system showing a significant improvement in the process kinetics in terms of reduced inhibition and increased maximum removal rate. The obtained kinetic parameters suggest that the two-phase sequencing batch system may find full-scale application, as the maximum removal rate k_{max} (~3 mg 4NP mgVSS^{-1} day^{-1}) is of the same order of magnitude of heterotrophic bacteria operating in wastewater treatment plants.

Keywords TPPB/SBR bioreactor · Xenobiotic biodegradation · 4-Nitrophenol · Industrial wastewater treatment

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Introduction

The application of biological processes to xenobiotic removal is a promising alternative to conventional chemical–physical (i.e. stripping and adsorption) treatment methods that transfer the target compounds from a diluted to a concentrated phase that has to be subsequently treated and disposed of. Biological processes are able, in principle, to attain complete mineralisation of many compounds, but the main limitation of biological xenobiotic destruction is the high concentrations that the biomass can experience, leading to a significant reduction in kinetic performance that is often not acceptable in practical applications (i.e. industrial wastewater treatment). The effects of substrate inhibition can be mitigated with a system that is able to optimise "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. To provide such balanced substrate delivery, the use of two-phase partitioning bioreactors (TPPBs) has been proposed (Collins and Daugulis 1996; Malinowski 2001; Daugulis 2001). TPPBs are based on the use of a water-immiscible and biocompatible organic solvent phase in contact with an aqueous phase containing

the microorganisms. The solvent is able to dissolve large amounts of the target compound(s) (due to the hydrophobic nature of most organic contaminants), which then partition into the aqueous phase at a concentration depending on the partition coefficient. With this configuration, even if the bioreactor is operated with high xenobiotic loads, the microorganisms are exposed to low levels that, in the majority of the cases, are sub-inhibitory. The substrate concentration in the aqueous phase is the result of a delivery process completely driven by the cellular metabolic processes; in fact, when the biodegradation occurs in the aqueous phase, xenobiotic transfers from the solvent to the water phase to restore the thermodynamic equilibrium. The amount of delivered substrate is always the optimal one because the transfer rate is dependent only on the biodegradation kinetics.

The concept of two-phase reactors can be practically realised both in continuous (operating at steady-state conditions) and discontinuous (operating in dynamic conditions) plants, but in the specific case of xenobiotic removal, the latter solution could be preferable. 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 operational flexibility, is a suitable technological solution in order to obtain a versatile microorganism culture able to develop metabolic pathways required in the degradation of xenobiotics (Ellis et al. 1996).

The advantages of combining the two-phase process with SBR technology is a promising area to be investigated as a possible strategy when xenobiotic removal has to be achieved in critical conditions characterised by very high influent concentrations. One typical example is the "remediation of stored xenobiotics" (unfortunately a quite common situation), which is the removal of toxic compounds stored in containers in large amounts arising from industrial closures or of the stockpiling of substances no longer usable (Daugulis 2001).

The objectives of this paper are to demonstrate the potential of a combined TPPB/SBR system in degrading xenobiotics and to determine the kinetic parameters leading to a mathematical model of the system. Kinetic parameter estimation also provides insights into any positive effects that may arise from employing the TPPB/SBR configuration. The target compound chosen for this work was 4-nitrophenol (4NP), which is a typical representative of substituted phenols found in many industrial effluents (arising from the manufacture of explosives, drugs, dyes, phosphororganic insecticides, pesticides and leather colouring). 4NP is also generated in aqueous matrices during formulation, distribution and field application of pesticides, making its environmental impact relevant for both wastewater and groundwater situations.

Materials and methods

Sequencing batch reactors

SBR reactors were glass vessels of 5 (SBR A) and 2 l (SBR B), respectively, with a thermostatically controlled water jacket to maintain the operating temperature at 20±0.5°C. Dissolved oxygen and pH were monitored online 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 achieved by a magnetic stirrer. Air was supplied by variable flow compressors through a glass diffuser.

The timing of the operational sequence and control strategies were automatically operated by a personal computer interfaced to the reactor. Specialised software was developed under the Labview-Windows 3.1 environment to manage the working cycle phases, driving of stirrer, compressors and pumps and dissolved oxygen (DO) and pH monitoring and control by on–off strategies. As a consequence, DO was maintained in the range of 3–4 mg/l and pH in the range of 7.5–8.

A typical SBR operating cycle lasted 12 h distributed as follows: fill, 30 min; reaction, 570 min; wastage, 3 min; settle, 92 min and draw, 25 min. The fill phase operated under mixed and aerated conditions. The exchange factor (added volume/total volume) was 0.5.

The smaller reactor was operated at the same operating conditions but with a two-phase configuration with a solvent/water ratio of 0.1 and 4NP concentration in the feed of 100–450 mg/l. The biomass utilised was taken from the conventional SBR (SBR A).

Analysis

Volatile and total suspended solids concentrations were determined according to standard methods (APHA 1998) for estimation of biomass.

4-Nitrophenol analysis was performed on samples filtered through syringe nylon membrane filters (0.2-μm pore size) and 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.

Bacterial culture

A mixed culture previously acclimatised 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 acclimatisation procedure are reported in previous papers (Tomei et al. 2003; Tomei and Annesini 2005).

To ensure the presence of required nutrients and micro-elements, in all cases, the feed consisted of a pure compound 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.

Chemicals

4-Nitrophenol was in granular form (purity≥98%) and was supplied by Fluka (Italy).

Liquid solvents 2-undecanone, 1-undecanol (purity 99%) and oleyl alcohol (purity 98%) were supplied by Sigma Aldrich (Germany). All other chemicals were commercial grade and supplied by Carlo Erba (Italy).

Test plan

Solvent selection The first part of the experimental activity was focused on solvent selection. Based on literature data available for phenols (Collins and Daugulis 1996, Vrionis et al. 2002), three solvents were utilised: 1-undecanol, 2-undecanone and oleyl alcohol. Partition coefficients were evaluated in batch tests performed in flasks (0.25-l volume) with distilled water at different solvent/water ratio in the range of 0.08–0.2. A thermostatically controlled bath was utilised to maintain the temperature at 20±0.5°C. The initial 4NP concentration was 100 mg/l, and aqueous phase concentrations were measured after a 1-h mixing time that was verified to be enough for reaching equilibrium conditions. Tests were repeated in tap water with the same procedure.

Biocompatibility 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 verify the removal rate of 4NP in water and in a solution saturated with the solvent after a contact time of 24 h. Operating conditions were T=20±0.5°C, 4NP concentration equal to 100 mg/l and biomass concentration $X \sim 4{,}000$ mgVSS/l.

Kinetic tests in the TPPB SBR The SBR B was operated with the TPPB configuration, and in the start up phase, the 4NP feed concentration was progressively increased from 100 to 450 mg/l. Kinetic tests were performed after each concentration step during the reaction phase of the working cycle. 4NP concentrations were measured on filtered samples taken from the aqueous phase of the TPPB and diluted for spectrophotometric reading. Sample filtration avoids interferences with microemulsions that could be present in the water phase. 4NP was measured at time intervals varying from 5 to 15 min, while VSS concentrations were determined at longer time intervals (30–40 min). The solvent-to-water ratio R was 0.1.

In a second aspect of the experimental work, in order to have a direct comparison of the performance of the conventional and two-phase system, the SBR B reactor was first operated in conventional mode and kinetically characterized; then, the solvent was added and kinetic tests were performed working with the two-phase system at the same feed concentration values utilised in the one-phase configuration.

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 was utilised:

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

where X and C_{NP} are the biomass and 4NP concentration, respectively. In this model, three fitting parameters, the rate constant k^* ($M_{NP}M_{VSS}^{-1}\ T^{-1}$) and the saturation and inhibition constants, K_s and K_I (ML^{-3}) are included. In order to give more direct information on the process kinetics (in particular on the maximum consumption rate and on the critical substrate concentration corresponding to the maximum removal rate), the classical form of the Haldane equation (Eq. 1) was rearranged in a different form:

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

In Eq. 2, $C^* = \sqrt{K_s \cdot K_I}$ is the critical 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 (a smaller value of β gives a larger 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 β and C^* in data fitting reduces some numerical problems that are often found with K_s and K_I in applying the classical form of the Haldane equation. The procedure to derive Eq. 2 from Eq. 1 is reported in Tomei and Annesini (2007).

The concentration profile is evaluated from the mass balance equation:

$$\frac{dC_{NPW}}{dt} = -r_s \quad (3)$$

for the conventional one-phase system where the subscript NPW indicates the 4NP in the aqueous phase.

For the two-phase system, with the assumption that equilibrium conditions are instantaneously reached, Eq. 3 is modified as follows:

$$\frac{dC_{NPW}}{dt} = -\frac{V_W}{(V_W + P \cdot V_O)} r_s \quad (4)$$

where P indicates the partition coefficient and the subscripts W and O indicate the aqueous and the organic phase respectively.

In Eq. 4, r_S is evaluated at the substrate and biomass concentration in the aqueous phase where the biochemical reactions occur.

Results

Solvent selection

The first screening of the solvents was done on the basis of literature data reporting organic solvents with satisfactory properties to be applied in TPPB reactors for phenol and aromatic compound biodegradation. The three compounds chosen were 1-undecanol, 2-undecanone and oleyl alcohol and were tested to evaluate their partition coefficient for 4NP. A first qualitative observation of the experimental data for oleyl alcohol showed a marked tendency for this solvent to form emulsions causing difficulties in separating the two phases and consequent uncertainty in spectrophotometric determinations. In contrast, 1-undecanol and 2-undecanone exhibited low emulsion-forming tendency, and further solvent evaluation was therefore restricted to these two compounds.

Experimental data of the partition coefficient tests in distilled water are reported in Figs. 1 and 2 for 2-undecanone and 1-undecanol, respectively, and good data correlation was observed in the two cases (correlation coefficient equal to 0.99 for undecanone and 0.98 for undecanol) with P values of 150 for 2-undecanone and 70 for 1-undecanol.

Since the anticipated application of the system is focused on wastewater treatment, the partition coefficient was also determined for 4NP solutions prepared in tap water, an environment that is more realistic in simulating actual wastewater systems. A significant decrease in the P values (30 and 14 for 2-undecanone and 1-undecanol, respectively), with respect to the values obtained in distilled water, was observed; however, the partition coefficient of 2-undecanone was still higher than that of 1-undecanol. Given very similar phase behaviour of the two solvents and water and the higher partition coefficient for 4NP by 2-undecanone, this solvent was subsequently tested for biocompatibility and utilised in the TPPB.

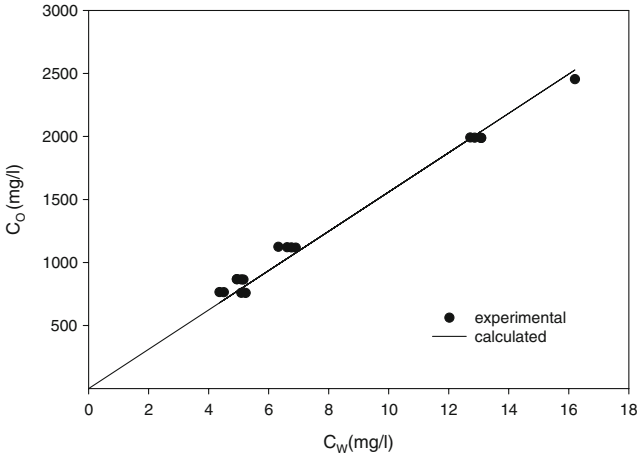

Fig. 1 4NP partition in the two-phase system undecanone-distilled water. C_O=concentration in the organic-phase, C_W=concentration in the aqueous phase

Biocompatibility

The biocompatibility tests were performed in order to evaluate the intrinsic kinetics of 4NP biodegradation in the presence of the solvent in the most unfavourable conditions, that is, in a saturated aqueous solution. This approach is simple to undertake and at the same time allows a rapid estimation of the solvent effects on the process rate. Typical profiles detected in the biocompatibil-

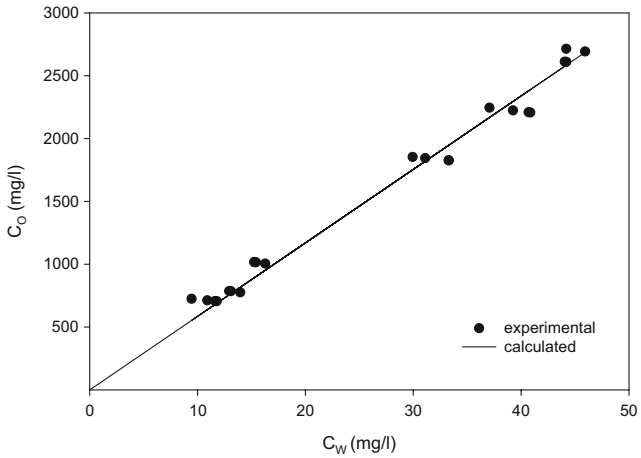

Fig. 2 4NP partition in the two-phase system undecanol-distilled water. C_O=concentration in the organic phase, C_W=concentration in the aqueous phase

ity tests are shown in Fig. 3 for two replicates. The experimental concentration profiles are reported vs. ($X \cdot t$). In this way, it is possible to provide a more direct comparison in terms of specific kinetics by taking into account any minor biomass concentration variations among replicates. It can be seen that, in the first part of the test at higher 4NP concentration, the degradation rates in the two cases are coincidental, while a slight decrease in rate is detected in the second part. In all cases, the time required for complete 4NP degradation (4NP concentrations < 1 mg/l) is practically the same. As a consequence, it was concluded that the tested solvent shows good biocompatibility characteristics and could now be tested in bioreactor operation.

Kinetic tests in the TPPB/SBR

During the start-up phase of the TPPB, the feed concentration was progressively increased from 100 to 450 mg/l. This was achieved over the course of several days by operating the SBR continuously at a fixed feed concentration until stable operating conditions (i.e. complete removal of the compounds in the same reaction time) were achieved. At this point, the kinetic tests were performed over the course of one cycle, after which the feed 4NP concentration was then increased. Because the biomass was already acclimatised to the compound, stable operating conditions were reached quickly, and it was not necessary to perform extensive acclimatisation procedures.

Typical concentration profiles are reported in Figs. 4 and 5 for feed concentration values of 300 and 450 mg/l, respectively, and it can be seen that the 4NP concentrations are always at sub-inhibitory levels, thus demonstrating potential application to more concentrated influent streams. Similarly the reaction time is very short, suggesting that the system could be operated with a reduced number of work cycles/day and/or higher 4NP influent concentration.

Data analysis was performed by fitting the experimental data with the proposed model (Eqs. 2 and 4). According to experimental VSS concentration values, it was assumed that the biomass levels remained practically constant throughout each run and, after a preliminary analysis showing that satisfactory data correlation was obtained for all tests with the same C^* and β values, the fitting was performed on k_{max}. Best fit values and related standard errors (SE) are reported in Table 1, and it can be seen that data correlation was satisfactory with SE always at least one order of magnitude lower than the parameter value. Correlation coefficients are in the range of 0.97–0.99. The fitted curves are also shown in Figs. 4 and 5.

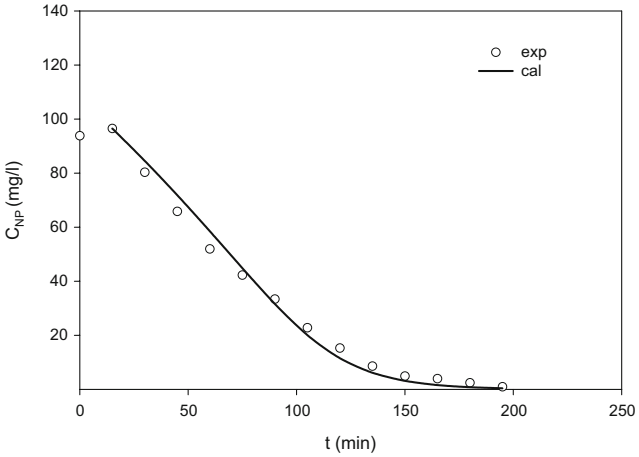

Fig. 4 Experimental and predicted concentration profiles for a kinetic test in the TPPB/SBR. 4NP feed concentration 300 mg/l. *Exp* Experimental data, *cal* calculated profile

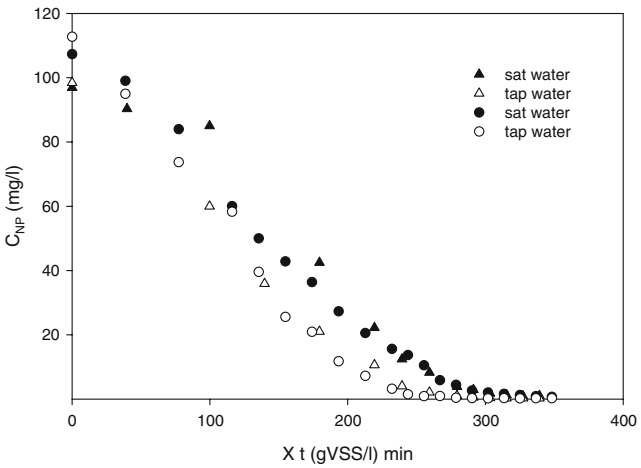

Fig. 3 Biocompatibility kinetic tests (two replicates). Initial concentration of 4NP=100 mg/l, X~4,000 mgVSS/l

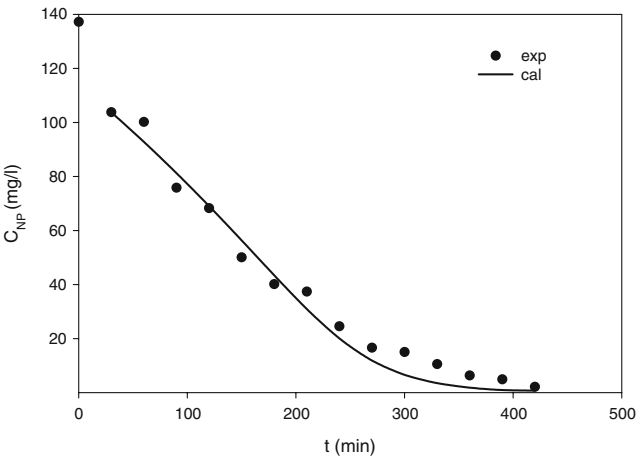

Fig. 5 Experimental and predicted concentration profiles for a kinetic test in the TPPB/SBR. 4NP feed concentration 450 mg/l. *Exp* Experimental data, *cal* calculated profile

Table 1 Best fit k_{max} values for the first series of kinetic tests in the TPPB [R=0.1 (β=0.6, C^*=50 mg/l)]

Influent 4NP (mg/l)	X (mg/l VSS)	k_{max} (mg4NP mgVSS^{-1} h^{-1})	SE
300	1,780	0.123	8.04×10^{-3}
300	1,780	0.108	4.67×10^{-3}
350	1,800	0.101	1.02×10^{-2}
350	1,800	0.111	4.36×10^{-3}
450	1,000	0.154	3.70×10^{-3}
450	1,000	0.160	3.74×10^{-3}

Kinetic test comparison: conventional and two-phase systems

In the second series of kinetic tests, the SBR B reactor was operated in conventional and TPPB configurations under the same operating conditions (mixing, aeration and work cycle, etc.) in order to provide a direct comparison of the process performance in the two cases. Figures 6 and 7 show the concentration profiles obtained in both cases for 4NP feed concentrations of 350 and 450 mg/l. As for Fig. 3, the concentration profiles are reported as C_{NP} vs. $X{\cdot}t$ in order to account for the effect of biomass concentration fluctuations.

An initial lag phase was observed in the conventional configuration mode. In contrast, in the two-phase system, the lag phase was not observed because the biomass is always exposed to lower (certainly sub-inhibitory) substrate concentrations, and the substrate/biomass ratio is low enough to allow the complete and rapid degradation of the available substrate that is gradually replaced.

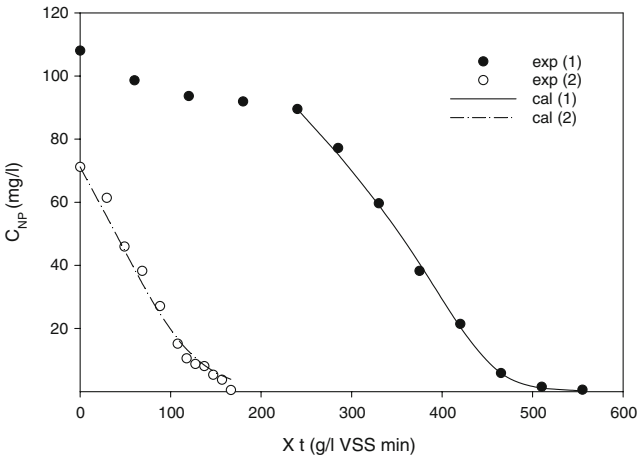

Fig. 6 Experimental and predicted concentration profiles for the kinetic test T1 performed in the SBR reactor operated in the conventional and two-phase configurations. 4NP feed concentration 350 mg/l. *Exp* Experimental data, *cal* calculated profile, *(1)* one-phase system, *(2)* two-phase system

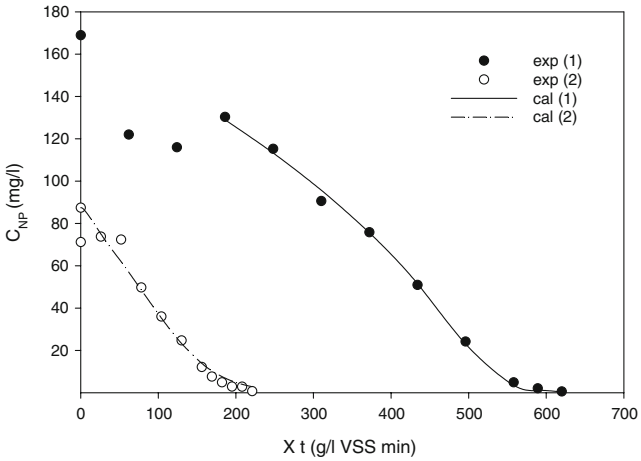

Fig. 7 Experimental and predicted concentration profiles for the kinetic test T2 performed in the SBR reactor operated in the conventional and two-phase configurations. 4NP feed concentration 450 mg/l. *Exp* Experimental data, *cal* calculated profile, *(1)* one-phase system, *(2)* two-phase system

Kinetic data analysis was performed for the two-phase system using the same procedure as was applied for the first series of data. For the conventional system, taking into account the complexity of the possible phenomena occurring in the lag phase and the uncertainty in their modelling, the initial data points of the concentration profile were not included in the kinetic analysis.

On the basis of the previous results reported in Table 1 for the two-phase system and in Tomei and Annesini (2008) for the conventional system, the two parameters β and C^* were assumed equal to 0.6 and 50 mg/l for the TPPB/SBR and 0.6 and 30 mg/l for the conventional SBR; the fitting was performed on k_{max} in both cases. Best-fit parameter values are shown in Table 2, while the fitted curves are also shown in Figs. 6 and 7.

Discussion

The combined TPPB/SBR configuration confirmed the benefits of this processing strategy over a single-phase system in the rapid degradation of 4NP. The performance of such two-phase systems is dependent on the solvent employed, which has to be selected for each specific case; however, in light of the fact that most xenobiotics are highly soluble in organic solvents and substantially less so in water, identifying solvents with high capacities for target molecules is now reasonably straight-forward. We found the two tested solvents, 1-undecanol and 2-undecanone, suitable for application to 4NP removal, but their partition coefficients showed a marked decrease when tap water was employed. A possible reason of this behaviour is given by the different pH values determined in distilled (pH ~5.1–

Table 2 Best-fit parameters values for the series of kinetic tests in the SBR bioreactor working with one-phase (subscript 1) and two-phase (subscript2) configurations

Test	Influent 4NP (mg/l)	X (mg/l VSS)	β	C^* (mg/l 4NP)	k_{max} (mg4NP mgVSS^{-1} h^{-1})	SE
$T1_1$	350	1,480	0.6	30	0.052	5.60×10^{-4}
$T1_2$	350	1,000	0.6	50	0.132	3.48×10^{-3}
$T2_1$	450	1,500	0.6	30	0.054	3.21×10^{-4}
$T2_2$	450	1,300	0.6	50	0.125	3.70×10^{-3}

5.8) and tap water (pH ~7.5–8.1) that cause different equilibrium distributions of 4NP species in the two phases. In fact, 4NP is characterised by an acid dissociation equilibrium, that is:

$$R-OH + H_2O \Leftrightarrow RO^- + H_3O^+ \quad (5)$$

with pK_a= 7.15; as a consequence, the ratio between undissociated and dissociated forms is given by:

$$\frac{[R-OH]}{[RO^-]} = 10^{(pK_a - pH)} \quad (6)$$

and the 4NP fraction present as undissociated form f_i is evaluated from:

$$f_i = \frac{[R-OH]}{[R-OH]+[RO^-]} = \frac{10^{pK_a}}{10^{pK_a}+10^{pH}} \quad (7)$$

As only the undissociated form will be extracted by the organic solvent, the partition coefficient increases with f_i, that is (according to Eq. 7) with a decrease in the pH.

In our case, the P decrease of about a factor of 5 detected in tap water with respect to distilled water for both solvents is in agreement with the ratio of f_i values calculated from the Eq. 7. Even though a significant reduction of the partition coefficient was observed in tap water for the two tested solvents, they are nevertheless suitable for use in a TPPB. The dependence of the partition coefficient on the pH is quite important. Even though it is usual practice to test the partition coefficients of solvents in distilled water, for biological processes, the pH values required for optimal growth conditions of the microorganisms are often around neutrality, so it is advisable to verify the solvent behaviour in conditions that are as close as possible to those of the experimental system.

Assessment of other properties, such as biocompatibility and emulsion-formation tendencies, however, generally still requires experimental effort. For the target compound, 4NP, 2-undecanone showed excellent results both in terms of partition coefficient (P=30 in tap water) and biocompatibility.

In the kinetic study of one- and two-phase configurations, the conventional system showed an initial lag phase that had already been observed in previous experiments when the SBR reactor was operated with short feed times (<60 min; Tomei et al. 2003; Tomei and Annesini 2005). This phenomenon could be attributable to a decrease in biomass activity due to a sudden change of the reaction environment caused by the short feed time (corresponding to a rapid increase of 4NP concentration to which the biomass is exposed) after a period of anaerobic stress (wastage, settling and effluent discharge of the previous cycle). Another possible explanation is the activation of storage phenomena observed with high ratios of substrate/biomass (as happens in the feed phase of the conventional system). According to Daigger and Grady (1982), storage occurs when the biomass, suddenly exposed to high substrate concentration, is not able to accomplish complete biodegradation of the available compound and is converted to storage polymers, which are accumulated in the cells as reserve compounds and subsequently metabolised. The observed lag phase could be the result of a temporary reduced activity of the biomass suddenly exposed to high xenobiotic concentration and/or consumption of storage polymers produced during the feed phase. This explanation was also supported by the absence of the lag phase in the TPPB reactor where the biomass is not exposed to sudden increase of xenobiotic concentration during the feed phase. Moreover, a significant reduction of the reaction time is obtained in the TPPB system that is also presumably attributable to the reduced substrate concentration to which the biomass is exposed during the whole test.

These results confirmed the main advantage of the two-phase system, i.e. the possibility of treating high concentrations of the xenobiotic without altering the biomass activity, and we were able to degrade feed concentrations of up to 450 mg/l. The marked reduction of the reaction time with respect to the single-phase system indicates that the TPPB could be potentially able to treat even higher substrate loadings.

Furthermore, it is worth noting that, for the two phase configuration, besides the reduction of the toxic effect on the biomass deriving from the exposure to sub-inhibitory substrate concentrations, a significant improvement in the process kinetics is also observed. From the kinetic analysis of the data reported in Table 2, this beneficial effect was

detected both in terms of reduced inhibition (increase of C^*) and increased maximum removal rate. It is also important to point out that the obtained kinetic parameters for the TPPB/SBR configuration are potentially scaleable for larger applications, since the k_{max} value is of the same order of magnitude as that assumed for heterotrophic bacteria operating in wastewater treatment plants.

From our results, it can be concluded that the TPPB/SBR configuration provided improved performance over a single-phase system, but some drawbacks were observed that have to be considered in practice. For example, the effluent turbidity and the associated loss of the biomass were increased due to the formation of microemulsions causing the entrapment and removal of microorganisms in the effluent. Occasionally, at the solvent–water interface, depending on the mixing conditions, biofilm formation was observed. To some extent, these emulsions and biofilm formation may be minimised with more accurate regulation of the mixing rate. Additionally, we found indirect evidence for the partial degradation of the selected solvent by the consortium based on a slight increase in oxygen consumption. This phenomenon was essentially limited to the second part of the reaction phase when the 4NP was almost depleted, suggesting that, in the SBR reactor, the effect can be minimised by more precise control of the reaction time to avoid long periods of target substrate depletion that could cause the use of the solvent as an alternative substrate. However, the 4NP was effectively degraded during the entire experimental period, and no appreciable solvent consumption was measured. The phenomenon of parallel solvent biodegradation without affecting the biodegradation kinetics of the target compound has also been seen before in MacLeod and Daugulis (2003), who successfully employed a bioavailable solvent in a TPPB for the biodegradation of polycyclic aromatic hydrocarbons.

The next step in the development of the TPPB/SBR system for 4NP degradation is to examine the potential of replacing the organic solvent phase with solid polymers that will be not only biocompatible but also non-biodegradable, as this approach has recently been shown to be effective with microbial consortia-degrading phenol (Prpich and Daugulis 2005; Prpich and Daugulis 2006). The potential of the two alternatives (liquid solvent or polymers) and the related advantages and disadvantages can then be evaluated and compared. In the case of polymers, the rate at which liquid/solid equilibrium is reached could be affected by low-adsorption/desorption rates arising from the fact that 4NP diffusivity coefficients may be limiting. In this case, mass transfer phenomena could be the limiting step that determines a reduction of the degradation kinetics and of the overall process performance.

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