2,4-Dichlorophenol removal in a solid–liquid two phase partitioning bioreactor (TPPB): kinetics of absorption, desorption and biodegradation

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The applicability of a sequencing batch two phase partitioning bioreactor (TPPB) to the biodegradation of a highly toxic compound, 2,4-dichlorophenol (DCP) (EC_{50} = 2.3–40 mg L^{-1}) was investigated. A kinetic study of the individual process steps (DCP absorption into the polymer, desorption and biodegradation) was performed and, based on favourable absorption/desorption characteristics (DCP diffusivity of 6.6×10^{-8} cm^2 s^{-1}), the commercial polymer Tone P787 (Dow Chemical), was utilized as the sequestering phase for TPPB operation. Batch kinetic biodegradation tests were performed in both single- and two-phase modes, and the Haldane equation kinetic parameters were estimated ($k = 1.3 \times 10^{-2}$ mgDCP mgVSS^{-1} h^{-1}, K_I = 35 mgDCP L^{-1} and K_s = 18 mgDCP L^{-1}), confirming the highly toxic nature of DCP. Consistent with these findings, operation of the single-phase system showed that for an initial DCP concentration of 130 mg L^{-1} the biomass was completely inhibited and DCP was not degraded, while the two-phase system achieved near-complete DCP removal. In sequencing batch mode the TPPB had a removal efficiency of 91% within 500 min for a feed of 320 mg L^{-1}, which exceeds the highest concentration previously degraded. These results have confirmed the effectiveness of the use of small amounts (5%, v/v) of inexpensive commercial polymers as the partitioning phase in TPPB reactors for the treatment of a highly toxic substrate at influent loads that are prohibitive for conventional single-phase operation, and suggest that similar detoxification of wastewater influents is achievable for other target cytotoxic substrates.

Introduction

Chlorophenolic compounds are of serious environmental concern because of their widespread occurrence in industrial wastewater [1] which arises from the production of pesticides, herbicides, dyes, pigments, phenolic resins and paper [2]. Because of their lipophilicity chlorophenols can penetrate cell membranes and bio-accumulate in aquatic organisms [3], are considered harmful for human health for their potential mutagenicity and toxicity, and are listed by the USA EPA as priority pollutants [4]. Several physical–chemical and biological methods have been used to remove chlorophenols from industrial effluents [1] but achieving complete mineralization has proved to be very difficult [5,6]. Biological methods are generally more efficient and cheaper than chemical methods and are in principle able to achieve complete mineralization; however, their efficacy in the case of chlorophenols is strongly affected by the cytotoxicity of such compounds.

In this paper the biological removal of a target compound, representative of chlorophenols, the 2,4-dichlorophenol (DCP), was investigated. DCP is a highly toxic compound characterized by EC_{50} values in the range of 2.3–40 mg L^{-1} determined on *Vibrio fisheri* [7] and on a mixed culture [8], respectively. Few papers on DCP biodegradation have been published in the last decade, with

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the majority employing more complex operational strategies with respect to conventional suspended biomass reactors: that is, bioaugmentation of immobilized cultures [9], biostimulation [10], sludge granulation [11], air lift bioreactors using pure strains immobilized on a honeycomb-like ceramic carrier [12,13] and combinations of chemical and biological treatment [14]. Influent DCP concentration treated in bioreactors are in the majority of cases $\leq$105 mg L^{-1} [10,11,13–15] with the only exception being Quan et al. [9] who reported the biodegradation of an influent at 250 mgDCP L^{-1} using a sequencing batch reactor (SBR) augmented with an immobilized mixed culture. It is clear that effective degradation of high-concentration DCP streams requires alternative operational strategies and innovative technological solutions to achieve high removal efficiencies needed for actual applications.

Two phase partitioning bioreactors (TPPBs) have proven to be an effective technology in reducing toxicity arising from high xenobiotic concentrations in biodegradation processes. TPPBs, which are characterized by a cell-containing aqueous phase, and a second immiscible phase (organic solvent or solid polymer), selectively sequester toxic molecules away from cells when exposed to high substrate concentrations, and deliver these substrates to the biocatalyst based on cell demand [16]. Moreover, the partitioning is driven entirely by the system seeking to maintain thermodynamic equilibrium between the two phases, with uptake by the sequestering phase during substrate addition, and release when the substrate concentration in the liquid phase is decreased due to biodegradation. In contrast to Granular Activated Carbon, which is manufactured to possess a large specific surface area for *adsorption* of organic molecules, the mechanism of molecular uptake by amorphous polymers is via *absorption* or 'dissolution' into the polymer structure, and is not dependant on surface area, only on the mass of polymers used, as the surfaces of polymer beads are smooth [17].

In the bioremediation of contaminated water, where mixed cultures are necessarily utilized, the use of polymers as the sequestering phase is advantageous as polymers are completely biocompatible and inert with respect to the biomass [18], avoiding possible parallel solvent biodegradation that could decrease performance in liquid–liquid TPPBs. Additional advantages of polymers are their exceedingly low cost in comparison to organic solvents and their ease of use, leading to the potentially easy retrofit of conventional biotreatment systems. Commercial polymers have proven to be effective in TPPBs for the treatment of phenolic compounds (i.e. 4-nitrophenol, 3,4-dimethylphenol) [19,20] but have not yet been tested for the biodegradation of highly toxic halogenated aromatics (chlorophenols). The objective of this study was to perform a series of tests on the different process steps involved (i.e. absorption, desorption and biodegradation) to determine the kinetics of each stage and its effect on the overall process rate, and to demonstrate for the first time the applicability of a solid–liquid TPPB system for the biological removal of DCP at high influent concentrations (92–320 mg L^{-1}). These values are representative of real industrial wastewater according to the concentration data of total phenols as reported in Ref. [21] for the chemical industry: phenolic resin production 400 mg L^{-1}, refineries 50 mg L^{-1}, naphthalenic acid production 12 mg L^{-1} and shale dry distillation 200 mg L^{-1}.

Materials and methods

Chemicals
DCP (purity > 99%) was obtained from Sigma–Aldrich (Italy). Tone^{TM} P787 (Dow Chemical Canada Inc.), a poly-caprolactone polyester (density 1.145 g cm^{-3} and melting point 60°C), in the form of roughly spherical beads ($\sim$4 mm diameter) was used. Before use, the polymer was washed with water several times to remove additives and contaminants that can often be present from polymer manufacturing processes.

Tone was chosen on the basis of the results of a preliminary screening test (data not shown) performed with different polymers according to the procedure reported in Ref. [20].

Bacterial culture
The biomass utilized in the experiments originated from a bacterial culture previously utilized for the biodegradation of 4-nitrophenol, and was progressively acclimatized to DCP. It was grown aerobically in mineral medium [22] with a feed initially consisting of DCP and sodium acetate (a source of easily biodegradable substrate) during the acclimatization period. DCP was increased stepwise from 30 to 100 mg L^{-1} and acetate was progressively decreased from 30 to 0 mg L^{-1}. The reactor was then fed with DCP as the sole carbon and energy source at an influent concentration increased up to 250 and 320 mg L^{-1} for the single- and two-phase operation modes, respectively.

Bioreactor
The lab scale SBR consisted of a 1 L glass vessel (0.8 L working volume) with a thermostatically controlled water jacket maintaining the temperature at 25 $\pm$ 0.5°C. Dissolved oxygen (DO) was continuously monitored by a WTW probe (CellOx 325). Customized software was used to manage the SBR working cycle phases, operation of the stirrer, compressors and pumps and DO monitoring and control in the range of 3–4 mg L^{-1} via an on–off strategy.

The SBR operating cycle lasted 12 hours and consisted of the following: feed 15 min, reaction 585 min, wastage 2 min, settling 90 min and draw 28 min. The feed phase was operated under mixed and aerated conditions, and the exchange ratio (added volume/total volume) was 0.5. Additional details of reactor operation are available in Tomei and Annesini [23]. When operating in two-phase mode, the SBR was modified as a TPPB by adding the polymer in the ratio of 5% (v/v). This value for the polymer fraction was in the lower part of the range (1–20%) of values reported in Prpich et al. [18]. A low value was chosen because it is preferable to operate with low polymer fractions to simplify the operability of the system and reduce the energy required for mixing.

Analysis
Biomass concentrations were measured as Volatile Suspended Solids (VSS) according to Standard Methods [24]. DCP was analysed via UV absorbance at 280 nm. In kinetic tests DCP analysis was performed on the supernatant of samples centrifuged for 4 min at 9500 rpm.

Absorption and desorption tests
To estimate the DCP uptake rate batch absorption tests with Tone were performed in duplicate at 25 $\pm$ 0.5°C, in 300 mL sealed glass

flasks with a liquid volume of 250 mL and polymer addition of 5% (v/v). The initial DCP concentration was in the range of 84–100 mg L^{-1}, and mixing was continuously provided by magnetic stirrers. The DCP concentration was measured at time intervals of 10–15 min. The variation of the liquid volume due to the sampling was negligible (<10% for all the samples in each test) because the absorbance reading was performed with micro-cuvettes (volume 1.5 mL). Long-term data (after 24 hours contact time) were considered representative of the equilibrium conditions and were utilized for partition coefficient (PC) determination. The same experimental procedure was used for desorption tests.

Biodegradation tests

Batch kinetic tests were performed in 250 mL flasks with a liquid volume of 200 mL in parallel in single- and two-phase systems (polymer/aqueous ratio ∼ 5%) operating with Tone, and utilizing the biomass from the SBR. The temperature was controlled at 25 ± 0.5°C, and the DCP and biomass concentrations were in the range of 90–130 mg L^{-1} and 2000–3000 mgVSS L^{-1}, respectively. The DCP solution was added to the flasks and rapidly mixed, after which the biomass was added. The flasks were continuously aerated and mixed by magnetic stirrers, and the DCP concentration was measured at time intervals of ∼5–15 min until no appreciable concentration decrease was observed. An abiotic control test was performed under the same operating conditions to evaluate the possible presence of DCP loss via air stripping.

Biodegradation tests were also performed directly in the bioreactor during the feed and reaction phases of the SBR work cycle operated in single- and two-phase configuration to evaluate the kinetics in an environment more representative of a real system. Feed DCP concentrations were in the range of 250–320 mg L^{-1}, and sampling and DCP concentration measurements were undertaken with the same procedures as used in the batch tests.

Polymer washing

To extract and quantify the residual amount of 4NP remaining in the polymer after the biodegradation tests the polymer was washed using a multi-step procedure with 10 mL of methanol per g of polymer for each washing step until the concentration in the methanol was negligible.

Modelling
Absorption tests

To model the abiotic kinetics of absorption in the polymer beads, a Fickian model is considered for diffusion into the beads and no resistance to mass transport in the liquid layer external to the particle surface is considered. This assumption is generally considered to be valid in two phase bioreactors operating with polymers, and a good experimental demonstration of its validity is given in a recent paper [25] with tests performed under different mixing conditions showing that the mass transfer was not externally controlled by the extent of mixing in the aqueous phase, but by substrate diffusion into the polymer.

For spherical particles, the unsteady state mass balance inside the polymer beads is:

$$\frac{\partial C_p}{\partial t} = \frac{D}{r^2}\frac{\partial}{\partial r}\left(r\frac{\partial C_p}{\partial r}\right) \qquad (1)$$

where C_p is the concentration inside the particle, D is the intra-particle diffusivity and r is the radial direction.

The initial and boundary conditions are:

$$0 < r < R \quad t \leq 0 \quad C_p = 0$$

$$r = 0 \quad t \geq 0 \quad \frac{\partial C_p}{\partial r} = 0$$

$$r = R \quad t \geq 0 \quad C_p = \text{PC} \cdot C$$

where R is the particle radius, C is the concentration of the solute in the liquid phase and PC is the partition coefficient defined as the ratio between the DCP concentration in the polymer and the aqueous phases. It is also assumed that the concentration at the particle surface is in equilibrium with the concentration in the bulk liquid phase.

The mass balance for the liquid phase can be written as:

$$-V\frac{dC}{dt} = -N_r|_{r=R}4\pi R^2 n_p \qquad (2)$$

where V is the volume of the liquid phase, N_r is the solute flux entering the polymer beads at the particle surface and n_p is the number of particles.

Eq. (2) can be rewritten as:

$$V\frac{dC}{dt} = -D\frac{\partial C}{\partial r}\bigg|_{r=R}\left(\frac{3V_p}{R}\right) \qquad (3)$$

with the initial condition:

$$t = 0 \quad C = Co$$

where Co is the initial concentration of the solute in the liquid phase.

Knowing the PC value, which is determined from the experimental data, Eqs. (1) and (3) can be analytically solved [26] to calculate the concentration profile in the liquid phase. Furthermore, the fitting of the experimental data with the model allows estimation of the diffusivity in the polymer beads.

Biodegradation process

The biodegradation process was modelled by the classical Haldane equation generally utilized for substrate inhibition:

$$r_s = v\frac{C}{C + K_s + (C^2/K_I)} = k \cdot X\frac{C}{C + K_s + (C^2/K_I)} \qquad (4)$$

where r_s is the removal rate and X and C are the biomass and substrate concentrations, respectively. In this model three fitting parameters, the rate constant k and the saturation and inhibition constants, K_s and K_I are included.

Substrate concentration decrease was utilized to follow the process kinetics because the biomass increase in a work cycle was not appreciable, being one order of magnitude lower than the biomass concentration in the system. The substrate data fitting was performed with the software package Scientist for Windows 3.0 (Micromath).

Results
Absorption and desorption tests

Absorption/desorption experiments constitute the first test to assess the possibility of using polymers as effective partitioning phases; desirable characteristics are high absorption capacity (PC) and, at the same time, fast uptake/release to ensure rapid
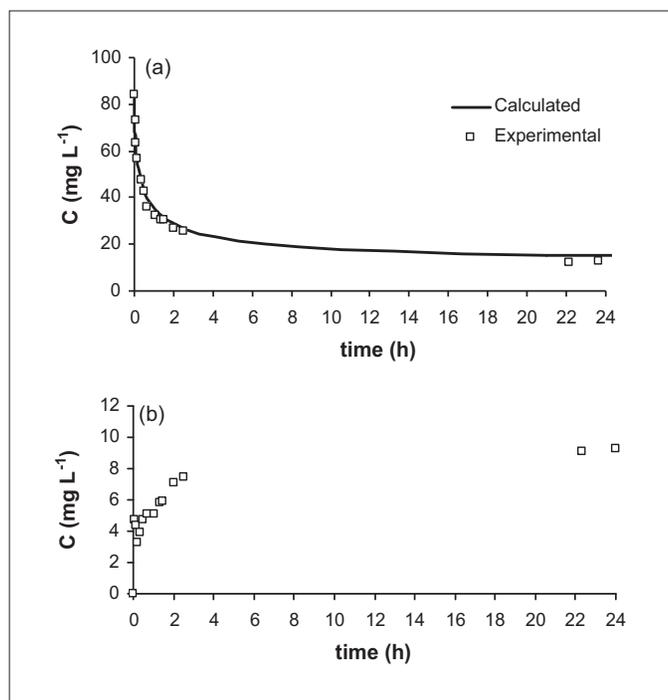
FIGURE 1

Experimental and calculated DCP concentration profiles in absorption **(a)** and desorption **(b)** tests with Tone; initial concentration 84 mg L^{-1}.

FIGURE 2

DCP concentration profiles in a single-phase and two-phase batch biodegradation test **(a)**; initial DCP concentration 92 mg L^{-1}; biomass concentration X = 1850 mgVSS L^{-1}; Tone 5% (v/v). The simulated curve is obtained by fitting of the data with the Haldane equation. Comparison between the concentration profiles detected in the two-phase biodegradation test and in an absorption test at the same initial DCP concentration and polymer amount **(b)**.

detoxification of the aqueous phase and adequate substrate delivery to the micro-organisms. Fig. 1a,b shows the DCP concentration profiles determined in the absorption and desorption tests, respectively, which allowed estimation of the PC value and diffusivity of DCP in Tone. These were found to be 96 and 6.6×10^{-8} cm^2 s^{-1}, respectively. The predicted concentration profile for adsorption, using these values, is also shown and confirms good fit to the experimental data (correlation coefficient $R \geq 0.97$).

Biodegradation kinetic tests

DCP biodegradation was investigated in both single- and two-phase systems with kinetic tests performed in batch flasks and in the TPPB–SBR. Batch tests were preliminarily performed to determine the Haldane equation parameters, and to evaluate the applied substrate concentration that the single-phase reactor could tolerate to avoid inhibition of the biomass. Fig. 2a shows the DCP concentration profile in the single- and two-phase batch tests corresponding to an initial substrate concentration of 92 mg L^{-1}. The contribution of air stripping to DCP removal by the abiotic control test demonstrated that this was negligible (data not shown), as practically no variation of DCP concentration over a five-hour period was observed. The kinetic parameters obtained by data fitting with the Haldane equation and Fig. 2a gave $k = 1.3 \times 10^{-2}$ mgDCP mgVSS^{-1} h^{-1}, $K_I = 35$ mgDCP L^{-1} and $K_s = 18$ mgDCP L^{-1} with very good correlation (correlation coefficient of 0.98); the model fit is also demonstrated by the solid line in this figure. Further, the results in Fig. 2a show the rapid, and extensive detoxification of the system in the two-phase case, relative to single-phase operation. To distinguish between merely absorption of DCP by the polymer and absorption/degradation, Fig. 2b shows a comparison between the experimentally determined DCP
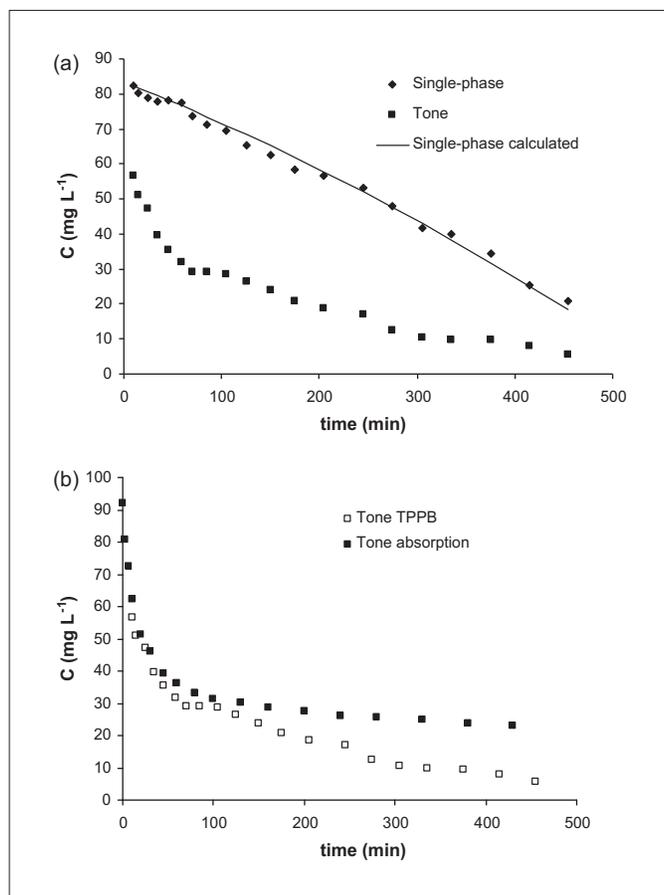
concentration profile in the biodegradation test and in an abiotic absorption test performed at the same initial concentration and percent of polymer, and demonstrates a significant additional removal due to DCP biodegradation.

To evaluate an upper concentration causing serious inhibition in the single-phase system, a second batch test at an increased initial concentration of 130 mg L^{-1} was performed whose concentration profiles are reported in Fig. 3. Poor performance of the conventional system can be seen, with practically negligible DCP removal, relative to the two-phase system.

Such an upper limiting substrate concentration for single-phase operation was also observed in the SBR as is shown by the DCP concentration profile and DO and Specific Oxygen Uptake Rate (SOUR) values for a kinetic test performed in the reactor at a DCP influent concentration of 250 mg L^{-1} reported in Fig. 4a,b, respectively. As the SBR was operated at an exchange ratio of 0.5, the 250 mg L^{-1} feed corresponds to an initial concentration of 125 mg L^{-1} in the SBR after feeding. As expected, the DCP concentration increases in the first 15 min as a result of the feed cycle of the SBR, and no subsequent appreciable DCP removal is observed. The saw-tooth curve in Fig. 4b is caused by the on–off
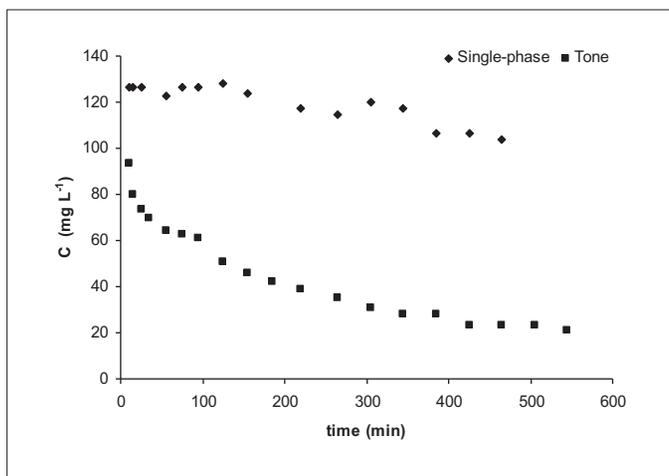
FIGURE 3

DCP concentration profiles in a single-phase and two-phase batch kinetic test; initial DCP concentration 130 mg L^{-1}; biomass concentration $X = 1890$ mgVSS L^{-1}; Tone 5% (v/v).

DO control, which allows a direct calculation of the oxygen consumption rate and an evaluation of the process kinetics in terms of SOUR (also reported in Fig. 4b) from the slope of the descending parts of the oxygen profile. Details concerning the SOUR evaluation procedure are reported in Tomei et al. [27]. In this case, as the DCP is not being degraded, the minimal oxygen consumption seen in this figure may be attributed to endogenous respiration.
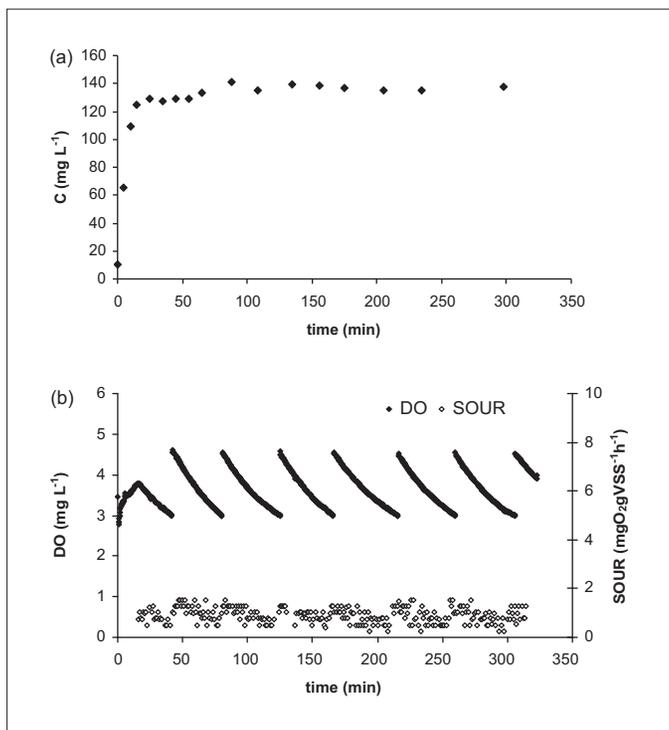

FIGURE 4

DCP concentration profile **(a)** and DO and SOUR values **(b)** detected in a kinetic test performed in single-phase SBR; influent DCP concentration 250 mg L^{-1}; biomass concentration $X = 2340$ mgVSS L^{-1}.
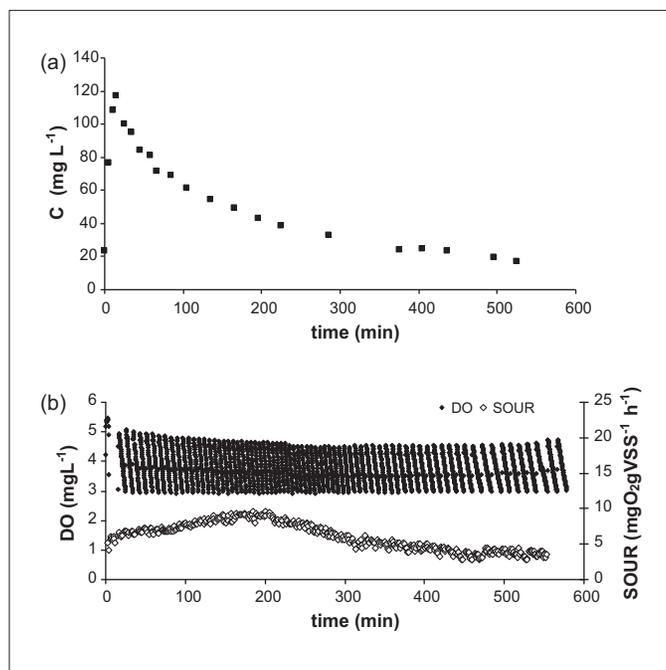

FIGURE 5

DCP concentration profile **(a)** and DO and SOUR values **(b)** detected in a kinetic test performed in a TPPB–SBR with Tone (5%, v/v) as the partitioning phase; influent DCP concentration 320 mg L^{-1}; biomass concentration $X = 2500$ mgVSS L^{-1}.

The reactor was then switched to operate in TPPB mode in the range of feed concentrations of 250–320 mg L^{-1} and in Fig. 5a,b the DCP concentration profile and the DO and SOUR values corresponding to the kinetic test performed at the higher feed concentration (320 mg L^{-1}) are reported. A clear improvement of the process performance is seen for the TPPB reactor, which was able to achieve high removal efficiency (>90%) even at a concentration that exceeded the upper value capable of being degraded in the conventional single-phase system.

Finally to evaluate the substrate mass balance, a multi-step washing procedure was used to remove DCP from the polymer utilized in the TPPB–SBR after 1 month of operation (∼60 work cycles) and it was found that 4% of the total DCP fed to the system was removed in the effluent, 5% was retained within the polymer and 91% was biodegraded.

Discussion

Absorption and desorption tests

The first step to evaluate the suitability of a polymer to be used as the partitioning phase in a TPPB is to determine the absorption capacity for the compound to be removed, which is expressed by the PC, and is the ratio of the concentrations of the compound in the solid and liquid phases at equilibrium. Experimental concentration data measured after 24 hours of contact time (a time verified to be long enough to reach equilibrium) were utilized to evaluate the PCs: a PC value for DCP of 96 was estimated, which is comparable to the partition data reported by Tomei et al. [20] for polymers utilized in solid–liquid TPPBs for the removal of other phenolic compounds.

The second parameter to characterize the sorption capability is the diffusivity of the target molecule in the polymer beads, which

was evaluated by fitting of the experimental data reported in Fig. 1a. Assuming a 2 mm radius for the solid particles, a diffusivity value of 6.6×10^{-8} cm^2 s^{-1} was calculated, a value that is consistent with literature data of diffusivities of phenolic compounds in polymers [17]. Furthermore, a marked reduction of DCP concentration of ~70% was obtained in the first 120 min (Fig. 1a), which demonstrate that the majority of the absorption process takes place in the first two hours of contact, a time interval that is comparable to the biodegradation time of phenolic compounds [23]. This confirms the approximately equal time constants for sorption/biodegradation and supports the suitability of the utilized polymer as the partitioning phase in a TPBB system. In addition to high absorption capacity, the polymers must be able to also provide rapid desorption to ensure prompt substrate delivery to the microorganisms; the desorption curve in Fig. 1b shows that, after 24 hours, the desorption for Tone was practically complete (experimental concentration ~ equilibrium concentration).

Biodegradation kinetic tests

The concentration curves reported in Fig. 2a which correspond to batch tests at an initial concentration of 92 mg L^{-1} show that the conventional system is still able to remove the compound even though inhibition is evident, particularly in the first part of the test during which a relatively low removal rate is observed. A quantitative estimation of the inhibition effect in the single-phase system is given by the kinetic parameters obtained by the data fitting with the Haldane equation; the calculated profile is also shown in Fig. 2a and gave the estimated Haldane parameters of $k = 1.3 \times 10^{-2}$ mgDCP mgVSS^{-1} h^{-1}, $K_I = 35$ mgDCP L^{-1} and $K_s = 18$ mgDCP L^{-1}. These correspond to a critical substrate concentration (i.e. the substrate concentration giving the maximum removal rate for the Haldane equation $C_{cr} = \sqrt{K_s \cdot K_I}$) of 25 mg/L, and confirm the highly toxic nature of DCP. The K_I and C_{cr}, values are comparable to those reported in Sahinkaya and Dilek [15] (44.5 and 24.7 mg L^{-1} for K_I and C_{cr}, respectively), which were obtained with an enriched culture degrading chlorophenols.

A comparison of the two concentration curves of the absorption and biodegradation tests reported in Fig. 2b shows that absorption alone does not account for the higher removal efficiency observed in the presence of the polymer, as DCP degradation can be clearly seen after an initial phase of DCP removal by polymer absorption.

In the second batch kinetic test at an initial concentration of 130 mg L^{-1}, whose concentration profiles are reported in Fig. 3, the single-phase system is significantly affected by the inhibitory nature of DCP, and negligible DCP removal is observed. By contrast, the two-phase batch system is able to achieve very effective (84%) DCP removal.

In Figs 4a and 5a the concentration patterns of the biodegradation tests obtained in the SBR bioreactor operated in conventional and TPPB mode, respectively, highlight the superior performance of the TPPB which achieved almost complete DCP removal at an influent concentration (320 mg L^{-1}), which is 28% higher than the limiting value (250 mg L^{-1}) observed with the conventional system. In addition, the oxygen consumption curves shown for the two SBR tests (Figs 4b and 5b) clearly demonstrate that biodegradation is occurring in the two-phase system, and not merely absorption. The SOURs were in the range of 0.5–1.5 mgO$_2$ gVSS^{-1} h^{-1} for the single-phase case (attributable, as previously indicated, to endogenous respiration) and 4–10 mgO$_2$ gVSS^{-1} h^{-1} in the two-phase system. The maximum SOUR value in the TPPB occurred when the DCP concentrations dropped below 40 mg L^{-1}, that is, the EC$_{50}$ value reported for mixed cultures. The significantly higher SOUR values in the TPPB system are a further indication of effective DCP biodegradation occurring, and a reduction in the DCP inhibition effect on the biomass. The duration of the dynamic DO and SOUR data for the SBR–TPPB also reflect the fact that the polymer is providing a gradual release of the substrate, driven by cellular metabolic activity, demonstrating the other crucial role played by the polymers, that of substrate desorption. The effective polymer performance was maintained for long-term operation as demonstrated by the very low residual amount retained in the polymer (5% of the total DCP amount fed to the system) after one month of operation. Furthermore, it is worth noting that the improved performance was achieved with only a 5% polymer fraction, a very small amount, suggesting that the system efficiency can be further improved by increasing the polymer phase ratio.

Conclusions

Our results can be summarized as follows:

- Biodegradation kinetic parameters ($k = 1.3 \times 10^{-2}$ mgDCP mgVSS^{-1} h^{-1}, $K_I = 35$ mgDCP L^{-1} and $K_s = 18$ mgDCP L^{-1}) for DCP were evaluated with reference to the Haldane equation in conventional single-phase batch tests, and confirmed the highly toxic nature of DCP.

- The biomass in the single-phase system was completely inhibited at a DCP influent concentration of 250 mg/L in the single-phase SBR system.

- High removal efficiency (>90%) was achieved in the TPPB system at a DCP influent concentration >300 mg L^{-1} thereby demonstrating competitive performance in comparison with more complex technologies (i.e. biomass immobilized on ceramic carriers).

- Inexpensive commercial polymers are an efficient substitute for organic solvents in TPPBs, and a very small amount (5%, v/v) was sufficient to detoxify DCP at influent loads that were prohibitive for the conventional single-phase system.

- The ease of use, and re-use, of polymers to detoxify influents suggest the possibility of implementing such an approach in actual conventional wastewater treatment processes which treat cytotoxic substrates.

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