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The use of used automobile tyres in a partitioning bioreactor for the biodegradation of xenobiotic mixtures

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Waste tyres were utilized as the sorption phase in a two-phase partitioning bioreactor (TPPB) for the biodegradation of a binary mixture of 2,4-dichlorophenol (DCP) and 4-nitrophenol (4NP). These compounds are extensively used in the chemical industry and are found in many industrial effluents. Although both compounds are toxic and are on the EPA list of priority pollutants, a higher inhibitory effect on microorganisms is exerted by DCP, and our experimental tests were focused on strategies to reduce its negative impact on microbial activity. Sorption/desorption tests for the DCP–4NP mixture were first performed to verify the related uptake/release rates by the tyres, which showed that the tyres had a higher capacity for DCP uptake and practically no affinity for 4NP. An acclimatized mixed culture was then utilized in a sequencing batch reactor (SBR) operated in conventional and two-phase mode. For the binary DCP-4NP mixture a significant reduction in DCP toxicity, and a concomitant enhancement in substrate removal efficiency (up to 83% for DCP and ∼100% for 4NP) were clearly seen for the TPPB operated with 10% and 15% v/v tyres, for influent concentrations up to 180 mg/L, with practically negligible biodegradation in the conventional single phase reactor. The long-term utilization of tyres was confirmed at an influent loading of 180 mg/L with a test performed over 20 work cycles showing an improvement of the removal performance for both compounds.

Keywords: re-utilization of tyres; mixtures of xenobiotics; two-phase partitioning bioreactors; sequential bioreactors; selective removal

1. Introduction

Two-phase partitioning bioreactors (TPPBs) have proven to be effective in reducing the toxicity of high xenobiotic concentrations in biodegradation processes.[1] TPPBs utilize an immiscible phase (liquid solvent or polymer) within the bioreactor, whose function is to sequester, and gradually deliver, toxic substrate molecules to the microorganisms. In this way the microenvironment of cells is favourably influenced by reduced xenobiotic concentrations in the aqueous phase, and the controlled re-release of the substrates, resulting in significantly enhanced biotreatment performance. In the bioremediation of contaminated water and soils, where mixed cultures are necessarily utilized, the use of polymers as the sequestering phase is highly advantageous as polymers are completely biocompatible and inert with respect to the microbial biomass.[2–4] A new opportunity for this technology in terms of environmental sustainability is to utilize waste polymeric materials as the partitioning phase. Recent experiments have demonstrated the feasibility of this strategy for the bioremediation of hydrocarbons employing used automobile tyres.[5] In a previous paper [6] we reported a first validation of the TPPB-tyres system for the bioremediation of xenobiotics: waste tyres in small

pieces (3–4 mm) were utilized as the sequestering phase in a TPPB for the degradation of 2,4-dichlorophenol (DCP). In this paper we explore the possibility of utilizing tyres for the selective removal of this compound in a mixture with 4-nitrophenol (4NP). These target compounds were chosen as they are extensively used in the chemical industry (i.e. production of pesticides and herbicides) and are found in many industrial effluents. Both compounds are toxic, and are contained in the EPA list of priority pollutants. A higher inhibitory effect on microorganisms is exerted by DCP being characterized by EC_{50} values of 2.4–40 mg/L [7,8] while a value of 64 mg/L [9] is reported for 4NP. The experimental tests were therefore focused on the selective removal of DCP from the mixture to reduce its negative impact and improve microbial activity. That is, in light of the significant differences in toxicity between the two substrates, the aim was to utilize a waste polymer that would specifically target the more toxic one, thereby effecting a greater positive impact on overall biodegradation effectiveness. The objectives of this work were therefore to demonstrate the possibility of usefully employing a waste polymer as the partitioning phase in a TPPB for selectively removing a specific compound in a mixture exploiting

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differences in affinity and, at the same time, to demonstrate the long-term possibility of tyre utilization without loss of effectiveness. The TPPB was operated in sequential mode, this configuration being especially advantageous in dealing with xenobiotic removal.[8,10,11]

2. Material and methods

2.1. Chemicals

4NP (purity >98%) was obtained from Fluka (Italy). DCP (purity >99%) and sodium acetate (purity 99.6%) were purchased from Sigma-Aldrich (Italy). All other chemicals were commercial grade and were from Carlo Erba (Italy). The partitioning phase consisted of shredded used automobile tyres (pieces of 3–4 and 10–15 mm) with a medium density of $0.8 \, g/cm^3$. The tyre pieces are obtained by cryogenic pre-treatment to below brittleness temperature, followed by grinding, and separation of the rubber from the steel and fibre fractions. Prior to use tyres were pre-treated with a multistep water washing to remove residual contaminants, which could be released during our experiments.

2.2. Biomass

The development of the mixed culture utilized in these experiments is detailed elsewhere.[10] An inoculum from this culture, previously adapted to 4NP, was acclimatized over a three-month period to DCP by switching from sodium acetate as the sole carbon and energy source. In the acclimatization phase the DCP concentration was gradually increased (from 60 to 100 mg/L) and sodium acetate was progressively reduced until complete DCP removal was obtained. The microbial consortium was then grown on a DCP and 4NP mixture whose concentration was gradually increased from 50 to 180 mg/L for each compound. In all cases, required nutrients and microelements were supplied by the addition of a mineral medium [12] in the proportion required to ensure a C:N:P ratio equal to 100:5:1 with respect to the DCP and/or 4NP carbon.

2.3. Bioreactor

The bioreactor consisted of a 1 L glass vessel (0.8 L working volume) with a thermostatically controlled water jacket maintaining the temperature at $25 \pm 0.5°C$. Air was supplied by a compressor through a glass diffuser and the dissolved oxygen level was continuously monitored by a WTW probe (CellOx 325), and controlled in the range of 3–4 mg/L by an on-off control strategy. The control system allows the continuous Oxygen Uptake Rate (OUR) evaluation as reported in detail in [10]. Feeding and effluent discharge were performed by peristaltic pumps (Cellai, Perinox SF3) through openings located in the reactor cover. Mixing was achieved by a magnetic stirrer that was able to obtain complete mixing conditions even in the presence

of tyres. The bioreactor was operating as sequencing batch reactor (SBR) with work cycles in the range of 12–18 h controlled by Labview-Windows 3.1 software and distributed as follows: feed 15 min, reaction 660–1020 min, settling 30 min and draw 15 min. The feed phase operated under mixed and aerated conditions. The exchange ratio (added volume/total volume) was 0.5. The SBR was operating as a TPPB by adding used tyres in the range of 7.5–15% (v/v).

2.4. Analytical methods

Analysis of 4NP and DCP was performed on aqueous samples after centrifugation at 10,000 rpm for 6 min; the supernatant was then analysed by UV absorbance at 400 and 280 nm for 4NP and DCP, 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. Volatile suspended solid (VSS) concentration was determined according to standard methods [13] as an estimate of the biomass concentration (X).

2.5. Absorption/desorption tests

The rates of sorption of the phenolic mixture by tyres was investigated in flasks containing 250 mL of a solution at 80 mg/L of each compound prepared with tap water at a tyres/solution ratio of 5% (v/v). The flasks were agitated by magnetic stirrers at $25 \pm 0.5°C$ for 24 h (a time estimated to be sufficient to reach equilibrium conditions, on the base of preliminary tests) and the remaining solute concentrations in the aqueous phase were determined at intervals of 10–15 min. Control tests were performed in parallel under the same operating conditions with tap water and tyres but without added solutes to take into account the possible presence of released substances from the tyres affecting the measurements. Absorbance readings of control samples were subtracted from the corresponding readings of the test samples. Desorption tests were carried out in fresh tap water using the same experimental procedure.

2.6. Partition tests

To determine the partitioning coefficients (PCs, defined as the ratio of the concentration in the solid and aqueous phases) for the two single compounds, parallel tests were performed in duplicate at $25 \pm 0.5°C$ with used tyres by adding 50 mL of tap water solution at 80 mg/L concentration of 4NP or DCP to known amounts of tyres (1, 2, 3 and 4 g) in 100 mL flasks, and mixing with magnetic stirring for 24 h. Parallel control tests were performed in tap water without tyres. The final concentration was measured and PCs were determined by mass balance of the absorbed compounds. DCP partition tests were also performed on tyres of different sizes in order to determine if there was an effect of the specific surface on the uptake/release.

Table 1. Summary of the operating conditions for single-phase (S1–S2) and two-phase (T1–T4) bioreactors; $4NP_{in}$ and DCP_{in} are the influent concentrations and X is the biomass concentration (mean value for the various work cycles).

Test	$4NP_{in}$ (mg/L)	DCP_{in} (mg/L)	X mgVSS/L	% tyre (v/v)
S1	100	100	2760	–
S2	180	180	2000	–
T1	180	180	2440	7.5
T2	180	180	2670	10
T3	180	180	2190	15
T4	180	180	2320	15

2.7. *Biodegradation tests*

Biodegradation rates were determined for the binary mixture in the bioreactor operated in conventional and two-phase modes with tyres as the partitioning phase. 4NP and DCP concentrations in the aqueous phase were measured at predetermined time intervals (5–20 min) during the feed and reaction phases. The VSS concentrations were also monitored but at longer time intervals (hours) due to the very low VSS variations during the tests. Specific oxygen consumption rate (sOUR = OUR/X) was continuously monitored during the reaction phase to evaluate the specific biodegradation rate of the two compounds in mixture as suggested by Orhon and Sozen [14] and Lepik and Tenno.[15] Reproducibility of the data was verified by performing subsequent work cycles (at least 10) under the same operating conditions. A summary of the operating conditions is reported in Table 1. New tyres were used for T1, T2 and T3 tests while tests T3 and T4 were carried out with the same tyres to determine the feasibility of tyre re-use.

3. Results and discussion

3.1. *Absorption/desorption and partition tests*

Absorption/desorption tests were performed on the binary mixture to investigate the behaviour of the two compounds in presence of tyres. Concentration profiles in the absorption and desorption tests with a 5% (v/v) tyre fraction are shown in Figure 1. Negligible sorption of 4NP occurred; therefore no subsequent desorption was observed for this compound. In contrast, for DCP a rapid decrease (about 80% of the final removal) was seen in the first 100 min of the experiment. Similarly, desorption occurred to a corresponding extent in the first 150 min of the test. The time intervals for both sorption and desorption are comparable to the biodegradation times of phenolic compounds [16] suggesting the suitability of tyres, from a mass transfer standpoint, as the partitioning phase in bioreactors for selective DCP removal.

An investigation of the mechanism involved in the uptake/release phenomenon observed with tyres was undertaken with the partition tests performed with different tyre sizes to determine whether adsorption (which is dependent on surface area) or absorption (dependent on tyre mass) was the predominant mechanism.
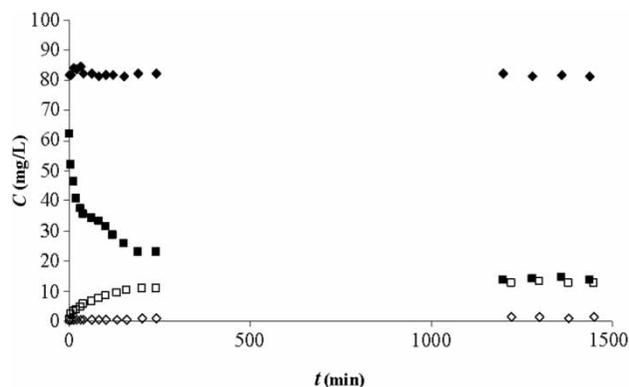

Figure 1. DCP and 4NP concentration profiles in sorption (closed squares and diamonds, respectively) and desorption (empty squares and diamonds, respectively) tests with 5% v/v tyres and initial concentrations of 80 mg/L.
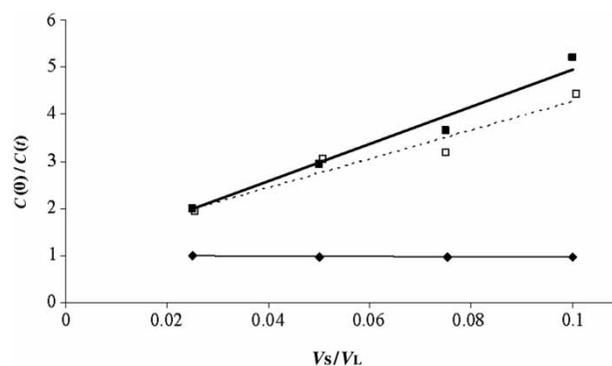

Figure 2. Partition test data for 4NP (closed diamonds and solid line) and DCP (closed squares and solid line for 3–4 mm size tyres and empty squares and dotted line for 10–15 mm size tyres). $C(0)$ = DCP initial concentration, $C(t)$ = DCP concentration at time t, V_L = liquid volume, V_S = solid volume.

Partition coefficients were evaluated from the mass balance equation:

$$V_L \cdot C_0 = V_L \cdot C(t) + V_S \cdot PC \cdot C(t), \qquad (1)$$

where V_L is the liquid volume, V_S the solid volume, C_0 and $C(t)$ are the concentrations in the liquid phase at time 0 (before tyre addition) and t (when equilibrium is reached), respectively. The linearized form of the equation:

$$\frac{C_0}{C_L} = 1 + \frac{V_S}{V_L} PC$$

is reported in Figure 2 as $C_0/C(t)$ vs. V_S/V_L for the two tests, the slopes of the straight lines give the PC values.

Experimental results showed that the partition coefficient for 4NP was $\sim$0 while values of 31 ($R^2 = 0.99$) and 28 ($R^2 = 0.96$) were calculated for DCP, respectively, for small and big tyre sizes. These values are of the same order as the PC values reported for commercial polymer previously utilized in TPPBs for the removal of phenolic compounds.[17]

The small difference of the PC values for the two sizes suggests that surface area available has a small impact on DCP sorption and therefore the predominant mechanism of uptake is absorption. These results are consistent with a recent study [18] which also showed that absorption, not adsorption, is the uptake mechanism for solutes by commercial amorphous polymers when operating above their glass transition temperatures.

3.2. Biodegradation tests

Biodegradation tests were performed on the mixture in both single- and two-phase bioreactors. The single-phase system

was able to achieve satisfactory removal efficiency > 95% for both compounds at a feed concentration of 100 mg/L for each substrate. Figure 3 shows the concentration patterns of the individual compounds and the oxygen consumption rates during test S1, and it can be seen that the less toxic compound, 4NP, was preferentially degraded while DCP removal was delayed. When the feed concentration was increased to 180 mg/L, 4NP was still almost completely degraded while the single-phase system was no longer able to remove DCP, whose concentration in the effluent dramatically increased as seen in Figure 4 which shows the concentration pattern in the feed and reaction phases. In the same figure the strong inhibitory effect of
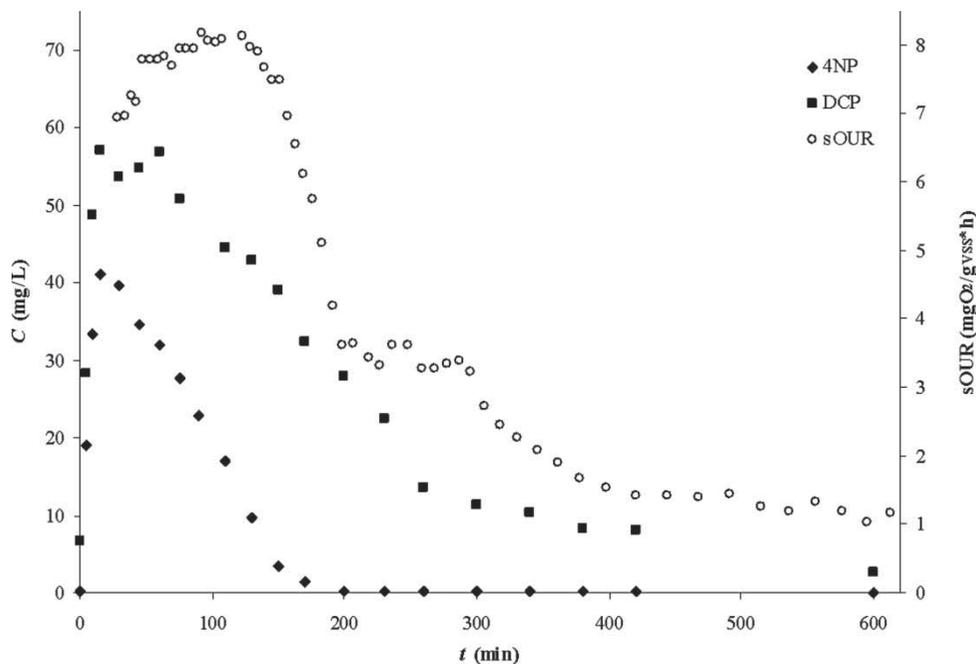

Figure 3. DCP, 4NP concentration profiles and specific oxygen consumption pattern in test S1 (single-phase, influent concentration 100 mg/L for each compound).
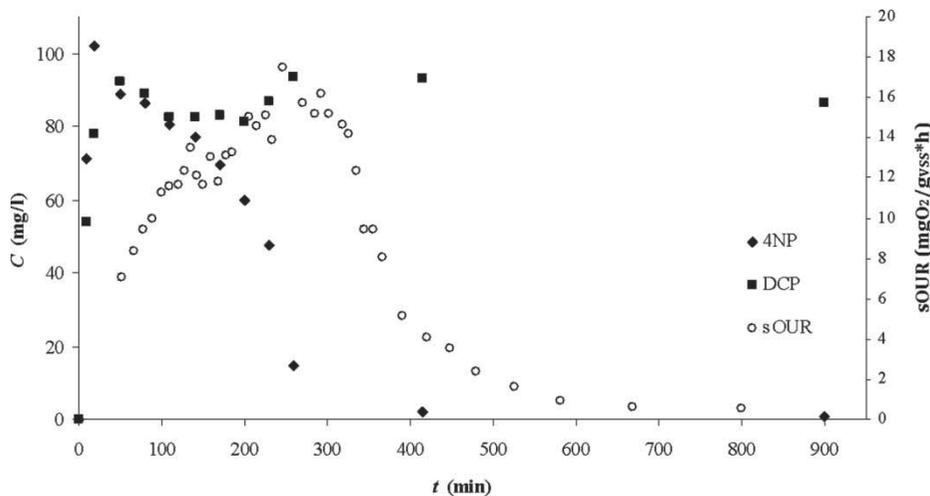

Figure 4. DCP, 4NP concentration profiles and specific oxygen consumption pattern in test S2 (single-phase, influent concentration 180 mg/L for each compound).
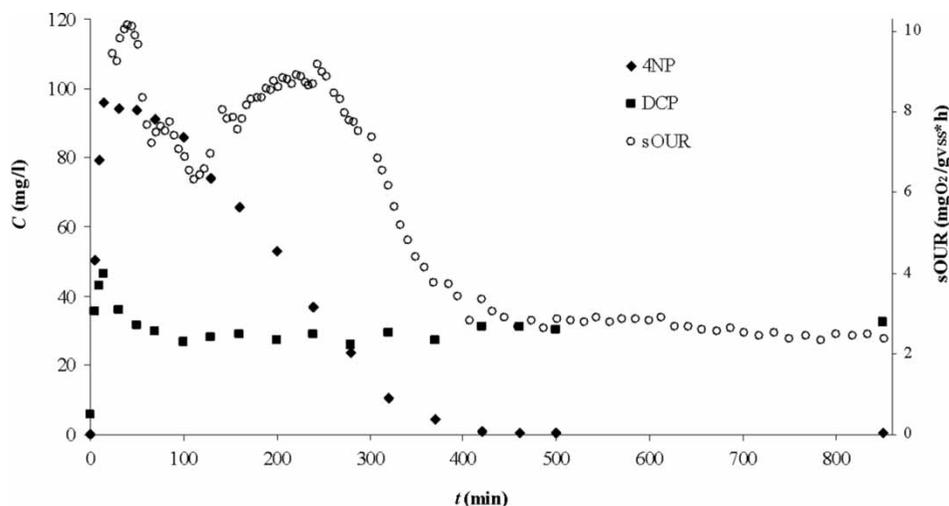
Figure 5. DCP, 4NP concentration profiles and specific oxygen consumption pattern in test T2 (two-phase, influent concentration 180 mg/L for each compound, 10%v/v of tyres).
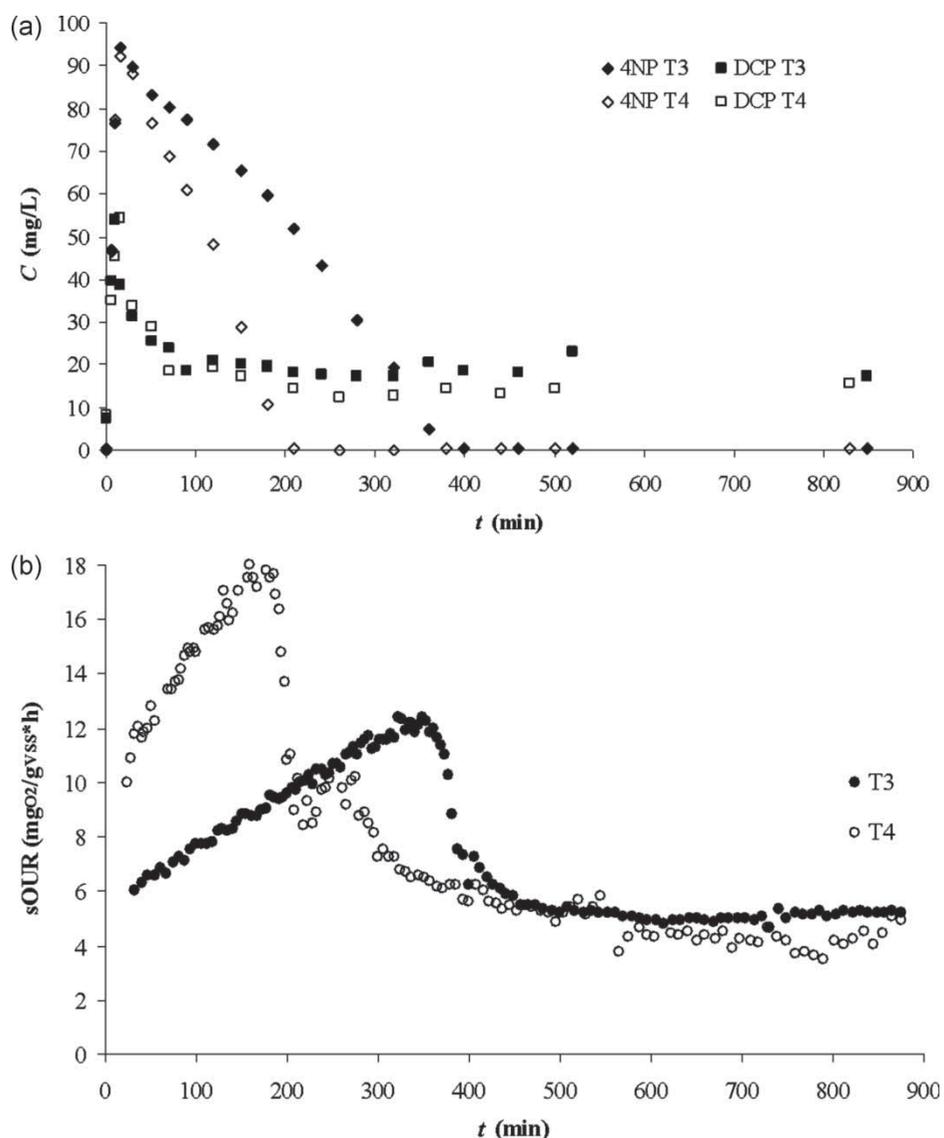

Figure 6. DCP and 4NP concentration profiles (a) and specific oxygen consumption (b) pattern in tests T3 and T4 (two-phase, influent concentration 180 mg/L for each compound, 15%v/v of tyres).

DCP is highlighted by the sudden decrease in the oxygen consumption rate, which reaches values close to zero after the completion of 4NP degradation with practically no DCP removal. After verifying the recovery of the reactor performance at reduced influent concentrations (100 mg/L for each compound) the bioreactor was then switched to work in two-phase mode utilizing tyres as the partitioning phase at a fraction of 7.5% v/v and influent concentrations of 180 mg/ for both 4NP and DCP. As expected from the sorption test, in the two-phase system practically no uptake of 4NP by the tyres was observed, while the DCP concentration was significantly reduced (∼40% removal efficiency). TPPB process performance can be improved by increasing the polymer/aqueous ratio, which was demonstrated by increasing the tyre fraction to 10% (test T2) and 15% v/v (T3). The results show a corresponding improvement of process performance with removal efficiencies of 64% and 81%, respectively. Concentration patterns and oxygen consumption rates are reported in Figure 5 for test T2 and in Figure 6(a) and 6(b) for test T3. The flat DCP concentration profile, in the second part of the tests T1-T3, is likely due to the slow biodegradation kinetics at low concentrations of

DCP, as also previously observed in single compound tests at approximately the same residual level [6] combined with the slow desorption rate. In light of the overall low residual level of DCP in the polymers, and therefore the low concentration driving force for release, along with slow microbial kinetics only the released amount is removed without appreciable variation of the concentration in the liquid phase. The low and relatively constant sOUR value seen in the final part of the test is indicative of the low substrate levels, and the correspondingly limited microbial activity.

In Figure 6 the results of a second work cycle (T4) of the two-phase system after two weeks of operation (20 working cycles) with tyres are reported, and show that good performance is maintained for DCP biodegradation, with even an improvement in 4NP removal. This finding confirms the robustness of the uptake/release mechanism for used tyres and the concomitant beneficial effect of sub-inhibitory conditions for the biomass. The specific oxygen consumption rates measured during the tests effectively demonstrated the better performance of the biomass operating for a prolonged period in the two-phase bioreactor in test T4: higher oxygen consumption rates are reached with a consequent improved removal efficiency for DCP and reduced reaction time of 55% in comparison with test T3 for complete 4NP removal.

An overview of bioreactor performance for the test conditions is shown in Figure 7, in which feed and effluent concentration are reported for both compounds and, for the two-phase bioreactor, the percent tyres employed for each test. Improved performance is evident with the TPPB system in terms of substrate loads handled (increase of 80%) and the flexibility of the TPPB system whose removal efficiencies and removal rates for both compounds can be easily improved by modifying the tyre fraction employed, as it is observed from the data reported in Table 2.

4. Conclusions

Our results confirm the possibility of utilizing a recycled material, such as used tyres, as the partitioning phase in a TPPB system operated with mixtures of toxic compounds. This demonstration was particularly relevant considering the high toxicity of the target compound (DCP), which
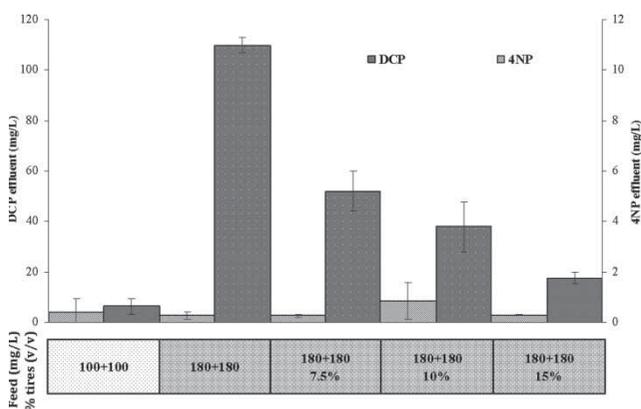

Figure 7. Overview of bioreactor performance: operating conditions (influent and effluent concentrations of the two compounds and percent of tyres) for the consecutive experimental periods. Standard deviations are calculated with the data of the work cycles performed in the corresponding period.

Table 2. Percent removal efficiencies and average specific removal rates observed in the single-phase and TPPB.

| Test | Removal efficiency % | | r_{4NP}[a] (mg 4NP/gVSS h) | r_{DCP}[a] (mg DCP/gVSS h) |
	4NP	DCP		
S1	99.6	95	5.41	1.86
S2	99	4.2	5.31	0.11
T1	99.8	40	5.65	1.68
T2	99.3	64	5.57	1.61
T3	99.7	80.6	6.14	2.47
T4	99.7	82.7	11.05	2.48

[a]Rates referred to the reported removal efficiencies.

is representative of chlorinated phenols. Sorption does not appear to be a limiting step in the overall process kinetics, and the TPPB operated with tyres was able to reduce the inhibitory effect even at influent loads of DCP prohibitive for the conventional single-phase reactor. Finally the high selective effect of this recycled material is particularly noteworthy, as it was able to specifically act on one of the compounds in the mixture. In the case under investigation this was advantageous because we could target and reduce the concentration of the more toxic compound. This feature could be usefully employed in other cases of interest, as for instance to selectively recover one compound in a mixture by a contact stage with a polymer prior to treatment. In light of these findings, work is in progress on the use of mixtures of polymers (commercial and/or recycled) specific for different compounds present in substrate mixtures.

References

[1] Daugulis AJ, Tomei MC, Guieysse B. Overcoming substrate inhibition during biological treatment of mono-aromatics: recent advances in bioprocess design. Appl Microbiol Biotechnol. 2011;90:1589–1608.

[2] Rehmann L, Prpich GP, Daugulis AJ. Bioremediation of PAH contaminated soils: application of a solid-liquid two-phase partitioning bioreactor. Chemosphere. 2008;73:798–804.

[3] Yeom SH, Daugulis AJ, Lee SH. Bioremediation of phenol-contaminated water and soil using magnetic polymer beads. Process Biochem. 2010;45(9):1582–1586.

[4] Tomei MC, Annesini MC, Rita S, Daugulis AJ. Two phase partitioning bioreactors operating with polymers applied to the removal of substituted phenols. Environ Sci Technol. 2010;44(19):7254–7259.

[5] Prpich GP, Rehmann L, Daugulis AJ. On the use, and re-use, of polymers for the treatment of hydrocarbon contaminated water via a solid-liquid partitioning bioreactor. Biotechnol Prog. 2008;24:839–844.

[6] Tomei MC, Annesini MC, Daugulis AJ. Solid–liquid two-phase partitioning bioreactors (TPPBs) operated with waste polymers. Case study: 2,4-dichlorophenol biodegradation with used automobile tires as the partitioning phase. Biotechnol Lett. 2012;34(11):2037–2042.

[7] Ren SJ, Frymier PD. Toxicity of metals and organic chemicals evaluated with bioluminescence assays. Chemosphere. 2005;58:543–550.

[8] Erol Nalbur B, Alkan U. The inhibitory effects of 2-CP and 2,4-DCP containing effluents on sequencing batch reactors. Int Biodeter Biodegr. 2007;60:178–188.

[9] Volskay VT, Grady CPL Jr. Respiration inhibition kinetic analysis. Water Res. 1990;24:863–874.

[10] Tomei MC, Annesini MC, Bussoletti S. 4-Nitrophenol biodegradation in a sequencing batch reactor: kinetic study and effect of filling time. Water Res. 2004;38:375–384.

[11] Elefsiniotis P, Wareham DG. Biodegradation of industrial-strength 2,4-dichlorophenoxyacetic acid wastewaters in the presence of glucose in aerobic and anaerobic sequencing batch reactors. Environ Technol. 2013;34:1167–1174.

[12] Williams TM, Unz RF. The nutrition of *Thiothrix*, Type 021N, *Beggiatoa* and *Leucothrix* strains. Water Res. 1989;23:15–22.

[13] APHA. Standards methods for the examination of water and wastewater. 20th ed. Washington, DC: American Public Health Association; 1998.

[14] Orhon D, Sözen S. Fate and effect of xenobiotics on biodegradation processes: basis for respirometric appraisal. Environ Technol. 2012;33(13):1517–1522.

[15] Lepik R, Tenno T. Determination of biodegradability of phenolic compounds, characteristic to wastewater of the oilshale chemical industry, on activated sludge by oxygen uptake measurement. Environ Technol. 2012;33(3):329–339.

[16] Tomei MC, Annesini MC. 4-nitrophenol biodegradation in a sequencing batch reactor operating with aerobic-anoxic cycles. Environ Sci Technol. 2005;39:5059–5065.

[17] Tomei MC, Rita S, Mosca Angelucci D, Annesini MC, Daugulis AJ. Treatment of substituted phenol mixtures in single-phase and two-phase solid-liquid partitioning bioreactors. J Hazard Mater. 2011;191:190–195.

[18] Craig T, Daugulis AJ. Polymer characterization and optimization of conditions for the enhanced bioproduction of benzaldehyde by pichia pastoris in a two-phase partitioning bioreactor. Biotechnol Bioeng. 2013;110:1098–1105.