Towards a continuous two-phase partitioning bioreactor for xenobiotic removal

M.Concetta Tomei, Domenica Mosca Angelucci, Andrew J. Daugulis

A prototype of a continuous two-phase partitioning bioreactor was investigated.

- The bioreactor contained coiled tubing of a selected extruded polymer, Hytrel 8206.
- Mass transfer and removal of a xenobiotic, 4-chlorophenol, were investigated.
- Removal efficiencies in the tubing wastewater stream were always > 96%.
- Presence of polymer tubing buffered increasing in organic load to the hybrid system.

ABSTRACT

The removal of a xenobiotic (4-chlorophenol) from contaminated water was investigated in a simulated continuous two-phase partitioning bioreactor (C-TPPB), fitted with coiled tubing comprised of a specifically-selected extruded polymer, Hytrel 8206. Wastewater flowed inside the tubing, the pollutant diffused through the tubing wall, and was removed in the aqueous bioreactor phase at typical biological removal rates in the C-TPPB simulated by varying aqueous phase throughput to the reactor. Operating over a range of influent substrate concentrations (500–1500 mg L\(^{-1}\)) and hydraulic retention times in the tubing (4–8 h), overall mass transfer coefficients were 1.7–3.5 \times 10^{-7} m s\(^{-1}\), with the highest value corresponding to the highest tubing flow rate. Corresponding mass transfer rates are of the same order as biological removal rates, and thus do not limit the removal process. The C-TPPB showed good performance over all organic and hydraulic loading ranges, with removal efficiencies of 4CP in the tubing wastewater stream always > 96%. Additionally, the presence of the Hytrel tubing was able to buffer increases in organic loading to the hybrid system, enhancing overall process stability. Biological testing of the C-TPPB confirmed the abiotic test results demonstrating even higher 4-chlorophenol removal efficiency (~99%) in the tubing stream.

1. Introduction

Solid-liquid two-phase partitioning bioreactors (TPPBs) have been demonstrated to be a powerful platform for xenobiotic treatment.
Nomenclature

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Definition</th>
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<tbody>
<tr>
<td>$A_t$</td>
<td>Tubing internal area ($m^2$)</td>
</tr>
<tr>
<td>$A_e$</td>
<td>Tubing external area ($m^2$)</td>
</tr>
<tr>
<td>$C_{ER}$</td>
<td>Substrate concentration in the reactor effluent (mg L$^{-1}$)</td>
</tr>
<tr>
<td>$C_{ET}$</td>
<td>Substrate concentration in the tubing effluent (mg L$^{-1}$)</td>
</tr>
<tr>
<td>$C_0$</td>
<td>Substrate concentration in the tubing influent (mg L$^{-1}$)</td>
</tr>
<tr>
<td>$C_P$</td>
<td>Substrate concentration within the polymer tubing (mg L$^{-1}$)</td>
</tr>
<tr>
<td>$C_R$</td>
<td>Substrate concentration in the reactor (mg L$^{-1}$)</td>
</tr>
<tr>
<td>$C_T$</td>
<td>Substrate concentration in the liquid inside the tubing (mg L$^{-1}$)</td>
</tr>
<tr>
<td>$F_t$</td>
<td>Flow rate in the tubing (L h$^{-1}$)</td>
</tr>
<tr>
<td>$F_R$</td>
<td>Flow rate to the reactor (L h$^{-1}$)</td>
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<tr>
<td>HRT</td>
<td>Hydraulic retention time</td>
</tr>
<tr>
<td>$k_0$</td>
<td>Overall mass transfer coefficient (m s$^{-1}$)</td>
</tr>
<tr>
<td>$k_R$</td>
<td>Mass transfer coefficient (reactor side) (m s$^{-1}$)</td>
</tr>
<tr>
<td>$k_T$</td>
<td>Mass transfer coefficient (tubing side) (m s$^{-1}$)</td>
</tr>
<tr>
<td>$P$</td>
<td>Polymer-water partition coefficient</td>
</tr>
<tr>
<td>$r_t$</td>
<td>Tubing internal radius (m)</td>
</tr>
<tr>
<td>$r_o$</td>
<td>Tubing external radius (m)</td>
</tr>
<tr>
<td>$V_{ER}$</td>
<td>Volume of the collected reactor effluent (L)</td>
</tr>
<tr>
<td>$V_{ET}$</td>
<td>Volume of the collected tubing effluent (L)</td>
</tr>
<tr>
<td>$V_P$</td>
<td>Volume of the polymer tubing (L)</td>
</tr>
<tr>
<td>$V_R$</td>
<td>Reactor liquid volume (L)</td>
</tr>
<tr>
<td>$V_T$</td>
<td>Internal tubing volume (L)</td>
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</tbody>
</table>

Subscript

- in: Influent
- out: Effluent

of operation is based on the membrane extraction and permeation of the inhibitory/toxic organic compounds, which are then biologically removed in a dedicated bioreactor, while the residual wastewater stream can be treated with different, specific processes suitable for the inorganic pollutants [8,9]. The extraction device utilized in EMBS is a membrane of silicon-rubber permeable to the organic species (i.e. aliphatic or aromatic compounds) but non-permeable to water and ionic species such as metals, provided in the form of parallel modules or continuous tubing, in the bioreactor [10]. The wastewater flows inside the tubing, and the organics diffuse through the membrane into the bioreactor, where they are biodegraded by highly specialized microbial cultures under controlled conditions. In this way, the biotreatment environment is not negatively affected by the characteristics of the original wastewater (i.e. extremes of pH, ionic strength, salts, metals), which can negatively affect biological process performance.

The same principle of operation could be applied in a continuous TPPB (C-TPPB) systems in which the tubing is comprised of a specifically-selected extruded polymer, thereby combining the advantages of high polymer affinity for target substrates, with the controlled reaction environments characterized by EMBS. The use of rationally-selected polymer tubing in C-TPPBs could extend the application to the treatment of broad classes of pollutants, arising from the “tailoring” of the polymer for specific contaminants as has recently been demonstrated using first principles’ thermodynamic methods [11]. Previous studies of EMBS have mainly been focused on the removal of VOCs [12,13], and the bioreactors were operated with a silicone rubber membrane, which was effective for these hydrophobic compounds, but could be limited by the solubility capabilities of the membrane used [14], and the limited capacity of this single type of material (silicone rubber) to sorb other types of important organic molecules such as phenols [15].

The complexity of C-TPPB operation, involving mass transfer in series with biological removal, requires an accurate characterization of the system to determine the mass transfer coefficients and to evaluate the response to operating range of conditions expected during operation. Performing this first characterization step under abiotic conditions eliminates potential interferences caused by the presence of biological materials, often possessing case-specific characteristics, which could increase the uncertainty of the results. Abiotic tests have been widely reported in the scientific literature to characterize the performance of different bioreactor configurations prior to undertaking biotic testing, i.e. for granite-based bioreactors for autotrophic nitrogen removal [16], down flow jet loop bioreactors [17], photobioreactors for microalgae cultures [18], air lift bioreactors [19], biotrickling filters packed with polyurethane foam for off gas treatment [20]. Abiotic characterization studies have been also performed prior to biotic operation for different types of membrane bioreactors i.e. submerged membrane bioreactors for hydrogen bioproduction [21], hollow fibers membrane bioreactors for syngas fermentation to biofuel [22], and EMBS [23,24].

The aims of this study are to operate a prototype C-TPPB to investigate the partitioning of a target xenobiotic compound, 4-chlorophenol, by operating a bioreactor containing coiled Hytrel 8206 tubing over a range of influent flowrates and substrate concentrations within the tubing. 4CP was selected to be representative of chlorophenols, contaminants of serious environmental concern because of their widespread occurrence throughout the environment [25]. 4CP is often chosen as a model non-growth substrate combined with cometabolites such as phenol or readily degradable substrates [26].

The first part of the study focused on tubing characterization in terms of substrate–polymer partition coefficient and mass transfer properties, followed by removal tests performed with varying flowrates through the external bioreactor to determine whether removal [1,2], and to be effective in reducing substrate toxicity for many classes of pollutants found in industrial wastewater. The self-regulating nature of substrate uptake and metabolic-driven substrate release characterizing their operation has been successfully demonstrated for hydrocarbons [3], substituted phenols [4], PCBs [5] and PAHs [6]. TPPB operation in the treatment of aqueous contaminants has mainly focused on batch and fed batch operation, and granular commercial polymers typically in the form of beads have been employed as the solid partitioning phase. One critical aspect of TPPBs is the flexibility in their operation provided by the use of a polymer, as the type, amount, and shape of the polymer can be modified and adapted to each specific case.

In terms of shape, the recently proposed possibility of extruding thermoplastic polymers into tubing opens new opportunities in operating TPPB bioreactors as continuous systems. This possibility has been exploited in a recent study on the bioproduction of benzaldehyde, which demonstrated the advantageous use of a Hytrel 3078 polymer tubing in a pervaporation system for continuous product recovery [7].

A similar process scheme could be applied to contaminated liquid streams containing groups of diverse types of pollutants including organic (potentially biodegradable) and inorganic (i.e. heavy metals, salts) components, which would normally be incompatible for treatment using a single approach as occurs in conventional treatment plants. The selective extraction/partitioning of target organic substrates, mediated by carefully selected polymer tubing, and separate targeted treatment, could provide a means of handling such diverse mixtures of contaminants. A similar strategy has been proposed and applied in extractive membrane bioreactors (EMBs) [8]. The EMB principle
the mass transfer properties of the tubing are potentially adequate to match simulated biodegradation rates. The feasibility of the proposed C-TPPB was confirmed with a biological test performed under the same operating conditions as the abiotic ones with a microbial culture acclimatized to the target compound.

2. Materials and methods

2.1. Target compound

4-chlorophenol (4CP), (CAS number 106-48-9, molecular weight 128.56 g mol\(^{-1}\) and purity >99%) was selected as the target compound and was purchased from Sigma-Aldrich (USA).

2.2. Analysis

Measurements of 4CP concentrations were performed on aqueous samples, after centrifugation (8 min at 13000 rpm), by using a spectrophotometer (PerkinElmer, Lambda 25), according to the procedure suggested for chlorophenols by da Silva and Laquipai [27]. From the UV-spectrum in the wavelength range of 190–410 nm (data not shown) the peak for 4CP was at 279 nm, thus this wavelength was utilized for the analysis.

Volatile suspended solid (VSS) concentration was determined according to standard methods [28] as an estimate of the biomass concentration in the bioreactor. Chloride concentration in the liquid phase of the bioreactor was measured with an ion chromatograph (DX-100 Dionex) according to standard methods [28].

2.3. Polymer tubing

Commercial polymer Hytrel 8206 was selected based on its high partition coefficient for 4CP reported for polymer beads, i.e. 303 according to Tomei et al. [29], and on its polymer processing ability for tubing extrusion. Du Pont, Canada kindly supplied extruded tubing, with internal and external diameters of 5 and 6 mm, respectively.

Multistep washing was carried out before use to remove any residual impurities from polymer fabrication, with the tubing first washed with methanol under vigorous stirring conditions, then 5 subsequent washing steps with distilled water. After pretreatment, the tubing was dried overnight in a fume hood.

2.4. Reactor

Mass transfer and removal tests were undertaken in the prototype reactor consisting of a glass vessel (working volume of 5 L) equipped with a magnetic stirrer and a thermostat for temperature control (25 ± 0.5 °C). The reactor contained a support for the spiral-wound tubing (length of 3.5 m), and the tubing/working volume ratio was 2% (v/v), i.e. of the same order of magnitude as for granular polymers previously employed in TPPBs [4,30,31].

An aqueous solution of 4CP was fed to the tubing by means of a peristaltic pump (Watson-Marlow, Cellai, Italy), suitable for low flow rates (in the range of 0.01–0.1 L h\(^{-1}\)) and the effluent was collected in a sealed glass flask.

In the abiotic tests, biological removal rates were simulated by applying different flow rates to the external reactor, and storing and analyzing samples of the reactor effluent. A biological kinetic test was also conducted to complete the feasibility study of the prototype. A schematic representation of the experimental apparatus is given in Fig. 1.

![Fig. 1. Schematic representation of experimental set up. VR=reactor volume; FT=Influent flow rate; FEffluent flow rate; S=storage tank. Subscripts: R=reactor; T=tubing.](image)

2.5. Test plan

2.5.1. Partition tests

Initially, small amounts of the polymer tubing were cut into small pieces (average size of 1–2 mm) and different amounts (0.1–0.3 g) were added to 20 mL of a 4CP solution (initial concentration of ~100 mg L\(^{-1}\)) in flasks, and kept under mixed conditions (320 rpm) for 24 h.

Liquid samples were collected after 4 and 24 h, and analyzed for 4CP. More details on the experimental procedure and data analysis are reported elsewhere [32].

Loaded polymer pieces, after the partitioning test, were added to fresh water for desorption, and the liquid phase was monitored for 4CP with the same procedure described above.

2.5.2. Mass transfer tests

In the first test MT1, performed under static conditions, the tubing (length = 3.5 m) was initially filled with a solution of 4CP (~500 mg L\(^{-1}\)) and the ends closed; then the reactor was filled with fresh water to completely immerse the tubing, and maintained under mixed conditions (320 rpm) for the next 2 days. The liquid concentrations inside the tubing and in the reactor were regularly monitored versus time. Only a few samples of the liquid inside the tubing were analyzed, in order to ensure negligible volume change in comparison to the internal tubing volume.

For the second test, MT2, the tubing was initially filled with a solution of 4CP (~500 mg L\(^{-1}\)), then it was continuously fed with the same fresh 4CP solution at a flow rate of 0.013 L h\(^{-1}\) (corresponding to a HRT of ~6 h). The 4CP concentration was regularly monitored both in the reactor and in the tubing, and the effluent from the tubing was collected and analyzed for the mass balance.

2.5.3. Abiotic removal tests

Starting with the tubing filled with the feed solution (range of nominal values 500–1500 mg L\(^{-1}\)), a hypothetical biological removal process was simulated by pumping a water stream to/from the bioreactor at different flow rates to mimic biological degradation of the target compound diffusing from the liquid inside the tubing through the polymer walls into the reactor, and being consumed (i.e. in this case removed from the bioreactor by aqueous
flow. Samples of the reactor and the tubing effluent were analyzed for 4CP concentration at time intervals of 15–30 min over the 6 h duration of the experiments. Effluent streams from the tubing and reactor were analyzed for 4CP periodically and at the end of the test, and the data were utilized for mass balance calculations.

Removal tests were performed under fully mixed conditions (320 rpm) at different reactor flow rates ($F_R$) (runs A.1–3), different 4CP load (run B.4–6), and different tubing flow rates ($F_T$) (runs C.7–9), according to the test plan reported in Table 1. The selected HRTs are in the range of values employed in biological processes for wastewater treatment, which are of the order of hours. Designations A.2, B.4 and C.8 (in italics in Table 1) indicate the same experiment, which is reported more times because its operating conditions are of relevance in the three series of tests.

### 2.5.4. Biotic removal test

The tubing was filled with a 4CP solution of ~500 mg L$^{-1}$, then a continuous flow of 0.013 L h$^{-1}$ at the same concentration was applied for 96 h. To ensure completely mixed conditions in the bioreactor agitation in the presence of biomass, the agitation speed was increased to ~400 rpm. An inoculum previously acclimated to 4-chlorophenol [31] was added to the bioreactor, which was operated at a biomass concentration of ~2 gVSS L$^{-1}$. A concentrated solution of a nutrient medium [33] was added daily to the external liquid phase of the bioreactor to ensure the optimal C:N:P ratio of 100:5:1. Two replicates of the test were conducted according to the operating conditions reported in Table 1 (run BIO), and with the same procedure as the abiotic removal tests by withdrawing periodic samples from the tubing, bioreactor and effluent tank. The biomass concentration was monitored daily, and, according to Caldeira et al. [34], biological removal of 4CP was evaluated by following the chloride concentration evolution in the bioreactor.

### 2.6. Mass balance

Mass balances allow quantification of the 4CP distribution during the test. At any time $t$, the compound is distributed in:

- the liquid phase in the reactor
  \[ V_R C_R \]  
  where $V_R$ is the reactor volume and $C_R$ is the 4CP concentration in the reactor at time $t$

- the inside tubing (evaluated assuming the average value of in and out concentrations at time $t$)
  \[ V_T C_T \]  
  where $V_T$ is the internal tubing volume and $C_T$ is the 4CP concentration at time $t$

- the effluent from the tubing, which was collected during the test from $t=0$ and $t=t$

  \[ V_T C_C \]
  where $V_T$ is the stored effluent volume and $C_C$ the related 4CP concentration at time $t$

  \[ V_T C_E \]
  where $V_T$ is the stored effluent volume and $C_E$ the related 4CP concentration at time $t$

The difference between the fed amount (including the initial mass in the tubing):

\[ F_T C_0 + V_T C_0 \]  
and the amounts 1, 2, 3, 4 (all measured) gives the amount absorbed into the polymer tubing $\Delta A$ in the time interval $0-t$.

For the biological test, term (4) was replaced by the amount of 4CP biologically degraded, estimated through the chloride balance in the liquid phase in the time interval $0-t$.

### 2.7. Evaluation of mass transfer coefficients

#### 2.7.1. Batch tests

Data from the batch test MT1 were correlated with a system of differential equations describing the mass transfer among the three phases in the system: i.e. the liquid phase in the tubing, the polymer tubing, and the liquid phase in the reactor.

The mass transfer between the liquid phases in the tubing and in the reactor is modeled as a two-step in-series process described as reported in the following.

Mass transfer tubing liquid phase–polymer:

\[ \frac{dC_T}{dt} = k_T a_{TP} \left( \frac{C_P}{P} - C_T \right) \]  
Mass transfer polymer–reactor liquid phase:

\[ \frac{dC_R}{dt} = k_R a_{PR} \left( \frac{C_P}{P} - C_R \right) \]  
Mass balance in the polymer:

\[ \frac{dC_P}{dt} = R_{TP} \frac{dC_T}{dt} + R_{RP} \frac{dC_R}{dt} \]  
where $P$ is the 4CP partition coefficient polymer tubing – water, $a_{TP} = A_{Ti}/V_T$, the specific internal tubing surface area, $a_{PR} = A_{Te}/V_R$, the specific tubing external area, $R_{TP} = V_T/V_P$ and $R_{RP} = V_R/V_P$, and $k_T$ and $k_R$ are the mass transfer coefficients. Boundary conditions for integration are:

at $t=0$ C$_P = 0$ C$_T = 0$ C$_0$

Data fitting was performed by Scientist 3.0 for Windows (Micromath). The overall mass transfer coefficient $k_O$ (related to the internal tubing area) from the tubing liquid phase to the reactor liquid phase can be estimated by:

\[ \frac{1}{k_{O TP}} = \frac{1}{k_{T TP}} + \frac{1}{k_{R TP}} \]  

### 2.7.2. Plug flow tests

In continuous fed tests, i.e. MT2 and all removal tests, the mass transfer coefficient was evaluated assuming that the liquid flowing inside the tubing was in plug flow, and the bulk liquid in the reactor is perfectly mixed. According to Freitas Dos Santos and Livingston [9], who operated with an extractive membrane bioreactor
to detoxify wastewater containing VOCs, the resulting expression for $k_O$ is:

$$k_O = \frac{F \ln \left( \frac{C_{t_m} - C_R}{C_{t_m} - C_{r_m}} \right)}{2 \pi r_L}$$

3. Results and discussion

3.1. Tubing characterization: partition coefficient

Results are shown in Fig. 2 and reported as the linearized form of the mass balance referred to 4 and 24 h. Details on the linearization procedure are reported elsewhere [32], and partition coefficients were determined both in sorption and desorption tests, with high correlation coefficients ($R^2 > 0.992$). It is interesting to observe that equilibrium was almost complete after 4 h, and only a small variation of $P$ (from 328 to 349 in sorption tests and from 381 to 313 in desorption tests) is detected after 24 h. The comparison with the granular Hytrel 8206 shows very close $P$ values (i.e. 303 vs 349 and 313) for beads and tubing pieces, respectively.

3.2. Tubing characterization: mass transfer properties

Mass transfer tests were carried out in two operation modes with respect to the state of liquid in the tubing, with the first (MT1) in static conditions i.e. there is no liquid flowing inside the tubing and the second (MT2) in dynamic conditions with a liquid flow...
inside the tubing. The second can be considered as a reference test for the following substrate removal tests, where, in addition to the internal tubing flow, an external liquid flow on the bioreactor side is applied. The results, reported in Fig. 3 and Fig. 4 for MT1 and MT2, respectively, show the change vs time of the 4CP concentration in the liquid phase inside the tubing and in the reactor.

In Fig. 3 the concentration in the polymer, evaluated from the mass balance, is also reported. The data show that, after 30 h, less than 1% of the initial amount of 4CP remains inside the tubing for both replicates. The residual 4CP amount is distributed between the liquid phase in the reactor (53%), and the amount absorbed in the polymer (46%), with only a very low fraction (~1%) still in the liquid inside the tubing.

The MT1 data have been correlated with the differential system of Eqs. (6)–(8), and the simulated concentration profiles, reported in Fig. 3, show a very good prediction of the experimental data ($R^2 \geq 0.98$ for all the concentration profiles). The best fit data for $k_T$ and $k_O$ are $2.6 \times 10^{-7}$ and $1.7 \times 10^{-5}$ m s$^{-1}$, respectively, which gives a $k_O$ equal to $1.4 \times 10^{-7}$ m s$^{-1}$.

Analogous results have been obtained for the MT2 test, and the evaluated mass transfer coefficient is $2.341 \times 10^{-7}$ m s$^{-1}$.

The $k_O$ values for MT1 and MT2 are in the range of values ($0.5–14.7 \times 10^{-7}$ m s$^{-1}$) reported for the selective extraction of phenolic compounds in tubular silicon rubber membranes immersed in an extracting solution [35], and therefore suitable for application for extractive bioreactors. In Fig. 4 the zoom of the bottom part of Fig. 4a, reported in Fig. 4b shows that ~24 h are sufficient to reach equal concentrations inside and outside the tubing. Previous studies on biological removal of substituted phenols in conventional single-phase and two-phase partitioning bioreactors demonstrated that this time is compatible (i.e. not limiting) with the biological reaction characteristic times [36].

3.3. Removal tests

Operating conditions for the removal tests were determined by specifying the external flow rate entering and leaving the bioreactor in order to achieve substrate removal rates in the range of values from 0.7 to 8 mg g$^{-1}$ VSS h$^{-1}$ [31,37] obtained in single and two-phase fed batch bioreactors degrading 4CP with a biomass concentration of 1.5–3 g L$^{-1}$. This criterion allowed simulation of an actual biological system.
In the first group of removal tests (A.1–3) the applied removal rate was varied by modifying the external flow rate in the range of 0.7–6.2 L h\(^{-1}\), i.e. over about one order of magnitude. Fig. 5(a-b) shows the concentrations in the effluent from the tubing while Fig. 5c displays the concentrations in the reactor. These concentration profiles suggest that steady state operation is reached after \(\sim 5\) h. In the three tests no appreciable difference in concentration was observed in the effluent from the tubing (see the zoom in Fig. 5b), thus, for the applied load, even the lowest removal rate is sufficient to reach removal efficiencies in the range of 95–96%. Concentrations in the reactor are always very low \(\leq 3.5\) mg L\(^{-1}\), and the best performance is found, as expected, for the highest external flow rate i.e. the highest removal rate.

In the second series of tests (B.1–3), the organic load was varied by modifying the influent concentration to the tubing from 500 to 1500 mg L\(^{-1}\) (nominal values), with the results shown in Fig. 6. Also in this case, \(5\) h are sufficient to reach steady state conditions. The 4CP concentration in the tubing effluent increases with the higher load from 20.7 to 47.3 mg L\(^{-1}\), but the related removal efficiencies are still very high in the range of 96–97%. Low concentrations in the reactor \((\leq 5\) mg L\(^{-1}\)) are observed even for the highest substrate load fed through the tubing. The system was able to respond to a three-fold increase in substrate load without a decrease in the removal efficiency. This finding confirms previous results obtained in the case of TPPBs using polymer beads to handle substrate surges [38,39].

Fig. 5. Variation of the external flow rate and biological removal test: 4CP concentration in the tubing effluent (a-b) and in the reactor (c) in A.1–3 and BIO tests.
In the third series of tests (C.7–9) the internal flow rate in the tubing was varied from 0.0095 to 0.023 L h⁻¹, which is equivalent to modifying the applied organic load from 5.2 to 10.3 mg₄CP h⁻¹ and to decrease the HRT from 8 to 4 h, with the results reported in Fig. 7. The time to reach steady state operation was always ∼5 h, and very stable concentrations in the reactor were achieved after 1.5 h with values in the range of 1.9–3.5 mg L⁻¹. The effluent tubing concentrations varied from 17 to 21 mg L⁻¹ with removal efficiencies ≥96%. The data do not show any influence of the explored HRT range on the reactor performance and the system is able to manage the two-fold increase in hydraulic load.

Mass transfer coefficients were evaluated for all the removal tests by applying Eq. (8) and the results are summarized in Fig. 8a. The kₒ values are in the range of 1.7–3.5·10⁻⁷ m s⁻¹, and minimal variations are observed for tests A and B, while in the third series of tests (C), as expected, an increase is observed with the increased flow rate in tubing.

The mass transfer coefficients during the removal tests are comparable to the values reported in Han et al. [35] for similar systems operated with silicon-rubber membranes. In the same Fig. 8(b), the calculated mass transfer rates for each test, and the biological removal rate evaluated at the steady state conditions from the biotic removal test (7.6 mg₄CP h⁻¹), are reported. It is observed that the mass transfer rates are always higher than the biological removal rate, thus confirming that there is no limitation due to the mass transfer resistance through the tubing walls relative to biolog-
ical reactions. Similar results have been reported in Pittman et al. [40] in a study investigating the relative rates of mass transfer and phenol biodegradation in a conventional TPPB bioreactor operating with Hytre 8206 polymer beads.

To complete the data analysis, a mass balance has been performed to evaluate the polymer tubing response under the tested operating conditions, and the results are reported in Table 2 in terms of 4CP distribution (amount and percent) at the end of each test. For an easier visualization, the percent distribution is also reported in graphical form in Fig. 9a, which also shows the removal efficiencies in all tests. The absorbed amounts of substrate into the polymer highlight the high adaptability of the polymer tubing in response to the different operating conditions. In tests A characterized by increasing removal rate, we observe a proportional decrease of the absorbed amount, while in tests B the absorbed amount proportionally increases by about one order of magnitude in response to the increased organic load. In tests C, with an increased hydraulic load, we observe an increase in the amount of 4CP absorbed into the polymer tubing followed by a decrease in C.9: this is consistent with the trend of the mass transfer coefficient, which in C.9 has the highest value. Higher mass transfer results in better substrate removal (see Table 2) with a consequent reduction of the amount of substrate absorbed by the polymer tubing. The achieved
Fig. 8. Mass transfer coefficient for the different tests (a) and mass transfer rates (b). The dashed line in (b) indicates the average biological removal rate of the BIO test.

Table 2
Removal tests: 4CP distribution at the end of the tests (6 h). Bold values indicate the amounts in the mass balance.

<table>
<thead>
<tr>
<th>Run</th>
<th>Unit</th>
<th>Absorbed into the polymer</th>
<th>In the liquid phase within the tubing</th>
<th>In the effluent leaving the tubing</th>
<th>In the aqueous phase within the reactor</th>
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<td>(mg)</td>
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<td>62.78</td>
<td>11.29</td>
<td>25.55</td>
<td>52.08</td>
</tr>
<tr>
<td>C.7</td>
<td>(mg)</td>
<td>3.95</td>
<td>21.98</td>
<td>3.26</td>
<td>9.91</td>
<td>35.37</td>
</tr>
<tr>
<td>C.8</td>
<td>(mg)</td>
<td>11.03</td>
<td>20.45</td>
<td>17.92</td>
<td>6.56</td>
<td>50.05</td>
</tr>
<tr>
<td>C.9</td>
<td>(mg)</td>
<td>6.47</td>
<td>20.2</td>
<td>17.7</td>
<td>6.5</td>
<td>49.3</td>
</tr>
</tbody>
</table>

removal efficiencies, referred to the tubing influent and effluent (i.e. the treated stream), are in the range of 95–97% for all tests.

A biological run was also performed to verify that the results of the abiotic characterization are applicable to biological systems, thus completing this first feasibility study of the C-TPPB. The employed culture was a microbial consortium acclimatized to 4CP and the biotic system was operated for 96 h (16 HRTs) i.e. a time long enough to ensure steady state conditions. An overview
Fig. 9. 4CP distribution in removal test: at the end of the tests, after 6 h, for abiotic tests (a) and at different times during the BIO test (b). 4CP concentration profiles in the tubing and in the bioreactor and removal rates for the two replicates: bars indicate standard deviation between replicates (c).
of the results is reported in Fig. 9c showing the concentration profiles in the tubing effluent and in the bioreactor, and the calculated removal rates vs. time. Concentration profiles in the first 6 h of the biological removal test have been also reported in Fig. 5 for comparison with the A1–3 test series, performed under the same operating conditions. Fig. 5a and b shows that tubing concentrations of 4CP are even lower than those measured in the abiotic tests, while in the liquid phase of the bioreactor the 4CP concentration after 6 h (~1 mg L⁻¹) is in the lower range of values observed for the abiotic tests (1–3 mg L⁻¹). The mass balance at different times of the BIO test, allowed determination of the 4CP distribution in the system, and the possibility of evaluating the biological removed amount, through the chloride measurement gives a precise estimate of the contribution of biodegradation to the observed 4CP removal. The data reported in Table 3, show a biological removal efficiency > 80% after 24 h, which increased in the following 90 h, reaching a value of 89%. It is worth noting that stable performance of the bioreactor was observed during the experiment: the removal efficiencies referred to the tubing effluent, reported in Fig. 9b, are >99% after 24 h and were maintained for the entire experiment. It is also worth noting that the removal efficiency can be improved by operating the bioreactor with a recycle in the tubing or by increasing the biomass concentration (cell recycle) but this may not always be advisable. In fact, too low concentrations in the bioreactors can give biological removal rates so low as to be incompatible with acceptable bioreactor volumes, and in common practice, when necessary due to low legal limits, the biological treatment of industrial wastewater is completed with a tertiary treatment step.

The very positive results obtained in the biotic test confirmed the data of the simulated kinetics in the abiotic tests, and completed a first validation step.

Table 3

<table>
<thead>
<tr>
<th>Time</th>
<th>Unit</th>
<th>Absorbed into the polymer</th>
<th>In the liquid phase within the tubing</th>
<th>In the effluent leaving the tubing</th>
<th>In the aqueous phase within the bioreactor</th>
<th>Biologically removed</th>
</tr>
</thead>
<tbody>
<tr>
<td>6 h</td>
<td>(mg)</td>
<td>4.31</td>
<td>21.30</td>
<td>3.76</td>
<td>4.67</td>
<td>51.20</td>
</tr>
<tr>
<td></td>
<td>(%)</td>
<td>5.1</td>
<td>24.9</td>
<td>4.4</td>
<td>5.5</td>
<td>50.1</td>
</tr>
<tr>
<td>24 h</td>
<td>(mg)</td>
<td>6.64</td>
<td>20.86</td>
<td>4.57</td>
<td>6.20</td>
<td>157.65</td>
</tr>
<tr>
<td></td>
<td>(%)</td>
<td>3.1</td>
<td>9.8</td>
<td>2.1</td>
<td>2.9</td>
<td>82.1</td>
</tr>
<tr>
<td>48 h</td>
<td>(mg)</td>
<td>9.64</td>
<td>20.06</td>
<td>5.15</td>
<td>6.47</td>
<td>331.48</td>
</tr>
<tr>
<td></td>
<td>(%)</td>
<td>2.8</td>
<td>5.4</td>
<td>1.4</td>
<td>1.7</td>
<td>88.9</td>
</tr>
<tr>
<td>72 h</td>
<td>(mg)</td>
<td>27.15</td>
<td>20.10</td>
<td>5.65</td>
<td>6.93</td>
<td>473.53</td>
</tr>
<tr>
<td></td>
<td>(%)</td>
<td>5.1</td>
<td>3.8</td>
<td>1.1</td>
<td>1.3</td>
<td>88.7</td>
</tr>
<tr>
<td>96 h</td>
<td>(mg)</td>
<td>38.89</td>
<td>20.14</td>
<td>6.50</td>
<td>8.17</td>
<td>617.07</td>
</tr>
<tr>
<td></td>
<td>(%)</td>
<td>5.6</td>
<td>2.9</td>
<td>0.9</td>
<td>1.2</td>
<td>89.4</td>
</tr>
</tbody>
</table>

4. Conclusions

This study is a first demonstration, performed with abiotic and biotic tests, that a tubing-based C-TPPB can be successfully applied for the partitioning and removal of inhibitory/toxic compounds, achieving residual 4-chlorophenol concentrations in the reactor in the range of 1–5 mg L⁻¹, even for the highest organic and hydraulic influent loads. This finding is explained in terms of the “buffer” effect of the polymer tubing, which allowed adaptation of the system in response to the increased organic load, as was previously demonstrated in the case of TPPBs using polymer beads. The mechanism of uptake-release characterizing the polymer in response to variations of the influent, already demonstrated effective in the case of polymer beads, is confirmed for the tubing. This feature and the high affinity for the substrate demonstrated by the selected polymer suggest applications to numerous other organic contaminants. Current work involves an extended characterization of the bioreactor operated with enhanced microbial cultures specifically acclimatized to the target compounds to demonstrate integrated transport/biodegradation over a wide spectrum of operating conditions, and the use of complex contaminant mixtures consisting of organics and heavy metals to confirm process performance under such challenging conditions.

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References
