



Treatment of synthetic tannery wastewater in a continuous two-phase partitioning bioreactor: Biodegradation of the organic fraction and chromium separation



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ABSTRACT

A continuous two-phase partitioning bioreactor has been tested for treating a synthetic leather tannery wastewater with the objective of achieving effective removal of the organic load and complete chromium separation. The bioreactor was operated with 5.5 m of Hytrel 8206 polymeric tubing fed with a synthetic tannery wastewater consisting of 4-chlorophenol (concentration in the range of 1000–2500 mg L⁻¹) and potassium dichromate (100 mg L⁻¹ as Cr (VI)), and immersed in a 4 L bioreactor containing the microbial culture acclimatized to the compound. This configuration prevents the direct contact between the toxic wastewater and the microorganisms themselves, and provides the gradual organic substrate delivery through the tubing walls. Abiotic partition and mass transfer tests were performed to investigate the transport of dichromate and 4-chlorophenol across the tubing into the bulk phase of the bioreactor. No appreciable mass transfer of dichromate was detected. During biotic testing, the influent organic load in tubing has been varied in the range of 19–94 mg h⁻¹ and the hydraulic retention time from 3 to 6 h. Achieved biological removal efficiencies were in the range of 89–95% for the highest applied loads. Process kinetics (which included consideration of both mass transfer and biological rates) were evaluated, and it was found that the increased load did not result in any decrease in 4-chlorophenol removal rate. This work has shown that the continuous two-phase partitioning bioreactor has significant potential in enhancing the biological treatment of tannery wastewater, which is a typical representative of industrial “hostile” wastewater.

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1. Introduction

The tanning industry is considered to be a major source of pollution producing noxious gases, such as hydrogen sulfide, as well as wastewater and solid wastes, which negatively affect ecosystems (Thanikaivelan et al., 2004). Leather manufacturing transforms raw skins into stable and finished leather products by means of three distinct processing units: 1) beam house operations, in which the skins are processed to clean and remove unwanted materials (e.g. hair, flesh) and are prepared for the subsequent tanning process, 2) the tanning step in which the hides are permanently stabilized against putrefaction, and 3) the post-

tanning process, in which the leathers are finished to provide them with the required aesthetic appeal and to improve their commercial value. In each of these operations, large amounts of water and chemical additives are used giving rise to characteristic pollutant loads. Water consumption is generally highest in the pre-tanning stages (15–22 L of water per kg of hide processed), but significant amounts of water are also consumed in the tanning and post-tanning processes, e.g. 1–2 and 2–4 L per kg of hide processed, respectively (Raghava Rao et al., 2003).

Due to the variability of the raw materials and the desired end-product characteristics, tanning processes can produce different amounts and kinds of complex and hazardous wastewaters. The effluent production generally varies over a broad range, i.e. 10–100 m³ per ton of rawhide (Lofrano et al., 2013), whose average composition can vary significantly. Organic substances (such as proteins, carbohydrates and lipids) present in tannery wastewater

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arise from the washed and treated skins, or are introduced during the tanning and post-tanning process as solvents, additives and biocides. The skins sorb only 20% of the chemicals used in the tanning process, with the remainder consisting of vegetable and synthetic tannins, oils, aldehydes, and zirconium, aluminum, titanium, and especially chromium salts entering the processing effluent (Chowdhury et al., 2015).

In the conventional treatment of tannery wastewater, tanning effluents are segregated and treated for chromium precipitation, then a physical treatment for chloride removal and a final biological treatment step, for removal of the residual organic load (Goswami and Mazumder, 2013) are applied. Unfortunately, this conventional approach can provide effluents that still do not meet the required discharge targets (Oller et al., 2011), and the presence of residual inorganic species such as chloride, sulphide, chromium etc., which are inhibitory for microbial growth, can limit traditional downstream biological treatment. For these reasons, tannery wastewater treatment still represents an important technological challenge. Advanced Oxidation Processes (AOPs), as a single treatment or as a pre- or post-treatment combined with biological processes, are among the most studied treatment strategies (Oller et al., 2011). Membrane processes can also be applied to treat tannery leather effluents in order to remove the salt content or to separate the biomass from the effluent after chemical–physical treatments (Cassano et al., 2001). An additional advantage of membrane processes is the recovery of chromium from residual effluents of leather tanning, which provides a significant economic advantage in terms of its reuse, besides the simplification of the wastewater treatment process (Cassano et al., 2007). De Gisi et al. (2009) applied Reverse Osmosis (RO) with a plane membrane as post-treatment to remove refractory organic compounds, thus obtaining a high quality permeate reusable within the production cycle.

Alternatively, a novel technological platform, based on two-phase partitioning bioreactors (TPPBs), has been recently proposed by Tomei et al. (2016) to treat industrial wastewater containing different pollutants including organic (potentially biodegradable), and inorganic species (i.e. heavy metals, salts). A modified TPPB has been configured by means of extruded polymer tubing immersed in a bioreactor, through which organic compounds can diffuse. A TPPB operated with mixed wastewater flowing through the tubing allows in principle the selective transport of toxic organic pollutants that are present “tubing side” to microbial cells located “bioreactor side”, thereby generating a residual metal concentrated stream within the tubing that could be reused in the tanning process. The biodegradable substances transferred from the wastewater, through the tubing walls, are biodegraded without any direct contact between the wastewater and the microorganisms themselves. In addition, due to the gradual substrate delivery regulated by the metabolic processes, cells are exposed to sub-inhibitory concentration levels. When required, as in the case of tannery wastewater, the biodegradation of the organic matter and the separation for recovery of the inorganic components can be achieved simultaneously in a single operation unit.

Chlorinated phenols including 4-chlorophenol (4CP) have often been found in tannery wastewater (Reemste and Jekel, 1997; Mwinjihija, 2011), associated with chromium. In the tanning operation, basic chromium sulphate [$\text{Cr}(\text{H}_2\text{O})_5(\text{OH})\text{SO}_4$] is the most widely used tanning agent; in hides/skins processing, the maximum utilization of chromium is usually 60–70%, and for this reason un-reacted chromium salts are found in very high concentrations in tannery wastewater (Goswami and Mazumder, 2013). Even if chromium VI (Cr (VI)) is not used in any step of the manufacturing process, its presence in the effluent from wastewater treatment plants arises from the oxidation of the trivalent to

the hexavalent state (Fuck et al., 2011). Cr (VI) is carcinogenic and is generally considered 1000 times more toxic than Cr (III) (ATSDR, 2008). Accordingly, in this study, 4CP has been chosen as the target organic contaminant to simulate a tannery wastewater containing chlorides, and a Cr (VI) salt (potassium dichromate) was added to simulate chromium content. With this strategy, a literature review was preliminarily carried out to determine a representative concentration range for 4CP and dichromate, in order to adequately simulate an industrial tannery wastewater.

The objective of this study was to demonstrate the feasibility of a continuous TPPB (C-TPPB) system for treating a leather tannery wastewater achieving effective removal of the organic load and complete chromium separation for subsequent recovery. Experiments were conducted using Hytrel 8206 polymeric tubing, because its high affinity for chlorophenols has already been demonstrated in a previous study on soil decontamination (Tomei et al., 2015). Abiotic partition and mass transfer tests were performed to investigate the transport of dichromate and 4CP from the tubing into the bulk phase of the bioreactor. Biological testing included an investigation of the effect of varying the organic and hydraulic loadings on C-TPPB performance. Overall process kinetics (including mass transfer and biological rates) were evaluated under all the applied loading conditions.

2. Materials and methods

2.1. Synthetic wastewater

The test solution was a synthetic wastewater simulating a real tannery effluent whose selected constituents were 4CP, (CAS number 106-48-9, purity >99%) as organic matter and chloride source, and potassium dichromate ($\text{K}_2\text{Cr}_2\text{O}_7$), (CAS number 7778-50-9, purity >99%) as a source of Cr (VI). The reagents used were purchased from Sigma-Aldrich (USA) and were of analytical grade.

2.2. Polymer tubing

Tubing made of Hytrel 8206 polymer was used for all experiments, according to previous results reported by Tomei et al. (2017) in the use of this material. The tubing (with internal and external diameters of 0.5 and 0.6 cm, respectively), was supplied and extruded by DuPont Canada, has a glass transition temperature of $-59\text{ }^\circ\text{C}$, a specific gravity of 1.17 and a flexural modulus (ASTM D790, at room temperature) of 80 MPa (Craig and Daugulis, 2014). Before use, a multistep washing with methanol and distilled water was undertaken according a procedure described elsewhere (Tomei et al., 2016) in order to remove any residual processing residues.

2.3. Microbial culture

A microbial consortium previously adapted to chlorophenols as described in Mosca Angelucci and Tomei (2015) and already utilized for the biodegradation of 4CP (Tomei et al., 2017), was cultivated with 4CP as the sole carbon and energy source for two months, with an organic loading rate within the range of 8–13 $\text{mg}_{4\text{CP}}\text{ h}^{-1}$. A lab-scale Sequencing Batch Reactor (SBR) was employed for growing and maintaining the biomass between each C-TPPB experiment. Additional details of SBR equipment and operation are reported in the Supplementary Material section.

2.4. C-TPPB-bioreactor

The C-TPPB reactor consisted of a glass vessel (working volume 4 L) interfaced to a dedicated control computer and equipped with magnetic stirring and temperature control ($T = 27 \pm 0.5\text{ }^\circ\text{C}$),

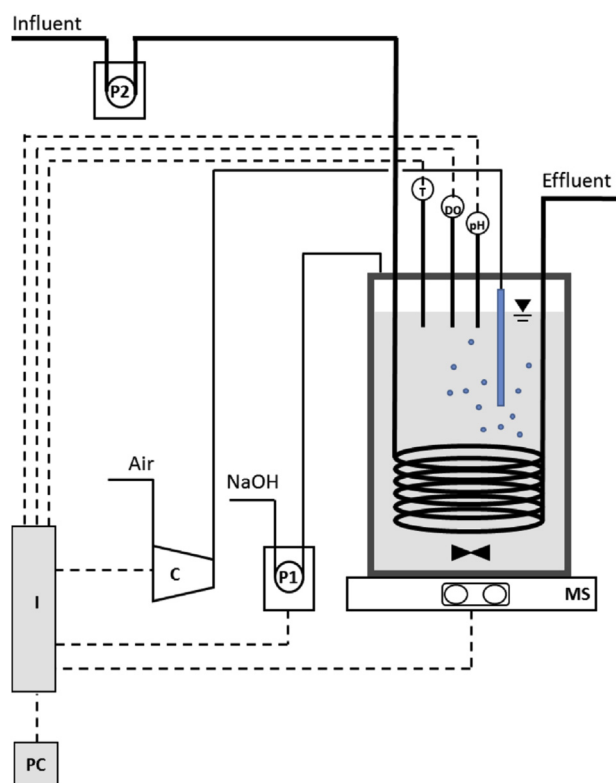


Fig. 1. Schematic diagram of the experimental apparatus and control system of C-TPPB (C: compressor; DO: Dissolved Oxygen sensor; I: interface; MS: magnetic stirrer; P1: pump for NaOH solution; P2: pump for tubing feeding; PC: personal computer; pH: pH sensor; T: Temperature sensor).

containing a support for the spiral-wound tubing (length of 5.5 m and external surface area of 1036.2 cm²). The (polymer tubing)/(liquid volume) ratio was 4% (v/v). The synthetic wastewater was pumped inside the tubing (with a flowrate set to give the required Hydraulic Retention Time (HRT) in the tubing) and a sealed glass flask was utilized to collect the tubing effluent. The liquid phase of the bioreactor contained the biomass where the biodegradation of the organics transferred from the tubing took place. A schematic diagram of the experimental apparatus is reported in Fig. 1. The pH was monitored and controlled (set point value 7.5) by dosing NaOH solution (1 M) through a peristaltic pump and an oximeter (Oxi 538, WTW) was employed for online Dissolved Oxygen (DO) monitoring and on-off control in the range of 3–4 mg L⁻¹. Air was supplied by a compressor via a glass diffuser.

Table 1

Operating conditions of the biotic tests. F_t : tubing influent flow rate; $4CP_t$: influent tubing concentration; $Cr(VI)_t$: influent tubing concentration; HRT_t = Hydraulic Retention Time in tubing; IOL: Influent Organic Load fed in tubing; X: biomass concentration in the bioreactor at the beginning of the test; BT-1000, BT-1500, BT-2500 indicate Biotic Tests at influent tubing concentration of 1000, 1500 and 2500 mg L⁻¹ respectively.

Run	F_t L h ⁻¹	$4CP_t$ mg L ⁻¹	$Cr(VI)_t$ mg L ⁻¹	HRT_t h	IOL ^a mg h ⁻¹	IOL ^b mg h ⁻¹	$Cr/4CP$ $\frac{mg_{Cr}}{mg_{COD}}$	X $g_{VSS} L^{-1}$
BT-1000	0.018	1000	100	6	18.0	19.2	0.06	1.00
	0.024			4.5	24.0	26.1		
	0.037			3	37.0	38.5		
BT-1500	0.018	1500	100	6	27.0	27.3	0.04	1.02
	0.024			4.5	36.0	38.3		
	0.037			3	55.5	58.4		
BT-2500	0.018	2500	100	6	45.0	46.0	0.02	1.18
	0.024			4.5	60.0	65.0		
	0.037			3	92.5	94.0		

^a Nominal value.

^b Experimental value.

DO data acquisition (performed at time intervals of ~15 s) allowed calculating the Specific Oxygen Uptake Rate (SOUR) according to the procedure reported in Tomei et al. (2004).

2.5. Test plan

2.5.1. Batch partition test for dichromate

To determine the partition coefficient of Hytrel 8206 for dichromate, a known amount (0.3 g) of small pieces (average size 2–3 mm) of Hytrel 8206 polymer tubing was added to a dichromate solution ($Cr(VI) \sim 50 \text{ mg L}^{-1}$) in a mixed (320 rpm) flask. Liquid samples were collected (after 4, 6 and 24 h), and analyzed for $Cr(VI)$ concentration.

2.5.2. Mass transfer test

Mass transfer properties have been investigated in a previous work (Tomei et al., 2016) for the Hytrel 8206 tubing with 4CP solution, but in order to study the effect of the presence of dichromate and its potential transport by the tubing, a mass transfer test was performed in the C-TPPB according to the following procedure. After filling the tubing with the feed solution, comprised of $\sim 1000 \text{ mg L}^{-1}$ of 4CP and dichromate to give $\sim 100 \text{ mg L}^{-1}$ of $Cr(VI)$, the reactor side was filled with tap water to completely submerge the tubing, and was maintained under mixed conditions (320 rpm) for the next 24 h. The tubing was then continuously fed for 24 h at a rate of 0.018 L h^{-1} , equivalent to a HRT of $\sim 6 \text{ h}$ in the tubing. This value was chosen based on the HRT range of values (1–30 h) reported in the literature for membrane bioreactors treating industrial wastewater (Freitas dos Santos and Livingston, 1995; Viero and Sant'Anna, 2008). The 4CP and $Cr(VI)$ concentration was regularly monitored both in the liquid phase of the reactor and in the liquid inside the tubing, and the effluent from tubing was collected and analyzed to allow the mass balance.

2.5.3. Loading tests: effect of hydraulic and organic loading

4CP biodegradation in the presence of influent variation was investigated on a synthetic wastewater containing dichromate at a concentration equivalent to 100 mg L^{-1} of $Cr(VI)$ and different 4CP concentrations (i.e. 1000–1500–2500 mg L⁻¹) continuously fed to the tubing, on a once-through basis. A biomass suspension was initially added to the C-TPPB to give a VSS (Volatile Suspended Solid) concentration of $\sim 1 \text{ g L}^{-1}$. A mineral salt medium (Williams and Unz, 1989), comprised of a deionized water solution of the following components (g L⁻¹) ($(NH_4)_2SO_4$, 5; $MgSO_4 \cdot 7H_2O$, 1; $CaCl_2 \cdot 2H_2O$, 0.5; K_2HPO_4 , 0.11; KH_2PO_4 , 0.85; $FeCl_3 \cdot 6H_2O$, 0.02; NaEDTA, 0.03, was daily dosed on the basis of the fed 4CP amount to give a ratio C:N:P of 100:5:1. In each test, the tubing was filled at the start

Table 2An overview of the composition of real tannery wastewaters (concentration units are mg L⁻¹).

pH	COD	TSS	Chloride	Sulphate	Sulphide	NH ₄ -N	Cr	References	Number
3–13	2000–20000	–	1500–28000	1000–7000	–	–	–	Panizza and Cerisola (2004)	#1
4.6–8.1	1100–3000	58–200	–	–	–	75–135	<0.001–0.36 ^{a,b}	Calheiros et al. (2007)	#2
8.0–8.2	3300–3600	800–1000	6000–7000	–	–	220–260	0.2–0.3	Di Iaconi et al. (2003)	#3
7.4–8	2188–3000	–	5438–5561	–	176–371	257–384	29–30.3 ^{a,b}	Szyrkowicz et al. (2005)	#4
7.2	2810	–	6400	–	89	130	62 ^a	Kurt et al. (2007)	#5
7.4	3700	–	6520	–	440	180	22 ^a	Apaydin et al. (2009)	#6
2.5–5.5	2000–7600	500–800	1200–2060	900–2270	2	80–160	120 ^a	De Gisi et al. (2009)	#7
9.6–11.5	2000–3560	655–2136	4500–12100	–	40–72	18–56	56–125 ^a	Ram et al. (1999)	#8
8–9	5000–5500	–	5000–6000	1800–2000	–	–	80–100 ^c	Fababuj-Roger et al. (2007)	#9
7–10	1760–3320	568–2132	1000–4549	–	14.8–424.5	–	23–122 ^a	Haydar and Aziz (2009)	#10
7.9–9.2	2533	1244	6528	–	860	118	258 ^a	Mandal et al. (2010)	#11

^a As total chromium.^b As Cr (VI).^c As Cr (III).

with synthetic wastewater at a fixed concentration, and then was fed continuously for 72 h. During this period, three loading steps (lasting 24 h each) were applied by increasing the tubing influent

flowrate, as indicated in Table 1. At the end of each loading step, (i.e. after 24 h), the feed to the tubing was temporarily stopped (for less than 1 h) and the bioreactor was switched to settling mode in order

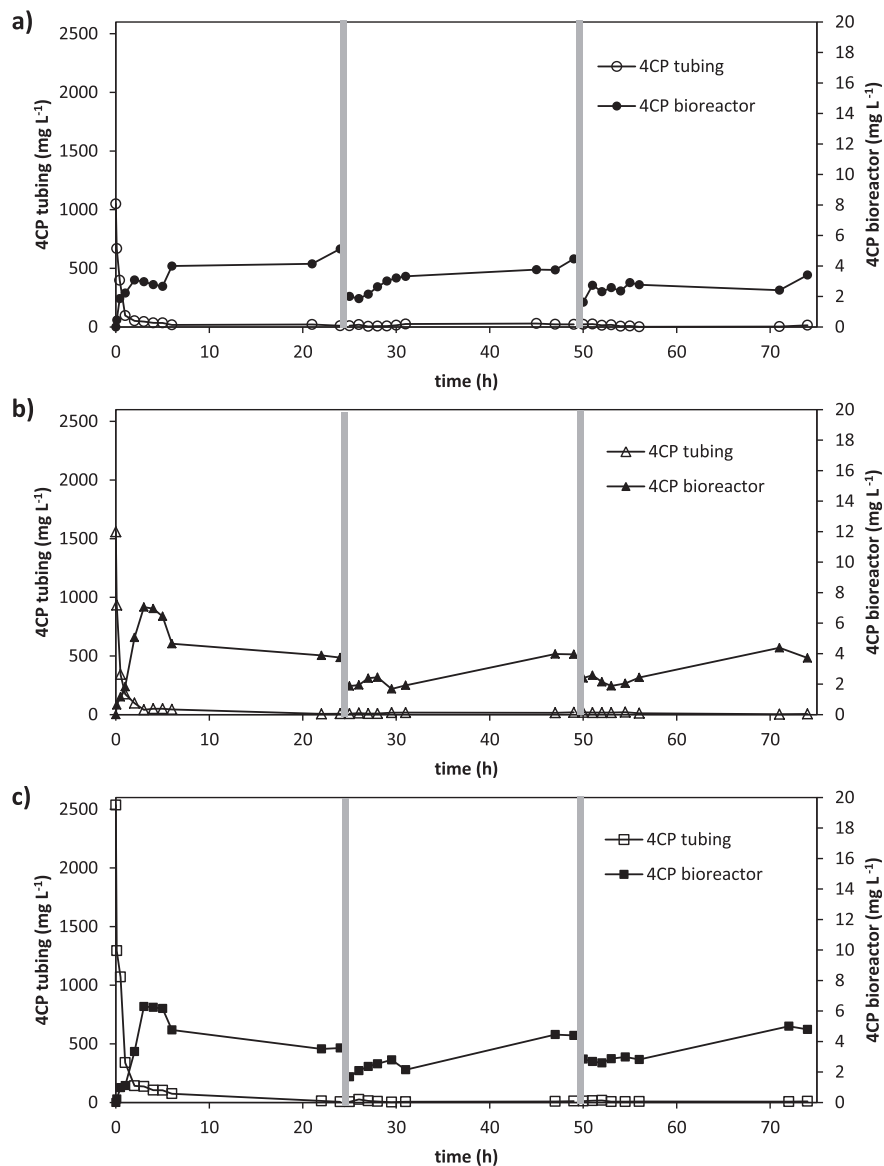


Fig. 2. 4CP concentration profiles in tubing and bioreactor vs. time during BT-1000 (a), BT-1500 (b) and BT-2000 (c) test. Grey bars represent settling period.

to settle the biomass and discharge 2 L of supernatant. Then the bioreactor was re-filled with fresh tap water, to avoid chloride buildup in the bioreactor liquid phase resulting in possible inhibition of the biomass. Samples from the tubing effluent, bioreactor, and effluent collection tank were analyzed for 4CP, Cr (VI) and chloride concentration at time intervals of 15–60 min in the first 6 h; then two last samples were taken at the end of each loading step (in the interval 20–24 h). The biomass concentration in the bioreactor was monitored daily and biological removal of 4CP was evaluated by following the chloride buildup in the bioreactor according to Caldeira et al. (1999).

2.6. Analysis

Analysis of 4CP were performed on supernatant after two subsequent centrifugation steps at 13,000 rpm (10 min) with a UV–visible spectrophotometer (PerkinElmer, Lambda 25) at $\lambda = 279$ nm. The Cr (VI) concentration was determined spectrophotometrically, according to Standard Methods (APHA, 2012), by adding H_2SO_4 and 1,5-diphenylcarbazide, producing a purple-violet colored complex whose absorbance was measured at a wavelength

of 540 nm. The chromium concentration of samples was determined by a calibration curve obtained with standard solutions of dichromate corresponding to Cr (VI) concentration in the range of 0.05 and 2 $mg L^{-1}$. The biomass concentration in the bioreactors was evaluated in terms of VSS concentration according to Standard Methods (APHA, 2012). An ionic chromatograph (DX-100 DIONEX) was used to determine the chloride concentration in the liquid phase of the bioreactor and in tubing by following the Standard Method (APHA, 2012). A pHmeter (pH700, EUTECH Instruments) was used for pH measurements in the bioreactor liquid phase.

3. Results and discussion

3.1. Composition of tannery wastewater

Table 2 provides a summary of the composition of tannery wastewater found in a preliminary literature analysis. Typical Chemical Oxygen Demand (COD) and Total Suspended Solid (TSS) values found in tannery effluents are up to thousands of $mg L^{-1}$, approximately five times higher than in municipal wastewaters (Fababuj-Roger et al., 2007). Characteristic COD values are within

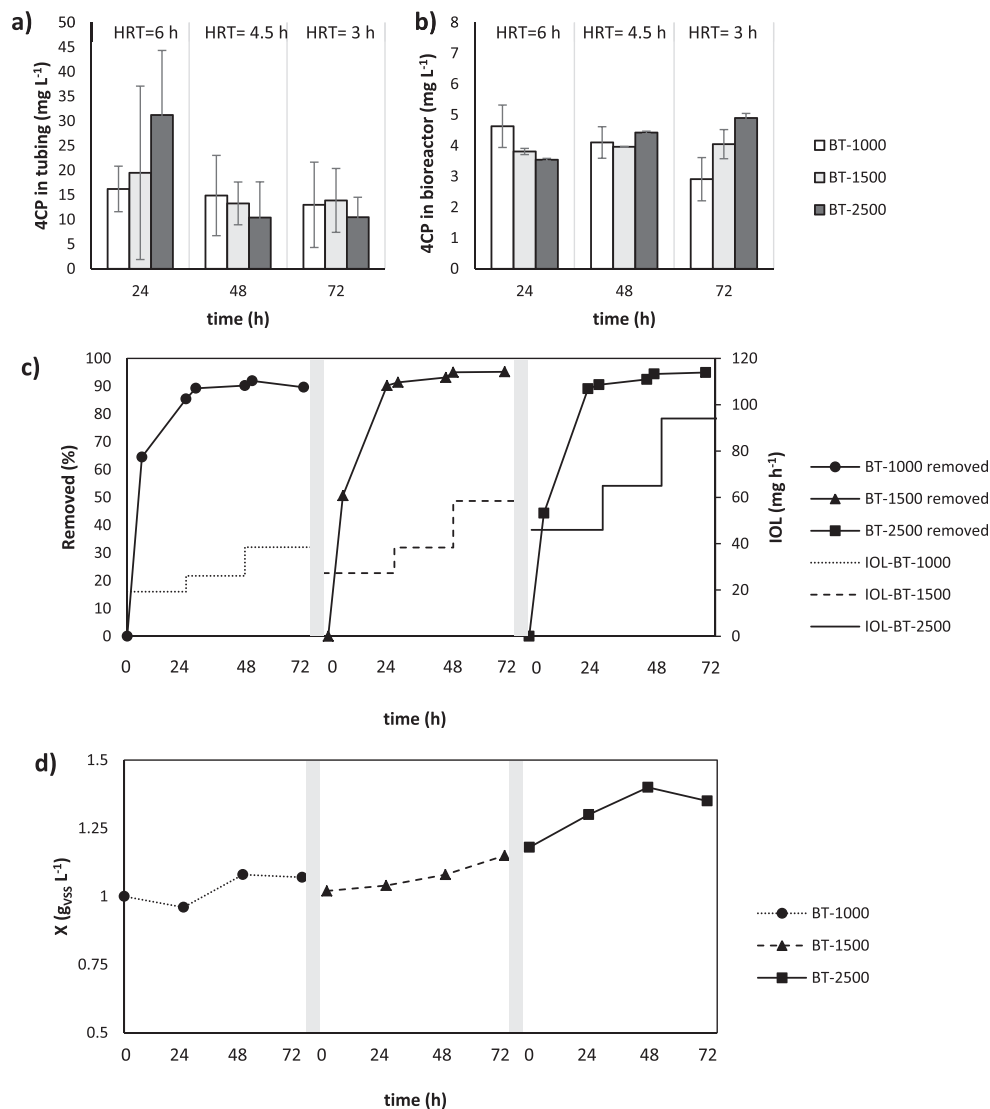


Fig. 3. Loading tests: average 4CP concentrations in the tubing effluent (a) and in the bioreactor (b) vs. HRT; 4CP biological removal (percent vs. time) (c); Biomass concentration vs time (d).

the range of 1100–20000 mg L⁻¹, but recurring values are in the range of 1000–6000 mg L⁻¹ as reported by Sundarapandiyan et al. (2010). Tannery streams are hypersaline, and pHs are variable and can be very acidic in the tanning process, while alkaline conditions prevail in the beamhouse stage. The chromium concentration varies widely depending on the selected tanning process: no chromium is detected for vegetable tannery wastewater (Panizza and Cerisola, 2004) or very low values (e.g. <0.001 mg L⁻¹) are reported by Calheiros et al. (2007) for wastewater effluents from post-tanning and finishing processes, listed as #1 and #2 in Table 2, respectively. In contrast, chromium levels have been detected in effluents collected in wastewater treatment plants located in industrial districts of tanneries based on both chrome and vegetable tanning (within the range of 0.2–120 mg L⁻¹, as reported in #3–7 of Table 2). Wastewater coming from tanneries based on chromium tanning processes (listed in #8–11 of Table 2) can present higher values up to 258 mg L⁻¹, as reported by Mandal et al. (2010).

According to the literature data, reporting the presence of chlorinated phenols and Cr (VI) in tannery wastewater (Reemste and Jekel, 1997; Mwinyihija, 2011), the synthetic wastewater employed in this study consisted of a solution of 4CP in the range of 1000–2500 mg L⁻¹ (equal to 1680–4200 mg_{COD} L⁻¹ with a conversion factor of 1.68 g_{COD} g_{4CP}⁻¹), and potassium dichromate in such an amount to give 100 mg L⁻¹ of Cr (VI). The corresponding range of values of the Cr/COD ratio is 0.06–0.02 mg_{Cr} mg_{COD}⁻¹, i.e. in the range of literature values (0.001–0.14) given by Lofrano et al. (2013).

3.2. Partition and mass transfer tests

Hytrel 8206 was selected on the basis of previous studies on the same pollutant-polymer pair in which the effective uptake-release of 4CP was demonstrated in both granular form and tubing. Partition coefficients determined in sorption tests for 4CP were 303 and

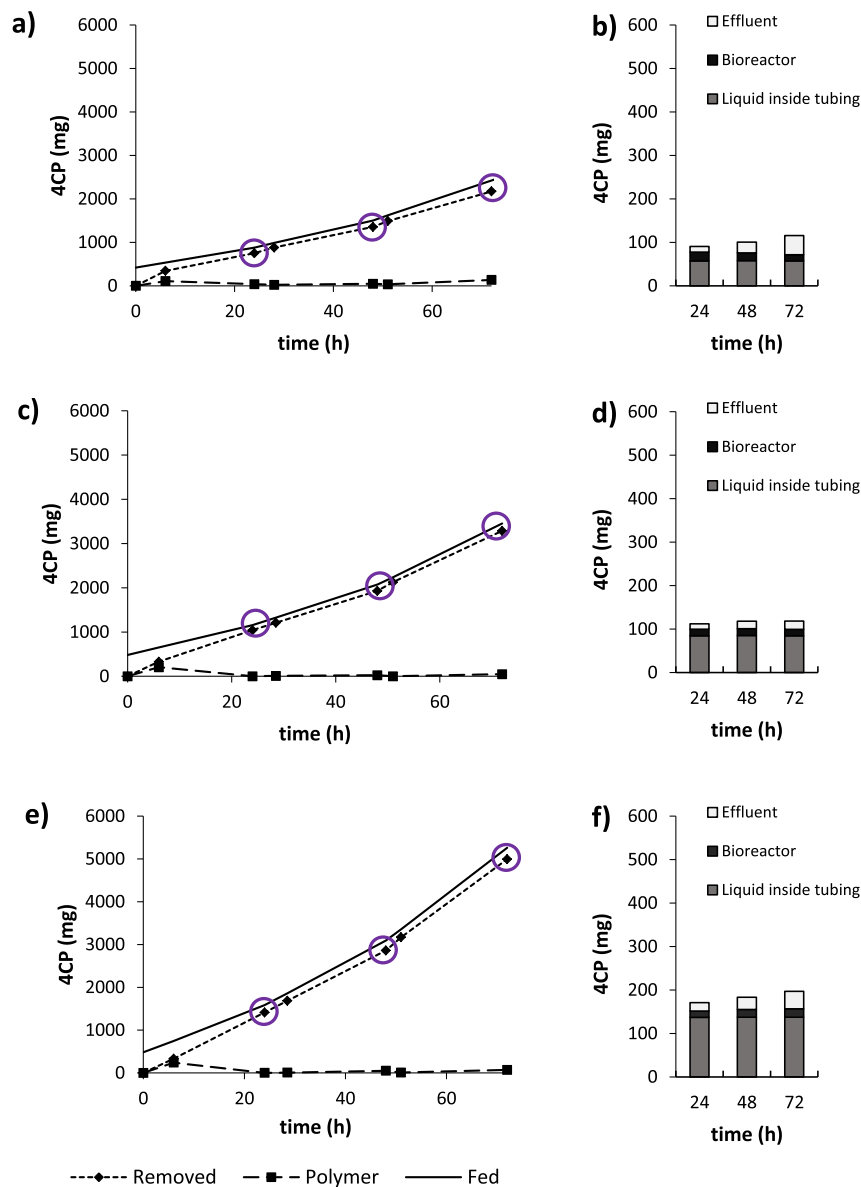


Fig. 4. Loading test mass balance: 4CP fed to the system, retained by polymer, and biologically removed and distribution of the residual amount among effluent, bioreactor and liquid inside tubing in BT-1000 test (a–b), BT-1500 test (c–d) and BT-2500 test (e–f). Circles in a, c and e indicate the points whose the detailed 4CP distribution is reported in b, d and f.

349 for granular beads (Tomei et al., 2015) and for tubing pieces (Tomei et al., 2016), respectively. Partition tests with dichromate showed that the tested polymeric tubing does not absorb this species in that there was no reduction in the concentration of Cr (VI) in the liquid phase put in contact with polymer pieces during the entire experiment (see Fig. S1 reported in Supplementary Material section).

A C-TPPB test confirmed this finding, as we continuously fed the tubing with the synthetic wastewater for 24 h, corresponding to an overall load of 429.2 mg_{4CP} and 42.7 mg_{Cr(VI)} per day, and the dichromate did not pass through the polymer tubing, as seen in Fig. S2 of Supplementary Material. Thus, the presence of the metal does not affect the 4CP transfer properties of the tubing. The high 4CP removal (>98%) from the synthetic tannery wastewater and the complete separation of dichromate achieved in C-TPPB, facilitate the chromium recovery for its subsequent reuse. In conventional tannery wastewater treatment plants, the mixing of different polluted liquid streams is a common practice and this can make difficult the chromium separation and recovery. For instance, the effectiveness of precipitation methods (one of the most applied metal recovery technique) strongly depends on the composition of the wastewater and is negatively affected by the presence of organic compounds (Hu et al., 2005). It is worth noting that the Cr (VI) concentration tested in this study is suitable for recovery according to the results of Hu et al. (2005) and Lasheen et al. (2014), who applied adsorbent nanoparticles (based on maghemite and magnetite respectively) to aqueous solutions at initial concentration of Cr (VI) in the range of 5–200 mg L⁻¹.

3.3. Loading tests

Three different 4CP influent concentrations were tested over a range of tubing influent flow rates (with a duration of 24 h) to

evaluate the effect of variations in the hydraulic and organic loading on the C-TPPB performance at nine operating conditions, as summarized in Table 1. The results are shown in Fig. 2 as 4CP concentration time-profiles detected in the tubing effluent and in the bioreactor in the BT-1000, BT-1500 and BT-2500 tests. As already observed for the mass transfer tests, no chromium was detected in the bioreactor during the entire experimental period, as also proved by monitoring the Cr (VI) concentration in both the tubing effluent and the bioreactor liquid phase. Cr (VI) concentrations in the tubing effluent remained practically constant and equal to the feed concentration for each loading test. Chromium concentration data vs. time are reported in Fig. S3 in Supplementary Material. Cr (VI) concentrations have been averaged for the entire duration of each test, thus obtaining the following mean values (\pm standard deviation): 101.1 \pm 1.8 mg L⁻¹, 100.0 \pm 1.3 mg L⁻¹, 100.5 \pm 1.2 mg L⁻¹, respectively in BT-1000, BT-1500 and BT-2500 test.

The removal of 4CP from the synthetic wastewater was effectively achieved for all the investigated loading conditions, and stable concentration values were reached after just 6 h from the start of each test as shown in Fig. 2. Data reported in Fig. 2 showed no significant differences in 4CP bioreactor concentrations with varying the Influent Organic Load (IOL), as they are all within the range of 3–5 mg L⁻¹, in spite of the wide range of applied IOLs, i.e. 19.2–94 mg h⁻¹. These IOL values are consistently higher than IOLs applied to single phase SBR used to develop and maintain the 4CP-degrading culture, that were within the range 8–13 mg h⁻¹.

The final 4CP concentrations in the tubing effluent (mean value in the interval time 20–24 h for each operating condition) are reported in Fig. 3a. Removal efficiencies of 4CP from the wastewater were in all cases \geq 98%. The influence of 4CP initial load on the tubing effluent concentration was detected only with a HRT of 6 h (corresponding to IOLs of 19.2, 27.3 and 46 mg h⁻¹ for BT-1000, BT-

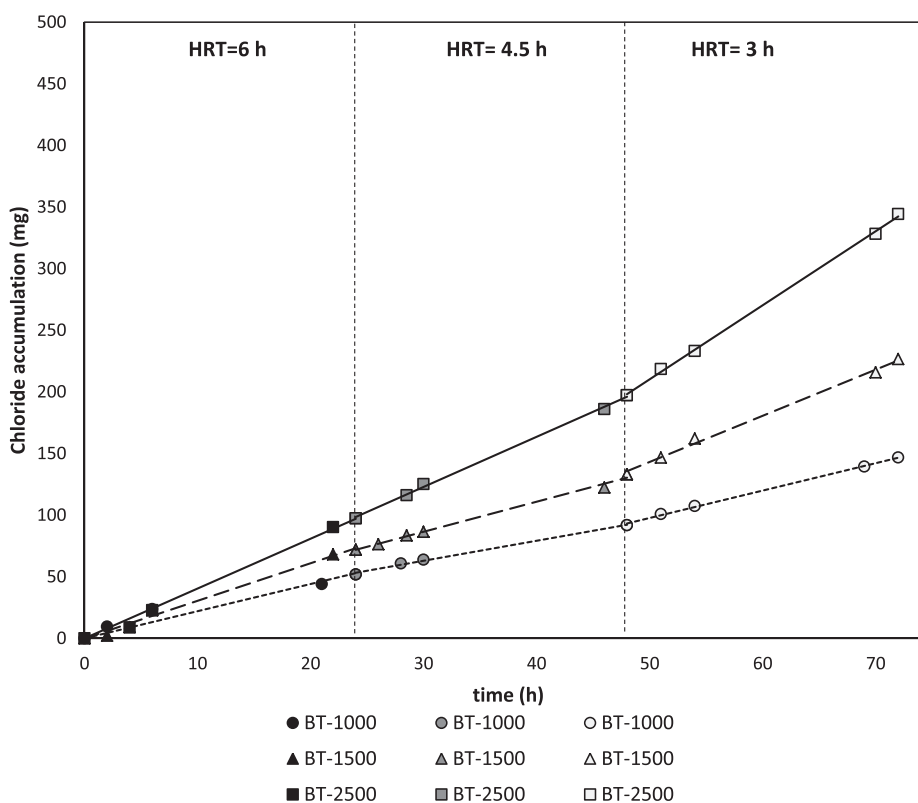


Fig. 5. Chloride accumulation during the loading tests.

1500, and BT-2500 test, respectively), after 24 h from the beginning of each test, as shown in Fig. 3a. In contrast, no consistent difference was found for the subsequent HRTs tested, possibly due to the progressive biomass adaptation. This result is also observed in the time profiles reported in Fig. 2 where no significant increase of 4CP concentration in the tubing effluent was detected after each increased load step (i.e. after settling indicated by the grey bars).

Fig. 3b shows the 4CP aqueous concentrations in the bioreactor (mean value in the interval time 20–24 h for each operating condition). Even in this case no consistent variation with increasing hydraulic and organic load is observed.

An estimate of the biodegradation rate of 4CP by the acclimated biomass was determined by means of a chloride balance for all the tests. The chloride concentration was measured in the liquid phase in the bioreactor and in the effluent from the tubing. This last measurement has been performed to evaluate the possible back-transfer from the reactor bulk phase to the liquid inside the tubing of Cl^- produced in 4CP biodegradation. Obtained values (data not shown) showed in all cases a negligible presence of chloride inside the tubing accounting for 0.1–0.2% of the amount in the bioreactor, thus the 4CP biodegradation has been evaluated referring to the chloride accumulation in the liquid phase of the bioreactor. Fig. 3c gives a summary of the applied IOLs and biodegradation efficiency obtained for the three series of tests and these vary within the range of 85–92%, 90–95% and 89–95% for the BT-1000, BT-1500 and BT-2500 tests, respectively. Increasing the 4CP influent concentration and the tubing flowrate, did not result in any decrease in biodegradation efficiency, indeed with higher IOLs, higher rates of removal were observed.

Biomass concentrations are reported in Fig. 3d showing increasing concentration with increasing loads from 1 to 1.2 gvss L^{-1} , suggesting favorable growth conditions achieved in the bioreactors due to the presence of the tubing. In fact, chromium toxicity has been eliminated (Cr (VI) was not detected in the bioreactor) and the average 4CP concentration measured in the bioreactor is in the range of 3–5 mg L^{-1} . These concentration levels are certainly sub-inhibitory, being lower than the EC_{50} values reported for 4CP, i.e. 95 mg L^{-1} (Polo et al., 2011) determined by respirometric tests on activated sludge, and 8.3 mg L^{-1} evaluated with the Microtox assay (Ribo and Kaiser, 1983).

In Fig. 4, the results of the mass balance highlight the distribution of 4CP in the system including the polymer tubing, the effluent reservoir, the liquid inside the tubing and the liquid phase of the bioreactor. The 4CP retained within the tubing itself at the end of each applied IOL is quite small for BT-1000 test, i.e. 4.3–5.6% of the total 4CP fed to the system and nearly negligible for the BT-1500 and BT-2500 test (0.1–1.2% and 0.1–1.6%, respectively). This finding is an additional confirmation that effective biodegradation of the target compound takes place as demonstrated by the negligible contribution of the absorbed amount to the calculation of substrate removal.

An overview of substrate degradation, estimated by the chloride buildup in the bioreactor, is shown in Fig. 5. A linear correlation of the chloride profile data is observed for each investigated condition, with a very good correlation coefficient R^2 always >0.99 . The linear trends demonstrate the achievement of non-inhibitory conditions in the bioreactor, suggesting that the 4CP degradation does not change with time (for each loading step) and it remains practically constant over a wide range of applied IOLs. Furthermore, for the BT-1500 and BT-2500 tests, an increased Cl^- accumulation corresponding to an increased 4CP consumption rate can be observed.

Respirometric data in terms of SOUR and specific substrate biodegradation rates (estimated from chloride accumulation in the bioreactor and reported on a COD basis) have been plotted vs. time

in Fig. 6a, b and c for the BT-1000, BT-1500 and BT-2500 tests, respectively. As expected, SOUR showed a progressive increase for increased IOLs. The same SOUR profile pattern is observed in the three tests with an initial increase followed by a decrease, which can be explained by the increase in the biomass concentration in the system. No consistent variation of the SOUR is detected after the second loading step in all the tests, while an increase is observed after the third loading step for BT-2500, which suggests effective biomass adaptation to the most severe feeding conditions. Similar considerations apply to the biodegradation rate, which is not negatively affected by the increased load in all cases.

4. Conclusions

A continuous TPPB operated with amorphous polymeric tubing for the treatment of “hostile” industrial wastewater is currently in

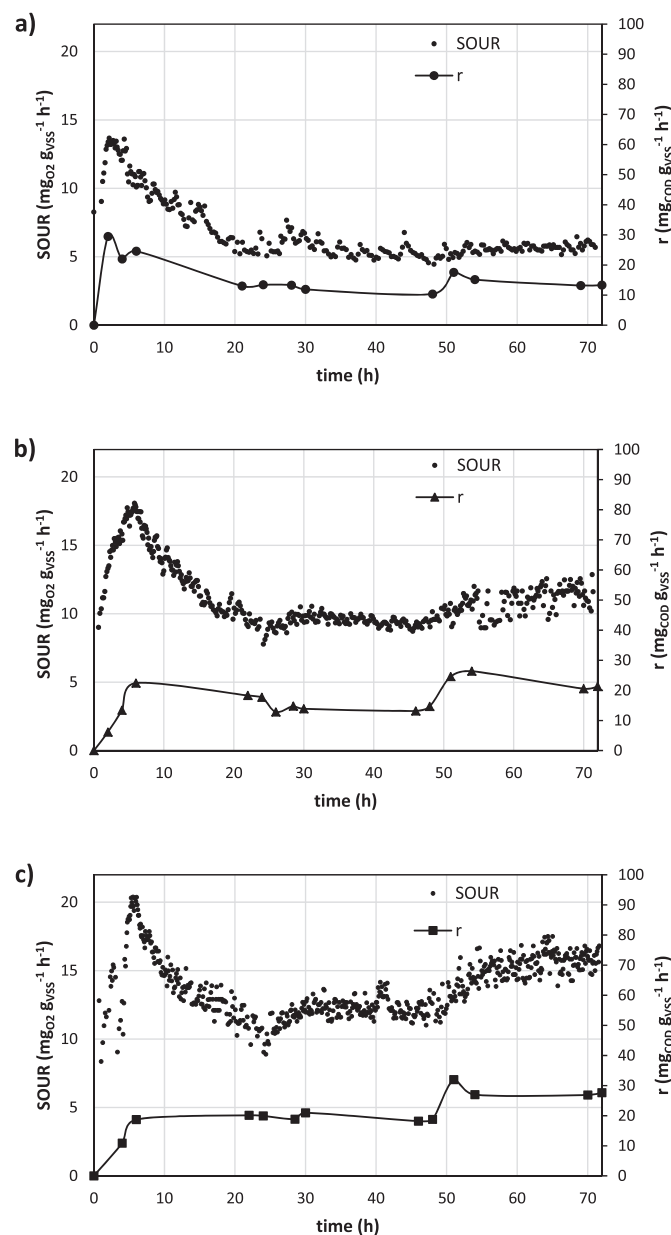


Fig. 6. SOUR and substrate consumption rate as a function of time in BT-1000 (a), BT-1500 (b) and BT-2000 (c) loading tests.

the development stage, and the present study indicates that this type of bioreactor has significant potential in treating wastewater from the tannery industry. The main attractiveness of the C-TPPB configuration with polymeric tubing, i.e. the feature to biodegrade toxic organic molecules such as 4CP without any contact between the bio-medium containing the microorganisms and the waste stream, has been successfully demonstrated in this case, by eliminating cell contact with the highly toxic Cr (VI) salt. Microbial activity was not affected by the influent wastewater composition in the range of investigated concentrations (i.e. 1000–2500 mg 4CP L⁻¹ and 100 mg Cr (VI) L⁻¹) and complete chromium separation allows its easy recovery from the tubing effluent. The robustness of the C-TPPB configuration has been demonstrated over a wide range of loading and 4CP removal was always higher than 98% even in presence of a 5-fold increased IOL. The demonstrated advantages of the proposed system fall within the basic principles of “reduce, reuse and recycle” required by a cleaner and eco-efficient improved production.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.jclepro.2017.03.135>.

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