



Comparison of bioaugmented EGSB and GAC–FBB reactors and their combination with aerobic SBR for the abatement of chlorophenols



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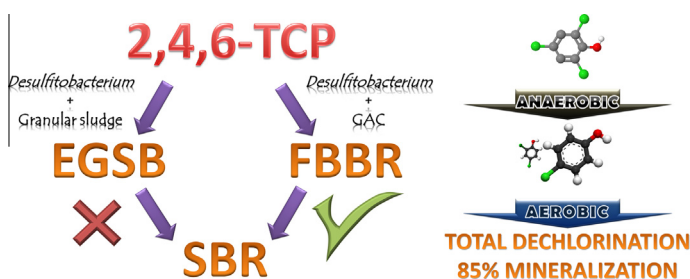
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HIGHLIGHTS

- Coupling bioaugmented GAC–FBBR and aerobic SBR was effective for 246TCP abatement.
- 246TCP was anaerobically dechlorinated to 4CP.
- Bioaugmentation with *Desulfotobacterium* spp. improves the stability.
- GAC–FBBR enhances the stability and dechlorination efficiency.
- 4CP and ecotoxicity were efficiently abated in the aerobic stage.

GRAPHICAL ABSTRACT



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ABSTRACT

The biological abatement of 2,4,6-trichlorophenol (246TCP) and its chlorinated degradation byproducts using anaerobic and aerobic biological reactors coupled in series has been studied. The performance of an anaerobic fluidized bed biofilm reactor (FBBR) and expanded granular sludge bed (EGSB) reactors bioaugmented with *Desulfotobacterium* strains was compared within a wide range of 246TCP loading rates. The bioaugmentation of an EGSB reactor with *Desulfotobacterium* strains enhanced the chlorophenols removal efficiency and the stability against high toxic shocks. The FBBR showed an even higher stability, but also improved the dechlorination efficiency and required a shorter start-up period than the bioaugmented EGSB reactor. Thus, it was selected as the preferred anaerobic system. The subsequent treatment of the effluents from the anaerobic reactors in an aerobic sequencing batch reactor allowed complete dechlorination and improved mineralization up to 85% TOC reduction with a substantial abatement of the ecotoxicity, which was diminished in more than 80%.

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1. Introduction

The development of effective solutions for the treatment of a wide diversity of industrial wastewaters containing highly toxic

chlorinated organic pollutants has gained a growing interest in the last decade. 2,4,6-Trichlorophenol (246TCP) is a well-known wood, leather and textile preservative and it has been widely used as a precursor in the synthesis of the biocides 2,3,4,6-tetrachlorophenol and pentachlorophenol until their prohibition by the Rotterdam Convention. It can be present in wastewater of the pulp industry resulting from the widely used ECF-type pulp bleaching process [1]. It has been also detected in groundwater [2] and in contaminated barrel-aged wine from *Quercus* sp. oak wood [3]. It

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has been described as genotoxicant [2] and the US-EPA has claimed about the high toxicity of 246TCP in contact with the human skin. Therefore, it has been included in the List of Priority Pollutants of the US-EPA and it is also listed in the European Directive 2006/11/EC on pollution caused by certain dangerous substances discharged into the aquatic environment of the Community (List I, organohalogen compounds). Besides its own high toxicity it is recognized as a precursor in the formation of some polychlorinated dibenzo-*p*-dioxins (PCDDs) and dibenzofurans (PCDFs) [4].

High rate anaerobic systems such as upflow anaerobic sludge blanket (UASB) reactor, expanded granular sludge bed (EGSB) reactor and fluidized bed biofilm reactor (FBBR) have been increasingly used for treating a wide range of industrial off-streams particularly toxicant-bearing wastewaters [5]. These systems can deal efficiently with high organic loads. Among the anaerobic high-rate technologies, the EGSB and FBBR systems have been claimed as highly efficient and cost-effective solutions. The former can be operated within a wide range of upflow rate ($2\text{--}10\text{ m h}^{-1}$), allowing the treatment of a diversity of wastewaters including those containing toxic pollutants. The second uses a fluidized media with high adsorption capacity, such as activated carbon, providing biomass support and toxicants uptake [6]. A comparison between these two technologies for the treatment of chlorophenols is still needed.

EGSB reactors and FBBRs have been used for treating chlorophenolic wastewaters [7]. The degradation of the resulting monoaromatics from dechlorination can be also achieved by these technologies [8]. Despite anaerobic cultures are capable of dechlorinating polychlorinated compounds [9], they show limitations for degrading monochlorophenols (MCPs) which then can remain in the final discharges [10]. In fact, these compounds have been described as the hardest aromatic compounds to be anaerobically biodegraded among 46 different aromatics [11]. Different intensification strategies have been evaluated as potential solutions. Bioaugmentation has been successfully applied for improving the anaerobic biodegradability of chlorophenols, reducing the acclimation time [12]. However, better knowledge on the dechlorination of polychlorinated compounds and the resulting MCPs upon anaerobic treatment is needed.

Despite the recalcitrant nature of MCPs, some efforts have been addressed towards the use of aerobic biological processes [13]. Among the different biological systems used for the breakdown of MCPs, sequencing batch reactors (SBR) have important advantages such as reduced space, easy control, nutrients removal and low energy requirements. The versatility of SBR allows modifying the operation and control strategies so that can be adapted to process a wide range of organic loads [14–16]. This technology has been evaluated for the treatment of different chlorophenols, analyzing the influence of the sequence, temperature and acclimation strategy, as well as the breakdown pathways of these compounds [14]. However, this system has shown a fairly low efficiency when dealing with polychlorinated species [7]. The combination of anaerobic and aerobic biological systems could be an interesting option for that kind of pollutants. The effective coupling of anaerobic and aerobic bioreactors has been claimed in the last years as a potential solution for different wastewaters [17].

The aim of this work is to compare the behavior of EGSB reactors and granular activated carbon (GAC)–FBBR bioaugmented with *Desulfitobacterium* spp. strains and to evaluate their combination with a subsequent aerobic SBR for the abatement of chlorophenols. The two-step anaerobic–aerobic biological treatment has been analyzed as a potential solution for the breakdown of 246TCP at relatively high loading rate (up to around $1300\text{ }\mu\text{mol L}^{-1}\text{ d}^{-1}$). The performance of the anaerobic reactors was evaluated in terms of chlorophenols conversion and overall dechlorination as well as stability towards toxic shocks of

246TCP. In addition, an innovative approach has been used for a better operation of an aerobic SBR as post-treatment system.

2. Materials and methods

2.1. Biodegradability and inhibition anaerobic tests

The specific methanogenic activity (SMA) tests were performed according to a previous work [10].

Anaerobic biodegradability of 246TCP, 2,4-dichlorophenol (24DCP) and 4-chlorophenol (4CP) was evaluated using 250 mL serum bottles inoculated with active granular sludge at 1.5 g L^{-1} of volatile suspended solids (VSS). The temperature and pH were set at $30 \pm 1\text{ }^{\circ}\text{C}$ and 7 ± 0.1 , respectively. The macro and micronutrients media were prepared according to Puyol et al. [10]. Bicarbonate was added at 4 g L^{-1} to provide alkalinity and the medium was supplemented with $100\text{ mg L}^{-1}\text{ Na}_2\text{S}\cdot 10\text{H}_2\text{O}$ and flushed for five minutes with $\text{N}_2\text{:CO}_2$ (80:20 v/v) in order to assure anaerobic conditions. 246TCP, 24DCP and 4CP were tested at concentrations up to 100 mg L^{-1} during 10 d. No extra carbon source was added. Anaerobic granular sludge from a full-scale UASB reactor treating brewery wastewater (Mahou, Guadalajara, Spain) was used as inoculum.

The amount of chlorophenols biosorbed onto the biomass was quantified following a partial detachment, extractive and digestive method. The sludge was centrifuged and washed and the chlorophenols were extracted with acetonitrile/water for determining the adsorbed fraction of chlorophenols. The chlorophenols absorbed inside the sludge were subsequently extracted by digestion of the cells with lysozyme [18].

Methanogenesis inhibition tests were performed in the same conditions as the biodegradability tests, but adding a standard carbon source for methanogenesis, consisting of a mixture of acetate:propionate:butyrate at 1:1:1 (by weight) equivalent to a COD of 4 g L^{-1} . Chlorophenols (246TCP, 24DCP and 4CP) were tested at concentrations up to 250 mg L^{-1} for 15 d. The previous acclimation of the sludge was studied as a determining factor. Experiments with non-acclimated microorganisms were conducted with the aforementioned brewery granular sludge. The biomass source for the acclimated sludge experiments had two different origins. Granular sludge adapted to 4CP and 24DCP was retrieved from a lab-scale EGSB reactor treating 24DCP for 180 d [10], whereas the sludge adapted to 246TCP was withdrawn from the non-bioaugmented EGSB reactor described above at the end of the experiments. Methane was measured by a bioprocess control automatic methane potential test system (AMPTS, Bioprocess Control, Sweden). The 50% effective concentration (EC_{50}) values were estimated by the following equation:

$$\text{SMA} = \text{SMA}_{\max} / [1 + (I/\text{EC}_{50})^n] \quad (1)$$

where SMA_{\max} represents the maximum SMA ($\text{g CH}_4\text{-COD g}^{-1}\text{ VSS d}^{-1}$), I is the chlorophenol concentration (μM) and n is the inhibition order (dimensionless). Experimentally measured SMA values were fitted to Eq. (1) by means of non-least square minimization of error using the Levenberg–Marquardt algorithm (Origin 8.0, OriginLab, Northampton, MA, USA).

2.2. Anaerobic continuous experiments

2.2.1. Reactors configuration

Long-term anaerobic experiments were performed at $30 \pm 1\text{ }^{\circ}\text{C}$ in three 5.4 L polymethylmethacrylate reactors of 10 cm internal diameter and 72.5 cm height. The three-phase separator was located 15 cm below the top of each reactor. Two of them were operated as EGSB reactors inoculated with 100 g VSS of an

anaerobic granular sludge retrieved from a lab-scale EGSB reactor enriched with 24DCP [10]. CO₂ was removed from biogas using a Mariotte flask with a 4 M NaOH solution trap, and methane was measured with a wet gas-meter (Schlumberger, Houston, TX, USA). The other reactor, operated as FBBR, was loaded with 1 kg of raw granular activated carbon (GAC) from Merck (Whitehouse Station, NJ, USA) characterized by a BET surface area (S_{BET}) of 917 m² g⁻¹, an external or non microporous area of 120 m² g⁻¹ and micro and mesopore volumes of 0.33 and 0.04 cm³ g⁻¹, respectively. Methane was not monitored in the FBBR. External recirculation in the EGSB and FBBR reactors was provided by means of peristaltic pumps for fluidizing both the granular sludge and GAC-biofilm at upflow rates of 4 and 10 m h⁻¹, respectively.

2.2.2. Synthetic wastewater

The synthetic feed was prepared as was described before for the anaerobic batch experiments. Sucrose, ethanol and yeast extract (3:2:0.1, expressed as COD) were used as carbon sources. The synthetic medium was dosed from a stock solution of 25 mM 246TCP (Sigma–Aldrich, St. Louis, MO, USA) in 0.1 M NaOH.

2.2.3. Bioaugmentation and start-up

One of the EGSB reactors (EGSB-B) and the FBBR were bioaugmented with three strains belonging to *Desulfitobacterium* genus (*Desulfitobacterium hafniense* PCP-1, *D. hafniense* TCP-A, and *Desulfitobacterium chlororespirans*). These species have been previously reported as very versatile bacteria capable of dechlorinating a wide range of chlorinated organics. Their specific growth rate is relatively elevated so that it is quite easy to grow them in batch and use for bioaugmentation. All of them are able of dechlorinating in *ortho*-position, but *D. hafniense* PCP-1 is even capable of achieving *para*-dechlorination [19]. These species were previously grown in a specific medium for *Desulfitobacterium* spp. until reaching the maximum biomass concentration (see [Supplementary Material](#)). Then, 350 mL of each culture were added to the EGSB-B and the FBBR reactors. Bioaugmentation was performed in batch mode during 12 d. Carbon source and 246TCP were added daily at 7.5 g COD L⁻¹ and 50 μM, respectively. The control EGSB reactor was not bioaugmented (EGSB-NB). The three reactors were started at an organic loading rate (OLR) of 7.5 g COD L⁻¹ d⁻¹, which was reduced to the half after 90 d of continuous operation for promoting MCPs biodegradation [10]. The 246TCP loading rate (246TCP-LR) was increased stepwise after the bioaugmentation from 50 to 1300 μmol L⁻¹ d⁻¹, in different operational stages.

2.2.4. Stability analyses

Two sharp increases of 246TCP-LR were applied to EGSB and FBBR reactors, a step one where the 246TCP-LR was raised to 5000 μmol L⁻¹ d⁻¹ and maintained for 22 h, and a pulse injection, allowing a 246TCP concentration of around 5000 μM inside the reactor. A stabilization time was let between both actions where the 246TCP-LR was set at 1300 μmol L⁻¹ d⁻¹. COD, chlorophenols concentration and methane production (in the EGSB reactors) were monitored.

2.2.5. Adsorption

The following mass balance serves to describe the sorption of 246TCP into the GAC of the FBBR:

$$Q \cdot C_0 - Q \cdot C = (V_T - V_C) \frac{\partial C}{\partial t} + M_C \frac{\partial q}{\partial t} \quad (2)$$

where Q is the inlet flow rate (L d⁻¹), V_T and V_C are volumes of the reactor and GAC, respectively (L), C_0 and C are the influent and effluent 246TCP concentrations (μM), M_C is the mass of GAC (g) and q is the amount of 246TCP adsorbed onto the GAC (μmol 246TCP g⁻¹ GAC).

Using the Freundlich equation to describe the adsorption equilibrium in Eq. (2) leads to:

$$(V_T - V_C) \frac{\partial C}{\partial t} + M_C \cdot K_f \cdot \frac{1}{n} \cdot C^{(\frac{1}{n}-1)} \cdot \frac{\partial C}{\partial t} + Q \cdot C - Q \cdot C_0 = 0 \quad (3)$$

Each stage corresponding to the different 246TCP-LR tested was separately simulated by using Scientist 3.0 software (Micromath, USA). Integration was performed by the EPISODE numerical method for Stiff systems. The boundary conditions for each stage result from the values obtained in the previous one.

2.3. Aerobic degradation tests

The biodegradability of the effluents from 246TCP treatment in EGSB-B and FBBR reactors was assessed by respirometry in a liquid-static-static (LSS) respirometer following the fast biodegradability test developed by Polo et al. [20]. The sample (1 L) was mixed with the biomass (350 mg VSS L⁻¹) and aerated for 24 h. The specific oxygen uptake rate (SOUR), COD and concentration of the main byproducts in the EGSB-B and FBBR effluents (246TCP, 24DCP, 4CP, acetate and propionate) were measured in each experiment. The stoppered reaction flasks were placed in a thermostatic bath and continuously stirred by magnetic bars.

The contribution of abiotic processes such as volatilization and adsorption onto the sludge flocs was evaluated. Adsorption of chlorophenols was measured after extracting the samples in Soxhelt following the US-EPA 8041 method. Volatilization tests were performed under identical operating conditions to those of the biodegradation experiments but in absence of biomass.

2.4. Aerobic long-term SBR experiments

Sequencing batch reactors of 2.1 L with a thermostatised water jacket to control the operating temperature were used. They were equipped with dissolved oxygen and pH probes. Peristaltic pumps were used for feed and discharge as well as for the addition of NaOH solution (3 M) used for pH control. Air was continuously supplied at 9 NL min⁻¹ through a ceramic diffuser and mechanical stirring was maintained. The experiments were conducted in a series of fixed-time stages in sequences of 12 h as follows: anoxic filling (1 h), aerated reaction (9.5 h), settling (1 h) and draw (0.5 h). The experiments were carried out at 25 ± 1 °C. HRT was studied between 16 and 80 h, and sludge retention time was fixed at 30 d. Biomass concentration in the reactors was maintained at around 2500 mg VSS L⁻¹. The inoculum was collected from a lab-scale SBR enriched with 4CP during 2 months at 390 μmol 4CP L⁻¹ d⁻¹.

2.5. Analytical methods

COD, total and volatile suspended solids (TSS and VSS), V_{30} , and sludge volumetric index (SVI) were determined following the APHA standard methods [21].

4-Chlorocatechol and 4CP were quantified by HPLC/UV (Prostar, Varian, USA) using a C₁₈ column as stationary phase (Microsorb MW-100-5) and a mixture of acetonitrile and milli-Q H₂O (40:60, by vol.) as mobile phase, whereas 246TCP and 24DCP were analyzed using a mobile phase consisting of acetonitrile and 0.025 M acetic acid (60:40, vol.). The flow rate was maintained at 1 mL min⁻¹ and a wavelength of 280 nm was used.

Total organic carbon (TOC) was measured using an OI Analytic Model 1010 TOC apparatus. Ecotoxicity determinations were carried out by the Microtox Acute Toxicity Test (SCI 500 Analyser) using a freeze-dried preparation of the marine bacterium *Vibrio fischeri* as described in ISO 11348-3. The results were expressed

in toxicity units (TU), calculated from the 50% inhibitory concentration (IC_{50}) values ($TU = 100/IC_{50}$).

2.6. Scanning electron microscopy (SEM)

The morphology of the granular sludge from the EGSB reactors and the biofilm supported on the GAC in the FBBR was analyzed by SEM with a Philips XL30 microscope. Samples were cut and fixed according to the method developed by Alphenaar et al. [22].

2.7. Fluorescence in situ hybridization (FISH)

Representative samples (around 0.03 g) of the anaerobic granular sludge and GAC-biofilm from the EGSB reactors and FBBR, respectively, were re-suspended in 890 μ L of a phosphate buffer solution (PBS, 130 mM NaCl, 10 mM Na_2PO_4/NaH_2PO_4 , pH = 7.2–7.4) and dispersed by sonication (average power 0.50 W, frequency 0.9 cycles s^{-1}). The FBBR samples were subsequently centrifuged at 500 rpm for 2 min to separate the GAC from the biofilm and recover the supernatant. Mixtures from EGSB and FBBR were fixed with formaldehyde (4% v/v final concentration) for 6 h, centrifuged 5 min at 13,000 rpm, the pellets washed with 1 mL PBS three times, and finally re-suspended in ethanol:PBS 50:50 v/v and conserved at $-20^\circ C$ before analyses. Specific probes for *Bacteria* (EUB-338), *Archaea* (ARC-915) [23], *Desulfitobacterium* spp. (Dsf326) and *D. hafniense* (Dsf576) [24] amended with Cy3 were selected. FISH was performed according to protocols described previously by Amann et al. [23]. After hybridization, the specimens were stained with 4',6'-diamidino-2-phenylindole (DAPI) (1 mg mL^{-1}). *Escherichia coli*, *Methanosaeta concilii* and *D. hafniense* PCP-1 were used as positive controls for EUB-338, ARC-915 and both Dsf326 and Dsf576, respectively. *D. hafniense* PCP-1 hybridization with NON338 [25] was used as negative control. Hybridized samples were observed using an epifluorescence microscope equipped with Cy3 and DAPI filters (Axioskop, Berkochen, Germany) and a digital camera. Counting was performed by taking pictures from 20 random fields for each sample, corresponding theoretically to around 1000 stained cells.

2.8. Data analysis

The results reported were the average values from duplicate runs. Control experiments were conducted for all the batch tests. The standard errors of the measurements were always lower than 5%.

3. Results and discussion

3.1. Biodegradability and inhibition anaerobic tests

Fig. 1 shows the time course of 246TCP, 24DCP and 4CP in the anaerobic batch experiments with non-acclimated granular sludge at different starting concentrations. 246TCP was almost completely converted in 10 d at starting concentrations below 300 μM . In the case of 24DCP an initial stage of high disappearance rate was observed due to biosorption, followed by biodegradation at almost constant rate even at the highest initial concentration tested. The main chlorinated by-product upon 246TCP and 24DCP biodegradation was 4CP, suggesting that the *ortho*-dechlorination is the predominant degradation pathway. This last species appears almost refractory to anaerobic biodegradation except at the lowest concentration tested (40 μM).

The inhibitory effect of 246TCP, 24DCP and 4CP on the methanogenic activity of acclimated and non-acclimated granular sludge has been analyzed by calculating the EC_{50} values. The decrease of

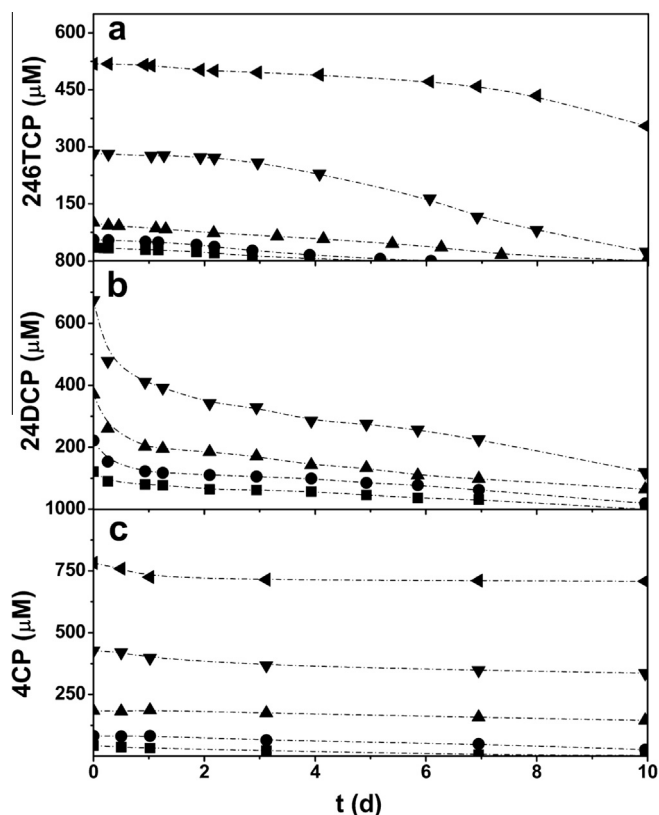


Fig. 1. Time-evolution of 246TCP (a), 24DCP (b) and 4CP (c) in the anaerobic batch experiments at different initial concentrations.

the number of chloride in the phenolic ring and the acclimation of the biomass reduce considerably the toxicity of chlorophenols towards methanogens. The EC_{50} values for non-acclimated biomass were 122, 276 and 311 $\mu mol L^{-1}$ for 246TCP, 24DCP and 4CP, respectively. The acclimation of the biomass increased these values up to 208, 798 and 948 $\mu mol L^{-1}$, respectively, indicating that methanogens can deal with much higher chlorophenols concentrations once the biomass is properly acclimated. It is therefore crucial to optimize the acclimation step for achieving high dechlorination efficiency and therefore ensuring a stable performance of a methanogenic anaerobic reactor.

3.2. Anaerobic reactors performance

The anaerobic reactors were operated for 248 d at different 246TCP loads, which were gradually increased along the successive operational stages for the aiming of acclimation. Table 1 summarizes the results obtained for the EGSB-NB and EGSB-B reactors. The high and stable values of COD removal efficiency and methane yield indicate the good performance of the EGSB system. Beyond the 8th stage, where the 246TCP loading rate acquired fairly high values, a decrease of the COD removal efficiency was observed although without significant effect on the methane yield. The analysis of the *Archaea* population in the granular sludge by FISH showed an increasing percentage of those microorganisms upon the testing time as can be seen in Fig. 2a and b. Previous works have reported a certain sensitivity of acetogenic bacteria and obligated hydrogen-producing acetogens, like propionate and lactate oxidizers, to the presence of chlorophenols [18,26]. It is therefore suggested that the decrease of the COD removal efficiency could be derived from partial inhibition of biological steps previous to methanogenesis.

Table 1
Performance of the anaerobic EGSB reactors.

Stage	t (d)	EGSB-NB						EGSB-B									
		TCP-LR ^a	4CP-D	24DCP-D	X_{TCP}	X_{COD}	η_{CH_4}	X_{CP}	Dec	TCP-LR	4CP-D	24DCP-D	X_{TCP}	X_{COD}	η_{CH_4}	X_{CP}	Dec
1	15	46 ± 6	27 ± 22	13 ± 2	>99	83 ± 1	20 ± 6	6 ± 3	65 ± 3	46 ± 10	9 ± 8	6 ± 38	70 ± 38	83 ± 1	29 ± 11	12 ± 6	55 ± 6
2	12	89 ± 5	81 ± 41	7 ± 1	>99	86 ± 2	36 ± 9	8 ± 6	67 ± 6	97 ± 7	25 ± 5	16 ± 1	>99	88 ± 2	50 ± 8	54 ± 17	79 ± 17
3	32	118 ± 17	117 ± 48	3 ± 0	>99	90 ± 2	33 ± 6	5 ± 4	67 ± 4	137 ± 16	73 ± 66	5 ± 1	>99	89 ± 3	45 ± 8	43 ± 26	83 ± 26
4	17	168 ± 25	127 ± 73	4 ± 1	>99	89 ± 3	37 ± 4	9 ± 8	75 ± 8	178 ± 10	139 ± 71	4 ± 1	>99	88 ± 2	39 ± 1	21 ± 19	74 ± 19
5	18	245 ± 36	247 ± 43	7 ± 1	>99	88 ± 2	36 ± 4	<5	66 ± 5	219 ± 29	206 ± 46	4 ± 1	>99	89 ± 2	41 ± 3	7 ± 6	69 ± 6
6	55	276 ± 36	282 ± 25	6 ± 1	>99	91 ± 1	41 ± 7	<5	66 ± 5	270 ± 32	277 ± 19	7 ± 1	>99	91 ± 1	54 ± 9	<5	65 ± 5
7	26	450 ± 40	295 ± 60	4 ± 1	>99	90 ± 1	27 ± 1	11 ± 6	78 ± 6	431 ± 34	384 ± 64	11 ± 1	>99	86 ± 2	37 ± 4	9 ± 6	69 ± 6
8	40	746 ± 57	749 ± 68	23 ± 4	98 ± 3	82 ± 3	32 ± 7	<5	62 ± 5	647 ± 89	605 ± 73	39 ± 1	99 ± 1	82 ± 2	48 ± 9	8 ± 5	64 ± 5
9	14	1023 ± 96	887 ± 66	51 ± 8	99 ± 2	75 ± 6	26 ± 7	7 ± 5	67 ± 5	1093 ± 40	662 ± 34	17 ± 15	95 ± 15	77 ± 6	48 ± 11	34 ± 7	74 ± 7
10	19	1318 ± 71	924 ± 174	64 ± 10	93 ± 11	72 ± 6	38 ± 5	18 ± 6	66 ± 6	1417 ± 86	684 ± 348	114 ± 23	83 ± 23	72 ± 6	35 ± 10	36 ± 6	61 ± 6

^a TCP-LR, influent 246TCP loading rate ($\mu\text{mol } 246\text{TCP L}^{-1} \text{d}^{-1}$); 4CP-D, 4CP discharge ($\mu\text{mol } 4\text{CP L}^{-1} \text{d}^{-1}$); 24DCP-D, 24DCP discharge ($\mu\text{mol } 24\text{DCP L}^{-1} \text{d}^{-1}$); X_{TCP}, 246TCP removal efficiency (%); X_{COD}, COD consumption efficiency (%); η_{CH_4} , methane yield (g COD-CH₄ g⁻¹ COD consumed); X_{CP}, chlorophenols removal efficiency (%); Dec, dechlorination efficiency (% chloride removed).

The fate of 246TCP and its degradation by-products is also summarized in Table 1. Fairly low percentages of chlorophenols removal (calculated as the difference between the inlet 246TCP molar concentration and the sum of the outlet molar concentrations of all the chlorophenols with respect to the inlet 246TCP molar concentration) were achieved (5–10%) in the non-bioaugmented EGSB reactor. However the overall dechlorination efficiency was maintained in general above 65% reaching more than 75% in some stages since the major chlorophenol in the exiting stream was by far 4CP (>80% on molar basis). The chlorophenols removal efficiency decayed somewhat as the 246TCP loading rate increased beyond 180–200 $\mu\text{mol L}^{-1} \text{d}^{-1}$ and seemed to recover moderately towards the end of the experiment suggesting some acclimation of the biomass.

The bioaugmentation of EGSB clearly improved the chlorophenols removal efficiency and allowed also a moderate increase of the methane yield although no significant effect was observed on COD reduction and overall dechlorination efficiency. The degradation pathway proceeded also through *o*-dechlorination of 246TCP to 4CP, but 4CP concentration in the effluent was lower than in the non-bioaugmented EGSB reactor, probably due to partial *p*-dechlorination of 4CP. To elucidate the differences found between the two reactors, the development of *Desulfotobacterium* genus was analyzed by FISH. The time course of *D. hafniense*, *D. chlororespirans* and the total *Desulfotobacterium* genus in EGSB reactors is shown in Fig. 2a and b. The presence of *D. hafniense* in the EGSB-NB was detected in the inoculum, since the biomass was previously adapted to 24DCP. The relative population of *D. hafniense* within the *Desulfotobacterium* genus in both reactors remained almost constant at around 30–35% of the total biomass, indicating a well-established population of this species in the granules. *D. chlororespirans* was only found in the EGSB-B after being externally added in this system by bioaugmentation. However, the relative abundance of this species seemed to decrease during the first 60 d of operation, when an important loss of the external layer of the granules was observed by SEM, probably caused by the toxic shock associated to the presence of chlorophenols (see Supplementary Material, Fig. S2). As the biomass was acclimating to the increasing 246TCP load, this layer seemed to be restored and *D. chlororespirans* appeared to establish within the microbial ecosystem, increasing its population in the granular sludge, thus leading to improved dechlorination.

Table 2 summarizes the operating conditions and performance of the FBBR. Both the chlorophenols removal and overall dechlorination efficiencies were much higher than the observed in the EGSB reactors. Almost complete disappearance of chlorinated species occurred during the first stages, probably due in great part to sorption onto the GAC-biofilm. In order to analyse more in depth the fate of 246TCP, its adsorption onto the supporting GAC was studied, assuming that the adsorption equilibrium follows the Freundlich equation as postulated by other authors for similar adsorbents [27]. Previous experiments showed that equilibrium was reached in less than 24 h (see Supplementary Material). The adsorption equilibrium tests were performed in stirred stoppered polyethylene bottles using GAC colonized with biofilms and the data were fitted to the Freundlich equation ($q = K_F C^{1/n}$, where q is the amount adsorbed, here in $\mu\text{mol g}^{-1}$, and C is the equilibrium concentration, μM). Values of 39.9 and 3.1 ($R^2 = 0.97$) were obtained for K_F and n , respectively. A more detailed explanation of the experimental procedure for adsorption experiments can be accessed in Supplementary Material. Simulation of the adsorption process on the GAC added into the FBBR was carried out by Eq. (3). The predicted values of the exit concentration of 246TCP by considering only adsorption onto GAC are depicted in Fig. 3 (line). According to the predicted curve saturation of GAC is accomplished in about 60 days (first three stages). However, the experimental

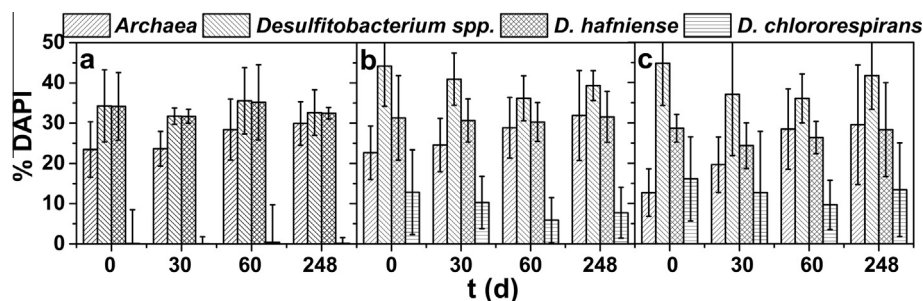


Fig. 2. Time course of Archaea and *Desulfitobacterium* spp. in EGSB-NB (a), EGSB-B (b) and FBBR (c) reactors.

Table 2

Performance of the anaerobic FBBR.

Stage	t (d)	FBBR						
		TCP-LR ^a	4CP-D	24DCP-D	X _{TCP}	X _{COD}	X _{CP}	Dec
1	15	41 ± 5	<1	<1	>99	19 ± 4	>99	>99
2	12	87 ± 19	<1	<1	>99	35 ± 7	>99	>99
3	32	106 ± 15	<1	<1	>99	35 ± 3	>99	>99
4	17	143 ± 17	<1	<1	>99	38 ± 10	>99	>99
5	18	185 ± 23	7 ± 5	<1	>99	67 ± 9	97 ± 9	99 ± 9
6	55	255 ± 27	33 ± 24	12 ± 7	>99	68 ± 5	83 ± 10	93 ± 10
7	26	375 ± 37	82 ± 28	6 ± 7	99 ± 1	82 ± 6	74 ± 4	90 ± 4
8	40	633 ± 122	264 ± 71	31 ± 35	98 ± 4	79 ± 5	51 ± 8	81 ± 8
9	14	933 ± 96	306 ± 65	45 ± 19	98 ± 2	79 ± 4	59 ± 8	84 ± 8
10	19	1078 ± 78	344 ± 122	57 ± 23	99 ± 1	80 ± 4	63 ± 9	85 ± 9

^a TCP-LR, influent 246TCP loading rate ($\mu\text{mol } 246\text{TCP L}^{-1} \text{d}^{-1}$); 4CP-D, 4CP discharge ($\mu\text{mol } 4\text{CP L}^{-1} \text{d}^{-1}$); 24DCP-D, 24DCP discharge ($\mu\text{mol } 24\text{DCP L}^{-1} \text{d}^{-1}$); X_{TCP}, 246TCP removal efficiency (%); X_{COD}, COD consumption efficiency (%); X_{CP}, chlorophenols removal efficiency (%); Dec: dechlorination efficiency (% chloride removed).

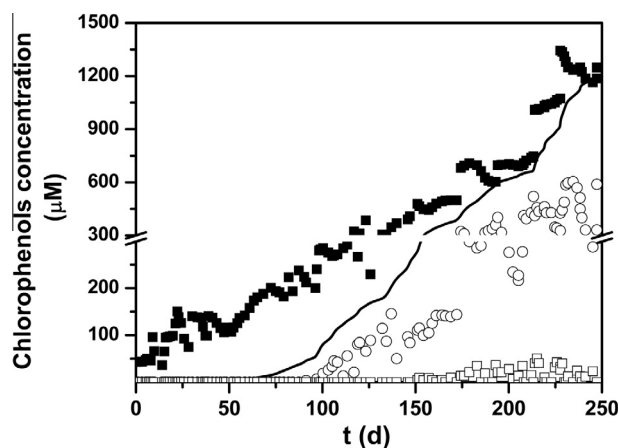


Fig. 3. Time course of inlet (■) and exit (□) 246TCP concentrations, and the total chlorophenols concentration (○) in the FBBR. The line depicts the predicted exit concentration of 246TCP by considering only adsorption onto GAC (Eq. (3)).

exit concentration of 246TCP continues being almost negligible up to the end of the 248 days of the experiment (see X_{TCP} values of Table 2). Thus, dechlorination is associated to biological activity and this is clearly more efficient in the FBBR than in the EGSB system. Explanation of these differences requires a detailed analysis of the microbial population contained in the biofilm attached to the GAC.

The biofilm was gradually developed upon the operation time acquiring a stable and mature form, as was evidenced by SEM images (see Supplementary Material, Fig. S3). Bacteria were preferably attached to carbon fissures at early stages, but finally they were able to cover the entire carbon surface. Colonization of *Desulfitobacterium* species started during the bioaugmentation

stage, reaching relative abundances of 28% and 16% of the biomass for *D. hafniense* and *D. chlororespirans*, respectively. Then, the presence of *Desulfitobacterium* decreased and the anaerobic community was gradually established and diversified, which gave rise to increasing COD reduction efficiencies. However, the increasingly high 246TCP-LR towards the end of the experiment favoured the selection of highly-resistant species with an increasing relative abundance of the halo-respiring-bacteria, mainly of *D. chlororespirans*, which finally achieved 16% of the total biomass. This species was more abundant in the biofilms from the FBBR than in the granules of the EGSB-B at the end of the experiments, reinforcing the idea of its important role in the higher dechlorination efficiency of the former system.

The dechlorination efficiency of both EGSB-B and FBBR reactors seems to be quite high if comparing with values reported in the literature. Maximum volumetric dechlorination rates of 2.59 and 2.75 mmol Cl⁻ removed L⁻¹ d⁻¹ have been achieved in the current work in EGSB-B and FBBR reactors, respectively. Other recent works reported values between 0.64 and 2 mmol Cl⁻ removed L⁻¹ d⁻¹ treating 246TCP and 24DCP with different anaerobic and partially-aerobic reactors [10,28–30]. The results taken as a whole indicated that the bioaugmentation with *Desulfitobacterium* spp., in the case of the EGSB-B and FBBR, and the use of GAC as an adsorbent support, in the later, increased considerably the reactor efficiency and therefore appear as promising and robust alternatives for the abatement of the toxicity associated to chlorine-containing species like chlorophenols.

A comparison between the stability of the EGSB and FBBR reactors was conducted by applying step and delta increases of 246TCP-LR. The step increase of the 246TCP-LR up to 5000 $\mu\text{mol L}^{-1} \text{d}^{-1}$ increased the exit 246TCP concentration up to 2500, 2000 and 1000 μM in the EGSB-NB, EGSB-B and FBBR, respectively, as can be seen in Fig. 4. The performance of the FBBR was recovered in 8 h, whereas 24 h were needed for the EGSB

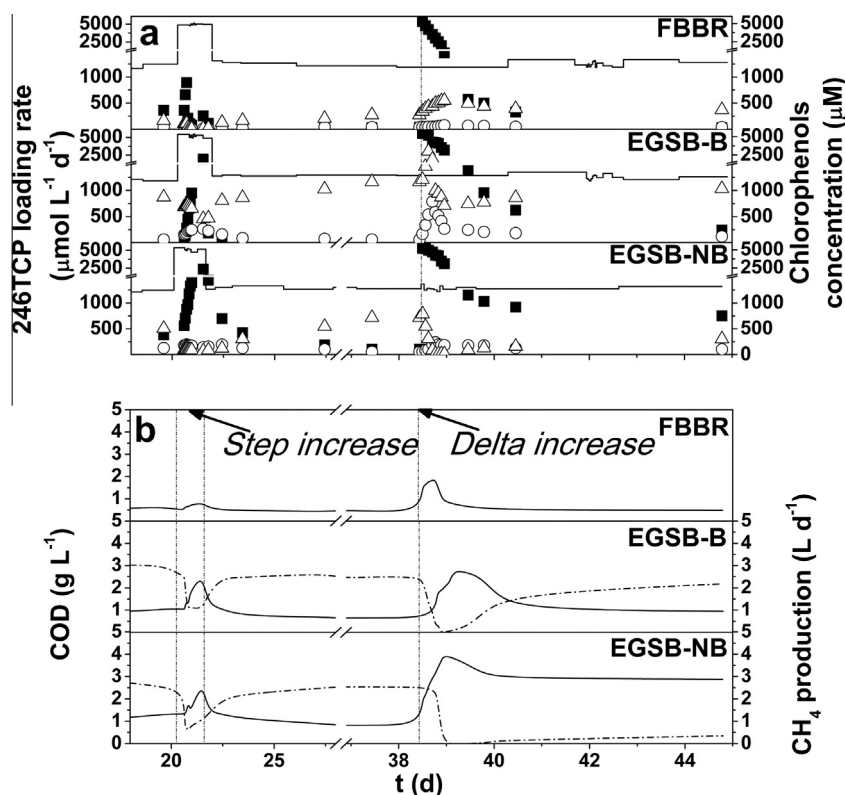


Fig. 4. Analysis of stability of the anaerobic reactors. (a) Time course of 246TCP-LR (continuous line) and the exit concentrations of 246TCP (squares), 24DCP (circles) and 4CP (triangles); (b) outlet COD values (continuous line) and methane production (dash line).

reactors. The increase of the 246TCP inlet concentration caused a competitive inhibition of 24DCP dechlorination, which explains the decrease of the 4CP concentration in the effluents. The exit COD increased above 2 g L^{-1} and the CH_4 production lowered below 1 L d^{-1} in both EGSB reactors, whereas the performance of the FBBR was not significantly altered as depicted in Fig. 4.

The delta increase of inlet 246TCP concentration caused more remarkable differences in the stability of the reactors. The CH_4 production was completely inhibited in the EGSB reactors and the exit COD increased up to values close to 4, 3 and 1.9 g L^{-1} in the EGSB-NB, EGSB-B and FBBR, respectively. After 6 d of the delta increase, the COD removal efficiencies were partially recovered reaching values of 23%, 75% and 87% in the EGSB-NB, EGSB-B and FBBR reactors, respectively. Exit 246TCP concentrations of 760, 240 and $25 \mu\text{M}$ were respectively measured.

The presence of *D. chlororespirans* must play a relevant role in improving the response of the reactors to toxic shocks. The EGSB-B reactor and the FBBR, which contain a stable population of this species, showed a better performance than the EGSB-NB reactor. This species has been characterized as a versatile bacterium in the use of both electron donors and acceptors for the reductive dechlorination process compared with its counterparts of the genus *Desulfitobacterium* [31]. It has also shown a slightly higher biomass yield and specific activity than its congeners [19,32] (see also Supplementary Material, Fig. S1).

The GAC in the FBBR could alleviate significantly the effect of the sudden increase of 246TCP by protecting the activity of the biofilm, thus maintaining the COD in the discharge below 2 g L^{-1} and allowing high dechlorination efficiency. Adsorption of chlorophenols in aqueous phase occurs into micro and mesopores of activated carbon [33], which cannot be occupied by bacteria because of size exclusion. Dechlorinating bacteria attached into the GAC can remove CP present in the liquid media or adsorbed on the

carbon surface, thus regenerating the adsorbent [34]. The combined action of biological reductive dechlorination and adsorption mitigates the toxic effect of CPs towards other microorganisms.

3.3. Aerobic biodegradability and ecotoxicity

Respirometric tests were carried out with the effluents from the EGSB-B reactor and FBBR in order to identify the best anaerobic system to be coupled with an aerobic step for the abatement of 246TCP and its degradation by-products. Table 3 summarizes the composition of the final effluents from the EGSB-B and FBBR experiments and the conversion values obtained in the subsequent aerobic biodegradability tests. Short chain organic acids were almost completely removed and 4CP was degraded by around 25%, leading to about 60% COD reduction. Aerobic degradation of 246TCP and 24DCP was negligible. Fig. 5 shows the respirometric profiles obtained in the biodegradability tests. The SOUR values observed for the FBBR effluents were initially much higher than those of the EGSB-B reactor, consistently with the higher concentrations of chlorinated species in the second case.

Table 3

Exit concentrations from EGSB-B and FBBR and conversion values achieved in the aerobic biodegradability tests after 24 h.

	EGSB-B exit (μM)	Aerobic conversion (%)	FBBR exit (μM)	Aerobic conversion (%)
COD (mg L^{-1})	1269	60	571	63
246TCP	73	0	21	0
24DCP	86	0	49	0
4CP	872	25	398	26
Acetate	9503	>99	4476	>99
Propionate	836	>99	394	>99

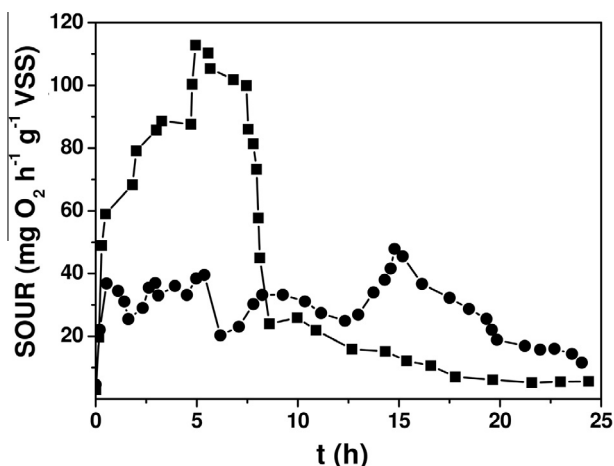


Fig. 5. SOUR profiles obtained for the effluents from the EGSB-B reactor (circles) and the FBBR (squares).

The Microtox® test yielded an increase of the ecotoxicity upon the anaerobic treatment experiment of Table 1 in the EGSB-B reactor (from the 23.4 T.U. initial value to 59.1 T.U. of the effluent). On the opposite, no change was observed in the case of FBBR (24.4 T.U. in the effluent). The ecotoxicity of both effluents was reduced by a 23% upon the aerobic biodegradability tests, which could be associated to the degradation of 4CP under aerobic conditions.

3.4. Aerobic sequencing batch reactor experiments

The aerobic respirometric test showed around 25% conversion of 4CP (see Table 3). Thus, an aqueous solution containing a 4CP concentration similar to the maximum discharge concentration detected in the resulting effluents from the anaerobic reactors (930 μM) was fed to the SBR. SBR was supplemented with nutrients (COD:N:P = 100:0.5:0.1) and operated at different HRT for the sake of learning on the potential coupling of anaerobic and aerobic treatment. The ability of the mixed bacterial cultures for using chlorophenols as carbon and energy sources is associated to the selection and enrichment in SBR of specialized populations present in the consortium [13]. As can be seen in Fig. 6a, 4CP was completely converted in the SBR within a wide range of HRT (16–80 h). The rate of disappearance increased with the HRT due to the decrease of the maximum 4CP concentration inside the reactor after the filling stage. The highest specific degradation rate (12.1 mg 4CP g⁻¹ SSV h⁻¹) was reached at an HRT of 80 h. Previous works have reported that the addition of cosubstrates and the use of operation strategies based on time control can increase the specific degradation rate up to 15.8 and 22.5 mg 4CP g⁻¹ SSV h⁻¹, respectively [13,14]. At 10 h HRT a concentration of 750 μM of 4CP was reached after filling, which caused the destabilization of the system (data not shown). A fraction of TOC showed to be refractory, representing about 35% at the lowest HRT tested. This residual matter can be partially associated to microbial or excreted products from 4CP degradation [35], whose accumulation decreases as the discharge volume from the SBR in every cycle increases. The remaining TOC can be related to the presence of refractory microbial products, whose concentration is significantly reduced at decreasing HRT due to the lower decay rate.

Recent studies have concluded that the filling stage exerts a great influence on the removal efficiency of toxic compounds, so that a high long-term stability can be achieved by controlling that stage [36]. However, the effect of the inlet flow rate (IFR) used for filling has not been studied so far. Thus, the SBR was operated at different IFR and volume exchange ratios (VER). The time course

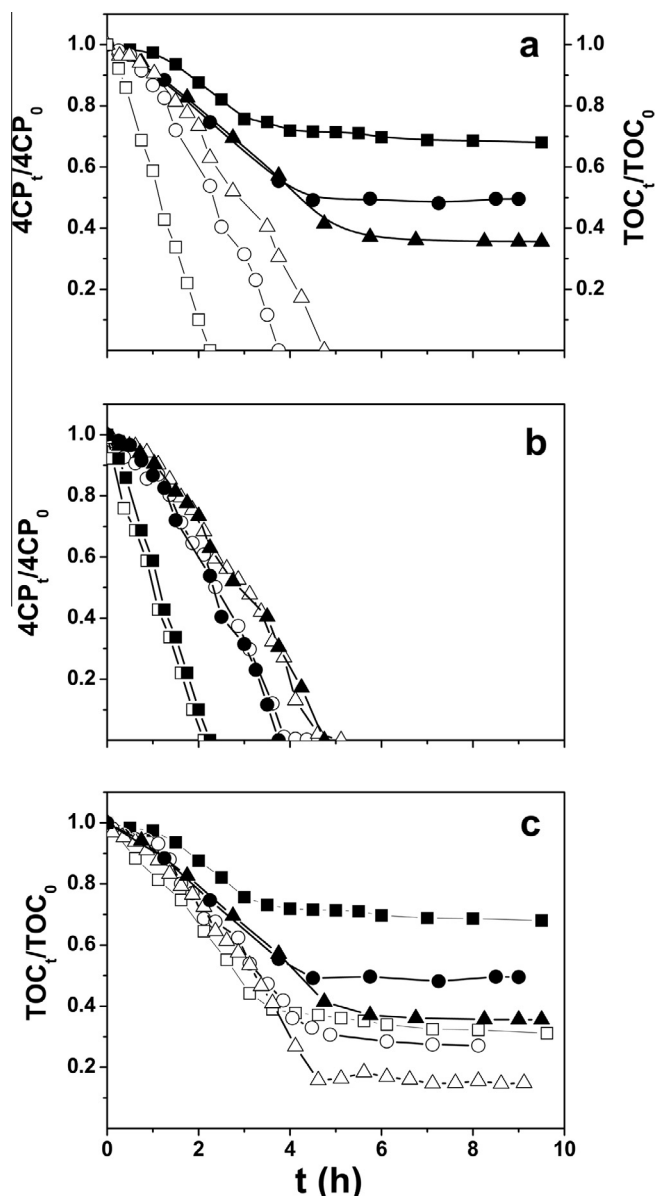


Fig. 6. Aerobic SBR performance. Time-evolution of 4CP (open symbols) and TOC (solid symbols) in the SBR operated at HRT of 80 (squares), 23 (circles) and 16 (triangles) h (a); and time-evolution of 4CP (b) and TOC (c) at VER values of 0.10 (squares), 0.35 (circles) and 0.50 (triangles) in the SBR operated at IFR = 0.10 (solid symbols) and 0.75 L h⁻¹ (open symbols).

of normalized 4CP and TOC during the aerobic stage is depicted in Fig. 6b and c. During these experiments the filling time was maintained constant at 30 min, since a too short filling time would reduce the removal rates finally causing a complete destabilization of the system [37]. A maximum VER of 0.5 could be applied, indicating that concentrations of 4CP above 470 μM at the end of the filling stage should be avoided since would lead to accumulation of 4CP destabilizing the SBR.

Decreasing the VER from 0.50 to 0.10 led to a reduction of the time required for complete conversion of 4CP from 4.75 to 2.25 h (Fig. 6b), since the maximum 4CP concentrations reached were 470 and 80 μM, respectively. Working at different IFR did not cause significant differences on the rate of disappearance of 4CP at any of the VER tested (Fig. 6b). However, the TOC removal rate was fairly improved when the IFR was increased from 0.10 to 0.75 L h⁻¹ at given VER (Fig. 6c). Although increasing the 4CP concentration

diminished the efficiency of the system, in this case, the increase of the VER from 0.1 to 0.5 at a given IFR reduced the TOC of the effluents from 50 to 7 mg L⁻¹. 4-Chlorocatechol was identified as the main intermediate of aerobic 4-CP biodegradation, which was completely removed along the cycle. The ecotoxicity of the effluents resulting at 0.5 VER and 0.75 L h⁻¹ IFR was around 4.5 TU, thus allowing concluding that a significant reduction of the ecotoxicity of the effluents from the FBBR can be achieved by further treatment in SBR.

4. Conclusions

The dechlorination capacity of EGSB reactors adapted to 246TCP can be enhanced by bioaugmentation with *Desulfotobacterium*. Particularly, *D. chlororespirans* plays a key role in the improvement of the stability towards toxic shocks. The use of GAC as an adsorptive biofilm carrier in FBBR serves as a protective barrier against the 246TCP toxicity. The protection improves the reactor stability and dechlorination efficiency compared with EGSB reactors working at similar operating conditions. The high ecotoxicity of 4CP resulted from the *o*-dechlorination of 246TCP is significantly reduced using an aerobic SBR, where the abatement of chlorophenols is achieved by VER and IFR optimization.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.cej.2014.07.134>.

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