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# 2,4,6-Trichlorophenol and phenol removal in methanogenic and partially-aerated methanogenic conditions in a fluidized bed bioreactor

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**Abstract: A fluidized bed bioreactor (FBBR) was operated for more than 575 days to remove 2,4,6-trichlorophenol (TCP) and phenol (Phe) from a synthetic toxic wastewater containing 80 mg $L^{-1}$ of TCP and 20 mg $L^{-1}$ of Phe under two regimes: Methanogenic (M) and Partially-Aerated Methanogenic (PAM). The mesophilic, laboratory-scale FBBR consisted of a glass column (3 L capacity) loaded with 1 L of 1 mm diameter granular activated carbon colonized by an anaerobic consortium. Sucrose (1 g COD $L^{-1}$) was used as co-substrate in the two conditions. The hydraulic residence time was kept constant at 1 day. Both conditions showed similar TCP and Phe removal (99.9 + %); nevertheless, in the Methanogenic regime, the accumulation of 4-chlorophenol (4CP) up to 16 mg $L^{-1}$ and phenol up to 4 mg $L^{-1}$ was observed, whereas in PAM conditions 4CP and other intermediates were not detected. The specific methanogenic activity of biomass decreased from 1.01 ± 0.14 in M conditions to 0.19 ± 0.06 mmol$CH_4$ $h^{-1}$ g$TKN^{-1}$ in PAM conditions whereas the specific oxygen uptake rate increased from 0.039 ± 0.008 in M conditions to 0.054 ± 0.012 mmol$O_2$ $h^{-1}$ g$TKN^{-1}$, which suggested the co-existence of both methanogenic archaea and aerobic bacteria in the undefined consortium. The advantage of the PAM condition over the M regime is that it provides for the thorough removal of less-substituted chlorophenols produced by the reductive dehalogenation of TCP rather than the removal of the parent compound itself.**


**Keywords:** chlorophenols; fluidized bed bioreactor; partially-aerated; methanogenic

## NOTATION

| | |
|---|---|
| 4CP | 4-Chlorophenol |
| COD | Chemical oxygen demand |
| DA | Diode array |
| DCP | 2,6-Dichlorophenol |
| DO | Dissolved oxygen |
| FBBR | Fluidized bed bioreactor |
| $I_{CH4}$ | Methane productivity |
| $L_{FB}$ | Liter of fluidized bed |
| M | Methanogenic condition |
| PAM | Partially-aerated methanogenic conditions |
| Phe | Phenol |
| SMA | Specific methanogenic activity |
| $SMA_c$ | Corrected specific methanogenic activity, expressed in terms of only anaerobic biomass |
| SOUR | Specific oxygen uptake rate |
| TCP | 2,4,6-Trichlorophenol |
| TKN | Total Kjeldahl nitrogen |
| vvd | Air flow rate (air volume reactor volume$^{-1}$ day$^{-1}$) |
| VSS | Volatile suspended solids |
| $\alpha$ | Alkalinity ratio |
| $\Delta Cl^-$ | Net Increase of chloride |
| $\eta_{COD}$ | Removal efficiency of organic matter |
| $\eta_{Phe}$ | Apparent removal efficiency of phenol |
| $\eta_{TCP}$ | Removal efficiency of 2,4,6-trichlorophenol |

## INTRODUCTION

Chlorophenols are ubiquitous pollutants in aquifers and wastewaters and considerable research has

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been devoted to their removal, because they are toxic, recalcitrant, and they accumulate in the trophic chain.[1] They are also recognized to be carcinogenic to rats and potentially carcinogenic to humans.[2] In particular, 2,4,6-trichlorophenol is also a precursor of carcinogenic substances such as dibenzo *p*-dioxins.[2] Chlorinated phenols are extensively utilized as pesticides, fungicides and disinfectants, and are important chemicals in a variety of industrial processes.[3] Several chlorophenol residues are released via chemical or biological transformation or degradation of several other groups of pesticides (for example pentachlorophenol, chlorinated phenoxy herbicides, etc) and compounds present in a variety of industrial wastewaters, such as bleaching effluents from the pulp and paper industry or effluents from the cleaning- and disinfectant-manufacturing industry.[4,5] Soils contaminated with wood preservatives very often show high contents of 2,4,6-trichlorophenol (TCP), among other chlorophenols, and also pose a threat to aquifer quality.[6]

Considerable research has been published concerning the treatment of chlorophenols-contaminated waters and wastewaters.[7] For instance, regarding TCP, most experiments report only partial removal and/or accumulation of lower-substituted chlorophenol compounds, such as 2,4-dichlorophenol and 4-chlorophenol, under anaerobic conditions.[8–10] Complete removal and degradation of TCP under anaerobic conditions by undefined consortia has been demonstrated in only a couple of cases.[11] On the other hand, complete aerobic mineralization of higher-substituted chlorophenols, such as TCP, is more difficult to achieve.[7] Only a minor proportion of reported experiments show complete degradation of TCP by undefined aerobic consortia.[12]

Partially-aerated methanogenic (PAM) bioreactors offer an interesting alternative for achieving a more complete removal of toxic compounds and their eventual metabolites, because they could provide more diverse catabolic pathways.[13,14] In particular, PAM bioreactors were successfully applied for the removal of *c* 100% of up to 68.5 mg $L^{-1}$ pentachlorophenol in an upflow anaerobic sludge blanket (UASB) reactor,[14] *c* 100% of an average of 32 mg $L^{-1}$ 2,4,6-TCP in an upflow reactor,[15] and 88% of 42.9 mg $L^{-1}$ perchloroethylene in an upflow packed bed reactor.[16]

The UASB has been the most commonly used bioreactor for the treatment of recalcitrant and toxic compounds in PAM conditions.[13] Other systems have used biomass immobilized in calcium alginate, chitosan, etc, in upflow reactors.[15,16] To the best of our knowledge, there are no reports of the use of partially-aerated methanogenic fluidized bed bioreactors for the treatment of 2,4,6-trichlorophenol-contaminated waters.[13] The fluidized bed bioreactor (FBBR) shows several advantages: it can carry a high concentration of biomass attached to a dense carrier, which cannot be easily washed out from the bioreactor and increases overall pollutant depuration rate; it provides a very large surface area for biomass attachment and wastewater/biocatalyst contact; high mass transfer rates are achieved; it allows for the treatment of low strength wastewaters; it has the ability to control and optimize the biofilm thickness; biomass carrier can be chosen for a specific application to enhance removal; recirculation of treated effluent means that the reactor shows an excellent hydraulic pattern that avoids plugging, shortcircuiting and dead zones; for the same reason an excellent dilution of influent with the effluent is achieved, which provides alkalinity (and consequently, some neutralization) and reduces the concentrations of pollutants and toxic substances (important for high organic wastewaters and/or toxic wastewaters).[17,18] Finally, regarding the potential application of this reactor to partially-aerated methanogenic operation, the resistance to oxygen exposure of bioparticles from an FBBR is reportedly greater than those of anaerobic suspended biomass and anaerobic granules.[19–22]

The objective of this work was to evaluate the feasibility of a partially-aerated methanogenic fluidized bed reactor for the removal of a mixture of TCP and phenol (Phe), using sucrose as co-substrate from a synthetic contaminated wastewater using an anaerobic undefined consortium.

## MATERIALS AND METHODS

### Fluidized bed bioreactor and experimental design

Two experimental regimes were used, namely M and PAM, using the same bioreactor. The laboratory-scale, mesophilic, methanogenic FBBR consisted of a glass column of 4.5 cm internal diameter, 185 cm length and 3 L of working volume; the fluidized bed was 1 L of *c* 1 mm diameter granular activated carbon as carrier, colonized by an anaerobic consortium. Before loading into the bioreactor, granular activated carbon was saturated with a solution containing 80 and 20 mg $L^{-1}$ of TCP and Phe, respectively, until equilibrium conditions were reached.

The experimental set-up of the bioreactor in the M regime is shown in Fig 1(A). It had a recirculation loop, and two peristaltic pumps (Perkin Elmer), the first one for effluent recycling and the second one for wastewater feeding. The experimental set-up of the bioreactor in the PAM regime was similar to that of the M regime, the only difference was the air sparger within the recirculation loop (Fig 1(B)). The hydraulic residence time for both regimes was kept constant at 1 day (fluidized bed volume basis). Average recirculation rates were 12.4 and 18.5 mL $s^{-1}$ in M and PAM conditions (see below for Phase definition) which translated into recirculation ratios of 1060 and 1600, respectively.

Five operational phases were distinguished: (i) Phase 1, start-up with anaerobic inoculum and methanogenic steady state using acetate and sucrose as carbon sources; (ii) Phase 2, phase of acclimation

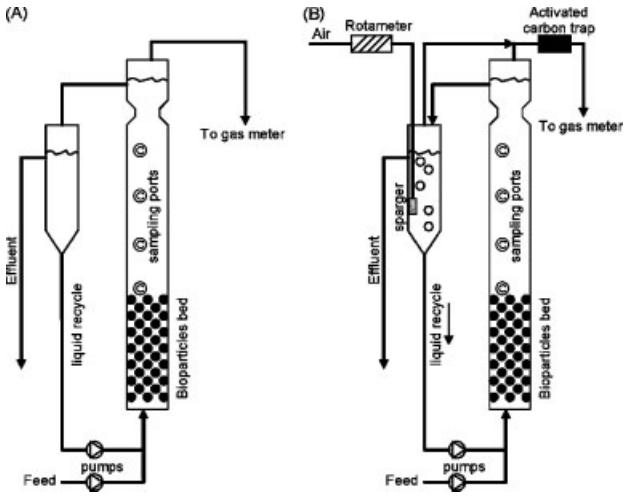


**Figure 1.** Diagram of the bioreactor set-up. (A) Methanogenic regime, (B) partially aerated methanogenic regime.

to TCP and Phe, using decreasing proportions of a synthetic wastewater containing sucrose and acetate as carbon source and increasing proportions of a synthetic toxic wastewater containing 1 g COD-sucrose $L^{-1}$, 80 mg TCP $L^{-1}$ and 20 mg Phe $L^{-1}$, in M conditions; (iii) Phase 3, M conditions with TCP, Phe and sucrose as co-substrate, (iv) Phase 4, PAM conditions with TCP, Phe and sucrose as co-substrate using 2 vvd aeration rate, and (v) Phase 5, PAM conditions with TCP, Phe and sucrose as co-substrate using 15 vvd aeration rate. In Phases 3–5 the influent concentrations of TCP, Phe, and sucrose in the influent were 80 mg $L^{-1}$, 20 mg $L^{-1}$, and 1 gCOD $L^{-1}$, respectively.

The evaluation variables were the removal efficiency of organic matter ($\eta_{COD}$), removal efficiency of TCP ($\eta_{TCP}$), apparent removal efficiency of Phe ($\eta_{Phe}$), appearance of less substituted chlorophenols, $\alpha$ parameter,[23,24] net increase of chloride ($\Delta Cl^-$), methane productivity ($I_{CH4}$), methane content in biogas (%$CH_4$), specific methanogenic activity (SMA) and specific oxygen uptake rate (SOUR) of the biomass. The $\alpha$ parameter is the ratio between the intermediate alkalinity (alkalinity titrated between pH 5.75 and 4.2) and partial alkalinity (titrated between original pH of the sample and 5.75); it approximately represents the ratio of volatile organic acids to bicarbonate alkalinity in anaerobic systems. Values of $\alpha$ lower than 0.5 correlate with robust methanogenic regimes, higher values of $\alpha$ are indicators of acidogenic excursions and instability in methanogenic reactors.[23,24]

## Wastewater and aeration conditions

In Phase 1, the reactor was fed a synthetic wastewater (pH 8.2) with the following composition (g $L^{-1}$): sucrose (0.94), glacial acetic acid (1.50), Fe $SO_4.7H_2O$ (0.012), $(NH_4)_2HPO_4$ (0.08), $K_2HPO_4$ (0.5), $NaHCO_3$ (1), $Na_2CO_3$ (1), and 1.1 mL $L^{-1}$ of a trace elements solution (EDTA—$Na_2.2H_2O$ (0.5), $FeCl_2.4H_2O$ (2), $NiCl_2.6H_2O$ (0.103), $CoCl_2.6H_2O$ (0.15), $(NH_4)_6Mo_7O_{24}.4H_2O$ (0.05), $ZnCl_2$ (0.058), $CuCl_2.2H_2O$ (0.042), $AlCl_3$ (0.03), and $MnCl_2.4H_2O$ (0.5)). In Phase 2, the reactor was fed a mixture of the synthetic wastewater and a synthetic toxic wastewater (the latter had the same composition as the synthetic wastewater except for only 1 g COD-sucrose $L^{-1}$ as co-substrate, 80 mg $L^{-1}$ of TCP, and 20 mg $L^{-1}$ of Phe, pH 8.4), in several stages with decreasing proportions of the synthetic wastewater and increasing proportions of the toxic wastewater (Table 1). After ending the acclimation, the reactor was fed with 100% of toxic wastewater (Phases 3–5). In Phase 4, the reactor was aerated with an air flow rate of 2 vvd, and in Phase 5 with 15 vvd.

**Table 1.** Operating conditions during acclimation phase (Phase 2)

| | Acclimation stage | | | | |
|---|---|---|---|---|---|
| Parameter | 1 | 2 | 3 | 4 | 5 |
| Time (days) | 10 | 9 | 8 | 8 | 13 |
| Synthetic wastewater (%) | 90 | 70 | 50 | 30 | 10 |
| Synthetic toxic wastewater (%) | 10 | 30 | 50 | 70 | 90 |
| 2,4,6-Trichlorophenol (mg $L^{-1}$) | 8 | 24 | 40 | 56 | 72 |
| Phenol (mg $L^{-1}$) | 2 | 6 | 10 | 14 | 18 |
| COD (mg $L^{-1}$) | 2450 | 2210 | 1780 | 1530 | 1210 |

## Analytical methods

The COD, pH, alkalinity, and chloride anion concentrations (titration method with $AgNO_3$) were determined in the influent and effluent according to the standard methods.[25] The chlorinated phenols (TCP, dichlorophenols, monochlorophenols and Phe) in the effluent, bioparticles, and activated carbon trap were analyzed by HPLC (Varian: UV-DA detector (280nm) model 9065, autosampler model 9300; Chromolith Column, model RP-18e, 100 mm × 4.6 mm; acetonitrile–water–acetic acid 60:39:1, flow rate 0.7 mL $min^{-1}$).[26] Carbon from the activated carbon trap and bioparticles of the fluidized bed were extracted as follows: 10 g were mixed with a mixture of acetonitrile–water–acetic Acid 60:39:1 (50 mL). The suspension was vortexed at maximum speed for 10 min and sonicated with an Astrason sonicator for 15 min. The supernatant was recovered by centrifugation. The procedure was repeated twice. About 150 mL of extract solution was recovered and concentrated in a Rotavapor to a volume of 10 mL. The concentrated extract was analyzed by HPLC for chlorophenols and phenol as mentioned above. Standards of TCP, all dichlorophenols and all monochlorophenols were from Sigma-Aldrich, Co. $CH_4$, $O_2$, and $CO_2$ in the biogas were analyzed by GC-TD (GOW-MAC 580 series). The chromatograph was equipped with an Alltech column, model CTR-1, 6″ × 1/4″. The operation temperatures were 28 °C, 200 °C, and 130 °C for the column, detector and

injector, respectively. Biomass content in the reactor was determined by total Kjeldahl nitrogen (TKN);[25] the conversion to VSS was calculated on the basis of the empirical formula $C_5H_7O_2N$ for the biomass with a factor of 0.124 mgN-TKN mgVSS-biomass$^{-1}$.[27] The SMA was determined as described by Sorensen and Ahring.[28] The SOUR was determined according to Kristensen *et al*.[29] The DO was measured using an oxygen meter (YSI INC, model 57, USA).

## RESULTS

Whilst the focus of this study was on Phases 2–5, the FBBR in Phase 1 operated at a volumetric organic loading 2.5 g COD $L_{FB}^{-1}$ day$^{-1}$ and showed a well developed methanogenic regime: pH 7.13 ± 0.18; $CH_4$ in biogas 82.5 ± 4.2% (v/v); COD removal efficiency 96.87 ± 1.74%; $\alpha$0.28 ± 0.03; $I_{CH4}$1.53 ± 0.32 L ($L_{FB}$ day)$^{-1}$; and bioparticle TKN 8.56 ± 2.43 mgTKN $g^{-1}_{\text{dry bioparticle}}$.[30]

### Phase 2: acclimation to 2,4,6-trichlorophenol and phenol

The organic removal efficiency ($\eta_{COD}$) in Phase 2 was high and almost constant (95.9 ± 2.3%). Chlorophenols were not detected in the effluent (Fig 2(A)). The $\alpha$ parameter was below 0.4 (Table 2), in the normal range reported in the literature for robust methanogenic regimes,[23,24] pH values (7.08 ± 0.14) were also in the typical range of methanogenesis.

The $I_{CH}$ decreased with the increased proportion of synthetic toxic wastewater in the influent (from 1.53 down to 0.34 $L_{biogas}$ $L_{FB}^{-1}$ day$^{-1}$, a 67% drop, Fig 2(A)), although methane content in the biogas was constant, around 85% in the whole phase.

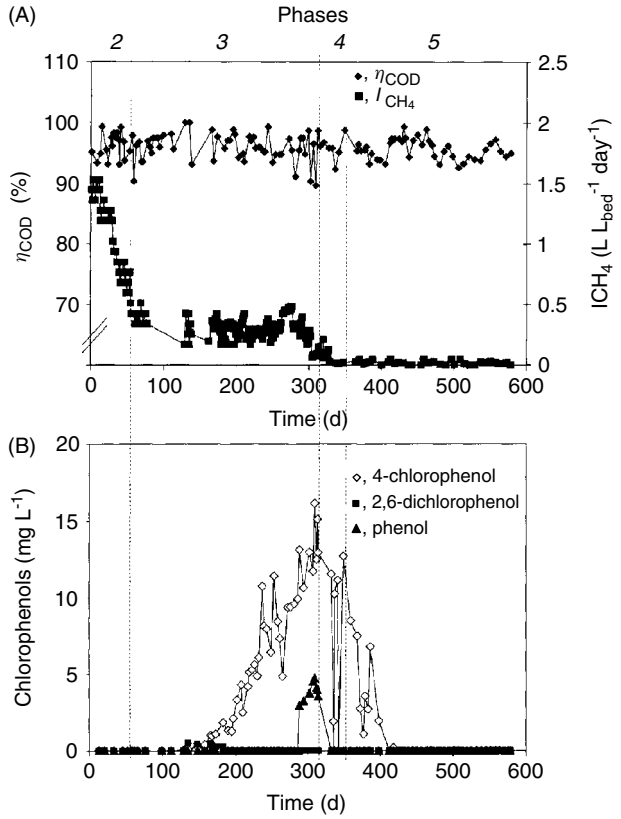


**Figure 2.** Performance parameters of the bioreactor: (A) COD removal efficiency ($\eta$COD) and $CH_4$ productivity ($I_{CH4}$). (B) chlorophenols in the effluent (4-chlorophenol, 2,6-dichlorophenol and phenol). 2, 3, 4, and 5: operational phases.

### Phase 3: methanogenic conditions with 2,4,6-trichlorophenol and phenol in the feed

An outstanding accumulation of 4-chlorophenol (4CP), starting on day 160 of operation, was observed, reaching a maximum of 16 mg $L^{-1}$ at about day 300 (Fig 2(B)). The $\eta_{COD}$ was 96.1 ± 2.2%, similar to that obtained in Phase 2. A decrease in $I_{CH4}$ was observed when a concentration of 12 mg 4CP $L^{-1}$ was reached in the reactor, although the percentage of methane in biogas was similar to Phase 2 (Table 2). An accumulation of Phe (up to 5 mg $L^{-1}$) occurred at the same time (Fig 2(B)). When 4CP concentration reached 16 mg $L^{-1}$ in the effluent, the highest concentration of Phe (~5 mg $L^{-1}$) was observed. Because of the transient accumulation of Phe, the apparent $\eta_{Phe}$ was slightly lower than that obtained in the acclimation phase (Phase 2). The detected phenol in the effluent could be due to the presence of this compound in the feeding as well as the phenol generated by the dechlorination of 80 mg $L^{-1}$ of TCP. Taking into account these two sources of Phe, the maximum concentration of this compound detected in the effluent (assuming zero removal of Phe) would have been 58.1 mg $L^{-1}$. Referring to this theoretical amount, the Phe accumulation reached 8% of that maximum.

Also, a transient accumulation of 2,6-dichlorophenol (DCP) appeared, although in a smaller concentration (1 mg $L^{-1}$) between days 140 and 180. This accumulation started at the same time as the accumulation of 4CP.

The $\alpha$ parameter was below 0.4 (Table 2), although it was greater than in Phase 2. pH values slightly increased with respect to Phase 2 (Table 2), even though both $\alpha$ and pH remained in the range suitable for methanogenesis.

The average TKN expressed in mgTKN $g^{-1}_{\text{dry bioparticle}}$ was 8.4. The average SMA and SOUR were 1.10 ± 0.14 mmol$CH_4$ $h^{-1}$ gTKN$^{-1}$ and 0.04 ± 0.01 mmol$O_2$ $h^{-1}$ gTKN$^{-1}$ respectively. These results suggest the presence of both facultative bacteria and methanogenic archaea in the biofilm of the bioparticles.

### Phase 4: partially-aerated methanogenic conditions: 2 vvd aeration rate

The $\eta_{TCP}$ averaged 99.9 + % in this phase, although the concentration of 4CP fluctuated between 0 and 12 mg $L^{-1}$ (Fig 2(B)). In contrast, the Phe completely disappeared. The $\eta_{COD}$ was 95.5 ± 2.1%, similar to values obtained in Phases 2 and 3. The $\Delta Cl^-$ increased

**Table 2.** Performance parameters of the bioreactor in the different phases of operation

| | Phase | | | |
|---|---|---|---|---|
| Parameter | 2<br>Acclimation to TCP/Phe | 3<br>M[a] | 4<br>PAM[b] | 5<br>PAM |
| $\eta_{TCP}$[c] (%) | 99.9+ | 99.9+ | 99.9+ | 99.9+ |
| $\eta_{Phe}$[d] (%) | 99.9+ | 98.81 | 99.9+ | 99.9+ |
| $\eta_{COD}$[e] (%) | $95.9 \pm 2.3$ | $96.1 \pm 2.2$ | $95.5 \pm 2.1$ | $95.2 \pm 1.6$ |
| 2,4,6-Trichlorophenol in the effluent ($mg\,L^{-1}$) | <DL[f] | <DL | <DL | <DL |
| Phenol in the effluent($mg\,L^{-1}$) | <DL | $0.69 \pm 1.52$ | <DL | <DL |
| $\Delta Cl^{-}$[g] ($mg\,Cl^{-}\,L^{-1}$) | | $18.4 \pm 11.8$ | $28.6 \pm 2.6$ | $30.6 \pm 3.4$ |
| pH | $7.1 \pm 0.1$ | $7.3 \pm 0.2$ | $7.6 \pm 0.1$ | $7.6 \pm 0.1$ |
| $\alpha$[h] | $0.30 \pm 0.08$ | $0.40 \pm 0.1$ | NA[i] | NA |
| $I_{CH4}$[j] ($L\,L_{FB}^{-1}\,day^{-1}$) | $1.06 \pm 0.33$ | $0.29 \pm 0.10$ | $0.04 \pm 0.02$ | $0.02 \pm 0.01$ |
| $CH_4$ in biogas (% v/v) | $85 \pm 3$ | $87 \pm 5$ | $0.02 \pm 0.01$ | $0.01 \pm 0.005$ |
| DO[k] ($mgO_2\,L^{-1}$) | NA | NA | $0.2 \pm 0.02$ | $0.5 \pm 0.02$ |
| SMA[l] ($mmolCH_4\,h^{-1}\,gTKN^{-1}$) | ND[m] | $1.097 \pm 0.135$ | ND | $0.189 \pm 0.058$ |
| SOUR[n] ($mmolO_2\,h^{-1}\,gTKN^{-1}$) | ND | $0.039 \pm 0.008$ | ND | $0.054 \pm 0.012$ |
| TKN[o] ($mgTKN\,g_{dry\ bioparticle}^{-1}$) | $8.06 \pm 1.43$ | $8.4 \pm 3.4$ | $14.2 \pm 1.3$ | $29.7 \pm 13.4$ |

[a] Methanogenic conditions; [b] partially-aerated methanogenic conditions; [c] removal efficiency of 2,4,6-trichlorophenol; [d] apparent removal efficiency of phenol; [e] organic matter removal efficiency; [f] below detection level; [g] net increase of chloride; [h] $\alpha$ parameter; [i] not applicable; [j] $CH_4$ productivity; [k] dissolved oxygen; [l] specific methanogenic activity; [m] not determined; [n] specific oxygen uptake rate; [o] total Kjeldahl nitrogen.

with respect to the corresponding value obtained in Phase 3 (Table 2).

### Phase 5: partially-aerated methanogenic conditions: 15 vvd aeration rate

Removals of both TCP and Phe were practically complete (Table 2). The 4CP concentration decreased until it was below the detection level of the method when the aeration rate was increased up to 15 vvd and kept constant at this value (Fig 2(B)).

The $\eta_{COD}$ was $95.2 \pm 1.6$, indicating that this parameter was unaffected by trichlorophenol or by oxygen sparging. The pH value increased slightly with respect to the previous phase, a fact that could be ascribed to bicarbonate alkalinity from the $CO_2$ produced in the aerobic degradation of sucrose. The $\Delta Cl^{-}$ increased with respect to previous phases ($30.6\,mgCl^{-}\,L^{-1}$), which agrees with the absence of 4CP in the effluent. This value of $\Delta Cl^{-}$ should be compared with a theoretical maximum $\Delta Cl^{-}$ of $43\,mg\,L^{-1}$ (assuming total dechlorination of $80\,mg\,L^{-1}$ of TCP).

The average TKN was $29.7\,mg_{TKN}\,g_{dry\ bioparticle}^{-1}$, which represented an increase of *ca* 300% with respect to the TKN measured in M conditions. Some methane was detected in the biogas in spite of air sparging in the whole phase (Fig 2(A), Table 2), although the $I_{CH4}$ was very low. The SMA decreased from $1.01 \pm 0.14$ in M conditions (Phase 3) to $0.19 \pm 0.06\,mmolCH_4\,h^{-1}\,gTKN^{-1}$ in PAM conditions (Phase 5), a decrease of 82%. The SOUR increased from $0.039 \pm 0.008$ in M conditions to $0.054 \pm 0.012\,mmolO_2\,h^{-1}\,gTKN^{-1}$ (39% increase).

The volatilized chlorinated compounds in our work were retained in an activated carbon trap; the amount of adsorbed compounds was lower than 0.05% of the total feeding of chlorophenols. In addition, the adsorption of xenobiotics onto the bioparticles was lower than 0.02% of TCP fed in the period (on organic chlorine basis, data not shown).

## DISCUSSION

In Phase 2 the $I_{CH4}$ dropped from 1.53 to $0.34\,L_{CH_4}\,L_{FB}^{-1}\,day^{-1}$, ie, *c* 77% reduction. This effect could be partially ascribed to the progressively lower organic loading rate during the acclimation phase since the toxic wastewater had 60% less organic substrate than the synthetic wastewater. Dissolved methane escaping in the effluent cannot account for the remaining 17% difference (0.5–1% at most). On the one hand, it seems that methanogenesis was somewhat affected by the increase of TCP and Phe in the feed in Phase 2, although TCP and Phe removal efficiencies did not decrease (comparing averages of Phases 2 and 3, Table 2). On the other hand, several studies have suggested that biological dechlorination is not necessarily coupled to methane production.[31,32] Kawahara *et al* observed that 2,4,6-TCP inhibited the methane production by nearly 20% when the culture was exposed to $80\,mg\,L^{-1}$ of TCP and by 60% when exposed to $100\,mg\,L^{-1}$.[33] These two lines of evidence could explain why methane production decreased 16.5% more than the theoretical decrease predicted by the mere decrease on COD load of the influent, while at the same time TCP removal efficiencies remained relatively high. It can be shown that $I_{CH4}$ in Phases 3, 4, and 5 were approximately 83, 11 and 6% of the maximum theoretical $I_{CH4}$, respectively.[34]

Interestingly, $I_{CH4}$ of Phases 4 and 5 were approximately 13.8 and 6.9% of that in Phase 3, respectively, based on data of Table 2. These values

plus the retained SMA of biomass in Phases 4 and 5 (Table 2) seem to confirm that the FBBR in this study operated in partially-aerated methanogenic conditions.

The SMA results show that methanogenic archaeabacteria were able to survive in the PAM conditions, although the exposure to oxygen significantly decreased the methanogenic activity of the consortium in Phase 5. The resistance to oxygen of this type of archaea has been reported in several works.[19,20,35] The SMA obtained under M conditions was 1.097 $\pm$ 0.135 mmol$CH_4$ $h^{-1}$ $gTKN^{-1}$ (Table 2), the results are greater than that reported previously for anaerobic sludge which was not exposed to xenobiotic compounds (0.070–0.808 mmol$CH_4$ $h^{-1}$ $gTKN^{-1}$).[28] James and co-workers obtained SMA values slightly superior to that reported in this work (2.24–2.49 mmol$CH_4$ $h^{-1}$ $gTKN^{-1}$) using anaerobic granular sludge from a UASB non-exposed to xenobiotics,[36] which allow us to suggest that the SMA reported in our study is very acceptable, taking into account that the microorganisms were also exposed to high concentrations of xenobiotics (TCP and Phe). The SMA of PAM conditions was *c* 82% less than that in M conditions, whereas the SOUR increased *c* 39% under PAM conditions (Table 2). The decrease in SMA in Phase 5 could be due to the double influence of the expected negative impact of oxygen on the methanogenic archaea activity and the increase of facultative or aerobic biomass in the bioparticles (biomass in bioparticles increased three-fold, from 8.4 to 29.7 mgTKN-biomass $g^{-1}_{\text{dry bioparticle}}$). A 'corrected' SMA ($SMA_c$, where the methane production is divided by only the anaerobic portion of biomass) of 0.47 mmol $CH_4$ (h g anaerobic TKN)$^{-1}$ can be calculated from SMA in Table 2, based on an estimation of the net increase of methanogenic biomass between Phases 3 and 5. In this way, the decrease in $SMA_{Phases3,5}$ ($\% \cong [1.097 - 0.47)/1.097] \cdot 100 = 57\%$) is possibly ascribed to a negative effect of aeration on methanogenic archaea. Moreover, the decrease in SMA ($82 - 57 = 25\%$) would be due to the effect of using overall biomass (methanogenic plus aerobic) in the denominator of the formula for SMA. The puzzling discrepancy between very low values of $I_{CH4}$ and low-to-moderate values of SMA in Phases 4 and 5 could be explained as follows: the two variables are not necessarily correlated since SMA is determined in a special batch test where all substrate is in principle available for methanogenesis, in the absence of competing oxygen and toxicants,[28] whereas $I_{CH4}$ is produced in actual conditions in a continuous reactor under partial aeration, where sucrose uptake is being channelled to both aerobic metabolism and methanogenesis in the presence of toxicants (Table 2, Phase 5).

The SOUR obtained in PAM conditions (0.054 mmol $O_2$ $h^{-1}$ $gTKN^{-1}$) was lower than the values reported for fluidized bed bioparticles from a completely aerated FBBR (0.385 mmol $O_2$ $h^{-1}$ $gTKN^{-1}$) which removed TCP and its by-products.[26] The specific respiration was *c* seven times lower in this study, which could be due to the fact that bioparticles studied by Campos-Velarde and co-workers 26 were exposed to lower concentrations of TCP than bioparticles of our work, and came from a completely aerated bioreactor.

Accumulation of Phe, 4CP, and DCP under methanogenic conditions (Phase 3) has been reported by several authors working with different chlorophenols in batch and continuous systems.[9,11,15,37] For instance, Gardin *et al*, working with TCP in a continuous upflow anaerobic sludge blanket (UASB) reactor loaded with an anaerobic consortium immobilized in $\kappa$-carrageenan, observed an accumulation of 4CP greater than 12 mg $L^{-1}$ during methanogenic operation.[15] Vallecillo *et al* experimented with an anaerobic FBBR fed with synthetic wastewater containing TCP and acetic acid as primary substrate. 4CP accumulated in the effluent, although the amount was not reported. Removal efficiencies of TCP averaged only 80% at influent TCP concentration of 25 mg $L^{-1}$,[9] significantly less than in the M and PAM (15vvd) conditions described here. Kennes *et al* showed accumulation of phenol when they treated 200 μmol 4CP in batch cultures under an anaerobic process.[11] Accumulation of 2,6-dichlorophenol in batch cultures under anaerobic conditions was observed by Takeuchi *et al* when treated with 2,4,6-trichlorophenol (4 μM).[37]

During PAM conditions (Phase 5), all the chlorinated compounds were removed in our study (Fig 2(B)). Gardin and coworkers also reported complete removal of TCP under PAM conditions, although they used a lower concentration of TCP in the influent (average: 32 mg $L^{-1}$) and a higher aeration rate (36–48 vvd) than in our study. They also supplemented with a mixture of organic co-substrates glucose, acetate and propionate (0.5 g COD $L^{-1}$ each). Unfortunately some of their results are not conclusive since they just tested two points with the highest concentration of TCP in the influent (100 mg $L^{-1}$) in the last phase of their experimentation which lasted only 13 days. They also observed an accumulation of 4CP in the same phase and could not conclusively show its removal under PAM conditions.[15]

Other researchers have also noted a better removal of chlorinated compounds under PAM conditions than under either anaerobic or aerobic conditions. For instance, Macarie and Guiot reported a degradation of 100% when they worked with 68.5 mg $L^{-1}$ of pentachlorophenol in PAM conditions in a UASB.[14] Tartakovsky *et al* observed higher mineralization efficiency of perchloroethylene using an upflow bed filter in PAM conditions (88%) than in M conditions (38%).[16]

It is well known that chlorophenols with a high degree of halogen substitution (penta-, tetra-, tri-chlorophenols) can be efficiently dechlorinated under anaerobic conditions,[8] however, very often the

dehalogenation is not complete and less-substituted chlorophenols (like 4CP) accumulate in the treated effluent, as was mentioned above.[6,8,10,11,15] On the other hand, the less-substituted chlorophenols (di- and mono-chlorophenols) can be transformed or biodegraded to $CO_2$, water and chloride under aerobic conditions.[26,38] So, accumulation of 4CP and Phe in Phase 3 of our work (M regime) and its thorough removal when the reactor was aerated in Phase 5 is consistent with results in the literature. This is exactly the core of the advantage of PAM conditions: they unite the best of both worlds, ie easier and faster reductive dechlorination of higher substituted chlorophenols by anaerobes and degradation by aerobic microbes of the produced lower substituted chlorophenols.

Another type of reactor tested to remove TCP was the series anaerobic–aerobic FBBR used by Campos-Velarde *et al*. Although they observed an efficient overall TCP removal of 95%, they needed two bioreactors.[26] Consequently, it would imply a more expensive set-up and operation than the current study in the case of any future application.

The low adsorption of chlorophenols onto the carbon in the trap and the bioparticles of the fluidized bed, plus the precaution of loading the FBBR with activated carbon that was previously saturated in a solution of $80\,mg\,TCP\,L^{-1}$ and $20\,mg\,Phe\,L^{-1}$, strongly suggests that removal of TCP and lower substituted chlorophenols was biologically-mediated.

## CONCLUSION

The biological fluidized bed reactor removed 99.9% of TCP, in both the methanogenic and partially-aerated methanogenic conditions. Interestingly, during the M phase, 4-chlorophenol and phenol accumulated up to $16\,mg\,L^{-1}$ and $5\,mg\,L^{-1}$ respectively. These metabolites were completely removed under partially-aerated methanogenic conditions. The advantage of the partially-aerated methanogenic reactor over the methanogenic relates more to the thorough removal of less-substituted chlorophenols produced by the reductive dehalogenation of the mother compound (2,4,6-trichlorophenol) rather than the removal of the parent compound itself.

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