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Fast Aqueous Biodegradation of Highly-Volatile Organic Compounds in a Novel Anaerobic Reaction Setup

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Abstract: The present work explores the biodegradation of some emerging pollutants (EPs) in an anaerobic slowly-agitated up-flow packed-bed reactor (USPBR) filled with biological activated carbon (BAC). Chlorobenzene (CB) and 2,4-dichlorophenoxyacetic acid (2,4-D) were selected as volatile organic compounds (VOC) and major constituents of many pesticides. Experiments carried out in continuous operation showed that bioconversion up to 90% was achieved for CB and 2,4-D, at space times below 0.6 h and 1.2 h, respectively, at ambient temperature. Overall, removal rates of 0.89 g L^{-1} d^{-1} and 0.46 g L^{-1} d^{-1} were obtained for CB and 2,4-D, respectively. These results revealed that the degradation of CB and 2,4-D in this anaerobic configuration of bioreactor is an efficient and fast process. The Michaelis–Menten model properly describes the degradation process for CB. Above initial concentrations of 100 mg L^{-1}, 2,4-D presented a considerable inhibitory effect over the biofilm. For this reason, a substrate inhibition factor was included in the Michaelis–Menten equation; the expanded model presented a good fitting to the experimental data, regardless of the inlet concentration. Therefore, USPBR-BAC combination showed to be a highly efficient system for the biodegradation of such compounds.

Keywords: anaerobic biodegradation; continuous fixed-bed reactor; biological activated carbon; chlorobenzene; 2,4-dichlorophenoxyacetic acid

1. Introduction

In the last decades, the planet has received the negative impacts of unbridled progress due to human activities, such as industry, transport, agriculture, and urbanization [1]. The increment in living standards and the rising needs of customers have increased water pollution by the so-called "emerging pollutants". Due to the possible impact of these substances for the environment and human health, even at very low concentrations (ng L^{-1} range), this area has becoming of growing interest for environmental researchers. This new class of pollutants involves pesticides, chlorinated aromatic compounds, pharmaceuticals and personal care products (PPCPs), metals, and many others. A frequent characteristic of all of them is that they are not yet nominally incorporated in habitual control programs at a global level, though they have indeed been added to the Candidates Contaminant List to prioritize their regulation in the immediate future [2–4]. The literature reports an increase of chemicals' worldwide production from 1 million to 400 million tons per year, between 1930 and 2000. Statistics reported by EUROSTAT (Statistical Office of the European Communities) in 2013 disclose

that, between 2002 and 2011, around 50% of the total chemical production can be included among environmental pernicious pollutants and over 70% of these are chemicals with a notable environmental footprint [1].

In this study, 2,4-dichlorophenoxyacetic acid (2,4-D) and chlorobenzene (CB) were chosen as model compounds to represent these micropollutants (Figure 1). 2,4-D is one of the most employed chlorinated phenoxy herbicides all over the world to control weeds in cultivated plants [5]. The US Environmental Protection Agency (USEPA) has established an enforceable regulation for 2,4-D, setting a maximum contaminant level (MCL) of 0.07 mg L^{-1} based on its toxicity category (I) of the most toxic according to the USEPA toxicity class [3]. Acute symptoms of exposure involve coughing, burning, dizziness, loss of muscle coordination, nausea, diarrhea, and vomit. Blood, liver, and kidney toxicity have also been observed after 2,4-D exposure [6]. In addition, 2,4-D damages the nervous system, resulting in loss of coordination, inability to walk, rigidity in the arms and legs, inflamed nerve endings, fatigue, stupor, coma, and occasionally death [7]. Its continuous application to crops may cause soil percolation and groundwater contamination, while the exposure of agricultural workers for extended periods of time can produce serious skin and eye irritation [8,9]. This chlorinated organic compound is poorly biodegradable. In fresh water, it can be mineralized by microbial pathways at concentrations below 1 µg L^{-1}, but it is not practically decomposed at concentrations above 1 mg L^{-1} [10,11].

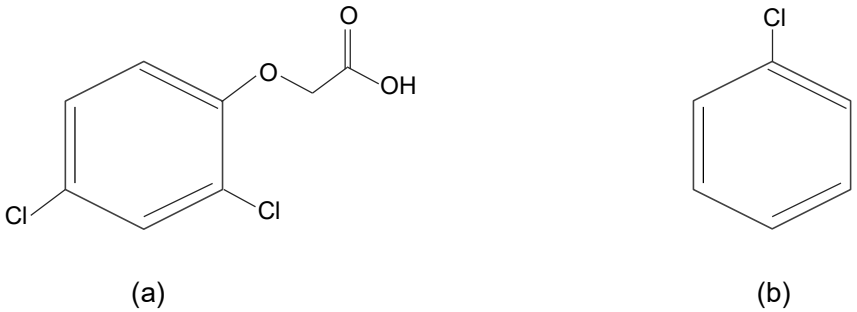

Figure 1. Chemical structure of 2,4-dichlorophenoxyacetic acid (**a**) and chlorobenzene (**b**).

On the other hand, pollution with CB is relevant due to its extensive use as an industrial solvent, carrier for pesticides, deodorant component, and chemical intermediate in many syntheses [12–14]. Its production, use, and inadequate removal lead to its presence in groundwater, surface water, and soil, implying serious threats to the public health and drinking water safety [15–18]. For humans, the contact with CB can irritate the skin, eyes, nose, and throat. The exposure to large amounts of CB can also provoke negative nervous system effects, including unconsciousness. Workers breathing vast quantities of CB can feel headaches, muscle spasms, and undesirable effects on the bone marrow. Other human health impacts related to repeated exposure to small amounts of CB over long periods of time are not known [19]. Thus, the USEPA has established an MCL for CB of 100-µg L^{-1} [20].

Currently, available technologies for the efficient removal of micropollutants from wastewater are based on physical, chemical, electrochemical, and biological methods [21–25], although the latter methods seem to be the most inexpensive and environmentally suitable treatment [26–29]. Biodegradability of this kind of micropollutants under aerobic conditions are well documented, but only few authors have studied the degradation under anaerobic conditions although it holds numerous advantages. Anaerobic treatments are technologically simple and relatively cheap. Furthermore, they consume little energy. Anaerobic microorganisms can remain unfed during long periods without any severe decline of their activity. Moreover, for anaerobic treatments, the nutrient demand is low and it tends to produce less possible toxic substances [30].

Recently, biofilms have become a focus of interest to the biodegradation of chlorinated compounds. Biofilms are suitable for the degradation of chemicals because of their highly concentrated microbial biomass and ability to immobilize pollutants [31]. In addition, immobilized bacteria have proven to be more efficient than suspended bacteria for biodegradation of these recalcitrant contaminants [32,33].

Moreover, biodegradation is facilitated by enhanced gene transfer between biofilm organisms so increases bioavailability of pollutants as an outcome of bacterial chemotaxis [34]. On the other hand, activated carbon (AC) has evidenced to be an effective adsorbent for the removal of a wide variety of micropollutants, but also an inert porous carrier, capable of distributing chemicals on its large hydrophobic internal surface, thus making them accessible to microorganisms [35]. The irregular shape and porous structure of granular activated carbon (GAC) particles can shield microorganisms from high fluid shear forces, and thus, can promote microbial colonization. In addition, its high adsorption capacity, due to its large specific surface area, increases the availability of organic substrates and nutrients to microorganisms at the media surface, and, therefore, can enhance the biodegradation efficiency [36]. Due to these properties, activated carbon has been vastly used in combination with microorganisms for treating wastewaters.

Previous studies developed by the authors have shown a high reduction of azo dyes, around 99% in very short space time (τ), 2.0 min [37,38]. The assays were conducted under continuous operation in packed-bed type reactors in an up-flow mode (UPBR), containing activated carbon (AC) or sludge carbonaceous material (SCM), with an immobilized anaerobic mixed culture. Initial clogging events were solved applying appropriate gentle agitation to the BAC (Biological Activated Carbon), resulting in an increment of Acid Orange 7 bioconversion up to 96% at a space time of 0.5 min [39]. This system's results demonstrated that the biodecolorization was higher than any other biological method by at least one order of magnitude.

Based on this evidence, the main objective of this research was to study the anaerobic degradation of CB and 2,4-D in a slowly-agitated up-flow packed-bed reactor.

2. Materials and Methods

2.1. Chemicals

2,4-dichlorophenoxyacetic acid (99%, Acros Organics, ref. 113631000) and chlorobenzene (99.99%, Sigma-Aldrich, St. Louis, MS, USA, ref. 319996) were used as received. Chlorobenzene was dissolved in 99.99% ethanol (Aldrich, ref. 121086.1214) to make a standard stock solution of 0.1 M. Sodium acetate (99%, Aldrich, ref.11019-1) was provided as the carbon source for the microorganisms. Activated carbon (AC) in granules of 2.5 mm was purchased from (ref. 1.02518.1000) and used as the support material in the USPBR. The activated carbon was crushed and sieved and grains of 0.3–0.7 mm were separated. Carborundum granules were obtained from Carlo Erba Reagents and utilized as inert solid diluent. The basal media included the following compounds (mg L^{-1}): $MnSO_4 \cdot H_2O$ (0.155), $CuSO_4 \cdot 5H_2O$ (0.285), $ZnSO_4 \cdot 7H_2O$ (0.46), $CoSO_4 \cdot 7H_2O$ (0.307), $(NH_4)6Mo_7O_{24}$ (0.285), $MgSO_4 \cdot 7H_2O$ (15.2), $Ca(NO_3)_2$ (19.93), $Fe_2(SO_4)_3$ (21.47), KH_2PO_4 (8.5), $Na_2HPO_4 \cdot 2H_2O$ (33.4), and K_2HPO_4 (21.75). All these chemicals were acquired from Sigma-Aldrich Company. Nitrogen (99.999% pure) was purged to maintain the anaerobic conditions.

2.2. Adsorption Experiments

The adsorption of CB and 2,4-D on the activated carbon was investigated under batch conditions. Essays were conducted over single component solutions. In the case of CB, the adsorption experiments were carried out in amber bottles where 100 mL of 300 mg L^{-1} was mixed with different weights of activated carbon (20–600 mg). In parallel, one blank control (CB solution without activated carbon) test was included to evaluate the potential loss of CB by stripping. For 2,4-D, the initial concentration varied between 50 and 300 mg L^{-1}, and 300 mg of activated carbon were added. In both set of experiments, the temperature and stirring were maintained constant at 35 °C and 200 rpm, respectively. Aliquots of clear supernatant were taken at different time intervals until equilibrium was attained.

2.3. Experimental Set-up for Anaerobic Biodegradation

The USPBR system and operating parameters used in this study are similar to those described elsewhere [39]. Figure 2 shows the experimental setup. The input feed was a 25 mg L^{-1} solution of each target compound containing 100 mg L^{-1} of sodium acetate as the substrate and the basal media containing microelements. The anaerobic conditions in the feed bottle were maintained by the bubbling of nitrogen. In addition, the feed was kept at 5 °C to prevent the growth of microorganisms. A gas chamber was placed before the entrance to the USPBR system at the same level of the bioreactor to capture the excess of N_2 contained in the feed solution. The feed flow rate was varied between 0.8 and 25 mL h^{-1} by a micro pump (Bio-chem Valve Inc., Boonton, NJ, USA, ref. 120SP2420-4TV). The reactors were assembled with an agitation system that allows the application of a manual stir (1 turn per day) in the BAC bed to avert clogging by excess biomass.

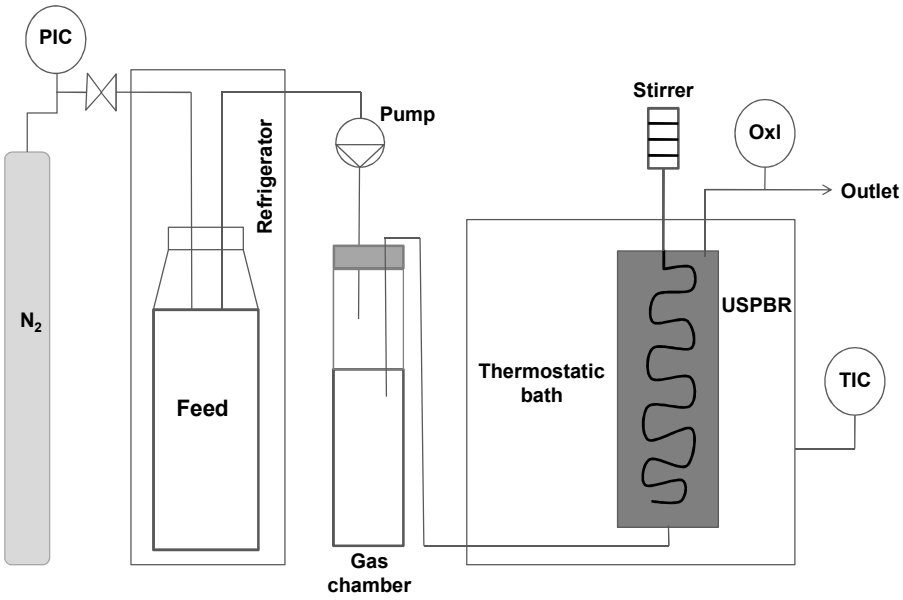

Figure 2. USPBR experimental setup.

The biological system was started using anaerobic sludge obtained from the municipal wastewater treatment plant from Reus (Tarragona, Spain). Initially, the raw sludge was filtered through a micro filter with a pore size of 20–25 µm to only collect single cells and/or spores. Then, the culture was adapted to grow with the selected pollutants, sodium acetate and the basal media. Culture 1 was fed with 25 mg L^{-1} of CB whilst culture 2 was fed with 100 mg L^{-1} of 2,4-D. To prepare the bioreactor, the cultures were pumped through the activated carbon bed for one week under anaerobic conditions at 35 ± 1 °C. During this period, a biofilm was formed and immobilized over the AC surface, resulting in the so-called BAC.

2.4. Analytical Methods

All samples were taken at the outlet effluent of the respective bioreactor, using sealing vials for this purpose, and were analyzed immediately employing the corresponding analytical method.

CB was analyzed by gas chromatography (GC) on an HP-5MS column (J&W Scientific, Folsom, CA, USA). The temperature program was as follows: 40 °C for 3.0 min, heated at 20 °C min^{-1} up to 100 °C, then increased at 65 °C min^{-1} up to 175 °C, and finally heated at 50 °C min^{-1} up to 270 °C and held for 0.5 min. The total chromatographic run time was 9.5 min. The carrier gas was helium. This analytical method is adapted from that described elsewhere [40].

2,4-dichlorophenoxyacetic acid was measured by high-performance liquid chromatography (HPLC) on a C18Hypersil ODS column (Agilent Technologies, Santa Clara, CA, USA). The mobile

phase was a mixture of methanol-acidified water (60:40) containing 4% acetic acid. The flow rate of the mobile phase was 1 mL min^{-1}, the injection volume was 20 µL, and the column compartment was set at 28 °C. The retention time for 2,4-dichlorophenoxyacetic acid under these conditions was approximately 10 min. The detection was performed at 240 nm with a diode array detector (DAD). This analytical method is based on that described elsewhere [41].

3. Results and Discussion

3.1. Adsorption Isotherms

Figure 3 shows the experimental isotherms for the adsorption of the CB and 2,4-D, from single component solutions over the activated carbon at 35 °C. The adsorption of organic compounds on GAC in water is often described by the Langmuir adsorption isotherm model [42,43]. Therefore, the Langmuir model was used to fit the experimental data through Equation (1):

$$Q_e = \frac{Q_L \cdot K_L \cdot C_e}{1 + K_L \cdot C_e} \quad (1)$$

where Q_e (mg $g_{AC}{}^{-1}$) and C_e (mg L^{-1}) are the concentrations of solute in the solid and liquid phases at the equilibrium, respectively. Q_L (mg $g_{AC}{}^{-1}$) is the maximum adsorption capacity, in accordance with the Langmuir model, and K_L (L mg^{-1}) is the Langmuir constant.

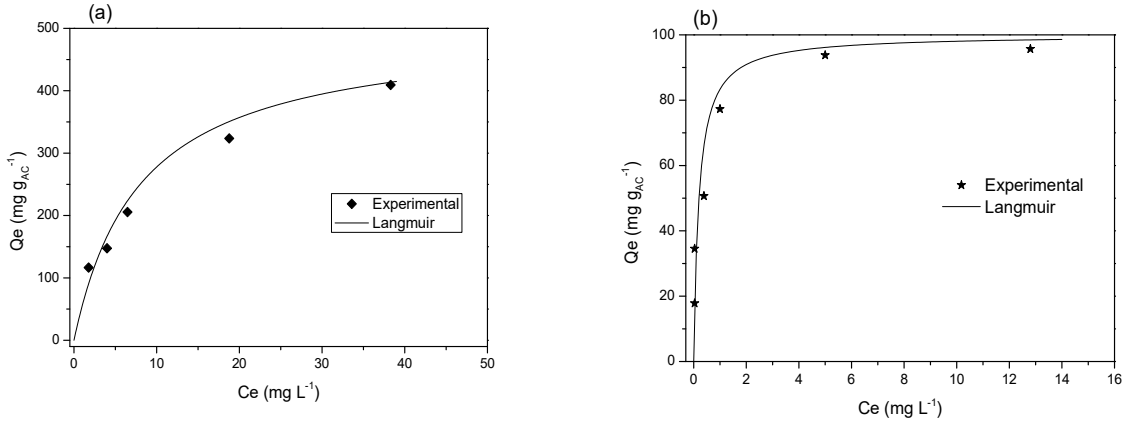

Figure 3. Equilibrium adsorption isotherms of CB (**a**) and 2,4-D (**b**) over activated carbon (35 °C). Symbols represent experimental data and lines are fitting to the Langmuir equation.

Figure 3 evidences that the experimental data showed good fitting with the model, so the maximum adsorption capacity of each compound (CB and 2,4-D) was estimated from Equation (1). The maximum adsorption capacity and the Langmuir constant values correspond to 417 mg g^{-1} and 0.15 L mg^{-1} for CB, and 100 mg g^{-1} and 5.00 L mg^{-1} for 2,4-D, with R2 > 0.99. In the blank control of CB, less than 3% of change in the concentration was found throughout the experiment, which proves that the stripping contribution can be neglected.

The adsorption isotherms of CB and 2,4-D correspond to type L of Giles classification, displaying a sharp initial increase and a concave curvature at low equilibrium concentrations followed by a well-defined plateau, i.e., the saturation limit, much more evident for 2,4-D in the range explored due to its lower adsorption capacity. This is distinctive of the high affinity between the adsorbate and adsorbent, without any strong competition of the solvent and almost insignificant interactions between the adsorbed molecules. Therefore, the adsorption of these compounds proceeds by the formation of a monolayer in the interval of concentrations used [44,45].

The maximum adsorption capacities measured for CB and 2,4-D are similar to those reported in the literature for different carbon adsorbents. Oliveira et al. [46] studied the adsorption of volatile organic

compounds in water over activated carbon/iron oxide magnetic composites. For CB, the maximum adsorption capacity was about 305 mg g^{-1} at 25 °C and experiments with pure activated carbon showed an adsorption capacity of 480 mg g^{-1}. Salman et al. [47] evaluated the adsorption potential of date seed based activated carbon for removing 2,4-D from aqueous solution. The equilibrium was best represented by Langmuir the isotherm model, showing a maximum monolayer adsorption capacity of 175.4 mg g^{-1} at 30 °C.

3.2. Biodegradation of Chlorinated Compounds

Continuous operation experiments were performed to study the biodegradation of CB and 2,4-D in USPBR under anaerobic conditions. In bed reactors, the critical factor is the load of the solid (either catalyst or adsorbent) rather than the reactor volume. Therefore, it is more adequate to consider conversion values as a function of space time instead of the true hydraulic residence time (HRT). Space time (τ, min) is defined by Equation (2):

$$\tau = \frac{m_c}{F_v \cdot \rho} \quad (2)$$

where m_c (g) is the load of the solid in the reactor, F_v (mL·min^{-1}) is the volumetric flow rate, and ρ (g mL^{-1}) is the density of the solution [37]. During the start-up, the flow rate was increased thrice to shorten the saturation time of the AC bed. Figure 4a,b show the acclimatization period for both components. As expected, an apparent complete conversion was initially observed due to the dominant role of adsorption. Once the bed was saturated, in near 48 h, the conversion dropped to practically zero because the lack of enough microorganisms to degrade the compounds. Then, the conversion progressively increased as the biofilm was created until reaching a stationary operation at a given conversion, which depends on the space time applied.

The starting and stabilization period was completed at a low space time (τ), 0.033 h (2 min), to prevent the excessive stressing of the newly created culture. As seen in Figure 4a,b, after some fluctuations during the first 50 and 85 days of continuous operation for CB and 2,4-D, respectively, the bioreactors reached a pseudo steady state and the processes were then monitored for another 20 days, approximately. As shown, the conversion was very low at this small space time, around 1 and 5% for 2,4-D and CB, respectively. These values represent poor removal rates of 940 mg L^{-1} d^{-1} (8.3 mmol L^{-1} d^{-1}) and 960 mg L^{-1} d^{-1} (4.3 mol L^{-1} d^{-1}) for CB and 2,4-D, respectively. The results indicated that a space time of 0.033 h was too short for achieving acceptable removal of the studied micropollutants. In addition, for 2,4-D, the feed concentration (100 mg L^{-1}) was demonstrated to be excessive in comparison to previous studies in other bioreactor configurations [48,49]. Thus, we decided to lower the feed concentration of 2,4-D to 25 mg L^{-1}, which was kept for the rest of the study. Once stabilized again the reaction system again, the experiments were continued (data not shown) while progressively increasing the space time. On the other hand, in the USPBRs used in previous studies [23], a decline of conversion was noted over the time, which was a result of the combination of biofilm aging and bed clogging [50]. Thereby, taking into account the large period necessary for the USPBRs to reach the steady state and to overcome this problem, a gentle and slow stirring of the BAC bed was applied. Under such conditions, Figure 4c,d show the performance of USPBRs in terms of the target conversion at different space times, with and without agitation of the BAC bed. Higher conversion values were achieved under agitation irrespective of the space time for both compounds. The results confirm that the excessive increase in biomass density probably constrains the biodegradation close to the AC surface. The agitation helps to remove the biofilm excess, and hence to maintain a practically constant amount of biomass in the bed, assuming no significant loss of activated carbon and a uniform operation.

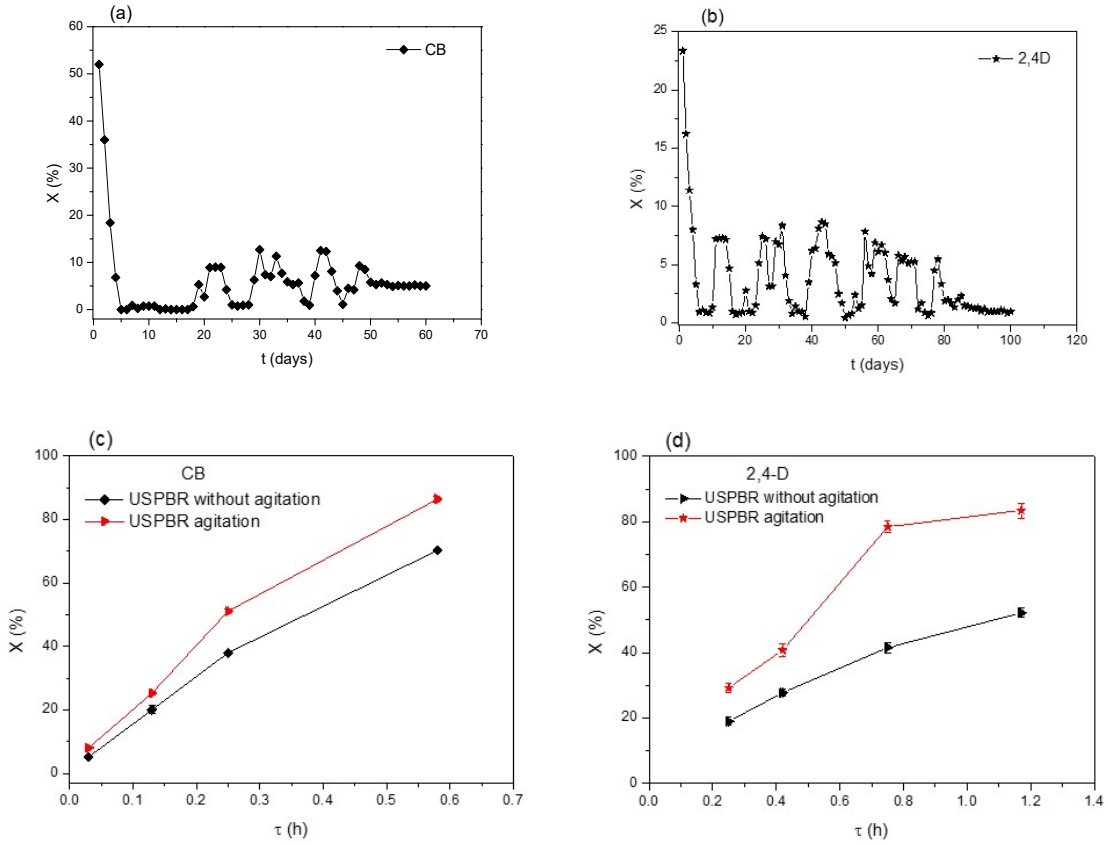

Figure 4. (**a**,**b**) Acclimatization and stabilization period of USPBRs for CB ([CB] in = 25 mg L^{-1}, T = 35 °C, τ = 0.033 h) and 2,4-D ([2,4-D]in = 100 mg L^{-1}, T = 35 °C, τ = 0.033 h). (**c**,**d**) Performance of USPBRs at different space times for CB and 2,4D ([CB]in or [2,4-D]in = 25 mg L^{-1}, T = 35 °C), with and without stirring.

As seen in Figure 4c, almost 90% of conversion was achieved at a space time of 0.58 h (35 min) for CB, with a removal rate of 890 mg L^{-1} d^{-1} (7.9 mmol L^{-1} d^{-1}). In comparison to other previously reported degradation processes for CB, it seems that anaerobic USPBR filled with BAC is faster than any other tested. Emanuelsson and coworkers [51] investigated the microbial community dynamics in a bioreactor inoculated with a pure bacterial strain (*Burkholderia* sp.) capable of degrading CB. The results revealed that 300 mg L^{-1} of CB was completely degraded with a removal rate of 347 mg L^{-1} d^{-1}. Jechorek et al. [52] explored the degradation of CB contaminated groundwater in a soil column packed with 13.4 kg of aquifer sediments colonized with a natural mixed culture of methanotrophic bacteria. The column was highly effective in the removal of CB, reducing the influent concentration of 25–30 mg L^{-1} to 0.04 mg L^{-1}, however, the removal rate was only of 16.5 mg L^{-1} d^{-1}. Wang and coworkers [53] studied the CB degradation by electro-heterogeneous catalysis in aqueous solution. The degradation efficiency was almost 100%, starting from an initial concentration of 50 mg L^{-1}, and the removal rate was up to 800 mg L^{-1} d^{-1}. Moreira et al. [54] studied the co-metabolic degradation of chlorobenzene with the fluorobenzene degrading wild strain, Labrys portucalensis. The biodegradation of 0.5 mM of CB was achieved at a rate of only 89 mg L^{-1} d^{-1} (7.95 μmol L^{-1} d^{-1}).

Similar trends were observed for 2,4-D removal. A conversion value of around 85% was achieved at a space time of 1.17 h (70 min), corresponding to a removal rate of 460 mg L^{-1} d^{-1}(2.1 mmol L^{-1} d^{-1}). This result illustrates that the degradation of CB is faster than the degradation of 2,4-D in USPBR, which is clearly related to the more complex structure of the latter. Anyway, a literature revision reveals that the anaerobic USPBR filled with BAC still compares very well with any other reaction configuration for treating 2,4-D. González et al. [55] studied the degradation of this herbicide by an indigenous *Delftia* sp. strain in batch and continuous systems. Complete degradation of 100 mg L^{-1}

of 2,4-D was achieved in 24 h under batch conditions, with a rate as low as 4.16 mg L^{-1} d^{-1}. In the continuous down-flow fixed-bed reactor using polyurethane foam cubes as support for immobilizing bacterial cells, the removal rate was again low at 21.7 mg L^{-1} d^{-1}. Quan et al. [56] conducted their experiments in a microcosm biofilm reactor operated in the fed-batch mode. The biofilm carriers could completely degrade an initial concentration of 65 mg L^{-1} of 2,4-D, as the single carbon source, the removal rate being 56.2 mg L^{-1} d^{-1}. Vroumsia et al. [57] studied the fungal bioconversion of 2,4-D and 2,4-dichlorophenol (2,4-DCP). After five days of cultivation, the best results were obtained with Aspergillus penicilloides for 2,4-D (100 mg L^{-1}), with an efficiency of 50% and a removal rate of 10 mg L^{-1} d^{-1}. Elefsiniotis and Wareham [58] examined the biodegradability of 2,4-D in a lab-scale sequencing batch reactor (SBR) that operated under anaerobic conditions. The results revealed that 100 mg L^{-1} of 2,4-D was completely degraded following an acclimatization period of 70 days, with a removal rate of 49.5 mg L^{-1} d^{-1}.

The high removal rate obtained in this study is attributed to the combination of different characteristics of the reactor system. Although packed-bed reactors using BAC have not been commonly employed for anaerobic degradation of these target compounds, the use of microorganisms supported on activated carbon must enhance the fusion of the good capacities of both to retain and/or degrade organic compounds, producing a synergistic effect between biofilm and support [59]. AC presents uncommon properties enhancing the degradation capacity of the system. Firstly, AC possesses a surface with outstanding properties to attach microorganisms; the porous arrangement of the carbon particles furnishes the protective environment needed by the microorganisms to easily colonize the surface to form the biofilm [60]. Secondly, the AC contains surface groups suitable for electron transfer thus intensifying its redox mediating capacity [61]. This mechanism has been established formerly [38,62] and confirms the key role of the AC as the redox mediator for the bioreduction of azo dyes. Whereby, the combined biodegradation–adsorption processes result in higher performance than expected for the processes applied separately.

Figure 5 assesses the performance of the USPBRs for long operation periods. The system demonstrated great robustness for CB and 2,4-D during the studied time, up to 40 days. For CB, a conversion around 87% was maintained with very small oscillations at a space time of 0.58 h, whilst for 2,4-D, the stable conversion was around 88% at a space time of 1.17 h.

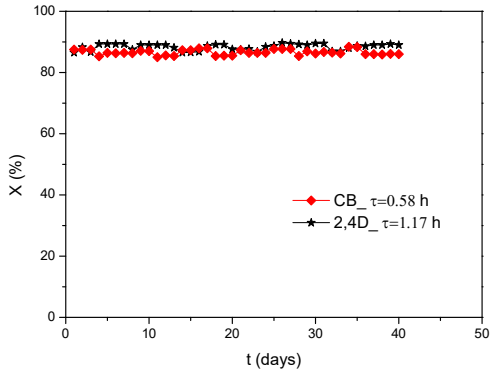

Figure 5. Stability of the USPBR system during long operation periods. ([CB]in or [2,4-D]in = 25 mg L^{-1}, T = 35 °C).

3.3. Modelling CB and 2,4-D Degradation in USPBRs

3.3.1. Reaction Rate

For design purposes, the kinetics of the biodegradation is needed so either the mass of the bed or the space time required to a given effluent could be estimated.

In the USPBR, the mole balance is given by Equation (3):

$$\frac{dF_P}{dm_c} = \frac{d(C_P \cdot F_v)}{d(\tau \cdot F_v \cdot \rho)} = -r_P \quad (3)$$

where F_P (mmol min^{-1}) is the molar flow rate of the micropollutant, m_C (g) is the load of AC in the bioreactor, r_P (mmol min^{-1} g^{-1}) is the reaction rate, C_P (mmol L^{-1}) is the concentration of micropollutant, F_v is the volumetric flow rate, τ (min) is the space time, and ρ (g L^{-1}) is the density of the solution. Assuming that the density of the solution is equivalent to the density of water and the flow rate of the micropollutant solution remains constant, the reaction rate reduces to Equation (4):

$$-\frac{dC_P}{d\tau} = r_P \quad (4)$$

3.3.2. Kinetic Models

The Michaelis–Menten model was expected to adequately describe the CB and 2,4-D anaerobic biodegradation in the USPBRs as long mass transfer and adsorption were faster steps. The kinetic rate, according to the Michaelis-Menten model, is expressed by Equation (5):

$$r_P = -\frac{k_1 \cdot C_P}{k_2 + C_P} \quad (5)$$

where k_1 ($mmol^{-1}$ min^{-1}) is the maximum rate of micropollutants degradation per unit mass of AC and k_2 (mmol L^{-1}) is the half velocity constant. The combination of Equations (4) and (5) were used to adjust the data displayed in Figure 4c,d. Fitting was conducted using MATLAB® R2012a. The algorithm applied a fifth-order Runge-Kutta subroutine to solve the differential equation and a nonlinear least-squares fitting algorithm (lsqnonlin; algorithm: Trust-Region-Reflective) provided an estimation of the values of the parameters included in the equation.

Table 1 shows the kinetic parameters estimated as described. The fitting results depicted in Figure 5 provide evidence of the goodness of the model that adequately describe the experimental data for CB degradation in USPBR (Figure 6a). On the contrary, significant deviation was obtained for 2,4-D at 100 mg L^{-1} although it is acceptable for the other two concentrations tested. Anyway, as expected, the maximum rate for CB (k_1 = 1.28 mmol g^{-1} min^{-1}) is higher than the maximum rate for 2,4-D (k_1 = 0.098 mmol $gcat^{-1}$ min^{-1}), reflecting the slower biodegradation of the latter as already commented above. Mathur and coworkers [63] studied the kinetics of the removal of mono-chlorobenzene vapour from waste gases using a trickle bed air biofilter. The Michaelis-Menten model was used as the macrokinetic determination method and the kinetic parameters obtained were k_1 = 0.121 g m^{-3} s^{-1} (0.064 mmol L^{-1} min^{-1}) and k_2 = 7.45 g m^{-3} (0.066 mmol L^{-1}). Zhou et al. [64] compared the performance and microbial communities of two differently inoculated biotrickling filters (BTFs) for treating CB. The kinetic parameters were calculated to understand the kinetic behaviour of BTFs and the Michaelis–Menten equation was employed for this purpose. The maximum degradation rate (k_1) was 83.61 g m^{-3} s^{-1} (0.74 mmol L^{-1} min^{-1}) for the BTF inoculated with a biomass suspension of Ralstonia pickettii L2 and an enriched activated sludge, whilst for the BTF inoculated only with the enriched activated sludge, k_1 = 36.64 g m^{-3} s^{-1} (0.33 mmol L^{-1} min^{-1}); indicating that the introduction of Ralstonia pickettii L2 had a positive effect on the CB degradation rates of BTF [64]. Taking into account the results mentioned above and to the best of our knowledge, the degradation of CB in our anaerobic USPBR is the fastest biodegradation process ever reported.

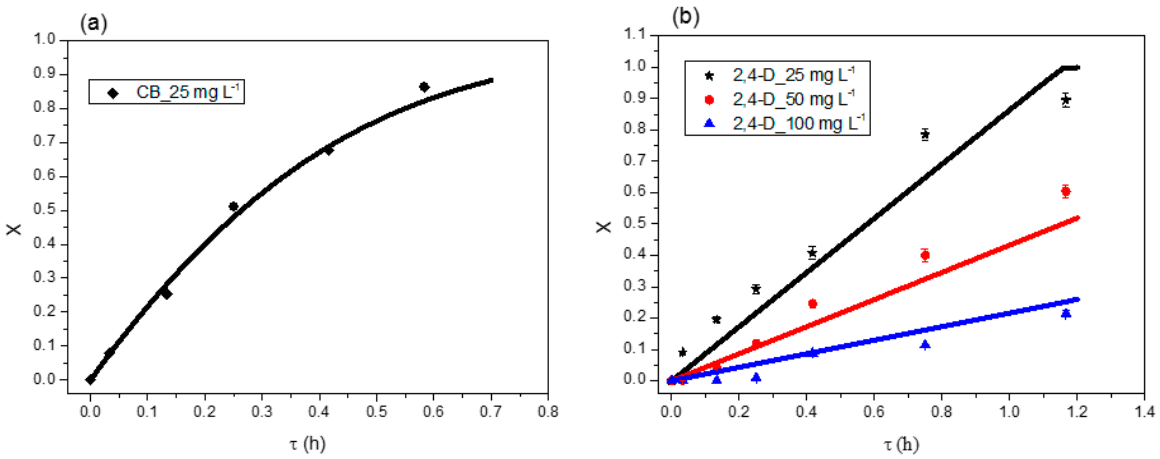

Figure 6. Kinetic modelling of CB (**a**) and 2,4-D (**b**) biodegradation in USPBRs. Lines represent fitting to the Michaelis-Menten model (T = 35 °C).

Table 1. Kinetic parameters for CB and 2,4-D anaerobic biodegradation in USPBRs based on the Michaelis-Menten model.

Compound	k_1 (mmol gcat^{-1} min^{-1})	k_2 (mmol L^{-1})	σ [a]
CB	1.28	0.32	0.003
2,4-D	0.098	0.00014	0.05

[a] Standard deviation associated to the model fitting: $\sigma = \sqrt{\frac{\sum(X - X^{MOD})^2}{n-1}}$, where n is the number of experimental points.

As previously commented, Figure 6b shows that the basic form of the Michaelis-Menten model displays a notable deviation mainly at the initial concentration of 100 mg L^{-1}; which indicates that 2,4-D have concentration-dependent inhibition impacts for microorganisms in the bioreactor. Therefore, the Michaelis-Menten model was extended with an inhibitor factor (ki) according to Equation (6) to express more properly the biodegradation process [65]:

$$r_P = -\frac{k_1 \cdot C_P}{k_2 + C_P + \left(C_P^2 / k_i\right)} \quad (6)$$

Figure 7 shows the fitting to the Michaelis-Menten model extended with a substrate inhibition factor while Table 2 lists the new kinetic parameters estimated. The recalculated kinetic constants, including the data for the three inlet concentrations (25, 50, and 100 mg L^{-1}), improve the predicted conversions, as the mean standard deviation lowers from 0.05 to 0.02. The clearest effect is on the data at the highest inlet concentration tested (100 mg L^{-1}) where the microorganisms are more exposed to inhibitory mechanisms or even toxicity effects not considered here.

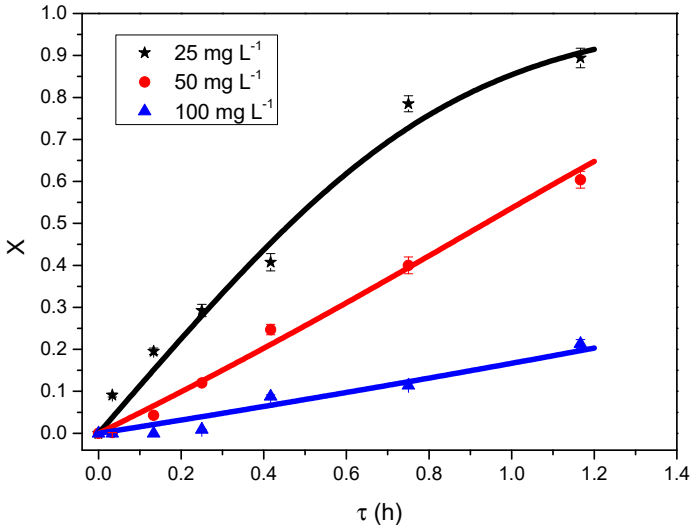

Figure 7. Fitting to the Michaelis-Menten model, including substrate inhibition, during 2,4-D biodegradation in USPBR. Continuous lines represent the fitting to the extended Michaelis–Menten.

Table 2. Kinetic parameters of the Michaelis-Menten with substrate inhibition model for 2,4-D anaerobic biodegradation in USPBR.

k_1 (mmol gcat^{-1} min^{-1})	k_2 (mmol L^{-1})	k_i (mmol L^{-1})	σ
0.55	0.19	0.071	0.02

4. Conclusions

A continuous USPBR reactor filled with BAC was employed for the first time for the anaerobic biodegradation of CB and 2,4-D. The application of gentle agitation in the BAC bed resulted in an increase of CB and 2,4-D bioconversion, assuring high conversion values (around 90%) at short space times of below 0.6 h and 1.2 h, respectively.

The Michaelis-Menten model provided good fitting to the experimental data of CB conversion, however, 2,4-D showed appreciable inhibition effects over the biomass at a high inlet concentration. Thus, a general model was assumed for the anaerobic degradation of 2,4-D in the USPBR-BAC system, based on the 2,4-D inhibition effect integrated on the Michaelis-Menten kinetics.

The high efficiency and removal rate obtained in this bioreactor configuration show that the use of activated carbon plays various critical roles as an efficient adsorbent for organics' excellent carrier material for the attachment of microorganisms, and as a redox mediator for enhanced CB and 2,4-D bio-reduction in the USPBRs. Thereby, in comparison to other continuous biological reactors, USPBR-BAC demonstrates the effectiveness of the process and their promising application for micropollutants removal.

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