Biological treatment of wastewater polluted with an oxyfluorfen-based commercial herbicide

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Abstract

Fluoxil-24 is a commercial herbicide based on oxyfluorfen (a hazardous non-soluble organochlorinated compound) and additional compounds used as solvents. The aim of this work is to study the biotreatability of this commercial herbicide in water through batch experiments performed at different temperatures (15, 20, 25 and 30°C) and initial concentrations (85, 150, 300 and 500 mg L⁻¹ of oxyfluorfen). Activated sludge from an oil refinery wastewater treatment plant was acclimated and used for biodegradation experiments. Two main mechanisms, volatilization and biodegradation, were observed to be responsible of the herbicide removal. *Fluoxil-24* removal efficiencies between approximately 40% and 80% were reached after 70 h, depending on the conditions used, and oxyfluorfen was not completely removed. Regarding the influence of the temperature, thermal inhibition problems appeared at 30°C, and the volatilization rate of solvents increased, causing oxyfluorfen to become unavailable for microorganisms. An increase of herbicide initial concentration did not clearly affect the herbicide removal efficiency, whereas it negatively affected the biological mechanism. The experimental results were fitted to a mathematical model that included both simultaneous mechanisms of volatilization and Monod biodegradation kinetics. The model was able to predict the experimental results, and the calculated model parameters confirmed the effect of the variables under study.

Keywords

Biodegradation, oxyfluorfen, herbicide, volatilization, kinetics.

1. Introduction

Although the use of agricultural herbicides is recommended, it causes currently great environmental and health impacts (Rio et al., 1997; Rodrigo et al., 2014; Özkara et al., 2016; Verma et al., 2014; Zhao et al., 2016). One of the most effective and widely used herbicides around the world is oxyfluorfen, a diphenyl-ether herbicide (Dos Santos et al., 2016). Oxyfluorfen has low solubility in water (0.116 mg L^{-1}) and low vapour pressure (0.026 mPa at $25 \text{ }^{\circ}\text{C}$) (Barba et al., 2017). Oxyfluorfen is usually marketed mixed with different organic substances, such as solvents (liquid hydrocarbons, acetonitrile, xylene, N-methyl pyrrolidine), to improve water solubility, enable easier management and ensure safe operating conditions because it is considered as hazardous substance.

Because of the above-described exposure, oxyfluorfen polluted soil and wastewater must be remediated. There are currently different alternative technologies for oxyfluorfen polluted wastewater remediation. Advanced oxidation processes have been shown to be potential technologies for oxyfluorfen removal from water (Rodrigo et al., 2014; Dos Santos et al., 2017a; Dos Santos et al., 2017b).

Regarding biological technologies, biodegradation of oxyfluorfen is characterized as being poor because the plants cannot metabolize this compound and microorganisms assimilate it slowly (Calderón et al., 2015; Barba et al., 2017; Sondhia, 2010). The number of published works regarding oxyfluorfen biodegradation is very low. Chakraborty et al. (2002) studied the degradation of oxyfluorfen by pure bacterial cultures of *Azotobacter chroococum* in a sterilized mineral salt medium. Other authors reported the degradation of this herbicide by using isolated bacterium from soil polluted with oxyfluorfen. Zhao et al. (2016) showed that *Chryseobacterium aquifrigidense*

could degrade 92.1% of oxyfluorfen at 50 mg L⁻¹ within 5 days, and Mohamed et al. (2011) identified 10 potential oxyfluorfen degraders from a polluted soil. Biodegradation of oxyfluorfen using activated sludge from a wastewater treatment plant has not been reported to date, and it is expected that a previous slow microbial acclimation step would be necessary. The complex chemical structure of oxyfluorfen, its molecular weight and the presence of functional groups such as chlorine are factors that could affect its microbial degradation. The chlorine substituents are rarely found in nature and increase resistance to microbial attack (Aislabie and Lloyd-Jones, 1995). All these features increase toxicity; therefore, microorganisms rarely use organochlorinated substances as a carbon source. As mentioned before, because of the low solubility of oxyfluorfen in water, this herbicide is usually applied with solvents and surfactants. If these chemicals were oxidized or evaporated, then microorganisms should require some physiological adaptations, such as biosurfactant production (Aislabie and Lloyd-Jones, 1995). This issue affects the microbial degradation of non-soluble compounds; thus, such degradation requires a longer time compared to the degradation of other compounds.

In this context, the main purpose of this work is to determine the feasibility of a biological treatment of an aqueous effluent polluted with a commercial herbicide composed of oxyfluorfen and auxiliary organic substances using an acclimated mixed microbial culture obtained from an oil-refinery wastewater treatment plant because the number of published works regarding oxyfluorfen biodegradation is very low. The influences of two variables (initial herbicide concentration and temperature) on the contributions of the different removal mechanisms, the removal efficiency and removal rate were studied. Moreover, a mathematical model that considered two removal mechanisms (volatilization and biodegradation) was used to understand the effects of

such variables and provide information regarding the feasibility of this process in practice.

2. Materials and methods

2.1. Chemicals and inoculum source

Cheminova Agro (Spain) supplied the commercial herbicide Fluoxil 24. This commercial herbicide contains 24% of oxyfluorfen as an active substance along with other compounds, such as xylene (<60%), cyclohexanone (<13%) and calcium dodecylbenzene sulphonate, to help to emulsify oxyfluorfen. Table S1 (supplementary material) shows the main physicochemical properties of these compounds. As seen in Table S1, oxyfluorfen is a hardly biodegradable compound, and its vapour pressure reaches a low value, indicating that this herbicide does not evaporate easily. In contrast, cyclohexanone, and especially xylene, are volatile and biodegradable compounds.

Activated sludge was collected from the biological reactor of an oil-refinery wastewater treatment plant (Puertollano, Spain). This microbial consortium was subjected to a standard acclimatization procedure described elsewhere (Moliterni et al., 2012). The sludge was fed with *Fluoxil 24* (with a concentration of oxyfluorfen of 200 mg L⁻¹) as a carbon source and a mineral salt solution that consisted of the Bushnell-Hass Broth (BHB) medium (Moliterni et al., 2012). The acclimatization period lasted several weeks.

2.2. Experimental set-up

The experimental system consisted of 0.75-L stirred batch glass reactors. All the experiments were conducted under aerobic conditions. The oxygen supply was provided because reactors were open to the atmosphere and agitated at 150 rpm. The total bulk volume was 500 ml, and the temperature was controlled by means of a thermostatic

chamber. The experiments were completed in the dark to prevent any photocatalytic degradation and the growth of algae. The tests were conducted at a pH 7.0 ± 0.2 .

2.3. Operational procedure

The reactors were fed with 500 ml of BHB medium and different amounts of *Fluoxil 24* to reach different initial concentrations of oxyfluorfen (the tested concentrations were 85, 150, 300 and 500 mg L⁻¹). Note that these oxyfluorfen concentrations are greater than those that can be found in the environment via the efficient and controlled use of herbicides (Mantzios et al., 2014); although this work considers that herbicide pollution can also be caused by possible spills or accidents resulting from their manufacture and transport. Furthermore, the reactors were inoculated with 5 ml of the acclimated microorganisms' culture. Samples were taken periodically (0, 2, 4, 8, 16, 24, 36, 48, 60 and 72 hours) to measure the concentration of different parameters (section 2.4). These experiments were also performed at different temperatures (15, 20, 25 and 30°C); thus, 16 batch biodegradation experiments were performed. All biodegradation experiments were performed in triplicate, and the results shown in section 3 are the average value of the three replicates. Error bars were included only in figures that plotted biomass measurements (biomass measurements are the ones that usually present the greatest experimental error compared to the rest of measurements).

Additionally, abiotic experiments (without microbial inoculation) under the same experimental conditions (different herbicide concentrations and temperatures) were also performed as reference tests in all cases.

2.4. Parameters monitored and analytical techniques

The following parameters were monitored in the samples: Total Organic Carbon (TOC) concentration, oxyfluorfen and xylene concentrations, liquid surface tension (ST), and

microbial biomass concentration. The oxyfluorfen and xylene concentrations were measure by HPLC (Jasco, Japan): the mobile phase (acetonitrile/water, 70/30 by volume) was pumped though a Kinetex 5 μm Biphenyl 100 Å, 150 mm $\times$ 4.5 mm column (Phenomenex, USA) with an isocratic flow rate of 0.6 ml min⁻¹; a UV wavelength detector was set at 220 nm, and the injection volume used for all samples was 20 μl . TOC was monitored using a MultiN/C 3100 analytic Jena analyser: organic carbon compounds were converted to carbon dioxide and then detected by a non-dispersive infrared detector. ST measurements were performed on a Nouy tensiometer (Lauda TD2).

Regarding the microbial culture, once the activated sludge was acclimated, microbial species were isolated and identified by using MS MALDI-TOF Axima Assurance by Shimadzu, as reported by Lovecka et al. (2015). The evolution of the microbial biomass concentration was analysed by direct optical density (OD) measurements at 600 nm in a Pharma Spec 1700 Shimadzu spectrophotometer, as described by Sadouk et al. (2008). Absorbance gives a reliable indirect measurement of microorganisms' concentration (Carboneras et al., 2017; Moser et al., 1996; Péquignot et al., 1998). A correlation curve between OD₆₀₀ and the real biomass concentration, measured as Volatile Suspended Solids (VSS), was developed and used to calculate the microbial concentration.

2.5. Mathematical modelling

A mathematical model was developed to predict the herbicide biodegradation process. The model describes the changes in TOC and microbial biomass concentrations versus time. Moreover, the evolution of the main model parameters as a function of the experimental conditions would contribute to the discussion of the influences of such

variables (herbicide concentration and temperature). The model is described by equations 1 and 2.

$$r_X = \frac{dX}{dt} = \mu_{max} \cdot \frac{(S-S_i)}{K_s + (S-S_i)} \cdot X - K_d \cdot X \quad (1)$$

$$-r_S = -\frac{dS}{dt} = K_L a \cdot S + \frac{1}{Y} \cdot \mu_{max} \cdot \frac{(S-S_i)}{K_s + (S-S_i)} \cdot X \quad (2)$$

Equation 1 describes the evolution of the microbial biomass concentration (X , mg_{VSS} L⁻¹) vs t (h) during the experiments according to Monod kinetics. Previous research studies have shown that Monod rather than first order kinetics (Cheyins et al., 2010) can describe organochlorine biodegradation. Equation 2 describes the evolution of S (herbicide concentration in water, expressed as TOC, mg L⁻¹). The depletion of S has been considered to be produced by two simultaneous mechanisms: volatilization and biodegradation. The volatilization mechanism was modelled by Mozo et al. (2012), and it corresponds to the first term on the right side in equation 2. The biodegradation was modelled as in previous works in our research group (Moliterni et al., 2012; Lacasa Fernández et al., 2016), and it corresponds to the second term on the right side on equation 2. This term considers that there is a biodegradable substrate fraction that is removed following Monod kinetics while an inert residual fraction (S_i) remains at the end of the experiment. The model parameters included in both equations are the following: μ_{max} is the maximum specific growth rate (h⁻¹), K_d is the microbial decay coefficient (h⁻¹), K_{La} is the surface mass transfer coefficient (h⁻¹), Y is the biomass yield (mg X mg⁻¹ S), and K_s is the half-saturation coefficient (mg S L⁻¹).

The abiotic tests did not include the biodegradation mechanism; thus, their results were used to estimate the volatilization mass transfer coefficient K_{La} in each test by a simple linear fitting to equation 3 (Mozo et al., 2012). Subsequently, to fit the equations to the

data set from biodegradation experiments, equations 1 and 2 were solved simultaneously using the Simplex algorithm and the protocol defined in the literature (Fernández et al., 2011). An initial set of values ($\mu_{\max}$, K_d , K_s and Y) were assigned to the parameters, and after several iterations, the values of the parameters that yielded the lowest sum of squared error (SSE) were selected. The SSE value was determined according to equation 4, where n is the number of data points, X_i and S_i are the calculated values of the functions at the i th measurement and $X_{\text{meas},i}$ and $S_{\text{meas},i}$ are the experimental values at the i th measurement.

$$-\frac{dS}{dt} = K_L a \cdot S \quad (3)$$

$$\text{SSE} = \sum_{i=1}^n \left((X_i - X_{\text{meas},i})^2 + (S_i - S_{\text{meas},i})^2 \right) \quad (4)$$

3. Results and discussion

3.1. Abiotic tests

Figure 1 shows the average concentration profiles of TOC (part a) and xylene (part b) obtained in the abiotic batch tests using four herbicide concentrations at 20°C. The lines indicate trends only. Abiotic tests were performed to determine the evolution of different parameters without the contribution of microbial mechanisms, such as biodegradation or bioadsorption. The results obtained at 15, 25 and 30°C are not shown.

Figure 1. Abiotic tests: average concentration profiles of TOC (a) and xylene (b) using four herbicide concentrations at 20°C. The lines indicate trends only.

Fig. 1a shows a slow TOC concentration decrease that is related to the evaporation of volatile compounds contained the commercial herbicide. Moreover, the volatilization is relatively fast during the first 15 h, whereas it becomes slower after this period. Additionally, Fig. 1b shows that xylene completely evaporates in 15 h. Finally, oxyfluorfen analyses (results not shown) indicated that its concentration remained approximately constant (approximately 5% deviation) during the abiotic experiments and no volatilization occurred. The behaviour observed in this group of experiments (20°C) was similarly observed at the other temperatures considered. According to these

results and the physical properties of the different compounds in the commercial herbicide (already indicated in section 2), it is deduced that xylene evaporated quickly (15 h) and cyclohexanone evaporated slowly during the whole batch tests duration, with the rest of non-volatile compounds remaining. In the case of monoaromatic compounds and low mass PAHs, previous works have indicated that volatilization can contribute significantly to their removal from the liquid phase in biological processes (Fatone et al., 2011; Yin et al., 2010). Niaz et al. (2015) reported that xylene easily vaporizes. When xylene is discharged to soil and water, it will ultimately vaporize into the environment. The change of the TOC slope profile at approximately 15 h was easily observed when a high herbicide concentration was used; in contrast, it was negligible in the case of low TOC initial concentrations.

3.2. Biodegradation experiments. Influence of the initial concentration.

Figure 2 shows the average concentration data of TOC during the biodegradation experiments performed at 20°C and using four initial concentrations of herbicide (black points). The TOC concentration data (white points) in the abiotic tests are also included as blank tests information. Finally, the average microbial concentration data during the experiments are also plotted. The results obtained at 15, 25 and 30°C are not shown.

Figure 2. Biodegradation experiments performed at 20°C and different initial herbicide concentrations (a: 85; b: 150; c: 300 and d: 500 mg L⁻¹). Concentration values of TOC (●, left axis) and microbial biomass (▲, right axis). The TOC concentrations in the abiotic tests (○, left axis) are also plotted. The lines indicate trends only.

Clear differences were observed between the abiotic tests and the biodegradation experiments in all cases. The microbial inoculation always caused higher TOC removal compared to the abiotic test, although a final approximately constant TOC level remained in all of the biodegradation experiments. No important differences between the abiotic tests and the biodegradation experiments were observed during the first 10 h; but subsequently, a fast TOC depletion in biodegradation experiments up to approximately 50 h was observed. Simultaneously, a conventional microbial growth profile was observed, with no apparent initial lag periods (except case *d*, that is, using the highest herbicide concentration), and the microbial growth stopped at approximately

30 h. As indicated in section 3.1, the behaviour observed in this group of experiments (20°C) was approximately the same as that observed at the other temperatures considered.

The xylene concentration was also measured in the same biodegradation experiments. Figure 3 shows the average xylene concentration data in the experiments performed at 20°C using the four initial concentrations of herbicide (black points). Additionally, the xylene concentration data in the abiotic tests (white points) are again included in each experiment as blank test information. The results obtained at 15, 25 and 30°C are not shown.

Figure 3. Xylene concentrations during the biodegradation experiments (■) and the abiotic tests (□) performed at 20°C.

Figure 3 shows that the xylene concentration profiles are nearly identical in both the abiotic tests and the biodegradation experiments, i.e., despite xylene being a readily biodegradable substance, the microorganisms did not metabolize xylene because it

evaporated quickly. Moreover, it could be supposed that the acclimated culture never metabolized xylene during the acclimation process because of its high volatility. This behaviour has been previously reported regarding simultaneous biodegradation and volatilization of hazardous aromatic substances (Mozo et al., 2010). These authors reported that the biodegradation of aromatic compounds requires the acclimatization of the microbial consortium, and this is only possible if the substrate is not removed rapidly via a non-biotic process. The behaviour observed in this group of experiments (20°C) was similarly observed at the other temperatures considered.

Figure 4 shows the average concentration data of oxyfluorfen during the biodegradation experiments performed at 20°C and at four initial concentrations of herbicide (black points and left axis). Additionally, surface tension data in the liquid medium (white points and right axis) in these experiments are plotted. The results obtained at 15, 25 and 30°C are not shown.

Figure 4. Oxyfluorfen concentrations (black points) and surface tension values (white points) during the biodegradation experiments at 20°C. The lines indicate trends only.

It can be observed that oxyfluorfen is efficiently (although not completely) removed in a relatively short retention time compared to similar herbicides biodegradation studies previously reported by our research group, in which several weeks of retention time were required (Carboneras et al., 2017). Most of the oxyfluorfen content (between 70% and 85% approximately) was removed in less than 10 h when initial concentrations at or below 300 mg L⁻¹ were used. In contrast, a higher retention time was necessary if a high initial concentration was used (500 mg L⁻¹), and the removal rate clearly decreased after 15 h. According to the results shown so far, it can be considered that the mixed microbial culture acclimated in the present work was able to consume oxyfluorfen; this observation represents a first successful conclusion because oxyfluorfen is known to be difficult to biodegrade (Calderón et al., 2015). The final acclimated microbial culture contained the following species: *Achromobacter denitrificans*, *Achromobacter xylosoxidans*, *Pseudomonas putida*, *Pseudomonas oryzihabitans* and *Brevibacterium casei*.

The oxyfluorfen concentration depletion in Figure 4 was found to be related only to the biodegradation mechanism because the abiotic tests proved that no volatilization occurred and the bioadsorption was considered to be negligible. Regarding this mechanism, it has been considered that hypothetical fast oxyfluorfen bioadsorption by the microbial biomass did not occur. According to a previous work reported, the maximum amount of oxyfluorfen that could be removed by bioadsorption is less than 3% (Chair et al., 2017).

However, it can also be observed that, despite the long retention times maintained in the experiments (up to 60 h), oxyfluorfen was not completely removed; apparently, there is a final residual constant oxyfluorfen concentration that cannot be degraded (values between 10 and 40 mgL⁻¹ depending on the experiments), and it is considered that the presence of such residual concentration does not correspond to a conventional biodegradation mechanism. Figure 4 also shows that the liquid medium surface tension increased during the experiments and reached a maximum value approximately after 25-30 h. This evolution was found to be related to the loss of volatile and water-soluble compounds of the commercial herbicide, such as xylene and cyclohexanone, as described in section 3.1. These compounds allow oxyfluorfen to be water-emulsified and thus to be bioavailable for microorganisms because oxyfluorfen is non-polar and its water solubility is very low. The volatilization of such compounds cause oxyfluorfen to become unavailable for the biodegradation process; consequently, it could not be removed by the microbial culture at times greater than approximately 35 h.

The surface tension slowly decreased again after approximately 50 h, probably because the biosurfactants were slowly yielded by the microbial culture. This behaviour has also been observed in our previous works regarding non-soluble hydrocarbon biodegradation (Moliterni et al., 2012). Although the expected biosurfactant production could help to remove oxyfluorfen completely, it is also expected that it would require long retention times as the surface tension decreased slowly and the oxyfluorfen concentration after 50 h was very low; thus, it would not be considered as an efficient process. Additionally, as previously explained, a residual TOC fraction remained after the experiments were completed in all cases. Regarding these TOC levels, and despite most of the oxyfluorfen being eliminated, it is possible that it was not completely oxidized but transformed into

intermediates (although not detected). Moreover, organic matter from microbial lysis could be present.

3.3. Biodegradation experiments. Influence of temperature.

Figure 5 shows TOC removal percentages in all experiments at different initial concentrations of herbicide and different temperatures: black bars denote the total removal percentage, and white bars denote the removal percentage via only biodegradation mechanisms. The dashed line denotes the percentage of remaining TOC after the experiments.

Figure 5. TOC removal efficiency after biodegradation experiments (%): total removal (black bars), removal only by biodegradation (white bars), and percentage of remaining TOC (dashed line).

Regarding the influence of the initial concentration, a similar behaviour was observed in all parts of Figure 5. No clear trend was found in the total TOC removal efficiency; thus, no clear influence of the initial concentration was observed. In contrast, the biodegradation efficiency appears to decrease at higher herbicide concentrations because the trend of the percentage values is decreasing in each case. In other words,

the biodegradation contribution decreased at higher concentrations while the volatilization contribution simultaneously increased. Indeed, a simple linear fitting of TOC profiles in abiotic tests clearly showed that the TOC volatilization rate increased at higher initial concentrations (results not shown). It has been considered that a deterioration of the biological contribution could be caused by the potential inhibition effect of high herbicide concentration (note the lag period for biomass growth in Figure 2d) or by the possible appearance of non-biodegradable intermediates as well as a higher volatilization rate. Substrate inhibition in herbicide biodegradation processes has been widely reported (Marques et al., 2015). Finally, it can be observed in every part of Figure 5 that an approximately constant fraction of the initial TOC remained after the experiments (dashed lines); thus, the influence of the initial concentration was not detected.

Regarding the effect of temperature, it can be evaluated by comparing the differences between parts a, b, c and d in Figure 5. As the temperature increases, higher total TOC removal percentages were achieved, whereas the residual TOC fraction decreased (note that dashed line is consistently low, and the average values of the residual TOC fraction were 46.5%, 35.5%, 28.4% and 25.6% at 15°C, 20°C, 25°C and 30°C respectively). Moreover, the contribution of biological degradation appeared to decrease at high temperature (note the decreasing trend of biodegradation removal percentages, white bars, in every part in Figure 5). It is well known that high temperature favoured volatilization. The slopes of TOC abiotic decreasing profiles obtained by linear fitting clearly increased at higher temperatures. The effect of temperature on the biological mechanism is not so clear. On one hand, microbial degradation rate increases with temperature; on the other hand, thermal inhibition effects could appear if temperature

reaches excessively high values, while simultaneously, high rate volatilization of solvents could cause oxyfluorfen to become unavailable for microorganisms.

Figure 6. (a) Oxyfluorfen concentrations during biodegradation experiments (C₄: 500 mgL⁻¹ oxyfluorfen) at different temperatures. (b) Oxyfluorfen removal efficiencies (%) in all biodegradation experiments after 70 h.

Figure 6a shows the average oxyfluorfen concentration profiles during the biodegradation experiments performed at different temperatures and at the maximum initial herbicide concentration (C₄: 500 mgL⁻¹ oxyfluorfen). It can be observed that oxyfluorfen was completely removed only at 15°C and a low concentration remained untreated after 70 h at the other temperatures. Indeed, there is no change in the oxyfluorfen concentration after 35 h. Thus, the same behaviour observed in Figure 4 is now observed again. Moreover, no clear differences can be observed between the decreasing concentration profiles; thus, a clear influence of temperature on the removal rate and removal efficiency cannot be detected. Figure 6b shows the oxyfluorfen removal efficiencies (%) in all biodegradation experiments after 70 h; the main result observed is that increases in both the initial herbicide concentration and the temperature negatively affected the removal efficiencies. As previously indicated, a potential inhibition effect of high herbicide concentration or the appearance of non-biodegradable

intermediates could damage oxyfluorfen biodegradation (Marques et al., 2015). Moreover, it has been supposed again that the influence of temperature is mainly related to thermal inhibition and to the increase in evaporation rate of volatile compounds in *Fluoxil 24*, which acted as solvents to become oxyfluorfen available for the microorganisms. The higher the temperature level, the faster the solvent evaporation; this phenomenon could cause the deceleration (even inactivation) of the biodegradation mechanism because oxyfluorfen was hardly bioavailable. This harmful effect also has been reported previously (Haritash et al., 2009).

3.4. Biodegradation experiments. Modelling results.

TOC data obtained from abiotic tests were used to calculate the K_{La} values by linear fitting according to Mozo et al. (2012). Despite the observation of a slight change in the slope at approximately 15 h (figure 1), only one average slope was considered during the whole volatilization process to model this mechanism in a simple manner. Table S2 includes the K_{La} values obtained in the different abiotic tests.

Regarding the biodegradation experiments, an accurate estimation of the model's parameters (μ_{max} , K_d , K_s and Y) requires an initial set of values. These initial values guarantee the convergence of the mathematical functions to obtain a minimal SSE. In the present work, initial Y values were estimated directly from the experimental results because Y can be approximately obtained from the ratio between the biomass growth and the biodegradable TOC consumption. The rest of initial values were chosen according to our previous works regarding the modelling of the biodegradation of organochlorines (Carboneras et al., 2017). The K_d values were allowed to vary between 0.005 and 0.035 h^{-1} , and the maximum K_s values were allowed to reach 100 $mg_{TOC} L^{-1}$. Table S2 shows the values of the model parameters obtained in all experiments. As seen

in Table S2, no mathematical convergence was possible in the case of biodegradation experiments using 500 mg L⁻¹ oxyfluorfen and 30°C.

The parameters obtained allowed for reproduction of the experimental data and validation of the model. Figure 7a shows (as an example) a comparison between the experimental data (points) and the model predictions (lines) in one case (using 85 mg L⁻¹ oxyfluorfen and 20°C). Figure 7b shows the variation of the $\mu_{\max}$ values as a function of the two variables (initial oxyfluorfen concentration and temperature); this information can also be easily checked in Table S2. It is considered that the evolution of the $\mu_{\max}$ values predicted by the model approximately agree with the discussion described in the previous sections. Regarding the influence of the initial concentration, it can be observed that $\mu_{\max}$ decreases when the initial oxyfluorfen concentration increases; thus, the biological mechanism becomes slower and its contribution to the total TOC removal is less important, as previously discussed in section 3.3. Regarding the influence of temperature, the $\mu_{\max}$ values initially increased (between 15°C and 25°C) but decreased again between 25°C and 30°C, that is, an excessively high temperature negatively affected the biodegradation process because of the reasons previously discussed in section 3.3.

According to the whole results discussed, it is clear that *Fluoxil 24* could be efficiently but not completely removed by the acclimated microbial culture, especially under high concentrations or temperatures. As a result, advanced oxidation processes, such as electro-oxidation, have been proposed as post-treatment in our previous works (Chair et al., 2017) to completely eliminate such a recalcitrant pollutant.

Figure 7. (a) Example of model validation ($C_1=85 \text{ mg L}^{-1}$ oxyfluorfen and 20°C): experimental data (points) and model prediction (line). Model parameters obtained: $K_{LA} = 0.007 \text{ h}^{-1}$, $\mu_{\text{max}} = 0.45 \text{ h}^{-1}$, $Y = 3.2 \text{ mg X mg}^{-1} \text{ S}$, $K_s = 75 \text{ mg S L}^{-1}$, $K_d = 0.025 \text{ h}^{-1}$. (b) μ_{max} values as a function of two variables (initial oxyfluorfen concentration and temperature).

4. Conclusions

Oxyfluorfen was nearly, but not completely, biodegraded because high concentrations (from 300 mg L^{-1}) and temperatures (from 25°C) negatively affected the biological process probably via inhibition effects as well as because the volatilization of solvents. A simple mathematical model that considered simultaneous volatilization and Monod kinetics was able to predict the experimental results, and the calculated model parameters confirmed the effects of the variables under study.

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