Accepted Manuscript

Title: Assessment of the mechanisms involved in the removal of emerging contaminants by microalgae from wastewater: A laboratory scale study

Author: Víctor Matamoros Enrica Uggetti Joan García Josep M. Bayona

Please cite this article as: Víctor Matamoros, Enrica Uggetti, Joan García, Josep M.Bayona, Assessment of the mechanisms involved in the removal of emerging contaminants by microalgae from wastewater: A laboratory scale study, Journal of Hazardous Materials http://dx.doi.org/10.1016/j.jhazmat.2015.08.050

Assessment of the mechanisms involved in the removal of emerging contaminants by microalgae from wastewater: A laboratory scale study

Víctor Matamoros[1][*] victor.matamoros@idaea.csic.es, Enrica Uggetti[2], Joan García[2], Josep M Bayona[1]

[1]Department of Environmental Chemistry, IDAEA-CSIC, c/Jordi Girona, 18-26, E-08034, Barcelona, Spain.

[2]GEMMA-Group of Environmental Engineering and Microbiology, Department of Hydraulic, Maritime and Environmental Engineering, Universitat Politècnica de Catalunya BarcelonaTech, c/ Jordi Girona, 1-3, Building D1, E-08034, Barcelona, Spain.

[*]Corresponding author.

Graphical Abstract
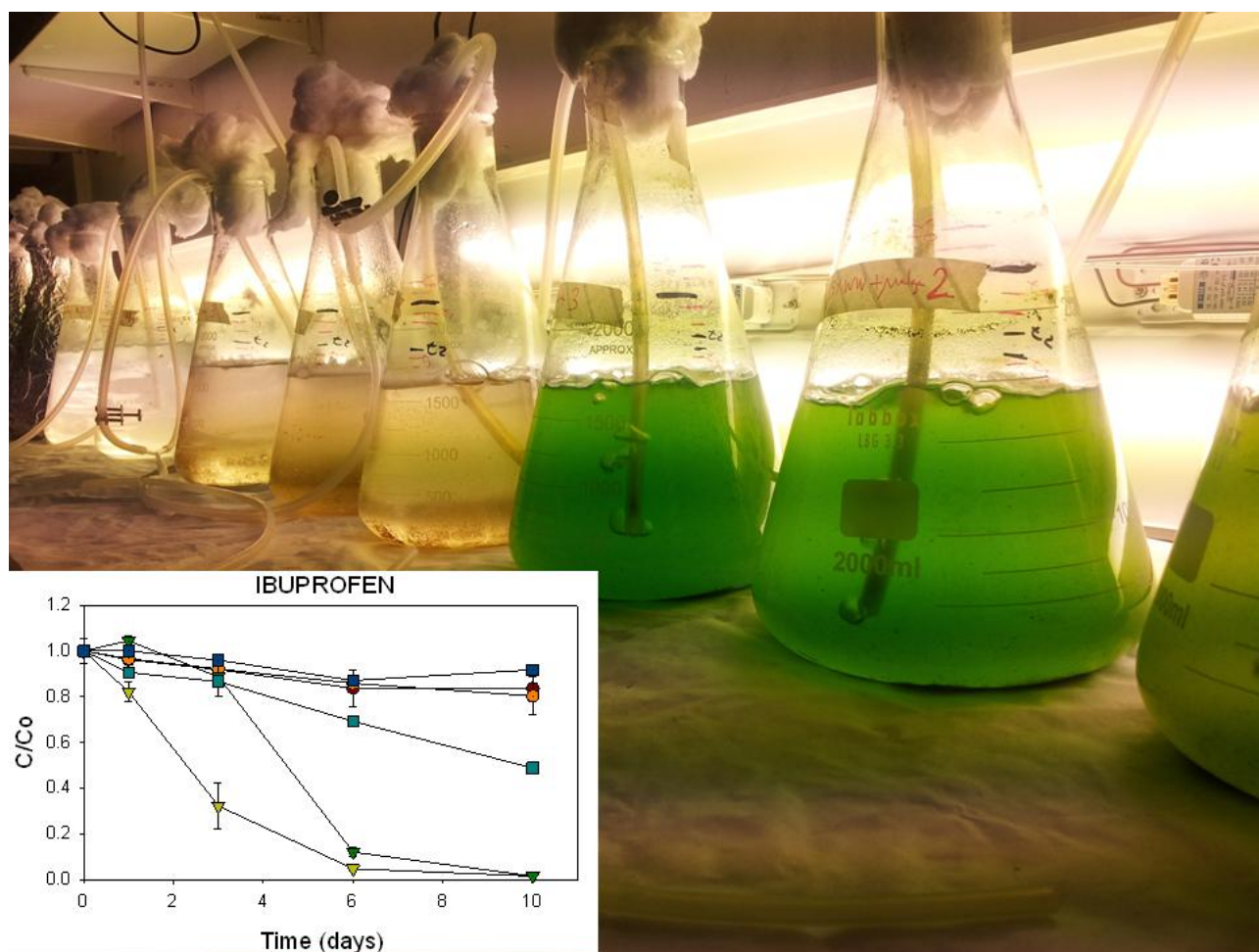
Highlights

The effect of microalage on the removal of emerging contaminants has been evaluated.

- Volatilization was relevant for compounds with a moderate Henry's law constant.
- Biodegradation was the main factor for the removal of caffeine and ibuprofen.
- Ibuprofen enantioselective biodegradation was observed.
- CA-ibuprofen and OH-ibuprofen followed the concentration decline of ibuprofen.

Abstract

Aerated batch reactors (2.5 L) fed either with urban or synthetic wastewater were inoculated with microalgae (dominated by *Chlorella* sp. and *Scenedesmus* sp.) to remove caffeine, ibuprofen, galaxolide, tributyl phosphate, 4-octylphenol, tris(2-chloroethyl) phosphate and carbamazepine for 10 incubation days. Non-aerated and darkness reactors were used as controls. Microalgae grew at a rate of 0.25 d^{-1} with the complete removal of N-NH$_4$ during the course of the experiment. After 10 incubation days, up to 99% of the microcontaminants with a Henry's law constant higher than $3 \cdot 10^{-1}$ Pa m^3 mol^{-1} (i.e. 4-octylphenol, galaxolide, and tributyl phosphate) were removed by volatilization due to the effect of air stripping. Whereas biodegradation was effective for removing ibuprofen and caffeine, carbamazepine and tris(2-chloroethyl) phosphate behaved as recalcitrant compounds. The use of microalgae was proved to be relevant for increasing the biodegradation removal efficiency of ibuprofen by 40% and reducing the lag phase of caffeine by 3 days. Moreover, the enantioselective biodegradation of S-ibuprofen suggested a biotic prevalent removal process, which was supported by the identification of carboxy-ibuprofen and hydroxy-ibuprofen. The results from microalgae reactors fed with synthetic wastewater showed no clear evidences of microalgae uptake of any of the studied microcontaminants.

Keywords: emerging organic contaminants; wastewater; removal; microalgae; biodegradation, volatilization.

1. Introduction

The presence of emerging contaminants (ECs) in the environment has already gained public awareness [1]. ECs are used daily in large quantities and include a wide variety of compounds such as pharmaceuticals, personal care products, plasticizers, flame retardants, surfactants, and certain pesticides, among others. Since conventional wastewater treatment plants (WWTPs) are not designed to treat this type of contaminants, many of these compounds occur at different concentrations in natural water bodies[2], where they may exert ecotoxicological effects even at relatively low concentrations [3, 4]. Among the better researched compounds, galaxolide has been found to inhibit the cellular multixenobiotic defense systems of marine mussels[5], tris(2-chloroethyl) phosphate has been shown to be carcinogenic, reproductive and neuro-toxic in animal cell cultures [6], and 4-octylphenol has high potential estrogenic effects on the amphibian *Xenopus laevis* [7]. As a consequence, known environmental effects of the occurrence of ECs in surface waters include the reduction of macroinvertebrate biodiversity in rivers [3] and behavioural changes in mosquito fish [4]. Therefore, it is of high importance to reduce their discharge into the aquatic environment.

Studies on the removal of these contaminants have mainly focused on conventional activated sludge WWTPs, but little attention has been paid to the effectiveness of natural engineered technologies such as microalgae-based wastewater treatment systems for removing ECs. Microalgae-based wastewater treatment technologies are relevant due to the resource recovery of algal biomass, for use as a fertilizer, as a source of products (e.g. paraffin, olefin, glycerol, protein, anti-oxidant, pigment, plastic, etc.) or as biofuel, but they also possess the advantage of providing a high-quality treated effluent [8]. Although the capability of microalgae wastewater treatment systems to remove organic matter and nutrients has already been studied, few such studies have focused on the removal of organic microcontaminants. Existing laboratory-scale studies dealing with microalgae's capacity to remove organic microcontaminants such as phenolic compounds, surfactants, biocides and

polycyclic aromatic hydrocarbons suggest that microalgae-based wastewater treatment systems may remove them by evaporation, photodegradation, biodegradation, and/or microalgae uptake [9-11]. Nevertheless, little attention has been paid to the processes involved in the removal of ECs and the effect of microalgae on such processes. Recent studies performed by de Godos et al [12] demonstrate that the use of microalgae systems such as high rate algal ponds (HRAPs) are suitable for removing tetracyclines. These results have been confirmed by our research studies in which HRAP pilot plants fed with real wastewater are capable of removing up to 90% of ECs. In this case biodegradation and photodegradation were postulated as the most relevant removal processes [13]. Nevertheless, there is a lack of knowledge regarding the processes involved in the removal of ECs in microalgae-based wastewater systems. To overcome this knowledge gap, laboratory-scale studies conducted under controlled environment conditions are imperative.

Our aim in this study was to quantify the effect of microalgae activity on the removal efficiency of 7 ECs in a laboratory-scale study. The ECs were selected on the basis of their concentration, their high frequency of detection in urban wastewaters and their physicochemical properties (i.e. caffeine, ibuprofen, galaxolide, tributyl phosphate, 4-octylphenol, tris(2-chloroethyl) phosphate and carbamazepine) (Table 1). Additionally, the identification of transformation products (TPs) and the assessment of the enantiomeric factor were used to ascertain the processes involved in the removal of ECs.

2. Material and Methods

2.1. Experimental design

In order to study the processes occurring behind the removal of microcontaminants in microalgae-based wastewater treatment systems, different reactor set-ups were assessed. The factors evaluated were microalgae (presence or absence), aeration (aerated and non-aerated), light-radiation (light *vs.*

darkness) and water quality (wastewater *vs.* synthetic wastewater). The set-up included aerated microalgae reactors fed with real wastewater (reactor 4) or synthetic wastewater (reactor 6), aerated control reactors fed with real wastewater (reactor 5) or synthetic wastewater (reactor 1), and control reactors in darkness (covered by aluminium foil) fed with real wastewater with and without aeration (reactors 2 and 3) (Table 2). The total number of experimental units was 18, which corresponds to 6 different types of reactors, with 3 replicates of each type.

All reactor systems consisted of containers of 2.5 L made of pre-cleaned glass. A standard solution containing the seven microcontaminants within methanol solution was added to each reactor (final water volume of 2 L) obtaining a final concentration of 5 µg L^{-1} (200 µL of spiking solution at 100 µg L^{-1} for each compound in methanol). Microalgae reactors were inoculated with a microalgae consortium obtained from an experimental high rate algal pond treating urban wastewater [14]. Main microalgae populations were made up by *Chlorella* sp. and *Scenedesmus* sp. Note that the inoculum also contained bacteria, however microalgae accounting for over 90% of the biomass as is usual in HRAPs [15]. The microalgae consortium was pre-acclimatised to the growth conditions for 10 days before the reactors were stocked with microalgae. The microalgae were inoculated to a concentration of approximately 100 mg L^{-1} dry weight (dw) biomass per reactor (250 mL of pre-acclimatised microalgae of approximately 1000 mg L^{-1} dw biomass). The experiments were simultaneously run for 10 days.

Wastewater reactors were fed with 25% of urban wastewater (75% of the solution was ground water and 25% urban wastewater) to reach a similar organic composition to that found in the mixed liquor of the HRAP [16]. The wastewater used for experiments was a primary treated effluent with an average composition as follows: Total Suspended Solids (TSS), 100±3 mg L^{-1}, total chemical oxygen demand (COD), 241±73 mg L^{-1}, NH_4-N, 47±6 mg L^{-1}. The groundwater composition was as follows: TSS, 10 mg L^{-1}, NH_4-N, 0.2 mg L^{-1}, and NO_3-N, 38 mg L^{-1}. The nutrient solution was based on a carbon-free synthetic wastewater containing the following (in mg L^{-1}): NaCl, 7; $CaCl_2$,

4; $MgSO_4 \cdot 7H_2O$, 2; K_2HPO_4, 21.7; KH_2PO_4, 8.5; Na_2HPO_4, 33.4; and NH_4Cl, 10 [17]. The reactors were setup in a temperature controlled growth room at 23 ± 5 °C and lit by fluorescent tubes at a photon flux density of 150 μmol m^{-2} s^{-1} in a 12 h light/12 h dark cycle (Philips Master TL-D, 36W/840). Aeration of the reactors was ensured by using an air flow of 50 L h^{-1} (EIHEM air pump, Germany).

2.2. Sampling strategy

Aqueous samples of 125 mL were taken regularly during experimentation at 0, 1, 3, 6, and 10 days. In order to keep the same water level in the reactors and compensate for any water loss by evaporation, the reactors were refilled with groundwater to a specific depth mark before sampling (groundwater used in the experimental design was stored in the fridge at 4ºC). All water samples were collected in 250 mL amber glass bottles, which were stored at 4 ºC until analysis. The sample holding time was less than 12 hours.

2.3. Microalgae identification and growth rate determination

25 mL of the collected water samples were examined at the optical microscope (Optiphot-Pol Nikon, Nippon) to identify the main populations.

Furthermore, in each water sample absorbance was measured at different wavelengths ($\lambda = 680$ and 800 nm) by optical spectrophotometry (Spectronic Genesis 8, Thermo Electric, UK) in order to obtain the optical density (OD). The microalgae growth rate (μ) was calculated by fitting the OD to an experimental function [18]:

$$\mu = (\ln(OD_{t,\,680}-OD_{t,\,800})-\ln(OD_{0,\,680}-OD_{0,\,800}))/t$$

where $OD_{0,\lambda}$ is the OD at the initial day, $OD_{t,\lambda}$ is the optical density measured on the day t.

2.4. Chemicals and reagents

Gas chromatography (GC) grade (Suprasolv) hexane, methanol, and ethyl acetate were obtained from Merck (Darmstadt, Germany). Analytical-grade hydrogen chloride was obtained from Panreac (Barcelona, Spain). Caffeine, ibuprofen (racemate form), carbamazepine, galaxolide, tributyl phosphate, tris(2-chloroethyl) phosphate, triphenyl phosphate, 4-octylphenol, atrazine D5, mecoprop D3, tonalide D3 and dihydrocarbamazepine were purchased from Sigma-Aldrich (Steinheim, Germany). Trimethylsulfonium hydroxide (TMSH) was obtained from Fluka (Buchs, Switzerland). Strata-X polymeric SPE cartridges (200 mg) were purchased from Phenomenex (Torrance, CA, USA) and the 0.7 μm glass fibre filters (ø 47 mm) were obtained from Whatman (Maidstone, UK).

2.5. Analytical methodology

Conventional wastewater quality parameters, including ammonium nitrogen (NH_4-N) and total suspended solids (TSS) were determined using the Standard Methods (APHA, 2001). All water samples were filtered and processed as previously reported[19]. A 50 mL sample was spiked with 100 ng of a surrogate standard (atrazine D5, mecoprop D3, tonalide D3, and dihydrocarbamazepine). The spiked sample was percolated through a previously activated polymeric solid-phase extraction cartridge (200 mg Strata X). Elution was performed with 10 mL of ethyl acetate. The eluted extract was evaporated under a gentle nitrogen stream until ca. 100 μL remained, at which point 100 ng of triphenylamine was added as an internal standard. After that, the vial was reconstituted to 300 μL with ethyl acetate.

Methylation of the acidic carboxyl group (derivatization step) was performed in a hot GC injector (270 ºC) by adding 10 μL of TMSH solution (0.25 mol L^{-1} in methanol) to a 50 μL sample before injection. Derivatized samples were injected into a TRACE GC–MS (Thermo-Finnigan, Dreieich,

Germany) in the electron impact mode (70 eV ionization energy) fitted with a 20 m × 0.18 mm, 0.18 μm film thickness Sapiens X5-MS capillary column coated with 5% diphenyl 95% dimethyl polysiloxane from Teknokroma (Sant Cugat del Vallès, Spain). Data processing and validation of the methodology are described elsewhere [19].

Enantiomeric composition of the all water samples was analysed as reported elsewhere [20]. Derivatized samples (TMSH) were injected at 280ºC in the split mode into a TRACE GC–MS (Thermo Fisher, Dreieich, Germany) in electron impact mode (70 eV ionization energy) fitted with an Astec Chiraldex chiral column (Whippany, NJ) coated with dimethyl-β-cyclodextrin as stationary phase (20 m × 0.25 mm × 0.12 μm film thickness). Acquisition was performed in single-ion monitoring (SIM) mode at 2 scans s^{-1}. The following ions (m/z) were monitored for enantiomeric ibuprofen determination: **161**/177/220.

2.6. Data analysis

The quantification of the removal percentage explained by each removal process was tentatively calculated from a comparison of the removal rates (Section 3.3.3) of the compounds taking into consideration the additive effect of all the studied factors. Sorption processes by the microalgae were not considered in this study since the incubation of the selected microcontaminants with microalgae and nutrients performed similar removal rates than those observed in the aerated control reactors without microalgae. Volatilization was estimated by comparing the aerated, covered control reactors (k2) and the non-aerated, covered control reactors (k3). Photodegradation was calculated from the uncovered and aerated control reactors (k1) and the covered and aerated control reactors (k2). Direct biodegradation due to the presence of bacteria in the wastewater was calculated from the rectors fed with wastewater (k5) and the uncovered control reactors (k1). The biodegradation enhancement by microalgae was estimated from the reactors fed with microalgae and wastewater (k4) and the aerated reactors fed solely with wastewater (k5). The direct effect of microalgae was

calculated from the reactors fed with nutrients and microalgae (k6) and uncovered aerated reactors (k1). In all of the cases the removal rate obtained from the reactors fed with wastewater and microalgae (k4) was used as a reference to quantify the removal percentage of each mechanism as follows:

Volatilization = (k2-k3)/k4 x100

Photodegradation = (k1-k2)/k4 x100

Spontaneous biodegradation = (k5-k1)/k4 x100

Biodegradation enhancement by microalgae = (k4-k5)/k4 x100

Direct microalgae effect = (k6-k1)/k4 x100

The experimental results were statistically evaluated using the SPSS v.13.0 package (Chicago, IL, US). Non-parametric statistics were applied for all statistical analysis according with the data set size. The Mann-Whitney U test was applied to compare differences between the experiments. Linear regressions between the pairs of variables (compound concentration *vs.* time) were performed using Pearson's correlation analysis. The statistical significance was defined as $p < 0.05$.

3. Results and Discussion

3.1 Reactors performance

Microscope observations showed that in microalgae reactors *Chlorella* sp. and *Scenedemus* sp. remained predominant during experiments. Fig. 1a shows the increase of microalgae biomass throughout the experimental period in the systems fed with wastewater and nutrients respectively. After 10 incubation days, the biomass weight was doubled in the reactors inoculated with microalgae, whereas no biomass development was observed in those reactors that were not inoculated with microalgae. The microalgae growth rate was 0.25 d^{-1}, which is quite low, but still within the range of values found in lab scale experiments treating urban wastewater (0.2-1 d^{-1}) [21, 22].

The removal of N-NH$_4$ was up to 99% after 10 incubation days, but the removal rate was higher in the reactors inoculated with microalgae as can be observed from Fig. 1b (removal rate of 0.63 *vs.* 0.48 d^{-1} for microalgae and wastewater reactors respectively). The main processes involved in this removal could be microalgae uptake, nitrification and ammonia volatilisation. Samorì et al [23] found similar N-NH$_4$ concentration decay in a batch culture system containing the microalgae *Desmodesmus communi*s and fed with wastewater. The pH increased from 7.0±0.3 at the beginning of the experiment to 8.6±0.1 at the end of the experiment in the microalgae reactors. The control reactors had a pH of 7.5±0.7 during the whole experiment.

3.2. Microcontaminants response curves

Because of the different physico-chemical characteristics of the studied compounds (Table 1), as well as the removal observed in this study, the microcontaminants can be grouped as follows: (i) volatile compounds (i.e. 4-octylphenol, galaxolide, and tributyl phosphate), (ii) biodegradable compounds (i.e. caffeine and ibuprofen) and (iii) recalcitrant compounds (i.e. carbamazepine and tris(2-chloroethyl) phosphate).

3.2.1. Volatile compounds

Fig. 2 shows that the decline of 4-octylphenol, galaxolide, and tributyl phosphate concentrations from the water depended almost exclusively on the aeration of the reactors. After 10 incubation days up to 90% of all these compounds were removed in all reactors except in those which were not aerated. This is explained by the higher tendency of these compounds to volatilize from the liquid solvent into the gaseous phase because of the air-stripping effect. In fact, all these compounds were neutral at the studied pH (7-9) with Henry's law constant values (tendency for volatilization) ranging from $3.23 \ 10^{-1}$ to $1.34 \ 10^{1}$ Pa m^3 mol^{-1}. Galaxolide, which presented the highest Henry law

constant (Table 1), was also the compound with the fastest concentration decay. Likewise, up to 14% of polycyclic musk fragrances such as galaxolide are removed by volatilization in conventional activated sludge WWTPs [24]. Moreover, these results are in agreement with Albargues et al. [9] who found that aerated microalgae batch reactors fed with a wastewater effluent were capable of removing 4-octylphenol up to 99% after 20 incubation hours whereas no removal was observed under non-aerated conditions. Therefore, volatilization due to the aeration processes should always be taken into consideration in these systems. The other studied compounds were not removed because they were present in an ionic form at working pH (i.e. ibuprofen) or they had low Henry law constant values (i.e. caffeine, 3.63 10^{-6} Pa m^3 mol^{-1}, carbamazepine, 1.1 10^{-5} Pa m^3 mol^{-1} and tris(2-chloroethyl) phosphate, 2.58 10^{-3} Pa m^3 mol^{-1}).

3.2.2. Biodegradable compounds

Fig. 3 shows the decreasing ibuprofen and caffeine concentration depended on the presence of microalgae and/or wastewater. After 10 incubation days, caffeine was removed more easily by the combined presence of microalgae and wastewater (99%) compared to the incubation solely in wastewater (86%) or microalgae (17%), whereas uncovered and covered control reactors without microalgae and wastewater did not show any removal. This may be explained by the presence of bacteria in the wastewater and the activity of microalgae. It has already been proven that these compounds are removed by biodegradation carried out by bacteria in laboratory scale studies, as well as in full-scale activated sludge WWTPs [25]. However, this is the first time that the enhancing effect of microalgae has been demonstrated (40% *vs.* 90% after 6 incubation days for caffeine and 15% *vs.* 60% removal after 3 incubation days for ibuprofen). Microalgae can increase the removal efficiency of ibuprofen and caffeine by either releasing exudates, which aid the biodegradation processes [26] or by microalgae uptake. Due to the negative charge of ibuprofen (pKa = 4.9) and the electrochemical negative charge of the microalgae cell walls [27], ibuprofen uptake can be

considered to be negligible at the working pH (pH = 7-9). Caffeine, which is neutral (pKa = 14) at the studied pH, could be removed from the reactors by either microalgae sorption or biodegradation. Nevertheless, due to the fact that microalgae reactors fed with nutrients were not capable of removing caffeine, it can be postulated that the main removal process of this compound is biodegradation and the microalgae uptake is unlikely. Zhang et al. [28] found that direct plant assimilation by *Scirpus validus* of caffeine was up to 60%. Although these results seem to be contradictory, it has to be taken into account that the microalgae present in our study were unicellular organisms while *Scirpus validus* is a macrophyte with a well-developed rizosphere capable of caffeine uptake.

The overall results are in agreement with those previously found for aquatic plants (*Salvinia molesta, Lemna minor, Ceratophyllum demersum,* and *Elodea Canadensis)*, although in these experiments the removal after 10 incubation days was lower than 40% since the reactors were fed only with nutrients [29]. Hence, the presence of bacteria in the wastewater dramatically increases the removal of caffeine and ibuprofen. Furthermore, the degradation experiments fed with wastewater revealed a lag phase of around 3 days for the ibuprofen concentration decay, but with the use of microalgae, this adaptation time was reduced. Therefore, the results showed that microalgae reduced the adaptation time required by bacteria to remove ibuprofen while simultaneously enhancing the biodegradation removal rate of caffeine, either by chemical exudates or oxygen release. Such a result is in agreement with previous results that prove that cyanobacterial/algal photosynthesis provides oxygen and organic exudates that help the pollutant-degrading heterotrophic bacteria [26].

3.2.3. Recalcitrant compounds

Fig. 4 shows that less than 20% of carbamazepine and tris(2-chloroethyl) phosphate were removed after 10 incubation days. In agreement with previously published studies, the photodegradation,

biodegradation and sorption into sludge of these compounds in activated sludge and biologically-based WWTPs seem to be negligible [30, 31]. Although some studies have demonstrated an active plant uptake of carbamazepine by rooted plants such as cucumber and *Thypa* sp. [32, 33], from our results, unicellular microalgae appear to be uncapable of carmabazepine uptake.

Overall our laboratory-scale studies have demonstrated that 4-octylphenol, galaxolide, and tributyl phosphate were removed efficiently by volatilization; ibuprofen and caffeine were removed by biodegradation; whereas carbamazepine and tris(2-chloroethyl) phosphate were not removed at all.

3.3 Kinetics

The decay rates of these microcontaminants in containers were fitted to a pseudo-first order removal kinetics model with Pearson correlation coefficients higher than 0.85 (p value < 0.05; Table 3) in all cases, except the covered and non-aerated controls, carbamazepine and tris(2-chloroethyl) phosphate setups. Table 3 shows the removal rates and removal efficiencies achieved after 10 incubation days for caffeine and ibuprofen. The ibuprofen removal rates obtained in this study by using microalgae and wastewater (0.448 ± 0.042 d^{-1}) were higher than those from previous studies carried out by using secondary-treated wastewater effluents and aquatic plants (0.109 ± 0.008 d^{-1} in reactors planted with *Lemma sp.*). The removal rates for caffeine were, however, similar in both studies (0.321 ± 0.035 d^{-1} in this study *vs.* 0.300 ± 0.01 d^{-1}) [34], and in all cases much higher than those observed from other studies in which aquatic plants were fed only with nutrients [29] (caffeine 0.014-0.135 d^{-1} and ibuprofen 0.016-0.182 d^{-1}). Hence, it can be postulated that the enhancing biodegradation effect provided by microalgae and carried out by bacteria may be at least as high as the effect from aquatic plants. Galaxolide and 4-octylphenol performed similar removal rates in all aerated reactors, 0.443 ± 0.084 and 0.310 ± 0.035 d^{-1} respectively, but no decline in concentrations were observed in the non-aerated systems. Galaxolide showed a significantly higher removal rate than 4-octylphenol (p<0.05), due to its higher Henry's law constant value (Table 1).

No decline in concentrations were observed for carbamazepine and tris(2-chloroethyl) phosphate in all setups.

3.4 Identification of transformation products

Fig. 5 shows the formation of two major degradation products from ibuprofen; carboxy-ibuprofen (CA-IB) and hydroxy-ibuprofen (OH-IB) in the microalgae reactors fed with nutrients. As is shown transformation products (TPs) were already present in the reactors fed with wastewater. In fact, CA-IB and OH-IB are the two major human metabolites of ibuprofen usually found in wastewater, with concentrations ranging from 29 to 63 $\mu g\ L^{-1}$ [35]. The reactors fed with 25% wastewater presented concentrations around 4 and 6 $\mu g\ L^{-1}$ of OH-IB and CA-IB respectively, which is in agreement with the concentrations found in raw wastewater. A part from that, they have also been described as the most abundant ibuprofen TPs after the biodegradation processes. Zwiener and Frimmel [36] described that in aerobic conditions both TPs can be formed, whereas anaerobic biodegradation processes only CA-IB is produced.

The predominance of OH-IB over CA-IB indicates that biodegradation occurs under prevailing aerobic conditions, which agrees with other studies [29, 36, 37]. The high oxygen concentration in the reactors (7-9 $mg\ L^{-1}$) is due to both microalgae release and air stripping. In this sense, the reactors fed with wastewater showed higher removal rate of CA-IB than OH-IB, probably due to the higher formation of the latter as observed in reactors fed with nutrients. This is in agreement with the results found by Ferrando-Climent et al. [35] who observed that CA-IB was removed more efficiently than OH-IB in batch experiment studies performed with activated sludge supplemented by nutrients and ibuprofen.

Although biodegradation of caffeine was observed, we did not identify any of their described TPs [38] which could be due to their low stability or poor sensitivity in the analytical method used. Further work involving the development of new analytical methods is necessary in this sense.

3.5 Assessment of removal processes

Up to 99% of 4-octylphenol, tributyl phosphate and galaxolide were removed by volatilization, while the other process was negligible (<5%). This is in agreement with the high tendency for volatilization of these compounds (high Henry's law constant values) as has been described in section 3.2.1. Photodegradation was not relevant for any compound (<5%), either because the compounds were not photodegradable (ibuprofen, caffeine, carbamazepine and tris(2-chloroethyl) phosphate) or because the effect of the aeration (air stripping) was much higher than that of the photodegradation, making the photodegradation undetectable (4-octylphenol, tributyl phosphate and galaxolide). Direct biodegradation by the presence of bacteria in wastewater reactors (reactor set 5) was the main factor for the removal of caffeine (59%) and ibuprofen (95%). The biodegradation rate of caffeine increased to 99% in the presence of microalgae. The ibuprofen removal rate constants were similar for both wastewater and wastewater-microalgae reactor sets (see Table 3). As has been mentioned previously, the main effect of microalgae in the removal of ibuprofen was the reduction of the lag phase from 3 days to an undetectable time period. No direct effect of microalgae was observed for any of the other studied compounds (<10%). In comparison with already published studies [34], the enhancement of the caffeine removal rate by the presence of microalgae in our study was found to be slightly higher than that found for the filamentous microalgae *Spirogyra sp.* (40 *vs.* 33%).

In conclusion this study has proved that air stripping is a relevant removal process for those microcontaminants with a Henry's law constant higher than $3 \ 10^{-1} \ Pa \ m^3 \ mol^{-1}$. Biodegradation, and its enhancement by the presence of microalgae is relevant for removing ibuprofen and caffeine, whereas carbamazepine and tris(2-chloroethyl) phosphate were not removed at all, even in the presence of microalgae.

3.6 Assessment of the ibuprofen enantiomeric ratio

Enantiomer composition differs in their biological and pharmacological activity as a result of their stereo-selective interaction with enzymes [39]. Since abiotic processes such as sorption and photodegradation are not enantioselective, the enantiomer composition has been postulated as a tracer of the biodegradation process [40]. Enantiomeric factor (EF) is defined as the quantitative ratio of the relative compositions of (R)- and (S)-enantiomers as follows:

EF = S-enantiomer/(S-enantiomer + R-enantiomer)

A racemic ibuprofen composition (EF=0.5) was used to spike the reactor systems, but the use of wastewater increased the EF to 0.6 in the reactors fed with wastewater. Fig. 6a shows the decrease of EF over time, with the reactors fed with both nutrients and microalgae showing the highest decline ratio, from 0.50 to 0.07, while the aerated control reactors (reactor set 1) showed no EF differences over time (EF = 0.5). Reactors fed with wastewater showed similar ratios, from 0.61 to 0.38. Although in all cases, except in the control reactors, the concentration decline fitted with EF decline (p value<0.05; Fig. 6b.), the highest EF decline was observed in the microalgae reactors fed with nutrients. This did not correlate with the highest removal efficiency observed in the reactors fed with wastewater. In fact, when enantiomer abundances were individually assessed only the S-enantiomer was removed in reactors fed with microalgae and nutrients whereas the abundance of the R enantiomer did not change. Conversely, in the reactors fed with wastewater the abundance of both enantiomers declined over time, but the abundance of S-enantiomer declined faster. Therefore, it can be postulated that EF can be used to assess the removal efficiency of ibuprofen, but the presence of microalgae increase the decline rate of the EF. These results are in keeping with other studies that evidence the enantioselective biodegradation of ibuprofen in conventional activated sludge WWTPs, aerobic constructed wetlands and laboratory-scale membrane bioreactors [20, 41, 42]. Moreover, Matamoros et al. [20] observed that this enantioselective degradation appears to be specific to aerobic biodegradation conditions and was not observed under anaerobic conditions. In

the current study, in which aerobic conditions prevailed, the enantioselective biodegradation has also been recorded. Nevertheless, the exclusive S-ibuprofen removal by microalgae systems fed with nutrients is probably explained by the supposition that enzymatic exudates released by microalgae are driving the ibuprofen biodegradation. Further studies will be needed to demonstrate this conclusively.

4. Conclusions

Laboratory experiments combining the presence of microalgae, synthetic and real wastewater allowed us to prove that air stripping is a relevant removal process for microcontaminants with a Henry's law constant higher than $3 \cdot 10^{-1}$ Pa m^3 mol^{-1}, such as 4-octylphenol, galaxolide, and tributyl phosphate. Biodegradation was the main factor for the removal of caffeine (99%) and ibuprofen (95%), whereas the enhancement of the biodegradation removal by microalgae was only observed for caffeine (40%). The main effect of microalgae in the removal of ibuprofen was the reduction of the lag phase from 3 days to an undetectable time period. CA-ibuprofen and OH-ibuprofen TPs from ibuprofen followed the concentration decline of ibuprofen, which confirmed that biodegradation was the main removal process. Moreover, ibuprofen enantioselective biodegradation was observed. Conversely, carbamazepine and tris(2-chloroethyl) phosphate were not removed at all, even in the presence of microalgae.

5. Acknowledgments

6. References

[1] K.E. Murray, S.M. Thomas, A.A. Bodour, Prioritizing research for trace pollutants and emerging contaminants in the freshwater environment, Environ. Pollut., 158 (2010) 3462-3471.

[2] M. Carballa, F. Omil, J.M. Lema, M. Llompart, C. García-Jares, I. Rodríguez, M. Gómez, T. Ternes, Behavior of pharmaceuticals, cosmetics and hormones in a sewage treatment plant, Water Res., 38 (2004) 2918-2926.

[3] I. Muñoz, J.C. López-Doval, M. Ricart, M. Villagrasa, R. Brix, A. Geiszinger, A. Ginebreda, H. Guasch, M.J.L. de Alda, A.M. Romaní, S. Sabater, D. Barceló, Bridging levels of pharmaceuticals in river water with biological community structure in the llobregat river basin (northeast spain), Environ. Toxicol. Chem., 28 (2009) 2706-2714.

[4] T. Henry, M. Black, Acute and Chronic Toxicity of Fluoxetine (Selective Serotonin Reuptake Inhibitor) in Western Mosquitofish, Arch. Environ. Contam. Toxicol., 54 (2008) 325-330.

[5] Z. Qiang, H. Dong, B. Zhu, J. Qu, Y. Nie, A comparison of various rural wastewater treatment processes for the removal of endocrine-disrupting chemicals (EDCs), Chemosphere, 92 (2013) 986-992.

[6] X. Li, W. Zheng, W.R. Kelly, Occurrence and removal of pharmaceutical and hormone contaminants in rural wastewater treatment lagoons, Sci. Total Environ., 445-446 (2013) 22-28.

[7] M.E. Hoque, F.d.r. Cloutier, C. Arcieri, M. McInnes, T. Sultana, C. Murray, P.A. Vanrolleghem, C.D. Metcalfe, Removal of selected pharmaceuticals, personal care products and artificial sweetener in an aerated sewage lagoon, Sci. Total Environ., 487 (2014) 801-812.

[8] R. Craggs, D. Sutherland, H. Campbell, Hectare-scale demonstration of high rate algal ponds for enhanced wastewater treatment and biofuel production, J. Appl. Phycol., 24 (2012) 329-337.

[9] M.R. Abargues, J. Ferrer, A. Bouzas, A. Seco, Removal and fate of endocrine disruptors chemicals under lab-scale postreatment stage. Removal assessment using light, oxygen and microalgae, Bioresour. Technol., 149 (2013) 142-148.

[10] A.K. Haritash, C.P. Kaushik, Biodegradation aspects of Polycyclic Aromatic Hydrocarbons (PAHs): A review, J. Hazard. Mater., 169 (2009) 1-15.

[11] L.E. de-Bashan, Y. Bashan, Immobilized microalgae for removing pollutants: Review of practical aspects, Bioresour. Technol., 101 (2010) 1611-1627.

[12] I. de Godos, R. Muñóz, B. Guieysse, Tetracycline removal during wastewater treatment in high-rate algal ponds, J. Hazard. Mater., 229-230 (2012) 446-449.

[13] V. Matamoros, R. Gutiérrez, I. Ferrer, J. García, J.M. Bayona, Capability of microalgae-based wastewater treatment systems to remove emerging organic contaminants: A pilot-scale study, J. Hazard. Mater., 288 (2015) 34-42.

[14] F. Passos, M. Solé, J. García, I. Ferrer, Biogas production from microalgae grown in wastewater: Effect of microwave pretreatment, Applied Energy, 108 (2013) 168-175.

[15] J. García, B.F. Green, T. Lundquist, R. Mujeriego, M. Hernández-Mariné, W.J. Oswald, Long term diurnal variations in contaminant removal in high rate ponds treating urban wastewater, Bioresour. Technol., 97 (2006) 1709-1715.

[16] J. García, R. Mujeriego, M. Hernández-Mariné, High rate algal pond operating strategies for urban wastewater nitrogen removal, J. Appl. Phycol., 12 (2000) 331-339.

[17] L.E. González, R.O. Cázares, S. Baena, Efficiency of ammonia and phosphorus removal from a colombian agroindustrial wastewater by the microalgae Chlorella vulgaris and Scenedesmus dimorphus, Bioresour. Technol., 60 (1997) 259-262.

[18] A. Michelle Wood, R.C. Everroad, L.M. Wingard, Measuring Growth Rates Microalgal Cultures, in: Robert Andersen, Provasoli-Guillard National Center for Culture of Marine Phytoplankton, West Boothbay Harbor, ME USA (Ed.) Algal Culturing Techniques, Elsevier Academic Press, 2005, pp. 269-288.

[19] V. Matamoros, E. Jover, J.M. Bayona, Part-per-Trillion determination of pharmaceuticals, pesticides, and related organic contaminants in river water by solid-phase extraction followed by

comprehensive two-dimensional gas chromatography time-of-flight mass spectrometry, Anal. Chem., 82 (2010) 699-706.

[20] I.A. Vasiliadou, R. Molina, F. Martínez, J.A. Melero, Experimental and modeling study on removal of pharmaceutically active compounds in rotating biological contactors, J. Hazard. Mater., 274 (2014) 473-482.

[21] B. Du, A.E. Price, W.C. Scott, L.A. Kristofco, A.J. Ramirez, C.K. Chambliss, J.C. Yelderman, B.W. Brooks, Comparison of contaminants of emerging concern removal, discharge, and water quality hazards among centralized and on-site wastewater treatment system effluents receiving common wastewater influent, Sci. Total Environ., 466-467 (2014) 976-984.

[22] M.E. Martínez, S. Sánchez, J.M. Jiménez, F. El Yousfi, L. Muñoz, Nitrogen and phosphorus removal from urban wastewater by the microalga Scenedesmus obliquus, Bioresour. Technol., 73 (2000) 263-272.

[23] G. Samorì, C. Samorì, F. Guerrini, R. Pistocchi, Growth and nitrogen removal capacity of Desmodesmus communis and of a natural microalgae consortium in a batch culture system in view of urban wastewater treatment: Part I, Water Res., 47 (2013) 791-801.

[24] N. Upadhyay, Q. Sun, J.O. Allen, P. Westerhoff, P. Herckes, Synthetic musk emissions from wastewater aeration basins, Water Res., 45 (2011) 1071-1078.

[25] Y. Luo, W. Guo, H.H. Ngo, L.D. Nghiem, F.I. Hai, J. Zhang, S. Liang, X.C. Wang, A review on the occurrence of micropollutants in the aquatic environment and their fate and removal during wastewater treatment, Sci. Total Environ., 473-474 (2014) 619-641.

[26] S.R. Subashchandrabose, B. Ramakrishnan, M. Megharaj, K. Venkateswarlu, R. Naidu, Consortia of cyanobacteria/microalgae and bacteria: Biotechnological potential, Biotechnol. Adv., 29 (2011) 896-907.

[27] X. Zhang, P. Amendola, J.C. Hewson, M. Sommerfeld, Q. Hu, Influence of growth phase on harvesting of Chlorella zofingiensis by dissolved air flotation, Bioresour. Technol., 116 (2012) 477-484.

[28] D.Q. Zhang, T. Hua, R.M. Gersberg, J. Zhu, W.J. Ng, S.K. Tan, Fate of caffeine in mesocosms wetland planted with Scirpus validus, Chemosphere, 90 (2013) 1568-1572.

[29] V. Matamoros, L.X. Nguyen, C.A. Arias, V. Salvadó, H. Brix, Evaluation of aquatic plants for removing polar microcontaminants: A microcosm experiment, Chemosphere, 88 (2012) 1257-1264.

[30] J. Meyer, K. Bester, Organophosphate flame retardants and plasticisers in wastewater treatment plants, J. Environ. Monit., 6 (2004) 599-605.

[31] V. Matamoros, L. Sala, V. Salvadó, Evaluation of a biologically-based filtration water reclamation plant for removing emerging contaminants: A pilot plant study, Bioresour. Technol., 104 (2012) 243-249.

[32] M. Shenker, D. Harush, J. Ben-Ari, B. Chefetz, Uptake of carbamazepine by cucumber plants " A case study related to irrigation with reclaimed wastewater, Chemosphere, 82 (2011) 905-910.

[33] A.V. Dordio, M. Belo, D. Martins Teixeira, A.J. Palace Carvalho, C.M.B. Dias, Y. Picó, A.P. Pinto, Evaluation of carbamazepine uptake and metabolization by Typha spp., a plant with potential use in phytotreatment, Bioresour. Technol., 102 (2011) 7827-7834.

[34] A. Garcia-Rodríguez, V. Matamoros, C. Fontàs, V. Salvadó, The influence of Lemna sp. and Spirogyra sp. on the removal of pharmaceuticals and endocrine disruptors in treated wastewaters, Int. J. Environ. Stud. International, (2014) 1-12.

[35] L. Ferrando-Climent, N. Collado, G. Buttiglieri, M. Gros, I. Rodriguez-Roda, S. Rodriguez-Mozaz, D. Barceló, Comprehensive study of ibuprofen and its metabolites in activated sludge batch experiments and aquatic environment, Sci. Total Environ., 438 (2012) 404-413.

[36] C. Zwiener, F.H. Frimmel, Short-term tests with a pilot sewage plant and biofilm reactors for the biological degradation of the pharmaceutical compounds clofibric acid, ibuprofen, and diclofenac, Sci. Total Environ., 309 (2003) 201-211.

[37] D. Reinhold, S. Vishwanathan, J.J. Park, D. Oh, F. Michael Saunders, Assessment of plant-

driven removal of emerging organic pollutants by duckweed, Chemosphere, 80 (2010) 687-692.

[38] V. Matamoros, E. Jover, J.M. Bayona, Advances in the determination of degradation intermediates of personal care products in environmental matrixes: A review, Anal. Bioanal. Chem., 393 (2009) 847-860.

[39] J. Caldwell, Stereochemical determinants of the nature and consequences of drug metabolism, J. Chromatogr. A, 694 (1995) 39-48.

[40] A.R. Ribeiro, P.M.L. Castro, M.E. Tiritan, Chiral pharmaceuticals in the environment, Environ. Chem. Lett., 10 (2012) 239-253.

[41] N.H. Hashim, L.D. Nghiem, R.M. Stuetz, S.J. Khan, Enantiospecific fate of ibuprofen, ketoprofen and naproxen in a laboratory-scale membrane bioreactor, Water Res., 45 (2011) 6249-6258.

[42] H.-R. Buser, T. Poiger, M.D. Müller, Occurrence and environmental behavior of the chiral pharmaceutical drug ibuprofen in surface waters and in wastewater, Environ. Sci. Technol., 33 (1999) 2529-2535.

Figure Captions
a)
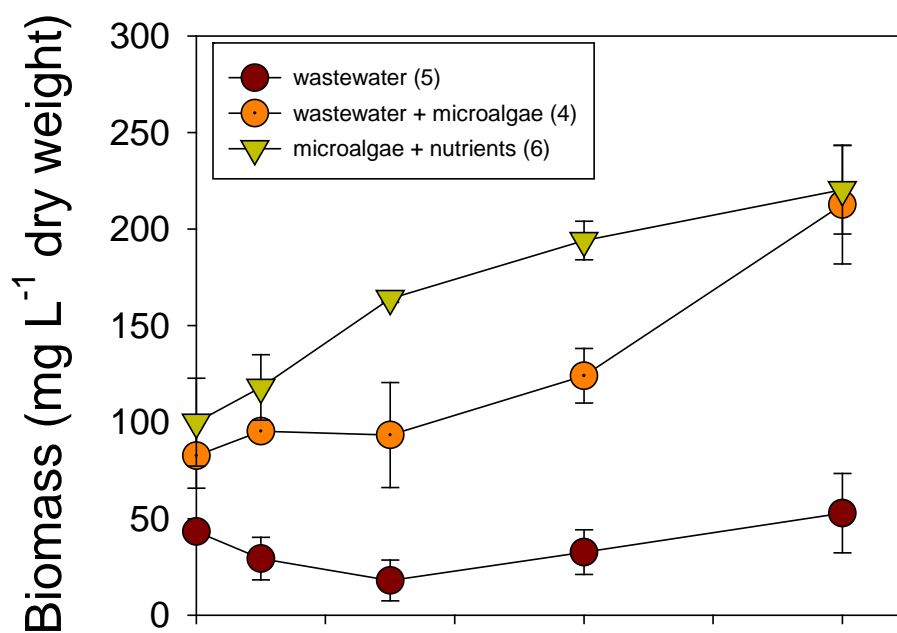
b)
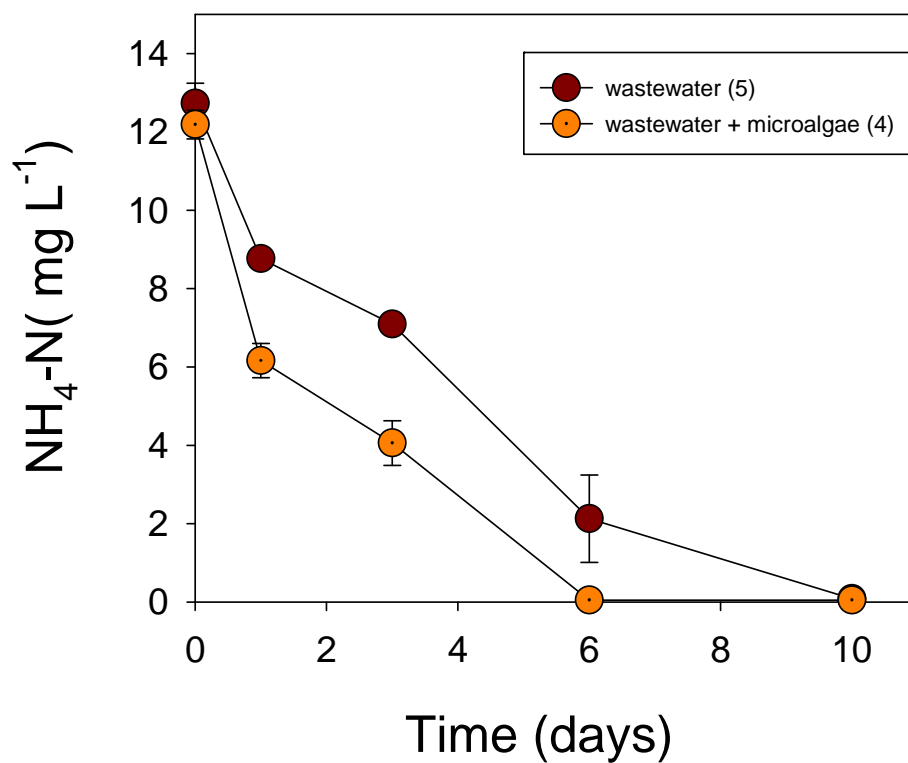
Figure 1. Algae biomass evolution (a) and NH$_4$-N concentration decline (b) over time in reactors fed with wastewater and nutrients.
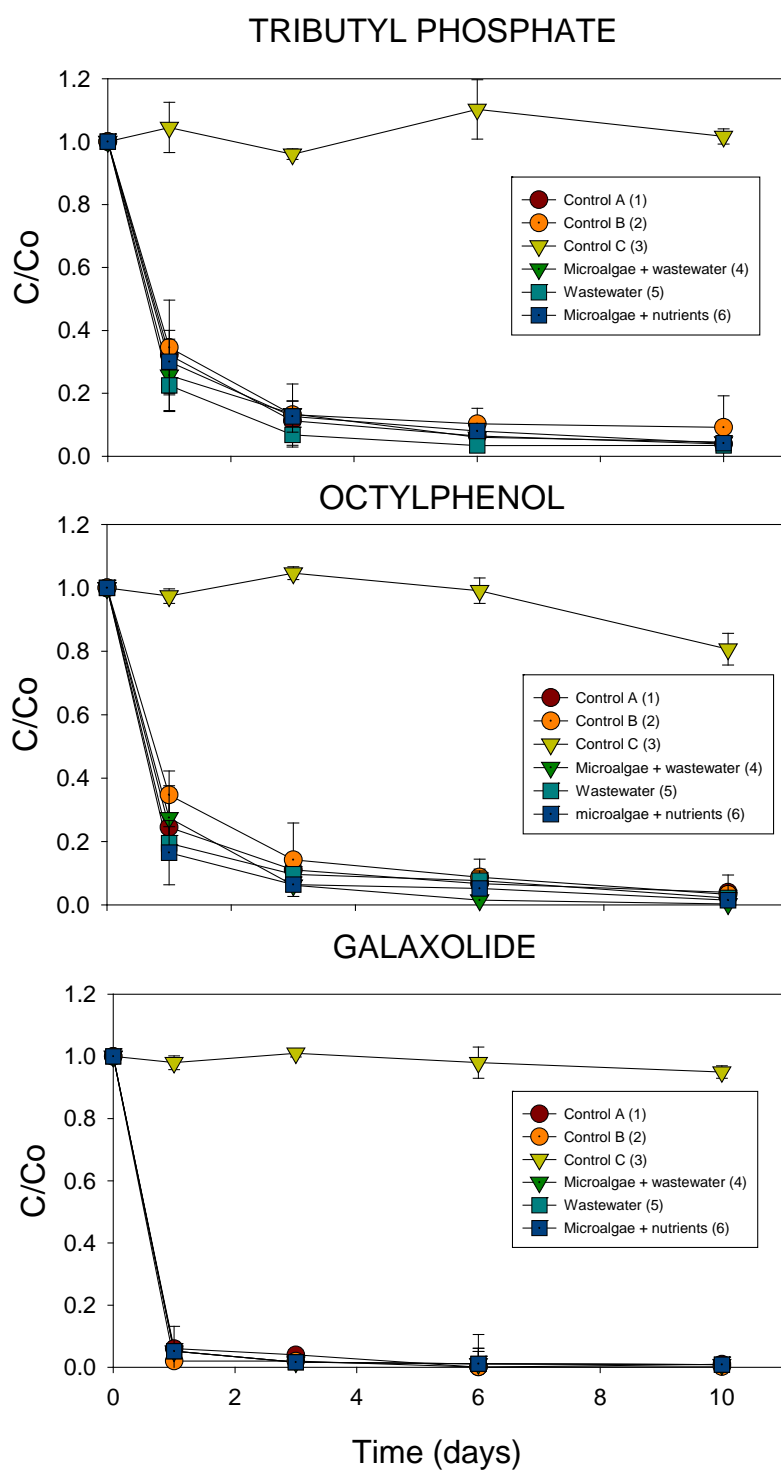
Figure 2. Response curve of the volatile compounds (tributyl phosphate, galaxolide and octylphenol) during the experimental period.
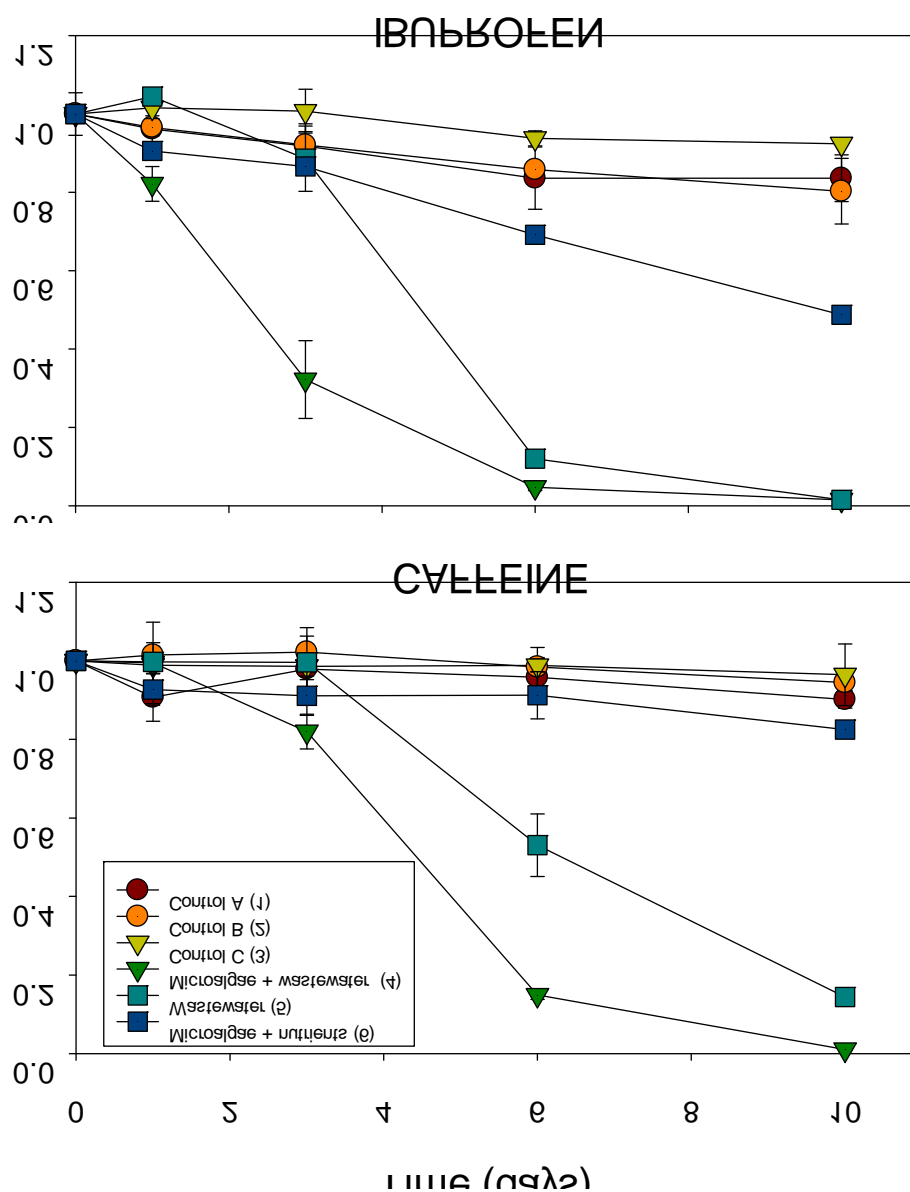
Figure 3. Response curves of the biodegradable compounds (ibuprofen and caffeine) during the experimental period. Notice that the same symbols also stand for the ibuprofen graph.
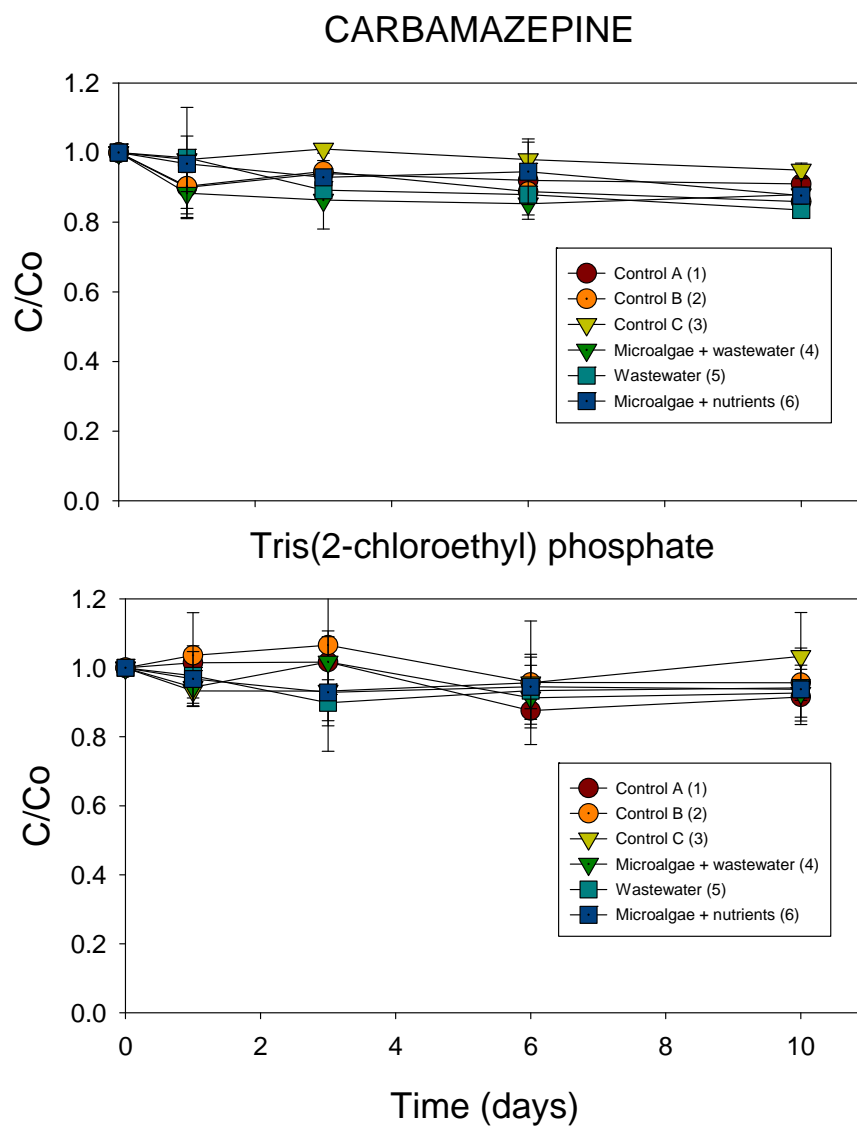
Figure 4. Response curves of the recalcitrant compounds (carbamazepine and tris(2-chloroethyl)phosphate) during the experimental period.
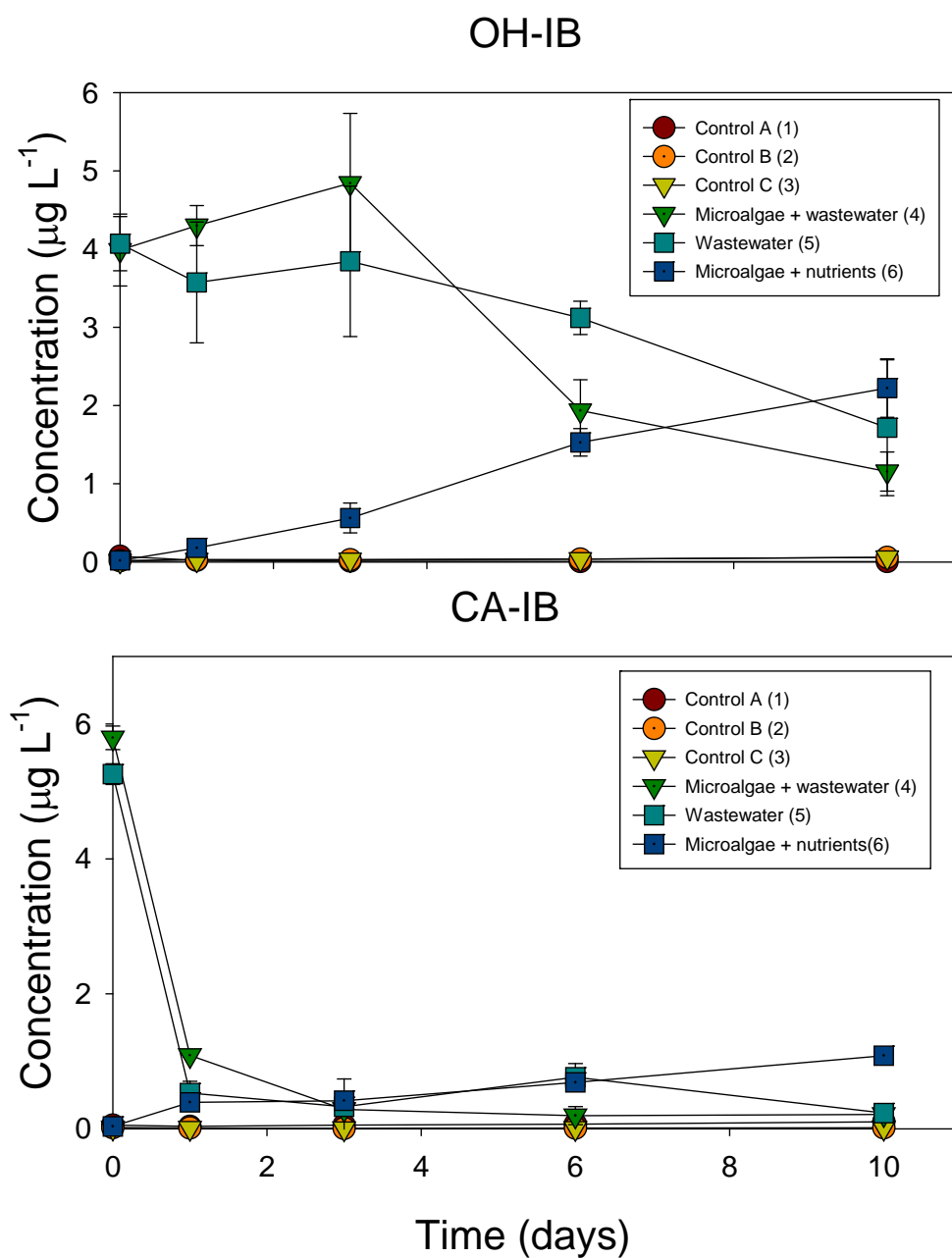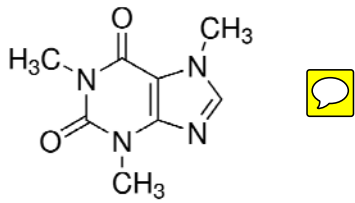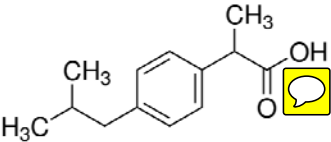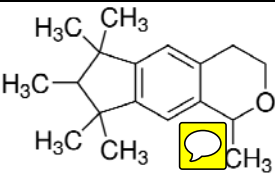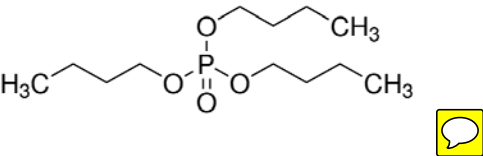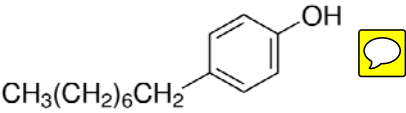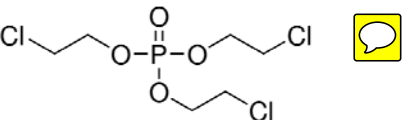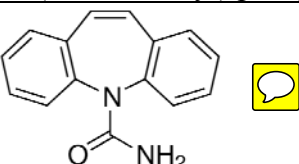
Figure 5. Evolution profiles of concentration of ibuprofen transformation products (CA-ibuprofen and OH-ibuprofen) during the experimental period.

a)

b)
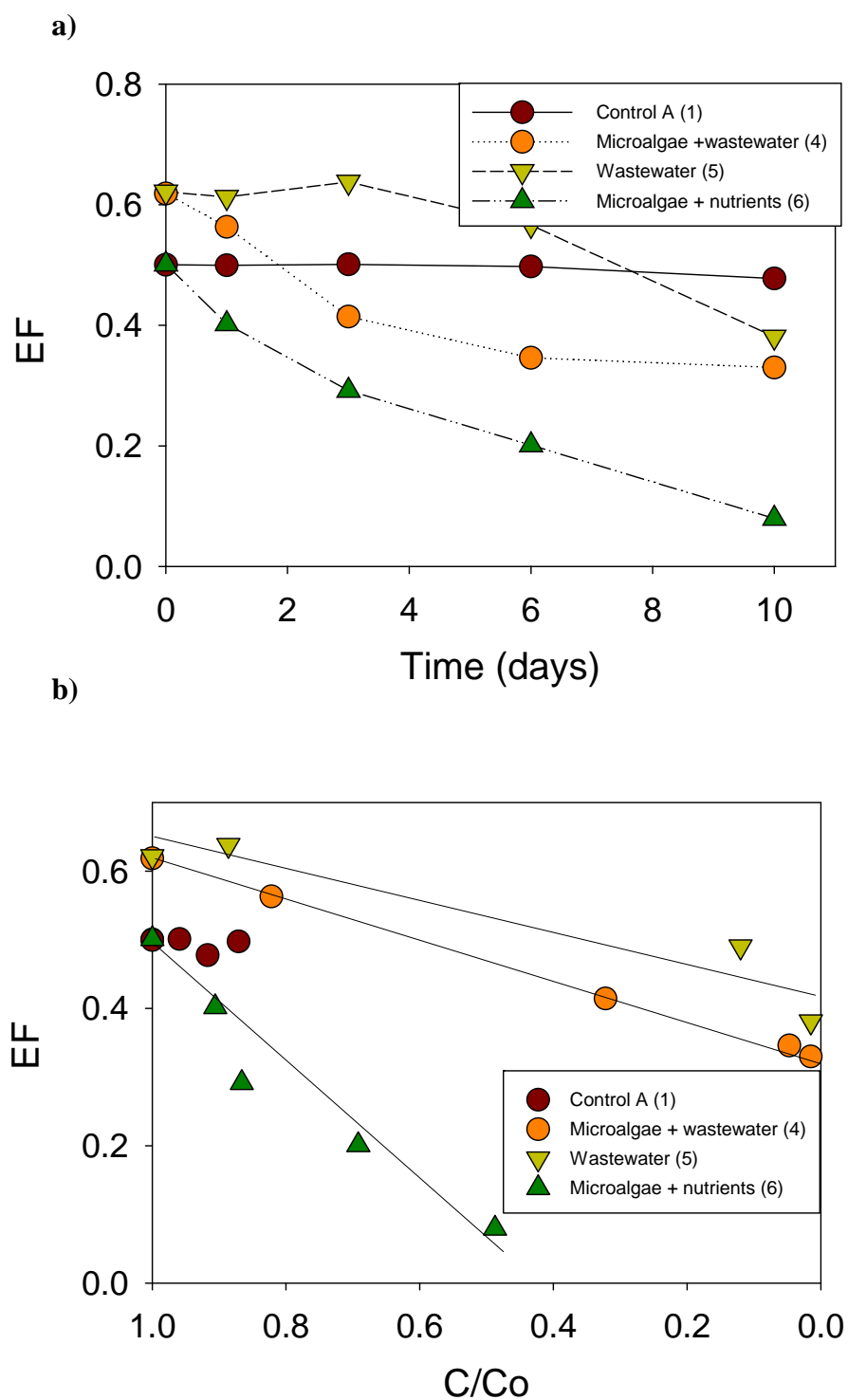
Figure 6. Enantiomeric fraction (EF) of ibuprofen in the different studied reactor sets during the experimental period (a) and its relationship with the concentration decline (C/Co) (b).

Tables

Table 1. Physico-chemical properties of the studied microcontaminants

Chemical structure/trade name	Log Kow	pKa	Henry's law constant (Pa m^3 mol^{-1}) *
caffeine	0.16	14	$3.63\ 10^{-6}$
ibuprofen	4.31	4.9	$1.54\ 10^{-2}$
galaxolide	6.26	-	$1.34\ 10^{1}$
tributyl phosphate	3.82	-	$3.23\ 10^{-1}$
4-octylphenol	5.28	10.4	$4.56\ 10^{-1}$
tris(2-chloroethyl) phosphate	1.63	-	$2.58\ 10^{-3}$
carbamazepine	2.25	13.9	$1.1\ 10^{-5}$

*Obtained from EPI suite software (bon estimation method)

Table 2. Experimental design indicating the water composition in each reactor set from 1 to 6.

Variables	1	2	3	4	5	6
Wastewater	-	-	-	+	+	-
Microalgae	-	-	-	+	-	+
Aeration	+	+	-	+	+	+
Light	+	-	-	+	+	+

Table 3. kinetic removal rate constants (in d^{-1}) and removal efficiency after 10 incubation days (in percentage, %) for compounds biodegradable compounds. Standard deviation is shown (n=3).

	Caffeine		Ibuprofen	
	k (d^{-1})	Removal (%)	k (d^{-1})	Removal (%)
Reactor set 1	0.006± 0.002	10±5	0.019±0.005	16±7
Reactor set 2	0.007±0.002	6±4	0.022±0.005	20±9
Reactor set 3	0.003±0.002	4±5	0.010±0.003	8±6
Reactor set 4	0.321±0.035	99±1	0.448±0.042	99±1
Reactor set 5	0.195±0.022	86±3	0.446±0.025	99±1
Reactor set 6	0.012±0.005	17±5	0.058±0.008	51±6