

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

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0. Abstract

Aerated batch reactors (2.5 L) fed either with urban wastewater or a nutrient supplemented solution were inoculated with microalgae (dominated by *Chlorella* sp. and *Scenedesmus* sp.) for the removal of 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 in the time course of the experiment. After 10 incubation days, up to 99% of the microcontaminants with a Henry's law constant higher than $3 \cdot 10^{-1}\text{ Pa m}^3\text{ mol}^{-1}$ (i.e. 4-octylphenol, galaxolide, and tributyl phosphate) were removed by volatilization due to the effect of air stripping. Whereas biodegradation was relevant for removing ibuprofen and caffeine, carbamazepine and tris(2-chloroethyl) phosphate behaved as recalcitrant. 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 predominant removal process, which was supported by the identification of carboxy-ibuprofen and hydroxy-ibuprofen. The results from microalgae reactors fed with nutrients 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 in the environment has already gained public awareness [1]. Emerging compounds are used in large quantities in our daily life 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 at relatively low concentrations [3, 4]. Of the better studied cases, 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 neural toxic in animal cell cultures [6], and 4-octylphenol has high potential estrogenic effects on amphibian *Xenopus laevis* [7]. As consequence, known environmental effects of the occurrence of emerging contaminants in surface waters are the reduction of macroinvertebrate diversity in rivers [3] and behavioural changes in mosquito fish [4]. Therefore, it is of high importance to reduce their discharge in to the aquatic environment.

Studies on the removal of these contaminants have mainly focused on conventional activated sludge WWTPs, but little attention has been paid on the effectiveness of natural engineered technologies such as microalgae-based wastewater treatment systems for removing emerging contaminants. Microalgae-based wastewater treatment technologies are relevant due to the resource recovery of algal biomass, for use as a fertilizer, as source of products or as biofuel, but they have also 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 studies have been 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 suggested that microalgae-based wastewater treatment systems may remove them by evaporation, photodegradation, biodegradation, or microalgae uptake [9-11], but little attention has been paid to the processes

involved in the removal of emerging contaminants 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 antibiotics such as tetracyclines. These results have been confirmed by our research studies in which HRAP pilot plants fed with real wastewater are capable of removing emerging contaminants up to 90%, 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 emerging contaminants in microalgae-based wastewater systems. To overcome this gap, laboratory-scale studies conducted under controlled environment conditions are mandatory.

Our aim in this study was to quantify the effect of microalgae activity on the removal efficiency of 7 emerging contaminants in a laboratory-scale study. The emerging contaminants were selected on the basis of their concentration, their high frequency of detection in urban wastewaters and their physicochemical properties (e.g. 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 are used to assess the processes involved in the removal of emerging contaminants.

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, a factorial experimental design was developed. The factors evaluated were microalgae (presence or absence), aeration (aerated and non-aerated), light-radiation (light vs. darkness) and water quality (wastewater vs. groundwater-nutrients). The factorial design gave us a total number of 16 possible combinations, from which only 6 were selected (table

2). Microalgae in darkness or without aeration were not included in the study. The total number of experiment was of 18, which corresponds to 6 different types of reactors, with 3 replicates per each type. All reactor systems consisted of containers of 2.5 L made of glass pre-cleaned. 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 $5 \mu\text{g L}^{-1}$ (200 μL of spiking solution at $100 \mu\text{g L}^{-1}$ for each compound in the methanol). Microalgae reactors were inoculated with a microalgae consortium obtained from an experimental high rate algal pond treating urban wastewater [14]. Main populations were made up by *Chlorella* sp. and *Scenedesmus* sp. Note that this inoculum also contained bacteria, however microalgae accounting for over 90% of the biomass as usually in HRAPs [15]. 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 quite similar organic composition found in the mixed liquor of the HRAP [16]. Wastewater used for experiments was a primary treated effluent with an average composition as follows: Total Suspended Solids (TSS), $100 \pm 3 \text{ mg L}^{-1}$, total Chemical Demand of Oxygen (COD), $241 \pm 73 \text{ mg L}^{-1}$, $\text{NH}_4\text{-N}$, $47 \pm 6 \text{ mg L}^{-1}$. The groundwater composition was as follows: TSS, 10 mg L^{-1} , $\text{NH}_4\text{-N}$, 0.2 mg L^{-1} , $\text{NO}_3\text{-N}$ 38 mg L^{-1} , $\text{PO}_4\text{-P}$ 3 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; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 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 $23 \pm 5 \text{ }^\circ\text{C}$ and supplied with light from fluorescent tubes at a photon flux density of $150 \mu\text{mol m}^{-2} \text{ s}^{-1}$ 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 correct for any water losses by evaporation, reactors were refilled with groundwater to a specific depth mark before each sampling (groundwater used in the experimental design was stored in the fridge at 4°C). All water samples were collected in 250 mL amber glass bottles, which were stored at 4 °C until analysis. The sample holding time was less than 12 hours.

2.3. Microalgae identification and determination of the growth rate

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]:

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

where $\text{OD}_{0,\lambda}$ is the OD at the initial day, $\text{OD}_{t,\lambda}$ 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 (racemic composition), 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 (\varnothing 47 mm) were obtained from Whatman (Maidstone, UK).

2.5. Analytical methodology

Conventional wastewater quality parameters, including ammonium nitrogen ($\text{NH}_4\text{-N}$) 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 $^{\circ}\text{C}$) 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 \times 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 were described elsewhere [19].

Enantiomeric composition of the all water samples was analysed as reported elsewhere [20].

Derivatized samples (TMSH) were injected at 280°C 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 \times 0.25 mm \times 0.12 μ m film thickness). Acquisition was performed in single-ion monitoring (SIM) mode at 2 scans s⁻¹. The following ions (m/z) were monitored for ibuprofen: **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 to 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 accounted to the presence of bacteria in the wastewater was calculated from the reactors 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:

$$\text{Volatilization} = (k_2 - k_3) / k_4 \times 100$$

Photodegradation = $(k_1 - k_2)/k_4 \times 100$

Direct biodegradation = $(k_5 - k_1)/k_4 \times 100$

Biodegradation enhancement by microalgae = $(k_4 - k_5)/k_4 \times 100$

Direct microalgae effect = $(k_6 - k_1)/k_4 \times 100$

Experimental results were statistically evaluated using the SPSS v.13.0 package (Chicago, IL, US). According with the data set size non-parametric statistics were applied for all statistical analysis. Mann-Whitney U test was applied to compare differences between experiments. Linear relationship between pairs of variables was performed using Spearman'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. maintained 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. Microalgae growth rate was 0.25 d^{-1} , which is quite low, but still in the range of values found in lab scale experiments treating urban wastewater ($0.2-1 \text{ d}^{-1}$) [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 with the microalgae *Desmodesmus communis* and fed with wastewater. pH ranged from 6.6 ± 0.3 at the beginning of the

experiment to 8.3 ± 0.1 at the end of the experiment in the microalgae reactors. Control reactors had a pH of 8.0 ± 0.7 along the whole experiment. ENRICA TIENES DATOS? TENGO ESTOS DATOS QUE HE PUESO, DE DONDE HABIAS COGITO LOS QUE ESTABAN ANTES?

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 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 $3.23 \cdot 10^{-1}$ to $1.34 \cdot 10^1 \text{ Pa m}^3 \text{ mol}^{-1}$. Galaxolide, which presented the highest Henry law constant (Table 1), was also the compound with the fastest concentration decay. Up to 14% of polycyclic musk fragrances such as galaxolide have been described to be removed by volatilization in conventional activated sludge WWTPs [24], so it is not unlikely that it may increase to 99% when aeration is forced as is the case in our study. 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 to remove 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, $3.63 \cdot 10^{-6} \text{ Pa m}^3 \text{ mol}^{-1}$, carbamazepine, $1.1 \cdot 10^{-5} \text{ Pa m}^3 \text{ mol}^{-1}$ and tris(2-chloroethyl) phosphate, $2.58 \cdot 10^{-3} \text{ Pa m}^3 \text{ mol}^{-1}$).

3.2.2. Biodegradable compounds

Fig. 3 shows that the decrease of ibuprofen and caffeine concentrations from the water was dependent 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%) than when this compound was incubated 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. In fact, it has already been proved 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 ($\text{pK}_a = 4.9$) and the electrochemical negative charge of the microalgae cell walls [27], ibuprofen uptake can be considered to be negligible at the working pH ($\text{pH} = 7-9$). Caffeine, which is neutral ($\text{pK}_a = 14$) 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 increases dramatically the removal of caffeine and ibuprofen. Furthermore, the degradation experiments fed with wastewater revealed a lag phase of around 3 days for ibuprofen concentration decay, but with the use of microalgae, this adaptation time was reduced. Therefore, the results showed that microalgae reduce the adaptation time required by bacteria to remove ibuprofen while enhance the biodegradation removal rate of caffeine, either by chemical exudates or oxygen release. In fact, this 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 onto 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 *Thypha* sp. [32, 33], from our results, unicellular microalgae seem not to be capable of carbamazepine uptake.

Overall these 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 Spearman correlation coefficients higher than 0.90 (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. Ibuprofen removal rates obtained in this study by using microalgae and wastewater ($0.448 \pm 0.042 \text{ d}^{-1}$) were higher than those from previous studies carried out by using secondary-treated wastewater effluents and aquatic plants ($0.109 \pm 0.008 \text{ d}^{-1}$ in reactors planted with *Lemna sp.*), but similar for caffeine ($0.321 \pm 0.035 \text{ d}^{-1}$ in this study vs. $0.300 \pm 0.01 \text{ d}^{-1}$) [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 $0.310 \pm 0.035 \text{ d}^{-1}$ respectively, but no concentrations decline 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 concentration declines were observed for carbamazepine and tris(2-chloroethyl) phosphate 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 $\mu\text{g L}^{-1}$ [35]. The reactors fed with 25% wastewater presented concentrations around 4 and 6 $\mu\text{g L}^{-1}$ of OH-IB and CA-IB respective, 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 produce CA-IB.

The predominance of OH-IB over CA-IB indicates that biodegradation occurs under prevailing aerobic conditions, which agree with other studies [29, 36, 37] and the high oxygen concentration in the reactors (7-9 mg L^{-1}) 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. 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%), neither because the compounds were not photodegradable (ibuprofen, caffeine, carbamazepine and tris(2-chloroethyl) phosphate) or because the effect of the aeration (air stripping) was as high as that of photodegradation, so no net result was detected (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 it 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 undetectable time period. No direct effect of microalgae was observed for any of the studied compounds (<10%). In comparison with other 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 $3 \cdot 10^{-1} \text{ Pa m}^3 \text{ mol}^{-1}$. 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:

$$EF = \frac{S\text{-enantiomer}}{S\text{-enantiomer} + R\text{-enantiomer}}$$

Racemic ibuprofen composition (EF=0.5) was used to spike 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, reactors fed with both nutrients and microalgae showed the highest decline ratio, from 0.50 to 0.07, whereas 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. Other studies have evidenced 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 may probably be explained on the supposition that enzymatic exudates released by microalgae are

driving the ibuprofen biodegradation. Further studies will be needed to conclusively demonstrate that.

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 $3 \cdot 10^{-1} \text{ Pa m}^3 \text{ mol}^{-1}$, 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 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.

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