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Comparative Assessment of Pharmaceutical Removal from Wastewater by the Microalgae *Chlorella sorokiniana*, *Chlorella vulgaris* and *Scenedesmus obliquus*

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Abstract

In view of risks associated with the discharge of pharmaceuticals in the aquatic environment, the objective of this work was to assess the removal of paracetamol, salicylic acid and diclofenac from water by a microalgae-based treatment. For a comparison purpose, the growth and kinetic parameters for the removal of drugs were determined for three different microalgae strains, namely *Chlorella sorokiniana*, *Chlorella vulgaris* and *Scenedesmus obliquus*. It was found that the drugs removal efficiency by these strains was related to their growth. Comparing the three pharmaceuticals, the salicylic acid was the most efficiently removed, especially by *S. obliquus* (>93% batch culture, >99% semicontinuous culture) and *C. sorokiniana* (>73% batch culture, >93% semicontinuous culture). Contrarily, paracetamol was the most poorly removed, the maximum efficiencies being those attained by *C. sorokiniana* (>67% batch culture, >41% semicontinuous culture). On the other hand, diclofenac was efficiently removed only by *S. obliquus* (>98% batch culture, >79% semicontinuous culture). For the three considered drugs, *C. vulgaris* was the strain showing the lowest removal capacity. The large differences here revealed between microalgae strains regarding their removal capacity of pharmaceuticals, pointed to the strain selection as a key issue for a successful application in wastewater treatment.

Keywords: emerging contaminants, wastewater treatment, phytoremediation, paracetamol, salicylic acid, diclofenac

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1. Introduction

Emerging contaminants (ECs) include a wide range of compounds and may be defined as naturally occurring, manufactured or man-made chemicals or materials that have been found or are suspected to be present in various environmental compartments and whose toxicity or persistence are likely to significantly alter the metabolism of a living being [1]. Among them, pharmaceuticals have received considerable attention with respect to their environmental fate and toxicological properties over the last decade [2]. Pharmaceuticals represent an especially worrying class since they were designed to cause a physiological response and their presence in the environment may affect non-target individuals and species [3]. This concern on pharmaceuticals presence in the aquatic environment has led to the recent consideration by European regulations within the Water Framework Directive (2000/60/EC) (WFD). The Commission proposal of 31 January 2012 foresaw the inclusion of three pharmaceuticals, namely diclofenac, 17-beta-estradiol (E2) and 17-alpha-ethinylestradiol (EE2) in the list of priority substances. Instead, by the EU Decision 2015/495, these compounds together with another estrogen (E1) and three antibiotics (azithromycin, clarithromycin and erythromycin) were finally included in the first watch list of substances to be monitored in all member states to support future reviews of the priority substances list [4].

Pharmaceuticals in domestic sewage or from hospital or industrial discharges end in municipal sewage treatment plants (STPs), but conventional wastewater treatments have been reported to be ineffective in the removal of such pollutants, with efficiency values of <5 to 40% [5]. In fact, STPs were not originally designed for the removal of pharmaceuticals due to the non-existence of limiting regulations on their discharge [6, 7]. Consequently, STPs are important sources of such pollutants in the aquatic environment [8, 9]. In this regard, Verlicchi et al. [10], who reviewed the occurrence of 118 pharmaceuticals in the influent and effluent of 244 STPs, found that the occurrence of some of them in the effluent discharged into surface water bodies may pose a medium-high (acute) risk to aquatic life. Among the studied pharmaceuticals, diclofenac was shown to have the highest average mass load (240 mg/1000 inhabitant) in the effluents of municipal STPs [10]. The removal efficiencies of diclofenac in conventional STPs have been reported to be about 17% [11], which translates into relative high concentrations in the corresponding effluents [12].

In the recent years, phytoremediation of waters by using photoautotrophic aquatic organisms such as algae has gained attention for the removal of both organic and inorganic pollutants [13–15]. Microalgae are characterized by high photosynthetic efficiency, high growth rates, wide adaptability and high potential to remove inorganic nutrients from the wastewater. The principal mechanism of algal nutrient removal is their uptake into the cell biomass [16]. The main advantages of using microalgae for nutrients removal during the tertiary treatment of wastewaters are the possibility of recycling the assimilated nitrogen and phosphorus into algal biomass as a fertilizer, as a source of products (e.g. paraffin, olefin, glycerol, protein, anti-oxidant, pigment, plastic, etc.), or as biofuel, and also the generation of an oxygenated high-quality effluent [17]. However, although the capability of microalgae wastewater treatments systems to remove organic matter and nutrients has been deeply studied, little is known about the removal of ECs, such as pharmaceuticals, by algae. In fact,

it has already been claimed the necessity of further studies on the removal of this sort of pollutants by algal systems [18].

In this context, the aim of this study was to determine and compare the potential of green microalgae *Chlorella sorokiniana*, *Chlorella vulgaris* and *Scenedesmus obliquus* to remove paracetamol, salicylic acid and diclofenac from water. The strains used in this work were selected since they are known to have fast growth rates and potential for wastewater treatment due to their tolerance to the severe environmental conditions found in municipal wastewater and some industrial wastewaters [19].

2. Materials and methods

2.1. Microorganisms and culture conditions

The microalgae strains used in this study were *C. sorokiniana* CCAP 211/8 K from UTEX Culture Collection of Algae, *C. vulgaris* SAG 221-12 from SAG Culture Collection of Algae and *S. obliquus* SAG 276-1 from SAG Culture Collection of Algae. These microalgae strains are among the most commonly used for wastewater treatment have high growth rates and are able to grow under a wide range of conditions [19], which motivated their choice for this study.

The inoculum of each strain was cultivated in 250-ml Erlenmeyer flasks in the standard culture medium Mann and Myers [20], which is composed of (per litre of distilled water): 1.2 g $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 1.0 g NaNO_3 , 0.3 CaCl_2 , 0.1 g K_2HPO_4 , 3.0×10^{-2} g Na_2EDTA , 6.0×10^{-3} g H_3BO_3 , 2.0×10^{-3} g $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, 1.4×10^{-3} g MnCl_2 , 3.3×10^{-4} g $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, 7.0×10^{-6} g $\text{Co}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$, 2.0×10^{-6} g $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$. The inoculum was kept inside a vegetal culture chamber, where growth occurred under controlled temperature ($25 \pm 1^\circ\text{C}$), irradiance in the range of photosynthetically active radiation ($175 \mu\text{E m}^{-2} \text{s}^{-1}$), photoperiod (12:12) and shaking (250 rpm).

Bubbling column photobioreactors (PBRs) with spherical bases (40 mm diameter and 300 mm height with 300 ml capacity) were used for the experimental setup, keeping an operating volume of 250 ml. In each PBR, the Mann and Myers culture medium was inoculated with the required volume of the corresponding pre-cultured microalgae in order to have an initial concentration of about 3×10^6 cells ml^{-1} .

During the experimental phase, the culture was aerated with filtered air (0.22- μm sterile air-venting filter, MillexFG50-Millipore), at a rate of 0.3 v/v/min, enriched with CO_2 at 7% v/v, which was injected on demand to keep a constant pH ($\text{pH} = 7.5 \pm 0.5$), as controlled by a pH sensor. The irradiance supplied during this phase was $370 \mu\text{E m}^{-2} \text{s}^{-1}$, which was provided by eight fluorescent lamps (58 W, 2150 lumen, Philips, France). The photoperiod was maintained in 12:12 h light/dark and the temperature in $25 \pm 1^\circ\text{C}$.

2.2. Experimental setup

PBRs were operated in batch mode until the end of the exponential growth phase and then under semicontinuous mode till the growth parameters remained constant at the steady state.

During the batch culture, an aliquot of 5 ml was daily taken from each PBR for the analytical determinations, this volume being replaced with distilled water to keep the operation volume. During the semicontinuous culture, 30% of the culture volume was daily harvested and used for analysis, this volume being replaced with fresh medium.

For each strain of microalgae used in this work (*C. sorokiniana*, *C. vulgaris* and *S. obliquus*), three treatments were conducted: (i) a treatment with inoculated culture medium and 25 mg l⁻¹ paracetamol (with *C. sorokiniana* PCS, *C. vulgaris* PCV, *S. obliquus* PSO), (ii) a treatment with inoculated culture medium and 25 mg l⁻¹ salicylic acid (with *C. sorokiniana* SCS, *C. vulgaris* SCV, *S. obliquus* SSO) and (iii) a treatment with inoculated culture medium and 25 mg l⁻¹ diclofenac (with *C. sorokiniana* DCS, *C. vulgaris* DCV, *S. obliquus* DSO). Also, the corresponding positive controls with inoculated culture medium (with *C. sorokiniana* CCS+, *C. vulgaris* CCV+ and *S. obliquus* CSO+) were run. The negative controls consisted of 25 mg l⁻¹ paracetamol (CP-), salicylic acid (CS-) or diclofenac (CD-) in culture medium with no microalgae. For each strain, experiments were run in triplicate and under identical conditions in all the PBRs. Paracetamol (C₈H₉NO₂, ≥99%) was supplied by Roic Pharma, salicylic acid (C₇H₆O₃, ≥99%) by Panreac and diclofenac (C₁₄H₁₀C₁₂NNaO₂, ≥99%) by Sigma-Aldrich.

Throughout the experiments, the growth of the culture was daily monitored by the determination of biomass concentration and cell density. The removal of pharmaceuticals was daily determined by the analysis of the remaining concentration of this drug in the culture medium. All analyses were conducted in triplicate.

2.3. Analytical methods

Biomass concentration (Cb) was determined by optical density at 680 nm (OD₆₈₀) by spectrophotometric (UV/visible spectrophotometer BECKMAN DU640) and verified by dry weight. Preliminary studies were conducted to determinate the relationship between dry weight and OD₆₈₀ for each strain; as shown in Eq. (1) for *C. sorokiniana*, in Eq. (2) for *C. vulgaris* and in Eq. (3) for *S. obliquus*:

$$OD_{C.S.680} = 5.1834 \times C_b + 0.0128, R^2 = 0.9983 \quad (1)$$

$$OD_{C.V.680} = 2.7933 \times C_b + 0.0317, R^2 = 0.9958 \quad (2)$$

$$OD_{S.O.680} = 2.0098 \times C_b + 0.0451, R^2 = 0.9915 \quad (3)$$

Dry weight measurements were performed by filtering 10 ml of culture through a 0.45 µm Whatman filter, which was then washed with 20 ml HCl (0.5 M) to dissolve precipitated salts. Then, the filtrate was dried in an oven at 80°C for 24 h. Additionally, the growth of the culture was measured as cell density (Nc) by cell counting with a Neubauer chamber.

The initial and remaining pharmaceuticals concentration in the culture medium was quantified by a Waters HPLC 600 equipped with a 2487 Dual λ Absorbance Detector. A Phenomenex Gemini-NX C18 column (5 μm , 250 mm $\times$ 4.6 mm) was used for the separation. The wavelengths of detection were 246 nm for paracetamol, 236 nm for salicylic acid and 276 nm for diclofenac. The mobile phase consisted of a mixture of acetonitrile to water (30:70, v/v) for the analysis of paracetamol and a mixture of acetonitrile to water to orthophosphoric acid (70:30:0.1, v/v/v) for salicylic acid and diclofenac. HPLC quality acetonitrile (CH_3CN) and orthophosphoric acid (H_3PO_4) from Prolabo Chemicals and ultrapure water obtained by a Millipore System were used for the preparation of the mobile phase. Before use, each mixture was passed through a Millipore 0.45- μm pore-size filter and degasified in an ultrasound bath for 30 min. Before analysis, all the samples were centrifuged twice at 7500 rpm for 10 min (SIGMA 2-16P centrifuge). For the chromatographic analysis, the mobile phase flow rate was 1 ml min⁻¹ and the injection volume was 100 μl .

2.4. Data analysis

Growth kinetics were resolved in OriginPro 8 using the classic model originally described by Verhulst [21] called logistic model, which has been proved to fit the growth of microalgae [22]. The logistic model fits to a sigmoidal curve that describes the relationship between microorganisms' growth and density in limited environmental conditions (Eq. (4)).

$$N = \frac{K}{1 + e^{a-rt}} \quad (4)$$

Where N (g l⁻¹) is the algal density at time t (h), K (g l⁻¹) is the carrying capacity (the maximum algal density reached in the culture), a is a constant in the logistic model that refers to the relative position from the origin and indicates the duration of the lag phase and r (d⁻¹) is the specific growth rate.

Furthermore, the kinetic curves for the removal of pharmaceuticals were fitted to the logistic model. In each case, the parameter K (g l⁻¹) is the maximum removal capacity by the microalgae in the culture. The parameter a is a constant in the logistic model that indicates the delay in the beginning of the target compounds removal and the parameter r (d⁻¹) is the specific removal rate.

Finally, differences among the strains with respect to the kinetic parameters of growth and removal of pharmaceuticals were compared by a non-parametric test using IBM SPSS Statistics 21. The comparison of means was performed by means of the U Mann-Whitney test. Significance was defined at $p \leq 0.05$.

For the removal of pharmaceuticals, the volumetric efficiency for each target compound was calculated as the difference between its average concentration in the influent (C_{inf}) and in the effluent (C_{efflu}) at every sampling day, considering the daily dilution rate of the corresponding operation stage (D) (Eq. (5)). During the batch culture these efficiencies were

cumulatively expressed as milligram per litre and as milligram per litre per day during the steady state of the semicontinuous culture:

$$\text{Volumetric efficiency} = (C_{\text{inf}} - C_{\text{efflu}}) \times D \quad (5)$$

The specific efficiency of the removed pharmaceuticals was calculated as the ratio between the volumetric efficiency and the biomass concentration (C_b) (Eq. (6)). Likewise, during the batch culture these efficiencies were cumulatively expressed as milligram per gram per biomass and as milligram per gram day during the steady state of the semicontinuous culture:

$$\text{Specific efficiency} = \frac{(C_{\text{inf}} - C_{\text{efflu}}) \times D}{C_b} \quad (6)$$

3. Results

3.1. Growth of the culture

The growth curves of *C. sorokiniana*, *C. vulgaris* and *S. obliquus* during the batch culture, of either the treatments or the controls, showed a typical sigmoidal growth of 8–10 days until reaching the steady state. On the other hand, during the semicontinuous mode, daily dilution rates produced instability and the growth rate declined throughout several days until the growth parameters remained constant during the steady state. This instability is a typical behaviour in the microalgae culture when the growth conditions change and it is related with an adaptation phase (**Figures 1–3**).

Figure 1. Growth curves of *C. sorokiniana* (CCS+ ●, PCS ○), *C. vulgaris* (CCV+ ■, PCV □) and *S. obliquus* (CSO+ ▲, PSO △) for the paracetamol treatments. Dots correspond to experimental data and continuous lines correspond to fittings by the logistic kinetic model during batch culture. Experiments were performed in triplicate and bars show standard derivations. Note: experimental points obtained during semicontinuous culture are connected with dashed lines.

Figure 2. Growth curves of *C. sorokiniana* (CCS+, ●; SCS, ○), *C. vulgaris* (CCV+, ■; SCV, □) and *S. obliquus* (CSO+, ▲; SSO, △) for the salicylic acid treatments. Dots correspond to experimental data and continuous lines correspond to fittings by the logistic kinetic model during batch culture. Experiments were performed in triplicate and bars show standard derivations. Note: experimental points obtained during semicontinuous culture are connected with dashed lines.

Figure 3. Growth curves of *C. sorokiniana* (CCS+, ●; DCS, ○), *C. vulgaris* (CCV+, ■; DCV, □) and *S. obliquus* (CSO+, ▲; DSO, △) for the diclofenac treatments. Dots correspond to experimental data and continuous lines correspond to fittings by the logistic kinetic model during batch culture. Experiments were performed in triplicate and bars show standard derivations. Note: experimental points obtained during semicontinuous culture are connected with dashed lines.

3.1.1. Growth of the culture under paracetamol addition

The microalgae growth curves of *C. sorokiniana*, *C. vulgaris* and *S. obliquus* under the presence of paracetamol, the corresponding positive controls and their respective fittings to the logistic kinetic model are represented as values of biomass concentration versus time in **Figure 1**. The

differences among the treatments were analysed according to growth kinetic parameters, as shown in **Table 1**.

	CCS+	PCS	CCV+	PCV	CSO+	PSO
Cb_0 (g l ⁻¹)	0.04	0.04	0.11	0.11	0.08	0.08
Nc_0 (cell ml ⁻¹)	3.20×10^6	3.20×10^6	1.21×10^6	1.21×10^6	8.35×10^5	8.35×10^5
Cb_m (g l ⁻¹)	1.41 ± 0.29	2.05 ± 0.03	2.48 ± 0.11	3.35 ± 0.08	3.33 ± 0.06	3.09 ± 0.20
Nc_m (cell ml ⁻¹)	$2.12 \times 10^8 \pm 0.49 \times 10^8$	$4.20 \times 10^8 \pm 0.22 \times 10^8$	$1.18 \times 10^8 \pm 0.09 \times 10^8$	$2.17 \times 10^8 \pm 0.20 \times 10^8$	$4.77 \times 10^7 \pm 0.01 \times 10^7$	$4.62 \times 10^7 \pm 0.20 \times 10^7$
a	3.77 ± 0.01	4.33 ± 0.21	4.47 ± 0.25	5.58 ± 0.03	5.45 ± 0.43	4.97 ± 0.31
K (g l ⁻¹)	1.40 ± 0.29	2.09 ± 0.02	2.60 ± 0.15	3.42 ± 0.15	3.46 ± 0.08	3.27 ± 0.15
r (d ⁻¹)	0.94 ± 0.06	0.96 ± 0.07	0.84 ± 0.06	1.08 ± 0.04	1.16 ± 0.07	1.12 ± 0.03
R^2	0.9935	0.9939	0.9971	0.9968	0.9874	0.9886

Cb_0 , initial biomass; Nc_0 , initial number of cells; Cb_m , maximum biomass; Nc_m , maximum number of cells; K , carrying capacity; a , constant of logistic kinetic model; r , microalgae growth rate; R^2 , correlation coefficient.

Table 1. Experimental data (Cb_0 , Nc_0 , Cb_m , Nc_m) and logistic model kinetic parameters (K , a , r) determined for the growth of positive controls and treatments with paracetamol of *C. sorokiniana* (CCS+, PCS), *C. vulgaris* (CCV+, PCV) and *S. obliquus* (CSO+, PSO), $n = 3$.

The addition of paracetamol increased the lag phase of the strains of the genus *Chlorella* compared with the positive controls ($p \leq 0.05$), as it can be seen for the values of the parameter a in **Table 1**. However, in the case of *S. obliquus*, the addition of the drug did not modify the beginning of the exponential growth phase compared with the positive control ($p > 0.05$). Furthermore, there were significant differences among the treatments with paracetamol, *C. vulgaris* showed a quite longer lag phase than *S. obliquus* and this one than *C. sorokiniana*.

At the end of the batch culture, the biomass concentration was increased above 49% by the presence of paracetamol in the *C. sorokiniana* culture (CCS+, 1.40 ± 0.29 g l⁻¹; PCS, 2.09 ± 0.02 g l⁻¹) and was increased above 31% in the *C. vulgaris* culture (CCV+, 2.60 ± 0.15 g l⁻¹; PCV, 3.42 ± 0.15 g l⁻¹) compared with their positive control, as shown in **Figure 1** and confirmed by K values in **Table 1**. However, *S. obliquus* culture was not significantly modified by the addition of the drug and the maximum algal density reached in the treatment (PSO, 3.27 ± 0.15 g l⁻¹) was similar to the positive control (CSO+, 3.46 ± 0.08 g l⁻¹). In spite of the different response of the strains to the presence of paracetamol, there were not significant differences between PCV and PSO, even though the value reached for the parameter K in the case of CSO+ was significantly larger than for CCV+. Still, the carrying capacity of the PCS treatment was significantly lower than for PCV and PSO.

Respect to microalgae growth rate (r), there was significant differences between the paracetamol treatment for *C. vulgaris* (PCV, 1.08 ± 0.04 d⁻¹) and the corresponding positive control (CCV+, 0.84 ± 0.06 d⁻¹). However, likewise the K parameter, the growth rate was neither modified in the case of *S. obliquus* treatment (PSO, 1.12 ± 0.03 d⁻¹) compared with the corresponding positive control (CSO+, 1.16 ± 0.07 d⁻¹). Also, no significant differences were detected in the case of *C. sorokiniana* (CCS+, 0.94 ± 0.06 d⁻¹; PCS, 0.96 ± 0.07 d⁻¹). In

addition, when comparing the treatments of the three strains, there were not significant differences between the paracetamol treatments of *C. vulgaris* and *S. obliquus* strains (PCV, PSO) despite there were significant differences between their respective positive controls (CCV+, CSO+).

3.1.2. Growth of the culture under salicylic acid addition

The microalgae growth curves of *C. sorokiniana*, *C. vulgaris* and *S. obliquus* under the presence of salicylic acid, the corresponding positive controls and their fittings to the logistic kinetic model, are represented as values of biomass concentration versus time in **Figure 2**. The differences among the treatments were analysed according to growth kinetic parameters, as shown in **Table 2**.

	CCS+	SCS	CCV+	SCV	CSO+	SSO
Cb_0 (g l ⁻¹)	0.04	0.04	0.11	0.11	0.08	0.08
Nc_0 (cell ml ⁻¹)	3.20×10^6	3.20×10^6	1.21×10^6	1.21×10^6	8.35×10^5	8.35×10^5
Cb_m (g l ⁻¹)	1.41 ± 0.29	2.05 ± 0.15	2.48 ± 0.11	3.02 ± 0.27	3.33 ± 0.06	4.33 ± 0.30
Nc_m (cell ml ⁻¹)	$2.12 \times 10^8 \pm 0.49 \times 10^8$	$3.15 \times 10^8 \pm 0.08 \times 10^8$	$1.18 \times 10^8 \pm 0.09 \times 10^8$	$1.76 \times 10^8 \pm 0.49 \times 10^8$	$4.77 \times 10^7 \pm 0.01 \times 10^7$	$6.97 \times 10^7 \pm 0.20 \times 10^7$
a	3.77 ± 0.01	4.16 ± 0.48	4.47 ± 0.25	7.99 ± 0.41	5.45 ± 0.43	4.20 ± 0.09
K (g l ⁻¹)	1.40 ± 0.29	2.14 ± 0.13	2.60 ± 0.15	3.09 ± 0.23	3.46 ± 0.08	4.71 ± 0.30
r (d ⁻¹)	0.94 ± 0.06	0.77 ± 0.12	0.84 ± 0.06	1.69 ± 0.13	1.16 ± 0.07	0.72 ± 0.01
R^2	0.9935	0.9912	0.9971	0.9929	0.9874	0.9883

Cb_0 , initial biomass; Nc_0 , initial number of cells; Cb_m , maximum biomass; Nc_m , maximum number of cells; K , carrying capacity; a , constant of logistic kinetic model; r , microalgae growth rate; R^2 , correlation coefficient.

Table 2. Experimental data (Cb_0 , Nc_0 , Cb_m , Nc_m) and logistic model kinetic parameters (K , a , r) determined for the growth of positive controls and treatments with salicylic acid of *C. sorokiniana* (CCS+, SCS), *C. vulgaris* (CCV+, SCV) and *S. obliquus* (CSO+, SSO), $n=3$.

Regarding the parameter a , the addition of salicylic acid increased significantly the lag phase of the strains *C. vulgaris* and *S. obliquus* compared with the positive controls. Also, *C. sorokiniana* treatment showed a higher a value than the positive control, in spite of the difference being not significant (**Table 2**). Comparing the treatments with salicylic acid, *C. vulgaris* showed a quite longer lag phase than *C. sorokiniana* and *S. obliquus*.

As it can be seen in **Figure 2**, the maximum algal density reached at the end of the batch culture was significantly higher in the treatments with salicylic acid for all strains here considered as compared with the positive controls. The *C. sorokiniana* treatment increased their biomass concentration above 52% (CCS+, 1.40 ± 0.29 g l⁻¹; SCS, 2.14 ± 0.13 g l⁻¹), *C. vulgaris* above 18% (CCV+, 2.60 ± 0.15 g l⁻¹; SCS, 3.09 ± 0.23 g l⁻¹) and *S. obliquus* above 36% (CSO+, 3.46 ± 0.08 g l⁻¹; SCS, 4.71 ± 0.30 g l⁻¹) over their respective positive controls at the end of the batch culture. However, under salicylic acid, the carrying capacity of *S. obliquus* was significantly larger than those of *C. sorokiniana* and *C. vulgaris*.

The *C. vulgaris* growth rate was significantly increased under the presence of salicylic acid in comparison with the positive control (CCV+, $0.84 \pm 0.06 \text{ d}^{-1}$; SCV, $1.69 \pm 0.13 \text{ d}^{-1}$). However, it was significantly reduced in the case of *C. sorokiniana* (CCS+, $0.94 \pm 0.06 \text{ d}^{-1}$; SCS, $0.77 \pm 0.12 \text{ d}^{-1}$) and *S. obliquus* (CSO+ $1.16 \pm 0.07 \text{ d}^{-1}$, SSO, $0.72 \pm 0.01 \text{ d}^{-1}$). Moreover, the growth rate of SSO was significantly lower than that of SCS and SCV.

3.1.3. Growth of the culture under diclofenac addition

The microalgae growth curves of *C. sorokiniana*, *C. vulgaris* and *S. obliquus* under the presence of diclofenac, the corresponding positive controls and their respective fittings to the logistic kinetic model are represented as values of biomass concentration versus time in **Figure 3**. The differences among the treatments were analysed according to growth kinetic parameters, as shown in **Table 3**.

	CCS+	DCS	CCV+	DCV	CSO+	DSO
$Cb_0 \text{ (g l}^{-1}\text{)}$	0.04	0.04	0.23	0.23	0.14	0.14
$Nc_0 \text{ (cell ml}^{-1}\text{)}$	3.39×10^6	3.39×10^6	3.53×10^6	3.53×10^6	3.40×10^6	3.40×10^6
$Cb_m \text{ (g l}^{-1}\text{)}$	1.53 ± 0.11	2.28 ± 0.03	1.69 ± 0.06	2.51 ± 0.13	1.27 ± 0.04	1.40 ± 0.05
$Nc_m \text{ (cell ml}^{-1}\text{)}$	$2.49 \times 10^8 \pm 0.22 \times 10^8$	$4.19 \times 10^8 \pm 0.04 \times 10^8$	$7.91 \times 10^7 \pm 0.19 \times 10^7$	$1.73 \times 10^8 \pm 0.22 \times 10^8$	$5.15 \times 10^7 \pm 0.38 \times 10^7$	$6.33 \times 10^7 \pm 0.32 \times 10^7$
a	3.31 ± 0.16	4.24 ± 0.00	2.60 ± 0.05	3.57 ± 0.12	3.30 ± 0.24	3.76 ± 0.37
$K \text{ (g l}^{-1}\text{)}$	1.58 ± 0.11	2.30 ± 0.03	1.96 ± 0.13	2.65 ± 0.10	1.34 ± 0.03	1.49 ± 0.05
$r \text{ (d}^{-1}\text{)}$	0.72 ± 0.04	0.96 ± 0.01	0.56 ± 0.00	0.74 ± 0.01	0.79 ± 0.03	0.81 ± 0.09
R^2	0.9907	0.9988	0.9804	0.9915	0.9890	0.9860

Cb_0 , initial biomass; Nc_0 , initial number of cells; Cb_m , maximum biomass; Nc_m , maximum number of cells; K , carrying capacity; a , constant of logistic kinetic model; r , microalgae growth rate; R^2 , correlation coefficient.

Table 3. Experimental data (Cb_0 , Nc_0 , Cb_m , Nc_m) and logistic model kinetic parameters (K , a , r) determined for the growth of positive controls and treatments with diclofenac of *C. sorokiniana* (CCS+, DCS), *C. vulgaris* (CCV+, DCV) and *S. obliquus* (CSO+, DSO), $n = 3$.

There were significant differences respect the parameter a ($p \leq 0.05$) between the positive control and the corresponding treatment of each strain of microalgae, reaching higher values in the case of the treatments with diclofenac. Therefore, the presence of diclofenac produced a delayed response in the beginning of the exponential growth phase compared with the positive control. Comparing the treatments with diclofenac, *C. sorokiniana* showed a longer lag phase than *C. vulgaris* and *S. obliquus*.

As it can be seen in **Figure 2**, the treatments with diclofenac achieved significantly higher biomass concentration than their respective positive controls. At the end of the batch culture, the *C. sorokiniana* treatment showed an increase of biomass concentration above 45% (CCS+, $1.58 \pm 0.11 \text{ g l}^{-1}$; DCS, $2.30 \pm 0.03 \text{ g l}^{-1}$), *C. vulgaris* above 35% (CCV+, $1.96 \pm 0.13 \text{ g l}^{-1}$; SCV, $2.65 \pm 0.10 \text{ g l}^{-1}$) and *S. obliquus* above 11% (CSO+, $1.34 \pm 0.03 \text{ g l}^{-1}$; SCS, $1.49 \pm 0.05 \text{ g l}^{-1}$) over their respective positive controls. The *C. vulgaris* treatment reached the highest

K value, which was significantly higher than those determined for the *C. sorokiniana* and the *S. obliquus* treatments.

With respect to microalgae growth rate, there were significant differences between the positive control and the corresponding treatment for the two strains of the genus *Chlorella* here used. The *C. sorokiniana* growth rate was significantly increased under the presence of this drug (CCV+, $0.72 \pm 0.04 \text{ d}^{-1}$; DCS, $0.96 \pm 0.01 \text{ d}^{-1}$). This significant increase was also confirmed for *C. vulgaris* (CCV+, $0.56 \pm 0.00 \text{ d}^{-1}$, DCV, $0.74 \pm 0.01 \text{ d}^{-1}$). However, no significant differences were determined in the case of *S. obliquus* (CSO+, $0.79 \pm 0.03 \text{ d}^{-1}$, DSO, $0.81 \pm 0.09 \text{ d}^{-1}$).

3.2. Removal of pharmaceuticals

The pharmaceutical concentration in each reactor was daily monitored and compared with the concentration of each pharmaceutical in the corresponding negative control. The concentration of the pharmaceuticals here studied decreased over the time in the treatments with microalgae, either with *C. sorokiniana* (PCS, SCS, DCS), *C. vulgaris* (PCV, SCV, DCS) or *S. obliquus* (PSO, SSO, DSO). Meanwhile, no concentration reduction was observed in the negative controls (CP−, CS−, CD−). Therefore, it may be assumed that the pharmaceuticals concentration decrease in the microalgae treatments was due to the removal by the microalgae.

3.2.1. Removal of paracetamol

The removal curves of paracetamol by each strain of microalgae and the corresponding fittings to the logistic kinetic model during the batch mode are displayed in **Figure 4(a)**. In addition, differences among the treatments were analysed according to removal kinetic parameters, as shown in **Table 4**.

Figure 4. Volumetric efficiency in the removal of paracetamol by *C. sorokiniana* (PCS, ●), *C. vulgaris* (PCV, ■) and *S. obliquus* (PSO, ▲) during batch culture (a). Dots correspond to experimental data and continuous lines correspond to fittings by the logistic kinetic model during batch culture. Volumetric efficiency in the removal of paracetamol (b) at the steady state of the semicontinuous culture. Experiments were performed in triplicate and bars show standard derivations.

Regarding the parameter a , there were no significant differences between *C. vulgaris* and *S. obliquus* in the lag phase for the removal of paracetamol. However, *C. sorokiniana* showed a significantly longer response at the beginning of the removal of this drug than the other two strains.

Paracetamol	PCS	PCV	PSO
<i>a</i>	4.49 ± 0.24	3.84 ± 0.01	3.19 ± 0.58
<i>K</i> (mg l ⁻¹)	17.62 ± 0.91	6.23 ± 0.02	10.41 ± 1.58
<i>r</i> (d ⁻¹)	1.01 ± 0.06	0.77 ± 0.01	0.86 ± 0.21
<i>R</i> ²	0.9941	0.9827	0.9766
Volumetric efficiency (mg l ⁻¹ d ⁻¹)	3.13 ± 0.22	0.95 ± 0.05	0.72 ± 0.07
Specific efficiency (mg g biomass ⁻¹ d ⁻¹)	2.68 ± 0.26	0.32 ± 0.02	0.37 ± 0.03
Salicylic acid	SCS	SCV	SSO
<i>a</i>	10.20 ± 3.16	4.09 ± 0.87	4.11 ± 0.16
<i>K</i> (mg l ⁻¹)	17.68 ± 0.96	6.44 ± 0.63	24.67 ± 0.32
<i>r</i> (d ⁻¹)	4.07 ± 1.21	0.84 ± 0.17	0.76 ± 0.03
<i>R</i> ²	0.9919	0.9947	0.9973
Volumetric efficiency (mg l ⁻¹ d ⁻¹)	6.98 ± 0.31	1.72 ± 0.15	7.55 ± 0.01
Specific efficiency (mg g biomass ⁻¹ d ⁻¹)	8.34 ± 1.21	0.67 ± 0.06	1.85 ± 0.02
Diclofenac	DCS	DCV	DSO
<i>a</i>	3.88 ± 0.62	3.23 ± 0.02	3.01 ± 0.38
<i>K</i> (mg l ⁻¹)	14.55 ± 0.73	15.52 ± 0.26	22.43 ± 0.20
<i>r</i> (d ⁻¹)	2.03 ± 0.33	1.44 ± 0.05	1.25 ± 0.19
<i>R</i> ²	0.9626	0.9755	0.9690
Volumetric efficiency (mg l ⁻¹ d ⁻¹)	2.18 ± 0.39	1.53 ± 0.32	5.66 ± 0.39
Specific efficiency (mg g biomass ⁻¹ d ⁻¹)	1.73 ± 0.38	0.97 ± 0.19	5.21 ± 0.18

Table 4. Logistic model kinetic parameters (*K*, *a*, *r*) determined for the removal of paracetamol, salicylic acid and diclofenac in the batch culture of *C. sorokiniana* (PCS, SCS, DCS), *C. vulgaris* (PCV, SCV, DCV) and *S. obliquus* (PSO, SSO, DSO). Volumetric efficiency and specific efficiency attained in the steady state of the semicontinuous culture. n=3.

The parameter *K* values in **Table 4** revealed that *C. sorokiniana* (PCS, 17.62 ± 0.91 mg l⁻¹) reached a carrying capacity 2.8 times higher than *C. vulgaris* (PCV, 6.23 ± 0.02 mg l⁻¹) and 1.7 times higher than *S. obliquus* (PSO, 10.41 ± 1.58 mg l⁻¹). In the same way, the removal rates revealed significant differences among the treatments, with *C. sorokiniana* showing the quickest removal (PCS, 1.01 ± 0.06 d⁻¹) and *C. vulgaris* the slowest one (PCV, 0.77 ± 0.01 d⁻¹), which is in agreement with the determined *K* values.

As a consequence of the different responses obtained for the removal parameters between the strains, at the end of the batch culture, efficiencies in the removal of paracetamol above 67% for *C. sorokiniana*, 21% for *C. vulgaris* and 40% for *S. obliquus* were achieved. These results evidenced a larger removal capacity of paracetamol by *C. sorokiniana*, followed by *S. obliquus*, and *C. vulgaris*.

The average volumetric efficiencies on the paracetamol removal by each strain at the steady stage of the semicontinuous culture are depicted as percentages in **Figure 4(b)**. The paracetamol volumetric efficiency reached values above 41% for *C. sorokiniana*, 12% for *C. vulgaris* and 9% for *S. obliquus*. Moreover, the ratios between the volumetric efficiency and the microalgae biomass are shown in **Table 4** as specific efficiencies. These results revealed that *C. sorokiniana* cells removed above 7.2 times more paracetamol than *C. vulgaris* and 8.4 times more than *S. obliquus* per gram of biomass. On the other hand, the paracetamol removal per gram of biomass was similar between *S. obliquus* and *C. vulgaris*.

3.2.2. Removal of salicylic acid

The removal curves of salicylic acid by each strain of microalgae and the corresponding fittings to the logistic kinetic model during the batch mode are displayed in **Figure 5(a)**. In addition, differences among the treatments were analysed according to removal kinetic parameters, as shown in **Table 4**.

Figure 5. Volumetric efficiency in the removal of salicylic acid by *C. sorokiniana* (SCS, ●), *C. vulgaris* (SCV, ■) and *S. obliquus* (SSO, ▲) during batch culture (a). Dots correspond to experimental data and continuous lines correspond to fittings by the logistic kinetic model during batch culture. Volumetric efficiency in the removal of salicylic acid (b) at the steady state of the semicontinuous culture. Experiments were performed in triplicate and bars show standard derivations.

In the case of *C. sorokiniana* there were significant differences respect the parameter *a*, which indicated that the beginning of the removal of salicylic acid had a delayed response as compared with the lag phase of *C. vulgaris* and *S. obliquus*.

The results obtained for the maximum removal capacity (*K* parameter) revealed that *S. obliquus* (SSO, 24.67 ± 0.32 mg l⁻¹) removed 1.4 times more salicylic acid than *C. sorokiniana* (SCS, 17.68 ± 0.96 mg l⁻¹) and 3.8 time more than *C. vulgaris* (SCV, 6.44 ± 0.63 mg l⁻¹). In spite of salicylic acid removal efficiencies at the end of the batch culture being above 73% by *C. sorokiniana*, 25% by *C. vulgaris*, 93% by *S. obliquus*, the removal rate of *S. obliquus* was significantly lower (SSO, 0.76 ± 0.03 d⁻¹) than that of *C. sorokiniana* (SCS, 4.07 ± 1.21 d⁻¹).

The average salicylic acid volumetric efficiencies by each strain at the steady stage of the semicontinuous culture are depicted as percentages in **Figure 5(b)**. The paracetamol volu-

metric efficiency did not showed significant differences between the strains *C. sorokiniana* and *S. obliquus*, reaching values above 93% for SCS and 99% for SSO. However, the salicylic acid volumetric efficiency of *C. vulgaris* (above 22%) was more than four times lower than by the other strains. Moreover, the obtained specific efficiencies revealed that *C. sorokiniana* removed above 12.4 times more salicylic acid than *C. vulgaris* and 4.5 times more than *S. obliquus* per gram of biomass (**Table 4**).

3.2.3. Removal of diclofenac

The removal curves of diclofenac by each strain of microalgae and the corresponding fittings to the logistic kinetic model during the batch mode are displayed in **Figure 6(a)**. In addition, differences among the treatments were analysed according to removal kinetic parameters, as shown in **Table 4**.

Figure 6. Volumetric efficiency in the removal of diclofenac by *C. sorokiniana* (DCS, ●), *C. vulgaris* (DCV, ■) and *S. obliquus* (DSO, ▲) during batch culture (a). Dots correspond to experimental data and continuous lines correspond to fittings by the logistic kinetic model during batch culture. Volumetric efficiency in the removal of salicylic acid (b) at the steady state of the semicontinuous culture. Experiments were performed in triplicate and bars show standard derivations.

The a values were similar ($p > 0.05$) for all the treatments, which indicated that the three strains showed the same delayed response in the removal of diclofenac. However, regarding the maximum removal capacity, there were significant differences between the treatment with *S. obliquus* (DSO, 22.43 ± 0.20 mg l⁻¹), which removed 1.5 times more diclofenac than by *C. sorokiniana* (DCS, 14.55 ± 0.73 mg l⁻¹) and 1.4 times more than *C. vulgaris* (DSO, 15.52 ± 0.26 mg l⁻¹).

Concerning the removal rate, the obtained results revealed significant differences among the treatments. The quickest removal rate was attained by *C. sorokiniana* (DCS, 2.03 ± 0.33 d⁻¹), with removal values 1.6 times higher than *S. obliquus* (DSO, 1.25 ± 0.19 d⁻¹) and 1.4 times higher than *C. vulgaris* (DCS, 1.44 ± 0.05 d⁻¹). Despite the differences between strains regarding the removal parameters, at the end of the batch culture, efficiencies above 65% for *C. sorokiniana*, 69% for *C. vulgaris* and 98% for *S. obliquus* were achieved.

The average volumetric efficiencies for the diclofenac removal in the steady stage of the semi-continuous culture are showed in **Figure 6(b)**. The volumetric efficiency for *S. obliquus* (above 79%) was 2.6 times higher than for *C. sorokiniana* and 3.7 times higher than for *C. vulgaris*.

Moreover, the ratios between the volumetric efficiency and the microalgae biomass are shown in **Table 4** as specific efficiencies. The determined values revealed that *S. obliquus* removed above 3.0 times more diclofenac than *C. sorokiniana* and above 5.4 times more than *C. vulgaris* per gram of biomass.

4. Discussion

In view of the obtained results, it may be inferred that the presence of paracetamol, salicylic acid and diclofenac modified the growth parameters of the strains here studied. In most of the treatments, the addition of the pharmaceutical increased the biomass concentration, which may be explained by the fact that these pharmaceuticals were an additional source of organic carbon. It is well known that the genus *Chlorella* and *Scenedesmus* can have a mixotrophic growth. However, *S. obliquus* did not show a significant increase of microalgae biomass under the addition of paracetamol or diclofenac. These results suggest that the other removal mechanisms, apart from metabolism, may be involved.

The fact that removal curves displayed a similar trend than growth curves points to the association between the microalgae growth and the removal efficiency of pharmaceuticals.

In view of the obtained results, it may be concluded that paracetamol was more efficiently removed by *C. sorokiniana*, either per litre or per gram of biomass (>67% batch culture, >41% semicontinuous culture), in spite of the biomass concentration reached in the culture being the lowest one among the three strains. Also, the removal rate by *C. sorokiniana* was the fastest one, in spite of showing the lowest growth rate among the paracetamol treatments. However, the addition of paracetamol in the *C. sorokiniana* culture produced the largest increase in the biomass concentration compared with the corresponding positive control (>49%).

On the other hand, *S. obliquus* showed the highest salicylic acid removal capacity at the end of the batch culture (>93%) and also at the steady state of the semicontinuous culture (>99%). However, the removal rate by *S. obliquus* was the lowest one among the salicylic acid treatments. The highest removal rate was reached by *C. sorokiniana*, which showed a removal per gram of biomass 4.5 times larger than *S. obliquus*. Furthermore, the increase of biomass under the addition of salicylic acid was above 52% in the *C. sorokiniana* treatment, while for *S. obliquus* was above 36%.

Regarding diclofenac, despite *C. sorokiniana* cells attained a higher removal rate and the higher growth rate, it may be stated that *S. obliquus* was the strain that reached the highest removal efficiency (>98% batch culture, >79% semicontinuous culture) with more diclofenac removed either per litre or per gram of biomass.

Comparing the three pharmaceuticals, the salicylic acid was more efficiently removed, with *C. sorokiniana* and *S. obliquus* showing the highest efficiencies. Contrarily, the paracetamol was the less efficiently removed. In all cases, *C. vulgaris* showed the lowest efficiencies for the three pharmaceuticals. These results may be related with the specific strain characteristics, the mechanisms involved in the removal and the particular properties of each pharmaceutical.

As in this work, published results on the removal of ECs by microalgae have revealed different efficiencies depending on the pollutant and on the microalgae strain. For example, Gattullo et al. [23] demonstrated that *Monoraphidium braunii* was able to remove up to 48% of bisphenol A with an initial concentration of 4 mg l⁻¹. de Wilt et al. [14] reported removal efficiencies by *C. sorokiniana*, grown in wastewater streams, up to 60–100% for diclofenac, ibuprofen, paracetamol and metoprolol. However, under identical conditions, the removal of carbamazepine and trimethoprim was incomplete and did not exceed 30% and 60%, respectively [14]. Wang et al. [24] studied the removal of phenol by *Chorella* sp. culture, obtaining removal efficiencies up to 100% from an initial concentration of 500 mg l⁻¹ in 7 days. Peng et al. [25] reported removals above 95% of progesterone by *S. obliquus* and *Chlorella pyrenoidosa*, nearly complete removal of norgestrel by *S. obliquus* and almost 40% of norgestrel by *C. pyrenoidosa*. Likewise, Hom-Díaz et al. [15] studied the elimination of the hormones E2 and EE2 from anaerobic digestate centrate by the microalgae *Selenastrum capricornutum* and *Chlamydomonas reinhardtii*. After 7 days of culture, these authors [15] determined removals above 88% for E2 and above 60% for EE2. Furthermore, Matamoros et al. [26] studied the capability of microalgae-based wastewater treatment systems to remove diclofenac, among other 25 emerging organic contaminants. These authors [26] determined diclofenac removal efficiencies above 82% under HRT of 4 days and above 92% under HRT of 8 days during the warm season (11–26°C, on a daily average). These efficiencies are higher than the here obtained under an HRT of 80 h and temperature of 25±1°C. Differences must be related, at least to some extent, to the fact that microalgae monocultures were used in this work while Matamoros et al. [26] worked with mixed microalgae strains present in the wastewater, mostly identified as *Stigeoclonium* sp., diatoms, *Chlorella* sp. and *Monoraphidium* sp.

5. Conclusions

Among the here considered strains, *S. obliquus* displayed the highest removal efficiency for salicylic acid and diclofenac, while *C. sorokiniana* did it for paracetamol. On the other hand, *C. vulgaris* showed the lowest efficiencies for the three pharmaceuticals. Comparing the three pharmaceuticals, the salicylic acid was more efficiently removed while paracetamol removal was the less efficient. These differences may be related with the specific strain characteristics, the mechanisms involved in the removal and the particular properties of each pharmaceutical. The obtained results pointed to the feasibility of using the microalgae here considered in bioremediation systems and revealed that this sort of studies are key for the selection of the strain, which depends on the application. Still, further research is needed to assess the mechanisms involved in the removal of pharmaceuticals by these strains.

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References

- [1] Sauvé S, Desrosiers M: A review of what is an emerging contaminant. *Chemistry Central Journal*. 2014;**8**:15. DOI: 10.1186/1752-153X-8-15
- [2] Evgenidou E, Konstantinou I, Lambropoulou D: Occurrence and removal of transformation products of PPCPs and illicit drugs in wastewaters: A review. *Science of the Total Environment*. 2015;**505**:905–926. DOI: 10.1016/j.scitotenv.2014.10.021
- [3] Santos L, Araújo A, Fachini A, Pena A, Delerue-Matos C, Montenegro M: Ecotoxicological aspects related to the presence of pharmaceuticals in the aquatic environment. *Journal of Hazardous Materials*. 2010;**175**:45–95. DOI: 10.1016/j.jhazmat.2009.10.100
- [4] Barbosa M, Moreira N, Ribeiro A, Pereira M, Silva A: Occurrence and removal of organic micropollutants: An overview of the watch list of EU Decision 2015/495. *Water Research*. 2016;**94**:257–279. DOI: 10.1016/j.watres.2016.02.047
- [5] Rigobello E, Dantas A, Di Bernardo L, Vieira E: Removal of diclofenac by conventional drinking water treatment processes and granular activated carbon filtration. *Chemosphere*. 2013;**93**:184–191. DOI: 10.1016/j.chemosphere.2013.03.010
- [6] Barceló D, Petrovic M: Reducing the environmental risk from emerging pollutants. In: *Trac-Trends in Analytical Chemistry*, editor. 1st EMCO workshop “Analysis and removal of contaminants from wastewater for the implementation of the Water Framework Directive (WFD)”; 20-21 October 2005; Dubrovnik, Croatia. 2006. p. 191–193. DOI: 10.1016/j.trac.2005.11.003
- [7] Bolong N, Ismail A, Salim M, Matsuura T: A review of the effects of emerging contaminants in wastewater and options for their removal. *Desalination*. 2009;**239**:229–246. DOI: 10.1016/j.desal.2008.03.020
- [8] Farré M, Pérez L, Kantiani L, Barceló D: Fate and toxicity of emerging pollutants, their metabolites and transformation products in the aquatic environment. *Trac-Trends in Analytical Chemistry*. 2008;**27**:991–1007. DOI: 10.1016/j.trac.2008.09.010

- [9] Pal A, He Y, Jekel M, Reinhard M, Gin K: Emerging contaminants of public health significance as water quality indicator compounds in the urban water cycle. *Environment International*. 2014;**71**:46–62. DOI: 10.1016/j.envint.2014.05.025
- [10] Verlicchi P, Aukidy M, Zambello E: Occurrence of pharmaceutical compounds in urban wastewater: Removal, mass load and environmental risk after a secondary treatment-A review. *Science of the Total Environment*. 2012;**429**:123–155. DOI: 10.1016/j.scitotenv.2012.04.028
- [11] Heberer T, Reddersen K, Mechlinski A: From municipal sewage to drinking water: Fate and removal of pharmaceutical residues in the aquatic environment in urban areas. *Water Science and Technology*. 2002;**46**:81–88.
- [12] Ashton D, Hilton M, Thomas K: Investigating the environmental transport of human pharmaceuticals to streams in the United Kingdom. *Science of the Total Environment*. 2004;**333**:167–184. DOI: 10.1016/j.scitotenv.2004.04.062
- [13] Combarros R, Rosas I, Lavin A, Rendueles M, Diaz M: Influence of biofilm on activated carbon on the adsorption and biodegradation of salicylic acid in wastewater. *Water, Air, and Soil Pollution*. 2014;**225**:1858. DOI: 10.1007/s11270-013-1858-9
- [14] de Wilt A, Butkovskiy A, Tuantet K, Leal L, Fernandes T, Langenhoff A, Zeeman G: Micropollutant removal in an algal treatment system fed with source separated wastewater streams. *Journal of Hazardous Materials*. 2016;**304**:84–92. DOI: 10.1016/j.jhazmat.2015.10.033
- [15] Hom-Díaz A, LLorca M, Rodríguez-Mozaz S, Vicent T, Barceló D, Blázquez P: Microalgae cultivation on wastewater digestate: Beta-estradiol and 17 alpha-ethynylestradiol degradation and transformation products identification. *Journal of Environmental Management*. 2015;**155**:106–113. DOI: 10.1016/j.jenvman.2015.03.003
- [16] Ritchmon A: *Handbook of Microalgal Mass Culture*. Boca Raton, Florida: CRC Press; 1986. 528 p.
- [17] Matamoros V, Uggetti E, García J, Bayona J: Assessment of the mechanisms involved in the removal of emerging contaminants by microalgae from wastewater: A laboratory scale study. *Journal of Hazardous Materials*. 2016;**301**:197–205. DOI: 10.1016/j.jhazmat.2015.08.050
- [18] Petrie B, Barden R, Kasprzyk-Hordern B: A review on emerging contaminants in wastewaters and the environment: Current knowledge, understudied areas and recommendations for future monitoring. *Water Research*. 2015;**72**:3–27. DOI: 10.1016/j.watres.2014.08.053
- [19] Beuckels A, Smolders E, Muylaert K: Nitrogen availability influences phosphorus removal in microalgae-based wastewater treatment. *Water Research*. 2015;**77**:98–106. DOI: 10.1016/j.watres.2015.03.018

- [20] Mann J, Myers J: On Pigments Growth and Photosynthesis of *Phaeodactylum tricornutum*. Journal of Phycology. 1968;**4**:349–355. DOI: 10.1111/j.1529-8817.1968.tb04707.x
- [21] Verhulst P: *Notice sur la loi que la population suit dans son accroissement* (A note on the law of population growth). Correspondance Mathématique et Physique. 1938;**10**:113–121.
- [22] Xin L, Hu H, Ke G, Sun Y: Effects of different nitrogen and phosphorus concentrations on the growth, nutrient uptake, and lipid accumulation of a freshwater microalga *Scenedesmus* sp. Bioresource technology. 2010;**101**:5494–5500. DOI: 10.1016/j.biortech.2010.02.016
- [23] Gattullo C, Baehrs H, Steinberg C, Loffredo E: Removal of bisphenol A by the freshwater green alga *Monoraphidium braunii* and the role of natural organic matter. Science of the Total Environment. 2012;**416**:501–506. DOI: 10.1016/j.scitotenv.2011.11.033
- [24] Wang L, Xue C, Wang L, Zhao Q, Wei W, Sun Y: Strain improvement of *Chlorella* sp for phenol biodegradation by adaptive laboratory evolution. Bioresource Technology. 2016;**205**:264–268. DOI: 10.1016/j.biortech.2016.01.022
- [25] Peng F, Ying G, Yang B, Liu S, Lai H, Liu Y, Chen Z, Zhou G: Biotransformation of progesterone and norgestrel by two freshwater microalgae (*Scenedesmus obliquus* and *Chlorella pyrenoidosa*): Transformation kinetics and products identification. Chemosphere. 2014;**95**:581–588. DOI: 10.1016/j.chemosphere.2013.10.013
- [26] Matamoros V, Gutiérrez R, Ferrer I, García J, Bayona J: Capability of microalgae-based wastewater treatment systems to remove emerging organic contaminants: A pilot-scale study. Journal of hazardous materials. Journal of Hazardous Materials. 2015;**288**:34–42. DOI: 10.1016/j.jhazmat.2015.02.002