Removal of pharmaceuticals from industrial wastewaters by microalgae culture

C. Escapa*, R.N. Coimbra, S. Paniagua, A.I. García, M. Otero

Department of Applied Chemistry and Physics, Institute of Environment, Natural Resources and Biodiversity (IMARENABIO), University of León, 24071 León, Spain

carla.escapa@unileon.es (C. Escapa), ricado.decoimbra@unileon.es (R.N. Coimbra),spanb@unileon.es(S. Paniagua),ana.garcia@unileon.es (A.I. García), marta.otero@unileon.es (M. Otero).

* Corresponding author: Address: Department of Applied Chemistry and Physics, Institute of Environment, Natural Resources and Biodiversity (IMARENABIO), University of León, Avenida de Portugal 41, 24071 León, Spain. Tel.: +34 987293378

E-mail address: carla.escapa@unileon.es (C. Escapa)

Abstract

This work aimed to study the removal of paracetamol (PAC) and salicylic acid (SaC) by*Chlorella sorokiniana*in view of its application for the treatment of wastewater from the pharmaceutical industry. For this purpose, a bubbling column photobioreactor was used for the cultivation of the microalgaein a standard culture medium containing either PAC or SaC.

The obtained results showed that the growth of the culture was significantly higher under the presence of the considered drugs than the positive control. On the other hand, the removal of pharmaceuticals in the steady state of the semicontinuous process showed efficiencies higher than 69% of paracetamol and 98% of salicylic acid. Under an irradiance of just 370 µE m^{-2} s^{-1}, the quantum yield reached high values for all the treatments due to the high biomass concentration achieved.Somehow, the quantum yield for SaC was 1.4 times higher than the other treatments.

It was concluded that microalgae may be applied for the removal of high concentrations of paracetamol and salicylicfrom wastewaters. The high removal rates here determined point to the possible application of microalgae cultures as bioremediation systems.

Keywords: *Chlorella sorokiniana*, paracetamol, salicylic acid, bioremediation

1. Introduction

Pharmaceutical industry generates a wide variety of wastewaters during manufacturing, maintenance and housekeeping operations. A pre-treatment process of these wastes is needed before to be mixed with conventional wastewaters to ensure an effective process. Conventional biological sewage treatment plants (STPs) are designed to eliminate carbon, nitrogen and phosphorus. Requirements for the elimination of the latter are different from those needed for active pharmaceutical ingredients (APIs), which pose high biological activity in a very low concentration,are mostly hydrophilic and have low adsorption rates[1]. Therefore, besides the nonexistence of limiting regulations [2,3], an effective elimination of these APIs is not possible in conventional STPs.

In addition, the presence of pharmaceuticals in the aquatic environment may cause physiological responses in organisms for which they were not intended, such as accumulation in tissues, reproductive damage, inhibition of cell proliferation and behavioural changes [4]. In fact, more than 50 pharmaceutical compounds have been detected during the last years in different aquatic environmental samples, due to the continuous improvement of the analytical techniques [5]. Given concern about pharmaceuticals, it is expectable that legislation on their discharge will come out in the near future.Among pharmaceuticals, paracetamol is easily accumulated in aquatic environment due to its relative high solubility and hidrophilicity. Moreover, it has been confirmed that 58-68% of paracetamol and their metabolites are excreted from the body during therapeutic use [6]. Likewise, salicylic acid removal from aqueous solutions has received a great deal of attention in recent years due to its toxicity and accumulation in the environment [7].

Wastewaters from the pharmaceutical industry are generally treated by chemical oxidation methods. The high operational cost associated, the harsh reaction conditions and the generation of secondary pollutants are important disadvantages of this sort of treatments [8]. Conversely, biodegradation treatments have been considered a low-cost option and environmentally friendly[9,10]. Despite there are preliminary studies about biodegradation of pharmaceuticals by microorganisms [7,8,11,12], to our best knowledge there is not information available about biodegradation of pharmaceuticals by microalgae.

Therefore, the aim of this study was to assess the feasibility of *Chlorella sorokiniana* to remove paracetamol and salicylic acid from water, in view of its application for the treatment of wastewater from the pharmaceutical industry. For this purpose, kinetics growth, removal rates of pharmaceuticals and the light energy efficiency of the cells were studied.

2. Materials and Methods

2.1. Microorganism and culture conditions

The microalgae strain used in this study was *Chlorella sorokiniana CCAP 211/8 K (UTEX Culture Collection).* Inoculum for the experiments was cultivated in the standard culture medium Mann and Myers [13]in 250 ml Erlenmeyer flasks. This pre-cultured was maintained inside a vegetal culture chamber, where the growth conditions remained constant, under controlled temperature (25±1ºC), irradiance (175 µE m^{-2} s^{-1}), photoperiod (12:12) and stirring (250 rpm).

Experiments were carried out in bubbling column photobioreactors (PBRs) with spherical bases (40 mm diameter and 300 mm height with 300 ml capacity), keeping an operating volume of 250 ml. To ensure the same initial concentration, all reactors were

inoculated with the same volume of pre-cultured microalgae (about $1.7x10^7$ cells ml^{-1}). Culture conditions were kept constant under controlled temperature (25±1ºC), irradiance (370 µE $m^{-2}s^{-1}$) and photoperiod (12:12), inside a vegetal culture chamber. Culture medium was the same used for the inoculum growth. PBRs were illuminated by 8 fluorescent lamps (58 W, 2150 lumen, Philips, France) and aerated at a rate of 0.3 v/v/min with CO_2 enriched at 7% v/v, which was injected on demand to keep a constant pH (pH = 7.5 ± 0.5) as controlled by a pH sensor. Before injection, the air was filtered through 0.2 µm sterile air-venting filter (Millex-FG50, Millipore).

2.2. Experimental set-up

Reactors were operated in batch mode until the end of the exponential growth phase, following by a semicontinuous mode until the culture parameters remained constant. Under semicontinuous mode, the daily dilution rate was 30% of the culture volume, which was replaced with fresh medium.

The experimentscarried out consisted of inoculated culture medium with 250 mg l^{-1} paracetamol (PC) or inoculated culture medium with 250 mg l^{-1} salicylic acid (SaC). In each case, trials also included positive controls with inoculated culture medium without drugs (C+) and the corresponding negative controlswith not inoculated culture medium and 250 mg l^{-1} PC(C_P-) or 250 mg l^{-1} SaC (C_{Sa}-). Three non-simultaneous replicates of each experiment were run under identical conditions.Paracetamol ($C_8H_9NO_2$, ≥99%) was supplied by Roic Pharma and salicylic acid ($C_7H_6O_3$, ≥99%) by Panreac. Physico-chemical properties of these pharmaceuticals are displayed in Table 1.

Throughout the experiments, the culture growth was daily monitored by biomass concentration and cell density. Also,to assess the pharmaceuticals removal, the concentration of paracetamol and salicylic acid was daily monitored. In addition, the

irradiance in the absence of cells in the central point inside of the PBR was measured to evaluate the light energy of the cells.

2.3. Analytical methods

Biomass concentration (C_b) was determined by optical density at 680 nm (OD_{680}) by spectrophotometric (UV/visible spectrophotometer BECKMAN DU640) and verified by dry weight. Preliminary studies were conducted to determinate the relationship between dry weight and OD_{680} as shown in Eq. (1):

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

Dry weight measurements were performed by filtering 10 mL of culture through a 0.45 µm Whatman filter and washing with 20 mL HCl (0.5 M) to dissolve precipitated salts. Then, the filtrate was dried in an oven at 80ºC for 24 hours. Additionally, the growth of the culture was measured as cell density (*Nc*) by cell counting with a Neubauer chamber.

A Waters HPLC 600 E equipped with a 2996 Photodiode Array Detector was used for the quantification of the target pharmaceuticals. The wavelengths of detection were 246 and 236 nm for paracetamol and salicylic acid, respectively. A Phenomenex Gemini-NX C18 column (5 µm, 250 mm x 4.6 mm) was used for the separation. The mobile phase consisted of a mixture of acetonitrile:water (30:70, v/v) for the analysis of paracetamol and a mixture of acetonitrile:water:orthophosphoric acid (70:30:0.1, v/v/v). HPLC quality acetonitrile (CH_3CN) from LAB-SCAN, orthophosphoric acid (H_3PO_4) from Panreac 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 during 30 minutes. For the chromatographic analysis, the mobile phase flow rate was 1 mL min^{-1}

and the injection volume was 50 μL. Before analysis, all the samples were centrifuged twice at 7500 rpm for 10 min (SIGMA 2-16P centrifuge).

The biomass extinction coefficient (K_a) was obtained from the average value of optical density in the visible range (400-700 nm) by spectrophotometric (UV/visible spectrophotometer BECKMAN DU640). The K_a is a function of the average optical density ($OD_{400\text{-}700}$), the biomass concentration (Cb) and the light path of the cell (ρ) (Eq. 2):

$$K_a = \frac{OD_{400-700}}{C_b \times \rho} \quad (2)$$

The average irradiance at which cells are exposed inside a culture (I_{av}) is a function of irradiance in the absence of cells (I_o), the biomass extinction coefficient (K_a), the biomass concentration (C_b) and the light path inside the reactor, by the formula defined by Grima et al. [14] (Eq. 3):

$$I_{av} = \frac{I_0}{(K_a \times \rho \times C_b)} \left(1 - exp(-K_a \times \rho \times C_b)\right) \quad (3)$$

The quantum yield (Ψ_E) is defined in microalgae cultures as the amount of biomass generated by the unit of radiation (usually a mole of photons) absorbed by the culture, which may be calculated by the expression defined by Grima et al. [14] (Eq. 4). The quantum yield is function of the volumetric biomass productivity (P_b) and the photon flux absorbed in the volume unit (F_{vol}). The photon flux absorbed through the reactor volume may be obtained from de average irradiance (I_{av}) on the culture volume basis on the Eq. 5 [14]:

$$\Psi_E = \frac{P_b}{F_{vol}} \quad (4)$$

$$F_{vol} = I_{av} \times K_a \times C_b \quad (5)$$

Considering the quantum yield defined by Grima et al. [14] and aiming to determine the relationship between the removal of pharmaceuticals and photon flux absorbed, a new

equation was set in this work. In this equation, the removal yield (Ψ_R, g removed E^{-1}) in microalgae cultures was defined as the amount of pharmaceutical removed by microalgae by unit of radiation absorbed by the culture (Eq. 6). Then, the removal yield was set as function of the volumetric removal efficiency (R_b, g removed m^{-3} s^{-1}) and the photon flux absorbed in the volume unit (F_{vol}, µE m^{-3} s^{-1}).

$$\Psi_R = \frac{R_b}{F_{vol}} \quad (6)$$

2.4. Statistical analysis

Growth kinetics were resolved in OriginPro 8, using the logistic model, a sigmoidal curve model which describes the relationship between a microorganism's growth and density in limited environmental conditions (Eq. 6).

$$N = \frac{K}{1+e^{a-rt}} \quad (6)$$

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 which indicates the relative position from the origin, and r (d^{-1}) is the specific growth rate.

Removal kinetics of pharmaceuticals were also resolved in OriginPro 8, using the logistic model.

Then, the software was also used to perform an analysis of variance, of growth and removal kinetic parameters on the removal of pharmaceuticals, using as mean comparison the Fisher LSD test.

3. Results and Discussion

3.1. Biomass growth

The microalgae growth curves, as biomass values versus time, and their fittings to the logistic kinetic model, including the positive control and the two treatments with drugs are represented in Figure 1.

The differences among the treatments were analysed according to growth kinetic parameters (Table 2).The parameter *a*, which refers to the relative position from the origin, showed significant differences ($p \leq 0.05$) between SaC and the other two treatments (C+ and PC). Therefore, it may be said that the addition of salicylic acid produced a delayed response of SaCin the beginning of the exponential phase. Also, the end of the exponential growth phase for SaC was reached in 12 days, while PC and C+ reached this stage within 7 days. Previous assays at an initial pharmaceutical dose of 25 mg l^{-1}did not shown a delayed response, either for salicylic acid or for paracetamol[15]. Therefore,the delay observed in this work may be due to the high dose of salicylic acid, which affects the growth response of the cells.

As seen in Figure 1, the treatments with drugs achieved higher biomass concentration than the positive control. The parameter *K*, which refers to the carrying capacity, revealed significant differences ($p \leq 0.05$) between the treatments. As it can be seen, the treatments with drugs achieved higher biomass concentration than the positive control.This may be explained by the fact that the drugs were an additional source of organic carbon (besides injected CO_2) and it is well known that the genus Chlorella can have a mixotrophic growth. Also, a higher biomass concentration was obtained under the presence of SaC, as compared with PC. According to properties in Table 1, a main question may be the pH speciation. For SaC, the unique species at pH=7.5 is the anionic form (100%). While for PC, the neutral form is the form having the largest concentration (99.4%). Therefore, the anionic SaC will be completely dissolved and easily available for microalgae.Respect to microalgae growth rate *r*, there were

significant differences ($p \leq 0.05$) between the treatments, showing the higher value for this parameter in the case of PC. Moreover, salicylic acid produced a delayed response in the beginning of the growth and also a lower growth rate than the positivecontrol. The results obtained respect parameter *K* and growth rate *r* are in accordance with those obtained at an initial dose of 25 mg l^{-1}in previous assays [15]. Hence, both under initial doses of 25 and 250 mg l^{-1}, higher carrying capacity and lower growth rate was achieved with SaC than with PC.

On the other hand, it must be highlighted that, under the semicontinuous operation mode, daily dilutions rates produced instability. As a consequence, as seen in Figure 1, the growth rate declined throughout several days until parameters remained constant during the steady state at lower values of biomass concentration. Growth rates obtained in this research (above 0.71 day^{-1} for C+, 0.95 day^{-1} for PC, 0.54 day^{-1} for SaC, as shown in Table 2)were quite higher than those reported by Kumar et al. [16] for the same specie (0.1 day^{-1}). Domínguez Cabanelas et al. [17], described growth rates for *Chlorella vulgaris* below 0.38 day^{-1} with different domestic wastewaters and a maximum biomass of 1.52 g l^{-1}. Nevertheless, Arbib et al. [18], reported growth rates for *Scenedemus obliquus* close to 1 day^{-1}.

3.2. Pharmaceuticals removal

In order to determine the removal of pharmaceuticals, the daily concentrations of paracetamol in PC and of salicylic acid in SaC reactors were compared to the concentrations of these pharmaceuticals in the corresponding C-. For these pharmaceuticals it was no observed any decrease of concentration in the respective C-. On the contrary, paracetamol and salicylic acid content decreased throughout time in PC and SaC reactors, respectively. Thus, this decrease was associated to the removal by *C.*

sorokiniana microalgae. The removal curves of paracetamol and salicylic acid and the corresponding fittings to the logistic kinetic model for the batch culture, are showed in Figure 2. These curves display a similar trend than growth curves given that the increase of removal rates is related with the increase of biomass concentration. Differences in the removal of the here considered pharmaceuticals were analysed according to the kinetic parameters of the logistic model (Table 3). There were significant differences ($p \leq 0.05$) respect to the parameter *a* between the two treatments. The beginning of the removal of salicylic acid in SaC had a delayed response. This response is related to the delay in the beginning of the exponential growth in this treatment. Regarding the maximum capacity of removal, parameter *K*, there were significant differences ($p \leq 0.05$), where SaC showed higher removal capacity. Moreover, the salicylic acid removal rate was much quicker than that of paracetamol. As seen in Figure 2, the maximum salicylic acid removal capacity was reached within only 4 days of culture time, as compared to eight days for paracetamol. In fact, the removal rates revealed significant differences ($p > 0.05$), with values 5.1 times greater for salicylic acid respect to those determined for paracetamol. In any case, at the end of the batch culture, efficiencies above 94% for salicylic acid and 48% for paracetamol were achieved. The removal rates obtained in previous assays at lower pharmaceutical initial concentration (25 mg l^{-1})were 1.0143 ± 0.0551 for paracetamol and of 4.0668 ± 1.2052 for salicylic acid, which maximum removal capacity was also reachedwithin only 4 days. The efficiencies at the end of the batch culture were above 73% for salicylic acid and 67% for paracetamol [15].

At this point, the relationship between the pharmaceuticals removal and the biomassgrowth throughout time was determined. The obtained results are shown in Figure 3, which evidences that there was a constant relationshipbetween the removal of paracetamol and the biomass concentration (48.11 ± 15.98 mg of paracetamol g^{-1}

biomass) throughout time. Differently, for salicylic acid there was an exponential increase up to 604.10 mg of salicylic acid g^{-1} biomass, as shown in the Figure 3. The constant relationship in the case of paracetamol and the exponential increase for salicylic acid are coincident with our previous results under lower pharmaceuticals initial concentration[15].

On the other hand, the average volumetric efficiencies for the pharmaceuticals removal in the steady stage of the semicontinuous culture are showed in Figure 4. The salicylic acid volumetric efficiency (98%) was 1.43 times higher thatof paracetamol (69%).The efficiencies reached in the previous assays at 25 mg l^{-1} dose were above 93 % for salicylic acid and 41% for paracetamol, which is quite lower than the one determined in this study.

No prior studies about pharmaceuticals removal by microalgae were found in the literature. On the other hand, information available about the utilization of other microorganisms for this purpose is scarce. Bacterial biodegradation of paracetamol has been studied by several authors [8,11,12], achieving high or complete degradations, even at initial concentration of 2,500 mg l^{-1} (70 h) by a species of genus Pseudomonas [11]. On the other hand, Combarros et al. [7] claimed that the biodegradation of salicylic acid by *Pseudomonas putida* achieved an efficiency of 100% (8h) and 94.7% (24 h) for initial concentrations of 100 and 500 mg l^{-1}, respectively.This decrease of efficiency with the increase of initial concentration found by Combarros et al. [10] has not been verified in this study for the elimination of salicylic acid by *C. sorokiniana* as compared with our previous results out under lower pharmaceutical initial concentration [3]. Hence, it could be inferred that *C. sorokiniana* may have a larger biodegradability potential than *Pseudomonas putida* under high concentrations expected in industrial wastewater.

3.3. Light energy efficiency of the cells

It was hypothesized that the presence of pharmaceuticals in the culture medium could modify the light energy efficiency of the cells. In order to verify this hyphothesis, the evolution of the light availability inside the culture and the quantum yield were determined. For an extinction coefficient value of 0.3161 g m^{-2}, the average irradiance inside the PBR (I_{av}) showed a gradual decrease corresponding to the increase of the biomass concentration, which reduce the irradiance available inside the culture (Figure 5). The results displayed a sharp decline during the first days due to the exponential biomass growth phase.Then, at the beginning of the semicontinuous culture, there was a progressive increase of the light available due to the decrease in the biomass concentrationcaused by the destabilization of the culture. Somehow, in the steady state of the semicontinuous cultureI_{av} showed a trend to stabilize throughout time. Therefore, the results showed an inversely proportional behaviour than growth curves(Figure 1). Despitethere were nothardly light in the central point of the PBR in the maximum growth phase (day 7 for C+ and PC; day 12 for SaC), the aeration allowedthe movement of the cells, which soalternate their position between illuminatedand dark areas.These results are in accordance with those obtained by Gómez et al. [19] for a *Muriellopsis*sp. culture, with anI_{av} value of 43 µE m^{-2} s^{-1} under semicontinuous operation with anI_o value of 1850 µE m^{-2} s^{-1}, which means an irradiance reduction of about 97%.

Results obtained during the steady state of the semicontinuous culture respect to the biomass generated by the unit of radiation (Ψ_E),are displayed in the Figure 6.There were significant differences ($p \leq 0.05$) among the $\Psi_E$determined for SaC and for the two other treatments, the Ψ_E corresponding to salicylic acid being 1.4 times higher. This evidences that the salicylic acid treatment produces higher biomass by unit of radiation

than the control and the paracetamol treatment. In any case, the here determined Ψ_E mean a higher efficiency than those obtained by Gómez et al. [19], whose I_o were 5 times higher than those here supplied.

The removal yield(Ψ_R)results during the steady state of the semicontinuous culture showed significant differences ($p \leq 0.05$) among the treatments with PC(1.72 ± 0.03 $g_{removed}$ E^{-1}) and SaC (2.45 ± 0.01 $g_{removed}$ E^{-1}). Despite these differences, the relationship between the quantum yield and the removal yield of each pharmaceutical showed a similar value, of 2.37 ± 0.10 for PC and of 2.72 ± 0.06 for SaC.Thisconfirms that the removal of pharmaceuticals was directly related to the microalgae productivity.

4. Conclusions

An increased biomass productivity of *C. sorokiniana* occurred under the presence of a relative high initial concentration of either salicylic acid or paracetamol (250 mg l^{-1}). Therefore it may be concluded that *C. sorokininana* was able to use these pharmaceuticals as an additional source of organic carbon. Consequently, in the batch culture, these pharmaceuticals were removed from the medium, although the salicylic acid removal rate was much quicker than that of paracetamol. Also, in the semicontinuous culture, both the volumetric efficiency and the light energy efficiency of cells were higher for salicylic acid than for paracetamol. In addition, the light available inside of the culture only had influence on the microalgae productivity, not on the removal of pharmaceuticals. The obtained results point to the promissory application of, *C. sorokiniana* as bioremediation system for the removal of paracetamol and salicylic from concentrated wastewaters.

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FIGURE CAPTIONS

Figure 1 Growth curves of *Chlorella sorokiniana* for: C+, PC and SaC. Dots correspond to experimental data and continuous lines correspond to fittings by the logistic kinetic model during batch culture. Note: experimental points obtained during semicontinuous culture are connected with dashed lines

Figure 2 Volumetric efficiency in the removal of paracetamol and salicylic acid of *Chlorella sorokiniana* during bath culture. Dots correspond to experimental data and continuous lines correspond to fittings by the logistic kinetic model

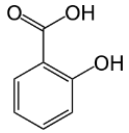
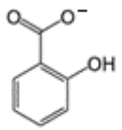
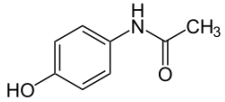
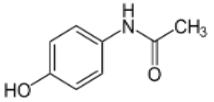
Figure 3 Relationship between the growth kinetics of *Chlorella sorokiniana* and pharmaceuticals removal kinetics in the batch culture

Figure 4 Volumetric efficiency in the removal of paracetamol and salicylic acid of *Chlorella sorokiniana* during the steady stage of the semicontinuous culture.

Figure 5 Average irradiance inside the culture of *Chlorella sorokiniana*

Figure 6 Quantum yield of *Chlorella sorokiniana* during the steady stage of the semicontinuous culture

Table 1. Physico-chemical properties of the pharmaceuticals used in this study

Pharmaceutical (formula)	Structure	Mw(g mol^{-1})	Sw(mg L^{-1})	pKa	log D	PSA (A^2)	$DS_{pH=7.5}$	
Salicylic acid ($C_7H_6O_3$)		138.12	2,240	2.97	1.98	57.5		100%
Paracetamol ($C_8H_9NO_2$)		151.16	14,000	9.48	0.91	49.3		99.4%

Source:http://www.chemspider.com/, last access: 17 April 2015

Abbreviations:

Mw = molecular weight

Sw = water solubility (25ºC)

$$pKa = pH + \ log_{10} \frac{[conjugated\ acid]}{[conjugated\ base]}$$

$$\log D = \frac{[solute]_{octanol}}{[solute]_{water}^{un-ionized}}$$

PSA= Polar surface area

$DS_{pH=7.5}$ = Dominant species at pH=7.5

Table 2. Logistic model kinetic parameters (*K*, *a*, *r*) of *Chlorella sorokiniana* growth and experimental data of their growth (Cb_o, Nc_o, Cb_m, Nc_m)

	C+	PC	SaC
Cb_0 (g l^{-1})	0.1387 ± 0.0000	0.1387 ± 0.0000	0.1387 ± 0.0000
Nc_0 (cell ml^{-1})	1.70 $x10^7$± 0.00	1.70 $x10^7$± 0.00	1.70 $x10^7$± 0.00
Cb_m (g l^{-1})	1.4097 ± 0.2697	1.7944 ± 0.1403	2.7515 ± 0.0835
Nc_m (cell ml^{-1})	2.03$x10^8$ ± 4.41$x10^7$	1.63$x10^8$ ± 6.01$x10^7$	3.84$x10^8$ ± 5.30$x10^6$
K (g l^{-1})	1.6460 ± 0.2501	1.8953 ± 0.0960	3.0221 ± 0.0345
a	2.9561 ± 0.1081	3.1113 ± 0.2131	4.1279 ± 0.2679
r (d^{-1})	0.7085 ± 0.0160	0.9467 ± 0.1334	0.5437 ± 0.0232
R^2	0.9841	0.9812	0.9928

Abbreviations: Cb_o= initial biomass; Nc_o= 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. Logistic model kinetic parameters (*K, a, r*) of *Chlorella sorokiniana* for the removal of pharmaceuticals in the batch culture. Volumetric efficiency and specific efficiency in the steady state of the semicontinuous culture

	PC	SaC
K (g l^{-1})	146.4567 ± 41.0142	234.3790 ± 0.6895
a	3.9833 ± 1.2664	14.8993 ± 0.0477
r (d^{-1})	0.9024 ± 0.4508	4.5736 ± 0.0661
R^2	0.9657	0.9990
Volumetric efficiency (mg l^{-1} d^{-1})	52.0806 ± 1.2273	74.2383 ± 0.4576
Specific efficiency (mg g $biomass^{-1}$ d^{-1})	101.1646 ± 9.0143	99.6191 ± 16.8436

Abbreviations: *K*= carrying capacity; *a*= constant of logistic kinetic model; *r*= pharmaceutical removal rate; R^2= correlation coefficient

Figure 1

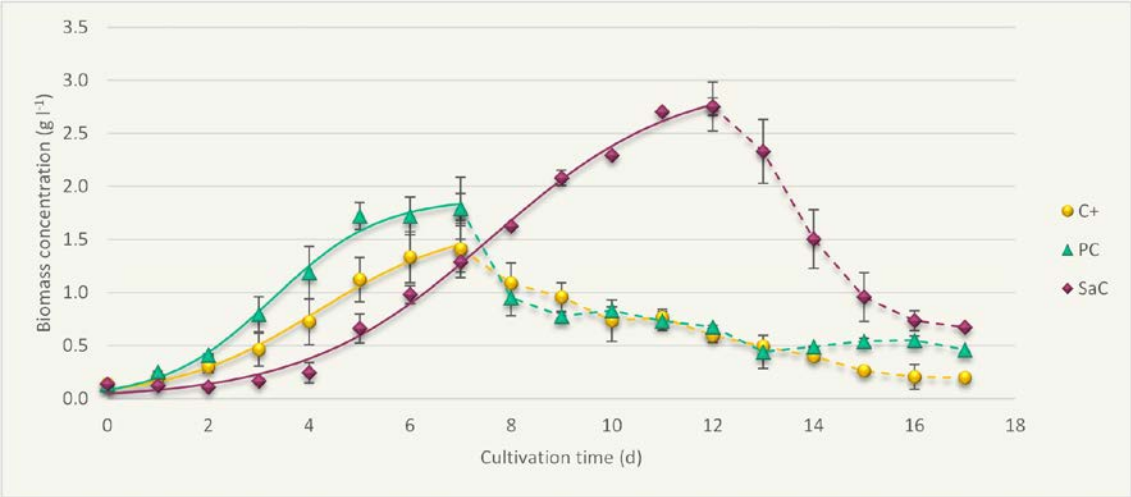

3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
Biomass concentration (g l-1)
0
2
4
6
8
10
12
14
16
18
Cultivation time (d)
C+
PC
SaC

Figure 2

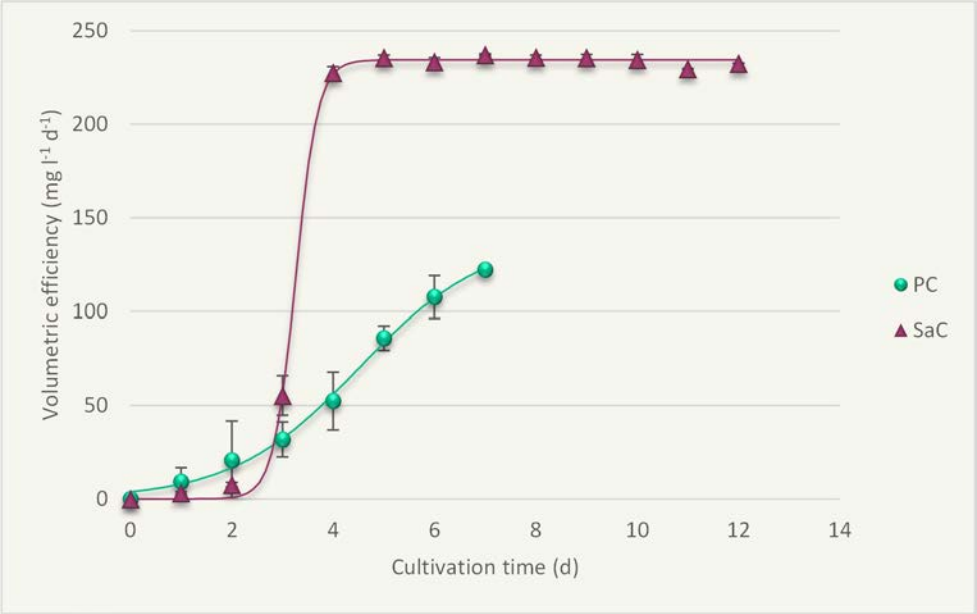

250
200
150
100
50
0
Volumetric efficiency (mg l-1 d-1)
0
2
4
6
8
10
12
14
Cultivation time (d)
PC
SaC

Figure 3

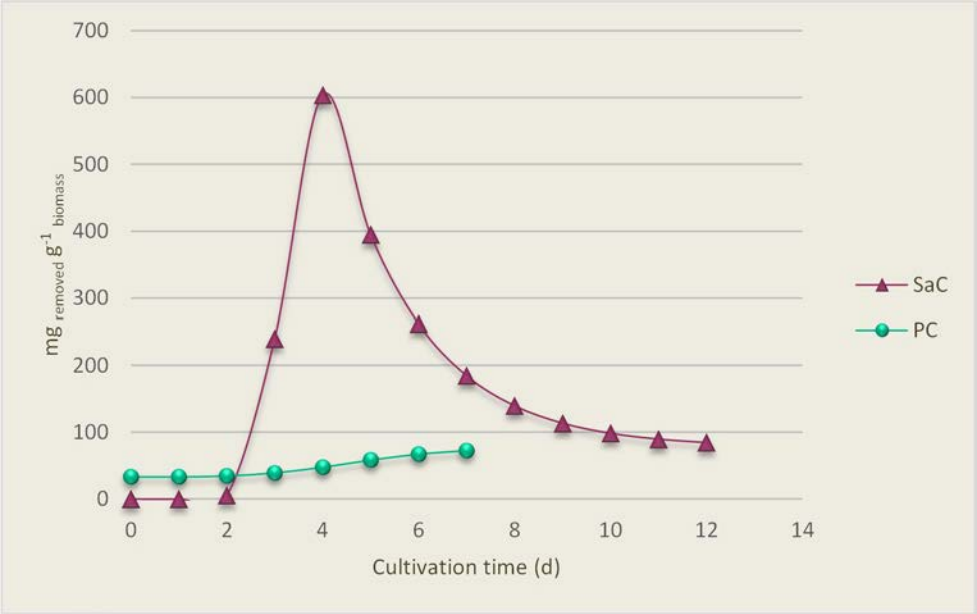

700
600
500
400
300
200
100
0
mg removed g-1 biomass
0
2
4
6
8
10
12
14
Cultivation time (d)
SaC
PC

Figure 4

Figure 5

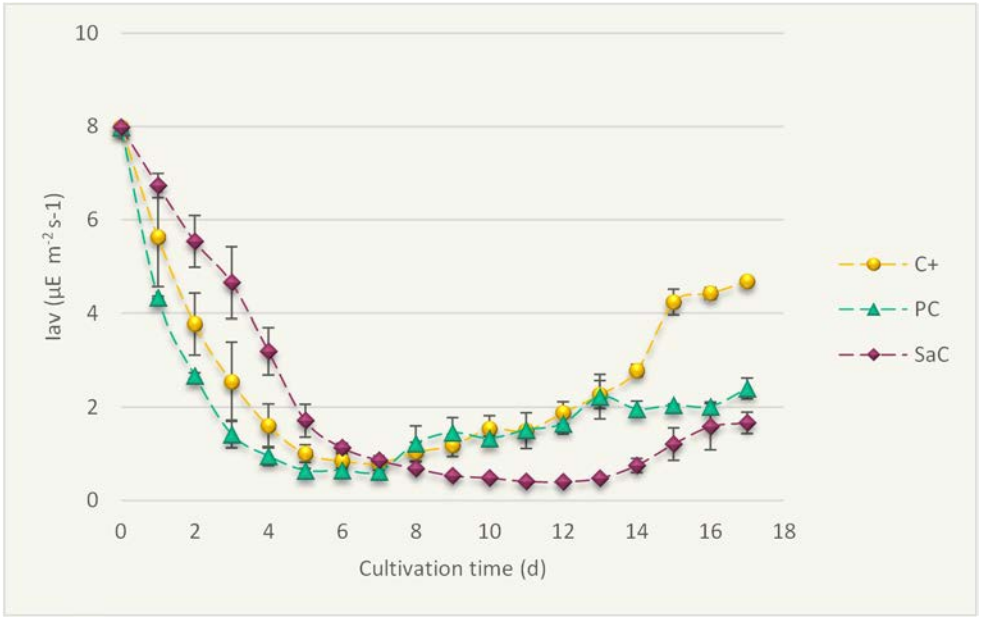

10
8
6
4
2
0
0
2
4
6
8
10
12
14
16
18
Iav (µE m-2 s-1)
Cultivation time (d)
C+
PC
SaC

Figure 6