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Utilization of Non-Living Microalgae Biomass from Two Different Strains for the Adsorptive Removal of Diclofenac from Water

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Abstract: In the present work, the adsorptive removal of diclofenac from water by biosorption onto non-living microalgae biomass was assessed. Kinetic and equilibrium experiments were carried out using biomass of two different microalgae strains, namely *Synechocystis* sp. and *Scenedesmus* sp. Also, for comparison purposes, a commercial activated carbon was used under identical experimental conditions. The kinetics of the diclofenac adsorption fitted the pseudo-second order equation, and the corresponding kinetic constants indicating that adsorption was faster onto microalgae biomass than onto the activated carbon. Regarding the equilibrium results, which mostly fitted the Langmuir isotherm model, these pointed to significant differences between the adsorbent materials. The Langmuir maximum capacity (Q_{max}) of the activated carbon (232 mg·g^{-1}) was higher than that of *Scenedesmus* sp. (28 mg·g^{-1}) and of *Synechocystis* sp. (20 mg·g^{-1}). In any case, the Q_{max} values determined here were within the values published in the recent scientific literature on the utilization of different adsorbents for the removal of diclofenac from water. Still, *Synechocystis* sp. showed the largest K_L fitted values, which points to the affinity of this strain for diclofenac at relative low equilibrium concentrations in solution. Overall, the results obtained point to the possible utilization of microalgae biomass waste in the treatment of water, namely for the adsorption of pharmaceuticals.

Keywords: emerging contaminants (ECs); sorption; wastewater treatment; bioremediation; algae

1. Introduction

Microalgae are photosynthetic microorganisms capable of using CO_2 as a carbon source. Thus, as the accumulation of CO_2 in the atmosphere is one of the most serious environmental issues to be faced nowadays, the possibility of using microalgae for its sequestration has received great attention [1]. Still, the implementation of CO_2 sequestration by microalgae is mostly limited by techno-economic constrains [2]. An option to increase the cost-effectiveness is the cultivation of microalgae in wastewater, which is a complex mixture that may serve as a source of nutrients and water [3]. This strategy allows for nutrient recycling with savings in microalgae cultivation costs and, simultaneously contributes to enhancing the sustainability of wastewater treatment [4,5].

Interest in microalgae-based wastewater treatment has increased in recent years since, while growing, these microorganisms are able to uptake pollutants like nutrients [6] and trace metals [7], but also emerging contaminants (ECs) such as pharmaceuticals [8–10]. The latter represent an especially worrying class of contaminants since they were designed to provoke a physiological response and their presence in the aquatic environment may affect non-target individuals. Among the different treatments proposed for the removal of pharmaceuticals from wastewater, microalgae-based systems have been proved to be effective either in close [8] or open [11] systems. Whatever the system configuration, biodegradation, together with bioadsorption and bioaccumulation, have been indicated as the main mechanisms for the removal of pharmaceuticals from wastewater [5].

Comparatively with research on the uptake of pharmaceuticals by growing microalgae in wastewater, the utilization of non-living microalgae biomass for the adsorptive removal of these pollutants is still in its early stages [12,13]. That is not the case of the well-known adsorption capacity of microalgae to remove other pollutants such as metals [14,15] or dyes [16]. Still, in the case of pharmaceuticals, a main advantage of the application of adsorption processes for their removal is that transformation products, which may be generated during treatments involving degradation [17,18], are not produced. On the other hand, the utilization of the residual microalgae biomass for the adsorption of pollutants from water following the extraction of lipids, has been pointed to as a feasible zero-waste strategy to improve the sustainability of microalgae cultivation [13].

In this context, the aim of this work was to study the adsorptive removal of diclofenac by non-living microalgae biomass of two different strains, namely *Scenedesmus* sp. (Chlorophyceae) and *Synechocystis* sp. (Cyanophyceae). For comparison purposes, a commercial activated carbon was used as a reference under the same experimental conditions as microalgae biomass. Diclofenac was selected as target pharmaceutical since it is a nonsteroidal anti-inflammatory drug (NSAID), it is widely consumed, it is one of the pharmaceuticals most frequently present in effluents from sewage treatment plants [19], and it is potentially toxic towards several organisms such as fish and mussels [20]. Moreover, concern about the presence of diclofenac in the aquatic environment has led to its inclusion in the first watch list (EU Decision 2015/495) to support future revisions of the list of priority substances within the Water Framework Directive (2000/60/EC) (WFD) [21].

2. Materials and Methods

2.1. Microalgae and Culture Conditions

Microalgae from two different genera were used in this work: (i) *Scenedesmus* sp. (SAG 276-1), which was purchased from the *Sammlung von Algenkulturen der Universität Göttingen* (Culture Collection of Algae at Göttingen University, international acronym SAG); and (ii) *Synechocystis* sp., which was isolated from natural freshwater in the surroundings of the province of León [22]. It is to note that the term microalgae was here used in a wide sense, since Cyanophyceae (commonly known as blue green algae) have prokaryotic cell structure like bacteria and, because of that, have also been named as cyanobacteria. An inoculum of each strain was maintained in Erlenmeyer flasks (250 mL) containing the standard medium Mann and Myers [23] and kept inside a vegetal culture chamber under controlled growth conditions: temperature (25 ± 1 °C), irradiance (175 μmol photons $m^{-2} \cdot s^{-1}$), photoperiod (12:12) and shaking (250 rpm). Then, the cultures were grown in bubbling column photobioreactors (PBRs) with an operation volume of 9 L. PBRs were kept in vegetal culture chambers under controlled conditions, namely at 27–30 °C, 16:8 photoperiod of light:darkness, and irradiance of 650 $\mu E \cdot m^{-2} \cdot s^{-1}$. The microalgae cultures were aerated with filtered air (0.22 μm sterile filters, Millex FG50 Millipore (Merck Millipore, Burlington, MA, USA)) at 0.3 v/v/min. Air was enriched with CO_2 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.

2.2. Adsorbent Materials and Adsorption Experiments

For the two different strains, the cellular suspension from each of the aforementioned cultures was centrifuged (7800 rpm, 7 min) to separate microalgae biomass from the culture medium. Then, the biomass was washed twice with distilled water, frozen and lyophilized. Before its use as a biosorbent, the lyophilized biomass was grinded and homogenized. For comparison purposes, a commercial activated carbon (PULSORB WP260 (Chemviron Carbon, Feluy, Belgium)), which was generously provided by Chemviron Carbon, was used in this work.

Diclofenac sodium ($C_{14}H_{10}Cl_2NNaO_2$, ≥99%) (Sigma-Aldrich, Madrid, Spain) was used in the adsorption experiments. The concentration of diclofenac in liquid phase was analyzed by a Waters HPLC 600 equipped with a 2487 Dual λ Absorbance Detector (Waters Corporation, Milford, MA, USA), a Phenomenex C18 column (Phenomenex España S.L.U., Madrid, Spain), (5 μm, 110 Å, 250 × 4.6 mm), a Rheodyne injector (Waters Corporation, Milford, MA, USA), and a 50 μL loop (Waters Corporation, Milford, MA, USA). The detection wavelength was 276.5 nm and the mobile phase consisted of acetonitrile:water:orthophosphoric acid (70:30:0.1, v/v/v), which was pumped at 1 mL·min^{-1}. For the mobile phase preparation, 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. Before use, the mobile phase mixture was passed through a Millipore filter (0.45 μm) and degassed by ultrasound application during 30 min. On the other hand, all the samples were centrifuged at 7500 rpm for 10 min (SIGMA 2-16P centrifuge (Sigma Laborzentrifugen GmbH, Osterode am Harz, Germany), before analysis.

The adsorption experiments were carried out under stirring and batch operation following a parallel approach (a reactor was run by triplicate for each desired time and/or adsorbent mass). Reactors were Erlenmeyer flasks (100 mL) containing a volume (V) of 50 mL of solution with a known initial concentration (C_i) of adsorbate, namely diclofenac, together with a known mass (m_{ads}) of each adsorbent. Since the adsorption behavior of an adsorbent towards a certain adsorbate is not known a priori, preliminary test were here settled at different C_i and m_{ads} for each material. These tests aimed at the selection of appropriate asorbent to adsorbate ratios for the subsequent kinetic and equilibrium experiments. The choice of the C_i and the m_{ads} for each material, which are specified in the following sections, was such to ensure: (i) a significant change of the adsorbate concentration in solution through adsorption experiments; and (ii) a final concentration of adsorbate that might be accurately and precisely determined by the analytic methodology used.

2.2.1. Adsorption Kinetics

For each adsorbent, adsorption kinetic experiments were first carried out in order to determine the time necessary to attain adsorption equilibrium (t_e). In each reactor, a diclofenac solution with C_i = 100 mg·L^{-1} was stirred at 250 rpm under controlled temperature (25 ± 1 °C) together with a known m_{ads}. In the case of *Scenedesmus* sp. and *Synechocystis* sp., 0.05 g of biomass were employed whereas 0.005 g of activated carbon were used in kinetic experiments. After stirring during the desired time (t), reactors were withdrawn, and a sample of the liquid phase was analyzed for the residual concentration of diclofenac (C_t). Three replicated reactors were run for each considered adsorbent and time. Furthermore, blanks (adsorbent + distilled water, without diclofenac in the aqueous phase) and controls (diclofenac solution with no adsorbent) were also run in triplicate. Throughout experiments, the pH of the solutions was not fixed at any initial value neither buffered, but stability in the values was observed along the kinetic experiments (7.0 ± 0.5).

At each t, the adsorbed concentration of diclofenac onto each adsorbent (q_t) was determined by a mass balance, as indicated by Equation (1):

$$q_t = \frac{(C_i - C_t)}{m_{ads}} \times V \quad (1)$$

Fittings of the obtained results to the pseudo-first order [24] and the pseudo-second order [25] equations were determined. Both the pseudo-first order (Equation (2)) and the pseudo-second order (Equation (3)) kinetic models are empirical rate equations based on the overall sorption rate:

$$q_t = q_e\left(1 - e^{-k_1 t}\right) \tag{2}$$

$$q_t = \frac{q_e^2 k_2 t}{1 + q_e k_2 t} \tag{3}$$

where k_1 (min^{-1}) and k_2 (g mg^{-1} min^{-1}) are the pseudo-first and the pseudo-second order rate constants, respectively, and q_e is the adsorbed concentration of diclofenac at the equilibrium.

2.2.2. Adsorption Equilibrium

After establishing the t_e from kinetic results, adsorption equilibrium experiments were conducted in order to determine the adsorption isotherms. For this purpose, experiments with different m_{ads} were carried out, each reactor containing 50 mL of a diclofenac solution with C_i = 100 mg·L^{-1}. Reactors were stirred at 250 rpm during the t_e and under controlled temperature (25 ± 1 °C). Equilibrium experiments were run with 0.05 g ≤ m_{ads} ≤ 0.5 g of *Scenedesmus* sp. biomass, 0.05 g ≤ m_{ads} ≤ 1.25 g of *Synechocysitis* sp. biomass, and 0.005 g ≤ m_{ads} ≤ 0.05 g of activated carbon. All the experiments were carried out in triplicate, including the corresponding blanks and controls. In each case, the amount of diclofenac adsorbed at the equilibrium (q_e) was determined as a function of the equilibrium concentration (C_e), according to the following mass balance in equation Equation (4):

$$q_e = \frac{(C_i - C_e)}{m_{ads}} \times V \tag{4}$$

In order to describe the equilibrium isotherms, the fittings of experimental results to the Freundlich [26] and the Langmuir [27] isotherm models, which are respectively expressed by Equations (5) and (6), were determined:

$$q_e = \frac{Q_{max} K_L C_e}{1 + K_L C_e} \tag{5}$$

where Q_{max} is the maximum adsorption capacity of the adsorbent material and K_L is the Langmuir constant, related to the adsorption energy.

$$q_e = K_f C_e^{\frac{1}{N}} \tag{6}$$

where K_f is the Freundlich constant and N is a constant related to the intensity of the adsorption process.

3. Results and Discussion

Controls carried out together with adsorption experiments allowed verifying that diclofenac concentration remained stable throughout the whole duration of the experiments. On the other hand, under the chromatographic operation conditions here used, results from blanks confirmed the absence of analytical interferences by the microalgae biomass or the activated carbon. Therefore, the decrease in diclofenac concentration observed in experiments was expected to be related just to adsorption onto the corresponding material.

The amount of diclofenac adsorbed with time onto biomass of the two microalgae strains considered is shown in Figure 1 together with results obtained for the commercial activated carbon. As can be seen, the adsorbed concentration of diclofenac onto the three adsorbent materials increased with time (t) until reaching the equilibrium. For the tree materials, the equilibrium was attained within 240 min, which was established as t_e.

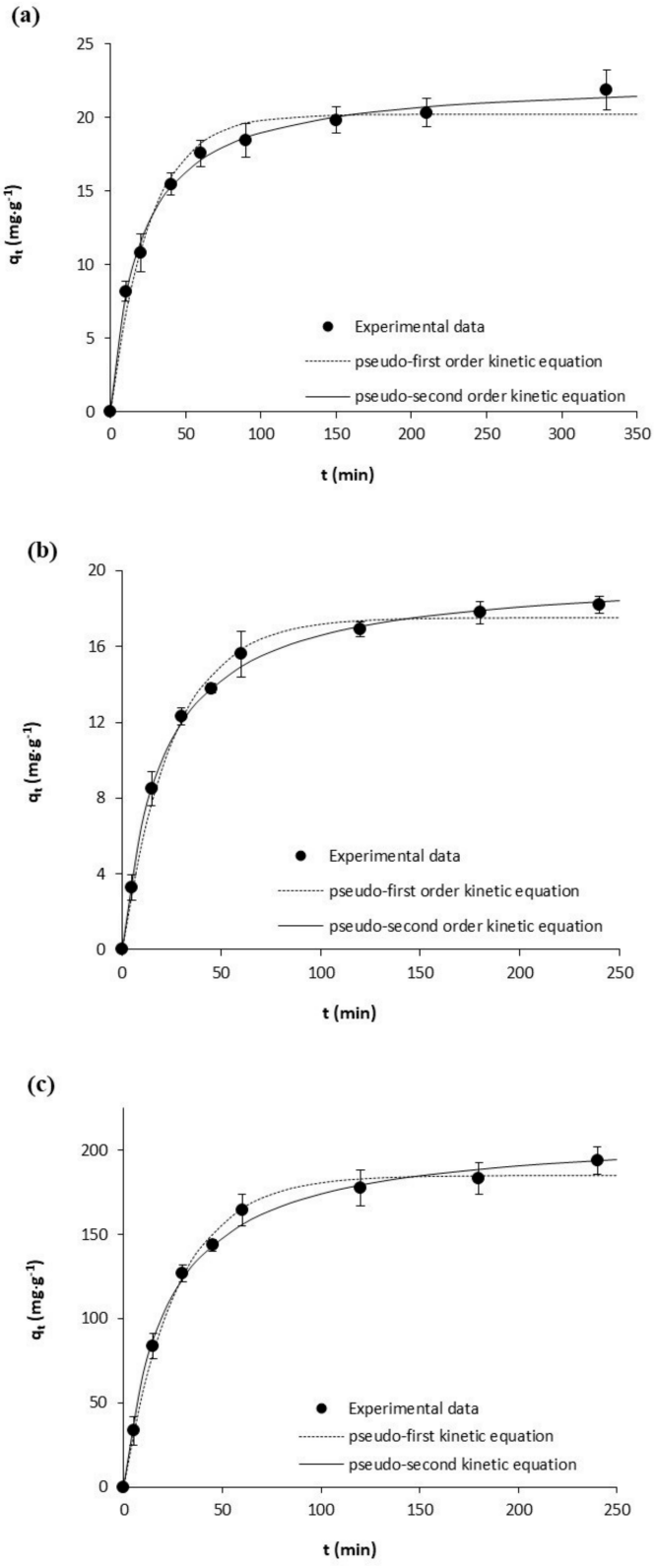

Figure 1. Kinetic results on the adsorption of diclofenac onto (**a**) *Scenedesmus* sp. biomass; (**b**) *Synechocystis* sp. biomass; and (**c**) activated carbon. Experimental data on the adsorbed concentration of diclofenac (q_t, mg·g^{-1}) versus time (t, min) are represented together with fittings to the pseudo-first and pseudo-second order kinetic equations. Notes: Error bars stand for standard deviation (N = 3). The scale of the axis has been adjusted for a better visualization of results.

Fittings of the experimental results to the pseudo-first and pseudo second-order kinetic equations are shown together with the experimental results in Figure 1. The kinetic parameters derived from these fittings are depicted in Table 1. Fittings to both equations were reasonably good, with $r^2 > 0.98$, with the pseudo-second kinetic equation describing results slightly better. Both the kinetic models here considered are based on the adsorbed concentration at the equilibrium (q_e). However, as can be seen in Figure 1, the pseudo-first order model is valid just at the initial stage of adsorption while the pseudo-second model provides good fitting over the whole time range. Hence, in the case of the k_1, values determined for the three materials were not significantly different, which points to the fact that the initial uptake of diclofenac adsorption by the activated carbon and the microalgae biomasses showed a similar rate. Then, differences in the kinetics occurred at a second stage, which was evidenced by the fitted values of the k_2 rate constants. These k_2 were equal for both microalgae strains and larger than that of activated carbon, which indicated that, on the whole, the adsorption kinetic was comparatively faster onto microalgae biomass.

Table 1. Parameters from the experimental results fittings to the kinetic (pseudo-first order kinetic equation and pseudo-second order equation) and equilibrium isotherm (Langmuir and Freundlich equilibrium isotherms) models considered.

Model	Parameter	*Scenedesmus* sp.	*Synechocystis* sp.	Activated Carbon
Kinetic Equations				
Pseudo-first order	k_1 (min^{-1})	0.0388 ± 0.0041	0.0393 ± 0.0024	0.0375 ± 0.0021
	q_e ($mg{\cdot}g^{-1}$)	20.19 ± 0.54	17.55 ± 0.30	184.90 ± 2.98
	r^2	0.981	0.9944	0.9951
	$S_{y.x}$	1.05	0.52	5.16
Pseudo-second order	k_2 ($g{\cdot}m^{-1}{\cdot}min^{-1}$)	0.0023 ± 0.0002	0.0025 ± 0.0002	0.00022 ± 0.00002
	q_e ($mg{\cdot}g^{-1}$)	22.64 ± 0.35	19.90 ± 0.34	210.80 ± 4.50
	r^2	0.9964	0.9968	0.9953
	$S_{y.x}$	0.45	0.40	5.09
Equilibrium Isotherms				
Freundlich	K_F ($mg{\cdot}g^{-1}$ $(mg{\cdot}L^{-1})^{-N}$)	3.48 ± 0.17	5.40 ± 1.01	43.55 ± 7.48
	N	2.36 ± 0.07	3.42 ± 0.61	2.80 ± 0.36
	r^2	0.9989	0.9424	0.9579
	$S_{y.x}$	0.26	1.78	15.23
Langmuir	Q_{max} ($mg{\cdot}g^{-1}$)	28.34 ± 1.19	19.76 ± 0.57	232.20 ± 7.41
	K_L ($L{\cdot}mg^{-1}$)	0.039 ± 0.005	0.143 ± 0.018	0.076 ± 0.007
	r^2	0.9941	0.9919	0.9932
	$S_{y.x}$	0.57	0.66	6.12

Note: r^2—Correlation coefficient; $S_{y.x}$—Standard error of the regression.

The diclofenac adsorption equilibrium isotherms using *Scenedesmus* sp. biomass, *Synechocystis* sp. biomass and activated carbon as adsorbents are shown in Figure 2.

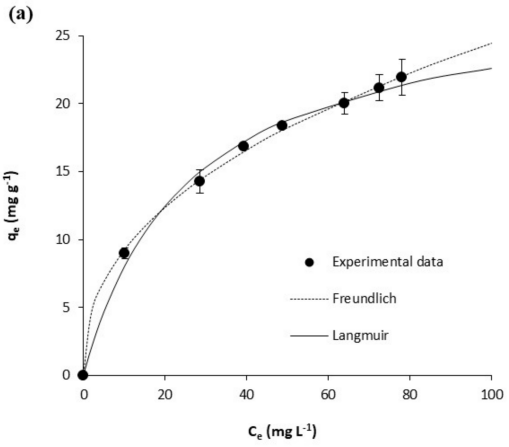

Figure 2. *Cont.*

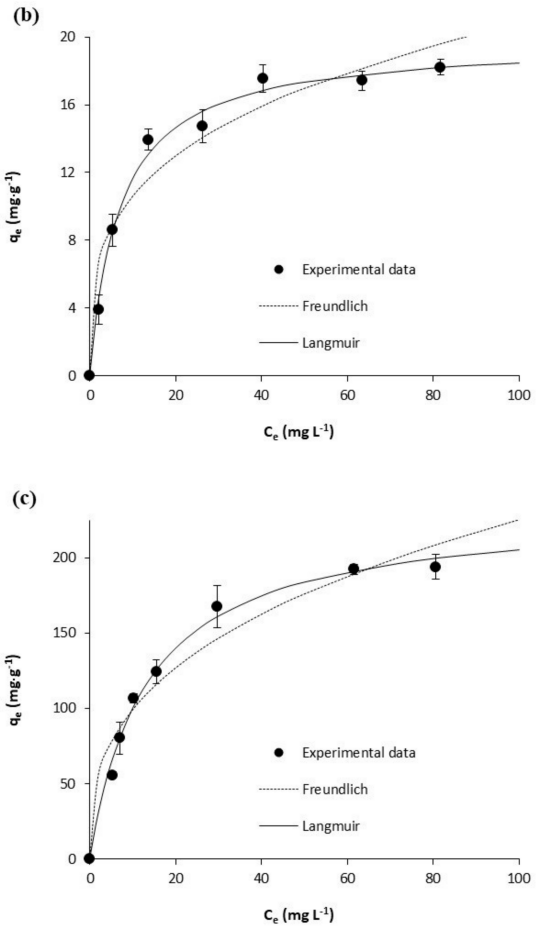

Figure 2. Equilibrium results on the adsorption of diclofenac onto (**a**) *Scenedesmus* sp. biomass; (**b**) *Synechocystis* sp. biomass; and (**c**) activated carbon. Experimental data on the equilibrium adsorbed concentration of diclofenac (q_e, mg·g^{-1}) versus the equilibrium diclofenac concentration in the liquid phase (C_e, mg·L^{-1}) are represented together with fittings to the Langmuir and Freundlich equilibrium isotherm models. Notes: error bars stand for standard deviation (N = 3). The scale of the axis has been adjusted for a better visualization of results.

Fittings of equilibrium experimental results to the Freundlich and Langmuir models are represented in Figure 2, the corresponding fitted parameters being depicted in Table 1.

In the case of diclofenac adsorption onto *Scenedesmus* sp. biomass, equilibrium results fitted the Freundlich and Langmuir isotherm models, with $r^2 > 0.99$ in both cases. However, for both the *Synechocystis* sp. biomass and the commercial activated carbon, equilibrium results were better described by the Langmuir isotherm.

Figure 2 makes evident that, at the equilibrium, the adsorptive removal of diclofenac by the activated carbon used here was larger than that of microalgae biomasses. On the other hand, the diclofenac adsorption capacity of *Scenecesmus* sp. was significantly larger than that of *Synechocystis* sp., which may be confirmed by Q_{max} values in Table 1. According to the Langmuir isotherm model [27], the Q_{max}, which is the maximum adsorption capacity, corresponds to the saturation of a monolayer of adsorbate molecules on the adsorbent surface, that is, when all the adsorption sites of the adsorbent are occupied by adsorbate molecules. Therefore, each adsorbent possesses a unique Q_{max} for each adsorbate and, in wastewater treatment applications, a larger value of Q_{max} implies that the adsorbent material will have a longer useful lifetime. Hence, Q_{max} is used for the prediction of the adsorbent performance in real systems and for the design of adsorbers at different scales [28]. In this work, the Q_{max} determined for activated carbon (232 mg·g^{-1}) was larger than that of *Scenedesmus* sp. and

Synechocystis sp. (28 $mg \cdot g^{-1}$ and 20 $mg \cdot g^{-1}$, respectively). In any case, the here obtained Q_{max} values for the adsorption of diclofenac onto microalgae biomass are higher than those determined for the adsorption of different polyphenols (8 $mg \cdot g^{-1} < Q_{max} < 19$ $mg \cdot g^{-1}$) onto non-living *Chlorella* sp. biomass [29] but lower than for the adsorption of acetaminophen onto *Synechocystis* sp. (52 $mg \cdot g^{-1}$). With respect to other materials used for the adsorptive removal of diclofenac from water, Table 2 shows recently Q_{max} published values for adsorbents of different nature. As may be seen, the range is quite large and comprises the here obtained Q_{max}.

Regarding the K_L, which points to the affinity of an adsorbent towards the adsorbate, the fitted value determined for *Synechocystis* sp. (0.14 $L \cdot mg^{-1}$) is within values obtained for the adsorption of polyphenols onto *Chlorella* sp. (0.09–0.022 $L \cdot mg^{-1}$) [29]. For the commercial activated carbon and *Scenedesmus* sp., the K_L determined was one order of magnitude lower than that of *Synechocystis* sp., as for the steeper isotherm of the latter (Figure 2). Therefore, although *Synechocystis* sp. displayed the smallest value of maximum adsorption capacity, this Q_{max} was attained at relatively low C_e of diclofenac in solution.

Table 2. Maximum adsorption capacities Q_{max} ($mg \cdot g^{-1}$) of different types of adsorbents used for the adsorptive removal of diclofenac from water (single non-competitive adsorption; T: 25 ± 2 °C; pH: 7 ± 2).

Adsorbent	Q_{max} ($mg \cdot g^{-1}$)	Reference
Activated onion skin	134	[30]
Metal azolate framework-6	503	[31]
Activated cork	79	[31]
Pyrolyzed pulp mill sludge	27	[32]
Granular activated carbon	36	[33]
Activated carbon from olive stones	11	[34]
Ionic liquid modified biomass	197	[35]
MIEX® resin	52	[36]
Molecular imprinted polymer	160	[37]
Powder activated carbon	301	[38]
Polymeric resin	39	[38]

To the best of our knowledge, there are not previous records in the literature on the adsorptive different performance of *Scenedesmus* sp. and *Synechocystis* sp. biomass observed in this work. It must be highlighted that, in the present work, microalgae biomass used was not previously modified neither subjected to thermal treatment. Thus, differences between the two strains regarding the adsorption of diclofenac may be related to their cell wall and biochemical composition. In fact, it has already being pointed out that the microalgae cell surface possesses a rich variety of binding possibilities for a whole range of chemical compounds [29].

Microalgae constitute a group of microorganisms that are easy to culture due to their high growth rates and productivities and, therefore, microalgae biotechnological applications are under expansion [29]. Among the strategies to reduce costs associated with the culture of microalgae is the utilization of flue gases as CO_2 supply and wastewater as nutrients and freshwater source [3]. In this way, microalgae could be used for the biosequestration of CO_2 while accomplishing wastewater treatment [39]. In any case, during cultivation, waste microalgae biomass is generated and a use should be given to this biomass within the actual circular economy context. Therefore, the utilization of microalgae biomass as adsorbent may be an option for increasing the sustainability of microalgae culture. Furthermore, such a use is especially interesting since it may be implemented after lipid extraction from non-living microalgae [13]. As diclofenac is among the pharmaceuticals within the first watch list in the European Union (EU) [21], the novel results obtained in this work on its uptake by non-living microalgae biomass point to the possible application of this biomass for the adsorptive removal of this sort of emerging contaminant. Promissory results obtained in the present work show that this is a new line of research that is worth to further exploring. In this sense, future studies are to be done on the application at real systems, in which fixed-bed microalgae adsorbers may be implemented by the immobilization of microalgae biomass. Although there are no published results for the removal

of pharmaceuticals, Saeed and Iqbal [40] immobilized a blue green microalga, namely *Synechococcus* sp. on loofa (*Luffa cylindrical*) sponge for the fixed-bed adsorptive removal of cadmium from water, a strategy that was later adopted by Chen et al. [41], who used *Scenedesmus obliquus* as biosorbent.

4. Conclusions

The microalgae non-living biomass of two different strains, namely *Scenedesmus* sp. and *Synechocystis* sp. was used for the adsorptive removal of diclofenac from water. Kinetic and equilibrium results were compared with those obtained by a commercial activated carbon under identical experimental conditions. Fittings of the kinetic experimental results to the pseudo-second kinetic equation showed that the rate of diclofenac uptake from aqueous solution was similar for both microalgae strains and faster than that of activated carbon. Regarding the equilibrium experimental results, the Langmuir isotherm model described the results for the three adsorbents. The fitted values of the Langmuir maximum adsorption capacity (Q_{max}) were 232, 28 and 20 mg·g^{-1} of diclofenac onto the activated carbon, *Scenedesmus* sp. biomass and *Synechocystis* sp. biomass, respectively. These values are within recently published Q_{max} for the adsorptive removal of diclofenac from water using different adsorbents. Differently from these adsorbents in the literature, microalgae biomass here used was neither modified nor treated, its use as biosorbent being an option to explore in view of a sustainable zero-waste strategy for the culture of microalgae.

Author Contributions: R.N.C., C.E. and M.O. conceived the work and the experimental design; R.N.C., C.E., N.C.V., G.N.-H. and M.O. performed the experiments and chemical analysis; R.N.C., C.E. and M.O. analysed the results and wrote the manuscript. All the authors approved the submitted final version.

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Conflicts of Interest: The authors declare the inexistence of conflict of interest. They also declare that the founding agents did not participate in nor decide on the design of the study, the chemical analysis, the interpretation of results, and the writing and publishing of the manuscript.

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