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Comparative assessment of diclofenac removal from water by different microalgae strains

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ABSTRACT

Diclofenac has recently been included in the first watch list of substances to be monitored in all member states so as to evaluate its future inclusion in the priority substances list by the Water Framework Directive. Therefore, in view of upcoming limitations on diclofenac discharge, the objective of this work was to assess its removal from water by a microalgae-based treatment. Moreover, considering microalgae application in wastewater treatment, it was aimed to verify if their nutrient removal capacity was affected by the presence of diclofenac. For a comparison purpose, three different microalgae strains, namely *Chlorella sorokiniana*, *Chlorella vulgaris* and *Scenedesmus obliquus*, were cultured in photobioreactors under identical controlled conditions. For the three strains, the addition of diclofenac meant an organic carbon source and provided higher biomass concentration. *C. sorokiniana* was the strain showing the largest increase of growth rate and microalgae density, which were above 25% and 31%, respectively, compared with the positive control. However, *S. obliquus* showed the highest efficiency in the removal of diclofenac (>79%) and nutrients (>87% nitrates, >99% phosphates) per litre and per gram of biomass. These results pointed to the potential application of microalgae for the removal of pharmaceuticals in the bioremediation of wastewater.

1. Introduction

The occurrence of micropollutants in the aquatic environment has become, during the last few decades, a global issue of increasing environmental concern. Micropollutants, commonly termed as emerging contaminants (ECs), consist of a vast and expanding array of anthropogenic as well as natural substances that are not currently covered by existing water regulations but are thought to be a threat to environmental ecosystems and human health [1]. Among ECs, pharmaceuticals and personal care products (PPCPs) 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], including humans [4]. This concern 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 ECs, namely diclofenac, 17-beta-estradiol (E2) and 17-alpha-ethinylestradiol (EE2) in the list of priority substances. However, by the Directive 2013/39/EU, these pollutants were finally included in the watch list of substances to be monitored in all member states to support future reviews of the priority substances list.

Diclofenac (2-(2-(2, 6-dichlorophenylamino)phenyl)acetic acid) is a non-steroidal anti-inflammatory drug (NSAID). It is sold as oral tablets or as a topical gel under the commercial names Acoflam, Algosenac, Almiral, Ana-Flex, Anthraxiton, Antiflam, Arcanafenac, Arthrex, Arthrifen, Arthtotec, Diclabeta, Diclac, Dicloabac, Diclodoc, Diclofenac–Ratiopharm, Diclofenbeta, Diclomex, Diclowal, Dicuno, Difen, Diklotab, Dolgit–Diclo, Eese, Effekton, Jutafenac, Monoflam, Motifene Dual, Rewodina, Sigafenac and Voltaren [5]. It is among the most consumed drugs in the world, although its consumption varies between and within countries from 195 to 940 mg per inhabitant and year [5]. Part of the consumed diclofenac is excreted in its original form so entering municipal wastewater, where its concentration reflects its consumption by the residents in the specific sewer system [5].

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 <5–40% [6]. In fact, STPs were not originally designed for the removal of pharmaceuticals due to the nonexistence of limiting regulations on their discharge [7,8]. Consequently, STPs are important sources of such pollutants in the aquatic environment [1,4]. In this regard, Verlicchi et al. [9], who reviewed the occurrence of 118 pharmaceuticals in the influent and

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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 [9]. The removal efficiencies of diclofenac in conventional STPs have been reported to be about 17% [10], which translates into relative high concentrations in the respective effluents [11]. Furthermore, an increase of pharmaceutical concentrations in receiving waters during summer due to the relative higher contribution of STPs' discharge to the river water flow may be expected [12].

The European Union (EU) has recognized the necessity of developing a strategic approach to water pollution by pharmaceutical substances, stating that until 2017 the Commission shall “propose measures […] with a view to reducing discharges, emissions and losses of such substances into the aquatic environment, taking into account public health needs and the cost-effectiveness” (Directive 2013/39/EU). Therefore, given social and political concerns at the EU about pharmaceuticals, and, specifically about diclofenac, it is expectable that legislation on its discharge will come out in the near future. However, research on their removal is at a very incipient state with most works on alternative to conventional treatments having been published within the last 5 years. Recently, Rivera-Utrilla et al. [13] published a review on treatments for the elimination of pharmaceuticals from water, and, in the actual context, they highlighted the necessity of studying and developing sustainable solutions. With this purpose, microalgae-based wastewater treatment technologies have emerged as a promising option [14–16].

A main advantage of microalgae application in wastewater treatment is that wastewaters constitute a nutrient source for microalgae [17–19] and one of the priority objectives in a wastewater treatment is the removal of high concentrations of nitrates and phosphates to meet the European Commission Directive 98/15/EEC requirements. Thus, microalgae wastewater treatment may be an option to reduce the costs associated to the production of microalgae based biofuels and/or CO_2 consumption [20,21]. In addition, the microalgae phytoremediation potential is well known and the feasibility of cultivating microalgae in wastewaters to remove organic carbon and inorganic nutrients have been extensively studied [18,19,22–24]. Among microalgae, the most studied genuses for wastewater treatment are *Chlorella* and *Scenedesmus* [17].

However, very few studies have been carried out on the performance of different species for the removal of pharmaceuticals. In this sense, Peng et al. [25] studied the biotransformation of progesterone and norgestrel in aqueous solutions by *Scenedesmus obliquus* and *Chlorella pyrenoidosa*; Escapa et al. [15] proved the capacity of *Chlorella sorokiniana* to remove salicylic acid and paracetamol from aqueous solution; and Hom-Díaz et al. [26] studied the removal of E2 and EE2 from anaerobic digester centrate by *Selenastrum capricornutum* and *Chlamydomonas reinhardtii*. However, to our best knowledge the removal of diclofenac from water by different microalgae species has not been assessed yet. Thus, the present study focuses on the evaluation and comparison of the removal of diclofenac from water by three different microalgae species, namely *Chlorella sorokiniana*, *Chlorella vulgaris* and *Scenedesmus obliquus* (homotypic synonymous of *Acutodesmus obliquus*).

2. Experimental

2.1. Microorganism and culture conditions

The microalgae strains used in this study were *Chlorella sorokiniana CCAP 211/8 K* (spherical cells with 3–5 μm diameter) from UTEX Culture Collection of Algae, *Chlorella vulgaris SAG 221-12* (spherical cells with 3–5 μm diameter) from SAG Culture Collection of Algae and *Scenedesmus obliquus SAG 276-1* (ovoid cells with 5–10 μm diameter) from SAG Culture Collection of Algae (Fig. 1). These microalgae strains are among the most commonly used for wastewater treatments, have high growth rates and are able to grow under a wide range of conditions [17], which motivated their choice for this study.

The corresponding inoculum of each strain was cultivated in 250 ml Erlenmeyer flasks in the standard culture medium proposed by Mann and Myers [27], which is composed of (per litre of distilled water): 1.2 g $MgSO_4 \cdot 7H_2O$, 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 $FeSO_4 \cdot 7H_2O$, 1.4×10^{-3} g $MnCl_2$, 3.3×10^{-4} g $ZnSO_4 \cdot 7H_2O$, 7.0×10^{-6} g $Co(NO_3)_2 \cdot 6H_2O$, and 2.0×10^{-6} g $CuSO_4 \cdot 5H_2O$. The inoculum was kept inside a vegetal culture chamber, where growth occurred under controlled temperature

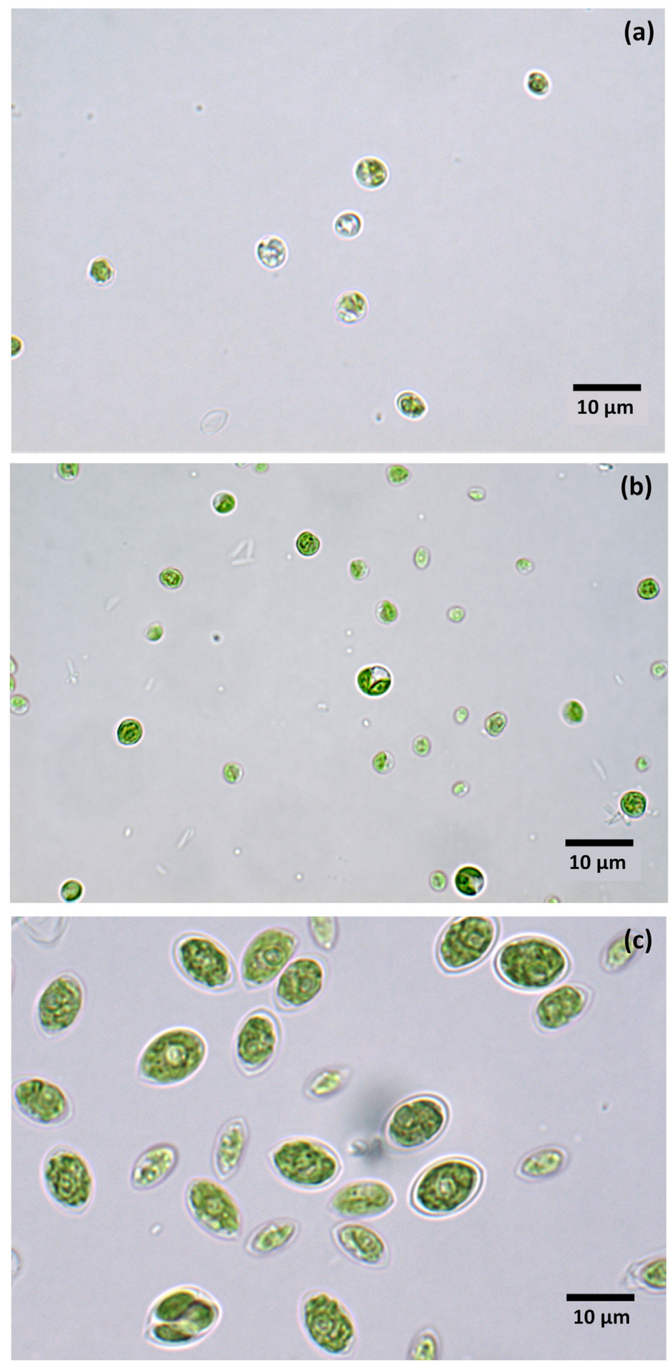

Fig. 1. Microscopic images of microalgal cells of *Chlorella sorokiniana* (a), *Chlorella vulgaris* (b) and *Scenedesmus obliquus* (c).