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Bioremediation of Cephalexin with non-living *Chlorella* sp., biomass after lipid extraction

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ABSTRACT

In this work, the removal of the Cephalexin by *Chlorella* sp., nonliving modified by extraction of lipids was evaluated. First, the microalga was grown to completing 20 days and later, the biomass of crop was centrifuged and the extraction of lipids was performed. Two adsorption experiments were performed: (1) with nonliving *Chlorella* sp. (control), and (2) the obtained biomass after lipid extraction. The high antibiotic removal, 71.19% and 82.77% (control), were obtained at the lowest initial concentration. The contact time between the biosorbent and the antibiotic was 2 h. The adsorption isotherm follows the Freundlich model and the obtained maximum absorption capacity was 63.29 mg of antibiotic/g of biosorbent for lipid-extracted biomass, while the control follows best to the Langmuir model with 129.87 mg/g in maximum absorption capacity. In summary, this biosorbent provides a potential alternative in the removal of Cephalexin.

1. Introduction

The use of antibiotics in agroindustry allows the treatment of different animal diseases (Alexandrino et al., 2017; Srinivasan et al., 2014). The manure from these treated animals increases the population of antibiotic-resistant bacteria in soil and crops (Marti et al., 2013). All antibiotics carry a potential risk on human health (Becerra-Castro et al., 2015; Gros et al., 2013), although their specific effects are unknown (Azanu et al., 2016).

The excessive use of antibiotics increased their presence in water sources, affecting the aquatic ecosystems and the quality of water (Andersson and Hughes, 2012; Gonzalez Ronquillo and Angeles Hernandez, 2017). In Spain, Estevez et al. (2012) found levels above 100 ng/L in irrigation water. Moreover, Rodríguez-Mozaz et al. (2015) reported values up to 131 ng/L in the Ter river in Girona.

These drugs generate more resistant bacteria (Bouki et al., 2013; Rodríguez-Rojas et al., 2013; Xu et al., 2015), especially in wastewater treatment plants (Everage et al., 2014; Huang et al., 2015b; Naquin et al., 2015). These engineering structures are not designed to eliminate antibiotics nor these microorganisms (Zhang et al., 2016). The concentrations of pharmaceutical products between 0.006 and 1.48 μg/L were found in the affluent of a wastewater treatment plant in Czech Republic, while values of 0.003–0.93 μg/L were found in the effluent (Golovko et al., 2014).

Additionally, water purification is affected by antibiotic-resistant bacteria in water sources (Wang et al., 2017). A concentration of antibiotic of 1.08 μg/L have been detected in the Pearl river, China (Huang et al., 2015a). These drugs have also been found in tap water of Guangzhou and Macao, with values of 1.0–679.7 ng/L and 2–37 ng/L respectively (Yiruhan et al., 2010). In Spain, the presence of 35 drugs with concentrations up to 1.20 μg/L was determined in the raw water of a drinking water plant (Huerta-Fontela et al., 2011). Therefore, the remediation of antibiotics from agroindustrial wastes become necessary in order to avoid introducing high concentration of these contaminants in water sources.

Different bioelectrochemical processes, such as magnetic ion exchange resin (MIEX), membranes, activated carbon, have been used to remove antibiotics from the wastewater (Genç and Dogan, 2015; Wang et al., 2017; Zhang et al., 2016). Other techniques, such as coagulation, flocculation, sedimentation, ultraviolet irradiation, activated sludge have been inefficient (Huerta-Fontela et al., 2011; Ma et al., 2015).

Nowadays, there are environmentally friendly methods for the treatment of wastewater from the field activities. Adsorption is an effective process to remove antibiotics from water (Hao et al., 2012; Wang et al., 2016). Microalgae, as *Chlorella* sp., are used for this purpose due their easy cultivation. These microalgae have been widely used to remove heavy metals (Wang et al., 2016; Zárate et al., 2017), dyes (Li et al., 2011a; Li et al., 2011b; Zhou et al., 2017), as well as carbon dioxide capture (Nascimento and Cabanelas, 2015), and the production of nanoparticles (Subramaniyam et al., 2016). Taking into account the important role of microorganisms degrading antibiotics, their removal by microalgae represents an alternative in the treatment

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of water from agroindustry (Roling and van Bodegom, 2014).

On the other hand, the microalgae's features makes them suitable for the production of biofuels such as biodiesel, bioethanol, biomethane, and biohydrogen (Nautiyal et al., 2017). The volume of waste generated by the extraction of biofuel can be high. It is possible to use this byproduct as biosorbent, adding value to the waste and improving the sustainability of the process (Nautiyal et al., 2017).

The aim of this work is centered on the evaluation of the adsorptive properties of algal biomass in media with high concentrations of antibiotic as the agroindustrial wastes. Therefore, a study of adsorption of Cephalexin on obtained biomass from nonliving *Chlorella* sp., after lipid extraction is presented. A discussion about Freundlich and Langmuir isotherms is carried-out, and the results were compared with reported studies with other substrates.

2. Materials and methods

2.1. Analytical methods

UV Visible spectrometry (Evolution 60S, Thermo Scientific) was used to follow the concentration of Cephalexin. The Cephalexin showed an absorption maximum at 298 nm (See electronic Supplementary material) and it was used for spectrometric measurements.

A stock solution (1000 mg/L) of Cephalexin (Genfar) and 0.1 mol/L of hydrochloric acid (Merck) was prepared. Solutions of 49.17, 95.42, 202.92, 296.67, 394.58 and 482.92 mg/L were obtained by serial dilution in deionized water of the stock solution. The calibration curve was obtained by plotting and fitting the absorbance versus the concentration (mg/L) of the abovementioned solutions – See electronic Supplementary material.

2.2. Preparation of lipid-extracted nonliving Chlorella sp.

The microalgae *chlorella* sp., were cultured in three pre-sterilized 4 L transparent bioreactors. The culture medium contained deionized water enriched with 13 mL of commercial fertilizer Nutrifoliar up to a total nitrogen concentration of 1 mmol/L. Then 170 mL of *Chlorella* sp., solution was added to obtain 0.100 absorbance units at a wavelength of 647 nm (UV–Vis spectrometer Evolution 60S, Thermo Scientific). Finally, sterile water was added up to 2.5 L. Cells were cultured at 27 ± 2 °C by 20 days with constant stirring using air pumps with flow 2.5 L/min (Power Life) and photoperiods day/night of 24 h with luminous intensity of 86.2 ± 5 μmol/m^{2}s (led lamps Ingeolux).

With the aim of obtaining the nonliving microalga; the light source was removed and the aeration suspended in one of the bioreactors after 20 days of culture, guaranteeing the death of the microalgae. After two days, the biomass was separated by decantation and centrifugation (Rotofix 32 A, Hettich) at 4000 rpm by 5 min. Finally, the *Chlorella* sp., was washed with distilled water. This procedure was repeated until a wet mass of 70 g was obtained, and dried in a drying and heating oven (FD 115-UL, Binder) at 60 °C for 18 h.

The total lipid amount from nonliving algae was extracted by a two-step procedure. In the first step, a modification of the protocol described by Bligh and Dyer (1959) and Guo et al. (2015) was performed. 10 mL of a chloroform-methanol (Merck) mixture in a 2:1 v/v ratio were added to 50 mg of dry seaweed and sonicated (VWR B1500A-MT, Ultrawave) for 1 h. The obtained extract was homogenized for 30 s after the addition of 0.9% w/v NaCl (Panreac) solution. Finally, the mixture was centrifuged for 8 min at 3000 rpm and the organic phase was filtered (1882-047, Whatman). Subsequently, the method described by Montes D'Oca (2011) was performed with a 150-mL Soxhlet extractor with hexane (Merck) for 5 h (syphon rate 8–10 changes per hour). The residual biomass was extracted and mixed with 150 mL of a 2:1 v/v chloroform:methanol mixture. The temperature was kept within the boiling range of each solvent, 61 °C for chloroform and 65 °C for methanol.

2.3. Adsorption equilibrium and removal of Cephalexin by nonliving Chlorella sp.

Chlorella sp. solutions have a non-zero absorption at 298 nm attributed to chlorophyll *A* and *B*, however this zone is an absorption minimum in the spectrum. In order to evaluate a possible matrix effect, a standard addition experiment was performed. A series of flasks were prepared containing 25 mL of each of the calibration solutions. Three treatments were considered: (1) without microalgae, (2) 50 mg of whole biomass and (3) 50 mg of lipid-extracted biomass. The flasks were stirred for 1 min, centrifuged, filtered and absorbance recorded. An ANCOVA test revealed no significant effect of the treatments on concentration of Cephaloxine ($p = 0.208$, $\alpha = 0.05$), i.e. there is no differences between the slopes of the three curves.

In this work, the efficiency of the biomass obtained after lipid extraction procedure was evaluated. A control experiment of the adsorption on the biomass without lipid extraction was performed with the aim of evaluating the cost/benefit of the process.

In a 100-mL container, 50 mg of treated dry microalgae and 25 mL of 482.92 mg/L Cephalexin solution were mixed. Then, the containers were then agitated in a digital orbital shaker (Sea Star) at 150 rpm. The absorbance at 298 nm was then recorded until the solution reached the equilibrium, i.e. constant absorbance. Each container was measured in the range of time: 15–300 min for the nonliving lipid-extracted biomass and 15–600 min for control. Finally, the equilibrium time and concentration were determined.

The removal percentage was determined as follow: 25 mL of Cephalexin solution was added to a 100-mL vessel with 50 mg of biosorbent at 27 °C. The vessels were shaken in triplicate at 150 rpm for 2 h. The absorbance at 298 nm was recorded before and after the agitation, and then, the concentrations were determined. These results were fitted to the Langmuir and Freundlich isotherms by least squares. The relation between the Cephalexin concentration and the absorbed quantity by the biosorbent was tested by a multilevel factorial experimental design. The significance of the factors was established by an analysis of variance (ANOVA) ($P < 0.05$) through a statistic program (Statgraphics Centurium II demo version).

The efficiency in the removal of antibiotic from the water was expressed as the percentage of removed antibiotic concentration in relation to the initial concentration:

$$\%Removal = \left(\frac{C_0 - C}{C_0}\right) * 100 \tag{1}$$

where, C_o is the initial concentration and C is the antibiotics concentration.

3. Results and discussion

3.1. Growth of Chlorella sp.

Fig. 1 shows the grown phases of the microorganism. Initially, the adaptation period took 2 days, where the culture medium conditioned the alga with an absorbance of 0.082. It continued an accelerated grown phase of 12 days, reaching the maximum absorbance value of 1.230. In this point, the cells took advantages of aeration, agitation, macronutrients (carbon, nitrogen, phosphorus, etc) and micronutrients (vitamins) duplicating their population in equal time intervals. The stationary phase came when the population density increases. The photosynthetic activity decreases as consequence of unavailability of nutrients and light. Additionally, the generated toxins by the metabolism of microalgae increase in the growth.

The behavior of the curve is similar to that observed by Praveenkumar et al. (2012) and Zárate et al. (2017). The former carried out batch cultures of *Chlorella* sp., while the latter cultured the microalga to use their strain in the heavy metals removal as Hg^{+2}.