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Enhanced wastewater bioremediation by a sulfur-based copolymer as scaffold for microalgae immobilization (AlgaPol)

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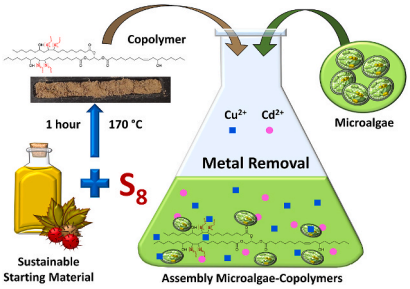
HIGHLIGHTS

- *C. sorokiniana* can tolerate the presence of S/CO copolymers.
- *C. sorokiniana* is able to use S/CO copolymers as support for immobilization.
- Biofilms of *C. sorokiniana*-S/CO copolymers are able to adsorb 50 mg L^{-1} of Cd^{2+}.
- These biofilms also adsorb mixtures of the heavy metals Cu^{2+} and Cd^{2+} (8 mg L^{-1}).
- Adsorption kinetics fit to a pseudo-second-order equation.

GRAPHICAL ABSTRACT



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ABSTRACT

In recent years, there has been an increasing concern related to the contamination of aqueous ecosystems by heavy metals, highlighting the need to improve the current techniques for remediation. This work intends to address the problem of removing heavy metals from waterbodies by combining two complementary methodologies: adsorption to a copolymer synthesized by inverse vulcanization of sulfur and vegetable oils and phytoremediation by the microalga *Chlorella sorokiniana* to enhance the metal adsorption. After studying the tolerance and growth of *Chlorella sorokiniana* in the presence of the copolymer, the adsorption of highly concentrated Cd^{2+} (50 mg L^{-1}) by the copolymer and microalgae on their own and the combined immobilized system (AlgaPol) was compared. Additionally, adsorption studies have been performed on mixtures of the heavy metals Cd^{2+} and Cu^{2+} at a concentration of 8 mg L^{-1} each. AlgaPol biofilm is able to remove these metals from the growth medium by more than 90%. The excellent metal adsorption capacity of this biofilm can be kinetically described by a pseudo-second-order model.

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1. Introduction

A primary concern for public and societal authorities is the contamination of environments, predominantly aquatic ecosystems. The increase of the world population causes higher need of fresh water, while provokes the development of polluting industrial activities and the proliferation of wastewaters (Plöhn et al., 2021b). These wastewaters contain high amounts of toxic compounds; while domestic runoffs are rich in nitrate, phosphate, or ammonium, industrial sewage can additionally contain heavy metals, pharmaceuticals, or polycyclic aromatic hydrocarbons (Ahmed et al., 2021). Their efficient removal of these pollutants is one of the main challenges in ecotoxicological research.

Microalgae emerge as a promising source to solve wastewater contamination. These microorganisms not only take up nutrients like nitrate, ammonium, and phosphate but can also reduce the levels of toxic compounds, such as heavy metals or pharmaceuticals (Gojkovic et al., 2019; León-Vaz et al., 2021a). Their fast growth in a wide range of environmental conditions makes microalgae a promising tool for wastewater bioremediation. Despite the numerous advantages of using photosynthetic microorganisms for industrial applications, the high cost of their cultivation is the main limitation for their development. Immobilization of the microalgae to different supporting materials (generation of an artificial biofilm) would reduce the costs of the process since it simplifies the harvesting of the biomass and improves cell resistance to adverse factors (Cao et al., 2022). Even though activated carbon is one of porous material most widely used as an adsorbent to remove wastewater pollutants ion, its high price makes it unsuitable as a biofilm support material for microalgae (Di Natale et al., 2007). Other carriers, such as Fe_2O_3, graphite, or chitosan, have been used as support for the immobilization of microalgae and studied to remove excess nutrients and soluble heavy metal ions from wastewater (Politaeva et al., 2020; Shen et al., 2020). Using new materials generated from agricultural or industrial wastes, able to form adsorbent materials to immobilize microalgae, would highly improve the sustainability of the process (Namal and Kalipci, 2019; Wang et al., 2021).

A new approach for the generation of sustainable adsorbent copolymers based on sulfur (S_8) and vegetable oils has recently been studied using inverse vulcanization. This technique uses the properties of sulfur obtained from the desulfurization of crude oil to react with olefinic compounds such as vegetable oils (Chung et al., 2013). These copolymers have demonstrated their ability to adsorb heavy metals, especially mercury (Tikoalu et al., 2020; Wadi et al., 2020; Worthington et al., 2017). Natural compounds, such as phenolic compounds, are able to modify the properties of these copolymers, providing them with properties such as increased antioxidant capacity and antimicrobial activity (Cubero-Cardoso et al., 2022b). Moreover, the surface of the material and its physical-chemical properties can be modified, turning these copolymers into excellent carriers of microorganisms and increasing their potential in wastewater treatment.

Combining copolymer and microalga-technology allows to improve the individual remediation efficiency of both remediation technologies and, at the same time, reduces the cost of the processes (Wang et al., 2021; Moreno-Garrido, 2008; Vasilieva et al., 2021). In this work, new biofilm (AlgaPol) composed of the green microalgae *Chlorella sorokiniana* and sustainable copolymers synthesized by inverse vulcanization was studied for its ability to remediate heavy metals. The adsorption of copper and cadmium ions was excellent in AlgaPol biofilm, and kinetic parameters of the process were studied. Therefore, this biofilm is a promising tool for removing contaminants from aquatic environments.

2. Materials and methods

2.1. Synthesis of copolymers

Hybrid copolymers have been synthesized by elemental sulfur (S) from the Cepsa petrochemical company and castor oil (CO) from Sigma-Aldrich. Synthesis of the S/CO: 80/20 (w/w) copolymers was carried out in an oil bath at 170 °C and was stirred with an IKA propeller stirrer and a paddle stirrer for 1 h at a constant temperature to finish the co-monomers conversion, as described by Tikoalu et al. (2020). Furthermore, a copolymer SS/CO was synthesized by adding NaCl as porogen during copolymer generation in a 2:1 mass ratio relative to the initial copolymer mass (Worthington et al., 2018). After finishing the reaction, the SS/CO mixture was washed four times with distilled water to remove excess NaCl.

2.2. Microalgal cultivation

The microalga *Chlorella sorokiniana* 211-32, provided by the *Institute of Plant Biochemistry and Photosynthesis*, was cultured in Tris-Acetate-Phosphate (TAP) medium (León-Vaz et al., 2021b). Microalgal pre-inocula were added to the medium at the initial concentration of 1 g L^{-1} dry weight for the adsorption experiments. The heavy metals $CuSO_4$ and $CdSO_4$ were added to the culture medium, at 50 mg L^{-1} of Cd^{2+} in the single heavy metal experiment, and 8 mg L^{-1} of Cd^{2+} and Cu^{2+} in the mixture experiment, and pH was adjusted to 6 before autoclaving. In each experiment, copolymers were added after autoclavation, and the biofilm alga-copolymer (AlgaPol) was cultured at 27 °C under continuous agitation (150 rpm) and light irradiation (150 μmol m^{-2} s^{-1}).

In tolerance experiments, the concentration of the S/CO and SS/CO copolymers was increased from 10 to 80 g L^{-1}, and 50 g L^{-1} was used for further assays.

2.3. Analysis of chlorophylls

Growth of *C. sorokiniana* was analyzed by measuring its chlorophyll (Chl) concentration. For total Chl determination, 1 mL aliquot of AlgaPol biofilm was sampled and harvested by centrifugation for 2 min at 13,400 rpm. Chl was extracted using 2 mL of methanol, sonicating the samples for 15 min at room temperature and heating the pellet for 15 min at 70 °C. Total Chl concentration was calculated spectrophotometrically using the calculations provided by Lichtenthaler and Buschmann (2001).

2.4. Electron microscopy

A scanning electron microscope (SEM) was used to analyze the morphology of the copolymers and the AlgaPol biofilm. In the case of the copolymers, the samples were covered with graphite using an EMITECH K250X carbon evaporator. The algae cells and copolymers were dehydrated by removing the supernatant with a centrifuge and increasing 30–100% ethanol concentrations each 30 min.

The captures were acquired using a FEI-QUANTA 200 Environment ESEM-EDS scanning electron microscope. Equipment worked under-voltage conditions of 20 Kv at a high vacuum, with Spot 4 at a working distance of 10 mm using the secondary electron detector (Everhardt Thornley Detector, ETD) to see the surface image.

2.5. FTIR characterization

Fourier Transform Infrared Spectroscopy (Buker IFS 66 v/S, DLATGS detector) was carried out on AlgaPol biofilm to characterize the presence of microalgal biomass in filtered copolymers. For these measurements, *C. sorokiniana* was cultured in a TAP medium as described in 2.2. The copolymers were added at a concentration of 50 g L^{-1} in 50 mL of culture medium. After 48 h, the copolymer was separated from the *C. sorokiniana* cell suspension by sieving (0.1 mm) and freeze-dried. The freeze-dried copolymers were mixed with KBr and analyzed by Diffuse Reflectance Infrared Fourier Transform Spectroscopy (DRIFTS) as described in Plöhn et al. (2021a). Spectra were recorded between 400 and 1800 cm^{-1}.

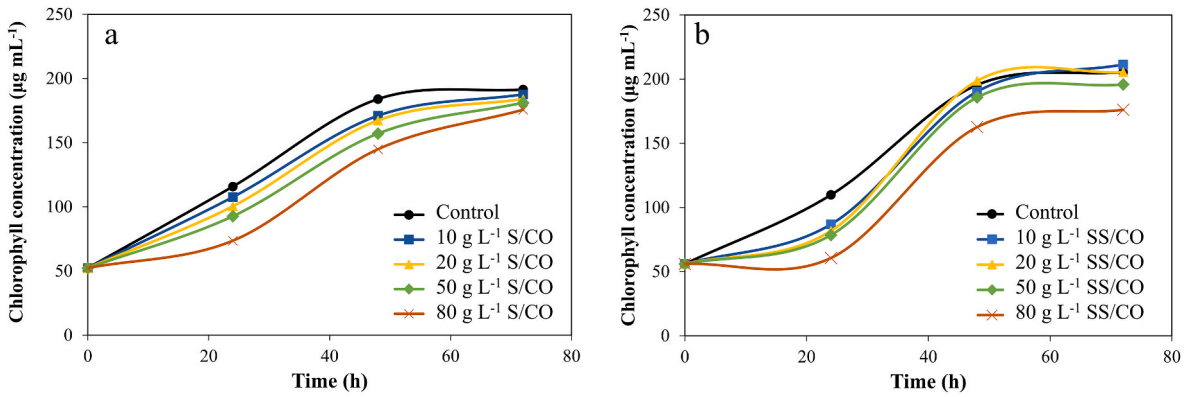

Fig. 1. Growth curves of the microalga *C. sorokiniana*, measured by total chlorophyll, in the presence of S/CO copolymer (a) or SS/CO copolymer (b) at different concentrations.

2.6. Adsorption studies

Batch adsorption experiments for cadmium ($CdSO_4 \cdot 8/3H_2O$, SIGMA ALDRICH) and copper ($CuSO_4 \cdot 5H_2O$, PANREAC QUIMICA SLU) metal with S/CO and SS/CO copolymers were made in triplicates. Microalgal pre-inoculum (initial concentration of 1 g L^{-1} dry weight) was diluted into 250 mL either in the presence of Cd^{2+} at a concentration of 50 mg L^{-1} (experiment 1) or in the presence of Cd^{2+} and Cu^{2+} both at concentrations of 8 mg L^{-1} solution (experiment 2). The heavy metals were added to the Erlenmeyer flask in the form of 100 mL of aqueous metal solution together with 5 g of S/CO or SS/CO copolymers and stirred at 400 rpm. As a control, 100 mL of the aqueous metal solution was added to only microalga or only copolymers.

2.7. Kinetic parameters of metal adsorption

The rate of metal adsorption provides valuable insights into the reaction pathways and the adsorption mechanism (Iqbal and Khera, 2015). The two most common models used to describe the reaction kinetics include pseudo-first-order and pseudo-second-order models. The pseudo-first-order kinetic model was proposed by Lagergren (1898). The general form of the model is expressed as follows:

$$\frac{dq_t}{dt} = k_1(q_e - q_t) \quad (1)$$

in which k_1 is the rate constant (min $^{-1}$), q_t is the amount of metal ion adsorbed at time t (mg/g) and q_e is the value at equilibrium (mg/g). After definite integration by applying boundary conditions $q_t = 0$ at $t = 0$ and $q_t = q_t$ at $t = t$, the integrated form becomes:

$$\ln q_e - \ln q_t = \ln q_e - k_1 t \quad (2)$$

The pseudo-second-order kinetic model proposed by Ho and McKay (1999) assumes that adsorption follows second order chemisorption. The general form can be written as follows:

$$\frac{dq_t}{dt} = k_2(q_e - q_t)^2 \quad (3)$$

in which k_2 is the pseudo-second-order rate constant (g/mg min), q_t is the amount of metal ions adsorbed at time t (mg/g) and q_e is the value at equilibrium (mg/g). The linear form can be expressed as follows:

$$\frac{t}{q_t} = \frac{1}{k_2 q_e^2} + \frac{t}{q_e} \quad (4)$$

2.8. Heavy metal determination

Trace elements were determined by Inductively Coupled Plasma Mass Spectrometry (ICP-MS, Agilent Technologies 7800) operated in He-HMI acquisition mode. The equipment was calibrated by standard solutions (Agilent Technologies). The sample was filtered and diluted with 2% nitric acid trace metal grade.

2.9. Statistical analysis

All measures were carried out in triplicates and represented as mean value ± SD. Significant differences were considered for values with $p < 0.05$. Statistical analyses were performed using STATISTICA 10.0 software (Dell, Round Rock, Tx, USA) by comparing mean values using a one-way analysis of variance (ANOVA).

3. Results and discussion

3.1. Tolerance of Chlorella sorokiniana to S/CO copolymers

The sustainable copolymers used in this study are based on sulfur and castor oil (S/CO copolymers) and, therefore, could be toxic for microalgae due to their antimicrobial properties (Cubero-Cardoso et al., 2022b). The growth of *C. sorokiniana* was analyzed in the presence of different concentrations of S/CO and SS/CO copolymers in the culture medium by measuring the total chlorophyll content of the culture. As seen in Fig. 1, the growth of the microalga was not impaired by the copolymer. The presence of S/CO (Fig. 1a) or SS/CO (Fig. 1b) copolymers at concentrations of 50 or 80 g L^{-1} induced a lag phase of 24 h in the *C. sorokiniana* cultures. However, at the end of the exponential phase, the chlorophyll concentrations did not show significant differences ($p < 0.05$) between control and cultures with copolymer (Fig. 1). Only SS/CO copolymer at the highest concentration of 80 g L^{-1} inhibited the growth of the microalga (Fig. 1b). Both copolymers, therefore, provide outstanding and harmless support for *C. sorokiniana* cells. Worthington et al. (2017) shown that a polymer synthesized with sulfur and 50% canola oil to capture mercury had neither toxicity before or after adsorption by human HepG2 and Huh7 cells. Similar biocompatibility has been observed in our study for S/CO and SS/CO copolymers against the microalga *C. Sorokiniana*. Poly (tetrafluoroethylene) or alginate are commonly used surfaces to immobilize microalgae. These systems are reported to increase the efficiency of microalgae in eliminating contaminants in wastewaters (Branco et al., 2016; Khatoon et al., 2021). However, the authors are unaware of any other study where microalgae are immobilized in copolymers obtained from industrial wastes. S/CO copolymers open novel and sustainable opportunities for microalgal bioremediation and biomass harvesting processes.

3.2. Immobilization of Chlorella sorokiniana in S/CO or SS/CO copolymers

Various microalgae strains have been immobilized to synthetic

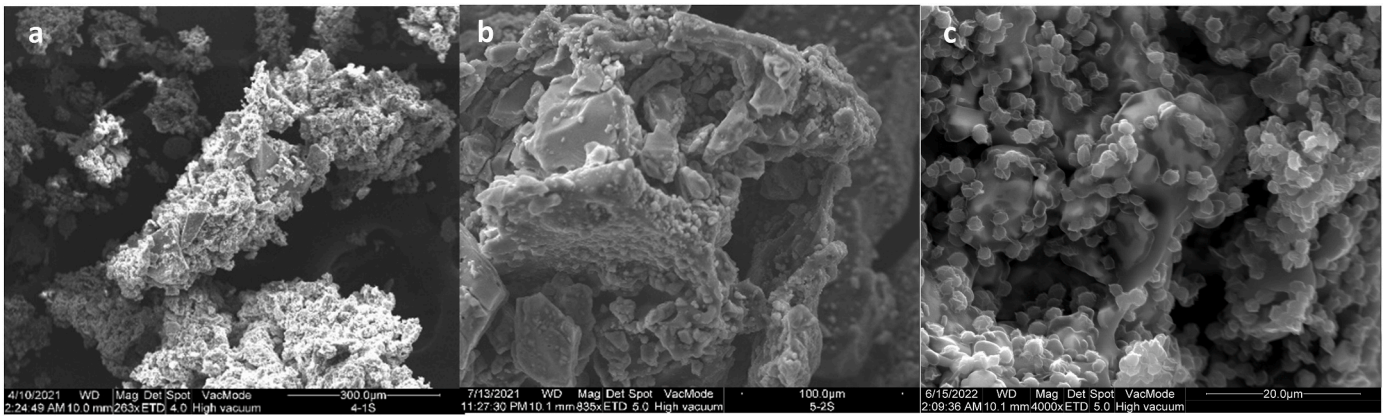

Fig. 2. Scanning electron microscope pictures of a) the S/CO copolymer, b) SS/CO copolymer, and c) biofilm consisting of *C. sorokiniana* cells and the SS/CO copolymer (AlgaPol) at a concentration of 50 g L^{-1} of copolymers in the culture medium.

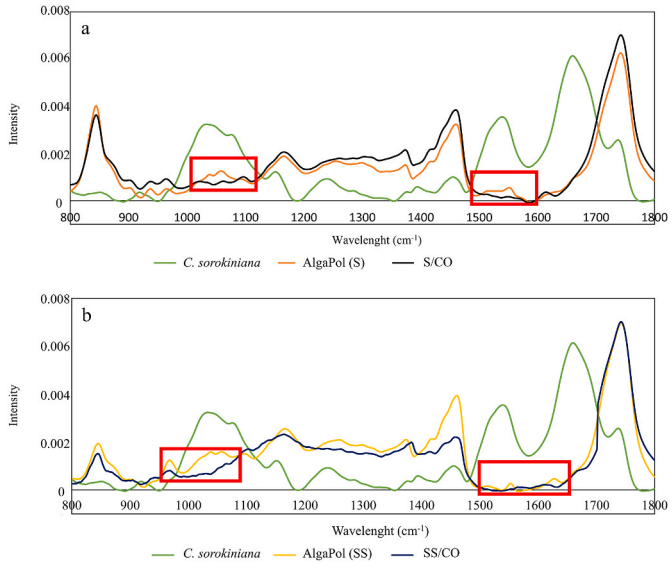

Fig. 3. FTIR spectra of *C. sorokiniana*, AlgaPol biofilm, and the S/CO copolymer (a) or the SS/CO copolymer (b) normalized to total area. Red squares highlight the bands characteristic of carbohydrates and proteins in biological samples. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

copolymers for different purposes, such as wastewater bioremediation or increased efficiency of harvesting processes (Moreno-Garrido, 2008; Safarik et al., 2020). In these studies, the microalgal cells were observed as smooth and spherical particles with a diameter of 3–5 µm (Tan et al., 2022). S/CO and SS/CO copolymers were analyzed by SEM microscopy (Fig. 2a and b). Pores generated by the salt in the SS/CO copolymer (Worthington et al., 2018) increased specific areas for microalgae immobilization. In other studies, small crystalline and smoother particles have been observed on the surfaces of the two copolymers identified as free sulfur (Hoefling et al., 2017; Tikoalu et al., 2020; Worthington et al., 2017). The immobilization and the capacity of the S/CO and SS/CO copolymers to support *C. sorokiniana* cells were observed by SEM after 48 h of microalgal cultivation in the presence of copolymers (Fig. 2c). As small round particles of less than 10 µm, the microalgae were observed to be attached to the surface of the entire copolymer, including the pores formed in the surface of SS/CO copolymer. Also, patches of free copolymer not occupied by microalgae were observed, which could allow the adsorption of metals (Fig. 2c).

To understand the interaction of the microalgae with the sulfur copolymers, Fourier Transform Infrared Spectroscopy (FTIR) analyses were performed on AlgaPol biofilm with *C. sorokiniana* (Fig. 3). Total area normalization in the 400-1800 cm^{-1} range of the raw FTIR data was carried out to compare the signals in all samples. FTIR analyses of the copolymer have been performed previously (Cubero-Cardoso et al., 2022a), as well as its characterization by thermogravimetric analysis (TGA), differential calorimetry (DSC), and ^{1}H NMR. The largest part of AlgaPol biofilm consisted of the copolymer, however, the regions of 1020–1060 cm^{-1} and 1500-1570 cm^{-1} (marked with red squares in Fig. 3a) are characteristic of carbohydrates and proteins in biological samples, respectively (Ferro et al., 2019); their increase, therefore, arises from the microalgae. We were not able to identify the microalgal lipid band (around 1700-1800 cm^{-1}) as the C═O bonds of castor oil covered this band within the copolymer. Additionally, the lipid content in the microalga *C. sorokiniana* is low when cultivated under standard conditions (Karimian et al., 2022). The peak at 850 cm^{-1} of the S/CO copolymer (Fig. 3a) had a higher intensity than the SS/CO copolymer (Fig. 3b). These peaks were attributed to C–H rocking vibrations in the vicinity of a C–S bond, which indicate a greater reactivity between sulfur and C═C. The porogen in the SS/CO could impede the interaction between C═C and sulfur (Cubero-Cardoso et al., 2022a). The presence of characteristic peaks for carbohydrates and proteins in the spectra of AlgaPol biofilm (Fig. 3) confirms that the microalga cells are adhered to the surface of the copolymers. *Saccharomyces cerevisiae* immobilized to Fe_3O_4 nanoparticles and *Thamnidium elegans* in *Phragmites australis* were also characterized by this technique (Azeez and Al-Zuhairi, 2022; Sayin, 2022). Thus, FTIR confirms that S/CO and SS/CO copolymers can be excellent carriers for *C. sorokiniana* immobilized cells.

3.3. Removal of a single metal through combining microalgae and copolymers

C. sorokiniana, as well as S/CO copolymers on their own, are known to uptake heavy metals from contaminated wastewaters (Cubero-Cardoso et al., 2022a; León-Vaz et al., 2021a). To analyze the adsorption of heavy metals of the AlgaPol biofilms, the removal of Cd^{2+} from the culture medium was compared between S/CO and SS/CO copolymers alone (Fig. 4a), *C. sorokiniana* alone (Fig. 4b), and AlgaPol biofilms (Fig. 4c and d) during 72 h of culturing. The initial concentration of Cd^{2+} was 50 mg L^{-1}, at this high concentration of heavy metals, most microalgae species are not able to grow. However, previous studies of our research group showed that *C. sorokiniana* could accumulate up to 11,000 mg kg^{-1} of Cd^{2+} in their living cells (León-Vaz et al., 2021a). Sulfur copolymers were shown to remove around 80% of total $Cd(NO_3)_2{\cdot}4H_2O$ in solution with this salt (100 mg L^{-1}) (Cubero-Cardoso et al., 2022a). Here, AlgaPol biofilms seemed to remove heavy metals from the growth medium in two different phases; a first initial period, where most of the Cd^{2+} was absorbed by the microalgal or

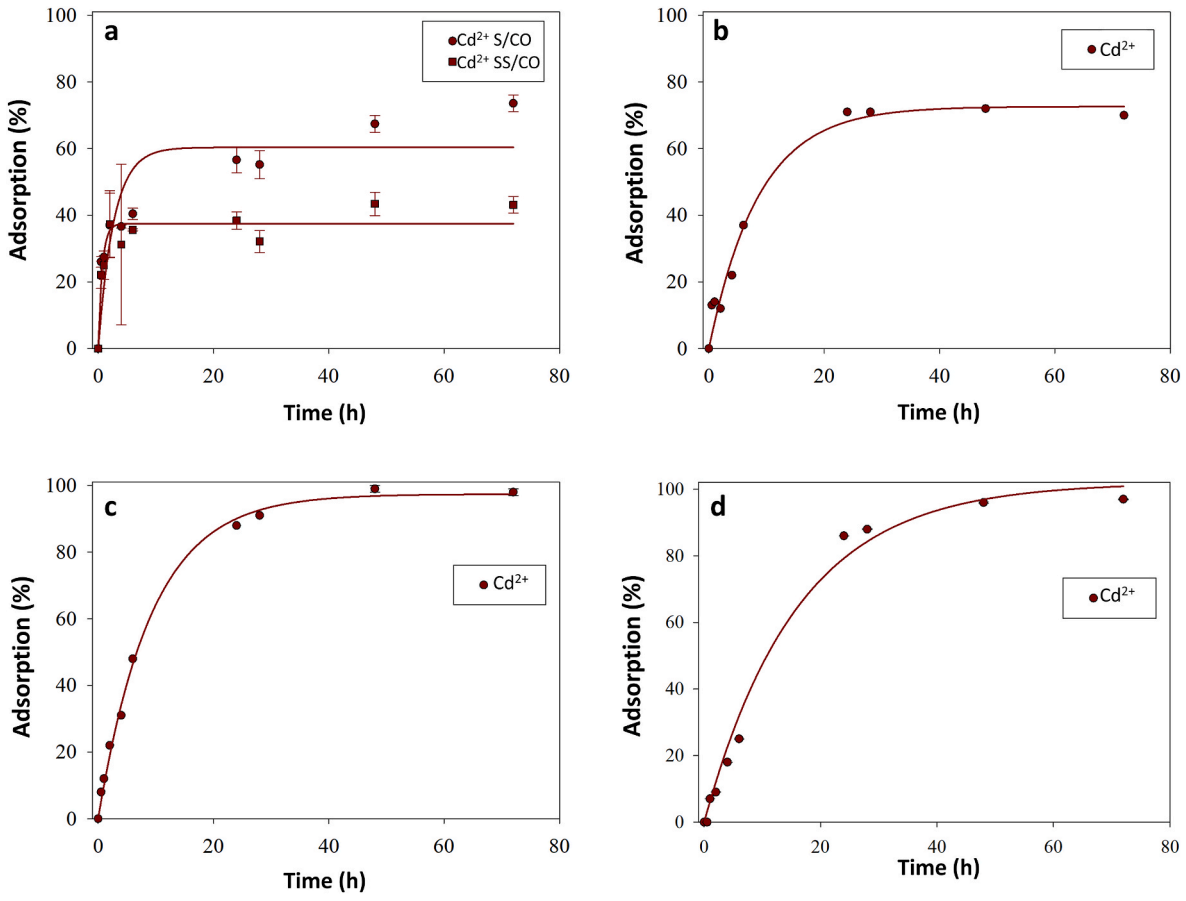

Fig. 4. Cd^{2+} adsorption of S/CO and SS/CO copolymers (a), *C. sorokiniana* (b), biofilm consisting of *C. sorokiniana* and S/CO copolymer (c), and biofilm consisting of *C. sorokiniana* and SS/CO copolymer (d).

copolymer surface; followed by a second phase, where the microalga was able to accumulate the Cd^{2+} ions in its cells (Fig. 4). The kinetic curves further showed that, in this second phase, the Cd^{2+} concentration in S/CO and SS/CO copolymers did not change (Fig. 4a). However, the first phase was shorter in assays with S/CO and SS/CO copolymers alone (5–6 h) than with *C. sorokiniana* (8 h) or the AlgaPol biofilm (15–20 h). As described in this work, the two-phase adsorption curve was also reported for other microalgae, including *Chlorella* and *Coelastrella* species, or inorganic activated carbon (de Franco et al., 2017; Plöhn et al., 2021a).

The removal efficiencies of S/CO and SS/CO copolymers were 75% and 45%, respectively, after about 8 h in a microalgal growth medium (Fig. 4a). In the *C. sorokiniana* culture for about 10 h, the adsorption of Cd^{2+} was logarithmic (Fig. 4b), and the removal efficiency then stagnated at 68% of the total Cd^{2+} concentration. The microalgae were, therefore, not only able to remove Cd^{2+} of the growth medium but also could grow under these conditions (León-Vaz et al., 2021a). Interestingly, in the AlgaPol biofilm with immobilized living microalgae, the removal of the growth medium increased up to 90% of the total Cd^{2+} concentration at 24 h, and 95% after 48 h (Fig. 4c and d). Cd^{2+} adsorption to the S/CO polymer biofilm was more efficient in the early phase of the experiment compared to the SS/CO polymer biofilm. The increased adsorption capacity of immobilized microalga in sustainable copolymers allows total uptake of high amounts of one of the most toxic heavy metals. Microalgae or microalgal biochar immobilized in loofa sponge or graphite were able to adsorb lower concentrations of Cd^{2+} (Jaiswal et al., 2021; Saeed and Iqbal, 2006). Studies using living cells to remove higher concentrations of heavy metals are likely lacking due to the high toxicity of Cd^{2+}. *C. sorokiniana* immobilized in S/CO copolymers therefore might provide one of the best systems for bioremediation processes using living microalgae, which are more efficient than dead cells (Chen et al., 2018) in highly contaminated environments, such as mining effluents (Pérez-Sirvent et al., 2016).

Table 1
Kinetic parameters derived from the adsorption of Cd^{2+} by either microalgae, copolymer, or the biofilms (AlgaPol) consisting of S/CO or SS/CO.

Surface	Cd^{2+}	Pseudo-first-order model			Pseudo-second-order model		
	$q_{e,\ exp}$ (mg g-1)	$q_{e,\ calc}$ (mg g^{-1})	k_1 (min^{-1})	R^2	$q_{e,\ calc}$ (mg g^{-1})	k_2 (g mg^{-1} min^{-1})	R^2
S/CO	0.69	0.420	0.0003	0.974	0.694	0.0061	0.986
SS/CO	0.41	0.115	0.0002	0.186	0.408	0.0223	0.985
AlgaPol (S)	1.14	1.109	0.0007	0.998	1.303	0.0014	0.995
AlgaPol (SS)	1.11s	0.778	0.0007	0.996	1.646	0.0004	0.932

A pseudo-second-order model best described the kinetics of Cd^{2+} adsorption in AlgaPol biofilm (independent of the copolymer used). S/CO had 41% more adsorption capacity (q_e) than SS/CO (Table 1). AlgaPol biofilms were adjusted for both kinetic equations, suggesting that cadmium removal is achieved through a physical and chemical adsorption process (Segneanu et al., 2022), visible by the fast surface adsorption of the copolymer and the microalgae, but also by the slower chemisorption of the microalgae observed in the second phase (Plöhn et al., 2021a). AlgaPol biofilms had around 75% higher q_e than the copolymers alone in the absence of microalgae. However, AlgaPol biofilms displayed a pseudo-second-order rate constant (k_2) lower than one of the

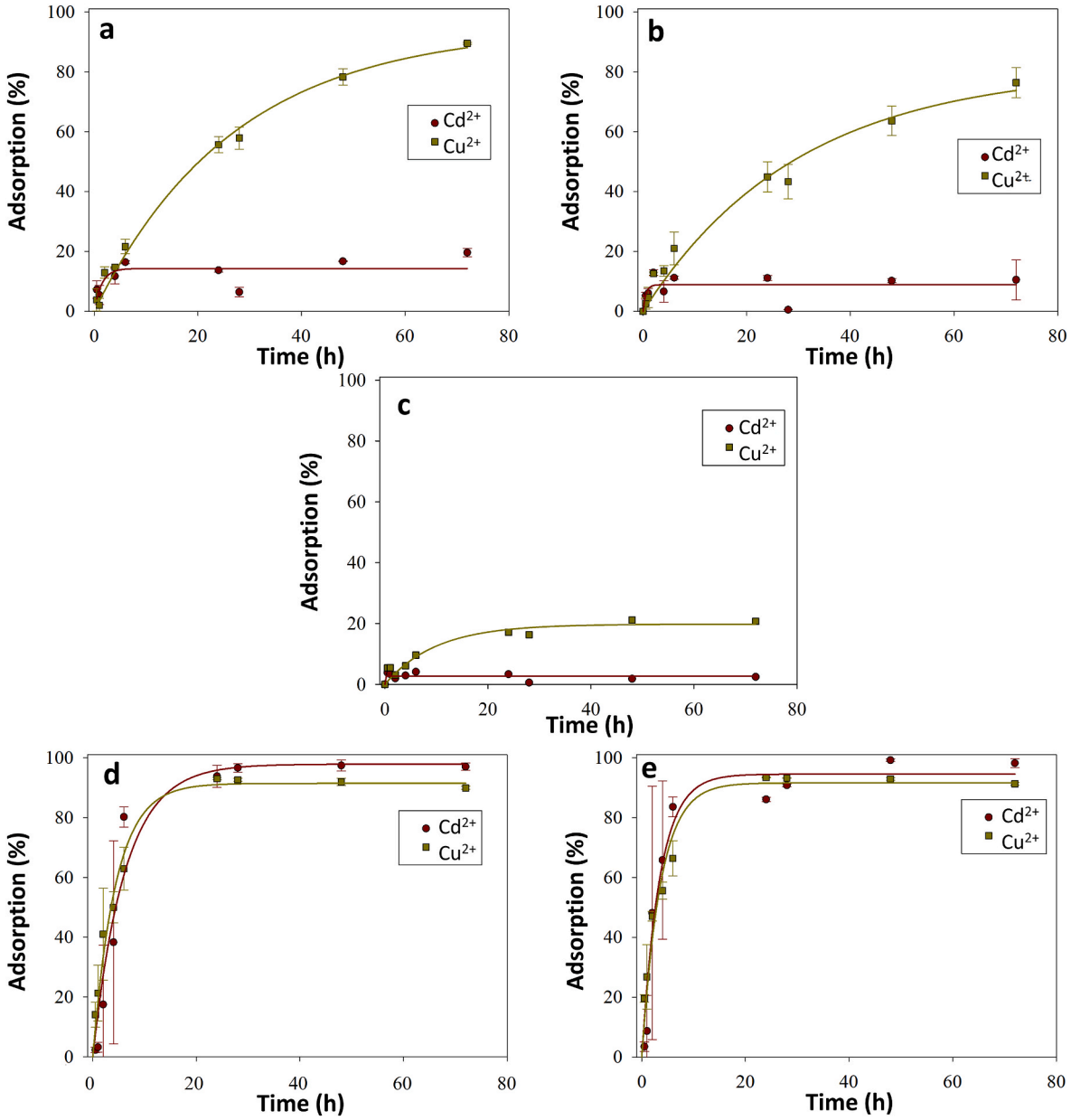

Fig. 5. Cu^{2+} and Cd^{2+} adsorption by S/CO (a) and SS/CO (b) copolymers, *C. sorokiniana* (c), biofilms consisting of *C. sorokiniana*-S/CO copolymer (d), and of *C. sorokiniana*-SS/CO copolymer (e).

copolymers alone (Table 1). In addition, S/CO alone exhibited a higher q_e, but lower K_2 than SS/CO; in the presence of microalgae, the opposite was observed (Table 1).

Table 2
Adsorption kinetics of Cd^{2+} and Cu^{2+} using copolymers and/or microalgae.

Surface	Metal		Pseudo-first-order model			Pseudo-second-order model		
		$q_{e,\ exp}$ (mg g^{-1})	$q_{e,\ calc}$ (mg g^{-1})	k_1 (min^{-1})	R^2	$q_{e,\ calc}$ (mg g^{-1})	k_2 (g mg^{-1} min^{-1})	R^2
S/CO	Cd^{2+}	0.046	0.022	0.0001	0.236	0.044	0.1686	0.979
SS/CO	Cd^{2+}	0.025	0.015	0.0003	0.478	0.025	1.6903	0.998
S/CO	Cu^{2+}	0.231	0.194	0.0003	0.814	0.316	0.0018	0.730
SS/CO	Cu^{2+}	0.197	0.187	0.0002	0.981	0.236	0.0035	0.952
AlgaPol (S)	Cd^{2+}	0.160	0.162	0.0013	0.943	0.213	0.0046	0.830
AlgaPol (SS)	Cd^{2+}	0.163	0.098	0.0005	0.749	0.183	0.0135	0.969
AlgaPol (S)	Cu^{2+}	0.144	0.127	0.0013	0.972	0.153	0.0422	0.997
AlgaPol (SS)	Cu^{2+}	0.148	0.120	0.0014	0.963	0.155	0.0542	0.998

3.4. Removal of several heavy metals in the mixture using the AlgaPol biofilm

Natural contaminated environments usually contain a mixture of several heavy metals, often at lower concentrations than those studied at a laboratory scale (Nowicka, 2022). In order to mimic natural conditions, *C. sorokiniana* cells immobilized to either S/CO or SS/CO copolymers were cultured in the presence of Cu^{2+} and Cd^{2+} ions (8 mg L^{-1} each) (Fig. 5d and e). The removal efficiencies of these AlgaPol biofilms were compared to the copolymers alone (Fig. 5 a and b) and to free microalgal cells (Fig. 5c). The S/CO and SS/CO copolymers alone adsorbed 20% and 10% of total Cd^{2+}, respectively (Fig. 5a and b) within the first hour. *C. sorokiniana* cells took up only 5% Cd^{2+} of the cadmium/copper mixture (Fig. 5c), then the uptake from the growth medium stagnated. The microalgae were decreased removal and had some preference for the metals in multimetallic solutions, as shown in different studies (Ferraro et al., 2021; Saavedra et al., 2018). AlgaPol biofilms (Fig. 5d and e) were able to remove 95% of Cd^{2+} from the medium within 8–10 h.

The removal of Cu^{2+} in the heavy metal mixture was more constant by the copolymers (Fig. 5a and b); 90% and 75% of total Cu^{2+}, respectively, was adsorbed by the copolymers within 72 h, while *C. sorokiniana* cells adsorbed about 20% of the Cu^{2+} ions within the first 20 h and then stagnated. Again, the AlgaPol biofilms (Fig. 5d and e) were highly superior to the individual components, 90% of Cu^{2+} was removed from the medium within 8–10 h. The enhanced adsorption in the AlgaPol biofilms points to a symbiotic effect: the copolymer takes up Cu^{2+} very fast within the first hours of cultivation, enabling the algae to adsorb Cd^{2+} (as shown in our single metal studies). This kinetic, between pseudo-first and pseudo-second-order model (Table 2) was also reported for activated carbon particles in a liquid-solid fluidized bed and Cu^{2+} ions (Lv et al., 2021).

Kinetic modelling on the adsorption behaviour of the copolymers indicated a significant correlation to second-order equations for Cd^{2+} (Table 2), similar to our previous results (Table 1). Nevertheless, Cu^{2+} removal follows rather a first-order equation due to the higher values for the correlation coefficient (R^2). The calculated adsorption capacity value was very similar to those determined experimentally. In addition, we observed that each copolymer had more than seven times more adsorption capacity (q_e) for Cu^{2+} than for Cd^{2+} (Table 2). Cu^{2+} removal showed a higher pseudo-second-order rate constant (k_2) in AlgaPol biofilm than Cd^{2+}, most likely because the copolymer adsorbs Cu^{2+} faster and therefore leaves Cd^{2+} available for the microalgae. No significant differences were observed between the adsorption rates of *C. sorokiniana* immobilized to S/CO or SS/CO copolymers, both copolymers provide outstanding systems for immobilizing this microalga and the reclamation of different heavy metals. The adsorption of specific heavy metals in a mixture differs in its binding specificity to microalgae (Sümeyye Hasanoğlu et al., 2021; Wang et al., 2019). A system combining *Pseudomonas hibiscicola* and peanut shell biochar was used to reduce high amounts of Ni^{2+} and nitrate, but only lower doses of Cu^{2+} and Cr^{6+} could be adsorbed (An et al., 2022). The bacteria-biochar system had improved properties compared to *Pseudomonas* or peanut shell biochar individually, similar to the AlgaPol systems described in this study. Further, it has been described that copolymers formed by vegetable oils are feasible to be implemented in large scale processes (Chalker et al., 2021). Sustainability of the process could be optimized, supporting circular economy, by obtaining oils from the cultivated microalgae as described in the study by Gupta et al. (2022). Various studies have been performed on reusing copolymers by washing them with acids and releasing the adsorbed metals (Chalker et al., 2021; Chen et al., 2020; Worthington et al., 2017). A future perspective would be the reuse of the copolymers once washed from metal and microalgae, the solution rich in metals and microalgae could be used to obtain triglycerides or other high-value compounds (Gupta et al., 2022; León-Vaz et al., 2023). Combining biological-inorganic/organic systems generates promising tools for the bioremediation of different contaminants in wastewaters.

4. Conclusions

Sustainable copolymers made of waste products were used in the presence or absence of a porogen to immobilize the green microalga *Chlorella sorokiniana*. The microalga was able to tolerate the antimicrobial properties of the sulfur-castor oil (80/20) copolymers and efficiently immobilized them within the scaffold. The novel biofilms (AlgaPol) were able to adsorb the heavy metal cadmium at high concentrations. A mixture of copper and cadmium was also removed from the growth medium far more efficiently than only algae or only polymers were able to. Kinetic studies support the fact that these systems have excellent potential to remove different contaminants from wastewater.

Author contributions

Conceptualization, A.L-V., J.C.-C., J.V., and J.U.; methodology, A.L-V., J.C.-C., and A.T-R.; resources, J.V., R.L., F.G.F., C.F., and J.U.; writing—original draft preparation, A.L-V., and J.C.-C..; writing—review and editing, J.V., F.G.F., R.L., C.F., and J.U.; supervision, J.V., and J.U.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

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