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Sustainable Microalgal Harvesting Process Applying *Opuntia cochenillifera*: Process Parameters Optimization

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Abstract: Microalgae harvesting by coagulation can use coagulant agents such as alum, synthetic polymers or biocoagulants. Biocoagulants have attracted the attention of researchers because they are natural, biodegradable, and promote high microalgal harvesting efficiencies. This study aims to optimize the harvesting of *Chlorella vulgaris* based on the dosage of the *Opuntia cochenillifera* extract and the choice of eluent for biopolymer extraction. The outdoor cultivation of *C. vulgaris* achieved a specific growth rate of 0.455 d^{-1} and a maximum biomass concentration of 1.28 g_{DW} L^{-1}. In order to harvest the microalgal biomass, the polymer present in the mucilage of *O. cochenillifera* was extracted using NaOH and HCl. Coagulation and sedimentation assays were performed with different coagulant dosages: 3.5, 5.9, and 8.2 g L^{-1}. The maximum harvesting efficiencies using the acid and alkaline extract coagulant solutions were 80.8% and 99.5%, respectively, with a dosage of 3.5 g L^{-1}. According to the results, the *C. vulgaris* biomass can be harvested with the mucilage from *O. cochenillifera* in acid and alkaline eluents. The application of this biocoagulant constitutes a sustainable solution for microalgal harvesting.

Keywords: biocoagulants; *Chlorella vulgaris*; microalgal harvesting; *Opuntia cochenillifera*; process optimization

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1. Introduction

Microalgae are photosynthetic unicellular and multicellular microorganisms living in almost all of Earth's ecosystems, as they can be found in oceans, rivers, lakes, and soils [1,2]. The classification of each specie is based on pigment composition and diversity of structural features. The great interest in these phytoplankton lies in their ability to produce bioactive compounds through photosynthesis. In this process, microalgae convert solar energy into chemical energy by fixing atmospheric CO_2 [3]. Therefore, the photosynthetic process makes microalgae cultivation an excellent option for capturing this air pollutant. Additionally, depending on the microalgal species, they can absorb dissolved inorganic carbon from the aquatic surroundings in the forms of H_2CO_3, HCO_3^- and CO_2 [4]. Microalgal biomass has many potential applications in several industries. In the cosmetic industry, bioactive compounds can be extracted from biomass and incorporated into several products [5]. Microalgal biomass can generate large amounts of lipids, proteins, and carbohydrates in a short time [5]. Microalgal protein can be converted into nutritional and pharmaceutical products, while lipids can be used to produce biodiesel [4]. Another microalgal application is nutrient removal from wastewater, promoting their treatment [6].

The major challenge of microalgal cultivation lies in the harvesting process due to the small cell size of microalgal cells (1–30 μm diameter) and their colloidal stability in

suspension. Li et al. [7] estimate that the cost involved in the harvesting process accounts for over 30% of the total production cost. Therefore, it is crucial to find an efficient harvesting method, taking into account the microalgal species, the maximum amount of biomass to be recovered, and the operational and energetic costs [8]. According to Udayan et el. [8], the selection of harvesting methods depends on the end product requirements, cell size and density, salt concentration, and moisture content. Therefore, two or more techniques can be combined.

Technologies for microalgal harvesting involve physical-, chemical-, biological- and electrical-based methods. The most common processes are coagulation/flocculation, gravity sedimentation, dissolved air flotation, electrical processes, centrifugation, membrane filtration, and magnetic separation [9,10]. However, some of these techniques can be energy-consuming (centrifugation), time-consuming (gravity sedimentation), or toxic to microalgal biomass (chemical coagulation/flocculation) [11]. Coagulation and flocculation are two of the most widely used methods for microalgal harvesting, as they are economical and allow the treatment of large amounts of diluted cultures. Coagulation and flocculation can be achieved in several ways, and various approaches for microalgal aggregation have been studied in recent years [12]. Coagulation consists of destabilizing the negative charge of colloidal particles (such as microalgae), whereas flocculation entails the aggregation of neutralized particles into flocs and their decantation over time [13]. The two methods combined with sedimentation or filtration can increase the overall recovery efficiency, promoting 100% recovered biomass [8].

For many years, chemical coagulants were used to harvest microalgae such as ferric sulfate, aluminum sulfate, and ferric chloride [14]. Due to contamination of the biomass by metallic ions and the degree of toxicity of some chemical coagulants (e.g., aluminum sulfate), which can cause damage to human health (e.g., Alzheimer's disease), biocoagulants have been increasingly used as alternative compounds for microalgae harvesting. Biocoagulants have attracted the attention of researchers because they are natural, biodegradable, inexpensive, non-toxic to algal biomass, and promote high coagulation efficiencies [13]. Biocoagulants comprise polysaccharides, proteins, and other functional groups that may be cationic, anionic, poly-ionic, or non-ionic [15].

The natural coagulants act by chemical adsorption and neutralization. The formation of hydrolyzed species of positive charge in the compound; then, the adsorption occurs at the surface of this particle suspension, and destabilization arises [13,16]. Biocoagulants/bioflocculants are obtained from living organisms, such as animals, plants, fungi, and bacteria [12]. According to Min et al. [12], fungi such as *Penicillium* cells or spores can be used to flocculate and recover *Chlorella vulgaris* biomass, resulting in a biomass recovery of over 98%. The aggregation resulting from the association between fungi and algae is called algal–fungal flocculation [12]. Biopolymers can be classified as anionic, which generally exhibit poor activation with microalgae, or cationic, that can neutralize or reduce the ionic surface charge of microalgae, resulting in a more efficient harvesting process [12].

The use of biocoagulants of plant origin, such as *Margaritaria discoidea*, *Moringa oleifera*, *Cocos nucifera*, *Jatropha curcas*, and *Luffa cylindrica*, and species from the genre Opuntia, such as *Opuntia ficus-indica*, have been shown to be efficient for biomass harvesting of microalgae [14,15]. Most fruit seed extracts have strong gel formation, which is able to remove the turbidity of water. As an example, the active coagulating ingredient in the *M. discoidea* and *M. oleifera* extracts was identified as water-soluble macromolecular hydrocolloidal polysaccharides [14]. They have a polymeric material with the hydroxyl group that easily forms hydrogen bonding in the aqueous medium. The seed of *M. oleifera* consists of water-soluble flocculating with a protein of cationic nature that acts as an active agent in the process of coagulation [17]. These biocoagulant/bioflocculant are hydrolyzed after dissolution in water and interact with microalgae (negative charge). The interaction destabilizes the surface charge of microalgae, allowing the microalgae cells to agglomerate and settle.

According to Udom et al. [18], mucilage extracted from cactus was shown to increase sedimentation rates of microalgae. For this reason, this work received an interest in evaluating microalgal harvesting by mucilage of cacti. The crop of *Opuntia* has a high potential to harvest microalgae, although little research has been carried out for this purpose. *Opuntia* has macromolecular polymers, which are flocculating agents. Some factors, such as media composition and environmental conditions, including temperature and pH, affect flocculant production by these cacti. Generally, species from the genre *Opuntia* have high flocculation efficiency and are cheap, sustainable, and environmentally friendly [19].

The infrared spectra for *Opuntia* sp., determined by Jadhan and Mahajan [20], indicate the presence of carboxylic acid groups that are negatively charged. The high-coagulation capability of the cactus is most likely attributed to the presence of mucilage, which is a viscous complex of carbohydrates. The same authors [20] also reported that galacturonic acid is another possible active ingredient that provides *Opuntia*'s coagulation/flocculation ability to remove the turbidity of wastewater. The mechanism relies on forming a bridge between the particles and the polymer, at which the particles in the solution establish linkages with the polymer. Considering that *Opuntias* sp. presented very satisfactory results in wastewater treatment and few studies were performed to harvest microalgae, this work aims to optimize the harvesting process of the microalga *Chlorella vulgaris* using a biocoagulant extracted from *Opuntia cochenillifera*. As far as it is known, *O. cochenillifera* has not yet been applied for this purpose. The specific objectives of the present study are to (i) evaluate the use of acidic and alkaline *O. cochenillifera* extracts as biocoagulants and (ii) optimize the biocoagulant dosage to determine the most efficient in harvesting *C. vulgaris* biomass.

2. Materials and Methods

2.1. Microalgae and Culture Medium

The *C. vulgaris* strains were obtained from the microalgae bank of the Laboratory of Environmental Biotechnology (LABAM) of the Federal University of Sergipe—Brazil. For the outdoor cultivation assay, the culture medium was composed of 744 mL of microalgal suspension, 1 L of BG-11 medium, 16 L of cooling water from a distiller in LABAM; the microalgal suspension had an initial concentration of 41.92 mg L^{-1}. The BG-11 medium was prepared as described by Rippka et al. [21]: 19 mg Na_2CO_3, 1500 mg $NaNO_3$, 5 mg $K_2HPO_4{\cdot}3H_2O$, 8 mg $MgSO_4{\cdot}7H_2O$, 22.65 mg $CaCl_2 \cdot 2H_2O$, 6 mg $C_6H_8O_7xFe_3{\cdot}$ NH_3, 0.736 mg $Na_2EDTA{\cdot}2H_2O$, 6.4 mg $C_6H_8O_7$ and a trace metal solution of 3.0 mg H_3BO_3, 2.0 mg $MnCl_2{\cdot}4H_2O$, 0.20 mg $ZnSO_4{\cdot}7H_2O$, 0.4 mg $Na_2MoO_4{\cdot}2H_2O$, 0.13 mg $CuSO_4{\cdot}5H_2O$ and 0.066 mg $Co(NO_3)_2{\cdot}6H_2O$. The cooling water from the distiller was reused to culture microalgae instead of freshwater, as its composition could benefit microalgal growth: 0.299 mg L^{-1} NO_3; 51.902 mg L^{-1} Cl; 1.358 mg L^{-1} SO_4; 30.62 mg L^{-1} Ca; 2.06 mg L^{-1} Fe; 0.049 mg L^{-1} ClO_2; 10.2 ppm total organic carbon. This characterization was conducted at the Water Treatment Station of the Sanitation Company of Sergipe, DESO in Aracaju-Se-Brazil, through ion chromatography (Chromatograph—Dionex—ICS 3000). The composition of the final culture medium in nitrogen was approximately 14.13 mg $N\text{-}NO_3$ L^{-1}, while phosphorus was 0.40 mg $P\text{-}PO_4$ L^{-1}.

2.2. Cultivation of C. vulgaris in an Outdoor Photobioreactor

C. vulgaris was grown in the previously described culture medium in an outdoor glass photobioreactor (W 42 cm × L 21.64 cm × H 22 cm) with 20 L capacity, built to provide enough sunlight for microalgal growth. Cultivation was performed at 27 ± 3 °C for 8 d, with a 12 h light:12 h dark photoperiod with a daily average solar irradiation of 4.500–5.102 kWh m^{-2}, according to Global Solar Atlas [22]. Atmospheric air was continuously supplied to the culture with an aeration rate of 0.361 vvm. Aeration was performed with atmospheric air through two SC3500 air pumps from Boyu (Chaozhou, China). Initially, the culture's pH was adjusted to 7.0 and measured once again at the end of the experiment using an S400 pH meter (Mettler Toledo, São Paulo, Brazil). At the end of

the cultivation process, coagulation/flocculation and sedimentation tests were conducted using this culture, as described in Section 2.5.

2.3. Microalgal Growth Monitoring

The microalgal growth was monitored daily along the 8 d of cultivation through the methodology: measurement of the culture's optical density at 750 nm (OD_{750}) using an SP 2000 UV spectrophotometer (Kasuaki, São Paulo, Brazil). A calibration curve was constructed between the optical density of the culture at 750 nm (OD_{750}) and the biomass concentration (X, g_{DW} L^{-1}), as represented in Equation (1). The biomass concentration was estimated through centrifugation of the culture, followed by filtration of the supernatant in microfiber filters with a pore size of 1.5 µm, and finally oven-drying at 60 °C overnight to eliminate the remaining moisture [23].

$$X = (3.11 \pm 0.09) \times OD_{750} + (0.08 \pm 0.02) \qquad R^2 = 0.9976 \tag{1}$$

The specific growth rate, µ (d^{-1}), at the exponential growth phase was determined as Equation (2), where X_1 and X_2 correspond to the biomass concentration at the beginning (t_1) and at the end (t_2) of this growth phase [24]. The average biomass productivity (P, g_{DW} L^{-1} d^{-1}) was calculated according to Equation (3), in which X_0 and X_f represent the biomass concentration at the beginning of the experiment (t_0) and at the end of the stationary phase (t_f) [25].

$$\mu = \frac{(\ln X_2 - \ln X_1)}{t_2 - t_1} \tag{2}$$

$$P = \frac{X_f - X_0}{t_f - t_0} \tag{3}$$

2.4. Coagulant Preparation: O. cochenilifera

The *O. cochenillifera* strain used in this study was obtained from the gardens of the Federal University of Sergipe, Brazil (10°55′22.1″ S and 37°06′03.9″ W). The *O. cochenillifera* leaves were pre-treated by cleaning the palms, removing all their spines, and crushing until a homogeneous paste was formed. In order to extract the biopolymer, 0.10 M NaOH and 0.10 M HCl solutions were used as eluents since their ionic strength maximizes the polymer recovery from the mucilage [20,26]. Therefore, the coagulant solutions were prepared by dissolving the cactus mucilage in the acid or alkaline solutions through homogenization in a magnetic stirrer at 100 rpm for 15 min. The solutions were filtered under a vacuum using a paper filter and a Büchner funnel and stored for coagulation/flocculation and sedimentation tests. Six different extract coagulant solutions were prepared: three acid extract solutions with 30, 50, and 70 g of mucilage per liter of eluent and three alkaline extract solutions with the same concentrations.

2.5. Coagulation/Flocculation and Sedimentation Tests

The coagulation/flocculation tests were performed in a jar test floc control (PoliControl, São Paulo, Brazil) by adding 40 mL of each extract coagulant solution to 300 mL of microalgal suspension collected from the photobioreactor (PBR). The experiments were conducted in triplicate for each coagulant dosage (3.5, 5.9, and 8.2 g L^{-1}) and for each eluent, with a total of 18 assays. After adding the coagulant, the suspensions were subjected to rapid mixing at 150 rpm for 3 min and a slow mixing step at 30 rpm for 15 min. The stirrer was then switched off, and the flocs were allowed to settle undisturbed for 30 min. Additionally, a control test was performed in the same conditions but without adding a coagulant. The optical density at 750 nm was monitored in three stages: at the beginning of the experiment, before the addition of coagulant, $(OD_{750})_1$; after coagulation/flocculation and before settling, $(OD_{750})_2$; and in the supernatant after settling $(OD_{750})_3$. The monitorization of the optical density allows the measurement of the light absorbed by the sample. The harvesting efficiency was calculated as represented in Equation (4) [27]. Considering that

the pH of the eluent solutions could influence the harvesting efficiency, it was of paramount importance to evaluate the hydrogenionic potential before, during, and after coagulation, flocculation, and sedimentation tests. The initial pH of the culture was adjusted to 7.0, and at the end of the 8 d of cultivation, the pH was 8.5. The acid and alkaline coagulant solutions that were adopted as eluents for the adsorption of the coagulant agent of the cactus had a pH of 5.5 and 12.5, respectively. The coagulant solutions to the microalgal suspension caused a shift in the culture's pH from 8.5 to 7.9 and 9.1, respectively.

$$\text{Harvesting efficiency } (\%) = \frac{(OD_{750})_1 - (OD_{750})_3}{(OD_{750})_1} \times 10 \quad (4)$$

After settling, the sedimentation kinetics was evaluated using a 250 mL measuring cylinder to observe the height of the flakes falling over time. In this last test, the decanting kinetics of the flakes was verified, generating a curve of the surface height versus the decanting time of the flakes. A linear adjustment was performed for each curve to determine the average speed of sedimentation, which corresponds to the symmetrical of the curve slope.

3. Results and Discussion

3.1. Assessment of C. vulgaris Growth in an Outdoor Photobioreactor

Figure 1 presents the time course of evolution of the microalgal biomass concentration over 8 d of cultivation. *C. vulgaris* grew exponentially at the initial stage of cultivation (0–4 d), with a specific growth rate of (0.455 ± 0.09) d^{-1}. However, growth inhibition was observed subsequently as the culture reached the stationary phase. In the growth curve, different growth phases are defined for microalgal cultures: lag, exponential, stationary, and decline phase. In this work, the lag phase was not evidenced, considering that the microalgal culture comes from a growing matrix in the bank of LABAM. The stationary phase was observed between the fourth and eighth day of cultivation. The biomass was harvested on the eighth day. Harvesting the biomass in the stationary phase could help improve the coagulation/flocculation process since microalgae produce substances such as extracellular polymeric substances (EPSs) that increase the aggregation of microalgal cells. For instance, according to Vu et al. [28], during the stationary phase, culture aging and an increase in EPS concentration can play a key role in the flocculation efficiency of *C. vulgaris*.

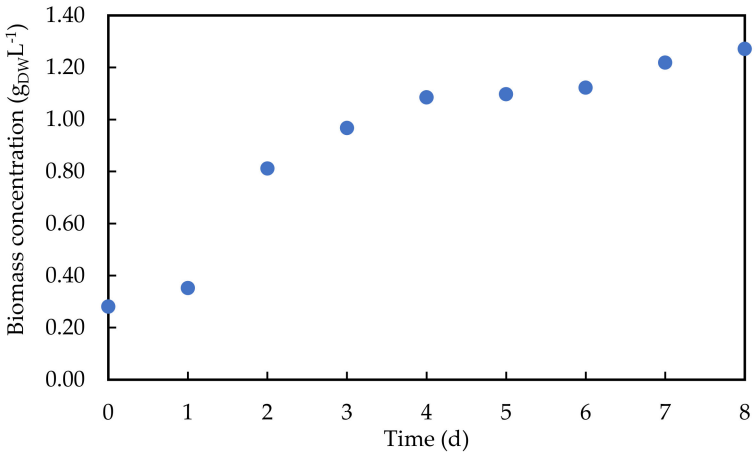

Figure 1. Time-course evolution of the microalgal biomass concentration.

The maximum biomass concentration was 1.28 g L^{-1}, while Wang et al. [29] achieved a maximum concentration of 75 mg L^{-1} in approximately 2 weeks of growing *Chlorella* in BG-11 medium with nitrogen and phosphorus contents of 45 and 4.5 mg L^{-1}, respectively, in an artificial climate chamber. Soto-Ramírez et al. [30] obtained a specific growth rate of 0.21 d^{-1} and biomass concentration of 0.99 g L^{-1} for 30 d of cultivation of *C. vulgaris*. In

this study, the culture was carried out at 20 °C with light-restricted conditions (incident irradiation of 400 μmol m^{-2} s^{-1}), although it had increased to 3 g L^{-1} of $NaNO_3$ of the BG-11 medium to ensure full nitrogen supply to the biomass growth of *C. vulgaris*. By comparing the cultivation conditions of this work with that of Soto-Ramírez et al. [30], the different results can be explained by the different experimental conditions, such as the lower concentration of nitrogen and phosphorus in the culture medium, since the BG-11 medium was diluted in the cooling water from the distiller, the shorter cultivation period, the use of solar radiation rather than artificial and consequent temperature variations. In other words, microalgae demonstrated efficiency in adapting to a culture system in an environment with nutrient restrictions and with high light intensity during the day to grow and develop.

The growth rate allows for defining the growth rate of *C. vulgaris;* according to the literature, the growth rate should be between 0.550 and 1.872 d^{-1} [31]. The average biomass productivity was 0.12 g L^{-1} d^{-1}, consistent with the results of Chang et al. [31]. In their study, microalgae grew slowly in the beginning (the maximum biomass productivity and the maximum specific growth rate were 0.192 g L^{-1} d^{-1} and 0.864 d^{-1}, respectively), due to an inadequate supply of carbon, as only CO_2 from atmospheric air was supplied to the *C. vulgaris* culture.

According to Feng et al. [32], microalgae such as *Chlorella* sp. and *Scenedesmus* sp. are characterized by high growth rates when cultured outdoors under natural sunlight. In their work, the culture of C. *vulgaris* achieved a specific growth rate of 1.48 d^{-1} and biomass productivity of 0.297 g L^{-1} d^{-1}, while, in this work, these kinetic parameters were lower. This reason may also be associated with nitrogen insufficiency and CO_2 limitation due to the aeration of atmospheric air.

3.2. Coagulation/Flocculation and Sedimentation of the C. vulgaris Culture

Table 1 shows the harvesting efficiencies after the coagulation/flocculation and sedimentation processes of *C. vulgaris* using the acid and alkaline *O. cochenillifera* extracts as coagulants. In these tests, the control assay indicated that microalgal sedimentation naturally occurred after agitation in the jar test without the addition of coagulants. In this assay, a harvesting efficiency of only 31.0% was obtained after 30 min of sedimentation. Regarding the experiments with coagulants, the increase in the coagulant dosages led to lower harvesting efficiencies after flocculation. Moreover, the acid coagulant provided a higher efficiency compared to the alkaline coagulant. However, after sedimentation for 30 min, the opposite was observed. The harvesting efficiencies for the acid coagulant ranged between 72.9% and 80.8%, with the highest value observed for the lowest coagulant dosage (3.5 g L^{-1}). Regarding the alkaline coagulant, the optical density was significantly lower after sedimentation compared to the remaining assays. Hence, the harvesting efficiencies were higher in this case, ranging between 99.3% and 99.5%. Moreover, after the sedimentation period, the results were very similar between the different coagulant dosages, allowing for inferring that the homogenous cactus solution was able to agglutinate the microalgal biomass, causing greater sedimentation. In summary, the *O. cochenillifera* extracts appear to be promising coagulants for microalgal biomass since both acid and alkaline coagulants significantly increased the harvesting efficiency after sedimentation compared to the control assay. Nevertheless, the alkaline extract coagulant appears to be the most suitable option for this purpose, which denotes a greater efficiency of the cactus extract at higher pH ranges after sedimentation.

Table 1. *Chlorella vulgaris* harvesting efficiencies after flocculation and sedimentation using acid and alkaline *Opuntia cochenillifera* extracts as coagulants.

Extract Coagulant Dosage (g L^{-1})	Eluent	$(OD_{750\ nm})_1$	$(OD_{750\ nm})_2$	$(OD_{750\ nm})_3$	Harvesting Efficiency (%) after Flocculation	Harvesting Efficiency (%) after Sedimentation
0 (control)	-	0.2030	-	0.1400	-	31.0%
3.5	0.10 M HCl	0.2030	0.0440	0.0390	78.32%	80.8%
5.9			0.0560	0.0430	72.41%	78.8%
8.2			0.0700	0.0550	65.51%	72.9%
3.5	0.10 M NaOH	0.2030	0.0440	0.0010	78.32%	99.5%
5.9			0.0670	0.0015	66.99%	99.3%
8.2			0.1270	0.0014	37.44%	99.3%

$(OD_{750\ nm})_1$: optical density before the addition of coagulant; $(OD_{750\ nm})_2$: optical density after coagulation/flocculation and before settling; $(OD_{750\ nm})_3$: optical density after settling.

Udom et al. [18] stated that the efficiency of plant coagulants (polymers) is due to variations in charge density and molecular weight, which are able to neutralize the charge and form bridges between algal particles, respectively. The same authors affirmed that high molecular weight cationic polymers might reverse the surface load of the algae and stabilize the suspension, while low molecular weight polymers may not cause flocculation. The high coagulation capacity of the *O. cochenillifera* extract is attributed to the presence of mucilage which is a viscous, cationic complex carbohydrate whose particles adsorb more in an alkaline solution than in an acid or salt solution. Mucilage is a slimy fluid that appears directly when the cactus is cut. This fluid is the active coagulant agent since it has the ability to precipitate ions and particles from aqueous solutions through bridging mechanisms, in which numerous negatively charged microalgae cells are adsorbed or neutralized onto the *Opuntia* chains [20,33]. Moreover, during flocculation, the microalgal cells in solution, being destabilized or not, collide and aggregate due to Van der Waals forces, forming flocs [12]. According to Min et al. [12], the polymer size and charge density may influence this process. In this study, the polymer in the alkaline solution might have led to the destabilization of the surface charge of microalgae allowing the cells to agglomerate and settle with a higher harvesting efficiency than the acid coagulant.

The pH is also an important factor that influences the surface charge of the coagulants and the stability of the microalgae cells in suspension, as well as the flocculation efficiency [34]. In this study, adding the acid and alkaline extract coagulant solutions to the microalgal suspension caused a shift in the culture's pH to 7.9 and 9.1, respectively. However, there was no variation in the hydrogenation potential during the coagulation/flocculation tests and after the biomass sedimentation. Therefore, the microalgal harvesting process occurred within these pH values. High pH can favor microalgal flocculation. According to Sakarika and Kornaros [10], microalgal flocculation can take place when the pH is higher than 9.0, either through the addition of a strong base or spontaneously (autoflocculation). Moreover, Teixeira et al. [33] observed that the harvesting efficiency of *C. vulgaris* using a bioflocculant (*Moringa* seed flour) increased with the pH, achieving a maximum of 89% when the pH was increased to 9.2. The higher harvesting efficiencies at higher pH could be associated with the formation of hydroxide–polymer precipitates, which constitutes a sweeping mechanism. According to Wang et al. [35], the precipitate sweeping of microalgae cells leads to cell aggregation, flocculation, and settling.

According to Dias et al. [36], green coagulants have a harvesting efficiency of >70% in acid or basic pH. For them, the greater percentages of biomass recovery depend on the kind of green coagulant and the dose, combined with pH adjustment and longer settling times. Adjustment to either acidic or basic values results in greater efficiency due to the favorable colloidal destabilization

Behera and Balasubrananian [37] reported the ability of *Opuntia ficus indica* to harvest the microalgal biomass of *Chlorella sp.* In their research, they used NaCl (3 N) as an eluent

for cactus mucilage and achieved a biomass removal efficiency of approximately 60% of *Chlorella sp*. after 4 h of sedimentation. They also used water as eluent, and the biomass removal efficiency was 40.5 % at the same time as sedimentation. It can be concluded that these eluents were not sufficient for the extraction of the coagulant compounds from the *Opuntia* species, although Vishali et al. [38] obtained better results than these authors when using NaCl as an eluent.

3.3. C. vulgaris Sedimentation Kinetics after the use of O. cochenillifera

After the jar test assays, the settling kinetics of the flakes was analyzed by observing the flakes settling over time in a measuring cylinder. Figures 2 and 3 represent the height of the flakes over time in these assays for each acid and alkaline coagulant dosage, respectively. In both figures, the control corresponds to the assay without coagulant.

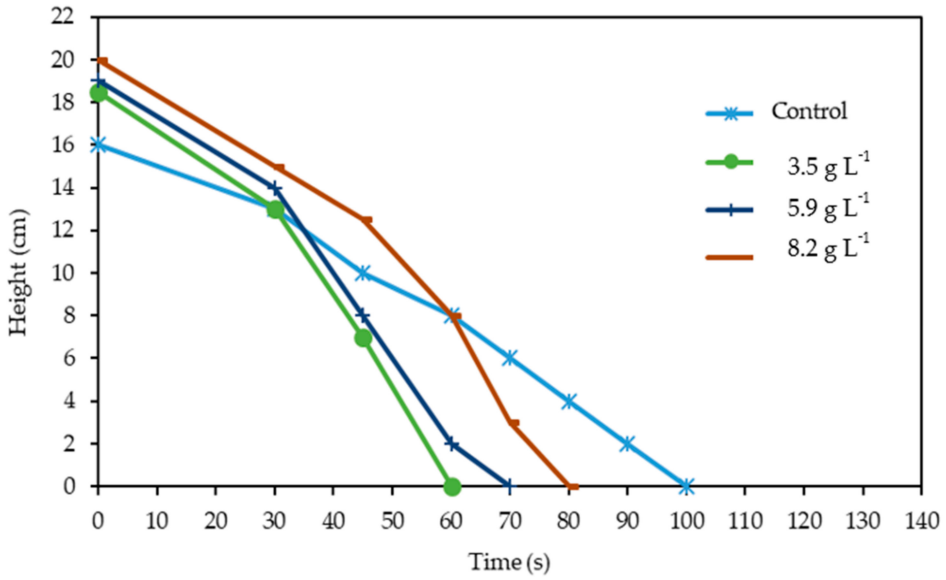

Figure 2. *Chlorella vulgaris* sedimentation kinetics after coagulation/flocculation with acid *Opuntia cochenillifera* extracts.

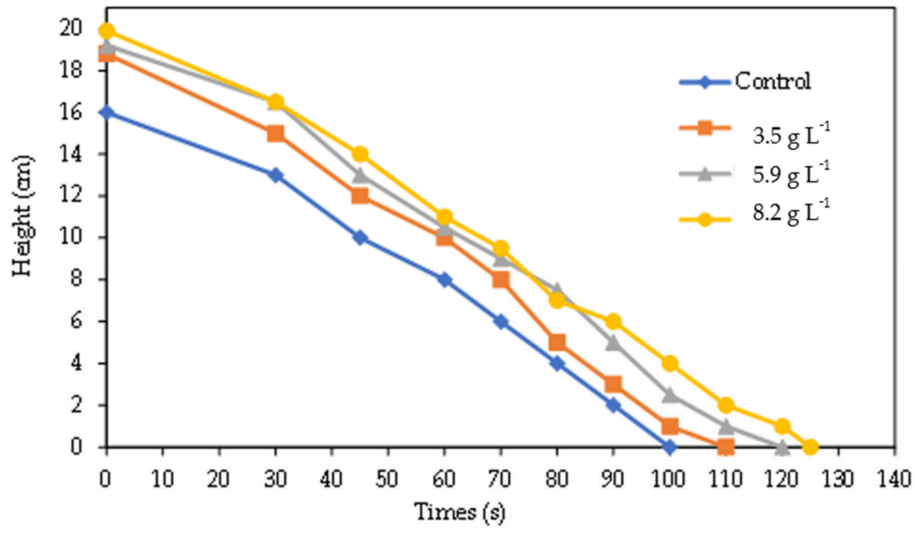

Figure 3. *Chlorella vulgaris* sedimentation kinetics after coagulation/flocculation with alkaline *Opuntia cochenillifera* extracts.

The starting point of the curves corresponds to the time point in which the flocs were still on the surface of the solutions. At this point, the floc height varies between samples due to the concentration of cactus mucilage in the solutions. Overall, the sedimentation kinetics differed between the assays with acid and alkaline coagulants. Lower settling times were observed when acid coagulants were used compared to the control and alkaline assays. Table 2 shows the average speed of sedimentation of the flakes over time in a measuring cylinder. The speeds were evaluated according to Figures 2 and 3. For each curve of the sedimentation kinetics of each graphic was performed the linear regression. The slopes of the regression lines are symmetrical to the value of the average falling speeds of the flakes for each concentration. Hence, it can be verified that the highest speeds are for the lowest dosages of acid extract coagulant solution. Generally, all dosages of acid extract coagulant solutions obtained higher sedimentation speeds than the alkaline extract coagulant solutions and the control. It can be observed that for the alkaline extract coagulant solutions, higher sedimentation speeds of the flakes than the one in control were observed. The sedimentation profile of suspended algae is influenced by the density, size, and shape of microalgal cells, as well as the type of coagulant. The particles settle down according to Stokes' Law; cells quickly reach their terminal velocity once the frictional force is balanced with the gravitational force. Evaluating the sedimentation kinetics realizes that the larger flakes reorganized in the graduated cylinders, leading to a shorter linear sedimentation portion and a faster sedimentation rate [39]. Hence, the kinetic tests performed with the microalgae and the *Opuntia* (biocoagulant) with the hydrochloric acid used as eluent were much faster than with the cactus mucilage in an alkaline medium, although the highest harvest efficiency occurred in an alkaline medium. The flocculation process determines the structure, density, and settling velocity of flocs, in which flocs acid extract coagulant-microalgae cells were with more compact structures and uniform than flocs alkaline extract coagulant-microalgae cells. In other words, larger particles are more dispersed in the solution; therefore, the sedimentation kinetics takes longer. Hence, the acid extract coagulant-microalgae cells had fast sedimentation but did not result in the highest removal efficiency. The structures of flocs perform the sedimentation kinetic, but not the highest harvesting microalgae cells.

Table 2. Average speed of flake sedimentation over time in a measuring cylinder.

Eluent	Extract Coagulant Dosage (g L^{-1})	Average Speed of Sedimentation (cm s^{-1})	Correlation Coefficient (R^2)
-	0 (control)	0.163 ± 0.008	0.98
HCl	3.5	0.30 ± 0.05	0.95
	5.9	0.29 ± 0.03	0.97
	8.2	0.25 ± 0.03	0.95
NaOH	3.5	0.181 ± 0.008	0.99
	5.9	0.172 ± 0.007	0.99
	8.2	0.166± 0.004	0.99

Even though the acid coagulant provided higher sedimentation velocities compared to the alkaline coagulant, the harvesting efficiency after 30 min of sedimentation was lower in this case, as mentioned in Section 3.2. The sedimentation profile of suspended algae is influenced by the density, size, and shape of microalgal cells, as well as the type of coagulant. The particles settle down according to Stokes' Law; cells quickly reach their terminal velocity once the frictional force is balanced with the gravitational force. Evaluating the sedimentation kinetics realizes that the larger flakes reorganized in the graduated cylinders, leading to a shorter linear sedimentation portion and a faster sedimentation rate [39]. A possible explanation for these results is that with the acid eluent, the biopolymer could have been partitioned into molecules with a wide range of molecular weights, which contributed to the formation of flocs with a less uniform size distribution. Hence, the larger flocs

might have a higher sedimentation speed, but a lower removal harvesting efficiency was obtained after sedimentation for 30 min since the smaller particles remained in suspension, influencing the optical density. On the other hand, using the alkaline eluent, the polymer might have been broken into molecules with a lower molecular weight. Therefore, smaller flocs might have been formed with a more uniform size distribution, taking more time to settle, which could explain the lower sedimentation speed. Hence, after sedimentation for 30 min, most of these particles were settled, leading to a higher harvesting efficiency.

In the experiments with the dosages of acid coagulant, the biomass sedimented faster than with the dosages of alkaline coagulant. This can be explained by the formation of well-defined flocs, which influences their mass and gravitational force. In addition to the action of the gravitational force on the flakes, the formation of better-defined colloids also implies a smaller number of particles dispersed in the solution, which facilitates the descent of the flocs with less friction [12]. This formation of colloids is given by the formation of bonds between the coagulants and the biomass.

4. Conclusions

C. vulgaris grew successfully with high biomass productivity in an outdoor open PBR using BG-11 medium diluted in cooling water from a distiller as a culture medium. Moreover, the present study confirms the positive coagulation properties of the biopolymer extracted from the mucilage of *O. cochenillifera* using acid (HCl) and alkaline (NaOH) eluents. The *C. vulgaris* harvesting efficiencies ranged between 72.9% and 80.8% using the acid coagulant at different concentrations and 99.3% and 99.5% using the alkaline coagulant, with the highest values observed for a 3.5 g L^{-1} dosage. Therefore, *O. cochenillifera* can be a cost-effective option to harvest microalgal biomass, as it is an efficient biocoagulant/bioflocculant, it is a plant of easy cultivation, which adapts to adverse conditions with the scarcity of water, high temperatures, and rotten soils, and it is not toxic to microalgae. The utilization of *O. cochenillifera* for algae harvesting can be considered to be one of the most promising and potentially sustainable approaches.

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