Bioresource Technology 311 (2020) 123526

Contents lists available at ScienceDirect

Bioresource Technology

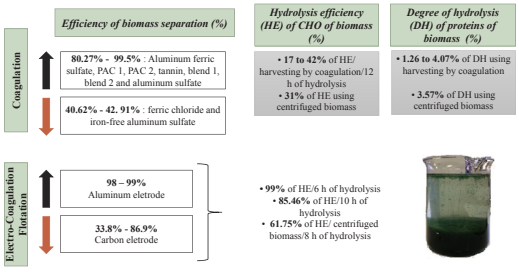
journal homepage: www.elsevier.com/locate/biortech

Effects of harvesting *Spirulina platensis* biomass using coagulants and electrocoagulation–flotation on enzymatic hydrolysis

Francine de Souza Sossella[a], Alan Rempel[a], Janayna Monroe Araújo Nunes[b], Gabriele Biolchi[c], Regina Migliavaca[d], Ana Carolina Farezin Antunes[c], Jorge Alberto Vieira Costa[b], Marcelo Hemkemeier[b], Luciane Maria Colla[a,*]

[a] Graduate Program in Environmental and Civil Engineering, University of Passo Fundo (UPF), Passo Fundo, Rio Grande do Sul 99052 900, Brazil
[b] Graduate Program in Food Science and Technology, University of Passo Fundo (UPF), Passo Fundo, Rio Grande do Sul 99052 900, Brazil
[c] Chemical Engineering Course, University of Passo Fundo (UPF), Passo Fundo, Rio Grande do Sul 99052 900, Brazil
[d] Enviromental Engineering Course, University of Passo Fundo (UPF), Passo Fundo, Rio Grande do Sul 99052 900, Brazil

GRAPHICAL ABSTRACT



ARTICLE INFO

Keywords:
Amylases
Microalgae
Physicochemical separation
Proteases
Saccharification

ABSTRACT

This study aimed to assess the harvesting of *Spirulina platensis* using coagulants and electrocoagulation–flotation (ECF) and to evaluate its influence on enzymatic hydrolysis. Using nine chemical coagulants, we obtained a biomass harvesting efficiency of up to 99.5%. Using ECF, the harvesting efficiency at the aluminum and carbon electrode was 98%–99% and 33.8%–86.9%, respectively. Hydrolysis efficiency (HE) with amylases varied from 17% to 42%, and the degree of hydrolysis (DH) with proteases varied from 1.26% to 4.07%, compared with an HE of 31% and a DH of 3.57% in the centrifuged biomass. Compared to an HE of 61.75% for the centrifuged biomass, and HE of 99% and 85.46% was obtained for the biomass harvested using the aluminum and carbon electrodes. The HEs with the electrodes were better than those with the alternative methods and centrifugation; hence, with some optimization, the biomass harvested could be used for enzymatic hydrolysis.

1. Introduction

Renewable fuels are economic, environmental, and social alternatives to fossil fuels, and biodiesel and ethanol, used alone or as mixtures, are the primary biofuels produced on a large scale. The use of microalgae biomass as a third-generation biofuel feedstock is a promising alternative; this could allow the production of high-value compounds (Gouveia et al., 2017). Microalgae can be cultivated in wastewater or saltwater as well as in nonagricultural areas, not competing with food production (Scaife et al., 2015). Further, microalgae are photosynthetically more efficient than terrestrial plants and are very effective at capturing atmospheric CO_2 (Yoza and Masutani, 2013).

* Corresponding author.
E-mail address: lmcolla@upf.br (L.M. Colla).

https://doi.org/10.1016/j.biortech.2020.123526
Received 24 March 2020; Received in revised form 8 May 2020; Accepted 9 May 2020
Available online 13 May 2020

A study on life cycle impacts, with emphasis on CO_2 emissions and land use, conducted by Gnansounou and Raman (2016) showed that the production of fuels from microalgae generates lower impact than that from conventional sources. Microalgae cultures can remove atmospheric CO_2 during photoautotrophic cultivation. Otherwise, the production of bioethanol and biogas can produce CO_2 via fermentation and biogas purification, respectively, but based on the concept of microalgae biorefinery, these gases can return to algae culture systems (Colla et al., 2019).

The most important factor currently hindering the viability of large-scale biomass production is cost. Research on biorefinery development has focused on cultivation and production; harvesting has attracted less attention (Aro, 2016). Harvesting and drying of microalgae has been estimated to contribute 20–30% to the net cost of biomass production; the small cell size of microalgae makes the harvesting process expensive, and this is one of the main barriers to production. Therefore, producing biofuel from microalgae and overcoming cultivation, harvesting and processing-related problems is important (Medipally et al., 2015; Aro, 2016).

Considering their efficiency, operational savings, and technical viability, flocculation and coagulation are considered superior methods for separation of microalgae from the culture medium; these methods are often used alone or in combination with other methods (Wan et al., 2015; Alam et al., 2015). However, some authors have indicated that these methods can induce unwanted changes in the cellular composition (Shuba and Kifle, 2018). The flocculant should be selected such that the subsequent downstream processing is not adversely affected by its usage. Flocculation and coagulation have advantages like high harvesting efficiency, medium costs and viable for large escale (Laamanen et al., 2016).

On the other hand, electrocoagulation–floatation (ECF) is a foam-flotation harvesting method that has been shown to be highly effective, rapid, and economic; further, it is a scalable separation methodology, but it has not been used industrially, however it depends on high conductivity to be economically viable (Landels et al., 2019; Shi et al. 2017; Shin et al., 2017). ECF combines the production of metal flocculant and microbubbles *in situ* using a gas-generating cathode and a metal floc-generating sacrificial anode (Landels et al., 2019). Inert anodes (Ti, TiO_2/RuO_2; C) can be used too; however, their efficiency is lower. Inert anodes are used to obtain biomass free from metal contamination, facilitating the process of obtaining biofuel (Fuad et al., 2018; Landels et al., 2019).

The mechanisms responsible for microalgae flocculation remain poorly understood (Shi et al., 2017). Hydrogen gas bubbles produced at the cathode plays an important role in microalgae harvesting by decreasing the floc density required for the flotation process (Fuad et al., 2018; Landels et al., 2019). Microalgae flocculation is achieved through charge neutralization. This surface charge can be measured by the Zeta potential (Shi et al., 2017; Landels et al., 2019). There are no clear mechanisms for the correlation between zeta potential and microalgae separation (Shi et al., 2017).

In the biotechnological production of bioproducts using microalgal biomass, it is common to use enzymes during the pretreatment steps for the hydrolysis of chains into small molecules. These enzymes vary according to the resistance of the cell wall of microalgae, as mentioned by Velazquez-Lucio et al. (2018). For the production of bioethanol, the carbohydrates are enzymatically converted by amylases to small chains of sugars, and after converted via fermentation by yeasts to bioethanol (Rempel et al., 2018). To obtaining biopeptides, proteases must to convert proteins in small peptides, being this application useful when the complete implementation of biomass in a biorefinery microalgae concept is being sought, involving various processes for the usage of all components of the feedstock, avoiding losses (Chandra et al., 2019; Trivedi et al., 2015; Sheih et al., 2009). Both enzymes mentioned (amylases and proteases), as well as the yeasts in the fermentation process, could be sensitive to the presence of substances (as residuals of the coagulants used) in biomass (Mishra et al., 2019).

As the particularities of microalgae influence the recovery process and the harvesting method must consider degree of interference in the processing and use of biomass, efficiency and cost, it is necessary to study a harvesting method that is applicable and meets the requirements for the use of biomass. This study aimed to assess and optimize the harvesting of *Spirulina platensis* using coagulation and electro-flocculation and to use the biomass in enzymatic hydrolysis using amylases and proteases.

2. Material and methods

2.1. Microorganism, inoculum preparation, and culture medium

Inoculum preparation and the experiments using *S. platensis* LEB 52 were conducted in Zarrouk's medium (Vonshak, 2002) diluted to 20% (Magro et al., 2017) under nonsterile conditions. The microalga was cultured in the semicontinuous mode in a 300-L raceway tank, with an actual volume of 218 L and an initial inoculum concentration of 0.20 $g{\cdot}L^{-1}$. Agitation was promoted using submersible pumps with a maximum flow rate of 2,500 L h^{-1}. The tank was placed in a greenhouse, made of transparent film, with controlled temperature (21 °C–35 °C), receiving uncontrolled, ambient light (Magro et al., 2017).

When the cells in the cultures reached the stationary phase, with a concentration of approximately 1.30 $g{\cdot}L^{-1}$, 30 L of the cell suspension per day was removed from the cultures for the coagulation tests. Water and Zarrouk media was added again in the tank to maintain the volume and ensure the operation of submerged pumps in the semicontinuous process of cultivation every 10 days approximately, providing nutrient replacement.

The cell concentration of the cultures was monitored by reading the absorbance at OD_{670} using a spectrophotometer and a calibration curve for the dry mass and absorbance of biomass (Costa et al., 2002). The pH and millivoltage of the cultures were evaluated using the potentiometric method, according to A.O.A.C. (2000).

2.2. Harvesting of Spirulina biomass using chemical coagulants

Cells from the culture medium were harvested using different coagulants. Two 22 Central composite designs (CCDs) were performed, one for each coagulant, being the control variables, the coagulant concentration, and pH (Table 1). The variable pH was fixed, while the variable concentration of the coagulant varied according to the type of coagulant used (Table 1). Two central points were used in each design. To choose the concentrations of the CCDs, preliminary tests were performed from 50 $mg.g^{-1}$ to a concentration that the coagulant acts to destabilize the loads and form the clots.

The commercial blends (B1 and B2) used are coagulants based on the cationic polymers in solution and inorganic coagulants. The CCD-related experiments were performed in triplicates using a 250-mL culture volume. pH was adjusted with glacial acetic acid (Table 1), and the coagulants were added according to the established concentrations along with mechanical stirring for 2 min at 200 rpm (2 *g*), followed by slow mixing at 20 rpm (0.01569 *g*) for 15 min for flocculation, and resting for 30 min. After coagulation, the biomass was removed and followed the drying procedure for subsequent analyzes. The supernatant was homogenized and a 10 mL sample was taken to measurements of residual biomass concentration in spectrophotometer at 670 nm. The biomass separation efficiency was calculated based on the initial concentration of the biomass and its final concentration in the supernatant after coagulation (Eq. (1)), where, C_0 is the initial concentration ($g.L^{-1}$) and C_f is the final concentration ($g.L^{-1}$).

$$Efficiency of biomass separation\,(\%) = \frac{(C_0 - C_f)}{C_0} \times 100 \quad (1)$$

Table 1
2^2 central composite designs used to study the influence of pH and coagulant concentration on harvesting of *Spirulina platensis* (coded and real values of pH and concentrations of each coagulant).

Experiment	Coded Values		Real Values									
	X_1 (pH)	X_2 (CC – mg.g^{-1})	pH	AS	FC	B1	B2	IfAS	PAC1	PAC2	AFS	Tannin
1	−1	−1	6.0	450	500	200	200	200	200	450	450	200
2	+1	−1	9.0	450	500	200	200	200	200	450	450	200
3	−1	+1	6.0	1,250	1,500	1,000	1,000	1,000	1,000	1,250	1,250	1,000
4	+1	+1	9.0	1,250	1,500	1,000	1,000	1,000	1,000	1,250	1,250	1,000
5	0	0	7.5	850	1,000	600	600	600	600	850	850	600
6	0	0	7.5	850	1,000	600	600	600	600	850	850	600

CC (concentration of coagulant); AS: Aluminum Sulfate; FC: ferric chloride; B1: Blend 1; B2: Blend 2; IfAS: Iron-free aluminum sulfate; PAC 1: Poly Aluminium Chloride 1; PAC 2: Poly Aluminium Chloride 2; AFS: Aluminum Ferric Sulfate.

According to the best results of the CCD for each coagulant, the coagulation and flocculation assays were performed using a 2-L culture volume. A control test was accomplished by performing biomass separation via centrifugation at 1,783*g* for 10 min to compare the biomass quality among the enzymatic hydrolysis types.

2.3. Harvesting of Spirulina biomass by electrocoagulation–flotation (ECF)

Two 2^2 CCDs with central points were accomplished, the variables studied being the pH and current density (CD) (the design in Table 4, in the result and discussion session). Among the different anode materials, aluminum (5.20×10^{-3} m^2) and carbon (9.67×10^{-3} m^2) were selected for evaluation. The cathode materials were carbon steel with same dimensions of anode. The electrodes (a gap of 10 mm) were vertically submerged in microalgae culture media and held in place using a clamp stand. The electric current and voltage (0.15–1.0 A, and 1.4–4.3 V depending of operational conditions) were supplied through a direct current power supply (New Dawer Model FCC5002-100W).

The initial pH was adjusted with HCL solution (2 mol/L). Agitation condition (150 rpm or 2.52 *g*) and electrolysis time (30 min) were maintained as constant. All ECF experiments were conducted at room temperature in cylindrical vessels (2.0 L). Experimental conditions were defined according to the literature and preliminary experiments (data not shown). The H_2 and O_2 (lesser extent) bubbles generated at the cathode and anode, respectively, caused the flotation of the flocs containing microalgae, which they were concentrated in the upper part of the cylindrical vessel. The clarified liquid was separated from the biomass by filtration. The biomass separation efficiency was in the same form as previously described (Eq. (1)).

2.4. Characterization of the biomass

The separated biomass was dried at 50 °C for 24 h and submitted for carbohydrate, protein, moisture, and ash content determination. Carbohydrate were determined according to the method described by Dubois et al. (1956); protein, Lowry et al. (1951); and ash, Instituto Adolfo Lutz (1985); the moisture content was determined at 105 °C (AOAC, 2000). For the analyses of carbohydrates and intracellular proteins of the microalga, the samples were submitted for cellular rupture using an ultrasonic probe (Ultronique, Indaiatuba, Brasil) with a micro titanium tip of 4-mm diameter. For this, 5 mg of biomass was added to 10 mL of distilled water and then sonicated for 5 min in 59-s cycles. Traces of coagulants in the biomass were evaluated using scanning electron microscopy (Tescan Veja 3LM) coupled with energy-dispersive X-ray spectroscopy (EDS) (Oxford), which produces surface images and chemically characterizes the sample.

2.5. Studies of enzymatic hydrolysis

Cellular disruption was accomplished to release intracellular compounds using the freeze–thaw method reported by Rempel et al. (2018). A 10% (w/v) *Spirulina* suspension was prepared in 0.2 M of sodium phosphate buffer at pH 5.5. The samples were then frozen for 24 h and thawed at 4 °C and heated in a thermostatic bath at 100 °C for 10 min to hydrate the starch present in the sample. Saccharification of the polysaccharides was performed using the free enzymes α-amylase (Liquozyme® Supra 2.2X) and amyloglucosidase (AMG® 300L), with the addition of 1% (v/v) of the *Spirulina* suspension after cell disruption. The enzymatic activity of α-amylase in starch was 0.92 U.mL^{-1} and in Spirulina carbohydrates it was 0.62 U.mL^{-1}. For amyloglucosidase it was 5.11 U.mL^{-1} in starch and 3.15 U.mL^{-1} in *Spirulina* carbohydrates (Rempel et al., 2018). The experiment was performed in 125-mL Erlenmeyer flasks placed in an orbital shaker at 150 rpm, with an actual volume of 50 mL and a temperature of 50 °C. Sampling was performed every 2 (biomass obtained by EFC) or 4 h (biomass obtained by coagulation) to determine the concentration of reducing sugar (RS) as per the 3–5-dinitrosalicylic acid (DNS) method (Miller, 1959), using a standard curve of glucose. The determination of reducing sugars was preceded by precipitation of the proteins using Carrez solutions I and II (containing 15% of potassium ferrocyanide and 30% sulfate or zinc acetate solution, respectively), in a 10-mL volumetric flask and centrifuged. All determinations were performed in duplicates. Interference of the coagulants in saccharification was measured as the saccharification efficiency, with an efficiency of 100% indicating total hydrolysis of the polysaccharides in reducing sugars.

Hydrolysis efficiency (HE) was calculated using the Eq. (2), where, HE (%) is the hydrolysis efficiency, RS_{AH} is the reducing sugars obtained after hydrolysis, $m_{biomass}$ (g) is the mass of *Spirulina* biomass used in hydrolysis, CHO (g.L^{-1}) is the percent of carbohydrates present in *Spirulina* biomass, V (L) is the final volume used in the hydrolysis process, and 1.1 is the conversion number of CHO to glucose (Hang et al., 1981).

$$HE(\%) = \frac{RS_{AH}\left(\frac{g}{L}\right)}{\left(\frac{m_{biomass(g)}CHO(g/L).(1.1)}{V(L)}\right)}.100 \quad (2)$$

Using the biomass obtained with chemical coagulants, protein hydrolysis was performed using the enzyme protease (Protemax® 580L). Parameters were set according to Lisboa et al. (2014), and a suspension of *Spirulina* containing 2% protein in 50 mL of bicarbonate-sodium carbonate buffer at pH 9.5 was prepared. For cell wall rupture, the same method reported before was used (Rempel et al., 2018). After cell disruption, 5 U·mL^{-1} of the protease enzyme was used for each test, where 1 U of enzymatic activity was defined as the amount of enzyme releasing 1 μg of tyrosine/min, expressed as U·mL^{-1}.

The samples were incubated in an orbital shaker at 180 rpm at 60 °C, the optimal temperature for enzyme activity. The samples were taken out at the initial time and final time, which was defined in 4 h of reaction. To determine the degree of hydrolysis (DH), 1 mL of the

hydrolysate was added 9 mL of 6.25% trichloroacetic acid solution (TCA) and rested for 10 min. Next, the sample was centrifuged for 5 min at 5000 rpm for the removal of insoluble material precipitated by TCA. The soluble protein content of the supernatant was determined using the method of Lowry et al. (1951), and the content was expressed as mg of albumin. DH was estimated according to the method described by Hoyle and Merritt (1994), with modifications, and expressed as the amount of soluble proteins in TCA before and after the addition of the enzyme relative to the amount of total protein present in the sample, calculated according to Eq. (3), where, SP_{t0} is the amount of soluble protein in 6.25% TCA before addition of the enzyme, SP_t is the amount of soluble protein at a given time after the addition of the enzyme, and P_{total} is the amount of total protein in the sample.

$$DH(\%) = \left(\frac{(SP_t - SP_{t0})}{P_{total}}\right). 100 \quad (3)$$

2.6. Data analysis

Data were analyzed using analysis of variance and the Tukey's test, with a 5% level of significance, to examine the differences between the means of each test. To evaluate the effects of the control variables studied in FFDs over the harvesting efficiency, the design of experiments methodology was used, which evaluates the effects of the study variables on the response (dependent variables). The effects of the variables, the interactions among the variables, and the statistical significance (p-value) were calculated. For data analysis, the software *Statistica* was used.

3. Results and discussion

3.1. Harvesting of Spirulina with chemical coagulants

3.1.1. Definitions of pH and coagulants concentrations using CCD

The cell concentration of the cultures used in the coagulation tests remained around 1.30 $g.L^{-1}$. Table 2 shows the mean removal efficiencies obtained from the CCDs performed for the nine coagulants tested, as well the estimated effects, regression coefficients, significance levels (p) and values of R^2 obtained for the models.

Only the effects of pH on the coagulation efficiency for the coagulants iron-free aluminum sulfate (IfAS) and aluminum ferric sulfate (AFS) were significant ($p < 0.05$). As the effect of the coagulant concentration on coagulation efficiency was not significant, the lowest concentration of coagulant was chosen, directly leading to a reduction in the process cost. For these coagulants (iron-free aluminum sulfate and aluminum ferric sulfate), the conditions of experiment 1 were selected (pH 6.0; the lowest concentrations tested: 200 $mg.g^{-1}$ for iron-free aluminum sulfate and 450 $mg.g^{-1}$ for aluminum ferric sulfate). The pH and coagulant concentration significantly influenced the coagulation efficiency ($p < 0.05$) of aluminum sulfate (AS) and PAC 2. Increasing the pH from the lower level (pH 6.0) to the higher level (pH 9.0) decreases the coagulation efficiency. The coagulant concentration showed an opposite effect; i.e, when the concentration was increased from the lower level (−1) to the higher level (+1), it resulted in an increase in the harvest efficiency. Therefore, for aluminum sulfate and PAC 2, it is advisable to use pH 6.0 and 1,250 $mg.g^{-1}$ of biomass for the separation of *S. platensis*.

The coagulants blend 1, blend 2, ferric chloride, tannin, and PAC 1 showed similar behavior in relation to the factors that presented significant effects. All indicated that the interactions between pH and concentration were significant in terms of coagulation efficiency ($p < 0.05$). The coagulants tannin, blend 1, and PAC 1 have the same profile, and we could conclude that the harvesting efficiency is higher when pH 6.0 was used and when the concentration of the coagulant was 1,000 $mg.g_{biomass}^{-1}$.

For ferric chloride (FC) and blend 2, when the pH was at the lower level, the efficiencies were similar for both concentration values. With the pH at the upper level, the efficiency was high when high concentration was tested. Thus, the pH can be used at level +1 (pH 9.0), provided that a high coagulant concentration is used, and at pH 6.0, thus enabling use at the lowest concentration of coagulant. For FC pH 6.0 and 500 $mg.g^{-1}$ of biomass and for blend 2 pH 6.0 and 200 $mg.g^{-1}$ of biomass were chosen.

Lama et al. (2016) mentioned that the dosage of coagulant is independent of the particle surface characteristics but must be high because the colloidal particles must be involved, forming large masses of precipitates. In general, the mechanism of bridging is observed with the use of polyelectrolytes; hence, the active fractions and the colloids are agglomerated. In the neutralization mechanism, generally observed with inorganic coagulants, the concentration of coagulant required is dependent on the number of charges that need to be neutralized; these are a function of the charge density of the cell surface as well as the surface–volume ratio of the cells, parameters that differ between species of microalgae. Hence, in the present study, it is natural that inorganic coagulants were more efficient at the high dosages because the *Spirulina* culture has a high number of negative charges to be neutralized.

Brennan and Owende (2010) indicated cell size as the main factor for ease or difficulty in harvesting. If only cell size was considered, in the present case, *Spirulina*, 20–100-μm long, could be considered as an easy-to-separate microalgae compared to *Chlorella*, which has a size of < 30 μm. However, in coagulation and flocculation, which works by destabilizing charges and aggregating cells, the charge must also be considered. Fig. 1 shows the relation of cell concentrations ($g{\cdot}L^{-1}$) and the millivoltages (-mV) measured during 30 days of cultivation of *Spirulina platensis* LEB 52 before starting the coagulation tests.

As the cell concentration increased, the millivoltage value (-mV) also increased. The consumption of the salts in the culture medium as nutrients for algal growth indicated a tendency of decrease of the charges that hinder separation. However, the increase the cell concentration causes an increase in the number of negative charges. This confirms that even the separation of the same species can be facilitated or hindered when conducted under different conditions, such as different cell concentrations or distinct culture phase, as it will have fewer or more charges to be destabilized. According to Shuba and Kifle (2018), the surface charge of microalgae cells is usually negative due to the ionization of functional groups in the microalgal cell walls and the adsorption of ions present in the culture medium. Coagulation and flocculation of microalgae are more effective at low pH; however, the processes are influenced by the growth phase, with better separation conditions at the end of the log phase and in the decline phase.

Henderson et al. (2008) observed that despite the similarity of surface areas (size) of the cells of *M. aeruginosa* and *C. vulgaris*, *C. vulgaris* demanded 3 times more coagulant compared to *M. aeruginosa*. *A. formosa* and *Melosira* sp. (a large filamentous diatom similar to *S. platensis*), which have cells relatively larger than *M. aeruginosa*, required 22 and 207 times more coagulant, respectively, than for *M. aeruginosa*. This is another indication that the demand for coagulant is related to the charge and not the cell size, as indicated by some studies. If separation efficiency were related to size, larger cells would require less coagulant compared to smaller ones. According to Lama et al. (2016), a flocculation method that is effective for one species may not be so for other species of microalgae, given the wide diversification of cell surfaces, sizes, shapes, and properties.

3.1.2. Chemical coagulation tests in the 2-L scale

In this step, sequential tests of coagulation were accomplished to obtain biomass to tests for enzymatic hydrolysis. The conditions of the *Spirulina* cultures used in the coagulation tests on 2 L scale are: cell concentration 1.31 ± 0.22 $g.L^{-1}$; pH 9.73 ± 0.21; millivoltage of −193.99 ± 7.18 mV and millivoltage after adjustment of pH to 6.0,

Table 2
Efficiencies of coagulation (%), estimated effects, regression coefficients, significance levels (p) and values of R^2 of the models obtained for each coagulant tested.

Exp.	Levels		Efficiencies of coagulation (%) for each coagulant								
	X_1	X_2	AS	FC	B1	B2	IfAS	PAC 1	PAC 2	AFS	Tannin
Exp. 1	−1	−1	74.01 ± 9.06^{b}	83.20 ± 7.57^{a}	40.65 ± 21.55^{b}	90.08 ± 4.04^{a}	71.53 ± 9.03^{a}	38.98 ± 6.39^{b}	64.01 ± 2.84^{b}	64.49 ± 2.64^{a}	45.05 ± 6.68^{b}
Exp. 2	1	−1	14.41 ± 0.84^{c}	22.57 ± 3.72^{c}	24.66 ± 6.15^{b}	45.29 ± 8.58^{b}	28.94 ± 8.28^{b}	9.04 ± 5.76^{c}	12.39 ± 0.00^{d}	41.39 ± 19.08^{a}	26.74 ± 3.36^{c}
Exp. 3	−1	1	99.54 ± 0.15^{a}	74.28 ± 6.87^{ab}	93.22 ± 5.53^{a}	88.03 ± 16.29^{a}	91.90 ± 8.04^{a}	92.09 ± 5.64^{a}	97.02 ± 4.33^{a}	75.38 ± 9.45^{a}	76.56 ± 3.53^{a}
Exp. 4	1	1	37.57 ± 18.69^{c}	62.47 ± 7.96^{ab}	28.46 ± 17.59^{b}	97.22 ± 2.96^{a}	26.39 ± 9.84^{b}	16.67 ± 0.49^{c}	26.25 ± 17.46 ^{cd}	50.98 ± 21.50^{a}	30.04 ± 3.17^{d}
Exp. 5	0	0	38.98 ± 5.08^{c}	61.68 ± 7.96^{ab}	58.81 ± 9.70^{ab}	92.37 ± 1.53^{a}	46.06 ± 5.99^{b}	14.41 ± 1.46^{c}	41.00 ± 9.75^{bc}	55.56 ± 1.31^{a}	66.41 ± 5.12^{a}
Exp. 6	0	0	35.31 ± 5.17^{c}	59.06 ± 14.70^{b}	49.32 ± 13.41^{b}	92.90 ± 1.20^{a}	31.02 ± 2.63^{b}	16.10 ± 1.69^{c}	33.63 ± 0.88 ^{cd}	50.11 ± 5.28^{a}	52.34 ± 5.47^{b}

		Estimated effects (EE), regression coefficients (RC), significance levels (p) for each coagulant and values of R^2 of the models								
		AS	FC	B1	B2	IfAS	PAC 1	PAC 2	AFS	Tannin
Mean	EE	49.97	60.34	49.19	84.31	49.30	31.21	45.72	56.32	49.52
	RC	49.97	60.34	49.19	84.31	49.30	31.21	45.72	56.32	49.52
	p	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
X_1	EE	−60.79	−36.22	−40.38	−17.79	−54.05	−52.68	−61.19	−23.75	−32.41
	RC	−30.39	−18.11	−20.19	−8.90	−27.02	−26.34	−30.59	−11.87	−16.21
	p	< 0.0001	< 0.0001	0.0001	0.0074	< 0.0001	< 0.0001	< 0.0001	0.0044	< 0.0001
X_2	EE	+24.34	+15.49	+28.18	+24.98	+8.91	+30.36	+23.44	+10.23	+17.40
	RC	12.17	7.74	14.09	12.47	4.46	15.18	11.72	5.12	8.70
	p	0.0067	0.0054	0.0029	0.0006	0.2272	0.0015	0.0018	0.1657	0.0100
$X_1.X_2$	EE	−1.18	+24.41	−24.39	+26.29	−11.46	−22.74	−9.57	−0.65	−14.10
	RC	−0.59	12.20	−12.51	13.49	−5.73	−11.37	−4.79	−0.33	−7.05
	p	0.8797	0.0001	0.0076	0.0003	0.1267	0.0108	0.1400	0.9269	0.0303
R^2		0.83	0.87	0.78	0.78	0.82	0.83	0.89	0.49	0.76
R^2adjusted		0.80	0.85	0.73	0.74	0.78	0.79	0.87	0.38	0.71

AS: Aluminum Sulfate; FC: ferric chloride; B1: Blend 1; B2: Blend 2; IfAS: Iron-free aluminum sulfate; PAC 1: Poly Aluminium Chloride 1; PAC 2: Poly Aluminium Chloride 2; AFS: Aluminum Ferric Sulfate. Equal letters in the lines indicate that there is no statistical difference between the means of the trials, $p > 0.05$.
Factors were considered significant when $p < 0.05$. p = significance level. X_1: pH; X_2: concentration (mg/g); p: significance level; EE: estimated effect. RC: regression coefficient. R2: determination coefficient.

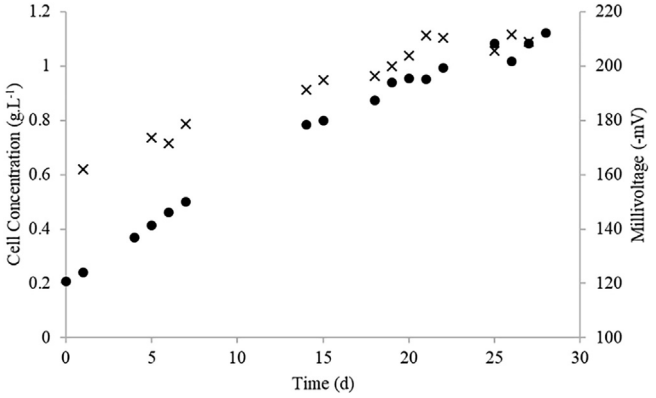


Fig. 1. Relation of cell concentrations ($g{\cdot}L^{-1}$) and the millivoltages (-mV) (•) and millivoltage (×) of the cultures.

36.74 ± 4.53 mV. Some coagulants presented low efficiencies of separation, which hindered the obtaining of biomass to the next studies of enzymatic hydrolysis. The mean efficiencies can be assessed based on Table 3, where it can be seen that efficiencies varied between CCD testing and scaling up. This can be justified by the period in which the tests were performed; the difference of 3 months between the completion of the CCD and the start of the scale up tests, possibly causing changes in biomass. Also, the mechanical stirring equipment used in the steps was different, as the test volumes differed from 250 mL to 2 L. The lower results were obtained with the coagulants, ferric chloride and iron-free aluminum sulfate, which also had low dosages when compared to the others. Thus, despite the low efficiency, the dosage represented lower costs. Differing statistically from the first group ($p < 0.05$) and with higher efficiencies are aluminum sulfate, blend 1, blend 2, PAC 1, PAC 2, aluminum ferric sulfate, and tannin. As for the concentration of the coagulant, the dosages of blend 2 and aluminum ferric sulfate were lower, which implies a resource saving.

3.2. Harvesting of Spirulina using electrocoagulation–flotation (ECF)

The pH and current density influenced the harvesting efficiency of *S. platensis* differently. (Table 4). The efficiencies were similar to those observed in literature (Luo et al., 2017), especially for aluminum electrodes.

Table 4
2^2 CCD (Central Composite Designs) used to study the influence of pH and Current Density (CD) on the harvesting of *Spirulina* platensis using aluminium and carbon electrodes (coded and real values of pH and CD to each electrode) and Harvesting Efficiencies (%) obtained.

Electrode material	Exp.	X_1 (pH)	X_2 (CD) (A/m^2)	Harvesting Efficiency (%)
Aluminium	1	−1 (4.5)	−1 (30)	99.9 ± 0.2
	2	+1 (5.5)	−1 (30)	98.0 ± 0.6
	3	−1 (4.5)	+1 (50)	98.8 ± 0.2
	4	+1 (5.5)	+1 (50)	98.8 ± 0.3
	5	0 (5.0)	0 (40)	98.4 ± 0.0
	6	0 (5.0)	0 (40)	98.45 ± 0.1
	7	0 (5.0)	0 (40)	98.9 ± 0.9
Carbon	1	−1 (4.0)	−1 (60)	43.7 ± 4.4
	2	+1 (5.0)	−1 (60)	35.7 ± 13.9
	3	−1 (4.0)	+1 (1 0 0)	83.5 ± 4.0
	4	+1 (5.0)	+1 (1 0 0)	33.8 ± 5.3
	5	0 (4.5)	0 (80)	86.9 ± 5.1
	6	0 (4.5)	0 (80)	90.5 ± 0.3
	7	0 (4.5)	0 (80)	83.3 ± 0.4

The pH and the interaction between current density (CD) and pH significantly affected the harvesting process using the aluminum electrode with the efficiencies of harvesting being between 98% and 99%. When the carbon electrode was used, lower range of efficiencies of harvesting were observed (33.8 to 86.9%), and all parameters studied influenced *S. platensis* harvesting ($p < 0.05$). For the aluminum electrode, the model of harvesting efficiency (%) was $98.896–0.458.X_1-0.464.X_1.X_2$ ($R^2 = 0.85$ and R^2adjusted = 0.79). For the carbon electrode, the model of harvesting efficiency (%) was $49.172–14.392.X_1 + 9.475.X_2 – 10.412.X_1.X_2$ ($R^2 = 0.94$ R^2adjusted = 0.91).

The lowest pH and the largest CD favored the harvesting of *S. platensis*. For both electrodes studied, the cathode generated hydrogen gas bubbles favoring the harvesting of *S. platensis*. Two mechanisms can act separately or together to neutralize microalgae charges: pH change and coagulant addition. For *S. platensis,* both mechanisms were observed, however it was clear that the flocculation with the Al electrode had the greatest efficiency and is therefore more suitable for harvesting this microalga. The decrease in pH caused the neutralization of *S. platensis* charges, which was more evident when the carbon electrode was used. Shi et al. (2017) observed a decrease in the charge of *Chlorella vulgaris*

Table 3
Separation efficiencies for each chemical coagulant in the harvesting of biomass to be used in the steps of enzymatic hydrolysis, chemical composition of dry biomass in comparison with the obtained by centrifugation, hydrolysis efficiencies of conversion of carbohydrates in reducing sugar (HE) and degree of hydrolysis of proteins (DH) for the biomasses obtained using several coagulants

Coagulation conditions and results			Biomass composition				Results of enzymatic tests using biomass		
Coag.	[Coag.] ($mg.g^{-1}$ of biomass)	Removal (%)	CHO (%)	Protein (%)	Ash (%)	Moisture (%)	RS 12 h ($g.L^{-1}$)	HE (%)	DH (%)
AS	1,250	97.80 ± 6.00^{a}	5.93 ± 0.67^{bd}	55.47 ± 3.77^{be}	30.66 ± 0.29^{bcde}	7.30 ± 0.26^{b}	3.14 ± 1.22^{a}	40.46 ± 15.77^{a}	1.84 ± 0.12^{a}
FC	500	40.62 ± 17.53^{b}	3.46 ± 0.70^{b}	28.23 ± 3.28^{d}	47.04 ± 0.72^{a}	8.48 ± 0.30^{ab}	1.94 ± 0.01^{a}	42.66 ± 0.23^{a}	2.01 ± 0.12^{a}
B1	1,000	99.40 ± 1.23^{a}	7.55 ± 0.78 ^{cd}	67.37 ± 6.29^{efg}	29.15 ± 0.78^{cde}	10.30 ± 0.28^{ab}	2.64 ± 0.58^{a}	26.73 ± 5.86^{a}	2.37 ± 0.33^{a}
B2	200	96.19 ± 6.57^{a}	10.49 ± 1.66^{ac}	70.44 ± 5.25 ^{fg}	23.60 ± 1.69^{e}	7.82 ± 1.53^{b}	3.37 ± 0.93^{a}	24.56 ± 6.79^{a}	1.26 ± 0.87^{a}
IfAS	200	42.91 ± 28.45^{b}	5.48 ± 1.52^{bd}	45.08 ± 0.73^{bc}	37.50 ± 1.79^{b}	12.33 ± 15^{a}	2.97 ± 0.18^{a}	41.28 ± 2.46^{a}	1.63 ± 0.22^{a}
PAC 1	1,000	95.68 ± 11.97^{a}	6.99 ± 1.64^{bcd}	63.27 ± 1.58^{eg}	30.54 ± 2.12^{bcde}	9.54 ± 0.82^{ab}	3.39 ± 0.55^{a}	37.01 ± 6.02^{a}	2.17 ± 1.13^{a}
PAC 2	1,250	98.77 ± 3.50^{a}	6.88 ± 1.28^{bcd}	36.23 ± 0.65 ^{cd}	34.26 ± 4.07^{bc}	8.24 ± 0.72^{ab}	3.27 ± 1.64^{a}	36.31 ± 18.25^{a}	4.07 ± 0.60^{a}
AFS	450	80.27 ± 23.79^{a}	8.05 ± 1.16 ^{cd}	69.49 ± 5.05 ^{fg}	32.09 ± 0.98^{bcd}	8.84 ± 0.23^{ab}	3.95 ± 1.01^{a}	37.52 ± 9.65^{a}	1.53 ± 0.91^{a}
Tannin	1,000	99.50 ± 0.62^{a}	12.09 ± 2.32^{a}	87.68 ± 5.43^{a}	24.27 ± 3.13^{de}	8.41 ± 0.41^{ab}	2.79 ± 0.26^{a}	17.60 ± 1.64^{a}	2.95 ± 1.37^{a}
Centrifuged	–	–	9.00 ± 0.64^{acd}	78.68 ± 6.96^{af}	27.79 ± 2.20^{cde}	10.79 ± 3.11^{ab}	3.70 ± 0.27^{a}	31.38 ± 2.31^{a}	3.57 ± 0.02^{a}

Coag.: coagulants; [coag.]: concentration of coagulants; CHO: carbohydrates; AS: Aluminum Sulfate; FC: ferric chloride; B1: Blend 1; B2: Blend 2; IfAS: Iron-free aluminum sulfate; PAC 1: Poly Aluminium Chloride 1; PAC 2: Poly Aluminium Chloride 2; AFS: Aluminum Ferric Sulfate. RS-12 h: reducing sugars formed in 12 h of hydrolysys HE: hydrolysis efficiency of sugars release in 12 h. DH: Degree of hydrolysys of proteins in 4 h of reaction. AS: Aluminum Sulfate; FC: ferric chloride; B1: Blend 1; B2: Blend 2; IfAS: Iron-free aluminum sulfate; PAC 1: Poly Aluminium Chloride 1; PAC 2: Poly Aluminium Chloride 2; AFS: Aluminum Ferric Sulfate. Equal letters in the columns indicate that there is no statistical difference between the means of the trials, $p > 0.05$.

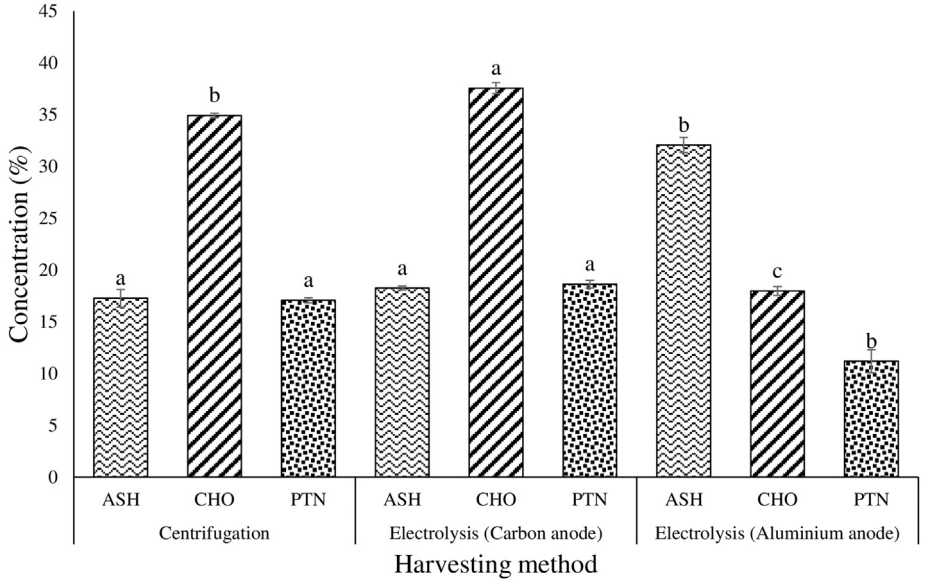


Fig. 2. Carbohydrate, protein and ashes percent in the biomass obtained by electro-coagulation flotation and centrifugation.

with a decrease in pH, and this also favored its removal from the medium. The efficiency of the electroflocculation process decreases with increasing pH as a function of aluminum hydroxide speciation. In alkaline pH levels, the formation of negatively charged aluminum hydroxide is promoted to $[Al(OH)]^{-4}$, which will not react with the negative charge of microalgae cells (Vandamme et al., 2011).

3.3. Characterization of biomasses obtained from chemical coagulation and electrocoagulation

3.3.1. Biomass obtained by chemical coagulation

For a better understanding of the composition of the biomass that were separated by the use of chemical coagulants, we performed the scanning electron microscopy in a 60-fold increase, being the images shown as shown in the Supplementary Material 1. The forms of the biomass differ from one method of harvest and another, since the bonds formed between the coagulants and the cells are different. The energy dispersive spectroscopy (EDS) showed that the nutritional components of the Zarrouk culture medium and types of coagulants influenced the composition of the biomass. Iron and aluminum were detected in the biomass harvested when these metals were used as coagulants. The biomass that was coagulated with the tannin presented low-grade aluminum.

The chemical composition of biomasses obtained with different coagulants are shown in Table 3. The high ash content for all samples indicated the presence of the coagulant and the salts of the culture medium which were also precipitated. Normally, the ash content of the *Spirulina* biomass is around 10% (Habib et al., 2008). Soares et al. (2020) also observed an increase in ash content using coagulants to separate microalgae from wastewater. Even the biomass separated by centrifugation had a high ash content, which can be explained as a function of obtaining the biomass by the semi-continuous mode performed in the microalgal cultivation. Thus, when the separation tests occurred, there was a considerable concentration of nutrients in the liquid medium, that were charged to the downstream steps and remained adhered to the biomass.

The presence of the coagulants and the salts of the culture media in the biomass would not be desirable, but to exclude this part, additional processes would be required that would add cost to the production of biomass and would diminish the environmental viability, since the most used process is the washing of the biomass with water or other solvents. In addition, one of the objectives of the work was to evaluate the application of enzymatic hydrolysis in this type of biomass. The high ash content interfered in the determination of carbohydrates and proteins, since even without biomass lipid determination, some biomass presented a sum of more than 100% in the chemical composition. The ash content, although elevated, is not likely to be mistaken, assuming that the ash and moisture determinations were gravimetric.

In the study by Laurens et al. (2012), large differences were found between the methods for the determination of proteins by colorimetric methods. According to the authors, the colorimetric procedure needs caution related to the accessibility of the proteins to Folin's reagent reaction, and the absence of interfering substances in the biomass. The list of interfering substances in the Lowry method is extensive and was published by Peterson (1979) and salts are reported as some of the substances responsible for interference, leading to an increase in the color intensity of the blank, or a decrease in the color intensity of the protein, or both. The determination of carbohydrates of microalgae by the phenol–sulfuric method can be influenced by lipid, protein, and pigment present, leading to an underestimation or overestimation of the total carbohydrate content (Templeton et al., 2012). Nevertheless, the carbohydrate content was expected to be low compared to the protein content, because of the cultivation was carried out in a semi-continuous mode, with periodic addition of nutrients in the medium, thus constantly maintaining the culture in an exponential phase of growth. A higher accumulation of carbohydrates, which constitute the nutritional reserves of the microalga, is expected in the phases of decline (Markou et al., 2012).

Despite the need to use 1 g of tannin per g of biomass, the separated biomass had one of the lowest ash contents and higher carbohydrate and protein contents, differing statistically from the others, which can be explained by the organic composition, thus, the coagulant may have protein and carbohydrate fractions that confer these characteristics to the biomass. The biomass coagulated with 500 $mg.g^{-1}$ of ferric chloride had the highest ash content and the lowest carbohydrate content, differing statistically from the others. The dosage was not high, but the low efficiency of coagulation, characterization, and also the orange color biomass indicated that the coagulant had detached from the *Spirulina* cells.

3.3.2. Characterization of biomass obtained by electrocoagulation–flocculation

Fig. 2 presents the comparison of carbohydrate, protein, and ashes in the biomass obtained by electro-coagulation flotation and

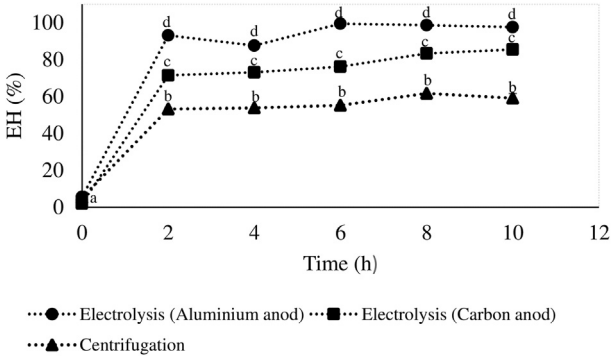


Fig. 3. Hydrolysis efficiency in biomass extract by different methods. Equal letters in the columns indicate that there is no statistical difference between the means of the trials, $p > 0.05$.

centrifugation.

The characteristics of biomass harvested with carbon electrodes and with centrifugation were similar. According to Guldhe et al. (2015), the ideal harvesting technique should not deteriorate the quality of microalgae biomass, and should not interfere with the extraction processes. As for the aluminum electrode, the high ash concentration showed the influence on the biomass composition. This can be explained by the presence of residual aluminum (EDS analyzes) in the biomass harvested.

3.4. Effects of chemical coagulants on the enzymatic hydrolysis of carbohydrates and proteins present in the biomass of Spirulina

For the biomasses obtained with chemical coagulation, the maximum concentrations of reducing sugars were reached within 12 h of reaction (Table 3). There was no significant difference between the values of the hydrolysis efficiency (Table 3). Possibly, the high ash content of the samples and consequently the low carbohydrate content were responsible for the low conversion into reducing sugar. The amount of enzyme used was not responsible for the low conversion, since the same conditions (1% for each enzyme) were used by Rempel et al. (2018) in a biomass with approximately 56% carbohydrates, achieving a hydrolysis efficiency of 99%. In this study the same concentration of enzymes was used, but for a lower substrate concentration.

For the assays with ferric chloride, aluminum ferric sulfate, PAC 1 and iron-free aluminum sulfate, the aluminum ferric sulfate presented satisfactory efficiency with respect to the biomass harvest, even with a lower dosage of 450 $mg.g^{-1}$ of biomass. The tannin, with organic characteristics and the highest carbohydrate content, although presented high efficiency in the coagulation stage, didn't presented good results in saccharification, did not reaching 20% conversion. Tannins, in general, present excellent antioxidant properties, which may have caused an inactivation of the enzymes. They are considered potent inhibitors of enzymes, due to their complexation with enzymatic proteins (Shirmohammadli et al., 2018; Reitzer et al., 2018).

The blends and PACs used also obtained median efficiencies, and blend 2 was used in the separation at a concentration of 200 $mg.g^{-1}$ of biomass, while blend 1 was used at 1,000 $mg.g^{-1}$ of biomass. This may indicate that this difference in concentration between the coagulants was not a determining factor for the efficiency of saccharification. However, if the economy of the process is prioritized, then the blend 2 is more appropriate.

The degree of hydrolysis of proteins (Table 3) was not statistically different for any of hydrolyzed samples. Other studies, such as that of Lisboa et al. (2014) achieved a 52.9% degree of hydrolysis working with *Spirulina* sp. LEB 18. The low degree of hydrolysis obtained for all the samples could be explained by the high ash content, which, as in the enzymatic saccharification stage (hydrolysis of the polysaccharides), may have hindered the action of the enzymes. Some factors that can be determinant in the hydrolysis efficiency are the type of pretreatment performed in the substrate, enzyme concentration, the substrate used, thermostability of the enzymes, pH of the medium and agitation. The low degree of hydrolysis can also be justified by the accessibility of the protease to the proteins. Pretreatment of the substrate increases the exposure of the peptide bonds and the adsorption of the enzymes to the protein, which may not have occurred in this type of biomass with the freeze–thaw pretreatment that was used (Carvalho et al., 2013). Thermostability, pH, and agitation were ensured during the test, controlling and monitoring the temperature and stirring of the assay and the pH through the use of the bicarbonate-carbonate buffer. The enzyme concentration, substrate concentration, and time were tested by Lisboa et al. (2014).

In this study, the highest degree of hydrolysis was obtained with the enzyme concentration of 10 $U.mL^{-1}$ (highest concentration tested), 5% of substrate (lowest concentration tested), and 4 h of reaction time. Under the conditions tested, when substrate concentration was increased, protein hydrolysis was inhibited. Even when using lower substrate concentration (2% protein) and an enzyme concentration of 5 $U.mL^{-1}$ and the same reaction time, the results were lower. Thus, the biomass that is separated by the processes of coagulation and flocculation, and consequently with high ash content, may require further specific analysis.

3.5. Effects of EFC on the enzymatic hydrolysis of carbohydrates present in the biomass of Spirulina

Fig. 3 presents the hydrolysis efficiency (HE) results obtained during the enzymatic saccharification process. It was observed that there was a variation in the period of obtaining the maximum saccharification efficiency between the samples. The biomass harvesting by aluminum electrolysis reached 99.41% of HE after 6 h, which corresponds to 19.66 ± 1.5 $g.L^{-1}$ of reducing sugars (RS). As well, the biomass obtained by electrolysis with graphite reached 35.35 ± 1.3 $g.L^{-1}$ of RS after 10 h, reaching 85.46% of HE.

In the centrifuged sample the highest HE obtained was after 8 h of hydrolysis, reaching 61.75% with RS of 23.72 ± 0.3 $g.L^{-1}$. Despite the fact that there was a maximum hydrolysis peak, both samples showed high efficiency for 2 h, which did not differ statistically during the other 8 h. From this it can be inferred that 2 h of hydrolysis would be suitable for the process, as it would allow a cost reduction.

The results showed that the hydrolysis efficiency for the biomass harvested by electrolysis was higher than that obtained by centrifugation. This result is attributed to electrochemical treatment, as verified by Daghrir et al. (2014). The authors observed morphological changes and significant rupture of the cell wall of the microalgae *Chlorella vulgaris* due the application of electrolysis, carried out for the extraction of lipids and proteins. The shape of the cell also changed from round to irregular as a result of the applied external potential. The use of lower current intensity combined with longer electrolysis time increased cell membrane porosity and permeability and it promoted the release of intracellular compounds to the surrounding environment.

Several studies have been carried out pertaining to bioethanol or biodiesel production by the use of microalgae biomass. Rempel et al. (2018) obtained a high percentage of carbohydrate, reaching 60% using the microalgae *S. platensis*. In this research, enzymatic hydrolysis was performed with free enzymes (amylases) at a concentration of 1%, obtaining a hydrolysis efficiency of 99.83% after 24 h. Alternatively, Shokrkar et al. (2017) used mixed microalgae biomass and the enzymes β-glycosidase/cellulase, α-amylase and amyloglucosidase, for hydrolysis at different addition times for each of them. The authors obtained 97.06% hydrolysis and concluded that when β-glycosidase/cellulase was used to hydrolyze microalgae biomass, total reducing sugar and glucose yield were much higher than those obtained without the

enzyme. The main carbohydrates identified in the microalgae biomass were cellulose and starch. Cellulose molecules are glucose polymers linked together by β-1,4 glycosidic bonds, as opposed to the α-1,4 and α-1,6 glycosidic bonds for starch. In enzymatic pretreatment of algae, the initial addition of cellulase facilitates the action of amylases in starch hydrolysis.

4. Conclusions

A high separation efficiencies of biomass using inorganic chemical coagulants (up to 99.5%) or electrocoagulation–flotation (ECF) were obtained (86.9 and 99% using carbon or aluminum electrodes respectively). The ECF had a comparatively lesser effect on the enzymatic tests. It was demonstrated that better hydrolysis results could be obtained with coagulation and ECF separated biomass than with centrifugation biomass. The biomasses separated by coagulation or ECF could be used in enzymatic processes even when presenting high concentrations of salts, and the culture mode (semi-continuous for this work) probably had a greater influence on the composition of the biomass and the enzymatic hydrolysis efficiencies than the separation method.

CRediT authorship contribution statement

Francine Souza Sossella: Data curation, Formal analysis, Investigation, Methodology, Writing - original draft. **Alan Rempel:** Data curation, Writing - original draft. **Janayna Monroe Araújo Nunes:** Data curation, Formal analysis, Investigation, Methodology, Writing - original draft. **Gabriele Biolchi:** Investigation, Methodology. **Ana Carolina Farezin Antunes:** Investigation, Methodology. **Jorge Alberto Vieira Costa:** Funding acquisition, Supervision. **Marcelo Hemkemeier:** Conceptualization, Project administration, Supervision, Writing - review & editing. **Luciane Maria Colla:** Conceptualization, Project administration, Writing - review & editing, analysis, Investigation, Writing - original draft.

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.

Acknowledgments

This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES) – Finance Code 001.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.biortech.2020.123526.

References

Alam, F., Mobin, S., Chowdhury, H., 2015. Third generation biofuel from Algae. Procedia Eng. 105, 763–768.
Aro, E.M., 2016. From first generation biofuels to advanced solar biofuels. Ambio 45 (1), 24–31.
Association of the Official Analytical Chemists (AOAC). 2000. Official methods of analysis of the Association of the Official Analytical Chemists International.
Brennan, L., Owende, P., 2010. Biofuels from microalgae - A review of technologies for production, processing, and extractions of biofuels and co-products. Renewable Sustainable Energy Rev. 14, 557–577.
Carvalho, M. L., JR., R.S., Rodríguez-zuñiga, U.F., Suarez, C.A.G., Rodrigues, D.S., Giordano, R.C., Giordano, R.L.C. 2013. Kinetic study of the enzymatic hydrolysis of sugarcane bagasse. Braz. J. Chem. Eng.. 30, 437–447.
Chandra, R., Iqbal, H.M., Vishal, G., Lee, H.S., Nagra, S., 2019. Algal biorefinery: A sustainable approach to valorize algal-based biomass towards multiple product recovery. Bioresour. Technol. 278, 346–359.
Colla, L.M., Margarites, A.C.F., Decesaro, A., Magro, F.G., Kreling, N., Rempel, A., Machado, T.S. (2019). Waste biomass and blended bioresources in biogas production. In Improving Biogas Production, Springer, Cham, pp. 1–23.
Costa, J.A.V., Colla, L.M., Duarte Filho, P., Kabke, K., Weber, A., 2002. Modeling of Spirulina platensis growth in fresh water using response surface methodology. World J. Microbiol. Biotechnol. 18, 603–607.
Daghrir, R., Igounet, L., Brar, S.K., Drogui, P., 2014. Novel electrochemical method for the recovery of lipids from microalgae for biodiesel production. J. Taiwan Inst. Chem. Eng. 45 (1), 153–162.
Dubois, M., Gilles, K.A., Hamilton, J.K., Rebers, P.A., Mith, F., 1956. Colorimetric method for determination of sugars and related substances. Anal. Chem. 28, 350–356.
Fuad, N., Omar, R., Kamarudin, S., Harun, R., Idris, A., Wakg, W.A., 2018. Mass harvesting of marine microalgae using different techniques. Food Bioprod. Process. 112, 169–184.
Gnansounou, E., Raman, J.K., 2016. Life cycle assessment of algae biodiesel and its co-products. Appl. Energy 161, 300–308.
Gouveia, L., Oliveira, A.C., Congestri, R., Bruno, L., Soares, A.T., Menezes, R.S., Filho, N. R.A., Tzovenis, I. Biodiesel From Microalgae. In: Gonzalez – Fernandez, C., Muñoz, R. 2017. Microalgae-Based Biofuels and Bioproducts: From Feedstock Cultivation to End Products. Woodhead Publishing.
Guldhe, A., Singh, B., Mutanda, T., Permaul, K., Bux, F., 2015. Advances in synthesis of biodiesel via enzyme catalysis: novel and sustainable approaches. Renewable Sustainable Energy Rev. 41, 1447–1464.
Habib, M. A. B., Parvin, M., Huntington, T. C., Hasan, M. R. 2008. A review on culture, production and use of Spirulina as food for humans and feeds for domestic animals and fish. FAO Fisheries and Aquaculture Circular. N° 1034. Roma, FAO.
Hang, Y.D., Lee, C.Y., Woodams, E.E., 1981. Production of alcohol from apple pomace. Appl. Environ. Microbiol. 42, 1128–1129.
Henderson, R.K., Parsons, S.A., Jefferson, B., 2008. Successful removal of algae through the control of zeta potential. Sep. Sci. Technol. 43, 1653–1666.
Hoyle, N., Merritt, J.H., 1994. Quality of fish protein hydrolysate from herring (Clupea harengus). J. Food Sci. 59, 76–79.
Instituto Adolfo Lutz. Normas analíticas do Instituto Adolfo Lutz. Métodos químicos e físicos para análise de alimentos. 3ª ed. V. 1. São Paulo, 1985.
Laamanen, C.A., Ross, G.M., Scott, J.A., 2016. Flotation harvesting of microalgae. Renewable Sustainable Energy Rev. 58, 75–86.
Lama, S., Muylaert, K., Karki, T.B., Foubert, I., Henderson, R.K., Vandamme, D., 2016. Flocculation properties of several microalgae and a cyanobacterium species during ferric chloride, chitosan and alkaline flocculation. Bioresour. Technol. 220, 464–470.
Landels, A., Beacham, T.A., Evans, C.T., Carnovale, G., Raikova, S., Cole, I.S., Goddard, P., Chuck, C., Allen, M.J., 2019. Improving electrocoagulation floatation for harvesting microalgae. Algal Res. 39, 101446.
Laurens, L.M.L., Dempster, T.A., Jones, H.D.T., Wolfrum, E.J., Van Wychen, S., Mcallister, J.S.P., Rencenberger, M., Parchert, K.J., Gloe, L.M., 2012. Algal biomass constituent analysis: method uncertainties and investigation of the underlying measuring chemistries. Anal. Chem. 84, 1879–1887.
Lisboa, C.R., Pereira, A.M., Ferreira, S.P., Costa, J.A.V., 2014. Utilisation of *Spirulina* sp. and chlorellapyrenoidosa biomass for the productionof enzymatic protein hydrolysates. Int. J. Eng. Res. Appl. 4, 29–38.
Lowry, O.H., Rosebrough, N.J., Farr, A.L., Randall, R.J., 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193, 265–276.
Luo, Y., Le-Clech, P., Henderson, R.K., 2017. Simultaneous microalgae cultivation and wastewater treatment in submerged membrane photobioreactors: a review. Algal Res. 24, 425–437.
Magro, F.G., Margarites, A.C., Reinehr, C.O., Gonçalves, G.C., Rodigheri, G., Costa, J.A.V., Colla, L.M., 2017. *Spirulina platensis* biomass compositionis influenced by the light availability and harvestphase in raceway ponds. Environ. Technol. 39, 1868–1877.
Markou, G., Angelidaki, I., Georgakakis, D., 2012. Microalgal carbohydrates: an overview of the factors influencing carbohydrates production, and of main bioconversion technologies for production of biofuels. Appl. Microbiol. Biotechnol. 96, 631–645.
Medipally, S.R., Yusoff, F.M., Banerjee, S., Shariff, M., 2015. Microalgae as sustainable renewable energy feedstock for biofuel production. Biomed Res. Int. 2015, 1–13.
Miller, G.L., 1959. Modified DNS method for reducing sugars. Anal. Chem. 31 (3), 426–428.
Mishra, S., Roy, M., Mohanty, K., 2019. Microalgal bioenergy production under zero-waste biorefinery approach: Recent advances and future perspectives. Bioresour. Technol. 292, 122008.
Peterson, G., 1979. Review of the Folin phenol protein quantitation method of Lowry, Rosebrough, Farr and Randall. Anal. Biochem. 100, 201–220.
Reitzer, F., Allais, M., Ball, V., Meyer, F., 2018. Polyphenols at interfaces. Advances In Colloid And Interface Science. 257, 31–41.
Rempel, A., Machado, T., Treichel, H., Colla, E., Margarites, A.C., Colla, L.M., 2018. Saccharification of *Spirulina platensis* biomass using free and immobilized amylolytic enzymes. Bioresour. Technol. 263, 163–171.
Scaife, M.A., Merkx-Jacques, A., Woodhall, D.L., Armenta, R.E., 2015. Algal biofuels in Canada: Status and potential. Renewable Sustainable Energy Rev. 44, 620–642.
Sheih, I.C., Fang, T.J., Wu, T.K., Lin, P.H., 2009. Anticancer and antioxidant activities of the peptide fraction from algae protein waste. J. Agric. Food. Chem. 58 (2), 1202–1207.
Shi, W., Zhu, L., Chen, Q., Lu, J., Pan, G., Hu, L., Yi, Q., 2017. Synergy of flocculation and flotation for microalgae harvesting using aluminium electrolysis. Bioresour. Technol. 233, 127–133.
Shin, H., Kim, K., Jung, J.Y., Bai, S.C., Chang, Y.K., Han, J.I., 2017. Harvesting of *Scenedesmus obliquus* cultivated in seawaterusing electro-flotation. Korean J. Chem.

Eng. 34, 62–65.
Shirmohammadli, Y., Efhamisisi, D., Pizzi, A., 2018. Tannins as a sustainable raw material for green chemistry: A review. Ind. Crops Prod. 126, 316–332.
Shokrkar, H., Ebrahimi, S., Zamani, M., 2017. Bioethanol production from acidic and enzymatic hydrolysates of mixed microalgae culture. Fuel 200, 380–386.
Shuba, E.S., Kifle, D., 2018. Microalgae to biofuels: 'Promising'alternative and renewable energy, review. Renewable Sustainable Energy Rev. 81, 743–755.
Soares, R.B., Martins, M.F., Gonçalves, R.F., 2020. Thermochemical conversion of wastewater microalgae: The effects of coagulants used in the harvest process. Algal Res. 47, 101864.
Templeton, D. W., Quinn, M., Van wychen, S., Hyman, D., Laurens, L. M .L. 2012. Separation and quantification of microalgal carbohydrates. J. Chromatogr. A. 1270, 225–234.
Trivedi, J., Aila, M., Bangwal, D.P., Kaul, S., Garg, M.O., 2015. Algae based biorefinery—how to make sense? Renewable Sustainable Energy Rev. 47, 295–307.
Vandamme, D., Pontes, S.C.V., Goiris, K., Foubert, I., Pinoy, L.J.J., Muylaert, K., 2011. Evaluation of electro-coagulation–flocculation for harvesting marine and freshwater microalgae. Biotechnol. Bioeng. 108 (10), 2320–2329.
Velazquez-Lucio, J., Rodríguez-Jasso, R.M., Colla, L.M., Sáenz-Galindo, A., Cervantes-Cisneros, D.E., Aguilar, C.N., Fernandes, B.D., Ruiz, H.A., 2018. Microalgal biomass pretreatment for bioethanol production: A review. Biofuel Res. J. 17, 780–791.
Vonshak, A., 2002. *Spirulina platensis (Arthrospira*): Physiology, Cellbiology and Biotechnology. Taylor & Francis, London.
Wan, C., Alam, M.A., Zhao, X., Zhang, X., Guo, S., Ho, S., Chang, J., Bai, F., 2015. Current progress and future prospect of microalgal biomass harvest using various flocculation technologies. Bioresour. Technol. 184, 251–257.
Yoza, B.A., Masutani, E.M., 2013. The analysis of macroalgae biomass found around Hawaii for bioethanol production. Environ. Technol. 34 (13–14), 1859–1867.