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Gracilaria chilensis: Bioethanol Production and By-Product Characterization

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Abstract

One of the most important challenges for the new global economy is to find new sources of bioenergy. Due to its photosynthetic efficiency and the possibility of biotransformation of its carbohydrates into bioethanol, seaweed plays an important role as a source of renewable biomass fuel.

This paper aims firstly to assess Gracilaria chilensis (red algae) as a substrate for bioethanol production by using anaerobic fermentation with Saccharomyces cerevisiae, and then to characterize the by-products of this biotransformation.

The main stage in the generation of solid byproducts is the hydrolysis with a yield about 50% w/w dry weigh. This by-product contains: fiber (42.7% w/w), proteins (39.95% w/w), carbohydrates (6.43% w/w), lipids (5.77% w/w), ashes (5.15% w/w) and a percentage of micronutrients P >K > Ca >Mg >Na in minor quantity than G. chilensis biomass.

Keywords: Seaweed; Bioethanol; By-products; Fermentation *Gracilaria chilensis*; Biotechnology

Introduction

The production of biofuels is generating worldwide interest in economic, environmental, institutional, and political circles, particularly in countries that are not self-sufficient in their supply of fossil fuel resources. As a result, there is a growing need to investigate local resources and diversify their energy-production technology.

Currently, there is a growing interest in combining the use of macroalgae in the food industry with the development of research into industrial bioenergy production. Seaweeds have several advantages, including the fact that they can be grown in three dimensions, which allow them to produce more biomass per hectare compared with terrestrial plants. In addition, macroalgal biomass generally has a high content of hydrolysable carbohydrates, which makes these algae an important raw material for bioethanol production [1].

Red algae are rich in polysaccharides (40% to 76% of dry weight), which are mostly distributed in the cell wall and in the form of carrageenan and agar as galactans and sulfated glucans [2]. Recent evidence suggests that the amount of bioenergy produced by red algae biomass is higher than any other source of biomass [3].

Some studies with *Gracialaria sp.* biomass for bioethanol production has been done, nevertheless both hydrolysis and fermentation methods are still under discussion because of long periods of sacharification (different acid and enzymatic conditions) as well as the need to improve the fermentative stages to achieve simpler processes with lower productive costs.

Chile has a large coastline that extends over 4,300 km, marine biomass (algae in particular) becoming the leading producer of read algae *Gracilaria chilensis*, which could be an excellent raw material for bioenergy generation.

The aim of the present research was to use *Gracilaria chilensis* as a new substrate for bioethanol production, applying the easiest hydrolysis method with the highest extraction yield of carbohydrate for the fermentation step with *Saccharomyces cerevisiae* and carry out the chemical characterization of by-products generated from bioethanol conversion processes.

Materials and Methods

Collection and size-fractionation of feedstock

Fresh samples of the macroalgae *Gracilaria chilensis* were collected in August 2010 in sector 41°51'07" south latitude and 73°58'30" east longitude in the X Region, Chile. The algae were washed and air-dried for 24 hours followed by oven incubation at 45 ± 1°C for 5 h. The dried algae was reduced in size to approximately 7 mm and stored at -20°C in polyethylene bags until use.

Chemical analysis

The moisture content of macroalgae samples was determined following the gravimetric method [4].

The total ash, lipids and proteins content was assessed according to standard methods [5]. Total carbohydrate content was determined using the phenol-sulfuric acid method [6] and fiber by difference.

Micronutrients analysis

The micronutrients: K, Mg, Ca, Na, of *G. chilensis* and solid by-product was carried out using an atomic absorption spectrophotometer. All measurements were performed using standard flame operating conditions, as recommended by the manufacturer and calibration of measurements was carried out using commercial standards. The determination of phosphorus was performed by employing molybdenum blue method at 730 nm [7].

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Acid thermal hydrolysis

For the acid hydrolysis, 50 g of dry macroalgae were suspended in 500 ml 1 M H_2SO_4 and macerated at 70°C with constant shaking about (200 – 400 rpm for 3 to 5 h). The mixture was cooled at room temperature and centrifuged at 6000 g for 10 min. The liquid phase was stored at 4°C for further analysis of the carbohydrates and proteins as described previously and the solid phase was dried at 45 °C until constant weight and screened through 45-35 mesh sieves. Proximate composition of powdered solid by-product was analyzed as described above.

Culture conditions and fermentation

S. cerevisiae (lyophilized commercial) was cultured on agar plates as described by Lee et al. [8]. An E-flask 250 mL containing 100 mL of sterile YEPD medium [9] was inoculated with *S. cerevisiae* and incubated at 37 °C with agitation (180 rpm for 24 h). Cell growth (g/L) was monitored by measuring at 600 nm (OD600).

Fermentation liquid hydrolysis (LH)

The Liquid Hydrolysis (LH) was diluted to a final concentration of carbohydrate 2% (w/v) and subsequently sterilized at 121 °C for 30 min and transferred into E-flask 1000 mL for anaerobic fermentation at pH 5.0. The mixture was inoculated with 10% (v/v) of *S. cerevisiae* pre-cultured and incubated at 27 °C in an orbital shaker at 250 rpm for 30 h.

The monitoring of the individual fermentation reactions was carried out at distinct time intervals over 40 h. The number of cell per mL was determined using a Neubauer chamber and yeast biomass content (g/L) by measuring the optical density OD600. The samples were centrifuged at 10,000 g for 2 min and supernatants were reserved for carbohydrates consumption analysis as described above and the ethanol produced (expressed in gL^{-1}) by Conway micro-diffusion technique [10].

Results and Discussion

Chemical composition

The proximate composition of *G. chilensis* showed a high carbohydrate content per dry weight (48.7% w/w) (Table 1). These results presented good concordance with those reported by Ortiz [11]. However, the carbohydrate content determined in this research is approximately 30 % w/w lower compared with those reported for *Gracilaria sp*. [12].

As it is well known, the algae carbohydrate quality and quantity strongly depend on different environmental physicochemical growth parameters, such as farming location, harvest season [13] and these can explain the differences between *Gracilaria* species.

The proximal composition of the by-product of hydrolysis shows an increment of percentage of fibers > proteins > lipids > ashes. The variation of the quantities of different components is explained the reduction of carbohydrates content (6.43% w/w) in the by-product (Table 1).

Micronutrients analysis of G. chilensis and by-product

The micronutrients analysis of *G. chilensis* showed the following composition order: Na > K > P > Mg > Ca (Table 1). Na and K present a positive correlation with other species of *Gracilaria* [14] and red algae [15].

Micronutrients content of solid by-product after acid thermal hydrolysis are lower than *G. chilensis* biomass, following the composition order P >K > Ca >Mg >Na (Table 1). The variation of percentages of these micronutrients can be explained the by chemical activity of H_2SO_4.

Characterization Hydrolysis liquid (HL) and by-products

The depolymerization of the sulfated carbohydrates present in four samples of *G. chilensis* was highly influenced by a gentle agitation of the biomass under acid thermal hydrolysis. This process led to two formations: a dark liquid that was used for fermentation and other corresponding to a solid by-product.

The hydrolysis average yield was 90.84 % w/w (Table 2) and higher comparing those carried out with a combined thermic process with autoclave [16] and enzymatic hydrolysis acid of *Gracilaria sp*. (80% w/w) reported by Wu et al. [12].

This hydrolysis process allows recovery of an average of about 46.88 % dry weigh of the solid biomass as a by-product with a low content of carbohydrate (6.43%). The carbohydrate content depletion increases the percentage of other components, and the microelements content shown a diminishing of their percentage under acid conditions, particularly the Na quantity (Table 1).

Fermentation monitoring

Hydrolysis liquid (HL) from four macroalgae *G. chilensis* samples with carbohydrate concentrations in the range of 22.33 ± 0.01 gL^{-1} were fermented with *S. cerevisiae*, The monitoring of ethanol-production is given in Figure 1. The carbohydrates presented a rapid degradation between 0 and 19 h, associated to the higher metabolic activity of the microorganism and a maximal ethanol production of 8.94 gL^{-1}. The remaining carbohydrate concentration was 1.91 % w/w of the initial substrate concentration. The fermentation average yield was 86.64% w/w (Table 2) with a production of 0.44g ethanol/g carbohydrate consumed. These results are very promising considering the low initial carbohydrate content and are in the same range as those reported by

Components (% w/w)	*G. chilensis*	By-product
Moisture	90.19 ± 0.92	96.05 ± 0.19
Ash	21.44 ± 0.33	5.15 ± 0.11
Lipids	0.48 ± 0.01	5.77 ± 0.09
Proteins	17.63 ± 0.11	39.95 ± 0.09
Fiber	11.79 ± 0.59	42.7 ± 0.43
Carbohydrates	48.66 ± 0.14	6.43 ± 0.41
Ca	0.21 ± 0.01	0.13 ± 0.01
Mg	0.24 ± 0.01	0.12 ± 0.01
K	5.37 ± 0.01	1.31 ± 0.01
P	1.74 ± 0.01	2.35 ± 0.01
Na	12.53 ± 0.03	0.06 ± 0.01

Values expressed in dry matter; Results expressed as mean ± SD of triplicates

Table 1: Chemical Composition of *G. chilensis* and by-product obtained from hydrolysis process.

Hydrolysis liquid (HL)	Hydrolysis Yield (% w/w)	Fermentation Yield (% w/w)
1	90.34	88.24
2	92.05	86.11
3	90.38	84.16
4	90.45	88.06

Table 2: Yield of hydrolysis and fermentation processes of *G. chilensis*

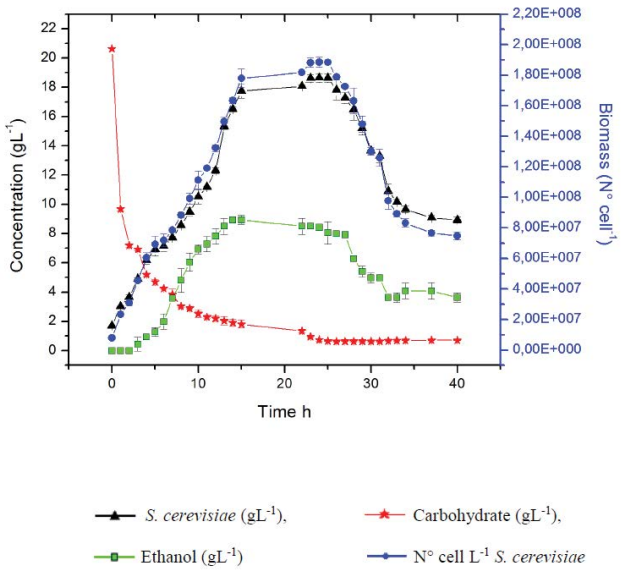

Figure 1: Monitoring of fermentation process of *Gracilaria chilensis* with *Saccharomyces cerevisiae*

Wu et al. [12].

As far the by-products of the fermentation process these were mainly remains of *S. cerevisiae* yeast with higher content of proteins (results are not shown).

Conclusions

This is the first report about *Gracilaria chilensis* bioethanol production and by-products with their corresponding characterization.

The production of bioethanol using *G. chilensis* was 0.18 g ethanol/g dry seaweed with a yield of 86.64 % w/w.

The mechanical agitation was essential to simplify the stage of hydrolysis to moderate acid and temperature conditions leading to a hydrolysis yield 91 % w/w.

The use of *G. chilensis* as substrate for bioethanol production allows to recovery almost 50% of seaweed as by-products which could have potential biotechnological applications.

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