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STUDY OF PRE-TREATMENT AND ENZYMATIC SACCHARIFICATION OF MICROALGAL BIOMASS FOR BIOETHANOL PRODUCTION

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ABSTRACT

Due to the growing demand for renewable fuel sources, microalgae have been attracting global interest as an alternative to the production of biofuels such as bioethanol. The production of bioethanol by microalgae is limited and depends on the steps of pretreatment necessary in the conversion of complex carbon molecules to simple sugars before the fermentation process. The objective of this study was to evaluate cell rupture methods to release the carbohydrates present in Spirulina platensis for later saccharification. The methods used were ultrasonic probe, heat treatment and freezing-thawing. The evaluation of the efficiency of these methods was carried out by determining the reducing sugars released after the saccharification of 10% (w/v) biomass solution in sodium phosphate buffer pH 5.5 at 50°C in a shaker table at 150 rpm, using amilolytic enzymes diluted 200-fold. Saccharification was evaluated in a 20% (w/v) solution in sodium phosphate buffer pH 5.5 at 50°C for 12 hours, using undiluted amylolytic enzymes. It was observed that the highest concentration of reducing sugars was obtained in the pre-treatment of freezing and thawing, resulting in 7.5 and 15.25 g/L of reducing sugars formed after 1 and 2 hours of hydrolysis respectively. Saccharification showed efficiency of approximately 75%.

1. INTRODUCTION

The increasing use of fossil fuels leads to increased carbon dioxide emissions into the atmosphere, so there is a great necessity to reduce this emission to avoid a negative impact on global warming (Alaswad et al., 2015). Based on that and in the high demand for energy in the future, there were

new areas of research and development of new sources and renewable forms to produce fuels, such as those generated through more sustainable sources, called biofuels (Milano et al., 2016).

Biofuels can be produced from several renewable raw materials, including microalgae. The use of microalgae biomass for the production of third-generation biofuels have many advantages over the production of first (soybean, castor bean, corn and sugar cane) and second generation (cooking oil and biomass waste), presenting as a sustainable alternative (Alaswad et al., 2015). This is due to its rapid growth which contributes to the reduction of the concentration of carbon dioxide in the atmosphere, the large capacity of cultivation, including waste water, besides there is no need for land during the cultivation, therefore, it will not compete with the production of food crops (Milano et al., 2016). One of the challenges for the production of bioethanol from microalgae biomass is the release of fermentable sugars from intracellular polysaccharides, requiring cell disruption steps to be performed the extraction of carbohydrate molecules and subsequent saccharification (Hernández et al., 2015). This study aimed to evaluate cell disruption methods to release the carbohydrates present in *Spirulina platensis* for subsequent saccharification.

2. METHODOLOGY

It was used *Spirulina platensis* grown in hydroponics greenhouse located at University of Passo Fundo RS, Brazil, background amid Zarrouk 20%, dry, containing 58% carbohydrates. The pretreatment assays used were: gelatinization at 100 °C for 10 minutes, ultrasonic probe - 10 cycles of 1 minute + gelatinization for 10 minutes, hydrothermal pretreatment at 121 ° C - 101 kPa for 20 min, freezing/thawing for 24 h each plus gelatinization for 10 minutes. The evaluation of the pre-treatment was carried out for determination of reducing sugars released after quick saccharification assay. During the test, it was used solids concentration of 10 % (w / v) dry biomass in 0.2M sodium phosphate buffer pH 5.5. The α-amylase (Liquozyme ® Supra 2.2, Novozymes) and amyloglucosidase (AMG ® 300 L, Novozymes) enzymes were added simultaneously and with 200 fold dilution, and kept at 50 ° C for 1 and 2 h. After the samples were centrifuged at 5000 rpm for 10 min and determined reducing sugars released, by the method of Miller (1959).

After choosing the best method of rupture, a saccharification assay was performed with dry biomass concentration of 20 % (w / v) in 0.2 M sodium phosphate buffer pH 5.5. The biomass was submitted to freezing/thawing (24h/24h) pretreatment plus gelatinization in a thermostated bath at 100 ° C for 10 min. The enzymes at the concentration of 0.8 % (v / v) α-amylase (Liquozyme® Supra 2.2, Novozymes) and 0.8 % (v / v) amyloglucosidase (AMG® 300 L, Novozymes) were added simultaneously, no diluted in reaction time of 12 hours at 50 ° C, pH 5.5 at 150 rpm in orbital shaker. Aliquots were taken every 2 hours for the determination of RS (reducing sugars) by Miller (1959). All the assays were performed in duplicate and the results were evaluated with analysis of variance (ANOVA) followed by tukey test with 95% confidence.

3. RESULTS AND DISCUSSION

Table 2 shows the amount of reducing sugars released after the pre-treatments in which the best pre-treatment was freezing and thawing followed by gelatinization, which presented the highest concentration of reducing sugars released after the test of hydrolysis.

Table 2. Averages of reducing sugar values and standard deviation of the samples

Test	Reducing sugars (g/L) after 1 h	Reducing sugars (g/L) after 2 h
1- Gelatinization – 100 °C per 10 minutes	3,72±0,29[a]	4,50±0,25[a]
2- Ultrasonic probe – 10 ciclos de 1	5,83±0,55[b]	6,79±0,10[b]
3- Heat treatment – 121 °C – 101 kPa per 20 minutes	5,98±0,24[b]	8,45±0,71[b]
4- Freezing and thawing followed by gelationization	7,50±0,57[c]	15,25±1,11[c]

In each column, averages followed by equal letters do not peresent significant differences among themselves at a 95% confidence level (average ± standard deviation).

During the freezing process and thawing process, the water present in the cell forms ice crystals during freezing, causing pretreatment (TUANKRIANGKRAI; BENJAKUL, 2010). Although the cell wall of the *Spirulina* microalgae is composed of mild polysaccharides as the peptide glycol without the presence of hemicellulose and lignin, which favors cell wall disruption (JOHN et al., 2011), the pretreatment release the carbohydrates stored in the cells of the microalgae.

Smichi et al. (2016) carried out the rupture in *Juncus maritimus* for the bioethanol production, comparing the results of two pre-treatments: freezing /thawing process and addition of 1% sulfuric acid solution. After cell disruption, a consortium of enzymes was used to perform the hydrolysis of the released carbohydrates. The freezing/thawing pretreatment showed a greater susceptibility to hydrolysis.

The Table 3 shows the release kinetics of reduced sugars formed during the saccharification of *Spirulina* biomass pre-treated by freezing/thawing plus gelatinization.

Table 3: Time course of-reducing sugar formed during saccharification of *Spirulina* biomass pre-treated by freezing/thawing+gelatinization.

Time	0 h	2 h	4 h	6 h	10 h	**12 h**
Reducing sugars (g/L)	8.71±1.1	50.53±0.46	55.05±1.3	60.22±0.13	69.48±0.08	**77.14±0.89**

*Results of media±standard deviation

Table 3 shows that at zero time there is already the formation of reduced sugars, a factor related to the usage of pretreatment, after 12 hours of enzymatic action it was possible to reach the maximum concentration of reducing sugars 77.14 g/L, which presents an efficiency of transformation of

carbohydrate in reducing sugars of 74.12%, since the biomass used had in its characterization 58% of CHO. Lee et al. (2013) studied the enzymatic saccharification of the residual biomass of the microalgae *Dunaliella tertiolecta* LB999, after lipid extraction. They used different types of enzymes, but amyloglucosidase presented a better saccharification efficiency, corresponding to a yield of 80.9% under optimal conditions of pH 5.5 and temperature of 55 ° C, without using a chemical pretreatment. The residual biomass presented approximately 51.9% of total CHO. Pancha et al., (2016) concluded that only the enzymatic hydrolysis is sufficient to obtain a saccharification of approximately 96% of the total carbohydrate content (45.23%) of the microalgae *Scenedesmus sp*. CCNM 1077.

5. CONCLUSION

The freezing/thawing pretreatment assay resulted in the higher susceptibility of the microalgal polysaccharides to enzymatic saccharification. The amylolytic enzymes used obtained polysaccharide conversion efficiency in simple sugars of approximately 75%, which can directly influence the final bioethanol yield after alcoholic fermentation.

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