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ENZYMATIC HYDROLYSIS OF THE MICROALGA *Chlorella sorokiniana*

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ABSTRACT

Microalgae are gaining attention as a potential source of fermentable sugars, since some species can accumulate high contents of intracellular starch. Therefore, the aim of this work was to study the enzymatic hydrolysis of the starch-rich green microalga Chlorella sorokiniana. Enzymatic and physical methods were evaluated for the disruption of the microalga's cell wall, and it was found that cellulases were not effective in disrupting this structure. After milling, the intracellular starch was easily hydrolyzed by fungal amylases without a gelatinization step, with a final glucose yield of 99 % after only 5 hours of hydrolysis. It was possible to increase the solids loading up to 25 % (m/m), promoting an increase of 350 % in the final glucose concentration with a yield loss of only 24 %.

1. INTRODUCTION

Microalgae research has been growing in number and importance in the last decades. Besides high added value products, such as carotenoids, polyunsaturated fatty acids and proteins, microalgae are being studied for the production of commodities, such as biodiesel (Chisti, 2007) and bioethanol (John et al., 2011). Besides bioethanol, glucose can be further upgraded into other molecules with the potential to substitute petroleum-derived chemicals (Bozell and Petersen, 2010). Under the right cultivation conditions, *Chlorella sorokiniana*, a green microalga with a high potential for industrial application (Morita et al., 2000), accumulated up to 30 % of its dry weight in the form of intracellular starch. Therefore, the aim of the present work was to study the enzymatic hydrolysis of this microalga in order to obtain a glucose-rich syrup.

2. MATERIALS AND METHODS

Chlorella sorokiniana was grown in Bold's Basal Medium in an orbital shaker at 225 rpm, 30 °C and 100 µmol.m^{-2}.s^{-1} for 20 days for starch accumulation. For the hydrolysis assays, two lab-made enzyme preparations were used: the fungi *Trichoderma reesei* RUT C-30 and *Aspergillus awamori* were cultivated to produce a cellulase-rich and an amylase-rich preparation, respectively. Hydrolysis assays were conducted initially at 50 °C, pH 4.8 and 1 % (m/m) solids loading in Eppendorf® tubes containing 1.25 mL of the hydrolysis mixture (enzymes, buffer and microalgal cells); microalgal cells were used fresh or freeze-dried with or without a previous milling treatment. Temperature and pH values were studied to maximize amylase activity and the new conditions of 60 °C and pH 4.0 were then used in hydrolysis experiments with solids loading varying from 5 % to 25 % (m/m), in flasks containing 12.5 mL of hydrolysis mixture. Glucose released from the hydrolysis experiments was analyzed using a biochemical analyzer (YSI 2700).

3. RESULTS AND DISCUSSION

Enzymatic hydrolysis has been proposed to rupture the microalgal cell wall (Gerken et al., 2013), making it possible for the amylases to hydrolyze the intracellular starch. Cellulose is said to compose the cell wall of microalgae from the genus *Chlorella* (Northcote et al., 1958), although there is evidence indicating otherwise (Blanc et al., 2010). Results from the characterization of the carbohydrate fraction of *Chlorella sorokiniana* indicate that, if cellulose is present, it only accounts for less than 2 % of the total dry weight of this microalga (Souza et al., 2017). Therefore, *Chlorella sorokiniana* cells were hydrolyzed with a mixture of cellulases, for cell wall disruption, and amylases, for starch hydrolysis. The influence of freeze-drying and milling in the hydrolysis process was also assessed, and the results are shown in Figure 1.

The enzyme mixture used was not able to fully disrupt *C. sorokiniana*'s cell wall, since the glucose yields obtained with both fresh and freeze-dried cells were low. Milling fresh cells was also not very effective for starch release, with a final glucose yield only slightly higher, of about 25 %. However, when dry cells were subjected to milling followed by enzymatic hydrolysis, yields were greatly enhanced, reaching 80 % of starch to glucose conversion in only 6 hours. The hydrolysis was attributed exclusively to the enzyme mixture produced by *A. awamori*, since the preparation containing both *T. reesei* and *A. awamori* gave the exact same results as the sole use of *A. awamori* enzymes and the enzyme mixture produced by *T. reesei* alone gave much lower yields, of about 20 % (data not shown).

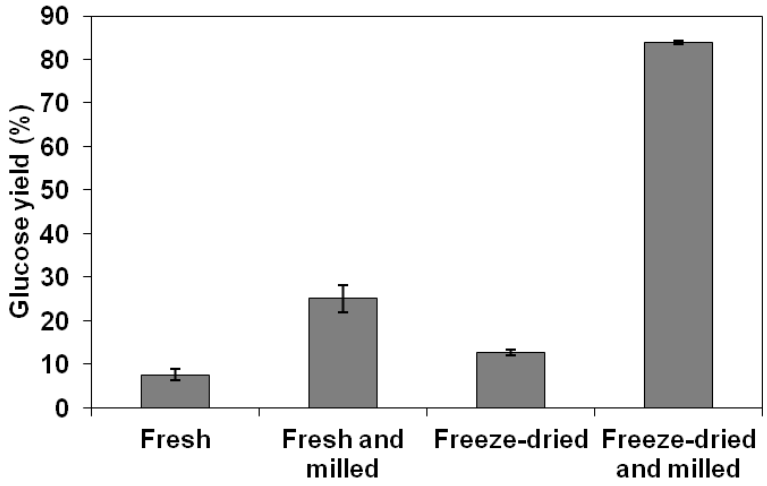

Figure 1. Glucose yield achieved after 6 hours of hydrolysis of C. sorokiniana cells with an enzyme mixture rich in cellulases and amylases produced by T. reesei and A. awamori

The increase in solids loading in hydrolysis process is important, since this will lead to lower water consumption, smaller reactors and a more concentrated product stream. However, increasing solids loading may lead to loss in hydrolysis yields due to problems in homogenization and mass transfer (Modenbach and Nokes, 2013). The hydrolysis of dried and milled *Chlorella sorokiniana* cells were evaluated with solids loading from 5 to 25 % (m/m) and the results are shown in Figure 2.

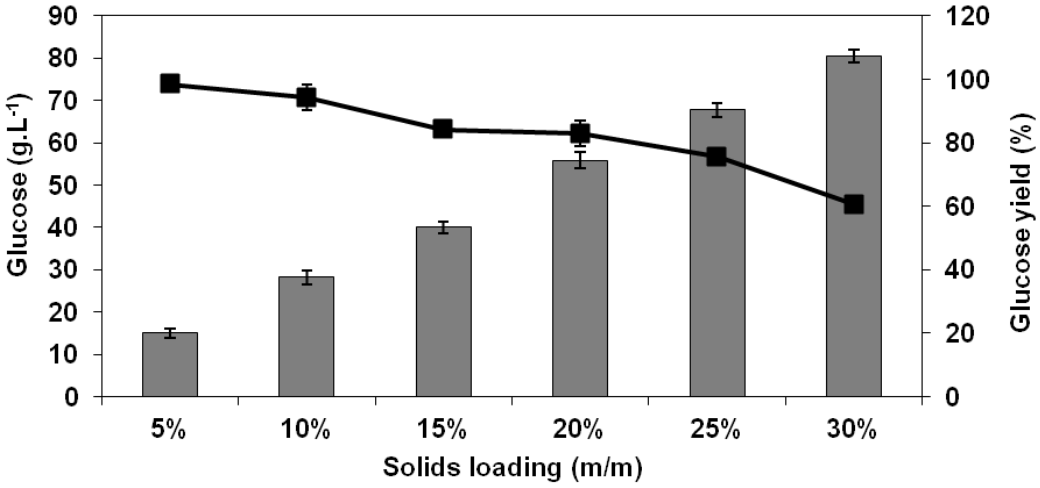

Figure 2. Glucose yield (squares) and concentration (columns) achieved after 5 hours of hydrolysis of dried althouand milled C. sorokiniana cells with an enzyme mixture produced by A. awamori

Even though the hydrolysis yield decreased 24 % when the solids loading increased from 5 to 25 %, the glucose concentration was increased by 350 %, indicating the potential of working in high solids loading. A further increase in the solids loading, to 30 %, resulted in a thick mixture with poor flowing properties, leading to a further reduction of the glucose yield to 63 %. However, in a scaled-up system with better mixing properties, this result could be further improved, with the possibility of reaching glucose concentrations higher than 90 g/L.

In all solids loading, high glucose yields were achieved within the first hours of hydrolysis, even though a gelatinization step was not present in the process and the temperature used for the hydrolysis was lower than the ones reported for microalgal starch gelatinization (Marsalkova et al., 2010). This high hydrolysis rate was attributed to the disruption of starch structure by the milling process used for cell wall rupture. Therefore, the chosen treatment method, although energy consuming, may be translated into an energy saving in the hydrolysis process due to the lower temperatures required when compared to the traditional starch hydrolysis process.

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