SECOND-GENERATION ETHANOL PRODUCTION USING GREEN COCONUT FIBER (GCF) FROM CHOLINE CHLORIDE PRETREATMENT

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ABSTRACT

In this study, GCF was pretreated with choline chloride ([Cho][Cl]) in an acidified medium with diluted sulfuric acid to enrich cellulose on the solid fraction and lignin on the liquid fraction. The aim was to evaluate the feasibility of obtaining 2G ethanol using a semi-simultaneous saccharification and fermentation (SSSF) strategy. The pretreatment was conducted in an autoclave (1 atm, 121°C) with dilutedsulfuric acid (1.0%, w/w), using a concentration of [Cho][Cl] of 75% (w/w) and incubation time of 50 min. The pretreated GCF was then submitted to the SSFS fermentation process. A preliminary study using 50 mg of a sodium lignin pretreated with [Cho][Cl], in diluted sulfuric acid, showed that was possible to solubilize 75% of lignin . The SSSF achieved a 23.2 g/L ethanol concentration with a productivity of 0.483 g/L.h and eficiency of 32.43% for the condition of 20% (w/v) solid loading.

Keywords: 2G ethanol. Pretreatment. High solid loading. Semi-simultaneous saccharification and fermentation (SSSF)

1 INTRODUCTION

Advances in the study of the use of lignocellulosic materials aim to avoid dependence on non-renewable energy resources – derived from petroleum. The great advantage of these lignocellulosic materials is that they are renewable and environmentally friendly raw materials. Additionally, these materials can help to mitigate current problems, such as the high price of fossil fuels and the worsening of the greenhouse effect. The use of lignocellulosic residues allows the production of products with high added value such as biofuels, biochemicals, bioplastics, etc. Green coconut fiber (GCF) is a lignocellulosic material produced worldwide. From this perspective, Brazil ranks fifth in the world in coconut production, and the Northeast region holds more than 73.0% of national production[1]. Brazilian coconut production reaches around 2.4 million tons, corresponding to 80.0% of total production in South America. Some countries such as India, Indonesia, the Philippines, and Brazil have active coconut industries to supply coconut products[2,3].

In the production of 2G ethanol, four steps are necessary. The first is pretreatment (chemical, mechanical, biological, etc.); the second, is enzymatic (or acid) hydrolysis; the third, is the fermentation process; and the fourth, is distillation[4,5]. Regarding pretreatments, recent studies have shown the use of ionic liquids, including choline chloride, as a promising alternative to improve biomass fractionation, focusing on the solubilization of hemicellulose and lignin; facilitating the access of cellulases to cellulose chains[6-10]. Therefore, in the present study, [Cho][Cl]) was used to enrich cellulose on the solid fraction and lignin on the liquid fraction. The aim was to evaluate the feasibility of obtaining 2G ethanol using a semi-simultaneous fermentation (SSSF) strategy.

2 MATERIAL & METHODS

In order to identify the affinity of lignin for [Cho][Cl], lignin solubilization tests were carried out (in duplicate) under different concentrations of [Cho][Cl]. This screening step was necessary to evaluate conditions with better delignification and check the solubility of [Cho][Cl] in an acidified aqueous medium. Based on this result, the concentrations of [Cho][Cl] were chosen in the GCF pretreatment. In this case, the sodium lignin came from GCF through pretreatment with sodium hydroxide and was obtained according to Padilha et al. (2020)[11]. Thus, although there may be differences in the solubility of the alkaline lignin used in the present study in [Cho][Cl] in an acidic medium when compared to the lignin present in the untreated biomass, it is assumed that this difference is not enough to modify the best result of 75% (w/w) [Cho][Cl] as shown below. Thus, initially, 50 mg of sodium lignin was mixed under different concentrations of [Cho][Cl] (0%, 10%, 25%, 50% 75%, 80%, and 90%, w/w) using acidified distilled water (H_2SO_4 1% (w/w)) as solvent. The lignin/[Cho][Cl] mixtures were placed on an orbital shaker (150 rpm) for 10 hours at room temperature (25°C). Then, they were centrifuged (1,500 × g) for 10 min and the supernatant was collected for further analysis. The solubilization capacity was evaluated using a spectrophotometer (Thermo Spectronic, Genesys 10 UV, USA) and ethanol as solvent (λ = 450 nm).

Based on Chen et al. (2018), GCF pretreatment was carried out by incubation with an aqueous solution of [Cho][Cl] + 1% (w/w) H_2SO_4 in an autoclave at 121°C and 50 min. The pretreated GCF was washed with running water and dried in an air circulation oven at 60°C. The SSSF of pretreated GCF was conducted according to Ribeiro et al. (2023)[13], using the yeast

Saccharomyces cerevisiae PE2. The solid loading from enzymatic hydrolysis ranged from 5% to 20% (w/v) (0.25-1 g in a useful volume of 5 mL), the pre-established enzymatic loading was 20 FPU per gram of biomass, the solvent was sodium citrate buffer (50 mM, pH 4.8) together with azide solution (0.01%, w/w). In this case, a pre-hydrolysis was carried out for 6 hours prior to the saccharification step, which was conducted simultaneously with fermentation for 48 hours (150 rpm, 40°C). The *S. cerevisiae* PE2 inoculum was fixed to reach 10^8 cells per mL in SSSF Erlenmeyer flasks.

3 RESULTS & DISCUSSION

As an initial step, the solubility of lignin in [Cho][Cl] was evaluated, since this was the ionic liquid used. From the preparation of [Cho][Cl] solutions, it was observed that at a concentration of 90% (w/w), crystals were formed, that is, they presented a heterogeneous phase. In this way, the maximum concentration achieved in the preparation of the solutions was 80% solubilization of [Cho][Cl] in an acidified aqueous medium. Thus, it was possible to extract lignin from the aqueous phase acidified by more than 77% due to the interaction of [Cho][Cl] (Figure 1), that is, a high solubility of lignin was noticed in this liquid matrix.

Smink et al. (2019)[14] investigated the relationship between the solubility of [Cho][Cl] and lactic acid to solubilize lignin. These authors realized that only the lactic acid solution provided greater lignin solubility. On the other hand, Francisco et al. (2012)[15] observed that lignin solubility increased with decreasing [Cho][Cl] concentration. As illustrated in Fig. 1, in the present study, an increase in lignin solubility was observed with increasing [Cho][Cl] concentration. It is important to highlight that the conditions, that is, the lignin quantification methodology, were very different from those presented by Francisco et al. (2012). In the present study, the fixed lignin mass was maintained and the concentration of [Cho][Cl] was changed. Furthermore, [Cho][Cl]/sulfuric acid was treated as a solvent that would perform the delignification function. It is important to highlight that the objective of evaluating the influence of [Cho][Cl] concentration on lignin solubility is that this lignin will be recovered in later stages and will be applied to oil removal.

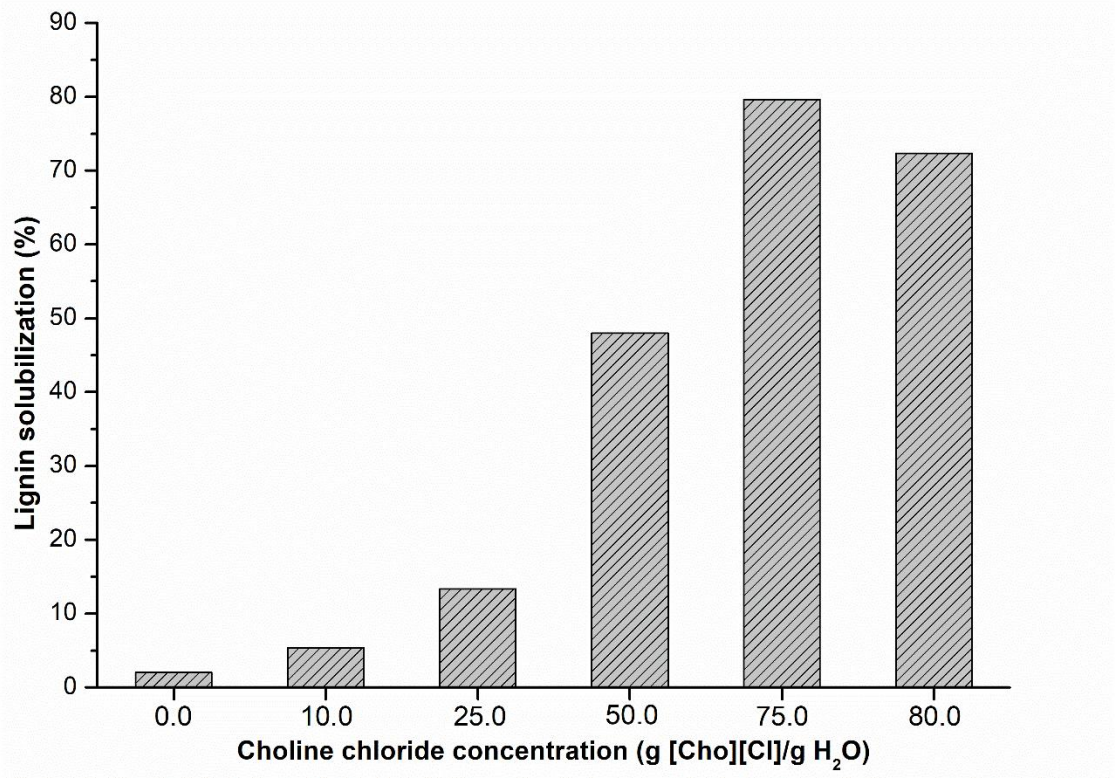

Figure 1 Extraction of lignin from GCF through a solution acidified with [Cho][Cl] at different concentrations (10 hours, temperature of 25°C).

Table 1 shows the values of ethanol concentration, productivity and fermentation efficiency for SSSF reached using GCF pretreated with choline chloride ([Cho][Cl]) at 75% (w/ m) in an acidified medium (1%, H_2SO_4, m/m) for 50 min in an autoclave.

Table 1 The SSSF results were obtained using GCF pretreated with choline chloride ([Cho][Cl]) at 75% (w/ m) in an acidified medium (1%, H_2SO_4, m/m) for 50 min in an autoclave.

Strategy	Solid loading (% w/v)	Ethanol concentration (g/L)	Ethanol productivity (g/Lh)	Efficiency (%)
SSSF	5.0	3.43 ± 0.28	0.07	19.21
	10.0	10.00 ± 0.18	0.21	28.00
	20.0	23.18 ± 0.72	0.48	32.43

As expected, the increase in solids loading up to 20.0% (w/v) favored an increase in concentration, productivity, and efficiency. It was observed that when increasing the solids loading from 5 to 20% (w/v) there was an increase in the ethanol concentration of approximately 7-fold. Thus as reported by Ribeiro et al. (2023)[13] for ethanol production to be feasible a high solid loading is necessary. However, the use of this strategy in SSSF brings some limitations as high viscosity, which lowers heat efficiency and mass transfer, and a lower interaction between substrate and enzyme. Of course, other strategies can be used to overcome these limitations such as the fed-batch one. For instance, using the GCF it was possible to reach 48.21 ± 1.13 g/L by adding 1.0% (w/v) Tween 80 to the nonisothermal-fed-SSSF with 30% (w/v) solid loading[13].

4 CONCLUSION

Pretreatment of GCF with [Cho][Cl] allows the removal of lignin and hemicellulose, which favors the enzymatic hydrolysis step. Thus, it was possible to use the SSSF strategy for reaching a 23.2 g/L ethanol concentration with productivity of 0.483 g/L.h, and efficiency of 32.43% for the condition of 20% (w/v) solid loading.

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