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Reuse of Residual Biomass of Cellulose Industry for Second Generation Bioethanol Production

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ABSTRACT

This study aimed at evaluating the potential of pulp mill residue (PMR) as a feedstock for ethanol production. The simultaneous saccharification and fermentation (SSF) process was operated using 8 gL^{-1} of a commercial strain of *Saccharomyces cerevisiae* JP1 under optimal proportions of cellulase cocktail (24.8 FPU/g cellulose of Cellic® CTec2) and cellulosic residue (200 gL^{-1}). After 48 hours of pre-hydrolysis at 50ºC and 200 rpm, the fermentation was carried out at 37 ºC, generating 48.5 gL^{-1} of ethanol in 10 hours and reaching a conversion efficiency of 53.3% from cellulose to ethanol and a volumetric productivity of 4.8 $gL^{-1}h^{-1}$ that is within the range of values of first generation ethanol production (5-8 $gL^{-1}h^{-1}$). These results showed that the pulp mill residue is an interesting and effective feedstock for the production of ethanol, which can be used for fuel purposes in the own pulp mills.

Indexing terms/Keywords

Ethanol; Pulp mill residue; Enzymatic hydrolysis; Simultaneous saccharification and fermentation; *Sacharomyces cerevisiae* JP1.

Academic Discipline And Sub-Disciplines

Biotechnology, Bioprocess engineering.

SUBJECT CLASSIFICATION

Biotechnology, Biochemistry, Bioprocess engineering.

TYPE (METHOD/APPROACH)

Biotechnology, Biochemistry, Bioprocess engineering.

INTRODUCTION

Cellulose pulp can be manufactured from feedstock containing cellulose fibers, generally wood, recycled paper and agricultural residues. The main steps in pulp mills comprehend raw material preparation and handling, pulp manufacturing, pulp washing and screening, chemicals recovery, bleaching and stock preparation [I]. The pulp mill industry implements several methods to dispose of residues generated during the different stages of their production process. The pulp mill provides an excellent platform for the production of cellulose fibers. In the future, large amounts of high value-added products, such as carbon fiber from lignin, nanocellulose and a variety of molecules produced within the biorefinery context are expected to be produced from this promising feedstock. Pulp mills also produce non-hazardous solid waste such as solid sludge derived from their pulping and bleaching operations. These materials comprise dirty wood chips or fibers as well as bark. The broken, low-quality fibers are separated out to become waste sludge. However, this solid waste, generated by mechanical and chemical degradation that occur during pulp manufacture and bleaching, presents high cellulose content. Furthermore, this residue, when compared to other lignocellulosic materials, exhibits a low level of lignin due to the delignification process that the wood goes through in the pulp mill industry [II]. The three main components of the residues generated in the cellulose industry (lignin, cellulose and hemicellulose), once integrated for proper use in a biorefinery, could be converted to products such as bio-oil, bio-coal and syngas that could serve as energy sources for this industry [III, IV].

There are several ways to convert pulp into a variety of high value-added products beyond those mainly generated nowadays (paper grade pulp, tall oil, and electricity). The cellulose and hemicellulose content of this solid waste presents potential to be converted into second-generation (2G) biofuels ("non-food"). Also, the use of the pulp mill residue is very attractive because its pretreatment is not necessary, since during the pulp mill processing a delignification stage is carried out, removing mostly lignin and a great part of hemicellulose present in the wood. The possibility of suppressing the pretreatment step stands as an advantage that contributes to reduce additional costs of the process, making the utilization of the pulp mill residue more competitive.

A key goal for the marketing of 2G biofuels is that all the sugars released during the stages of pretreatment and hydrolysis are fermented into ethanol. Thus, the efficiency of lignocellulosic biomass hydrolysis requires the use of efficient cellulase cocktails, which are mainly produced by filamentous fungi, specially from the genus *Trichoderma*. However, high production costs, along with inhibition problems to which these enzyme preparations are subjected to, are factors that must be considered when looking for the development of an efficient fermentation process. Even though technologies for the production of 2G ethanol are relatively young, it is expected that they acquire good potential for reducing costs and increasing the production efficiency levels. Depending partly on oil prices, the development of these technologies plays an important role in shifting the transport sector towards more sustainable energy sources in a near future. Nevertheless, technical and economic hurdles have to be faced before they can be widely employed. Saccharification processes and separate or simultaneous fermentation are low-cost routes to the utilization of different lignocellulosic feedstocks [V] and recent research has focused on them.

In a simultaneous saccharification and fermentation process (SSF) for 2G ethanol production, enzymatic hydrolysis and sugar fermentation occur in a single reactor. SSF presents many advantages once compared to separate hydrolysis and fermentation (SHF), such as: higher hydrolysis efficiency, reduction of contamination risks, decrease in the complexity, as well as in the process cost, and reduction of the cellulase inhibition by its hydrolysis products [VI, VII].

Within this context, this study aims at evaluating the potential of pulp mill residue for the production of 2G ethanol through simultaneous saccharification and fermentation process (SSF), combining commercial cellulases and yeasts.

MATERIALS AND METHODS

Feedstock

Fibria Celulose S.A. (Aracruz, ES, Brazil) kindly provided the feedstock used in this work – the pulp mill residue (PMR) – as well as the analysis of its composition, containing cellulose, hemicellulose and lignin.

Enzyme Cocktail

The commercial enzyme cocktail used in the hydrolysis of PMR was Cellic® CTec2, acquired from Novozymes. FPase activity was measured according to a methodology adapted from Eveleigh *et al.* (2009) [VIII], using filter paper Whatman no. 1 cut into strips of 1 x 6 cm as a substrate in a 60-minute hydrolysis. The activity quantification was carried out in triplicate and was expressed in µmol of product per minute per milliliter of enzyme cocktail (FPU/mL). Its result, 325.5 ± 10.3 FPU/mL, was used to standardize the enzyme loading in the enzymatic hydrolysis stage.

Microorganism

The industrial strain *Saccharomyces cerevisiae* (JP1) from Agro Japungu (Santa Rita, PB, Brazil) was employed for the production of second generation ethanol during fermentation. The fermentative agent was kept in solid medium with pH 5.0, containing 20 gL^{-1} glucose, 10 gL^{-1} yeast extract, and 15 gL^{-1} agar, incubated for 24 hours at 30 ºC and stocked under refrigeration at 4 ºC. Cells were activated and propagated in the medium described by Danesi et al. (2006) [IX], containing 20 gL^{-1} glucose, 10 gL^{-1} yeast extract, 1.25 g L^{-1} urea, 1.1 g L^{-1} KH_2PO_4, and 40 $mL.L^{-1}$ of mineral solution with citric acid. To the inoculum, 5% (v/v) of the pre-inoculum were added.

Both pre-inoculum and inoculum steps were performed in 500 mL conical flasks containing 200 mL of culture medium inoculated with the yeast. The flasks were mechanically stirred in a rotary shaker (New Brunswick™ Innova® 44) at 37 ºC and 200 rpm for, respectively, 24 hours and 8 hours, aiming at achieving a cell concentration of 8 gL^{-1}. The cells were centrifuged at 3,000 rpm for 20 minutes and suspended aseptically into the fermentation medium.

Simultaneous Saccharification and Fermentation

SSF were performed in triplicate at 37 ºC and 200 rpm in 500 mL conical flasks containing 125 mL of fermentative medium in which the concentrations of nutrients were the same as those used for the microorganism growth (except for glucose that would come from hydrolysis). The fermentation started when the yeast was added, what occured after 48 hours of hydrolysis. The previous enzymatic hydrolysis was carried out at 50 ºC and 200 rpm in a rotary shaker (New Brunswick™ Excella® E25) containing a 25 mM citrate buffer solution with pH 5.0. The solid: liquid ratio was 1:4 (grams of solid per milliliters of liquid) and the enzyme loading was 24.8 FPU/$g_{cellulose}$ (20.1 FPU/g_{solid}) as optimized elsewhere.

The flasks were closed during fermentation to avoid oxygen entrance and, therefore, meet biochemical requirement of an anaerobic environment. Only a small tube was kept opened to enable CO_2 way out through a slightly acidified solution by hydrochloric acid.

Weight of the fermentation system was monitored at appropriate time intervals and its reduction was exclusively due to the release of CO_2 generated from glucose along with ethanol. Thus, based on the stoichiometry of the fermentation, it was possible to build the kinetic profile for ethanol, whose concentration was calculated indirectly (equivalent ethanol).

Additionally, samples were collected at the beginning and at the end of SSF stages and were centrifuged at 12,000 rpm for 10 minutes. The supernatants were used to evaluate total reducing sugar through 3,5-dinitrosalicylic acid method [X], while glucose, galactose, cellobiose and ethanol were quantified through HPLC (Shimadzu, Hi-Plex H column) using refraction index detector (RID).

RESULTS AND DISCUSSION

Characterization of Pulp Mill Residue

Pulp mill residue is composed of 80.9% (w/w) of cellulose, 16.2% (w/w) of hemicellulose and 1.7% (w/w) of lignin, according to Fibria Celulose S.A analysis. It can be observed that this biomass is rich in cellulose when compared, for example, to sugarcane straw from different Brazilian regions, which presents approximately 44.3% of this component [XI], and to pretreated rice husk, as reported by Reyes *et al.* (1998) [XII]. In their research, it was indicated a maximum cellulose percentage of 53.2% after treatment with H_2O_2.

Additionally, after evaluating lignin content in this lignocellulosic material, it becomes clear that there is no need to perform any pretreatment for removal of this component due to its low proportion in the feedstock composition (1.7%). Hence, PMR utilization becomes even more interesting and promising within the context of biorefinery since the process cost decreases once execution of previous treatments are not required.

Simultaneous Saccharification and Fermentation

The enzymatic hydrolysis profile in terms of total reducing sugar (TRS) was built at 50ºC and 200 rpm with an enzyme loading of 24.8 FPU/g $_{cellulose}$ and a solid:liquid ratio of 1 g: 4 mL. The results are shown in Figure 1. The time of pre-hydrolysis was settled on 48 hours because it approximately corresponds to the moment when the TRS concentration becomes invariable, with roughly 60% conversion efficiency of cellulose to glucose. This steady concentration of TRS before the cellulose source is depleted might be related to the generation of inhibitory products and their effects on the enzyme activity, since the lignin content is very low to prevent the accessibility of the enzymes to the fibers.

After the accomplishment of the pre-hydrolysis step, the yeast was inoculated in the fermentation and the kinetic profile of equivalent ethanol production quantified through the weighing method was build up, as also shown in Figure 1. The SSF process was carried out in a rotary shaker for 10 hours.

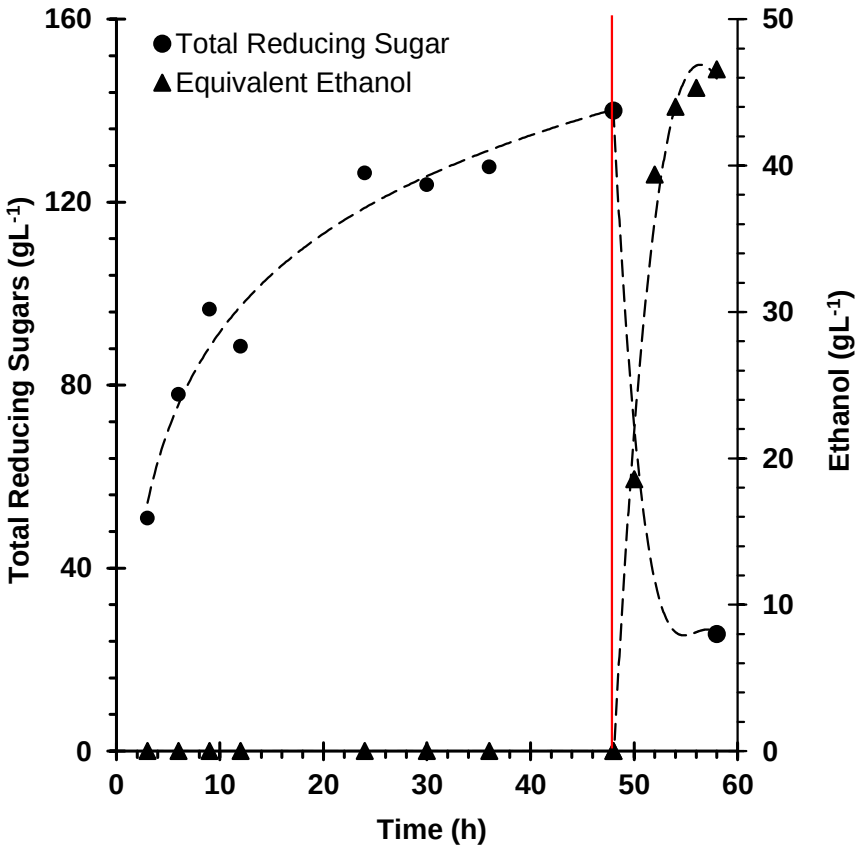

Figure 1. Pre-hydrolysis of pulp mill residue at 50 ºC and 200 rpm with an enzyme loading of 24.8 FPU/g $_{cellulose}$ and a solid:liquid ratio of 1:4, followed by simultaneous saccharification and fermentation at 37 ºC and 200 rpm

At the end of SSF, 46.6 gL^{-1} of ethanol were produced, according to the weighing. Table 1 shows the results obtained from the withdrawn samples at the beginning and at the end of the process.

Table 1. TRS, glucose, galactose, cellobiose and ethanol in SSF

Time (h)	TRS (gL^{-1})	Glucose (gL^{-1})	Galactose (gL^{-1})	Cellobiose (gL^{-1})	Ethanol (gL^{-1})
0	118.4	93.4	9.9	18.3	2.3
10	25.6	1.5	7.2	13.9	50.8

In a comparison between the weighing method and the concentrations exhibited in Table 1, it can be observed that they are similar. Therefore, this simple way of evaluating the alcoholic fermentation process is effective and the kinetic profile of equivalent ethanol (Figure 1) can be considered correct and trustworthy.

Conversion efficiency of the SSF process was 53.3% (considering that all cellulose would be converted into glucose and all glucose into ethanol) and a volumetric productivity of 4.8 $gL^{-1}h^{-1}$ of 2G ethanol was achieved after 10 hours of fermentation. Furthermore, considering the fact that, after 5 hours of fermentation, the process was almost stable, the volumetric productivity could reach approximately 8 $gL^{-1}h^{-1}$ of ethanol, as observed in Figure 1.

Important researches reported in the literature were taken into consideration in order to make a comparison to ethanol concentrations and volumetric productivities in SSF processes. Silva *et al.* (2011), for example, working with residual wood chips of cellulose industry, reached 28.7 gL^{-1} of ethanol and a volumetric productivity of 0.52 $gL^{-1}h^{-1}$ using 4 gL^{-1} of yeast *Saccharomyces cerevisiae*, 20 % (w/w) of solid load and 30 FPU/g_{solid} [IV].

Santos *et al.* (2010), on the other hand, produced ethanol from sugarcane bagasse by *Zymomonas mobilis*. From a cell concentration of 4 gL^{-1}, using an enzyme loading of 25 FPU/g and a solid content of 30% (w/w), they achieved approximately 60 gL^{-1} and a volumetric productivity of 1.5 $gL^{-1}h^{-1}$ [VI].

Kádár *et al.* (2004) made a comparison of simultaneous saccharification and fermentation processes between two yeast strains, in which one of them was *Saccharomyces cerevisiae*. Working with paper sludge, they achieved an ethanol concentration of 9.0 gL^{-1} with a conversion of cellulose into ethanol of 59.7% and a volumetric productivity of 0.125 $gL^{-1}h^{-1}$ after 72 hours of fermentation and 24 hours of pre-hydrolysis [VII]. It can be seen that the time of the entire process is higher when compared with the one used herein, which was about 58 hours.

Hence, the results reported in the present work are meaningful in producing ethanol. Furthermore, the ethanol concentration achieved in this research corresponds to approximately 300 L/ton of pulp mill residue. According to BNDES (Banco Nacional de Desenvolvimento Econômico e Social) and UDOP (União de Produtores de Bioenergia), ethanol yield from sugar cane, which is the mostly employed raw material for ethanol production, is 80 L/ton [XIII]. Thereby, it becomes even clearer the potential of pulp mill residue for ethanol production.

Finally, this work is very promising considering that ethanol can be used worldwide as a renewable energy source in replacement of fossil fuels. The more efficient use of this residue largely produced by cellulose industry can aggregate value to this feedstock. Additionally, it can increase the profits of industry since new high value-added products could be generated, expanding the products range of cellulose factories. Researches like the one here presented can be very relevant for this field.

CONCLUSION

Pulp mill residue is a cellulose-rich material, which is very interesting in the context of biorefinery. Additionally, pretreatments stages are not required for this feedstock because of its low lignin content, an advantage that allows reduction of production costs, making it suitable for large-scale processes. When evaluating the fermentability of this residue to produce ethanol, it was observed a great potential, achieving an ethanol yield of approximately 300 L/ton of pulp mill residue. This is a very promising result, since ethanol is widely regarded as an alternative renewable energy source for the future. Furthermore, the accomplishment of this research is positive for cellulose industries due to the possibility of adding value to pulp mill residue, besides increasing the profit and the portfolio of cellulose factories.

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