



POTENTIAL OF EUCALYPTUS BARK FOR BIOFUELS PRODUCTION

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Abstract

Eucalyptus bark has been identified as novel source of biomass for sustainable production of biofuels. In this work, *Eucalyptus nitens* bark (EBN) was characterized and evaluated for ethanol production. For that, an experimental design was carried out to optimize organosolv delignification process in which the variables temperature (170-200 °C), time (30-90 min) and percentage of ethanol (50-80 %) were studied. Organosolv process was suitable for the fractionation of *E. nitens* bark in which up to 93 % of glucan recovery and 50 % of delignification were achieved. After organosolv pretreatment, delignified *E. nitens* samples bark were submitted to simultaneous saccharification fermentation (SSF) using an industrial *Saccharomyces cerevisiae* strain. Results obtained from SSF assays showed that the variables temperature and time of delignification process had a significant influence on ethanol production. The organosolv pretreatment improved the ethanol yield from 36 to 96 %. The process proposed in this work shows a suitable use of bark residue for biofuel production.

INTRODUCTION

The increasing worldwide population coupled with the industrialization and the development of emerging economies results in increasing energy demand. Environmental concerns related to this industrialization promotes the search of alternative, sustainable



and clean energy sources. In this context, bioethanol is the most used biofuel worldwide and a suitable process and renewable raw materials (as lignocellulose biomass) are necessary for its sustainable production. For this lignocellulose-to-ethanol process, a pretreatment is mandatory (enabling the selective separation of the main components, including cellulose, hemicelluloses and lignin) in the scope of biomass biorefinery (Romaní et al., 2011). In this sense, delignification process can increase the cellulose content in the pretreated biomass which is the great importance to obtain higher glucose concentration in the hydrolysates. In this context, literature has reported on the delignification of several lignocellulosic biomasses by organosolv treatments (Romaní et al., 2011; Domínguez et al., 2014) using organic solvents (including ethanol, methanol, acetone, glycerol, etc.), or mixture of solvent-water (with or without addition of catalysts) (Martín et al., 2011).

During the chemical manufacturing of eucalyptus pulp, large amounts of wastes are produced, such as leaves, branches, barks, cross-cut ends and out-of-specification wood chips, which are left in the field to enrich the soil or burned for electricity or heat production (Lima et al., 2013; Moshkelani et al., 2013). Among these residues, bark represents the 10-12% of the total biomass harvested (Lima et al., 2013). Therefore, Eucalyptus bark has been recently identified as novel source of biomass with potential for bioethanol production (Lima et al., 2013).

The aim of this study was the valorization of *Eucalyptus nitens* bark as raw material for bioethanol production by organosolv pretreatment. Experimental design was proposed for evaluation of operational conditions of process (temperature, time and ethanol-water percentage) on fractionation of *E. nitens* bark and to improve the ethanol production by simultaneous saccharification and fermentation.

METHODOLOGY

Raw Material and organosolv pretreatment

Eucalyptus nitens bark (ENB) was kindly provided by a local pulp mill (ENCE, Pontevedra, Spain). ENB was submitted to organosolv delignification process with ethanol-water solutions in a 2 L stainless steel Parr reactor. The independent variables studied (Table 1) were temperature (170-200 °C), time (30-90 min) and percentage of ethanol (50-80 %).



Table 1. Experimental Doehlert design expressed in terms of the dimensional variables temperature, time and ethanol and dimensionless variables x_1 , x_2 and x_3 .

Run	Dimensional independent variables			Dimensionless, normalized, independent variables		
	Temperature (°C)	Time (min)	Ethanol (%)	x_1	x_2	x_3
1	200	60	65	1	0	0
2	170	60	65	-1	0	0
3	192.5	34.0	65	0.5	-0.866	0
4	192.5	86.0	65	0.5	0.866	0
5	192.5	51.3	77.2	0.5	-0.289	0.817
6	192.5	68.7	52.8	0.5	0.289	-0.817
7	177.5	51.3	77.2	-0.5	-0.289	0.817
8	177.5	86.0	65.0	-0.5	0.866	0
9	177.5	34.0	65.0	-0.5	-0.866	0
10	177.5	68.7	52.8	-0.5	0.289	-0.817
11	185	42.7	52.8	0	-0.577	-0.817
12	185	77.3	77.2	0	0.577	0.817
13	185	60	65	0	0	0
14	185	60	65	0	0	0
15	185	60	65	0	0	0

Simultaneous Saccharification and Fermentation of delignified biomass

The delignified solids were subjected to Simultaneous Saccharification and Fermentation (SSF) experiments in orbital shaker at 35 °C (150 rpm). The solution containing nutrients (20 g/L of peptone and 10 g/L of yeast extract) and pretreated solids were sterilized separately in autoclave at 121 °C for 15 min. Enzyme concentrates (Cellic Ctec2 and Cellic Htec2) were kindly provided by Novozyme (Denmark). Enzymatic activities were determined and corresponded to 120 FPU/mL and 1690 IU/mL, respectively. SSF started after mixing the nutrients and 10 % of pretreated solids and adding the inoculum and enzymes to the media. All the SSF experiments were carried out using 25 FPU of Cellic Ctec2/g of substrate of and 8 UI of Cellic HTec/FPU of Cellic Ctec2 and inoculated with 5 mg of fresh yeast /mL



Analytical methods

EBN and delignified EBN were assayed for moisture (TAPPI T-264-om-88 m), extractives (TAPPI T-264-om-88m method) and ashes (T-244-om-93 method). Cellulose, xylan, arabinan and acetyl groups were calculated using the HPLC determination of the glucose, xylose, arabinose and acetic acid contained in liquors from the TAPPI T13m assay. The acid insoluble residue content was measured by gravimetric determination of the solid from the TAPPI T13m assay. All the analyses were carried out in triplicate. Samples from chemical analysis composition, hydrolysates and SSF assays were analyzed by HPLC with a refractive index detector (Jasco) and, Aminex HPX-87H (BioRad, USA) column eluted with 0.005 M H₂SO₄, flow rate of 0.6 mL/min at 60 °C.

RESULTS

Chemical composition of EBN (expressed in g per 100 g of wood oven-dry basis) was as follow: 45.3 % of glucan, 12.7 % of xylan, 1.2 % of arabinan, 2.8 % of acetyl group, 22.0 % of Klason lignin and 5.0 % of extractives. As seen, EBN is composed mainly by polysaccharides (closed to 60%) followed by Klason lignin (21.96 %). Therefore, the high polysaccharide content shows its potential as raw material for bioconversion into chemicals and fuels.

After organosolv process, pretreated ENB was characterized to evaluate the effect of organosolv process on biomass fractionation. Chemical composition of delignified ENB is listed in Table 2. The glucan varied from 50.3 to 65.3 g glucan/100 g of pretreated EBN. The highest glucan content (run 1) also resulted in the minimum Klason lignin content obtained (17.99 %). This increment of glucan allowed an enrichment of 1.44-fold higher glucan respect to the raw material. The glucan recovery varied from 73.9 to 92.5 % showing the selective of organosolv pretreatment to retain the cellulose in the solid phase. On the other hand, xylan (main component of hemicellulose) was solubilized in the range of 14.4-73.5 %. The xylan solubilization was influenced by temperature and percentage of ethanol. As general trend, xylan content was lower than 10 % at temperature > 192.5 °C and percentage ethanol-water < 77% (Table 2). This fact could be related to typical behavior of hydrothermal treatment, influenced by high water content (Novo et al., 2011).



Table 2. Main results obtained from organosolv process (chemical characterization of delignified eucalyptus bark) and from simultaneous saccharification and fermentation of delignified eucalyptus bark (ethanol concentration and ethanol yield)

Run	Solid Yield (%)	Glucan (%)	Xylan (%)	Klason Lignin (%)	Ethanol max (g/L)	Ethanol yield (%)
1	61.7	65.3	6.4	18.0	28.3	82.6
2	77.2	52.8	14.0	21.1	10.0	36.1
3	68.4	58.0	9.3	19.8	16.9	55.6
4	56.7	64.6	7.4	18.8	32.5	96.1
5	65.2	55.9	13.5	21.9	10.6	36.1
6	53.5	65.3	6.3	19.8	23.8	69.5
7	77.7	50.3	14.0	21.2	9.3	35.2
8	69.4	60.4	10.4	19.8	17.1	54.0
9	74.3	53.8	14.5	20.2	8.9	31.5
10	59.1	56.6	8.7	21.4	15.9	53.4
11	61.4	58.4	7.9	20.4	18.7	61.1
12	64.4	54.7	9.5	21.1	14.5	50.6
13	63.9	56.8	9.1	21.2	16.7	56.0
14	63.6	57.3	10.8	20.6	17.2	57.3
15	63.3	56.5	9.8	20.1	16.5	55.6

Klason lignin content remained in a range from 17.99 to 21.94 g of Klason lignin/100 g of delignified EBN which corresponded to delignification percentage between 25-52%.

Results of ethanol production by SSF were also shown in Table 2 revealing a significant increase of ethanol yield from 32 to 96 % depending of operational conditions. The SSF profiles of ethanol yield (data no shown) displayed a clear difference among experiments, achieving a 77 % of ethanol yield within 10h of process (Run 4). These results showed a high improvement of ethanol production from Eucalyptus bark, which was influenced by an increase of temperature and time (Table 2). Few works in the literature have studied the pretreatment effect on enhancing of Eucalyptus bark saccharification, (Lima et al., 2013).



This is the first work, as far as we know, showing a suitable process for the efficient bioethanol production from Eucalyptus bark (ethanol yield of 96 %).

CONCLUSIONS

In this work, a by-product from pulp and paper industry (eucalyptus bark) was characterized and processed by an alternative organosolv process showing promising results on enhancement of ethanol production by simultaneous and fermentation process.

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