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S06-002

ETHANOL PRODUCTION FROM FRACTIONATED *EUCALYPTUS* WOOD

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Abstract

Eucalyptus globulus wood (EGW) is a lignocellulosic material with high cellulose and hemicellulose content, suitable for the simultaneous production of hemicellulosic and cellulosic ethanol. Processing of EGW by autohydrolysis yields a liquid phase rich in hemicellulosic-derived compounds (13.73 kg of xylooligosaccharides (Xylo-O)/ 100 kg of raw material). The liquid phase was processed by membranes, achieving a concentrated-liquor of 52.9 g of Xylo-O/L. The liquor from membrane processing was hydrolyzed with sulphuric acid, detoxified and fermented. The maximal concentration of ethanol from liquid phase was 19.3 g/L (volumetric productivity Q_p=0.19 g/Lh and yield $Y_{P/S}$=0.38 g/g). The solid phase from autohydrolysis was submitted to organosolv delignification, obtaining a solid with 81 kg of cellulose/100 kg of delignified solid. The simultaneous saccharification and fermentation (SSF) of delignified solid was carried out, achieving 62.7 g/L of ethanol with cellulose conversion to ethanol of 92% (based on the cellulose content of delignified solid).

Keywords: Autohydrolysis, Bioethanol, *Eucalyptus*, Organosolv

INTRODUCTION

The bioethanol from lignocellulosic materials (LCMs), also called second generation ethanol, is becoming into an interesting alternative to fossil fuels, due to its renewable character, large availability and low cost (Sánchez and Cardona, 2008).

Eucalyptus globulus wood (EGW) is a LCMs, which are mainly composed by structural components (cellulose, hemicelluloses and lignin), forming a complex structure which makes difficult its accessibility by enzymes or microorganisms.

The selective separation of LCMs components and their utilization allow a biomass refinery approach (FitzPatrick et al., 2010). The LCMs are submitted to successive processing stages, achieving an integral fractionation. Hydrothermal treatments (as autohydrolysis) cause the hemicellulose solubilization into derived compounds (mainly oligosaccharides). The spent solid from autohydrolysis is enriched in cellulose and lignin (Gullón et al., 2012). This solid can be subjected to organosolv process in which the lignin is removed, leaving a substrate with high cellulose content and very suitable for enzymatic hydrolysis (Romaní et al., 2011). The bioethanol from LCMs is obtained following three mainly steps: i) pretreatment of the raw material, ii) enzymatic hydrolysis or acid hydrolysis of polysaccharides or oligosaccharides into sugars, iii) fermentation of sugars into ethanol.

The aim of this work is provide an evaluation about ethanol manufacture from EGW. The autohydrolysis treatment was proposed as the first step of biorefinery, obtaining a high recovery of hemicellulose-derived compounds in liquid phase. The autohydrolyzed solid was subjected to organosolv delignification, achieving a solid enriched in cellulose. The two fractions (solid and liquid) were converted into ethanol by SSF for cellulosic ethanol and acid hydrolysis followed by fermentation for hemicellulosic ethanol.

METHODOLOGY

The overall view of methodology used in this work can be seen in the Figure 1.

Analysis of the Raw Material and Processed Solids and Liquid Fraction

EGW samples were milled, air-dried, homogenized and stored until its use. The raw material and the solids from autohydrolysis and organosolv processes were analyzed by NREL standards (LAP-002, LAP-003). For the determination of oligosaccharides, the liquor from autohydrolysis was submitted to post-hydrolysis analysis (4% w/w sulphuric acid, 121ºC for 20 min) and aliquots of liquor were analyzed for monomeric sugars determination.

Autohydrolysis Process

EGW was submitted to autohydrolysis and a liquid/solid ratio of 8 was used in a stainless steel reactor of 3.75 L. The blend reacted was heated up to reach the desired temperature (193 ºC). After cooling the reactor, solid and liquid fractions were separated by filtration. The solid was denominated autohydrolyzed solid (AS).

Organosolv Delignification

The AS was subjected to organosolv process, with an ethanol-water solution (47.7%) and a liquid/solid ratio of 8 during 60 minutes at 198 ºC in a 1 L reactor. The mixture was cooled and the delignified solid (DS) was recovered and washed.

Membrane Processing

The liquid phase from autohydrolysis was processed by membranes as described by Rodríguez-López et al., 2012. The volume treated in each batch was 8 L and the initial to final volume ratio was 6.

Acid hydrolysis, detoxification and xylose fermentation

The liquor from membrane processing was submitted to post-hydrolysis with 1% w/w sulfuric acid at 131ºC during 18 minutes (Garrote et al., 2001) to convert the oligosaccharides into monosaccharides. Then, the hydrolyzate was neutralized to pH 5 using $BaCO_3$. The acetic acid was removed by ionic exchange (cationic and anionic

resins). The xylose conversion into ethanol was carried out by *Pichia stipitis* T Pignal (provided by Spanish Collection of Type Cultures, ATCC 57376). Cells were grown in 100 mL media, containing: glucose, 10 g/l; peptone, 5 g/l; malt extract, 3 g/l and yeast extract, 3 g/l. The fermentation of the detoxified hydrolyzate was carried out in a 250 mL Erlenmeyer flaks and incubated in an orbital shaker at 30ºC and 100 rpm.

Simultaneous Saccharification and Fermentation of Delignified Solid

The DS was used as substrate in SSF. The assay was carried out at 35ºC in an orbital shaker, 100 rpm. The commercial enzymes used in this study were Celluclast 1.5L and Novozym 188 (provided by Novozymes) and were added as follow: loading 8.9 FPU/g of substrate and 10 UI/FPU, respectively. The liquid to solid ratio of SSF was 6 g of liquid/g DS. The yeast used in this experiment was *Saccharomyces cerevisiae* (CECT 1170). The cells were grown in a media containing 10 g/L glucose, 5 g/L peptone and 3 g/L extract malt and yeast during 24 h, 32 ºC and 150 rpm.

RESULTS

Structure of Experimental Scheme

The experimental procedure is shown in the Figure 1. The conditions of each stage were chosen based on our experience (Garrote et al., 2001) and in previous work not shown in this manuscript. The *Eucalyptus globulus* wood was submitted to autohydrolysis under non-isothermal conditions (193 ºC), in order to achieve fractionation effects (related to the selective removal of hemicelluloses). The results confirmed that the cellulose and the lignin were almost quantitatively retained in autohydrolyzed solid. On the other hand, xylooligosaccharides (Xylo-O) were the main hemicellulose-derived component, reaching up a solubilization of 78 %.

Membrane processing of autohydrolysis liquors was carried out, to achieve the concentration of oligosaccharides and the removal of compounds with low molecular weight. A high concentration of oligosaccharides in liquor will give a high concentration of ethanol. This fact is very valuable from a technical and economical point of view.

In order to improve the enzymatic digestibility of cellulose, the AS was submitted to organosolv delignification. The operational conditions were chosen according to previous optimization (Romaní et al., 2011). The data showed that 90 % of cellulose remained in the solid fraction whereas 75.5 % of lignin was removed.

As a whole, the processing of 100 kg or raw material would result in the recovery of 77.4 kg of valuable fractions in three streams.

Simultaneous Saccharification and Fermentation of Delignified Solid

The DS was used as substrate in SSF. The glucose released by the enzymes is simultaneously fermented into ethanol by yeast, thus reducing inhibition from glucose. Figure 2 shows the ethanol concentration, achieving 62.3 g/L with cellulose conversion to ethanol of 90 %. These results were obtained with a low load of enzyme (8.9 FPU/g of substrate).

Fermentation of Concentrated-Hydrolyzate

The fermentation of concentrated-hydrolyzate is shown in Figure 3. The maximum concentration of ethanol (19.3 g/L) was reached at 48 h. The Q_P was 0.19 g/Lh and the yield value ($Y_{P/S}$) was 0.38 g/g.

CONCLUSIONS

The processing method described in this work enabled an efficient fractionation of EGW. Hemicelluloses were converted into mono-and oligosaccharides, whereas autohydrolyzed wood was extensively delignified using uncatalyzed ethanol-water solutions. Delignified solid was highly susceptible to SSF. Membrane processing and detoxification by ion exchange of liquors from autohydrolysis provided a xylose-media without acetic acid, suitable for xylose-fermentation into ethanol. The overall yield of ethanol obtained was 22.1 kg/100 kg o.d. raw material. In order to reduce costs, further research is necessary, focused on finding alternative methods of detoxification (by enzymes).

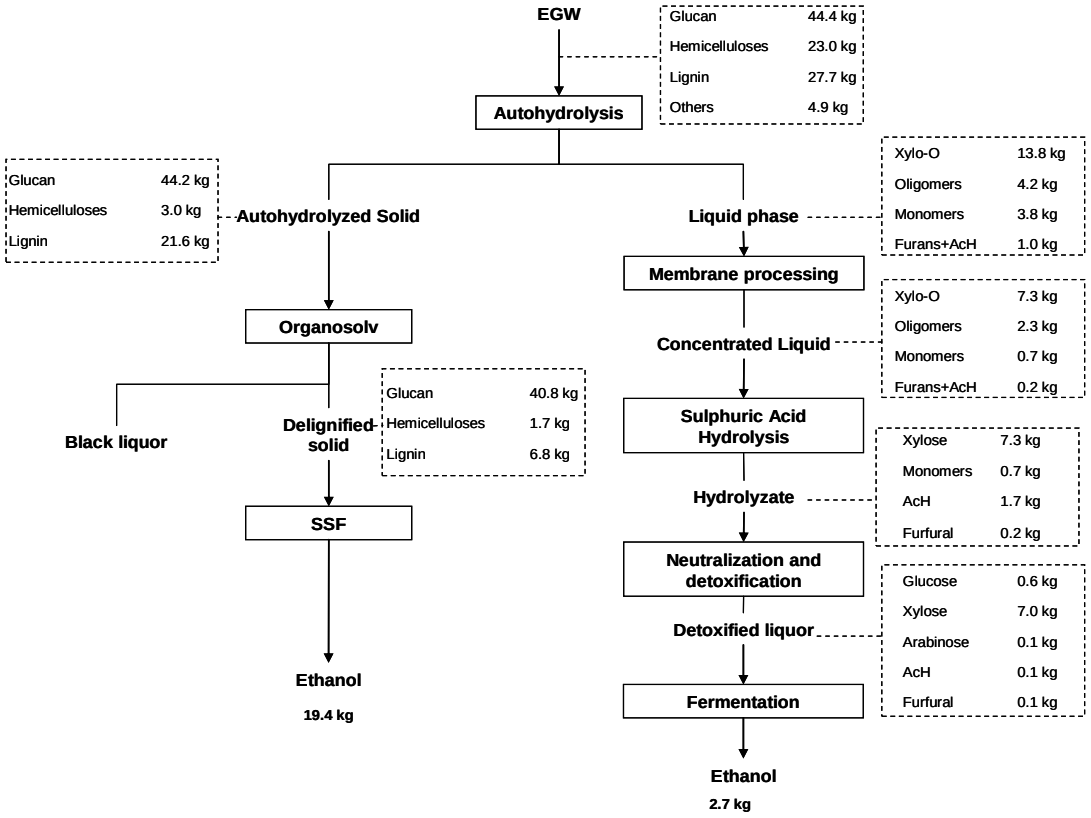

Figure 1. Experimental scheme of processing *Eucalyptus globulus* wood (100 kg oven-dry)

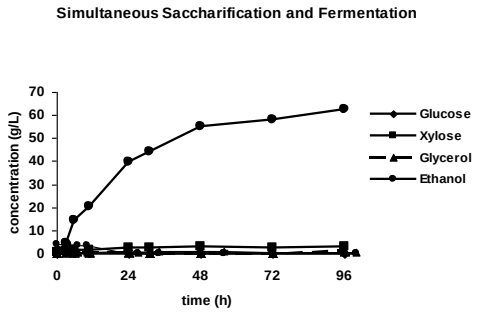

Figure 2 Simultaneous saccharification and fermentation of delignified solid.

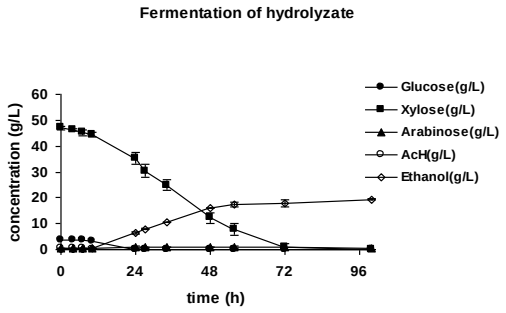

Figure 3. Concentration profiles determined in the fermentation

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S06-030

SECOND GENERATION ETHANOL PRODUCTION FROM SUGARCANE BAGASSE HYDROLYSATE BY A NOVEL ISOLATE XYLOSE-FERMENTING YEAST FROM BRAZILIAN FOREST

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Abstract

In last decades, biofuels have gained a significant momentum to be a sustainable alternative of gasoline. Among the lignocellulosic materials explored for second generation ethanol production, sugarcane bagasse (SB) generated by sugar-ethanol industries has proven as a promising feedstock for the ethanol production in countries like Brazil. The carbohydrate skeleton of SB can be hydrolyzed into sugar monomers which can be subsequently fermented for ethanol production. Dilute acid hydrolysis of SB released 10.9 $g.L^{-1}$ xylose in addition to other sugars and inhibitors. This hemicellulosic hydrolysate after concentration by vacuum evaporation followed by detoxification with overliming showed 30.89 $g.L^{-1}$ xylose along with other products (0.32 $g.L^{-1}$ glucose, 2.31 $g.L^{-1}$ arabinose, 1.26 $g.L^{-1}$ acetic acid etc). Fermentation efficiency of the detoxified hydrolysate was compared with the synthetic medium consisted of commercial sugars for the ethanol production by *Candida shehatae* UFMG 52.2, a novel isolate from Brazilian forests. After 24 hours of fermentation, maximum ethanol production (8.56 $g.L^{-1}$, productivity 0.36 g. L^{-1}. h^{-1}) and (9.48 $g.L^{-1}$, productivity 0.395 g. L^{-1}. h^{-1}) was achieved from detoxified hydrolysate and synthetic medium respectively. The significant productivities obtained in both experiments