EUCALYPTUS WOOD AND CHEESE WHEY VALORIZATION FOR BIOFUELS PRODUCTION

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Abstract
In this work, two raw materials (Eucalyptus wood and cheese whey) were used for ethanol production. Eucalyptus wood was hydrothermally pretreated at 233 ºC in order to increase the enzymatic saccharification of cellulose. Pretreated Eucalyptus wood mixed or not with cheese whey were used as substrates for ethanol production by simultaneous saccharification and fermentation (SSF) using two *Saccharomyces cerevisiae* strains (industrial Ethanol Red® and laboratory CEN.PK113–7D). The use of cheese whey mixed with Eucalyptus wood increased 1.3 and 1.5-fold the ethanol concentration in comparison with Eucalyptus without cheese whey using *S. cerevisiae* Ethanol Red® and CEN.PK113–7D strains, respectively. Higher ethanol concentration was obtained by Ethanol Red® than ethanol produced by CEN.PK113-7D with cheese whey supplementation (93 g/L and 65 g/L corresponding to 94 % and 66 % of ethanol yield, respectively). Results obtained in this work showed an interesting strategy for the valorization of two raw materials in order to produce high concentrations of ethanol.

Keywords: *Eucalyptus globulus wood*, Cheese Whey, Bioethanol, *Saccharomyces cerevisiae* strains.

Introduction

Production of biofuels from renewable materials is a promising alternative to replace fossil fuels [1]. Bioethanol is the most used biofuel for transportation in worldwide [2]. The main raw materials used for bioethanol are maize (starch) and sugar cane (sucrose). However, direct competition of these raw materials with food use promotes the searching of other raw materials. In this sense, lignocellulosic materials and/or agro-food wastes can be used as carbon source for bioethanol production. *Eucalyptus globulus* wood (EGW) is a lignocellulosic material with high cellulose content, suitable for the production of cellulosic ethanol [3]. However, a pretreatment able to break down the recalcitrant structure of lignocellulosic materials is necessary to increase the accessibility of cellulose to enzymatic hydrolysis. Hydrothermal treatment (also known as autohydrolysis) is considered an environmentally friendly treatment since waster is used as solvent. This pretreatment allows the recovery of two fractions a liquid phase enriched with oligosaccharides and solid phase composed of cellulose and lignin. On the other hand, cheese whey is mainly composed of lactose and protein, also used for ethanol production [4] and/or for the nutritional supplementation of lignocellulosic to ethanol fermentations [5].

The objective of this work was the valorization of eucalyptus wood and cheese whey for ethanol production at high concentrations using two *Saccharomyces cerevisiae* strains.

Material and Methods

Analysis of raw materials. Eucalyptus wood was milled, air-dried, homogenized and stored until its use. Eucalyptus wood and solid phase after autohydrolysis were analyzed by NREL standards (LAP-002, LAP-003). Cheese whey was analyzed for lactose concentration by HPLC.

Autohydrolysis treatment. EGW was submitted to autohydrolysis and a liquid/solid ratio of 8 was used in a stainless-steel reactor of 2 L. The blend reacted was heated up to reach the desired temperature (233 ºC). After cooling the reactor, solid and liquid fractions were separated by filtration. The solid (pretreated Eucalyptus wood) mixed or not with cheese whey was used as substrate for SSF assays

Simultaneous Saccharification and Fermentation assays (SSF). SSF assays were carried out at 35 ºC in an orbital shaker, 100 rpm. The commercial enzymes used in this study were Cellic CTec2 (cellulase) and Saphera (β-galactosidase) (kindly provided by Novozymes). Percentage of substrate employed in the SSF assays were 25 % of pretreated Eucalyptus wood and 6 % of cheese whey. The enzyme loading was 24 FPU/g of pretreated Eucalyptus for Cellic CTec2 and 20 UI/g of cheese whey for Saphera. The yeast strains used in the SSF experiments were *Saccharomyces cerevisiae* Ethanol RED ® and CEN.PK 113–7D. The cells were grown in a media containing 20 g/L of glucose, 10 g/L of peptone and 10 g/L of yeast during 15 h, 30 ºC and 200 rpm. Cells were collected and added to SSF experiments. Samples were withdrawn and analyzed by HPLC for ethanol concentration.

Results

Figure 1 a) and b) showed the main results (ethanol concentration and ethanol yield) obtained in SSF assays at 120 h of fermentation. Performance differences were observed between *S. cerevisiae* strains, being more satisfactory the results obtained by Ethanol Red®. Higher ethanol concentrations were obtained when the cheese whey was mixed with Eucalyptus wood due to the increment of total sugars. Moreover, the addition of cheese whey improved the ethanol yield obtained by laboratory strain. Ethanol Red® consumed practically all the sugars, while 17 g/L of glucose and 29 g/L of galactose were not consumed by CEN.PK 113–7D (data not shown). The ethanol productivity at 48 hours for Ethanol Red® was 1.7 g/(L.h) with Cheese Whey and 1.0 g/(L.h) without Cheese Whey. On the other hand, the ethanol productivity of CEN.PK 113–7D at 48 hours was 1.2 g/(L.h) with Cheese Whey and 1.1 g/(L.h) without Cheese Whey. The results reported in this work can be favorably compared with reported data in literature [6], in which 73.1 g/L of ethanol at 120 h of fermentation were obtained from mixture of 14.5 % liquefied corn kernels with amylases and 4.5 % of corn residue from furfural manufacturing. In this work, the mixture of residues was shown as interesting alternative to increase cellulosic ethanol concentration.

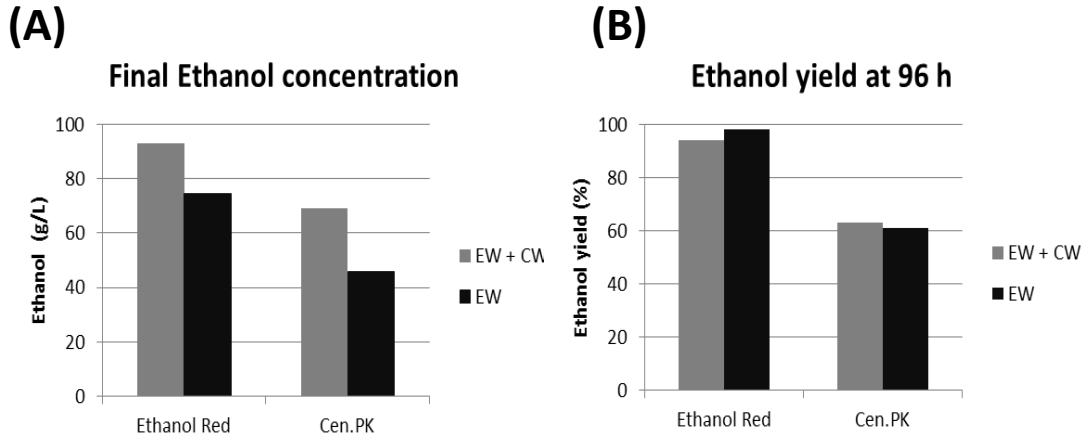

Figure 1 Simultaneous saccharification and fermentation of Eucalyptus wood (EW) mixed or/not with Cheese whey (CW) using different strains, Saccharomyces cerevisiae Ethanol Red® and Cen.PK 113–7D: (A) Final Ethanol concentration (B) Ethanol yield at 96 h, in different medium composition, Eucalyptus Wood (EW) and Cheese Whey (CW).

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