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Lignocellulosic bioethanol production with revalorization of low-cost agroindustrial by-products as nutritional supplements

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ABSTRACT

During the pretreatment of lignocellulosic biomass for second generation bioethanol production, fermentation inhibitors are released. To overcome this, the use of a robust industrial strain together with agro-industrial by-products as nutritional supplementation was proposed to increase ethanol productivity and yields. Two factorial experimental designs were carried out to optimize fermentation of hydrolysate from autohydrolysis of *Eucalyptus globulus*. The most influential variables on ethanol production were cheese whey and $K_2O_5S_2$ (potassium metabisulfite) supplementation. Nutrient addition effect was demonstrated using the whole slurry from autohydrolysis in two process configurations (separate hydrolysis and fermentation, SHF and simultaneous saccharification and fermentation, SSF). Comparing the supplemented SHF and SSF assays with non-supplemented, 2.3 and 7.4 fold higher ethanol concentrations were obtained, respectively. In the case of SSF, 50.4 g L^{-1} of ethanol concentration and 92.2% of ethanol conversion were attained, demonstrating an improved fermentation performance in industrial lignocellulose fermentations.

1. Introduction

Bioethanol from lignocellulosic materials (LCMs), also called second generation bioethanol, is considered a promising alternative to first generation bioethanol (obtained from feedstock such as sugar cane, corn, wheat or barley) (García et al., 2014). The LCMs, as *Eucalyptus globulus* (EGW) wood, are renewable and widespread raw materials constituting the world's largest bioethanol renewable resource. An efficient bioethanol production process from lignocellulose involves an effective pretreatment of LCM to improve the accessibility of cellulose and thus enhance the enzymatic saccharification (Buruiana et al., 2014; Singh and Bishnoi, 2013). One interesting approach is to use the whole slurry from treatment, since allows economical and industrial benefits: washing steps are avoided, water consumption is lower and the sugars from liquid phase can be used, increasing ethanol concentration (Tengborg et al., 2001). However, during the pretreatment step some compounds (such as furans, phenolic compounds and weak acids) are produced. Furfural, hydroxymethylfurfural and phenolic compounds result from degradation of pentoses, hexoses and lignin, respectively and the acetic acid is released in the deacetylation of hemicelluloses (Klasson et al., 2013). These compounds have an inhibitory effect on the microorganisms used for hydrolysate fermentation (reviewed by Palmqvist and Hahn-Hägerdal, 2000a).

In this sense, the success of lignocellulosic biomass fermentations is necessarily dependent on the ability of the yeast strains to cope with the different stresses imposed during these processes. In biomass-based fermentations, yeast cells, besides having to tolerate the presence of the above-referred inhibitors, are also exposed to nutrient starvation and the absence of oxygen (Pereira et al., 2011a). To overcome the problem of toxicity, several strategies have been considered such as a biological or chemical detoxification step prior to fermentation (reviewed by Palmqvist and Hahn-Hägerdal, 2000b) or the improvement of resistance of the organism itself (Heer and Sauer, 2008). Another possible strategy can be the optimization of fermentation environment in order to minimize the toxic effects of inhibitors (Huang et al., 2011). Supplements utilization is an alternative to facilitate the microorganism growth and fermentation in an inhibitory medium (Tomás-Pejó et al., 2012). Taking into account that the nutrient sources in the lignocellulosic hydrolysate are present in low amounts, it is important to optimize the nutritional supplementation for the improvement of fermentation performance. Several authors have reported that the use of supplements

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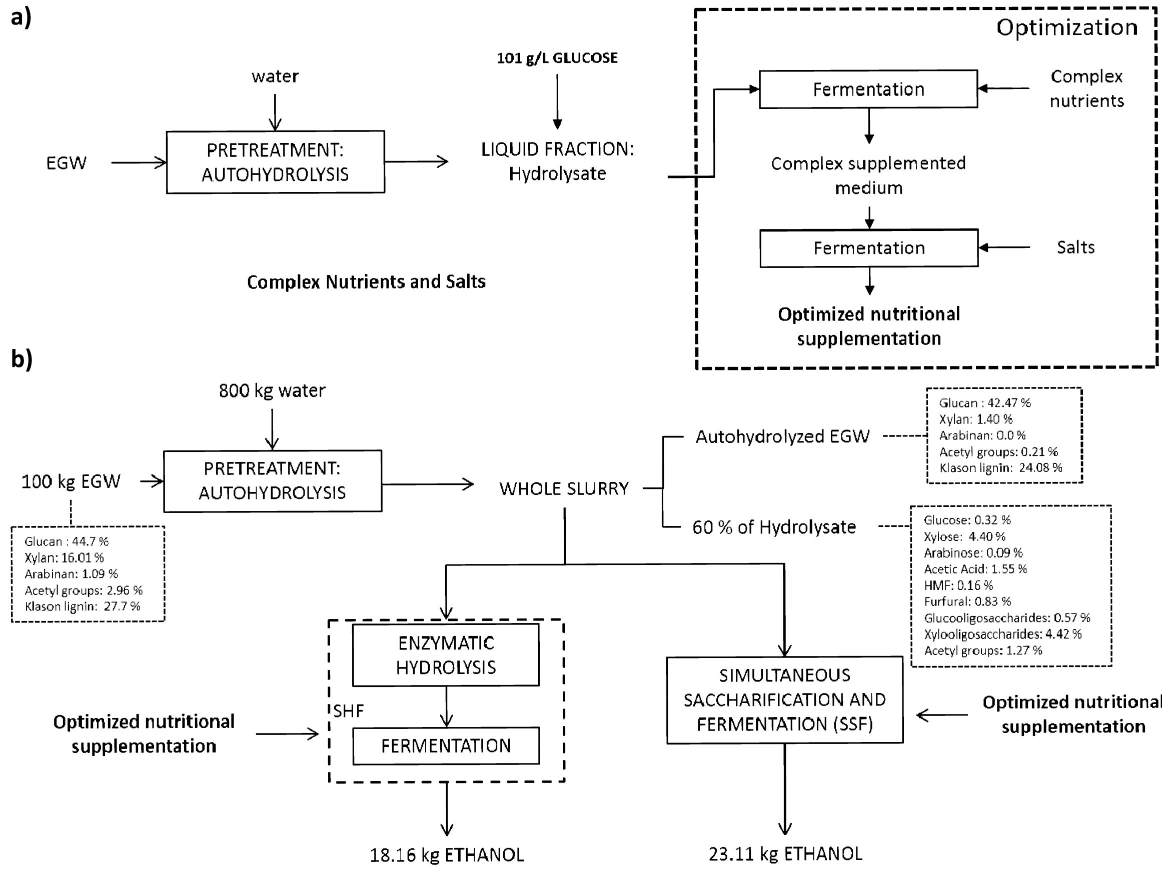

Fig. 1. (a) Steps for supplementation and fermentation medium optimization using EGW hydrolysate (b) Mass balance and application of the optimized supplementation to whole slurry fermentations.

is able to increase the production yield and productivity of ethanol, with or without stress conditions (Pereira et al., 2010; Jørgensen, 2009; Dragone et al., 2004). In this context, the use of residues or by-products generated in the agro-food industry is an attractive approach since it contributes to re-valorization of these residues and increases the overall economic process feasibility. Different agro-industrial residues, such as corn steep liquor (CSL), raw yeast extract (RYE), cheese whey (CW) and Urea, have been individually studied as supplements for fermentation processes supplying complex nutrients and nitrogen to the medium (Jørgensen, 2009; Salgado et al., 2009; Gullón et al., 2008). On the other hand, nutrients in minor amounts and supplied in salts, as magnesium, calcium and zinc have influence in the rate of sugar fermentation and are necessary as cofactors for several metabolic pathways (Pereira et al., 2010).

In this context, this work aims for the intensification of EGW hydrolysate fermentation processes combined with re-valorization of agro-industrial residues as low-cost nutritional supplements, in order to attain faster and higher ethanol production. For that, a previously selected industrial robust strain of *Saccharomyces cerevisiae* PE-2 (Pereira et al., 2014) was used for efficient fermentation. The addition of nutritional sources was evaluated, optimized and demonstrated in multiple process configurations such as separate hydrolysis and fermentation (SHF) and simultaneous saccharification and fermentation (SSF) of pretreated *Eucalyptus globulus* wood.

2. Materials and methods

2.1. Diagram representation of experimental work

Fig. 1 shows the structure of experimental work carried out in this study. The raw material used was obtained from autohydrolysis treatment of EGW. To study supplement effect on inhibitory medium, fermentations were performed using only hydrolysate (containing the inhibitory compounds) instead of the whole slurry. Despite the whole slurry being the most close to reality approach, the occurring of enzyme inhibition by sugars, previously analyzed in recent works (Romaní et al., 2014), could interfere with the experimental results, masking inhibitor and/or supplement effects and interfering with the models obtained in the experimental design. The hydrolysate was complemented with 101 g L^{-1} of glucose and employed for optimization of nutritional supplementation (complex nutrients and salts) in a two-step approach as represented in Fig. 1a. Finally, the optimized nutritional supplement was added in the saccharification and fermentation of whole-slurry fermentations (hydrolysate + autohydrolyzed EGW) for ethanol production, as represented in Fig. 1b.

2.2. Autohydrolysis of *Eucalyptus globulus* wood: hydrolysate and pretreated solid

EGW was previously analyzed by Pereira et al. (2014), containing (expressed in g/100 g of raw material on dry basis, average values of three replicate ± standard deviation): 44.7% ± 0.81 of glucan; 16.0% ± 0.35 of xylan; 1.1% ± 0.05 of arabinan; 3.0% ± 0.28 of acetyl groups; 27.7% ± 0.6 of Klason lignin and 2% ± 0.2 of extractives. EGW was submitted to autohydrolysis treatment. EGW was mixed with water at liquid-to-solid ratio (LSR) of 8 kg water kg^{-1} of oven-dry raw material in a pressurized reactor (Parr Instruments Company, Moline, IL). The autohydrolysis treatment was carried out at 150 rpm and heated, following the heating profile (Garrote et al., 2008) up to reach the desired temperature (T_{MAX} = 210 °C). The operational conditions were chosen on the basis of reported