

# Optimization of low-cost nutritional supplementation for lignocellulosic ethanol fermentation

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Bioethanol from lignocellulosic materials (LCM), also called second generation bioethanol, is considered a promising alternative to first generation bioethanol. An efficient production process of lignocellulosic bioethanol involves an effective pretreatment of LCM to improve the accessibility of cellulose and thus enhance the enzymatic saccharification. 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 [1]. However, during the pretreatment step some compounds (such as furans, phenolic compounds and weak acids) are produced. These compounds have an inhibitory effect on the microorganisms used for hydrolysate fermentation [2]. To overcome this, the use of a robust industrial strain together with agro-industrial by-products as nutritional supplementation was proposed to increase the ethanol productivities and yields.

*Eucalyptus globulus* wood (EGW) was submitted to autohydrolysis treatment under non-isothermal condition,  $T_{max}=210$  °C and liquid to solid ratio 8g/g [3]. Two factorial experimental designs were carried out to optimize fermentation of hydrolysate from autohydrolysis of EGW. For this, a study of complex nutritional supplementation of hydrolysate was carried out using a Box-Behnken design. The complex nutrients used in this work were: corn steep liquor (CSL), raw yeast extract (RYE), cheese whey (CW) and Urea. Moreover, the effect of salts addition to the supplemented hydrolysate was evaluated using a Plackett-Burman design.

In order to evaluate the impact of optimized nutritional supplementation by experimental designs on overall process yields, two strategies of saccharification and fermentation (SHF and SSF) were conducted. SHF and SSF assays were carried out at 15.5 % of solid loading in an orbital shaker at 150 rpm, pH 4.8, using commercial enzymes (Cellic CTec2 and HTec2) at enzyme to substrate ratio 22.5 FPU/g and 500 U/g, respectively. The strain used in this work was *Saccharomyces cerevisiae* PE-2, isolated from Brazilian bioethanol industry and previously shown to withstand EGW-hydrolysate inhibitory conditions [4]. 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 of ethanol concentration and 92.2 % of ethanol conversion were attained, demonstrating an improved fermentation performance in industrial lignocellulose fermentations.

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