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DESIGN OF AN INTEGRATED PROCESS FOR BIOETHANOL PRODUCTION FROM LIGNOCELLULOSIC BIOMASS

Ph.D. Thesis

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ABSTRACT

The aim of the present thesis was the design of an integrated process of bioethanol production from lignocellulosic materials, which are an important alternative source for 2nd generation bioethanol production because they do not compete for the same resources of the food industry and are cheaper than sugar-starch raw materials. Sweet sorghum (SS) is considered an excellent raw material for bioethanol production, due to its high biomass yields and fermentable sugar content (9-25%). Sorghum bagasse (SB), the solid residue after extraction of sugars, is rich in cellulose (40%) and hemicelluloses (35.5%).

The bioconversion of lignocellulosic material to ethanol consists of the following steps: (a) pretreatment (physical, chemical or biological) for the release of cellulose from hemicellulose and lignin, (b) enzymatic hydrolysis of the polymers for the production of fermentable sugars and (c) bioconversion of the produced sugars to ethanol.

For the bioconversion of sweet sorghum and sorghum bagasse to ethanol the fungi *Fusarium oxysporum* F3 and *Neurospora crassa* DSM 1129 were used, because they possess the abilities to produce the required enzymes for the hydrolysis of lignocellulosic materials and also ferment their hydrolysis products (hexoses and pentoses) to ethanol. The optimum conditions for growth and cellulases and hemicellulases production of fungus *F. oxysporum* F3 when grown on submerged cultures are: (a) carbon source 10:1 (w/w) mixture of corn cobs and wheat bran and (b) nitrogen source ammonium phosphate. The optimum conditions of the fungus *N. crassa* DSM 1129 when grown on solid state cultures are: (a) carbon source 5:1 (w/w) mixture of wheat straw and wheat bran, (b) nitrogen source ammonium sulfate, (c) initial pH of 5.0 and (d) initial moisture percentage of 70.5% (w/w).

The above fungi were able to ferment the whole spectrum of sugars and polysaccharides that are present in sweet sorghum into ethanol. Ethanol yields during sweet sorghum bioconversion by the fungi *F. oxysporum* and *N. crassa* reached 54.2 and 59.6% of maximum theoretical, respectively, in the presence of the yeast *S. cerevisiae* 2541 cells.

Following, the hydrothermal pretreatment of SB was studied, at various temperatures (160°-210°C) and treatment times (3-30 min), with the addition of dilute sulfuric acid (0-4g/100g SB), resulting in high hemicellulose degradation at the more intense conditions. The concentrations of the inhibitors (furfural, 5-

hydroxymethyl furfural and formic acid) formed during hydrothermal pretreatment were kept at low levels, compared to the literature. The hydrothermal pretreatment conditions with maximum sugar yields and minimum inhibitor formation were 210^{o}C for 10mins and addition of 2g acid per 100g SB.

The enzymatic hydrolysis of sorghum bagasse showed that the cellulolytic and hemicellulolytic system of *F. oxysporum* F3 is more efficient than the one produced by the fungus *N. crassa* DSM 1129. Synergism between the two enzymatic systems was observed during the hydrolysis of untreated and hydrothermally pretreated sorghum bagasse.

Ethanol production from untreated SB was kept at low levels by both fungi. Combing the hydrothermal pretreatment of SB (210^{o}C, 10 min, 2g sulfuric acid/100g SB) and the addition of commercial cellulases (6 FPU/g SB) at the fermentation step resulted in substantial increase in ethanol yield. The maximum ethanol production by *N. crassa* was 27.6 g/L (31.5 g/100g total solids), or 82.3% of maximum theoretical yield. Ethanol production by the fungus *F. oxysporum* F3 seemed to be affected by the presence of inhibitors formed during pretreatment. Using mixed cultures of *F. oxysporum* F3 and the yeast *S. cerevisiae* 2541 ethanol production reached 13.6 g/L (16 g/100g total solids) or 40.5% of theoretical yield.

Finally, the wild type strain of *F. oxysporum* F3 was genetically engineered to improve its rate of sugar assimilation and ethanol production. The engineered strain FF11, in which the enzymes phosphoglucomutase (involved in hexose metabolism) and transaldolase (of pentose phosphate pathway) were constituently expressed, showed increased bioconversion yields to ethanol of sweet sorghum, untreated SB and hydrothermally pretreated SB, compared to the wild type strain F3.