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Evaluation of dilute acid and alkaline pretreatments, enzymatic hydrolysis and fermentation of napiergrass for fuel ethanol production

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ABSTRACT

Napiergrass (*Pennisetum purpureum* Schum.) is a promising low cost raw material which does not compete with food prices, has attractive yields and an environmentally friendly farming. Dilute sulfuric acid pretreatment of napiergrass was effective to obtain high yields of sugars and low level of degradation by-products from hemicellulose. Detoxification with $Ca(OH)_2$ removed inhibitors but showed sugars loss. An ethanol concentration of 21 g/L after 176 h was found from the hydrolyzate using *Pichia stipitis* NBRC 10063 (fermentation efficiency 66%). An additional alkaline pretreatment applied to the solid fraction remaining from the diluted acid pretreatment improved the lignin removal. The highest cellulose hydrolysis values were found with the addition of β-glucosidase and PEG 6000. The simultaneous hydrolysis and fermentation of the cellulosic fraction with *Saccharomyces cerevisiae*, 10% (w/v) solid concentration, β-glucosidase and PEG 6000, showed the highest ethanol concentration (24 g/L), and cellulose hydrolysis values (81%). 162 L ethanol/t of dry napiergrass were produced (overall efficiency of 52%): 128 L/t from the cellulosic fraction and 34 L/t from the hemicellulosic fraction.

1. Introduction

Lignocellulosic materials are a promising carbon source for ethanol production because of its wide availability, low cost and little competence with foods. *Pennisetum purpureum* Schum. (napiergrass or elephant grass) is a perennial grass, which has higher dry matter yields compared to other grasses. Moreover, what makes it more attractive is its adaptability to low fertility yields, its high resistance to drought and low water requirements [1].

The bioethanol production from lignocellulosic biomass is a complex process, which requires a pretreatment step to hydrolyze the hemicellulose and to remove lignin, leaving

Abbreviation: APS, solid residue left after the acid hydrolysis (acid pretreated solid); AAPS, acid and alkaline pretreated solid; DM, dry matter; DW, dry weight; HMF, hydroxymethylfurfural; SSF, simultaneous saccharification and fermentation.

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the cellulosic surface exposed to the enzymatic hydrolysis. This pretreatment step is a limiting step in the lignocellulosic bioethanol production. There is a wide variety of pretreatments such as chemical (dilute acid or alkaline hydrolysis), physicochemical (steam explosion, ammonia fiber explosion -AFEX-), physical or biological pretreatments [2]. The diluted acid pretreatment hydrolyses the hemicellulose efficiently [3]. However, this method is not effective enough in the lignin removal and many inhibitor compounds of yeast fermentation (principally acetic acid, which could be removed using lime) and cellulytic enzymes (phenolic compounds) are produced during the hydrolytic reaction [4–6]. In some cases, it can be implemented a detoxification step of the liquid fraction as well as another pretreatment step, such as an alkaline one in order to remove a larger fraction of lignin. An alkaline pretreatment with NaOH, makes the biomass to swell, increasing the surface area, decreasing the crystallinity and disrupting the lignin structure [7].

Among the detoxification methods the most studied is the overliming with $Ca(OH)_2$, which is based on the precipitation of the toxic components as well as instability of some inhibitors at high pH. The weakness of this method is the carbohydrate degradation due to high temperature and pH [8,9].

The enzymatic hydrolysis of cellulose is another limiting step in the lignocellulosic bioethanol production. In contrast to the chemical hydrolysis, it is highly substrate specific, achieving high yields of glucose. However, cellulases can carry out non specific binding to lignin, so some alternative techniques like the utilization of surfactants, which have the ability to reduce these bindings, can improve the enzymatic yields. Another issue to be considered in enzymatic hydrolysis, is the inhibition caused by the substrate. This problem can be overcome, by using techniques of fermentation in simultaneous with the enzymatic hydrolysis step [10–12].

An ideal fermenting microorganism should achieve high ethanol yields and productivity, and be tolerant to high ethanol concentration. There is a wide variety of fermenting microorganism that includes bacteria, yeast and fungi species. Yeast strains, such as *Saccharomyces cerevisiae*, are currently used in the industrial processes due their high conversion efficiency and adaptability under fermentation conditions. However, this strain is unable to metabolize the main hemicellulose sugar monomers. The yeast *Pichia stipitis* can be used instead since it efficiently ferments xylose to ethanol [13].

The aim of this work was to evaluate the potential of an experimental culture of napiergrass for fuel bioethanol production. To that end, it was studied its response to a biomass conversion process to ethanol which includes steps of diluted acid and alkaline pretreatments, detoxification of the hemicellulose hydrolyzates, different cellulosic enzymatic hydrolysis strategies, and fermentation of simple sugars from the hemicellulosic (with high xylan content) and cellulosic (with high glucan content) fractions. Fig. 1 shows the strategy used.

2. Materials and methods

2.1. Raw material

Pennisetum purpureum Schum. (~30 kg) was given by the Departamento de Producción Vegetal, EEMAC, Facultad de Agronomía, Universidad de la República, Paysandú, Uruguay. It was dried (final humidity 4%) and ground (particle size <0.5 mm, average particle size 0.3 mm).

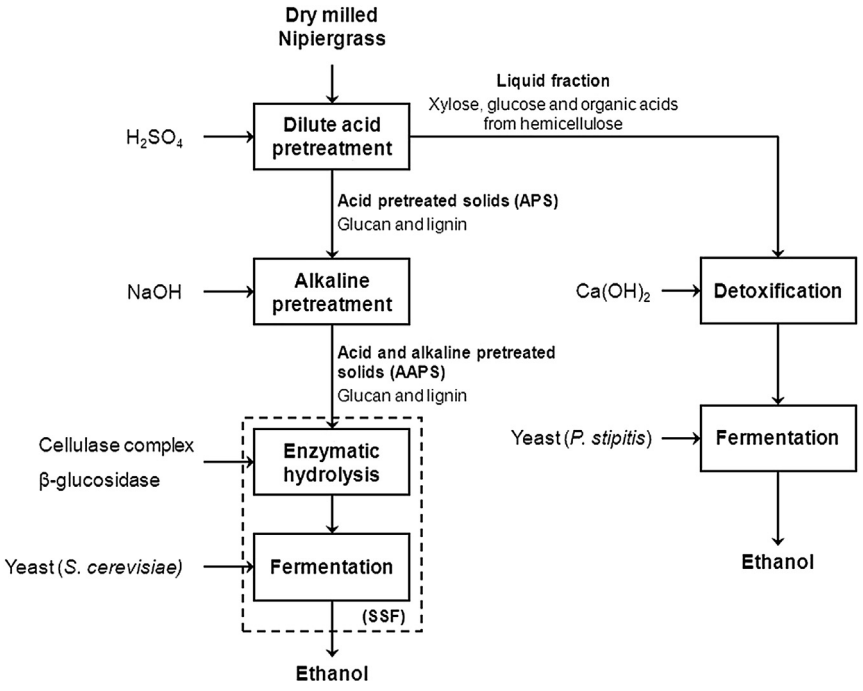

Fig. 1 – Strategy of ethanol production from napiergrass.