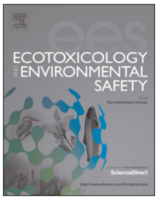
Ecotoxicology and Environmental Safety ∎ (∎∎∎∎) ∎∎∎–∎∎∎

Contents lists available at ScienceDirect

Ecotoxicology and Environmental Safety

journal homepage: www.elsevier.com/locate/ecoenv



Steam pretreatment of *Saccharum officinarum L.* bagasse by adding of impregnating agents for advanced bioethanol production

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ARTICLE INFO

Article history:
Received 19 February 2015
Received in revised form
14 July 2015
Accepted 28 July 2015

Keywords:
Sugar cane bagasse
Sulfur dioxide
Hydrogen peroxide
Steam explosion
Enzymatic hydrolysis

ABSTRACT

The main byproduct of the sugarcane industry, *Saccharum officinarum L.* bagasse (sugarcane bagasse, SCB), is widely used as lignocellulose biomass for bio-ethanol (EtOH) production. In this research study, SCB was pretreated by steam explosion (SE) method using two different impregnating agents: sulfur dioxide (SD) and hydrogen peroxide (HP). As matter of fact, the use of impregnating agents improves the performance of SE method, increasing the concentrations of fermentable sugars after enzymatic saccharification, and decreasing the inhibitor compounds produced during the steam pretreatment step. The aim of this study was to investigate and compare the use of the two impregnating agents in various SE-conditions in order to optimize pretreatment parameters. For every pretreatment condition, it has been evaluated: concentration of fermentable sugars, glucose and xylose yields, and the effects of the inhibitor compounds on enzymatic hydrolysis step. The obtained results allow to improve the efficiency of the whole process of bio-EtOH synthesis enhancing the amount of fermentable sugars produced and the eco-sustainability of the whole process. Indeed, the optimization of steam pretreatment leads to a reduction of energy requirements and to a lower environmental impact.

1. Introduction

Sugarcane (*Saccharum officinarum* L.), is a perennial grass belonging to the family of *Poaceae* that is used to produce sugar, accounting for almost 70% of the world's production (Table S1).

In the agricultural industries, sugarcane is crushed to extract the juice in order to produce sugar or it is fermented to obtain EtOH. The bagasse is the lignocellulose residue that remains after the juice extraction. The annual world production of sugarcane is about 1.6 billion tons, and it generates 279 million metric tons (MMT) of bagasse that, in the majority, remained unused and left to natural degradation or burnt in the field, causing severe environmental aggression and wastage of resource (Rashidi et al., 2015; Chandel et al., 2012). In recent years, there has been an increasing trend towards a more efficient utilization of SCB for bio-EtOH production process, one of the most promising substitute for petroleum-based gasoline (Thatoi et al., 2014). SCB contains (% w/w dry basis) appreciable amount of cellulose (35–45) and hemicelluloses (26–36), which can be de-polymerized by chemical or enzymatic hydrolysis in sugars monomers, mainly in glucose (Desouky et al., 2014). The fermentation of these sugars in bio-EtOH is carried out by microorganisms, including bacteria, yeasts and fungi (Dasgupta et al., 2013).

The bagasse also consists of a low lignin content (11–25) and it is a definite advantage in the process of bio-EtOH production. Lignin indeed forms a protective shield around cellulose and hemicelluloses, protecting polysaccharides from enzymatic degradation (Pandey et al., 2012). An upstream pretreatment is so necessary to promote the physical separation of lignocellulose matrix in lignin, hemicellulose and cellulose and to make more accessible cellulose and hemicellulose to attack by enzymes during hydrolysis step (Haghighi Mood et al., 2013). Therefore, a low amount of lignin allows an easier separation through the pretreatment method (Sun et al., 2014).

The SE is among the widest method used for the pretreatment

Abbreviations: AA, acetic acid; EtOH, ethanol; F, furfural; FA, formic acid; Gly, glycerol; HMF, hydroxymethylfurfural; HP, hydrogen peroxide; LA, levulinic acid; Lac, lactic acid; SCB, sugar cane bagasse; SD, sulfur dioxide; SE, steam explosion; WI, without impregnation; WIS, water insoluble solid

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http://dx.doi.org/10.1016/j.ecoenv.2015.07.034
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Please cite this article as: Verardi, A., et al., Steam pretreatment of *Saccharum officinarum L.* bagasse by adding of impregnating agents for advanced bioethanol production. Ecotoxicol. Environ. Saf. (2015), http://dx.doi.org/10.1016/j.ecoenv.2015.07.034

of lignocellulose biomass. Compared to other pretreatment technologies, the SE pretreatment is effectively able to overcome the recalcitrance of lignocellulosic biomass. As a matter of fact, the deconstruction of plant cell walls is a key step in the production of bio-EtOH (Brouder et al., 2011). This technique is also reported in the literature as effective on a wide variety of biomass substrates: wood chips, macro alga, oil palm cake and fiber, wheat straw, hemp, corn silage, and banana grass (Xu and Huang, 2014). However, SE process offers several attractive features when compared to other pretreatment technologies. These include: the potential for significantly lower environmental impact, lower capital investment, and more potential for energy efficient. For example, the conventional mechanical methods require 70% more energy than SE to achieve the same particle reduction (Hendriks and Zeeman, 2009). Other advantages include: a less hazardous process conditions and chemicals; a complete sugar recovery; the possibility of using high chip size, and an easier scale up for industrial production (Alvira et al., 2010).

In SE process, high temperature steam (160–260 °C) and pressure (0.69–4.83 MPa) is introduced into a sealed chamber containing lignocellulose material. After a period of time ranging from seconds to several minutes, the material is exposed to atmospheric pressure, so the pressure is suddenly reduced and the biomass undergo as an explosive decompression (Tutt et al., 2014).

SE pretreatment combines mechanical forces and chemical effects. The mechanical effects, due to sudden reduction of pressure, cause separation of lignocellulose matrix in individual fibers (hemicelluloses, cellulose and lignin) with minimal loss of material (Chen and Liu, 2014).

The chemical effects are due to the high temperature promoting the hydrolysis of acetyl groups that are included in hemicellulose (Alvira et al., 2010). These acidic groups could catalyze the further hydrolysis of the hemicelluloses by a process called “auto hydrolysis” or “auto-cleave” steam pretreatment (Hendriks and Zeeman, 2009). The soluble sugars produced by auto hydrolysis process of the hemicelluloses are primarily xylose, and further mannose, arabinose, and galactose (Carrasco and Baudel, 2010). In the pretreatment of lignocellulose biomass fermentation, inhibitors are released (Palmqvist et al.,1997), including organic acids (AA), furan derivate (F and HMF) and lignin degradation products such as phenolic, aliphatic and aldehydes compounds (Palmqvist and Hahn-Hägerdal, 2000). These products may also strongly inhibit the cellulose hydrolysis by cellulose enzyme (Jing et al., 2009).

In this study two different impregnating agent as SD (SO_2), and HP (H_2O_2), have been tested in order to optimize the performance of the SE-step.

The addition of SD is an effective pretreatment method for softwood, hardwood, and agricultural residues. The impregnation of SD, prior to pretreatment, results in lower process temperatures and in short reaction times (Shi et al., 2011).

The adding of SD supplies the acid medium favorable to plant carbohydrate hydrolysis. For this reason, SD-impregnation, prior to steam pretreatment, enhanced the carbohydrate hydrolysis rate by increasing the accessibility of cell walls through the formation of fractures and the removal of hemicelluloses during the steaming of the substrate, while reducing the depolymerization of oligomers and increasing of monomers in the water-soluble stream (Shevchenko et al., 2000). The purpose of the pretreatment with HP was, therefore, the delignification. HP is a chemical oxidizing which is commonly used as a disinfectant. Its oxidizing ability allows to detach and solubilize lignin, loosening the lignocellulose matrix, increasing the amount of cellulose available for hydrolysis by enzymes. Hydroperoxyl and hydroxyl radicals which are generated by decomposition of HP initiate delignification (Eriksson et al., 2010). The use of HP is considered “environmental benign” and the chemicals costs, compared to the use of other effective pretreatment chemicals, are lower (Cardona et al., 2010). The main disadvantage of using these impregnating agents is their production of inhibitor compounds. SD-pretreatment generates fermentation inhibitors, in particular HMF and F (Yang and Wyman, 2007) and the high oxidative degradation of lignin by HP can lead to the accumulation of aliphatic, aldehydes and phenolic inhibitory products (Ximenes et al., 2011).

Thereby, the purpose of this study was to investigate the improvement of SE efficiency based on the use of these two different impregnating agents, comparing the concentrations and yields of fermentable sugars (glucose and xylose) after enzymatic saccharification, and the effects on enzymatic hydrolysis of the inhibitor compounds produced during steam pretreatment step.

2. Material and methods

2.1. Experimental design

In order to optimize steam-explosion pretreatment of SCB, four parallel pretreatment conditions were tested: one without any impregnation (WI), a second with 2% of SD, a third one with 1% of HP, and a fourth one with 0.2% of HP. SCB steam pretreatment produce a slurry material in which it is possible to distinguish the solid (water insoluble solid, WIS) and liquid fractions that can be separated by filter press (Palmqvist et al., 1996). The WIS fraction consists of cellulose, lignin and the remaining hemicellulose, while the liquid fraction contains the hydrolyzed hemicellulose (monomer and oligomer sugars, AA), sugar degradation products (such as furanics, LA or FA), and phenolic compounds (released from lignin). The hydrolyzability of the part of material was assessed by using the solid washed fractions. The enzymatic hydrolysis was performed in water bath at 45 °C, with glass flasks. The test were carried out at two WIS concentrations: 2 wt% WIS and 10 wt% WIS.

2.2. Raw material

The raw material, SCB, was obtained from the sugar plant, located in Seranna, São Paulo, Brazil, with the following composition (% of dry weight, DW): 69.9% of carbohydrates and 23.6% of lignin (Table S2). The composition of raw material was obtained by the National Renewable Energy Laboratory (NREL) methods (Sluiter et al., 2010). The material has been stored in plastic buckets at 5 °C. The dry matter content of SCB was initially 91.61%.

In order to impregnate the bagasse with SD, the raw material was put in plastic bags. and then SD-gas has been added from a gas container corresponding to 2.0% of weight based on the water content of the bagasse. The bag has been then sealed and the gas left to distribute and impregnate the bagasse. The bag is let it settle for about two hours at room temperature. The same procedure was used to impregnate bagasse contained in a plastic bucket with 1% and 0.2% of HP by weight based on the water content of the bagasse. The buckets has been covered and kept at room temperature. The pretreatment with these kinds of impregnating agents was compared with a pretreatment of SCB without use of impregnating agents.

2.3. Steam pretreatment

The pretreatment has been performed in 10 l steam reactor followed by a flash tank to accumulate the pretreated bagasse. Bagasse equivalent to 600 g of dry matter was loaded into the preheated reactor. The bagasse was treated by saturated steam produced by an electric boiler at 200 °C for 5 min, and at 210 °C for 15 min. The SE conditions were shown in Table S3. These

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