Industrial Crops and Products 89 (2016) 425–433

Contents lists available at ScienceDirect

Industrial Crops and Products

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

Simultaneous saccharification and fermentation of cactus pear biomass—evaluation of using different pretreatments

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ARTICLE INFO

Article history:
Received 18 November 2015
Received in revised form 10 May 2016
Accepted 13 May 2016
Available online 6 June 2016

Keywords:
Cactus pear
Ethanol
Pretreatment
SSF
Enzymatic hydrolysis

ABSTRACT

Lignocellulosic biomasses of two species of cactus pear (*Opuntia ficus indica* and *Nopalea cochenillifera*) were tested for the production of ethanol. Their ability to grow in water-stressed regions makes them eligible for reducing the production cost of biofuels in drought regions. The biomass was pretreated using three different strategies: alkaline hydrogen peroxide, alkaline using NaOH, and acid using H_2SO_4 followed by alkaline delignification with NaOH. Analyses of the composition, crystallinity, and enzymatic hydrolysis were performed on the material before and after pretreatment. An experimental design was used to evaluate the influence of temperature and initial cellulose concentration on the process of simultaneous saccharification and fermentation (SSF) using the pretreated material, which showed the best performance in previous tests, and two strains of *Saccharomyces cerevisiae* (PE-2 and LNF CA-11). Biomass characterization showed a content of 31.55% cellulose, 17.12% hemicelluloses, and 10.25% lignins for *N. cochenillifera* and 34.86% cellulose, 19.97% hemicelluloses, and 15.72% lignins for *O. ficus indica*. The alkaline pretreatment provided the best enzymatic saccharification yields. The SSF experiments resulted in the greatest cellulose-to-ethanol yields using strain PE-2 with the pretreated *N. cochenillifera* (93.81%) at 40 °C and an initial cellulose concentration of 4%. *N. cochenillifera* showed better yields than *O. ficus indica*, and the PE-2 strain performed better than CA-11. These results suggest a good potential for cactus pear cladodes in the production of cellulosic ethanol.

1. Introduction

Cactus pear, originally from Mexico (Stintzing et al., 2001), has been cultivated in arid and semi-arid regions of Brazil since the late 19th century (Santos et al., 2016). In northeast Brazil, one can find three main species: two from the genus *Opuntia* (*O. ficus indica* and *Opuntia* sp.) and *Nopalea cochenillifera*. *Opuntia ficus indica* is considered the most productive and most resistant to dry regions; however, it is less palatable and has lower nutritional value. *N. cochenillifera* is more nutritious and appreciated by cattle but exhibits lower drought resistance. The moisture content of fresh biomass has been reported in the literature to be between 88 and 95% (Kuloyo et al., 2014; Santos et al., 2016).

Cactus pear has been attracting the interest of many researchers because of its wide availability, absence of an off-season, and low technology demand for cultivation and harvesting and because it is widespread and highly drought resistant (Griffith, 2004; Inglese et al., 2002). The reason for the high resistance to an arid environment amongst members of the *Cactaceae* family is Crassulacean acid metabolism (CAM). The CAM cycle consists of nocturnal CO_2 fixation into malic acid when there is low evaporative demand and stomata are open. During the day, stomata are closed, and the stored malic acid is decarboxylated back to CO_2, which is used for carbohydrate production through photosynthesis (Ting, 1985). Cacti can, therefore, survive in water-stressed regions efficiently using the limited soil water to produce biomass representing an important resource in drought areas for both wildlife and humans (Nobel, 2002).

Among its applications, it is worth mentioning the use of cactus pear for food and feed, as sources of energy, in the manufacture of pharmaceuticals and cosmetics, and in soil protection and conservation (Feugang et al., 2006). However, in Brazil, cactus pear has been mostly used only as cattle forage (Santos et al., 2016). The exploitation of these species using a biorefinery strategy by integrating the extraction of value-added chemicals and the production

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http://dx.doi.org/10.1016/j.indcrop.2016.05.028
0926-6690/

of fuel from the residual fibre appears to be an interesting strategy to be studied.

Although lignocellulosic ethanol technology has been significantly improved, the high production cost compared with fossil fuels still limits its commercialization (Chen and Fu, 2016; Thangavelu et al., 2016). Reduction in production costs can be achieved by using regionally cultivated biomass due to the important contribution of the transportation cost of low bulk density biomass on the final product (Haque et al., 2014). Therefore, cactus pear represents a potential source for biofuel production in arid and semi-arid regions in the world including northeast Brazil (Santos et al., 2016).

An efficient pretreatment step is important to change the structure of the lignocellulosic biomass to improve ethanol production from lignocellulosic biomass. The goal of this step is to remove the hemicellulose and lignin barrier around the cellulose fibres and to open the crystalline structure of the cellulose, making the cellulosic fibres more accessible to enzymatic attack (Bouza et al., 2016; Mosier et al., 2005). Physical, biological, chemical, and physicochemical strategies can be used (Lienqueo et al., 2016).

Following the pretreatment, the next steps comprise the release of monomeric sugars from cellulose and hemicelluloses and their conversion into ethanol using a microorganism (Paulova et al., 2015). Simultaneous saccharification and fermentation (SSF) has been proposed as a method for cost reduction of lignocellulosic ethanol because during this process, cellulase catalyses the hydrolysis of cellulose into glucose, which is simultaneously fermented into ethanol in a single reactor. This strategy reduces the potential for microbiological contamination, reduces equipment costs, and prevents enzyme inhibition by the sugar and cello-oligosaccharides produced (Fox et al., 2012), which increases the overall yield.

Recent studies have investigated the application of the cactus biomass in the production of biofuels including lignocellulosic ethanol (Kuloyo et al., 2014; Santos et al., 2016). However, further analysis of lignocellulosic biomass composition and the effect of different pretreatment methods on the fibres are still required to determine the biomass potential for ethanol production.

This study was proposed to evaluate the use of the lignocellulosic fraction of cactus pear, which is an important crop in drought regions, for the production of ethanol by studying all of the steps from pretreatment to fermentation. Different pretreatment strategies (alkaline hydrogen peroxide, alkaline NaOH, and acidic H_2SO_4 followed by alkaline delignification with NaOH) were tested using cladodes of two species of cactus pear (*Opuntia ficus indica* and *Nopalea cochenillifera*) to provide, after enzymatic hydrolysis, fermentable sugars for the production of cellulosic ethanol. The process of simultaneous saccharification and fermentation (SSF) using pretreated biomass to produce ethanol was also evaluated.

2. Material and methods

2.1. Raw material

The cactus cladodes of the two species tested in this study (*N. cochenillifera* and *O. ficus indica*) were kindly provided by Aquafrutas II farm (Touros, RN, Brazil). They were harvested from a thornless variety commonly found in the region. After the cladodes were selected and washed to remove impurities, they were cut into small pieces approximately 3 cm × 3 cm × 1 cm and taken to a forced air circulation oven where they were held at 50 ± 1 °C for at least 48 h or until the material was sufficiently dry to be milled. The dried material was then ground with a knife mill, and the fine particles passing through a 20-mesh (0.841 mm) sieve were collected. The material was then successively washed with tap water (at approximately 70 °C) to remove soluble materials and was subsequently dried under similar conditions to those previously mentioned. The material was finally placed in a sealed container and stored in a dry place at room temperature to avoid degradation.

2.2. Microorganisms

A sample of *S. cerevisiae* (strain LNF CA-11) was kindly provided by LNF Latino Americana. The strain PE-2 used in this study was obtained from the culture stock of the Biochemical Engineering Laboratory of the Department of Chemical Engineering, UFRN. The microorganisms were maintained in YEPD medium in a Petri plates with the pH adjusted to 5.0. After inoculation, the plates were left for 48 h at 28–30 °C for colony growth and were then stored at 4 °C (Ruiz et al., 2012). Both strains were unable to ferment C5 sugars.

2.3. Pretreatment

Three strategies for pretreatment were tested using lignocellulosic biomass. The alkaline hydrogen peroxide route used a 7.5% (v/v) H_2O_2 solution with the pH adjusted to 11.5 using 5 M NaOH (Saha and Cotta, 2007). The solution was mixed with lignocellulosic material (4%, w/v) in a 5-L beaker and stirred with an impeller at 100 rpm at room temperature for 1 h. Due to the heat released by the lignin oxidation, the mixture temperature tends to increase during the pretreatment and typically exceeds 80 °C. However, no temperature control strategy was used. The pretreatment in alkaline medium was carried out at 121 °C (same temperature reported in the literature for cactus pear (Kuloyo et al., 2014; Santos et al., 2016)) for 30 min using a 0.5% NaOH solution (w/v) containing 10% (w/v) lignocellulosic material. The acidic pretreatment was performed using a 0.5% (w/v) sulphuric acid solution containing 10% (w/v) lignocellulosic biomass and by exposing the mixture to a temperature of 121 °C for 30 min. The obtained material was then autoclaved a second time for delignification according to the alkaline pretreatment methodology described above.

After pretreatment, the lignocellulosic material was subjected to repeated washings with tap water (approximately 10% w/v biomass) to remove recalcitrant compounds formed by the degradation of the lignins. Washing continued until the filtrate pH was close to that of the tap water (approximately 6) and the colour disappeared.

2.4. Analytical methods

Pretreated and non-treated biomasses were characterized according to procedures described in the literature. Extractive (Gierlinger et al., 2004), cellulose (Sluiter et al., 2008), hemicellulose (Sluiter et al., 2008), lignin (Sluiter et al., 2008), and ash (Sluiter et al., 2008) contents were determined as described in the literature. The content of pectin in the non-treated materials was also analysed (Rangana, 1979). All analyses were performed in triplicate for data reproducibility except in the case of pectin, which was analysed in duplicate because of the large amount of material needed.

The high-performance liquid chromatography (HPLC) analysis to determine the polysaccharide monomers was conducted using a Shimadzu LC-10ADVP chromatograph equipped with a degasser (DGU-14A), a column heating furnace (CTO-10ASvp) and a refractive index detector (RID-10A). The mobile phase was 5 mM sulphuric acid with a flow rate of 0.6 mL/min. The chromatography was conducted in a Shim-pack SCR-101H column at 65 °C preceded by a pre-column model Shim-pack SCR-H both from Shimadzu. Before being injected into the HPLC, the samples were filtered through a 0.2-μm membrane.

To determine the best route, the total biomass recovery, the cellulose recovery and the delignification (percentage of lignin