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Microwaves as a pretreatment for enhancing enzymatic hydrolysis of pineapple industrial waste for bioethanol production

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ABSTRACT

The pineapple industry generates significant amounts of residues which are classified as lignocellulosic residual biomass. In the present paper, microwaves are studied as a pretreatment to improve pineapple waste saccharification. Different microwave (MW) powers (10.625, 8.5, 6.375, 4.25 and 2.125 W/g) and exposure times (1–20 min) were applied to the solid part of the waste before enzymatic hydrolysis. Infrared thermography was used to assess temperature evolution and structural modifications were evaluated by Cryo-SEM. Sugar content and fermentation inhibitors (phenols, furfural and hydroxymethylfurfural) were also determined. MW increased sugar yield as long as intermediate powers were used (up to 6.375 W/g). However, high powers and longer treatments resulted in sugar degradation and/or a decrease in the efficiency of the enzymatic hydrolysis process. Temperature records indicated that thermal sugar degradation may occur in those cases. The presence of fermentation inhibitors have been confirmed and related to prolonged MW treatments. Microscopic observations suggested that mild microwave pretreatments may promote microstructural changes that enhance enzyme performance, whereas harsher treatments could increase tissue compactness and reduce the effectiveness of the enzymatic treatment. It is concluded that microwave pretreatments using the appropriate energy supply and exposure time enhances saccharification efficiency, potentially improving further bioethanol yield.

1. Introduction

The global energy problem and the search for solutions based on sustainable and environmentally friendly renewable energies (Sun and Cheng, 2002) such as biomass and others (EC, 2009) has promoted bioethanol to be a clear alternative to fossil fuels. In this scenario, second-generation bioethanol, i.e. the produced from the fermentation of lignocellulosic biomass (waste and energy crops), deserves especial attention. Unlike first-generation bioethanol, second-generation of this biofuel helps diversify energy supplies without competing in the global food market (Rutz and Janssen, 2008; Bacovsky, 2010). Furthermore, the use of waste as a source for bioethanol production would also add up value to the whole manufacturing process.

The food industry generates significant amounts of residues which are a potential source for bioethanol production. In particular, pineapple production increases annually, and reached 24.79 million tons in 2013 (FAOSTAT, 2016). In addition, the industrialization of these fruit (juice, cannery,

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minimally processed) generates significant amounts of residues which consist mainly of the peel, core and crown of the pineapple. Pineapple waste usually represents about 50% (w/w) of the total processed fruit (Ketnawa et al., 2012), although some authors have even suggested values up to 80% (Ban-Koffi and Han, 1990). On the one hand, the liquid phase of this residue contains a high content of fermentable sugars (glucose, fructose, and sucrose) (Nigam, 1999). On the other hand, the solid phase is a lignocellulosic material which, apart from lignin, consists of cellulose and hemicellulose, polymers which are potentially hydrolyzable into fermentable mono- and disaccharides (Abdullah and Mat, 2008). Consequently, pineapple industrial waste has been investigated as an interesting source for ethanol (Ban-Koffi and Han, 1990; Nigam, 1999; Tanaka et al., 1999; Ruangviriyachai et al., 2010) and other metabolites production such as citric acid (Imandi et al., 2008).

However, bioethanol production from lignocellulosic biomass continues to be a challenge due to the complexity of this material in which cellulose and hemicellulose are densely coated by a hard-to-degrade lignin cover (Taherzadeh and Karimi, 2007). Hydrolysis of cellulose (polymer of D-glucose units linked by β-1,4-glycosidic bonds) and hemicellulose (complex heteropolysaccharide polymer that consists of pentoses, hexoses and uronic acids) could yield fermentable sugars to be used in bioethanol production (Scheller and Ulvskov, 2010). Lignocellulose would need to be disrupted in order to expose cellulose and hemicellulose to further chemical or enzymatic hydrolysis. Therefore, a pretreated lignocellulosic matrix becomes an essential prerequisite to obtain ethanol.

Nowadays, different physical, physicochemical, chemical and biological pretreatments, as well as a combination of all of them, are being assayed for pretreating lignocellulose (Sun and Cheng, 2002). Most of the conventional pretreatments require high temperatures usually reached by convection or conduction heating (Liu and Wyman, 2005). This creates a high energy cost that reduces the efficiency of the process. Therefore, there is a need for alternative methods to conventional pretreatments, among which microwaves have been suggested (Hu and Wen, 2008). The use of microwaves enables a volumetric, targeted and faster heating of the product than conventional heating, since there is direct contact between the product and the electromagnetic field generated by the microwave (De la Hoz et al., 2005). Furthermore, Xiong et al. (2000) showed that the use of microwaves could change the ultrastructure of cellulose, degrade lignin and hemicellulose and facilitate hydrolytic enzymes to access the lignocellulosic substrate (Kitchaiya et al., 2003; Zhu et al., 2005).

In this context, the aim of the present study was to investigate microwaves as an alternative pretreatment in order to improve the enzymatic hydrolysis yield of pineapple industrial waste.

2. Materials and methods

2.1. *Sample preparation*

A total of 20 pineapple fruits (*Ananas comosus* [L.] Merr., MD-2 cv.) were obtained from a tropical fruit importer and selected on the basis of their external factors such as the absence of injuries, ripeness and weight. Pineapples were first washed in a sodium hypochlorite solution (0.1%) for 5 min. Next, a pineapple cutter was used to remove the crown and separate the pulp. The resulting waste, consisting of the peel and core, were cut into smaller pieces and pressed in a screw press at 2.5 bar (Vincent Corporation model CP-4), the resulting press ratio being 0.49 (kg pressing cake/kg liquid phase). Liquid phase was removed from the original pineapple waste since it already contains simple sugars which would be directly fermentable (Nigam, 1999). In addition, sugar degradation can take place during microwave heating, for which only the solid part or press cake was subjected to subsequent microwave (MW) pretreatment and hydrolysis. Thus, the resulting press cake (*solid pineapple waste*) was grinded in a blender (Solac Inox Professional 1000 W Mixer), introduced in glass flasks (40 g each) and kept frozen (−22 °C) until the experiments were conducted. The resulting product was named *grinded solid pineapple waste*.

Experiments were performed on 40 g of thawed grinded solid pineapple waste to which distilled water had been added in 1:1 (w/w) proportion, resulting in the final sample identified as *reconstituted pineapple waste*. Distilled water was added to the press cake in order to enhance the microwave pretreatment effect as well as to avoid calcinations, as suggested by some authors (Azuma et al., 1984; Ooshima et al., 1984).

2.2. *Microwave pretreatment*

Microwave (MW) pretreatment was carried out in a microwave oven provided with a turntable plate (LG MH63340F/MH6340FS) with a frequency of 2.45 GHz. Samples were introduced in microwave intended plastic containers. The samples were treated at the following nominal powers: 170, 340, 510, 680 and 850 W, which resulted in the applied nominal powers: 2.125, 4.25, 6.375, 8.5 and 10.625 W/g; and exposure times from 1 up to 6, 8, 10, 14 and 20 min, respectively. Time exposure limits were defined by the appearance of calcinations or scorching. The power absorbed by the sample at these nominal power levels was estimated by heating 1 kg of distilled water from 10 °C up to 20 °C at 170, 340, 510, 680 and 850 W, according to the international standard IEC 60705 (1999). A thermocouple (HIBOK-14, sensor type K, sensitivity 39 $\mu V\,{}^{\circ}C^{-1}$, accuracy ±0.1 °C) was used for temperature measurements. Experiments were performed in triplicate and results showed an average (and standard deviation) of 129 (3) W for the 170 W, 247.4 (1.2) W for the 340 W, 336 (2) W for the 510 W, 485.5 (1.3) W for the 680 W and 602.0 (0.9) W for the 850 W. Corresponding absorbed powers in W/g were then estimated as 1.61 (0.04), 3.09 (0.02), 4.2 (0.03), 6.07 (0.02) and 7.53 (0.01). Finally, the pH of the samples was adjusted to 5 by adding NaOH 1 N (Panreac Química, S.L.U.). Water loss due to microwave processing was determined by weight difference and restored before proceeding with saccharification. Experiments were conducted in triplicate.

2.2.1. *Evolution of microwave heating by infrared thermography*

In order to estimate the temperatures reached during MW pretreatments, an infrared thermocamera Testo 870-1 (Testo AG) with a spectral infrared range of wavelength from 7.5 to 14 mm, 9 Hz frame rate and detector with 160 × 120 pixels, was used. In order to compare the difference in the heating undergone by the pineapple waste due to the different MW powers and exposure times applied, an image of the bottom surface of the container was taken just after each microwave pretreatment. Testo IRSoft software was used for image analyses, which allowed the study of microwave heating evolution,