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Research Paper

Ultrasound-assisted enzymatic hydrolysis of sugarcane bagasse for the production of fermentable sugars

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The effects of ultrasound irradiation on enzymatic hydrolysis of sugarcane bagasse were evaluated to obtain fermentable sugars. The influences of temperature, enzyme concentration and moisture content were evaluated with and without ultrasound irradiation. The hydrolysis yield achieved using ultrasound irradiation was significantly higher than that without. The highest amount of fermentable sugars obtained in the presence of ultrasound was 0.26 g [sugar] g^{-1} [dry sugarcane bagasse], which was around twice the value obtained without, at the temperature of 50 °C, 10% mass of enzyme and a moisture content of 75% (dry basis), after 240 min of reaction. The ultrasound irradiation appears to be a promising technology to be used in enzymatic reactions due to its positive effects on the reaction yield.

1. Introduction

Cellulose is the most abundant carbohydrate component of biomass (Yang et al., 2010) and accounts for about the half of the total bagasse on a dried basis (Ma, Xue, Yu, & Wang, 2012). Conversion of lignocellulose into biofuels (particularly ethanol) and other useful chemicals has recently been attracting increasing attention (Shaikh, Adsul, Gokhale, & Varma, 2011). Ethanol can be produced from abundant and renewable biomass sources such as agricultural wastes, including maize straw and also sugarcane bagasse (Chen, Zhao, & Xia, 2008; Maeda et al., 2011). The fermentable sugar obtained from lignocellulosic biomass is an alternative to the use of refined glucose, because it can be produced economically.

The lignocellulosic biofuel process could be made more economic by developing cost-effective pretreatment and hydrolysis strategies. The effective conversion of lignocellulose to sugar and its recovery, can be regarded as the key to the success of this technology (Shaikh et al., 2011). The enzymatic

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hydrolysis of cellulose using cellulase as biocatalyst is regarded as the most promising technique for converting lignocellulosic compounds into fermentable sugars such as glucose, which can be used as a cheap carbon source for ethanol or other bioproducts (Su et al., 2012).

However, little attention has been paid in the design of intensified enzyme-based processes for hydrolysis of lignocellulose, for example, the use of a solid-state enzyme reaction, which is defined as the reaction involving solids (lignocellulosic) in absence (or near absence) of free water but where the substrate must possess enough moisture to support the action of the enzymes. The main advantage of this procedure is the fact that the process is carried out in the absence of a free aqueous phase, resulting in minimum water consumption and reduced effluent being produced. As the amount of water can be adjusted to a minimum during the extraction of the hydrolysed sugars, the resulting solution is more concentrated than traditional enzyme hydrolysis, although there are also disadvantages associated with mass-transfer (Concept adapted from Pandey, 2003).

The reaction rates and mass transfer have been improved by using ultrasound, which can be considered an auxiliary source of energy (agitation and/or heating) in enzymatic reactions by the formation of micro bubbles during sonication treatments, due to the cavitation phenomenon, which involves a local increase of temperature and pressure at the solid–solvent interface. It is known that ultrasound pretreatment application may significantly increase the conversion of starch materials into glucose as well as overall biofuel yield (Ma et al., 2012; Nikolic, Mojovic, Rakin, Pejin, & Pejin, 2010; Vilkhu, Mawson, Simons, & Bates, 2008).

Ultrasound technology has potential for structural and functional modification on the properties of cellulose (Sindhu et al., 2013). Ultrasound is commonly used for pretreatment of wood wastes (Kunaver, Jasiukaityte, & Cuk, 2012)and sugarcane bagasse (Velmurugan & Muthukumar, 2012), whereas less attention has been paid for the ultrasound-assisted hydrolysis (Leaes et al., 2013; Li et al., 2012; Sulaiman, Ajit, & Chisti, 2013; Werle et al., 2013). Werle et al. (2013) verified that ultrasound irradiation enhance the yield of fermentable sugar after the acid hydrolysis of palm leaves, but at the best of our knowledge, there is no work reporting the ultrasound-assisted enzymatic hydrolysis of lignocellulosic materials.

Based on these aspects, aim was to evaluate the advantages of using ultrasound irradiation in the enzymatic hydrolysis of sugarcane bagasse under solid-state reaction conditions. For this purpose, process variables such as temperature, cellulase concentration and moisture content were evaluated during the enzymatic hydrolysis of sugarcane bagasse with, and without, ultrasound irradiation.

2. Material and methods

2.1. Materials

The sugarcane bagasse was obtained in a local factory (Santa Maria-RS). After being collected, the samples were immediately dried at 60 °C during 3 days, ground and sieved; the collected the particles being those that passed through the sieve of 16 mesh and were retained in the sieve of 32 mesh. The enzyme used in this study was the cellulolitic complex from *Trichoderma reesei* (NS50013) kindly donated by Novozymes Latin American, Brazil.

2.2. Equipment and experimental procedure

The experimental setup consists of an ultrasonic bath (Unique Inc., model USC 1800A, Brazil, BR) equipped with a transducer having longitudinal vibrations. The ultrasonic unit has an operating frequency of 40 kHz and a maximum-rated ultrasound power output of 132 W. The ultrasonic transducer (surface area of 282.2 cm^2) is fitted at the bottom of the bath horizontally along the length of bath, corresponding to an ultrasonic intensity of 0.46 W cm^{-2} (Werle et al., 2013).

Initially, an Erlenmeyer flask containing 5 g of dry bagasse was maintained in the ultrasonic bath up to 240 min at specified temperature, moisture content and enzyme concentration. The amount of fermentable sugar released was assessed at 0, 60, 120, 180 and 240 min. Because of the difficulties of sampling the solid material, a new reaction was started at each time and the whole content of the reaction was sampled for sugar extraction. For comparison, the same experiment was carried out with and without ultrasound irradiation. In the tests without the use of ultrasound irradiation the ultrasonic bath was used as thermostatic bath.

2.3. Effect of process variables on the hydrolysis

The influence of temperature on the enzymatic hydrolysis of sugarcane bagasse was determined in the range of 40–70 °C, maintaining the cellulase concentration and moisture content at 10% mass and 75% (dry basis), respectively. The effect of cellulase concentration on the hydrolysis was evaluated in the range of 5–15% mass, maintaining the temperature and moisture content at 50 °C and 75% (dry basis), respectively. The effects of moisture content on the hydrolysis were evaluated in the range of 55–95% (dry basis), maintaining the temperature and enzyme concentration at 50 °C and 10% mass, respectively. Sodium acetate buffer 0.1 M pH 5.2 was used to adjust the moisture content of bagasse (Balsan et al., 2012).

2.4. Fermentable sugar determination

After the reaction, the fermentable sugars were extracted from the hydrolysed sample using distilled water at solid to liquid ratio of 0.1 (dry basis). The extraction was carried out at 30 °C under orbital shaker agitation of 150 rpm during 30 min. After the extraction, the solution was filtered by vacuum filtration (Whatman qualitative filter paper, grade 1) and the supernatant used to determine the amount of fermentable sugars by the 3.5-dinitrosalicylic acid method (Miller, 1959). All results were expressed as mass of sugar (glucose equivalent) per mass of dry material (Werle et al., 2013).

3. Results and discussion

The experiments were carried out in order to evaluate the influences of temperature, cellulase concentration and