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Ethanol production by solid-state saccharification and fermentation in a packed-bed bioreactor

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ABSTRACT

In this work was presented a sequential strategy to optimize and scale-up the production of ethanol by solid-state saccharification and fermentation using rice bran as substrate. In the first step, fermentation was carried out in Erlenmeyers to study the influence of eight variables by means of a Plackett Burman design. After the choice of most significant ones, a central composite rotational design (CCRD) for three independent variables (rice bran concentration, moisture content and inoculum size) was conceived to optimize the ethanol production in a packed-bed bioreactor. From Erlenmeyers to packed bed bioreactor the process was scaled-up 10 times. Maximum ethanol production in the packed-bed bioreactor was 135 ± 10.8 g kg^{-1} at inoculum size, rice bran concentration and moisture content of 10% v/v, 62.5% w/w and 65% w/w, respectively. The ethanol yield obtained in the packed-bed bioreactor was similar to that in the erlenmeyers flasks (138.7 g kg^{-1}), validating the strategy adopted for optimization and scale-up.

1. Introduction

Overall energy consumption is increasing with the growing world population and rapid industrial growth, reducing fossil fuels reservoirs and increasing environment problems caused by greenhouse gases accumulation [1–3]. For this reason, countries worldwide have paid much attention to the non-fossil energy, especially, the cleaning renewable biomass fuels [3–5]. Lignocellulosic biomass has been considered as the ideal feedstock for biofuel production as it does not compete with food resources and can potentially reduce carbon dioxide emission by up to 75% compared to fossil fuels [1,6–8].

Second generation biofuels from lignocellulosic biomass or agricultural wastes have advantages, for instance, most second generation biofuels are considered to be able to deliver substantial greenhouse gases emissions reductions when compared with petroleum. Development of second generation biofuels has been challenging, because it is need ensure low-cost and stable feedstocks, minimizing land use and changes caused by demand for biomass feedstocks and optimizing bioethanol production technologies [9–11].

The effective conversion of agricultural residue to sugar and its recovery can be regarded as the key to the success of this technology. Enzymatic hydrolysis is reported 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 production [12]. Enzymatic hydrolysis of agricultural residues is mainly accomplished with simultaneous saccharification and fermentation processes in liquid media [13]. Therefore, more energy to concentrate the ethanol produced due to low solid content (substrate) and a large quantity of waste water is required [14].

Solid-state saccharification and fermentation is defined as the simultaneous hydrolysis and fermentation in absence (or near absence) of free water, it can overcome the above problems because of the many benefits such as high substrate concentration and product yield, simple and controllable operation, less effluent wastewater, and less energy consumption. The main advantage of this procedure is the fact that the process is conducted in the absence of free aqueous phase, resulting in minimum water consumption and thus a low effluent production. As the amount the water can be adjusted to a minimum during the extraction of ethanol produced, the resulting solution is more concentrated that traditional liquid fermentation [12,15]. Some studies had

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demonstrated that solid-state saccharification and fermentation of cellulosic materials can effectively reduce the production cost, improving the commercial application of bioethanol [13,15].

However, it is fundamental to define a strategy to optimize and scale-up of solid-state saccharification and fermentation process. Solid-state fermentation, unlike submerged fermentation, has many challenges to be solved before the scale-up. One of these difficulties is the choice of bioreactor, since it provides the environment for growth and activity for the microorganisms, which cause the biological reaction. The most used bioreactors for solid-state fermentation are tray, packed bed and rotary drum [16]. The packed-bed reactor seems to be a promising bioreactor configuration, since they are capable of supporting microbial growth for long culture periods under low shear conditions, due to the immobilisation of cells within macroporous matrices.

Based on these aspects, the main objective of this work was to define a strategy to optimize and scale-up the ethanol production using rice processing residue as substrate by solid-state saccharification and fermentation. In the first step, fermentations in erlenmeyers flasks were carried out select the most important variables. In the second one, the optimization and scale-up were accomplished in a packed-bed bioreactor.

2. Material and methods

2.1. Materials

The rice husk and bran were obtained in a local rice milling (Santa Maria-RS, Brazil), which were maintained under refrigeration (4 °C) until the experiments. The composition (%wt) of rice bran was determined using the methodology described by Van Soest et al. [17]: Lignin (5.4), hemicelluloses (22.9), cellulose (9.2), fat (16.5), starch (30.8) and protein (15.2). Corn steep liquor (CSL) was obtained from Ingredion (Mogi Guaçu, Brazil). Soybean bran was obtained in a local market. The enzymes used in this study were the cellulolytic complex from *Trichoderma reesei* NS50013 (Novozymes Latin American) and the amylolytic complex Spirizyme Fuel (Novozymes Latin American).

2.2. Microorganism and inoculum

A commercial strain of *Saccharomyces cerevisae* (Fleischmann) was used [18]. Cell production for pre-inoculum was carried out in Erlenmeyers with 10 mL of medium containing ($g.L^{-1}$): sucrose (20.0), yeast extract (5.0), K_2HPO_4 (5.0), NH_4Cl (1.5), KCl (1.15) and $MgSO_4 \cdot 7H_2O$ (0.65). The medium was inoculated with 1 g of dehydrated yeast and incubated in orbital shaker (INNOVA 44R, New Brunswick Scientific), at 30 °C, 150 rpm for 24 h.

2.3. Experimental procedure for solid-state fermentations

The experiments for optimization of ethanol production were carried out in two steps. In the first one, the fermentations were performed in Erlenmeyers containing 100 g of dry solid substrate, where the effects of eight variables were studied. The second step consisted in the selection of most significant effects of first one to optimize the process in a packed-bed bioreactor containing 1 kg of dry substrate (scale-up of 10 times).

For the first step, 100 g of dry solid substrate, which was composed by different proportions of rice bran and rice husk (rice husk was used only as support) were charged into 500 mL Erlenmeyer flasks. Afterwards, the solid substrate was supplemented (CSL and soybean bran) and the moisture content adjusted at specified level. Each Erlenmeyer was covered with hydrophobic cotton and autoclaved at 121 °C for 20 min. Preliminary experiments showed that no changes in moisture content of the substrate after autoclaving were detected. After cooling, the enzymes (amylase and cellulase) and microbial cells were inoculated and incubated at specified temperature for 24 h in a chamber with temperature and humidity control (POL-EKO, model KK 350). The variables investigated in this step were: temperature (30–40 °C), rice bran concentration used in the solid substrate (50–75% w/w), inoculum concentration (5–15% v/v), moisture content (60–80% w/w), CSL concentration (5–15% w/w), soybean bran concentration (5–15% w/w), amylase concentration (0.2–0.6% v/w) and cellulase concentration (0.2–0.6%v/w). All concentrations were defined for a dry mass of 100 g. The effect of variables was investigated by a Plackett-Burman design (PB16).

In the second step was employed a packed-bed bioreactor that consists of a cylindrical stainless bed connected to a thermostatic bath for temperature control. It was filled with 1 kg of dry solid substrate and supplemented with soybean bran and CSL. The moisture content was corrected to desirable level and autoclaved at 121 °C for 20 min. After cooling, the enzymes (amylase and cellulase) and microbial cells were inoculated and incubated at 36 °C for 24 h. A central composite rotational design (CCRD) for three independent variables was conceived to investigate the influence of rice bran concentration in the solid substrate (41.5–83.5% w/w), moisture content (39.8–90.2% w/w) and inoculum (1.6–18.4% v/v).

2.4. Fermentable sugar and ethanol determination

After the fermentations, ethanol and sugar were extracted from the solid material following methodology described by Canabarro et al. [19]. After the extraction, 1 mL of supernatant was used to determine the amount of fermentable sugars by 3.5-dinitrosalicylic acid method [20]. A liquot of 5 mL of supernatant was used for determination of ethanol content using an alcolyzer at 25 °C (Alcolyzer Wine M/WE – Wine Analysis System - Anton Paar). The results were expressed in g of ethanol per kg of dry substrate.

2.5. Statistical analysis

All the results were analyzed using the software Statistica® 7.0 (Statsoft Inc., Tulsa, OK, USA), considering a significance level of 90% for Plackett-Burman design and 95% for the CCRD.

3. Results and discussion

3.1. Selection of variables in erlenmeyers

Table 1 present the results of the PB16 to select the most significant variables on ethanol production by solid-state saccharification and fermentation in Erlenmeyers. The ethanol concentrations ranged from 149.2 (run 8) to 17.7 g kg^{-1} (run 2), with great variation among the runs. Central points of PB16 presented low experimental variation, showing a good reproducibility of data. From data of Table 1 it was evident that the studied process variables affect the production. Aiming to select the most significant variables, data of Table 1 were used to determine the effects of independent variables on the response, which are presented in Table 2.

The media supplements (CSL and soybean bran) did not influence the ethanol production in the evaluated range. Concerning the enzymes used to convert substrate (starch or cellulose) into fermentable sugars, amylase showed a positive effect, whereas cellulase was not significant. This can be due to fact that the main sugar source in the solid material is starch (cellulose content is about three times lower) and, by this reason, the contribution of amylase in comparison with cellulase activity to provide sugar for