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ENZYMATIC HYDROLYSIS OF SUGARCANE BAGASSE IN ROTATING DRUM REACTOR

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Abstract. *The high yields are obtained in biological conversion of cellulosic biomass to fuels and chemicals and they generate vital products, an economical success and a potential for very low costs. Enzymatic hydrolysis that converts lignocellulosic biomass to fermentable sugars may be the most complex step in this process. A major requirement in cost-efficient lignocellulosic biomass processing is to employ reactor that will ensure, with a maximal conversion of the cellulose using a minimal enzyme dosage. The aim of this work is to use a reactor to process a large amount of biomass, and to promote an environment with better ability to transfer heat and mass differently than is obtained in processes performed Erlenmeyer flasks. However, at high solid loadings, the viscosity of the reaction mixture will be very high and other factors should also be considered, such as mixing and mass transfer limitations for the product inhibition. The present paper deals the analysis of the enzymatic hydrolysis process of sugarcane bagasse performed in rotating drum reactor (working volume of 4 L), using bagasse with 10 w/v and cellulase enzyme. The results of the experiments showed an increase in the glucose concentration (g L^{-1}) produced in the reactor in comparison to those realized in erlenmeyer flasks (control), because the mass transfer and mixing allow a large contact area of the enzyme with the substrate (sugarcane bagasse) in the reactor.*

Keywords: rotary drum reactor, sugarcane bagasse, enzymatic hydrolysis, high solid loadings

1. INTRODUCTION

Currently, the international energy system is very dependent on fossil fuels (coal, oil and gas), because global energy consumption about 80% originates from these sources; consumption shows an annual growth of 2% (average of 20 years) and annually increases by 3.1% over the past five years. This is a situation that deserves to change, not only by the gradual depletion of fossil fuel reserves, as well as negative effects on the environmental that result from their use, as global warming. The search for alternative fuels has conducted some countries to choose biofuels due to the recent interest in biomass energy, generating fuel liquids, as ethanol produced by the fermentation of sugars (first generation ethanol). It is mainly extracted from sugarcane sugar, maize, sugar beet and others. Another way to the ethanol production is by the hydrolysis of cellulosic biomass with the generation of glucose, which can be fermented to produce ethanol (second generation ethanol) (Ogeda & Petri, 2010).

In the last 15 years, an increase on efforts has been made towards a more efficient utilization of renewable agro-industrial residues, including sugarcane bagasse (Soccol, *et al.*, 2010). This has occurred due to the renewable nature, low cost and local availability of lignocellulosic biomass. The Brazilian sugarcane system of agroenergy is considered as the most efficient system. Therefore, in order to meet wider needs, a significant increase in the production of ethanol would be possible only if the basic knowledge necessary for the development of technologies that will be capable to obtain energy from lignocellulosic materials present in sugarcane. Although the chemical hydrolysis of biomass from sugarcane is a consolidated methodology under laboratory conditions, its large-scale application is not yet

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economically viable in Brazil (Soccol, *et al.*, 2010). Thus, research focusing the enzymatic hydrolysis of cellulosic biomass has been performed in several research centers, to allow this residue is able to compete economically with corn ethanol and petroleum-derived gasoline (Galbe & Zacchi, 2002; Lynd *et al*., 2008; Sun & Cheng, 2002).The advantages of the enzymatic hydrolysis of cellulose over chemical hydrolysis methods are lower utility (cooling water, gas, electricity), disposal costs and no corrosion issues for the equipment (Sun & Cheng, 2002). The production of fuel ethanol from lignocellulosic biomass includes biomass pre-treatment, cellulose hydrolysis, fermentation of hexoses, separation, effluent treatment, and depending upon the feedstock, gathering, which may have an additional cost (Soccol *et al.*, 2010).

The enzymatic hydrolysis of cellulose to glucose by cellulases is one of the major steps involved in the conversion of lignocellulosic biomass to biofuel yield. This hydrolysis by cellulases, a heterogeneous reaction, currently has some major limitations, most importantly a reduction in dramatic rate at high degrees of conversion. To render the process economically viable, increases in hydrolysis rates and yields are necessary and require improvement both in enzymes (via protein engineering) and processing, i.e. optimization of reaction conditions, reactor design, enzyme and substrate cocktail compositions, enzyme recycling and recovery strategies (Bansal *et al*., 2009).

One factor that influences the overall rate of reaction is the mass transfer resistances, including boundary layer resistance and resistance to internal enzyme diffusion. At the start of the hydrolytic reaction in a stirred batch reactor, the overall reaction rate is determined by the rates of three events in sequence: (i) the external enzyme mass transfer rate through the stagnant liquid film layer adjacent to the substrate solid, (ii) the rate of enzyme adsorption at the substrate surface, and (iii) the rate of cellulase catalysis. During this dynamic interactive process, local mass transfer barriers caused by stagnant film layer and changing substrate structure could become a significant factor in the reaction rate determination. Also, enzyme inhibition depends largely on the local cellobiose and glucose concentration, which again depends on the overall mass transfer efficiency inside a reactor (Gan & Taylor, 2003).

The aim of the present study was to evaluate the performance of a rotating drum reactor (RDBs) in the enzymatic hydrolysis of sugarcane bagasse. This type of equipment has a potential to provide better heat and mass transfer characteristics than solid state fermentation (SSF) bioreactors with static beds, whereas it provides gentler agitation that seen for bioreactors with the internal stirrers. Furthermore, the absence of internal moving parts for mixing makes a simpler design, construction and operation, and the low pressure-drop across the bioreactor reduces the operating costs associated with the aeration system. The gentle agitation associated with the tumbling motion of the substrate bed minimizes the damage to the substrate particles (Hardin, *et al.*, 2002). Since this equipment model allows the use of a higher substrate load, the experiments were performed in the reactor using 10% (w/v) of sugarcane bagasse and 2 different concentrations of commercial cellulase enzyme. The ranges considered for the pretreatment conditions were determined based on the results of a previous study (Cruz, *et al*., 2013; Rezende, *et al*., 2011) that assessed the pretreatment of bagasse.

2. MATERIAL AND METHODS

1. Substrates

The sugarcane bagasse samples used in the experiments are from the harvest (2011/2012) and provided by Raizen Group (São Paulo State, Brazil). Prior to analysis and pretreatment, the sugarcane bagasse was dried at 45 °C for 48 h and left at room temperature for 24 h and then stored in plastic containers at room temperature until its use (Rabelo, 2010).

2. Pretreated sugarcane bagasse

The sugarcane bagasse was initially pretreated with a dry sugarcane bagasse/1% H_2SO_4 solid-liquid ratio of 1:16 to solubilize the hemicellulose. The bagasse solid fraction was separated from the hydrolysate by filtration and abundantly washed with distilled water to eliminate the acid excess before oven drying at 60°C for 24 hours. After this pretreatment, the samples were treated with the alkaline solution (NaOH) of 1% w/v to promote the delignification. The suspension containing sugarcane bagasse was autoclaved for 40 min at 1 atm and 120 ºC. Consequently, the samples were washed with hot distilled water to eliminate alkaline excesses until a neutral pH and solid were dried in an oven for more 24 hours at 60°C (Rezende, *et al*., 2011). The determination of the carbohydrate content of the pretreated bagasse samples was done through Thermal Analysis (TG/ DTG and DTA). Thermogravimetric Analysis (TGA) was applied for a semi quantitative analysis of hemicellulose, cellulose and lignin content in biomasses. Cruz, *et al*., 2013 showed the results of the chemical composition of the bagasse that was used in this study.

3. Enzyme Hydrolysis

The enzymatic hydrolysis experiments were performed in Erlenmeyer flask (control) and in the reactor. The experiments in Erlenmeyer flasks (0.5 L) were carried out using 0.1 Kg (dry weight) L^{-1} of pretreated sugarcane bagasse

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(05L) an enzyme load (Accellerase 1500® cellulose enzyme Danisco, Rochester, NY, USA) of 7 and 10 FPU g^{-1} dry biomass. The suspensions were incubated at 200 rpm, 323.15 K and pH 4.8 (sodium citrate buffer, 50 mM). During hydrolysis, the samples were collected at times of 2, 4, 6, 12, 24, 48 and 72 h and inactivated by increasing the temperature to 80°C for 15 min. Finally, the glucose concentration was determined using a kit based on the glucose oxidase reaction (reagent GOD PAD). Cellulase activity was measured by the filter paper method as described by Ghose (Ghose, 1987) and its value was of 25 FPU mL^{-1}.

4. Equipment - Rotating drum reactor

A rotating drum reactor employed in the experiments was made of stainless steel with a diameter of 0.45 m, a length of 0.25 m and a total internal volume of 38 L (Salles, 2013). Four straight baffles were longitudinally installed to move the bagasse, thereby to improve the mixture. On the outside of the inner cylinder was installed a heat exchanger, responsible for maintaining the temperature of the reaction media in 323.15 K. The reactor also presents temperature sensors installed at the outside of the drum (Figure 1). The working volume was 10% of the total capacity of the equipment. In the reactor, the suspensions were incubated at 15 rpm and pH 4.8 (sodium citrate buffer, 50 mM) for 72h. The procedure for removal of the samples and the determination of glucose were in the same control flasks.

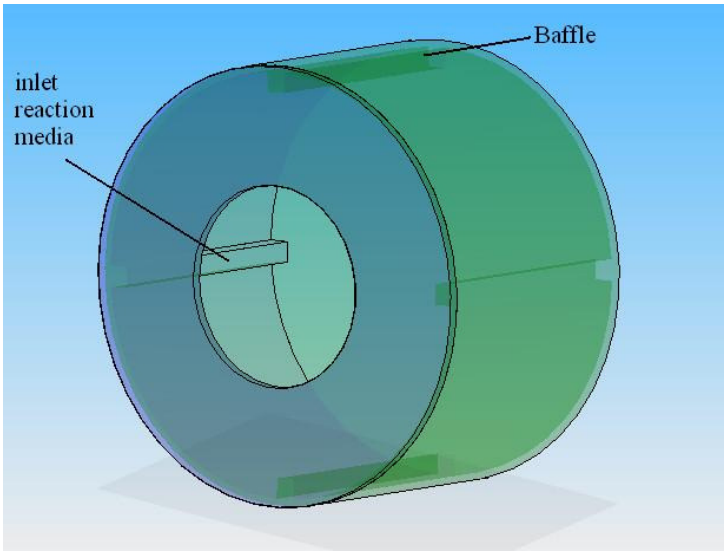

Figure 1. Geometric scheme of the rotating drum reactor.

5. Fundamental calculations of rotating drum reactor

The effect of rotation rate on the mass transfer between the bed and headspace is related to the flow regimes that correspond to the several rotation rates. The flow regimes proposed by Mellmann (2001) for a rotating drum without internal elements are the basic ways Slipping, Cascading and Cataract. As the speed of rotation of the drum is increased, the regime of flow of the particles changes. At very low speeds the particles exhibit slumping flow in which the solid bed periodically slips back down the wall. At higher speeds the material on the top of the bed tumbles back down the face of the bed in a continuous cascade. At still higher speeds material is thrown into the air before landing again (cataracting). These regimes are divided into sub-forms depending on the degree of filling (f) defined as the fraction of the section cross cylinder which is filled with the particulate material and number of Froud (Fr). Although, there is a division into basic flow forms, such amounts are for orientation and are dependent on the particular bed material used (Mellmann, 2001).

A characteristic criterion for the motion of solids in rotating drum reactor is the Froude number (Fr) as the ratio of centrifugal force to gravity. The centrifugal force is related to the inner radius of the cylinder, hence this criterion is also named the peripheral Froude number and calculated from Equation (1).

$$Fr = \frac{\omega^2 R}{g} \tag{1}$$

where ω - drum angular velocity, R - radius of the drum; g - gravity acceleration. The angular velocity (ω) is calculated by the frequency or rotational velocity (F), as in Eq. (2).

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$$\omega = 2\pi F \tag{2}$$

The degree of filling, as the portion of the cylinder cross-section occupied by the bed, is determined by the filling angle (Eq. 3).

$$f = \frac{1}{\pi}\left(\varepsilon - \sin\varepsilon\cos\varepsilon\right) \tag{3}$$

The filling angle corresponds to the half bed angle of the circular segment occupied with solids. Assuming a flat bed surface, its distance from the axis of rotation is calculated from $r_0 = R\cos\varepsilon$. Thus, the width (chord) of the solid bed is given by $S = 2R\sin\varepsilon'$ and the maximum bed depth at mid-chord amounts to $h = R - r_0$ (Mellmann, 2001).

Schlünder & Mollekopf proposed the following equation for the determining the number of the rotations required for the perfect mixing (N), as in Eq. (4) (Grajales *et al.*, 2012).

$$N = 16Fr^{0.2} \tag{4}$$

Beyond a certain critical speed (N_c) there is sufficient centrifugal force to hold the bed against the wall, thus it is convenient to relate the flow regimes to fractions of the critical rotational speed (N_C), as in Eq. (5) (Mitchell, *et al.*, 2006).

$$N_C = \frac{42}{\sqrt{D}} \tag{5}$$

where D is the drum diameter.

6. RESULTS AND DISCUSSION

7. Characterization of the pretreated sugarcane bagasse

Data for the bagasse composition (cellulose 63.0%; hemicellulose 3.5%; lignin 23.5% and ashes 4.4%) indicated, as expected for pretreated material, low hemicellulose content. Percentages of cellulose, hemicellulose, lignin and ashes were calculated on a dry weight basis. The cellulose amount increased after pretreatment, ranging from 35% content (bagasse *in nature*) to 63% under pretreatments using 1% NaOH. Most of the hemicellulose fraction was removed using acid, as shown by its percentage decrease from 25% to 3.5% for bagasse *in natura*.

8. Fundamental calculations of rotating drum reactor

The operating conditions of the process were: rotation rate was 15 rpm and filling degree, f=0.23. These conditions, the value of the Froude number was Fr = 5.6 x10^{-2}. Thus, based on the values obtained in this work, the fluid flow regime in the reactor was Cascading and the sub-form was cascading. For this type of motion (Cascading), the height of the arch bed increases with increasing in rotational speed. The sub-form cascading characterized by an arcuate surface and higher particle velocities present in free surface and also a flow regime that promotes good mixing of solids. This sub-form is predominant in reactors rotary drum (Xavier *et al*., 2009).

Other characteristics of the reactor of this work are the number of rotations calculated using Equations 4 and 5 (N_c = 62) for the conditions applied in this study. As the speed of rotation of the drum was 15 rpm, this means 25% below the critical value, a rolling flow began with a flat surface, and consequently, over time there was a cascading flow characterized by a curved surface.

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9. Enzymatic Hydrolysis

Figure 2 shows the results obtained from the experiments in Erlenmeyer flasks (control) and the rotating drum reactor.

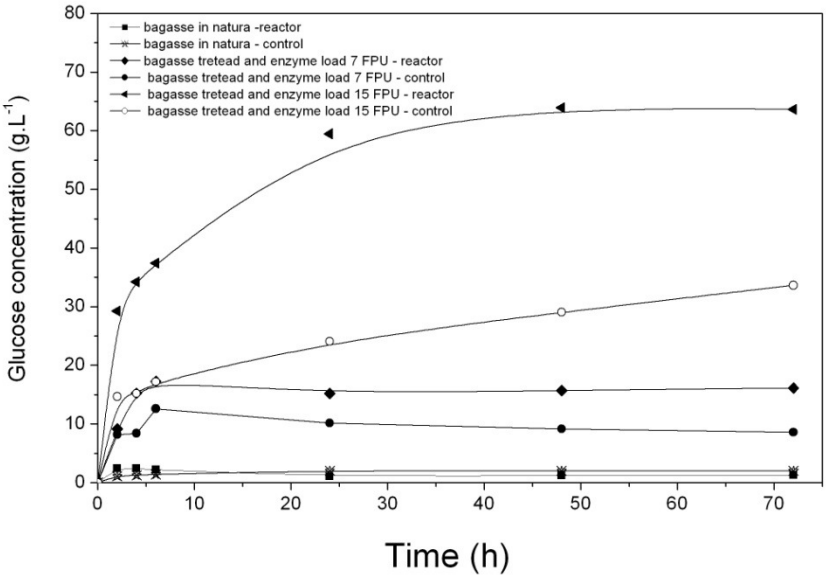

Figure 2. Comparison of the hydrolysis profile of untreated bagasse and pretreated varying the concentration of enzyme.

Through the figure 2, it can be seen that in all the experiments, the process of cellulose hydrolysis was completed in 24 hours. After this period, there was little variation in the amount of glucose produced. This occurred, in the case of biomass without pretreatment because the enzyme did not have access to the substrate. In other cases, the pretreated biomass, the enzyme concentration was insufficient (for example in experiments with enzyme concentration (7 FPU mL^{-1}) or due to problems related to product inhibition.

After the experiments with biomass without pretreatment was conducted tests with a minimum concentration of enzyme or 7 FPU mL^{-1}. The range of concentration of enzyme used in the process varies 7 to 33 FPU mL^{-1} (Sun & Cheng, 2002). Therefore, the experiments were performed in Erlenmeyer flasks and in the reactor with enzyme concentration of 7 FPU g^{-1}dry bagasse.

Santos & Gouveia (2009) observed in experiments with sugarcane bagasse pretreated with concentration of 186.16 g L^{-1} in bottles flasks using a enzyme concentration of 17.2 FPU g^{-1}dry bagasse (Celluclast 1500), that after 46 hours, the process stabilized. To solve this problem, the authors added more 2.10^{-3} L of enzyme in the process after 46 hours in an attempt to increase the conversion of the enzyme. However, there was not process improvement, the authors attributed this to the presence of lignin in the reaction medium, which results in a slow conversion and a high glucose concentration and inhibits the action of Beta-glycosidase enzymes. For the reduction of these effects of inhibition during hydrolysis, some procedures have been suggested by Sun & Cheng (2002) as the removing sugars (glucose) during hydrolysis semi-continuous process or by ultrafiltration of the reaction medium.

Due to the low levels of glucose concentration obtained in the experiment at a concentration of enzyme 7 FPU g^{-1}dry bagasse it was decided to increase the amount of enzyme load to 15 FPU g^{-1}dry bagasse. The increase in enzyme concentration also caused an increase in the concentration of glucose. In function of the bioreactor, the yield was much higher due to a better mass transfer and heat, and a mixture inside the equipment in relation to the Erlenmeyer flasks. There was no significant change in the results in both experiments after 24 hours of cultivation, showing that the hydrolysis process stabilized. One possible factor for this occurrence is the product inhibition (glucose).

Wang, et al (2011) observed a decrease of conversion of cellulose to high substrate concentration, and concluded that this does not occurred neither due to loss of enzyme activity nor inhibition of the final product. Instead, the authors linked this result with the change of a capacity of adsorption of the enzyme in higher solids loading. The lower binding capacity of cellulase is possible to lead to a lower surface coverage of enzyme on cellulose, thus may influence the hydrolysis of cellulose. Instead, it may be related to the change of adsorption capacity at high solid loading. The authors did not discard the influence of other factors, such as the slower three-dimensional diffusion of enzymes in solution, and some substances in enzyme solution maybe also related to the decreased sugar yield. Cellulase adsorption to cellulose has been thought to be much related to the hydrolysis of cellulose, and the results showed that more studies are needed to better understand the mechanism of adsorption of enzyme on cellulose.

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Pereira *et al.*, (2011) investigated the enzymatic hydrolysis in a bioreactor type STR (mechanically stirred), capacity of 1 L, (Model Biostat B-plus, Sartorius) equipped with turbine impeller. In this study, the sugarcane bagasse and enzyme concentration was 0.1 Kg L^{-1} and 10 FPU g^{-1}dry bagasse (*Trichoderma reesei* RUT C30 and *Aspergillus awamori* 2B.361 U2/1), respectively. The authors obtained a releasing of glucose of 27 g L^{-1} in 40 hours, and this value was lower than that those obtained in the present study with the bioreactor.

O'Dwyer *et al*., (2006) performed the experiments using enzymatic hydrolysis corn stover and different concentrations of enzymes (*Trichoderma reesei* - from 0.25 to 50 FPU g^{-1}dry straw). Glucose has a binding affinity equal to the enzyme that may cause the cellulose inhibit for the glucose enzyme bond. Another explanation is not presented for the preference and irreversible binding of lignin. Data obtained ranged from 10 to 100 g L^{-1} in 72h.

3. CONCLUSIONS

The efficiency of the use of the rotary drum reactor in the enzymatic hydrolysis process was verified in the present study. The access of the enzyme to the substrate has been enabled due to a better mass transfer.

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