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# OPTIMIZATION OF SOLID-LIQUID EXTRACTION OF ETHANOL OBTAINED BY SOLID-STATE FERMENTATION OF SURGARCANE BAGASSE

N.I. CANABARRO[1], C. ALESSIO[1], J.F. SOARES[1], J.V.S. CORRÊA[1], N. SUSIN[1], W. PRIAMO[2] e M.A. MAZUTTI[1]

[1]Universidade Federal de Santa Maria, Departamento de Engenharia Química
[2]Departmento de Tecnologia de Alimentos, IFRS - Campus Sertão
E-mail para contato: claudianp@msn.com

ABSTRACT – Solid-state fermentation has arrived as an alternative to reduce the amount of waste water in ethanol fermentation. However, the recovery of ethanol from solid medium should be investigated, since depending of experimental condition used in the extraction, significant difference in the results can be obtained. In this work was investigated the influence of temperature (30-50°C), solid to liquid ratio (0.1-0.4 wt%) and orbital agitation (50-180 rpm) in the recovery of ethanol from sugarcane bagasse at different fermentation conditions of moisture content (50-80%) and ethanol amount (5-20 wt%). The highest recovering efficiency was 99,8% at 30°C, initial ethanol amount of 10 wt%, orbital agitation of 100 rpm and moisture content of 60%. The main contribution of this work was to demonstrate that the amount of water used in the extraction is lesser than that used in traditional liquid fermentation, making possible to obtain a more concentrated broth, saving with water treatment and energy.

## 1. INTRODUCTION

Brazil is known as the greatest sugarcane producer in the worldwide, which is the basis for ethanol production. In recent years, the efforts in research and development have been directed toward reducing the input energy and cost for production of bioethanol as the most promising biofuel according Moukamnerd *et al* (2010).

For practical production, the total energy balance throughout the entire process including the pre- and post-fermentation must be considered, because biomass transportation of biomass and waste water treatment, respectively, require much energy, cited by Luo *et al*. (2009). Most of the conventional ethanol production methods involve liquid fermentation and exhaustion of fermentation broth and residue, mentioned by Sree *et al*. (1999), which require large amounts of energy and cost for treatment. In addition, because the water content of fermentation residues is high, a considerable amount of energy is required to dry them before incinerating or recycling them as fertilizers, according to Jain *et al*. (2013).

To reduce the amount of waste water in ethanol fermentation, solid-state fermentation (SSF) is one of the preferable options. In the process of bread dough where the water content is about 50%, it is known that yeast is quite active and produce ethanol and carbon dioxide. The application of solid-state fermentation for ethanol production from biomass, however,

requires the regulation of sugar and ethanol contents in the fermentation mixture below suitable levels because the high osmotic pressure and high ethanol content can decrease the fermentative activity of yeast, like the studies of Moukamnerd *et al*. (2010). In addition, SSF presents potential advantages such as: smaller volumes of fermentation mash, less requirement of water, physical energy requirement, among others.

In recent years, some researches were published focusing the ethanol production by solid-state fermentation. The researchers Lin *et al*. (2013) evaluated the production of ethanol from sugarcane bagasse by simultaneous saccharification and fermentation using a pilot rotary drum reactor (capacity of 100L). These authors demonstrated that the performance and the chosen SSF operating conditions for the drum reactor scale-up was as effective as those attained from flask runs and the use of rotary drum reactor for cellulosic ethanol production under SSF operating conditions is simple to scale up and shows commercial potential. Chu *et al*. (2012) employed solid-state fermentation of enzymatic hydrolysis residue and available to enhance ethanol production and cellulose-to-ethanol conversion. Kwon *et al*. (2011) and Yu *et al*. (2008) studied the ethanol production using sweet sorghum by solid-state fermentation. Moukamnerd *et al*. (2010) developed a consolidated continuous solid-state fermentation system for ethanol production by simultaneous saccharification and fermentation of raw corn starch. Wang *et al*. (2010) developed and validated a model of a rotating drum bioreactor with capacity of 5 $m^3$ for ethanol production by solid-state fermentation. Sree *et al*. (1999) used solid substrate fermentation to produce ethanol from various starchy substrates like sweet sorghum, sweet potato, wheat flour, rice starch, soluble starch and potato starch by simultaneous saccharification and fermentation process.

Although the studies reported above demonstrated that solid-state fermentation can be effective for ethanol production, this kind of fermentation present an inherent difficulty that is the separation of product from solid fermented material. Studies carried out for enzyme recovery demonstrated that depending of experimental condition used in the extraction, significant difference in the results can be obtained by Bender *et al*. ( 2008). Several studies are performed to optimize the extraction process of products of commercial interest (Gharekhani *et al*., 2012; Palmeira *et al*., 2012).Taking into account that the studies reporting the ethanol production did not focus in the extraction process, then the main objective of this work was to investigate the influence of temperature (30-50°C), solid to liquid ratio (0.1-0.4 wt%) and orbital agitation (50-180 rpm) in the recovery of ethanol from sugarcane bagasse at different fermentation conditions of moisture content (50-80%) and ethanol amount (5-20 wt%).

## 2. MATERIAL AND METHODS

## 2.1. Raw Material and Chemicals

In this work was used sugarcane bagasse obtained in a local distillery, dried at 60°C for 72 hours, ground and sieved, collecting the particles that passed through a sieve of 16 mesh. The samples were then stored at room temperature under nitrogen atmosphere prior to the extraction. The ethanol was purchased from Vetec – Brazil with 99.8% purity.

## 2.2. Experimental Procedure for Ethanol Extraction

As the objective of the work was to evaluate the influence of different parameters in the extraction of ethanol from sugarcane bagasse after the fermentation, it is important to study the procedure from a raw material that present known amount of ethanol and moisture content. For this reason, it was decided do not carry out the fermentations because is not possible determine the real amount of ethanol produced in the medium due to loss by evaporation and adsorption in the solid material, leading to errors in the determination of recovery efficiency. To overcome this difficulty, it was simulated a fermentation with different moisture content (ranging from 50 to 80%) and ethanol amount (5-20 wt%), obtaining a solid-material with known moisture and ethanol content, making possible the correct determination of recovery efficiency.

For this purpose, 50 g of dry bagasse were used in each experiment, being corrected the moisture at speci-fied level using deionized water and sterilized at 121°C for 20 min (to simule real fermentation condition). Af-terwards, a determined amount of ethanol was added to the autoclaved solid medium and manually homoge-nized for 10 minutes. The resulting solid material was sealed and maintained under rest for 60 minutes to guarantee a satisfactory adsorption of ethanol into solid bagasse.

After the "simulated fermentation" described above, the solid material was used for ethanol extraction, where a determined amount of deionized water was added aiming to reach the specified solid to liquid ratio, following incubation at determined temperature and orbital agita-tion. The amount of ethanol recovered was assayed in the supernatant after centrifugation at 4°C, 15.000 rpm for 15 min. The recovery of ethanol was evaluated at 0, 2, 4, 6, 8, 10, 15, 30 and 60 minutes of extraction.

Different fermentation conditions such as moisture content (50-80%) and ethanol amount in the fermented bagasse (5-20 wt%), as well as different extraction con-ditions such as temperature (30-50°C), orbital agitation (50-180 rpm) and solid to liquid ratio (0.1-0.4 wt%). The influence of variables was analyzed considering the methodology of one-factor-one-time.

## 2.3. Determination of Ethanol

After the extractions, an aliquot (5 mL) of supernatant was used for direct determination of ethanol content using the Alcolyzer Wine M/WE – Wine Analysis Sys-tem (Antoon Par). The results obtained, expressed in terms of ethanol % v/v, was converted to ethanol wt% and the percent recovery of ethanol determined, taking into account the initial amount added.

## 3. RESULTS AND DISCUSSION

## 3.1. Influence of Fermentation Parameters on Recovery of Ethanol

Table 1 presents the results concerning ethanol re-covering for different moisture contents of fermented sugarcane bagasse. The results are demonstrating that for high moisture content of solids the recovery is more efficient, since 98,9% of ethanol added to bagasse was recovered when the moisture content was 80%, whereas only 87,2% was recovered at moisture content of 50%.

This result is interesting from an industrial viewpoint, since fermentations with high moisture content are preferable for yeast growth, due to the high water activity of solids. In practice, the results obtained here are showing that will be possible to obtain high recovering of ethanol at experimental conditions that present good characteristics for microbial growth. For fixed-bed bioreactors, it is difficult to maintain the moisture content at high level, due to loss by evaporation. However, the use of rotating drum bioreactors can be an effective alternative, due to possibility to replace the water evaporated in the process.

**Table 1 -** Influence of moisture content on recovering of ethanol from bagasse

| Run | Moisture content (%) | Ethanol recovered (%) |
|-----|----------------------|------------------------|
| 1 | 50 | 87,2 |
| 2 | 60 | 90,2 |
| 2 | 70 | 98 |
| 4 | 80 | 98,9 |

Fixed variables: 30°C, solid to liquid ratio of 0.1 wt%, orbital agitation of 100 rpm and initial ethanol amount of 10 wt%.

Other variable important during the recovering the ethanol from fermented sugarcane bagasse is it the amount produced by the microorganism, which can vary greatly in the fermentation, depending of the experimental condition used. In this sense, Table 2 presents the results concerning ethanol recovering at different ethanol amount in the medium. The efficiency of recovering decreased conform the ethanol amount was increasing in the medium. For sake of comparison, the amount recovered when the amount of ethanol added was 5 wt% was around 20% higher than adding 20 wt% was added. The results presented here are indicating that the recovering will be decrease for high production of ethanol in the medium.

**Table 2** - Influence of initial amount of ethanol on recovering from bagasse

| Run | Ethanol amount (%) | Ethanol recovered (%) |
|-----|---------------------|------------------------|
| 1 | 5 | 84,6 |
| 2 | 10 | 77,1 |
| 3 | 15 | 95,4 |
| 4 | 20 | 71,2 |

Fixed variables: 30°C, solid to liquid ratio of 0.1 wt%, orbital agitation of 100 rpm and moisture content of 60%.

## 3.2. Influence of Extraction Parameters on Recovery of Ethanol

Table 3 presents the results that expressing the effect of solid to liquid ratio on ethanol recovering from fermented sugarcane bagasse. Increasing the solid to liquid ratio was verified a significant increase in the amount of ethanol recovered, reaching 94,3% of recovering for solid to liquid ratio of 0.4 wt%. These results are also attractive on an industrial viewpoint since less water is used during the extraction of ethanol, resulting in more concentrated broth, reducing energy cost in the distillation and in the water treatment. The results presented in Table 3 are showing an advantage of SSF that is the possibility to adjust the amount of water to obtain a concentrated broth (Martins et al. 2011). This point should be taking into account in the analysis of economical

feasibility of an industrial plant for ethanol production by solid state fermentation.

**Table 3 -** Influence of solid to liquid ratio on ethanol recovering from bagasse

| Run | Solid to liquid ratio (wt%) | Ethanol recovered (%) |
|-----|------------------------------|------------------------|
| 1 | 0.1 | 29,9 |
| 2 | 0.2 | 57,2 |
| 3 | 0.3 | 77,1 |
| 4 | 0.4 | 94,3 |

Fixed variables: 30°C, initial ethanol amount of 10 wt%, orbital agitation of 100 rpm and moisture content of 60%.

Table 4 presents the results concerning the influence of temperature in the extraction of ethanol from sugarcane bagasse. In a general way, the temperature did not present significant alterations in the amount of ethanol recovered. The lower temperature evaluated (30 °C) presented the higher ethanol recovering, about 84,7%. This result was possible because ethanol is very soluble in water, in a manner that low amount of water is sufficient to solubilize most of ethanol present. Obviously, the solid matrix is complex and part of ethanol stay adsorbed in internal structure, leading to decrease the recovery efficiency. In practice, the result obtained is desirable, because the extraction can be accomplished industrially at room temperature, without the necessity to use energy to adjust the temperature in the extraction.

**Table 4 -** Influence of temperature on ethanol recovering from bagasse.

| Run | Temperature (°C) | Ethanol recovered (%) |
|-----|-------------------|------------------------|
| 1 | 30 | 84,7 |
| 2 | 40 | 42,3 |
| 3 | 50 | 81,0 |

Fixed variables: initial ethanol amount of 10 wt%, orbital agitation of 100 rpm, solid to liquid ratio of 0.3 wt% and moisture content of 60%.

Table 5 presents the results that expressing the effect of orbital agitation on ethanol recovering from fermented sugarcane bagasse. The results demonstrated that no significant alteration in the recovery efficiency was obtained for orbital agitation ranging from 100 to 180 rpm. However, for orbital agitation of 50 rpm, was verified a decreasing in the efficiency around 10%, probably due to evaporation of ethanol during the extraction.

**Table 5 -** Influence of orbital agitation on ethanol recovering from bagasse.

| Run | Orbital agitation (rpm) | Ethanol recovered (%) |
|-----|--------------------------|------------------------|
| 1 | 50 | 87,1 |
| 2 | 100 | 99,8 |
| 3 | 150 | 99,2 |
| 4 | 180 | 97,0 |

Fixed variables: 30°C, initial ethanol amount of 10 wt%, orbital agitation of 100 rpm, solid to liquid ratio of 0.3 wt% and moisture content of 60%.

The better condition for ethanol recovering were the ethanol amount of 10 wt%, solid to liquid ratio of 0.3 wt%, orbital agitation of 100 rpm, moisture content of 60% and temperature of 30°C, in this condition the ethanol recovering was 99,8%. These results can be improved if the orbital agitation and moisture content were increased, as can be observed in the influence of moisture content (Table 1) and the influence of orbital agitation (Table 5).

## 4. CONCLUSIONS

In this work was demonstrated that the extraction process is an important step during the production of ethanol by solid state fermentation. At the optimized condition at 30°C, the initial ethanol amount of 10 wt%, orbital agitation of 100 rpm and moisture content of 60% promoted an ethanol recovery of 99,8%. Even at optimized condition, the recovery of ethanol was not total, demonstrating that is important to define the extraction conditions before the fermentation. Other important results presented here are related to the influence of solid to liquid ratio on the recovering efficiency, where was demonstrated that the amount of water used in the extraction is lesser than that used in traditional liquid fermentation, making possible to obtain a more concentrated broth, saving with water treatment and energy for ethanol concentration.

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