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FED-BATCH LIQUEFACTION OF SUGARCANE FOR ENDOGLUCANASES PRODUCTION

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ABSTRACT

This paper reports on the application of pretreated and liquefied sugarcane bagasse as inducer for endoglucanases production by Aspergillus niger. *Two* fed-batch strategies for solids feeding during the sugarcane bagasse liquefaction were evaluated. *The most favorable liquefaction condition for enzymatic production* was initiated with 8% *w/v* solids loading *and adding* 6, 3 and 3% of fresh solids after *2, 4 and 6 h to reaches a final solids loading of* 20% w/v. *The highest value of enzymatic activity (409.2 ± 7.6 IU/L) was obtained from the cultivation with 6% w/v of solids. The results demonstrate that liquefied bagasse can be used as a cultivation medium in submerged fermentations of* A. niger *and its composition influences the induction of endoglucanases production.*

1. INTRODUCTION

Cellulosic ethanol produced from lignocellulosic biomass is a promising alternative to fossil fuels. However, enzyme cost is one of the major challenges to cellulosic ethanol competitiveness. A recent study demonstrated that use liquefied biomass to fungal cultivation increases the cellulase production significantly (Cunha, 2014). The use of liquefied bagasse as a carbon source reduces the disadvantages of using insoluble carbon substrates in a large-scale process and provides soluble oligosaccharides that are considered the direct inducers of cellulases production. Besides, the use of agro-industrial residue is an interesting strategy for the reduction of cellulases production costs. Thus, the aim of this work was to study different fed-batch strategies for the liquefaction of pretreated sugarcane bagasse and carry out cultivations of *A. niger* using these liquefied materials as inducer of the endoglucanases production.

2. MATERIALS AND METHODS

2.1. Materials

The steam exploded sugarcane bagasse was kindly donated by the Sugarcane Research Center (CTC, Brazil). The steam explosion was conducted at 12 bar for 15 min. The substrate used for liquefaction was milled and sieved in order to obtained particles smaller than 1.4 mm. A cellulase mixture, Cellic CTec2, was kindly provided by Novozymes A/S (Denmark).

2.2. Microorganism

Aspergillus niger wild type A12 strain, from Embrapa Food Technology collection (Rio de Janeiro, Brazil) was maintained at -18°C in a 30% (w/w) glycerol/water solution. The microorganism was activated in potato dextrose agar (PDA) for 5 days at 32 ◦C.

2.3. Liquefaction reactions

The hydrolysis experiments were conducted in a 0.5 L stirred tank reactor equipped with two Elephant Ear impellers at 50^{o}C, under agitation of 500 rpm in a fed batch mode. The reactions were carried out with 20% w/v solid load at 50^{o}C in sodium citrate buffer, 50 mM and pH 4.8. The enzymatic loading was 10 FPU/g_{solid} and the enzyme was completely added into the solution at the beginning of liquefaction according to 20% w/v solid. Samples were withdrawn at regular intervals for quantification of reducing sugars and glucose using the DNS method (Miller, 1959) and enzymatic kit (Glicose Liquiform, Labtest), respectively.

2.4. Submerged fermentation with liquefied sugarcane bagasse

The microorganisms were inoculated at 10^{7} spores/mL in a total volume of 100 mL of liquefied bagasse and nutrient medium prepared according to Cunha et al. (2012). The medium and liquefied were sterilized by autoclaving at 121°C for 15 min and their ratio was varied considering the desired final solids content. For some cultivation conditions, the initial glucose concentration was adjusted to 10.0 g/L. The cultivations were kept in an orbital incubator shaker for 72 h at 200 rpm and 32 ^{o}C. After cultivation, the broth was filtered, centrifuged at 4 ◦C and 12000 rpm for 20 min. The cultivations were carried out in triplicate. Endoglucanase activity was measured with 4% w/v carboxymethylcellulose (CMC) in 50 mM sodium citrate buffer pH 4.8 (Ghose, 1987). One unit of endoglucanase activity was defined as the amount of enzyme that released 1µmol of reducing sugar per min, using the DNS method. All enzymatic analyses were carried out in triplicate and reported as IU/L.

3. RESULTS AND DISCUSSION

3.1. Fed-batch strategies used in the liquefaction of sugarcane bagasse

Two feeding conditions of liquefaction were analyzed and they are described in Table 01. The glucose and reducing sugars (RS) concentrations were monitored over time and the results are shown in Figure 1.

Table 1. Substrate feeding strategies.

Liquefaction	Substrate feeding (% w/v) Time (h)			
01	5 / 0	5 / 2	5 / 4	5 / 6
02	8 / 0	6 / 2	3 / 4	3 / 6

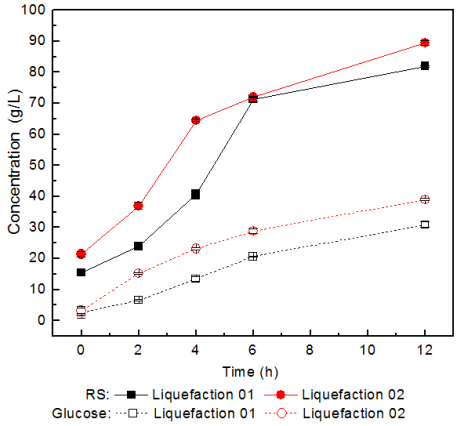

Figure 1. Reducing sugars (RS) and glucose concentrations along liquefaction time.

The Liquefaction 02 resulted in a higher glucose concentration. This difference may be justified by the ratio of enzyme/substrate at the beginning of the reaction. The amount of substrate in contact with enzymes in Liquefaction 02 was bigger than in Liquefaction 01 during the first 4 h. The second feeding condition contributed to a higher conversion to glucose and resulted in a different oligosaccharide profile.

3.2. Endoglucanases production using liquefied sugarcane bagasse

The two liquefied materials were used as soluble carbon substrate in *A. niger* cultivations. Figure 2 shows the endoglucanases activities obtained from cultivations with solids loading varying from 0.2 to 6.0% w/v. The cultivations with 0.2, 2 e 4% w/v were also carried out setting the initial glucose concentration to 10.0 g/L.

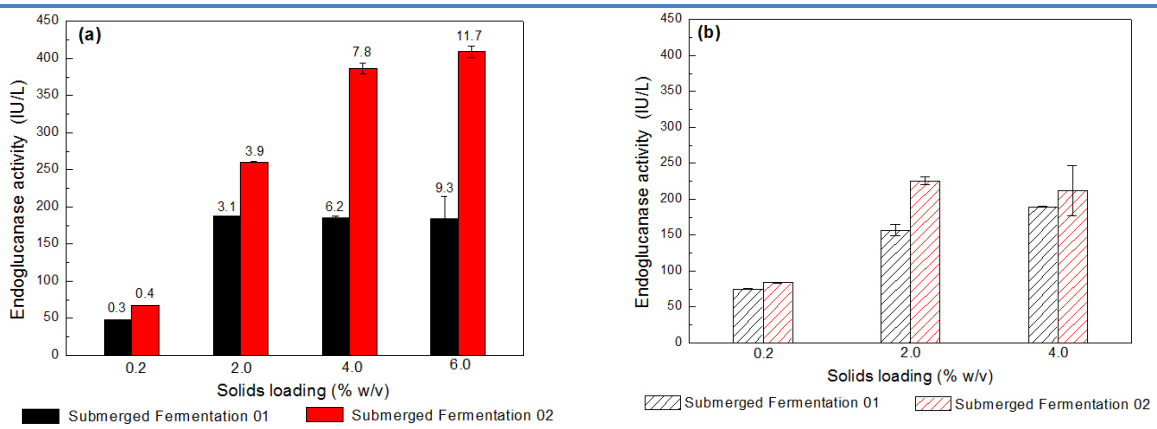

Figure 2. Endoglucanses activities using the liquefied: (a) Cultivations without glucose addition. The numbers above the bars correspond to the initial concentration of glucose in g/L in the medium. (b) Initial glucose concentration fixed in 10.0 g/L.

Endoglucanases production increases with increasing solids content. This trend is justified by the higher concentrations of oligosaccharides, which act as direct inducers of cellulase production, and glucose, the immediate carbon source for cell growth. The liquefied obtained from second feeding strategy (Liquefaction 2) resulted in higher endoglucanases activities and the highest value was 409.2 ± 7.6 IU/L. These results may indicate that the oligosaccharides profile present in this material was more favorable to induce the enzymatic synthesis. Besides, it was verified that by setting the initial glucose concentration at 10.0 g/L did not improve the endoglucanases production.

4. Conclusion

This study demonstrated the potential of using liquefied sugarcane bagasse as inducer of enzyme production. The liquefaction releases soluble saccharides that act as a trigger for further induction resulting in higher production yields. Further studies are needed to optimize the liquefaction conditions in order to obtain a liquefied material with ideal composition.

5. REFERENCES

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