ANAIS

I WORKSHOP DO PROJETO TEMÁTICO FAPESP

Proc.: 08/56246-0

BIOPROCESS SYSTEMS ENGINEERING (BSE) APPLIED TO THE PRODUCTION OF BIOETHANOL FROM SUGARCANE BAGASSE

05 A 07 DE JULHO DE 2010

Departamento de Engenharia Química

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São Carlos - SP

REALIZAÇÃO

Departamento de Engenharia Química – UFSCar

Empresa Brasileira de Pesquisa Agropecuária - EMBRAPA

Projeto financiado pela

FAPESP

APRESENTAÇÃO

Este "I Workshop do Projeto Temático" tem como principal objetivo a apresentação de propostas e de resultados obtidos durante o primeiro ano de desenvolvimento do Projeto Temático: **"Bioprocess Systems Engineering (BSE) Applied to the Production of Bioethanol from Sugarcane Bagasse"**, financiado pela Fundação de Amparo à Pesquisa do Estado de São Paulo – FAPESP (Processo 2008/56246-0), no bojo do programa FAPESP/PRONEX/BIOEN, com vigência de junho de 2009 a julho de 2013. O projeto, proposto conjuntamente pelo Departamento de Engenharia Química da UFSCar e pelo grupo de Bioprocessos da Embrapa Instrumentação Agropecuária, incorpora atualmente colaborações com outros laboratórios e instituições como Instituto de Catálisis y Petroleoquimica (Consejo Superior de Investigaciones Científicas, Espanha), Institute of Resource and Energy Technology (Technishe Universität München, Alemanha), Programa de Engenharia Química da COPPE/UFRJ e do Grupo de Intensificação, Modelagem, Simulação, Controle e Otimização de Processos da UFRGS. O projeto é coordenado pelo Prof. Dr. Roberto de Campos Giordano.

O tema do projeto foi subdividido em **cinco subprojetos interligados**, que buscam promover o conhecimento aprofundado do tema e o desenvolvimento de tecnologia para a produção de bioetanol a partir de bagaço da cana-de-açúcar:

a) Desenvolvimento, implementação e validação de um ambiente computacional integrado amigável, permitindo simulação, otimização, avaliação econômica, análise de CO_2, análise de dados cinéticos e automação de biorreator para processos de produção de etanol lignocelulósico.

b) Cultivos de microrganismos a partir do banco da Embrapa (*Aspergillus sp.*), para a produção de celulases e xilanases usando reatores trifásicos não convencionais, incluindo bagaço pré-tratado no meio.

c) Pré-tratamento físico-químico do bagaço: explosão a vapor, remoção da hemicelulose e deslignificação. Produção de substratos para rotas de produção de bioetanol via fermentação de hexoses.

d) Determinação das condições (sub-)ótimas para a produção de etanol a partir da celulose.

e) Avaliação da produção de etanol a partir da hemicelulose usando enzimas livres e imobilizadas.

CULTIVATION OF *Aspergillus niger* FOR CELLULASE PRODUCTION USING A NON-CONVENTIONAL PROCESS

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The technological advances needed to increase efficiency in the second generation ethanol production are directly related to research and development on enzyme production processes. Currently, the cultivation of filamentous fungi for enzyme production has been mostly performed in conventional stirred tank bioreactors as submerged fermentation (SmF). Nevertheless, the importance of non-conventional reactors, like the airlift bioreactor has grown in recent years due to its high oxygen transfer rates, lack of mechanical seal, lower cost and lower power consumption compared to conventional reactors. In this context, the objective of this work is to evaluate the cultivation of *Aspergillus niger* for the production of cellulase using non-conventional triphasic reactors in the presence of sugarcane bagasse. The main technical-scientific issue of this work is related to the feasibility of obtaining an efficient process for production of cellulases using a system that combines the advantages of solid state fermentation (SSF) and SmF in combined process.

The first step of this work was to evaluate the influence of inoculum preparation for the production of cellulolytic enzymes by *Aspergillus niger*. In order to compare the proposed methodology of using a initial phase of fungi growth as SSF for inoculum preparation, three different inoculums were prepared and the results on fermentation in shaker were compared. The first inoculum was prepared by cultivating the fungi during 24 h in a solid medium (sugar cane bagasse), followed by addition of liquid medium containing 30 $g.L^{-1}$ of glucose (called In 1). The second inoculum was prepared like the first one, but using a liquid medium without glucose (called In 2). Finally, for the third inoculum, fungi was grown directly in a liquid medium containing 30 $g.L^{-1}$ of glucose (called In 3). Liquid medium used in the preparation of all three inoculuns was the basic nutrient medium proposed by Mandels (1976). In this step, it was also evaluated the influence of glucose (10 $g.L^{-1}$) on the medium composition of the fermentation (Mandels medium plus 1% sugar cane bagasse). The experiments were conducted in Erlenmeyer flasks agitated at 200 rpm at 32°C during 96 h. The agent of fermentation was a strain of *Aspergillus niger* Aliquots of 5.0 mL were collected at intervals of 24h and cellulolytic activities were determined in international unit (IU) in the presence of CMC at 50°C (Ghose, 1987) and glucose was analyzed by the DNS method (Miller, 1959).

It was observed that in both fermentations where inoculum was prepared in medium containing 1% w/v of bagasse without glucose (In 2), enzyme production was significantly lower than when the inncolum was prepared in a medium containing glucose (In 1 and 3) (Figure 1). Also, in both fermentation conditions, when the inoculum was prepared with a initial growth phase as SSF (Inoculum 2) resulted in higher enzyme production when compared to the conventional inoculum (In 3). All fermentations containing glucose in the medium (Figure 1A), resulted in higher enzyme production when compared to the ones carried out in a medium without glucose (Figure 1B). There was also a difference in the fungi morphology according to the type of inoculum preparation, cultures whose inoculum were prepared in SSF the fungi grew dispersedly (Figure 2A) and in the conventional inoculums growth was in pellets (Figure 2B). The highest CMCase activity of 1150 $IU.L^{-1}$ was obtained in the experiment with In 2, and medium containing 1% w/v of bagasse and 10 $g.L^{-1}$of glucose. This production was of same order of magnitude of the values obtained by Ahamed and Vermette (2008), whom obtained cellulases activities of 4700 $IU.L^{-1}$ from mixed cultures of *Trichoderma reesei* RUT-C30 and *Aspergillus niger* LMA in fed-batch cultivations utilizing a stirred tank bioreactor.

These conditions will be reproduced in triplicate with the final validation of the results obtained in an 5.0 L airlift bioreactor. The next steps consist in evaluating the influence of bagasse feeding and operational parameters of pneumatic bioreactor such as air flow rate in the production of cellulolytic enzymes by *Aspergillus niger*.

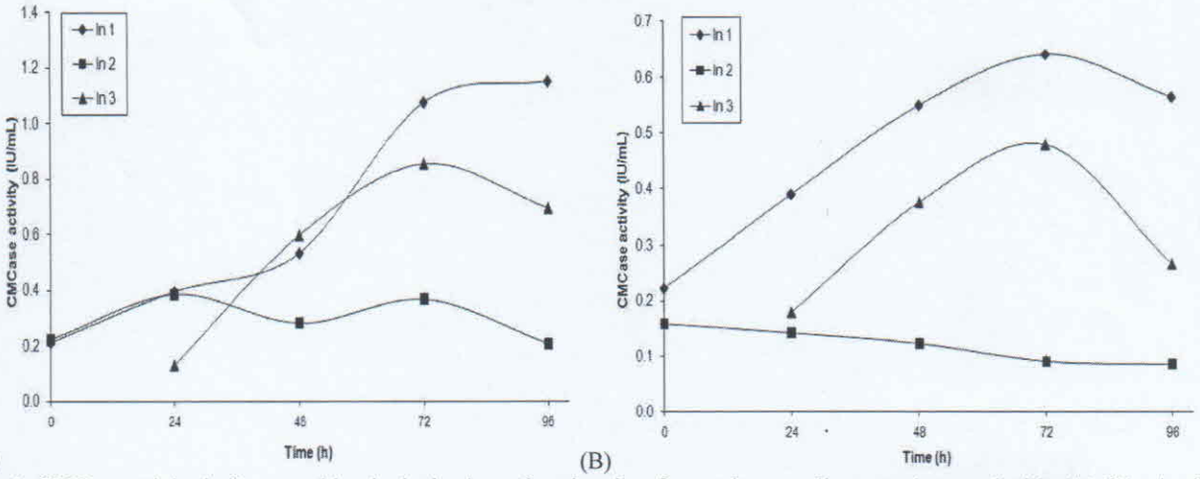

Fig. 1. CMCase activity in fermented broth obtained as a function time from culture medium supplemented with: (A) 1% w/v of sugar cane bagasse and 10 $g.L^{-1}$of glucose and (B) only 1% w/v of sugarcane bagasse. Results are shown for Inoculum1: prepared by cultivating the fungi during 24 h in a solid medium (sugar cane bagasse) in the presence of 30 $g.L^{-1}$ of glucose in added medium; Inoculum2: prepared by cultivating the fungi during 24 h in a solid medium (sugar cane bagasse) without glucose im added medium and Inoculum3: prepared by cultivating the fungi in a liquid medium containing 30 $g.L^{-1}$ glucose.

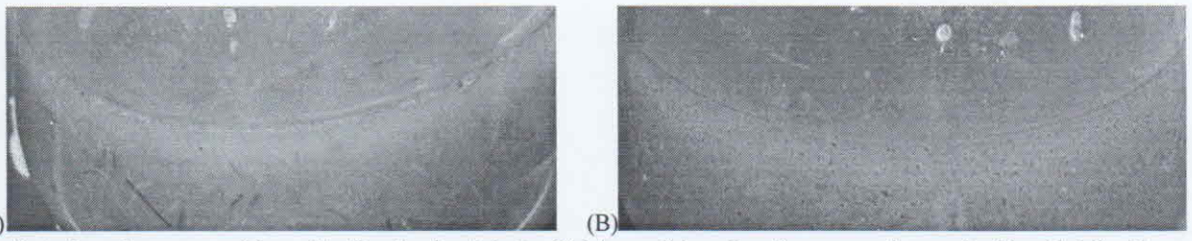

Fig. 2. A- Inoculum prepared by cultivating the fungi during 24 h in a solid medium (sugar cane bagasse) with added liquid medium with 30 $g.L^{-1}$ glucose. B- Inoculum prepared by cultivating fungi in a liquid medium with 30 $g.L^{-1}$ glucose.

References

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