Original Article

CULTIVATION OF *Aspergillus niger* ON SUGARCANE BAGASSE WITH VINASSE

CULTIVO DE Aspergillus niger *EM BAGAÇO DE CANA-DE-ACÚCAR COM VINHAÇA*

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ABSTRACT: Solid-state cultivation (SSC) involves growth of microorganism in absence or near-absence of free water, employing a natural or inert support. Ethanol production in Brazil from molasses or sugarcane juice generates large volumes of vinasse and sugarcane bagasse, a liquid nutrient medium and a potential carry for SSC, respectively. Consecutively to use the wastes, experiments were set up on packed bed column-reactor with sugarcane bagasse impregnated suspension of *Aspergillus niger* and vinasse with 80% moisture, 25°C, aeration flow-rate of 0.4L/min of water-saturated air, for 6 days. The results hint the efficiency of the SSC in this situation, with 1.45g of total acid per g of dry bagasse per day. The purpose is an alternative to right the major residues from sugarcane processing.

KEYWORDS: State-solid cultivation. Waste. Agro-industrial. *Aspergillus niger*.

INTRODUCTION

Solid-state cultivation (SSC) has been defined as the microbial growth occurring on the absence or near-absence of free water, employing a natural or an inert support impregnated with nutrient solution (MITCHELL et al., 1999; PANDEY; SOCCOL; MITCHEL, 2000; GAMARRA et al., 2010; SINGHANIA et al., 2009). In recent years, SSC has shown much promise in development of several bioprocesses due to low investment and energy consumption of agro-industrial wastes as a solid matrix (WU et al., 2010).

Many microorganisms are able to grow on solid substrates, but only filamentous fungi can grow to a significant extension in absence of free water because SSC resembles the natural habitat of these microorganisms (SINGHANIA et al., 2009). Solid-state fermentation has contributed to biotech industries due to its potential applications in the production of biologically active secondary metabolites, apart from feed, fuel, food, industrial chemicals and pharmaceutical products. It has also emerged as an attractive alternative to submerged fermentation (PANDEY; SOCCOL; MITCHEL, 2000; RODRÍGUEZ-COUTO; SANROMÁN, 2006; SINGHANIA et al., 2009).

In SSC, the filamentous fungus *Aspergillus niger* has been used to produce several enzymes and it has become an alternative for citric acid production using agro-industrial residues, such as sugarcane bagasse (KUMAR; JAIN, 2008). Citric acid has been very widely used as an acidifying agent and antioxidant in food, beverages and pharmaceutical industries, usually produced by submerged processes (KUFORIJI; KUBOYE; ODUNFA, 2010; KUMAR et al., 2003).

The use of lignocellulosic materials available in agro-industrial wastes as a source of raw material or solid support for citric acid production is interesting because their renewable nature and abundance (KHOSRAVI-DARANI; ZOGHI, 2008). These processes present several advantages, i.e., low solid waste management, biomass energy conservation, production of high value products and low bacterial contamination (KUMAR; JAIN, 2008; PRADO et al., 2005). The use of agro-industrial residues as support for SSC is an alternative economically important (SANTOS et al., 2008). For example, sugarcane bagasse is generated in large quantities during sugarcane processing and its utilization as a carrier for SSC could allow the production of value added bio-products (KHOSRAVI-DARANI; ZOGHI, 2008).

Brazil is the one of the greatest producers of sugar from sugarcane in the world. In 2004, the country produced approximately 5 billion ton of sugarcane that were used to produce sugar and ethanol. Bagasse is a by-product resulting from juice extraction and consists of water (46-52% w/w), fiber (43-52%w/w including cellulose 50%, hemicelluloses 25% and lignin 25%) and relatively small quantities of soluble solids (2-6% w/w) (PANDEY; SOCCOL; MITCHEL, 2000; KHOSRAVI-DARANI; ZOGHI, 2008; MAZUTTI et al., 2006). Therefore, sugarcane bagasse is produced abundantly in Brazil, it has been generally used as fuel. Another important waste of ethanol

Received: 03/05/11
Accepted: 25/01/12

production, which is generated from distillation processes, is generally known as vinasse. This wastewater is one of the most recalcitrant since it contains the remaining non-volatile organic matter after the fermentation-distillation process through molasses or sugarcane juice. The vinasse treatment reported including mainly anaerobic digestion and concentration, corresponding to waste of nutrients as potassium, nitrogen and organic molecules (FREIRE; CORTEZ, 2000; NAVARRO; SEPÚLVEDA; RUBIO, 2000). Cultivation of *A. niger* on sugarcane bagasse with vinasse could allow the utilization of the main residues from sugarcane processing. However, there are no studies reporting the utilization of vinasse as moistening liquid of supports for SSC.

The aim of this study was to evaluate the cultivation of *A. niger* on sugarcane bagasse moistened with vinasse, improving the findings about this solid-state cultivation and management of wastes from sugarcane processing.

MATERIAL AND METHODS

Microorganism

Inoculum of *Aspergillus niger* CCT 4355 was maintained on 50 mL of the media synthetic in the Laboratory of Applied Microbiology (LABMAC/CCA/UFSCar), according to KUMAR et al., 2003. The media synthetic was prepared with 15-20% sucrose, 0.25%, ammonium nitrate (NH_4NO_3), 0.1%, potassium phosphate (KH_2PO_4), 0.025%, magnesium sulfate ($MgSO_4$) and 0.004%, copper sulfate ($CuSO_4$), pH 4.0 and sterilized at 121ºC for 20 min. Fungi suspension was adapted for seven days under constant agitation (150 rpm) before the SSC experiments on sugarcane bagasse.

Solid support

Bagasse and vinasse were obtained on sugarcane industries from region of Araras/SP, Brazil. Bagasse was classified Tyler sieves, using particles with diameter between 0.59 and 1.17mm. Vinasse was treatment with acid hydrolyzes for availability of sugars (BASTOS et al., 2009). Bagasse particles and vinasse were sterilized at 121ºC for 20 minutes and solid material was moistened with vinasse and inoculums suspension.

Solid-state cultivation

The experiments were set up on packed bed column-reactor with solid support (sugarcane bagasse) impregnated with suspension of *Aspergillus niger* and vinasse at 80% moisture (wet basis), 25 ± 3ºC and air flow-rate 0.4L/min of water-saturated air, according to Figure 1 (BASTOS, 2006).

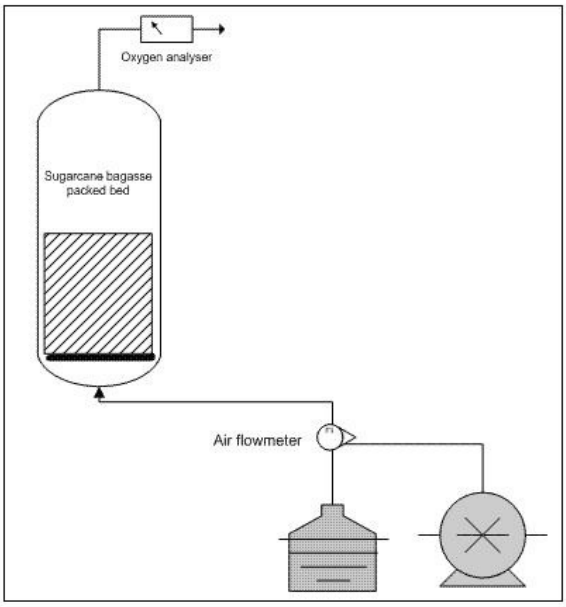

Figure 1. SSC system of *Aspergillus* niger on sugarcane bagasse with vinasse, detailing one packed-bed column.

Each day, one packed-bed column was collected for evaluation. Firstly, solid medium moisture was measured by gravimetric method at 105°C for 24h and pH was determined by potentiometer using 2g of solid medium in 20mL of distilled water (BASTOS, 2006). After this, fungal extract was obtained with distilled water (1g solid for 15mL water), in orbital shaker at 30°C for 24h.

In the fungal extract, total acid and glucose were measured by neutralization method with NaOH (AOAC, 1995) and by enzymatic method glucose oxidase-peroxidase with LABORLAB® KIT (GAMARRA et al., 2010), respectively. Oxygen demand was estimated by overall mass balance on the column (GOWTHAMAN et al., 1995; THIBAULT et al., 2000).

RESULTS AND DISCUSSION

Figure 2 presents the profiles of total acid and glucose from fungal extract. These data showed maximum growth and acid productivity in about 3 days (approximately 1.1 g/g), as previously described by Kumar et al., 2003.

Glucose profile showed a rapid consumption of initial glucose, production by fungal hydrolases and stability after 4 days of cultivation. Sugarcane bagasse is generally used as a fuel because consists of cellulose, hemicelluloses and lignin (KHOSRAVI-DARANI; ZOGHI, 2008). Therefore, solid medium presents low monosaccharide content and *Aspergillus niger* needs to hydrolysis of the structural polysaccharides. Moreover, vinasse presents low content of mono or disaccharides, consisting of high molar organic molecules, potassium and nitrogenous compounds. In this context, the glucose profile represents a steady-state because there is a production due to hydrolytic enzymes activity and consumption by fungi.

Microorganisms present average oxygen demand of 0.24µmol/g.min during the first 6 days of cultivation. From our results of oxygen demand and glucose profile indicate suitable oxygen supply in the packed-bed columns and non-limiting conditions (GOWTHAMAN et al., 1995). In aerobic cultivation, oxygen transfer to the microorganisms is undoubtedly the most important phenomenon to sustain the microbial activity (THIBAULT et al., 2000).

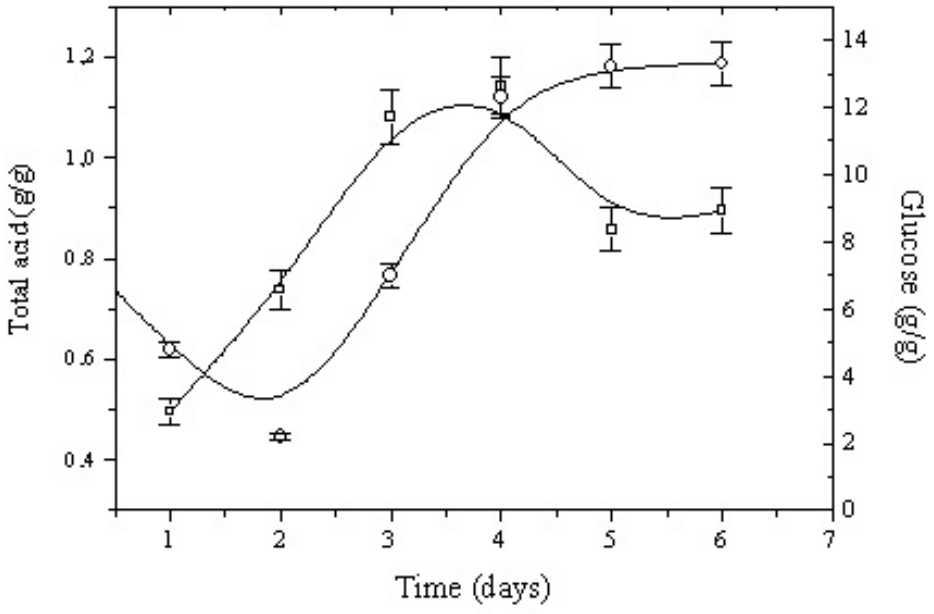

Figure 2. Profiles of total acid (□) and glucose (○) from fungal extract from SSC of *Aspergillus niger* on sugarcane bagasse impregnate with vinasse

Results of pH and moisture medium are shown in Figure 3. Vinasse as moistening agent maintained pH at acid range (about 3.9) and acid production did not considerable change it.

Moisture of solid medium changed between 70 and 80% and there was a slight decreases until the 4th day of cultivation. This reduction could be related to enzymatic hydrolyses during the fungus growth, since the packed bed system was fed with saturated air. In the SSC processes, microorganisms grow within or on the surface of solid particles, which are surrounded by a thin liquid film (THIBAULT et al., 2000). However, the slight increase in moisture does not seem to have influenced the process, nor led a variation of the film liquid thickness. Further studies about water mass balance on this packed-bed would allow a better understanding of the system.

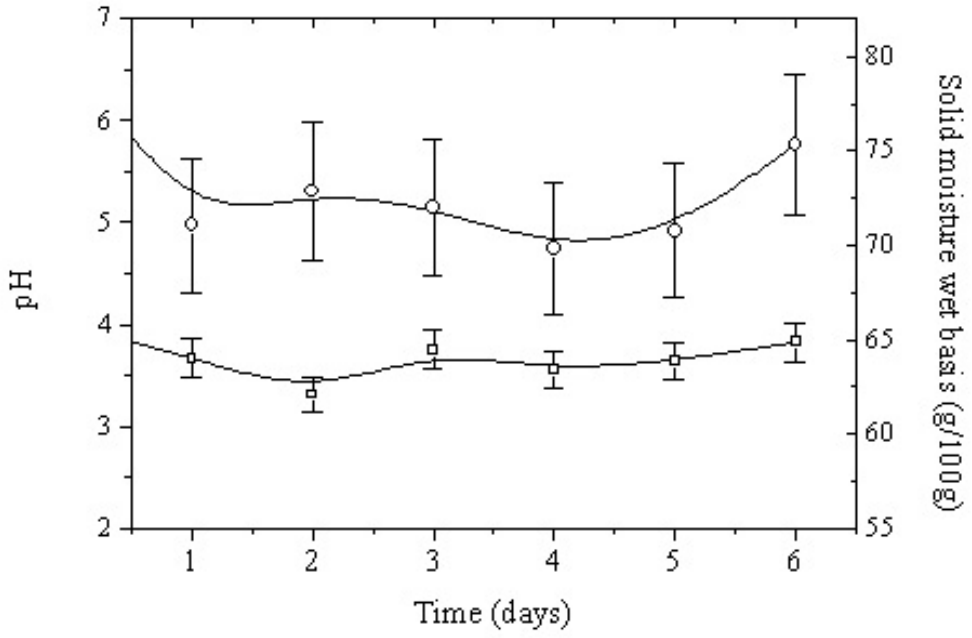

Figure 3. Profiles of pH (□) and solid moisture (○) of solid medium during SSC of *Aspergillus niger* on sugarcane bagasse impregnate with vinasse

Experimental overall productivity of total acid was 1.45g per g of dry bagasse per day. Acid and glucose production, in addition to solid moisture profiles demonstrate the feasibility of using this system to grow *Aspergillus* on SSC with sugarcane bagasse and vinasse. The results also showed the satisfactory use of two wastes from sugarcane processing. This proposed bioprocess could minimize the environmental impact due to the use vinasse from the ethanol production, very common waste products of ethanol production in the South-East region of Brazil.

CONCLUSION

In summary, the data showed that *Aspergillus niger* can use sugarcane bagasse impregnated with vinasse in solid-state cultivation. This system could represent an alternative to conventional submerged processes for obtaining bioproducts from filamentous fungi as *Aspergillus niger.*

ACKNOWLEDGEMENTS

The authors gratefully acknowledge the financial support given by CNPq and CCA/UFSCar, Brazil.

RESUMO: O cultivo em estado sólido (SSC) envolve o crescimento de microrganismos na ausência total ou parcial de água livre, tendo suporte natural ou sintético. A produção de etanol no Brasil por melaço ou caldo de cana-de-açucar gera alta quantidade de vinhaça e bagaço de cana, um meio líquido nutritivo e uma fonte potencial para o SSC, respectivamente. Então o uso de resíduos em experimentos é colocado em reator de coluna com bagaço de cana impregnado com suspensão de Aspergillus niger e vinhaça com 80% de umidade, 25ºC, aeração de 0.4 L/min de ar saturado de água, por 6 dias. Os resultados apontam a eficiência do SSC nestas condições, com 1.45 g do total de ácido por g bagaço seco por dia. A proposta é uma alternativa para empregar os resíduos gerados no processamento da cana-de-açúcar.

PALAVRAS-CHAVE: Cultivo em estado sólido. Resíduos. Agroindustrial. *Aspergillus niger.*

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