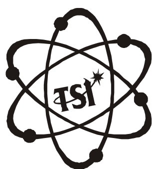
April 2010 ISSN : 0974 - 7427 Volume 4 Issue 1

BioCHEMISTRY

An Indian Journal



Trade Science Inc.

Regular Paper

BCAIJ, 4(1), 2010 [40-43]

A novel way for xylitol bioproduction

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Received: 10th February, 2010 ; Accepted: 20th February, 2010

ABSTRACT

Among the possible biotechnological alternatives to perform xylitol production, the Submerged Fermentation (SmF) method has widely been investigated ultimatelty, using different configurations. However, little is known on the use of the Solid-State Fermentation (SSF) to carry out such a bioprocess. Taking into account the well-known advantages of this mode of operation over the SmF, this study aims at evaluating the potential of Solid-State Fermentation (SSF) for this bioprocess. SSF runs have been done at 30ºC, under oxygen limited conditions, in polypropylene sachets, using sugarcane bagasse as inert support, commercial xylose as substrate and Candida guilliermondii as the fermenting yeast.

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KEYWORDS

Xylitol bioproduction;
Solid-state fermentation;
Sugarcane bagasse;
Residue;
Biotechnology.

INTRODUCTION

Xylitol is an alternative high added-value sweetener of great interest to the food industry and the biomedical sector. It is largely used in the prevention and treatment of several pathologies[1]. Research has been done to produce xylitol by biotechnological route as an alternative to the expensive chemical process of production. An optimized biotechnological process could be a cheaper way to produce xylitol, because it would take place under mild conditions and utilize not purified hemicellulosic hydrolyzates[2].

Currently, the research-work has been focused on the use of microorganisms, especially yeasts, for xylitol production by Submerged Fermentation (SmF) from hemicellulosic hydrolyzates. In this case, the microorganism, through a complex, integrated multi enzymatic system, uses the xylose present in hydrolyzate to get energy and, under certain conditions, allows accumulating xylitol in the fermentation medium. Hydrolyzates from different lignocellulosic residues, among which sugarcane bagasse[3,4] and rice straw[5,6], have been satis-

factorily used as alternative media.

The Solid-State Fermentation (SSF) has recently received special attention because of several advantages, mainly on engineering aspects, among which reduced water activity and formation of gradients of temperature and nutrient and product concentrations. Bioprocessing of agro-industrial residues in SSF has often been found very efficient[7]. Many studies about the application of SSF are focused in adding value to agroindustry residues, which have been extensively used as physical support or source of nutrients in SSF[8]. It differs significantly from SmF, mainly in terms of production of enzymes and secondary metabolites, mixing and diffusion[9].

Even in face of many process advantages as use of low cost residues, higher productivities, low energy requirements, lower wastewater production, extended stability of products and low production costs, little is known on the use of the SSF to carry out xylitol production. This article aims at evaluating SSF potential using an agro-industrial residue (sugarcane bagasse) as an inert support for xylitol production.

MATERIALS AND METHODS

Preparation of the inert support

The sugarcane bagasse was initially ground passing through a 14 mesh (1.41 mm) standard Tyler sieve and retained in a 35 mesh (0.5 mm) sieve. After separation and screening, it was washed with distilled water and dried at 100°C up to constant weight.

Microorganism maintenance and inoculum preparation

Cells of the yeast Candida guilliermondii FTI 20037, belonging to the culture collection of the Department of Biotechnology of the Engineering College of Lorena (University of São Paulo), were maintained at 4°C in a medium containing agar malt extract (Merck, Darmstadt, Germany).

To prepare the inoculum, yeast cells were cultured at 30°C and 200 rpm for 24 h in 250 ml-Erlenmeyer flasks containing 50 ml of a cultivation medium composed of 30 g/l xylose, 3 g/l (NH4)2SO4, 0.1 g/l CaCl2.2H2O and 10% (w/v) rice bran extract, placed in an incubator, model G25-KC (New Brunswick, Edison, NJ). After growth, the cells were recovered by centrifugation, model CU-500 (Damon/IEC, Needham, MA) at 2000 × g for 20 min, washed and resuspended in isotonic solution (de-ionized water) in order to get a highly concentrated cell suspension. To obtain always the same inoculum (1.0 g/l wet weight), cell concentration of this suspension was determined and, then, appropriate aliquots were added to the fermentation medium.

Fermentation conditions

All the fermentations were performed at 30°C in polypropylene sachets inside a greenhouse containing 7 g of sugarcane bagasse embedded with 70 ml of a previously-inoculated fermentation medium composed of 50 g/l xylose, 3 g/l $(NH4)_2SO_4$, 0.1 g/l $CaCl_2.2H_2O$ and 10% (w/v) rice bran extract.

One sachet was taken after every 24 h for analyses. All the experiments were carried out in duplicate, and the standard deviations never exceeded 8%; therefore, no additional statistical analysis was considered to be necessary.

Solid-liquid extraction ("Leaching")

The samples were transferred from the sachets to 250 ml-Erlernmeyers containing 50 ml of distilled water (as extraction solvent) and incubated for 30 min at 30ºC in a greenhouse, model G25 – KC (New Brunswick, Edison, NJ), rotating at 200 rpm. After vacuum filtration through qualitative filter (14 µm-pore diameter), the filtrate was analyzed by HPLC.

Analytical determinations

Cell concentration was determined by optical density (OD) measurements at 640 nm, using a spectrophotometer, model DU 640B (Beckman Coulter, Fullerton, CA). A previously-constructed calibration curve was used to relate the OD measurements to dry cell concentration of samples of both this suspension as well as that used for inoculum.

After sample filtration through Sep Pak C18 filter, xylose and xylitol concentrations were determined by HPLC, model LC-10-AD (Shimadzu, Tokyo, Japan), equipped with an Aminex HPX-87H (300 × 7.8 mm) column (Bio-Rad, Hercules, CA) and a refractive index RID 6A detector, under the following conditions: injection volume of 20 µl, column temperature of 45°C,

0.01 N H_2SO_4 as the mobile phase used at a flow rate of 0.6 ml/min.

The microphotograph of the support surface was obtained using an optical microscope, model N107/T (Coleman, Santo André, SP, Brazil).

Fermentation parameters

The xylitol volumetric productivity (QP, g l-1 h-1) was calculated as the ratio of xylitol concentration at the end of the run (Pf, g/l) to the fermentation time (t, h).

The yield of xylitol on consumed xylose (YP/S, g/g) calculated as the ratio of Pf and the difference between the starting and the final xylose concentrations.

RESULTS AND DISCUSSION

The results of xylose SSF by *C. guilliermondii*, in terms either of xylose consumption or xylitol formation can be seen in Figure 1, while Figure 2 shows the corresponding progressive increase in cell concentration.

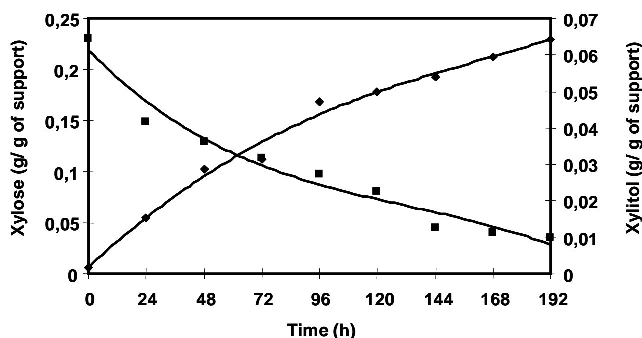

Figure 1 : Time behaviors of xylose consumption and xylitol production during SSF

They are very similar to the results obtained by SmF[10], demonstrating the potential of this promising mode of operation. In special the cell grow behavior, at the beginning of fermentation there was low cell growth, but at the end it accelerated.

The decreasing pH behavior illustrated also in Figure 2 was indeed expected by the fact that cells were metabolically active during the bioprocess and then consumed $(NH_4)_2SO_4$ as the nitrogen source and xylose as the carbon source. It was in fact previously demonstrated that the former activity is responsible for continuously release of protons and the latter for CO_2 development[11].

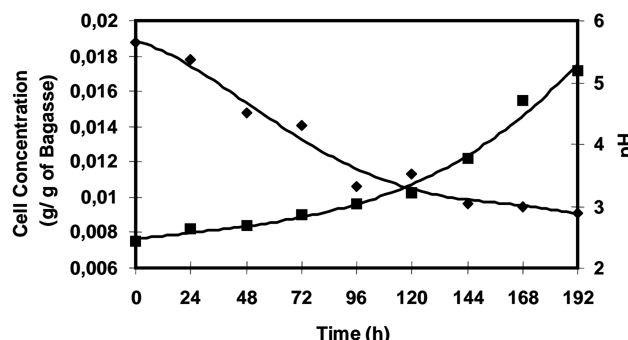

Figure 2 : Time variation of cell concentration and pH during SSF

Preliminary results (not shown) suggest that xylitol production can be increased using a buffer solution that allows SSF to proceed at constant pH. The pH decrease was quicker in this process comparing to the SmF operation. This is probably due to the occurrence of oxygen limited conditions at SSF that stimulated the consumption of both nitrogen and carbon sources. For this reason, futures attempts will be made using external oxygen supply to increase xylitol production by SSF.

Optical microphotographs were done at the end of the runs (192 h) (Figure 3), in order to verify the cell growth during SSF as well as to demonstrate the potential of the sugarcane bagasse as inert support. It can be seen that cell growth was effective, as demonstrated by the uniform cell growth onto the whole surface of bagasse fibers.

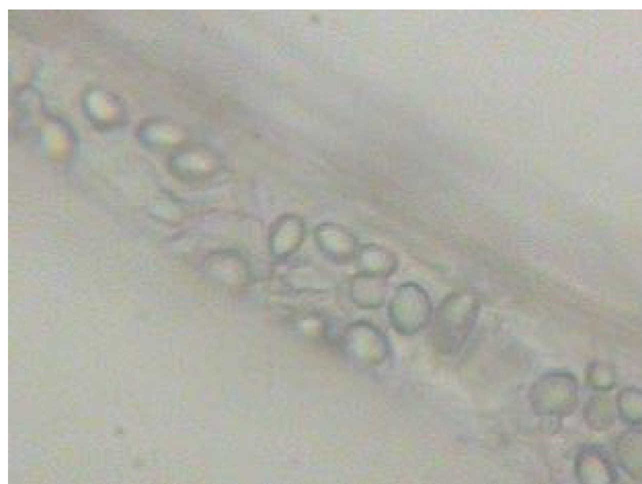

Figure 3 : Optical microphotograph of candida guilliermondii cells (400X) on sugarcane bagasse done after 192h of fermentation

No reliable comparison can be made between the theoretical xylitol yield on consume xylose (YP/S =

0.917 g/g) estimated on the basis of the knowledge on the metabolism of C. guilliermondii[12] and the value obtained in this work (0.338 g/g), because the former was obtained in SmF. Therefore, an alternative way to estimate the theoretical yield for SSF is needed to compare its performance with the one of SmF.

Another fermentation parameter that can be use to make a comparison is the volumetric productivity that reached in this study a value (QP = 0,066 g L^{-1} h^{-1}) one order of magnitude lower than that obtained by our research group for SmF at same initial xylose concentration using cells immobilized in the same support[10]. These results suggest that not only that the SSF process is remarkably slower than the SmF one, but also that the mechanism of cell adhesion could be completely different. As mentioned earlier, the oxygen limited conditions inside the solid material could have increased the consumption of nitrogen and carbon sources, thus acidifying the medium. Therefore, although superficial growth could have been somehow promoted, the anaerobic conditions inside the support could have limited xylitol formation.

CONCLUSIONS

Although xylitol production by SSF using sugarcane bagasse as an immobilizing support is presently unsatisfactory compared to that ensured by SmF, in SSF the cell growth was effective and uniform onto the whole surface of bagasse fiber. This result suggests that the use of this novel methodology could be successful. Experiments using optimized oxygenation conditions to SSF process will be carried out as well as the study of this process in continuous mode.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge the financial support of FAPESP (Fundação de Amparo à Pesquisa do Estado de São Paulo), CAPES (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior) and CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico), Brazil.

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