V SIMPÓSIO DE BIOQUÍMICA E BIOTECNOLOGIA
05 a 07 de agosto de 2015, Londrina – PR

Zymomonas mobilis immobilized on loofa sponge: Ethanol and Levan production by support recycling

Vidiany Aparecida Queiroz Santos[1], Michel da Silva Fonseca[1]; Mário Antônio Alves da Cunha[1], Crispin Humberto Garcia Cruz[2] e Maria Del Carmem Montes Horcasitas[3]

[1]Universidade Tecnológica Federal do Paraná - UTFPR - Departamento de Química - CEP - 85503-390 Pato Branco, Paraná, E-mail: vidianysantos@utfpr.edu.br.
[2]Universidade Estadual Paulista - UNESP - Departamento de Engenharia e Ciência de Alimentos - CEP - 15054-000 São José do Rio Preto, São Paulo.
[3]Centro de Investigação e estudos avançados do IPN - CINVESTAV - Departamento de Biotecnologia e Bioengenharia, Cidade do México, México.

ABSTRACT

The Zymomonas mobilis is considered a high technological potential for the ethanol and levan production, because showed low biomass and high tolerance to ethanol. The aim of this study was to evaluate the levan-ethanol production by Zymomonas mobilis immobilized on loofa sponge by immobilization support recycling. The immobilization was realized directly on the reactor with changes in the design of support fermentation and the fermentation cycles were repeated till the production of levan and ethanol dropped to considerably lower level. It was observed higher ethanol (34.64 g.L^{-1}) and levan (26.40 g.L^{-1}) production during all fermentation cycles. The maximum substrate consume was 45% and the final pH was between 3.3 and 3.5. It was also found that the microorganism remained viable and showed levan and ethanol production until the last recycle day.

Keywords: immobilization; biopolymer; bioethanol.

INTRODUCTION

The *Zymomonas mobilis* has shown a great potential for levan and ethanol production. However, its efficiency may be impaired due to free cells remain highly exposed to adverse conditions during fermentation (BEHERA et al., 2010). Regarding this difficulty, immobilized cells have been intensively investigated during the last years by levan and ethanol production, because offer advantages over conventional cell suspension. Among these advantages include a higher cell concentration in the immobilization support, repeated batch operations and an improvement in the process efficiency and productivity (GENISHEVA et al., 2011). Several supports for cell immobilization has been evaluated and showed good results, such as alginate rice husk, sugarcane or sorgun bagasse, wheat; corn cobs, and loofa sponge (KANDYLIS et al., 2010; BEHERA et al., 2010; YAO et al., 2011).

Loofa sponge is a natural and fibrous material basically composed of cellulose, hemicellulose and lignin forming an interlaced structure with high porosity. In front of these characteristics, loofa sponge has been studied as immobilization support by production of different compounds (OGBONNA et al., 1994; PHISALAPHONG et al., 2007). Therefore the aim of this study was to evaluate the levan-ethanol production by *Zymomonas mobilis* immobilized on loofa sponge by immobilization support recycling.

MATERIAL AND METHODS

Microorganism and culture conditions

Zymomonas mobilis CDBB-603 was obtained from the National Collection of Microbial Cultures CINVESTAV-IPN-MEXICO. The culture was grown and maintained in liquid medium containing in $g.L^{-1}$: 10.0 yeast extract, 10.0 peptone and 20.0 glucose. For the inoculum cultivation, used in all experiments, it was inoculated 10% (w/v) of seed material into flasks containing the same medium and incubated at 30°C in orbital shaker (100 rpm) during 24 hours. The fermentation medium contained: yeast extract 5.0 $g.L^{-1}$, KH2PO4 1.0 $g.L^{-1}$, (NH4)2SO4 1.0 $g.L^{-1}$ and MgSO4. 7 H2O 1.0 $g.L^{-1}$, with initial pH 4.95.

Immobilization and Fermentation

The immobilization was realized directly on the reactor with changes in the design of support fermentation. The immobilization support was attached to a stainless steel wire to prevent pieces of loofa sponge interrupt the agitation medium. It was used the loofa sponge concentration cited before but the immobilization medium was similar at the fermentation medium with addiction of glucose 100 $g.L^{-1}$. Then the reactor was inoculated with seed 10% (w/v) and incubation during 24 hours at 30ºC and 200 rpm agitation. After the medium was removed washed with distilled water and added fresh fermentation medium. The bioreactor used in this study was in-house-designed with capacity of 500 mL jar. reactor and 6 cm in diameter and 16 in height, containing 200 mL of fermentation medium without pH control, and 200 rpm only for medium homogenization; without oxygenation and at 30°C. At the end of each fermentation cycle the medium was removed of reactor and then transferred a similar volume of fresh medium for a next cycle of cultivation. The cycles were repeated till the production of levan and ethanol dropped to considerably lower level. All experiments were carried out without pH control.

Analytical methods

The cells were removed by centrifugation at 12.000 rpm and 4ºC during 10 minutes and the supernatant was used for levan, ethanol and reducing sugar analysis. The ethanol concentration was measured by gas chromatography. Reducing sugars were measured by dinitrosalicylic acid method by previously hydrolysis of sucrose with hydrocloridric acid (HCl) concentrated solution for 9 minutes in boiling water and neutralization with sodium hydroxide (NaOH) 40% solution. The cell growth in the support was determined by optical density at 600 nm. The levan concentration was determined as fructose units according Borsari et al. (2006) with adaptations. All determinations were made in triplicate.

RESULTS AND DISCUSSION

The support reusing fermentation was performed in reactor (200 mL) during 10 days. The condition used was: sucrose 250 $g.L^{-1}$; fermentation medium without pH control at 30 °C and agitation of 200 rpm for homogenization of the medium, but without oxygenation.

The immobilized cells on loofa sponge showed good levan and ethanol production, during 10 fermentation cycles reusing the support. According showed in table 1, there was an increase in ethanol production until the 8^{th} cycle with maximum of 34.64 $g.L^{-1}$ and yield 0.32 $g.g^{-1}$ (Table 1). These results were higher to those observed by Chandel et al. (2009) using *Saccharomyces cerevisiae* V3 immobilized on bagasse and obtained 22.85 $g.L^{-1}$ and 0.44 $g.g^{-1}$ ethanol yield also after eight cycles of reuse.

Table 1. Fermentation parameters by *Zymomonas mobilis* CDBB-603 immobilized on loofa sponge during ten fermentation cycles.

Batch cycle	pH	SC (%)	EP ($g.L^{-1}$)	EY ($g.g^{-1}$)	LP ($g.L^{-1}$)	LY ($g.g^{-1}$)
1	3.43±0,07	45.43±0,18	29.71±0,38	0.26±0,81	16.04±0,79	0.14±0,09
2	3.59±0,12	37.69±0,24	18.52±0,54	0.19±0,06	24.27±1,60	0.25±0,10
3	3.39±0,17	25.22±0,14	20.94±1,32	0.33±0,13	26.07±1,35	0.41±0,76
4	3.40±0,00	27.69±0,01	23.15±0,78	0.33±0,13	18.28±0,23	0.26±0,33
5	3.45±0,16	28.27±0,48	27.87±0,83	0.39±0,03	26.40±2,13	0.37±0,51
6	3.33±0,12	27.22±0,11	23.19±0,41	0.34±0,18	24.96±0,71	0.36±0,57
7	3.33±0,12	41.57±0,29	28.77±1,68	0.27±0,54	22.02±1,40	0.21±0,80
8	3.46±0,05	43.03±0,04	34.64±1,14	0.32±0,79	25.01±1,26	0.23±0,62
9	3.46±0,05	36.11±0,18	32.20±0,28	0.35±0,99	24.27±0,38	0.26±0,93
10	3.51±0,24	45.22±0,02	33.70±0,67	0.29±0,64	24.36±0,02	0.21±0,19

SC: Substrate consume; EP: Ethanol production; EY: Ethanol yield; LP: Levan production; LY: Levan yield.

The levan production during ten cycles remained almost constant with reduction only in the second and fourth cycles. However, the fifth cycle showed the highest production values with 26.4 $g.L^{-1}$ and yield was 0.37 $g.g^{-1}$ (Table 1). Better results were obtained by Shih et al. (2010) using *Bacillus subtilis* immobilized in alginate beads and found high production in the third cycle (70.6 $g.L^{-1}$) with initial pH control, supplementation of medium with organic nitrogen during the five reuse cycles each with 72 hours. These authors observed that the beads remained stable during the five cycles of fermentation.

The low yield observed for ethanol and levan production could be due to reduced rate of substrate conversion during the 24 hours of fermentation, where the maximum consumption of 45.43% was obtained in the first cycle and the eighth 43.03% (table 1). The latter was the best for the ethanol production and productivity. This low rate of substrate conversion was possibly affected by the initial pH of medium, which was not controlled before of starting fermentation (4.95) and without control during the process. Due this initial pH (4.95) may have occurred a rapid reduction of culture medium pH. Below 3.5 the pH is inhibitory factor for the growth and production for *Zymomonas mobilis.*

This factor may lead to decrease of initial pH of the fresh medium and thus affect the consumption of substrate and production. With this low initial pH, the microorganism can be inhibited by high acidity generated, which would explain the low conversion rates observed

during the 3 to the 7 cycle (table 1). The others recycles, 8, 9 and 10 showed a higher rate, possibly due to the death of microorganisms in the support, and this could occur with reduction in acidity of the medium during the last recycles.

Immobilized cell at the end of ten fermentation cycles was 0.27 $g.L^{-1}$ and free biomass of each cycle was between 0.62 and 2.42 $g.L^{-1}$. These results show that there was low fixation of bacteria in the support, which may have been caused by two factors. Firstly due to low affinity of the strain studied with the support, because not used any pretreatment to enhance adhesion ability. And yet, due the microorganism do not be flocculant and therefore the ability of adhesion to the substrate is restricted.

On the other hand, high growth of free biomass on the medium was observed for all fermentation cycles indicating that immobilization by adsorption on solid surfaces occurs naturally with detachment of immobilized cells to grow medium which help in production, leading to a mixed culture.

CONCLUSION

The immobilization of *Zymomonas mobilis* CDBB 603 in loofa sponge was not very efficient due to the low affinity of the organism for the support and the not flocculant capacity, but there was good ethanol and levan production. It was also found that the bacterial remained viable until the last day to recycle. Therefore, the not flocculant bacteria can be used immobilized, requiring only adjustments to the support to increase adhesion.

Financial support: Capes

REFERENCES

BEHERA, S.; RAY, R.C.; MONHANTY, R.C. Comparative study of bioethanol production from mahula (*Madhuca latifolia*) flowers by immobilized cells of *Saccharomyces cerevisiae* and *Zymomonas mobilis* in calcium alginate beads. **Journal of Science Industrial Researce**, v.69, p. 472-475, 2010.

BORSARI, R.R.J.; CELLIOGI, M.A.P.C.; BUZATO, J.B.; SILVA, R.S.S.F.Influence of carbon source and the fermentation process on levan production by *Zymomonas mobilis* analyzed by the surface response method. **Science Food Technology**, v. 26, p.604-609, 2006.

CHANDEL, A.K.; NARASUB, M.L.; CHANDRASEKHAR, G.A.; MANIKYAMA VENKATESWAR, L.R. Use of *Saccharum spontaneum* (wild sugarcane) as biomaterial for cell immobilization and modulated ethanol production by thermotolerant *Saccharomyces cerevisiae* VS3. **Bioresource Technology**, v. 100, p. 2404-2410, 2009.

GENISHEVA, Z.; MUSSATTO, S.I.; OLIVEIRA, J.M.; TEIXEIRA, J.A. Evaluating the potential of wine-making residues and corn cobs as support materials for cell immobilization for ethanol production. **Industrial Crop Production**, v. 34, p. 979-985, 2011.

KANDYLIS, P.; DROUZA, C.; BEKATOROU, A.; KOUTINAS, A. Scale-up of extremely low temperature fermentations of grape must by wheat supported yeast cells. **Bioresource Technology**, v. 101, p.7484-7491, 2010.

MILLER, G.L. Use of Dinitrosalicylic acid reagent for determination of reducing sugar. **Analytical Chemistry,** v, 31, n. 3, p. 426-428, 1959.

OGBONNA, J.C.; LIU, Y.C.; LIU, Y.K.; TANAKA, H. Loofa (*Luffa cylindrica*) sponge as carrier for microbial cell immobilization. **Journal Fermentation Bioengineering** 78: 437-442, 1994.

PHISALAPHONG,M.; BUDIRAHARJ, R.; BANGRAK, P.; MONGKOLKAJIT, J.; LIMTONG, S. Alginate-loofa as carrier matrix for ethanol production. **Journal Bioscience Bioengineering**, v.104, p.214-217, 2007.

SHIH, I.L.; CHEN, L.D.; WU, J.Y. Levan production using *Bacillus subtilis* natto cells immobilized on alginate. **Carbohydrate Polymers**. v. 82, p.111-117, 2010**.**

YAO, W.; WU, X.; ZHU, J.; SUN, B.; ZHANG, Y.Y.; MILLER, C. Bacterial cellulose membrane - A new support carrier for yeast immobilization for ethanol fermentation. **Process Biochemistry**, v. 46, p. 2054-2058, 2011.