Vol.49, n. 3 : pp. 371-374, May 2006
ISSN 1516-8913 Printed in Brazil

BRAZILIAN ARCHIVES OF
BIOLOGY AND TECHNOLOGY
A N I N T E R N A T I O N A L J O U R N A L

Influence of Ultrasound on Sorbitol Release by *Zymomonas mobilis* Grown on High Sucrose Concentration

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ABSTRACT

This work investigated the effect of applying low intensity ultrasound on sorbitol release by Z.mobilis cultures grown on 200 g/L sucrose medium up to 48 h. The best sorbitol production was 36.09 g/L in 36 h culture. Ultrasound irradiation did not alter the sorbitol values detected after disrupting the cells with 20 minutes treatment.

Key words: *Zymomonas mobilis;* sorbitol, ultrasound

INTRODUCTION

Sorbitol is an alcohol sugar found in nature at high concentrations in various fruits and at lower concentrations in some algae and has applications in confectionary, chewing gums, candy, desserts, ice cream, diabetic foods and a wide range of food products. It is also used in pharmaceutical products, sorbose, ascorbic acid, propylene glycol, synthetic pasticizers and alkyd resins among others (Silveira and Jonas, 2002).

Zymomonas mobilis a gram-negative bacterium discovered in the late 1970s as the fastest and most efficient ethanol producer using sucrose, glucose or fructose as sole carbon and energy sources, has commercial potential for the production of other economically valuable by-products including sorbitol (Doelle et al. 1993).

Sorbitol production by the *Z. mobilis* occurs through the action of the glucose-fructose-oxidoreductase enzyme (GFOR) present in the bacterium periplasm that simultaneously converts mixtures of glucose and fructose into gluconolactone and sorbitol, respectively Sprenger (1996). Silveira and Jonas (2002) reported that the oxidoreductase enzyme presents an important physiological function for the osmotic regulation of the cell when grown in nutrient medium on high sugar concentrations. The enzyme produces sorbitol which works as compatible solute as its accumulation is linked to high sugar concentration outside the cell. Concomitantly with sorbitol accumulation, an exponential phase of cellular growth was observed by Loos et al. (1994) in their studies using high glucose and other sugar concentrations.

As sorbitol accumulated is excreted from the cell, an alteration in the cell membrane permeability may improve the release of this product. The application of low intensity ultrasound could interfere in the membrane permeability accelerating the transfer of substances and promoting cell growth and propagation. This

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indicates that ultrasound has a promising application in fermentation engineering (Lanchun et al. 2003).

Products of economic interest, such as proteins, enzymes, polyols and others, when stored inside the cells, lead to reduced production efficiency. Sinisterra (1992) reported that application of ultrasound irradiation to microorganisms caused cell damage or rupture, due to transitory acoustic cavitation or create micro currents that favored mass transfer through the membrane/cell wall by the stable cavitation. The use of ultrasound to release biomolecules has been reported by various authors with biotechnological application (Vargas et al. 2004; Levinia et al. 2000; Mason et al. 1996 and 2000).

The objective of this study was to investigate the influence of culture time on sorbitol production by *Z. mobilis* in high sugar concentration, and the effect of ultrasound on the release of sorbitol to the culture medium.

MATERIALS AND METHODS

Microorganism and Fermentation

The microorganism used in this study was the *Zymomonas mobilis* ATCC29191 preserved in medium containing (g/L): glucose 100; yeast extract 10; $(NH_4)_2SO_4$ 2; KH_2PO_4 3; $MgSO_4.H_2O$ 0.3; peptone 0.5 and $FeSO_4$ 0.2. Medium for fermentation was 200 g/L sucrose supplemented with salts above mentioned.Batch fermentations were carried out in 250 mL erlenmeyer flasks with 50 mL fermentation medium at 30°C without agitation for times 12, 18, 24, 36 and 48 h. The inoculum was standardized at 0.2 g/L. The process was monitored by quantifying the biomass, sugar consumption and sorbitol production

Reducing sugars (RS) and total reducing sugars (TRS) were quantified by the cupro-arsenate method of the Somogy (1945) and Nelson (1944). Total reducing sugars were determined after hydrolysis by 0.1N HCl according to Amorim (1982). Glucose was used as the standard sugar. Biomass was determined after centrifugation of the fermentation medium. The cells were re-suspended in NaCl solution at 0.9% (w/v) and the optical density was read at 605 nm. Sorbitol concentration was analyzed by high pressure liquid chromatography (HPLC) with a refractive index detector and HPX aminex column (8 um, 300 x 7.8 mm), at 55°C using ultra pure water as eluent at a flow rate of 1mL/min.

Culture ultrasound irradiation

After defining the best sorbitol production time, ultrasound irradiation was applied using an ultrasonic Processor 20 kHz, model GE 130 PB/70W (Sonics and Materials) equipped with a 9.5 mm diameter probe-type wave guide. Acoustic power was manually controlled below 40 W, and the waveguide probe immersed at a depth of 2cm in 50 mL of culture medium with continuous irradiation. After irradiation for different periods (0, 10, 20 and 30 minutes) at amplitude 40 W, the samples were centrifuged at 8000 g for 20 min under refrigeration. The sorbitol content was determined in the supernatant. Cultures without treatment were used as control in the experiments (time 0).The irradiated and control cells were stained by Gram and observed by an optical microscope Nikon, model Labophot 2H III.

Statistical analysis of the results

The means of the sorbitol results and the ultrasound effect were compared by the Tukey test at level of 5% probability.

RESULTS AND DISCUSSION

The influence of culture time on sorbitol production and the cell growth in the different periods was analyzed and the results shown in Table 1.

As shown in Table 1, there was a significant increase in cell production up to 24 h but after 24 h no increase in the cell concentrations were observed. Highest sorbitol production occurred in the 36 h culture period with a production of 38.09 g/L sorbitol, presenting a productivity of 1.34 $g.L.h^{-1}$ that was not statistically different from the 24 h time period. In the 48 h fermentation period, there was a significant drop in sorbitol production and the value of 20.22 g/L did not differ statistically from that of 18 h.

Table 1 - Total reducing sugar (TRS), Sugar consumption, sorbitol production and cell growth by *Zymomonas mobilis* at different fermentation times.

Time(h)	TRS (g/L)	Consumption (g/L)	Sorbitol (g/L)	Cells (g/L)
0	229.90	0	0	0.2
12	158.50	71.40	11.36[c]	0.15[c]
18	137.46	92.45	24.27[b]	0.65[b]
24	123.93	105.98	36.98[a]	1.60[a]
36	84.25	145.65	38.09[a]	1.64[a]
48	35.41	198.76	20.22[b]	1.65[a]

Means with same letters not differentiate at 0.05 significance

Martinez et al. (1998) assessed sorbitol and glycolic acid synthesis by the GFOR enzyme of *Z. mobilis* and concluded that the time at which the enzyme presented most conversion activity of the substrate to sorbitol was close to 36 h and that beyond this period the enzyme activity remained constant. Barros et al. (2002) also studied the *Z.mobilis* grown on 200 g/L sucrose medium treated with and without yeast invertase at different time periods and also obtained the best sorbitol production in the 36 h in both media. Cells growth was highest at 24 h but after this time the growth ceased.

The effect of time on the sorbitol production by *Z. mobilis* is shown in Fig. 1.

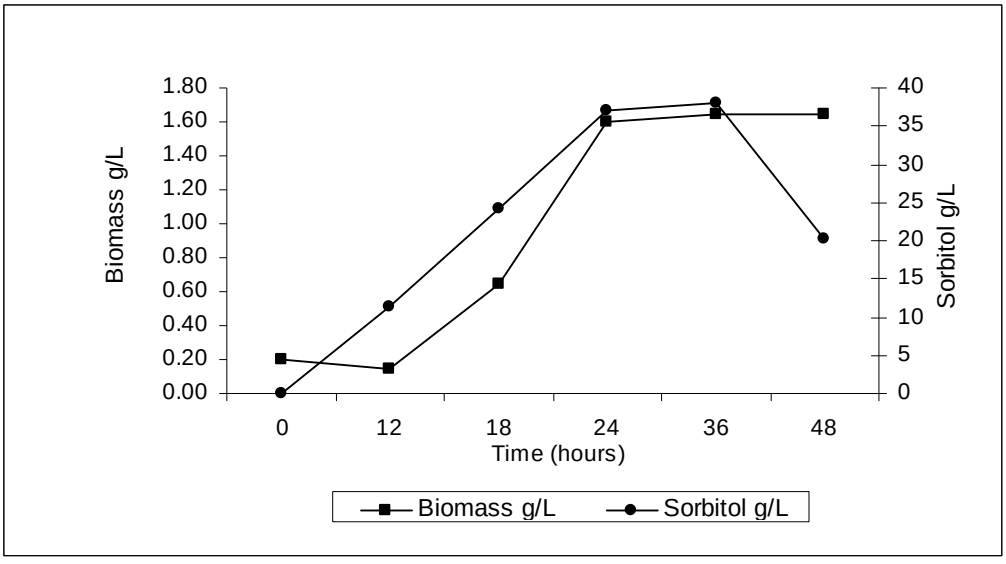

Figure 1 - Biomass and sorbitol production by *Zymomonas mobilis* at different fermentation times.

To verify whether all the sorbitol produced by *Z. mobilis* was released into the extracelular medium, the 36-h culture with the highest sorbitol production was subjected to ultrasound irradiation for different time. Apparently there was no statistically significant differences in the concentration of sorbitol present in the medium after applying ultrasound. This to the conclusion that all the sorbitol produced and that accumulated intracellularly was excreted in the medium after the 36 h culture period. The results are presented in Table 2.

Table 2 - Effect of sonication time on the sorbitol release by *Zymomonas mobilis*.

Sonication time (min)	Sorbitol (g/L)
0	38.09[a]
10	38.38[a]
20	39.30[a]
30	37.70[a]

Means with same letters not differentiate at 0.05 significance

RESUMO

A bactéria *Zymomonas mobilis* produtora de etanol, produz também vários subprodutos quando crescida em meio de sacarose, entre esses o sorbitol. O sorbitol é produzido pela enzima glicose-frutose oxidorredutase (GFOR) presente no periplasma da bactéria, a função fisiológica da enzima é estabelecer a regulação do equilíbrio osmótico, quando a célula é crescida em meio com altas concentrações de açucares. A enzima produz sorbitol e este é acumulado, como um soluto compatível à alta concentração de açúcar fora da célula. Este trabalho investigou efeito da aplicação de ultra-som de baixa intensidade na liberação de sorbitol de células de *Zymomonas mobilis* crescida em meio com sacarose a 200 g/L até 48 h de fermentação. A melhor produção de sorbitol foi de 36,09 g/L em 36 h de cultivo. A irradiação ultra-sônica não alterou os valores de sorbitol detectados e o ultra-som levou ao rompimento das células após 20 min de tratamento.

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Received: September 09, 2004;
Revised: January 13, 2005;
Accepted: February 03, 2006.