Vol. 52, n. 1 : pp. 153-155, January-February 2009
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 the Accumulation of Phosphate and Magnesium Ions in the Yeast Cells on the Ethanol Productivity in Batch Ethanol Fermentation

Rafael Almud Villen [*], Walter Borzani "*In Memoriam*" and Antonio Sacco Netto
[1]*Instituto Mauá de Tecnologia; Centro de Pesquisas; Praça Mauá 1; 09580-900; São Caetano do Sul - SP - Brasil*

ABSTRACT

The accumulation of phosphate and magnesium in the yeast cells is not necessary to assure the ethanol productivity of batch ethanol fermentations. To avoid the decrease of the ethanol productivity it was sufficient to use a fermentation medium containing calculated concentrations of phosphorus and magnesium sources in order to maintain practically constant the phosphorus and magnesium initial contents of the biomass during the fermentation.

Key words: Batch ethanol fermentation, accumulation of phosphate and magnesium, ethanol productivity

INTRODUCTION

Several papers have been published reporting the results of studies related to the accumulation of phosphate and magnesium ions in yeast cells (e.g. Knotkova and Kotyk, 1981; Booth and Guidotti, 1997; Walker and Maynard, 1997; Pestor et al., 2003). No information was found, however, about the influence of such accumulation on the ethanol productivity of ethanol fermentation processes. The aim of this work is to study the above influence in the particular case of batch ethanol fermentation.

MATERIALS AND METHODS

Bakers' compressed yeast (*Saccharomyces cerevisiae*) was used as inoculum. The fermentation media were prepared dissolving D-glucose (120-140 g/L), KH_2PO_4 (6.0 g/L), urea (2.5 g/L), yeast extract (2.5 g/L) and $MgSO_4.7H_2O$ (1.3 g/L) in distilled water. Other fermentation media (see Table 2) containing lower concentrations of KH_2PO_4 and $MgSO_4.7H_2O$ were also used.

The tests were carried out in 15-L Biolafitte fermentor under the following experimental conditions: initial volume of inoculated medium, 12.0 L; volume of antifoam (silicone), 0.5 mL; initial yeast cells concentration (dry matter), 10-13 g/L; initial pH, 4.5; temperature, 32.0±0.5°C; impeller speed, 300 min^{-1}; no air was supplied. Some tests were carried out in unstirred 250-mL Erlenmeyer flasks containing 125 mL of inoculated medium.

Prior to the analytical determinations, a fermenting medium sample (400 mL) was filtered (membrane pores diameter, 1.2 μm) in order to obtain the

[*] Author for correspondence

medium aqueous phase and the biomass. The concentrations of glucose, ethanol, phosphorus and magnesium were measured in the aqueous phase. The biomass was washed with distilled water (250 mL), dried (105°C; 4 h) and then used to measure its contents of phosphorus and magnesium. Glucose and ethanol concentrations were determined by the dinitrosalicylic acid (Miller, 1959) and dichromate (Joslyn, 1970) methods, respectively. Gravimetric methods (Cunniff, 1995) were used to measure the concentrations of phosphorus and magnesium in the aqueous phase and in the biomass.

The biomass concentrations (dry matter) were measured as by Borzani and Jurkiewicz (1998).

RESULTS AND DISCUSSION

Table 1, presenting the results obtained in a typical test, show that both phosphorus and magnesium accumulated in the biomass: at t = 2 h, the phosphorus and magnesium contents of the biomass were, respectively, 145% and 75% higher than the corresponding initial values; at fermentation completion the above percentages were 117% and 65%, respectively.

Several batch tests were then carried out using the fermentation media whose compositions are presented in Table 2.

Table1 - Results of a batch fermentation experiment.

T (h)	X (g/L)	S (g/L)	E (g/L)	C_P (g/L)	C_M (g/L)	α_P (%)	α_M (%)
0	12.6	140.6	0	1.41	0.140	1.06	0.20
0.5	13.0	131.2	3.5	1.26	0.131	--	--
1.0	13.9	115.0	11.2	1.18	0.123	2.41	0.29
1.5	15.2	95.6	16.4	1.13	0.114	--	--
2.0	17.1	74.1	25.7	1.08	0.097	2.60	0.35
2.5	18.7	50.6	34.8	1.06	0.091	--	--
3.0	20.7	31.9	44.4	1.03	0.086	2.41	0.34
3.5	21.7	12.5	51.9	1.03	0.082	--	--
4.0	22.4	0	54.4	1.02	0.080	2.30	0.33

t = time; X = biomass concentration (dry matter); S = glucose concentration; E = ethanol concentration; C_P = phosphorus concentration; C_M = magnesium concentration; α_P = phosphorus content of the biomass; α_M = magnesium content of the biomass.

Table 2 - Fermentation media compositions.

Medium N°	Concentrations (g/L)				
	G	U	Y	P	M
1	130	2.50	2.50	6.00	1.30
2	130	2.50	2.50	3.00	0.65
3	130	2.50	2.50	0.51	0.23
4	130	2.50	2.50	0.26	0.12
5	130	2.50	2.50	0	0

G = glucose. U= urea. Y = yeast extract.
P = KH_2PO_4. M = $MgSO_4.7H_2O$

It must be pointed out that the initial concentrations of KH_2PO_4 and $MgSO_4.7H_2O$ of medium n° 3 were calculated taking account of the biomass growth, in order to maintain the phosphorus and the magnesium contents of the yeast cells practically constant during the experiments. The results of the above experiments (see Table 3) clearly show that the ethanol productivity decreased only when the initial values C_P and C_M were not sufficient to maintain practically constant the initial values of α_P (1.09%) and α_M (0.19%) (media n° 4 and 5). In other words, the ethanol productivity was not affected by the accumulation of phosphorus and magnesium in the biomass

Table 3 - Influence of the medium composition (see Table 2) on the ethanol productivity (Q).

Medium N°	Q (g/L.h)	
	BF (*)	EF (*)
1	11.7	8.5
2	11.7	8.4
3	11.8	8.4
4	10.4	7.7
5	9.4	7.2

(*) Tests carried out in Biolafitte fermentors (BF) and in unstirred Erlenmeyer flasks (EF).

To avoid the seduction in the ethanol productivity it was sufficient to assure the constancy of the phosphorus and magnesium contents of the biomass during the experiment by using a fermentation medium presenting suitable initial concentrations of phosphorus and magnesium sources.

ACKNOWLEDGMENTS

The authors acknowledge the technical assistance of Douglas Dalla Justina e Renato Piplovic.

RESUMO

O acúmulo de fosfato e magnésio nas células de levedura não é necessário para assegurar a produtividade em etanol de fermentações alcoólicas descontínuas. Para evitar a diminuição da produtividade em etanol, foi suficiente utilizar um meio de fermentação contendo concentrações calculadas das fontes de fósforo e de magnésio de modo a manter praticamente constantes, durante a fermentação, os teores iniciais de fósforo e magnésio da biomassa.

REFERENCES

Booth, J.W. and Guidotti, G. (1997). Phosphate transport in yeast vacuoles. *J. Biol. Chem.*, **272**, 20408-20413.

Borzani, W. and Jurkiewicz, C.H. (1998). Variation of the ethanol yield during very rapid batch fermentation of sugar-cane blackstrap molasses. *Braz. J. Chem. Eng*., **15**, 225-233.

Cunniff, P. (1995). *Official Methods of Analysis of AOAC International*, 16 th ed. Arlington: AOAC International.

Joslyn, M.A. (1970). *Methods in Food Analysis.* New York: Academic Press.

Knotkova, A. and Kotyk, A. (1981). Dependence of phosphate transport in yeast of glycolitic substrates. *Folia Microbiol. (Praha)***, 26**, 377-381.

Miller, G.L. (1959). Use of dinitrosalicylic acid for determination of reducing sugars. *Anal. Chem*., **31**, 426-428.

Pestor, N.A.; Kulakovskaya, T.V. and Kulaev, I.S. (2003). Polyphosphates and exopolyphosphatases of the yeast *Saccharomyces cerevisiae* mitochondria under the conditions of phosphate hypercompensation. *Doklady Biochem. Biophys*., **389**, 126-129.

Walker, G.M. and Maynard, A.L. (1997). Accumulation of magnesium ions during fermentation metabolism in *Saccharomyces cerevisiae*. *J. Ind. Microbiol. Biotechnol*., **18**, 1-3.

Received: October 05, 2006;
Revised: July 18, 2007;
Accepted: May 20, 2008.