CHEMICAL ENGINEERING TRANSACTIONS Volume 21, 2010
Editor J. J. Klemeš, H. L. Lam, P. S. Varbanov
 ISBN 978-88-95608-05-1 ISSN 1974-9791
DOI: 10.3303/CET1021223

Production of Bioethanol by Simultaneous Saccharification and Fermentation of Corn Meal by Immobilized Yeast

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The production of bioethanol by simultaneous saccharification and fermentation (SSF) of corn meal using immobilized cells of Saccharomyces cerevisiae var. ellipsoideus yeast in a batch system was studied in this work. The yeast cells were immobilized in Ca-alginate by electrostatic droplet generation method. The kinetics of the SSF process with immobilized yeast was assessed with various initial glucose concentrations. In addition, the effect of media supplementation with the yeast activators such as mineral salts, $ZnSO_4 \times 7H_2O$ and $MgSO_4 \times 7H_2O$ and vitamins, Ca-pantothenate, biotin and myo-inositol was investigated. The system was compared with the SSF system with free yeast. The immobilized system was found superior to the free system since higher ethanol concentration and process productivity were obtained. The maximum increase in ethanol concentration was achieved in immobilized system when mineral salts were added. In this case, the ethanol concentration of 9.70% (w/w) was achieved after 38 hours of fermentation. Addition of the magnesium and zinc contributed to the achievement of a high productivity of the batch SSF of corn meal with immobilized S. cerevisiae var. ellipsoideus yeast, while still preserving a physical and chemical stability of Ca-alginate gel beads. High productivity of the system was attributed to the cell protection from inhibition by ethanol that was achieved by immobilization, and the activation of the yeast metabolism that was accomplished by the minerals.

1. Introduction

Bioethanol produced from renewable biomass, such as sugar, starch, or lignocellulosic materials, is one of the alternative energy resources, which is both renewable and environmentally friendly. Although, the priority in global future ethanol production is put on lignocellulosic processing, which is considered as one of the most promising second-generation biofuel technologies, the technology of utilization of lignocellulosic material for fuel ethanol is still under improvement (Mojović et al.; 2009, Balat and Balat, 2009).

Bioethanol accounts for more than 94% of global biofuel production, with the majority coming from sugar cane (Brazil) and corn (USA) (Licht, 2007). In Serbia, starch-based raw materials such as corn are the most abundant agricultural products which can be

Please cite this article as: Mojović L., Rakin M., Vukašinović M., Nikolić S., Pejin J. and Pejin D., (2010), Production of bioethanol by simultaneous saccharification and fermentation of corn meal by immobilized yeast, Chemical Engineering Transactions, 21, 1333-1338 DOI: 10.3303/CET1021223

used for bioethanol production since the average annual corn yield in Serbia is approximately 40% higher than the calculated domestic needs (Nikolić et al., 2009; Nikolić et al. 2010).

The production of bioethanol by simultaneous saccharification and fermentation (SSF) of corn meal using immobilized cells of Saccharomyces cerevisiae var. ellipsoideus yeast in a batch system was studied in this work. The yeast cells were immobilized in Ca-alginate by electrostatic droplet generation method. The kinetics of the SSF process with immobilized yeast with various initial glucose concentrations was assessed and the effect of media supplementation with particular minerals and vitamins was also investigated. The immobilized system was compared with the SSF system with free yeast.

2. Materials and Methods

2.1 Materials, enzymes and microorganisms

Corn meal obtained by dry milling process was a product of corn processing factory ("RJ Corn Product", Sremska Mitrovica, Serbia). The corn meal consisted of particles with diameter 0.2-1.7 mm (95% or more particles pass through a 1.70 mm sieve). Termamyl® SC, a heat-stable α-amylase from Bacillus *licheniformis* (enzyme activity was 133 KNU/g) was used for corn meal liquefaction and SAN Extra L, Aspergillus niger glucoamylase (activity 437 AGU/g) was used for corn meal saccharification.

The enzymes were gift from Novozymes, Denmark. *Saccharomyces cerevisiae* var. *ellipsoideus* was used for the fermentation of hydrolyzed corn starch. The culture originated from the collection of BIB-TMF, Belgrade, and was maintained on a malt agar slant. Improvement of the ethanol production was investigated by adding different yeast activators: mineral salts $ZnSO_4 \times 7H_2O$ (0.3 g/l) and $MgSO_4 \times 7H_2O$ (2.0 g/l) and vitamins Ca-pantothenate (30.0 mg/l), biotin (64.0 μg/l) and myo-inositol (350.0 mg/l). All vitamins and minerals used were of analytical grade.

2.2 Immobilization and cultivation procedure

S. cerevisiae var. *ellipsoideus* yeast was immobilized in Ca-alginate using an electrostatic droplet generation method (Nikolić et al., 2009).

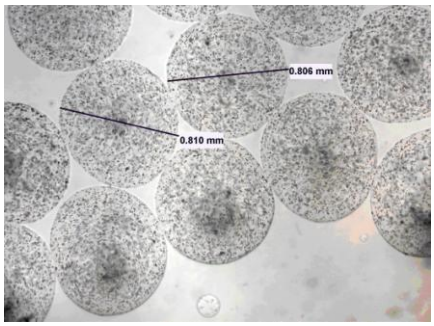

Figure 1: Yeast S. cerevisiae var. ellipsoideus entrapped in Ca-alginate.

The Ca-alginate microbeads with immobilized cells had an average diameter of 0.8 mm, and are presented in Figure 1. Number of viable cell was approximately 5·107 CFU/ml

of beads and the media was inoculated with 2% (by volume) of immobilized yeast at the beginning of fermentation.

2.3 Corn meal hydrolysis and fermentation

100 g of corn meal were mixed with water at the weight ratio (hidromodul) 1:3, respectively and 60 ppm of Ca2+ (as $CaCl_2$) ions were added. The liquefaction was carried out at 85 °C and pH of 6.0 for 1 h by adding 0.026% (v/w, volume of enzyme per weight of starch) enzyme Termamyl® SC. The liquefaction and SSF process were performed in flasks in thermostated water bath with shaking (150 rpm), as described previously by Mojović et al. (2006).

During the corn meal hydrolysis and fermentation, the content of reducing sugars, calculated as glucose, was determined by 3.5-dinitrosalicylic acid (Miller, 1959). The ethanol concentration was determined based on the density of alcohol distillate at 20 ° C and expressed in weight % (w/w) (AOCS Official Methods, 2000). Indirect counting method i.e. pour plate technique was used to determine the number of viable cells (Nikolić et al., 2009). Samples with and without (control) the addition of supplements were tested simultaneously under the same experimental conditions in order to make comparisons.

3. Results and Discussion

3.1 Effect of initial glucose concentration

The ethanol production and glucose consumption during the fermentation of corn meal hydrolyzates with various initial glucose concentrations (150, 176 and 200 g/l) are presented in Figure 2. As shown in Figure 2, the ethanol concentration gradually increased during the fermentation with initial glucose concentrations of 150 and 176 g/l. However, lower ethanol concentrations were obtained at the initial glucose concentration of 200 g/l, because the substrate and product inhibition took place. The maximum values of ethanol concentration, ethanol yield, percentage of the theoretical yield of ethanol and volumetric productivity during 74 h of fermentation were achieved at the initial glucose concentration of 176 g/l. Since high values of the ethanol concentration of 8.90% (w/w), the ethanol yield of 0.51 g/g, the percentage of the theoretical yield of ethanol of 81.88%, the volumetric productivity of 2.34 g/(l·h) and the total amount of utilized glucose of 81.76% were achieved after 38 h of fermentation with initial glucose concentration of 176 g/l, it is reasonable to reduce fermentation time to 38 h. The initial glucose concentration of 176 g/l was selected as the optimal one for SSF with immobilized yeast.

In a previous study reported by Mojović et al. (2006), a lower initial glucose of 150 g/l concentration was chosen for the SSF process with free yeast, suggesting that the immobilization itself contributed to the protection of yeast to toxic effects of the ethanol. Immobilized cells are considered to be more tolerant against ethanol since the matrix provides a protective environment against ethanol toxicity as reported by Ciesarová et al. (1998) and Verbelen et al. (2006).

According to the obtained results, the yeast S. *cerevisiae* var. *ellipsoideus* cells entrapped in Ca-alginate showed good physical and chemical stability, and no substrate and product diffusion restrictions were noticed.

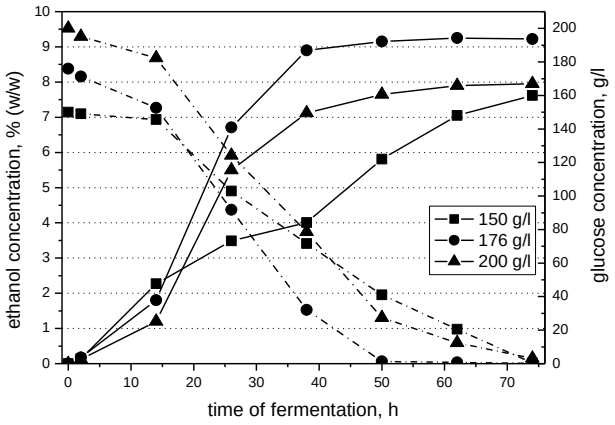

Figure 2: Time course of glucose consumption and ethanol production from corn meal hydrolyzates by immobilized cells of S. cerevisiae var. ellipsoideus with various initial glucose concentrations. Solid - ethanol concentration, dashed - glucose concentration.

3.2 Effect of media supplementation

Figure 3 presents the effect of addition of mineral salts ($ZnSO_4 \times 7H_2O$ - 0.3 g/l + $MgSO_4 \times 7H_2O$ - 2.0 g/l), vitamins (Ca-pantothenate 30.0 mg/l + biotin 64.0 µg/l + myo-inositol 350.0 mg/l) and a mixture of these mineral salts and vitamins on the ethanol concentration achieved at the end of fermentation with immobilized cells of S. *cerevisiae* var. *ellipsoideus*.

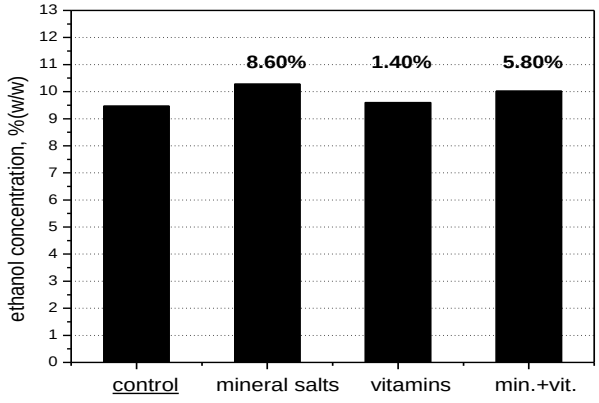

Figure 3: Effect of addition of mineral salts, vitamins and a mixture of mineral salts and vitamins on ethanol concentration after 38 h of SSF process of corn meal by immobilized cells of S. cerevisiae var. ellipsoideus. The numbers above the bars represent the percentage of the increase in the ethanol concentration.

Inoculum's concentration was 2 % (w/v, weight of inoculum per volume of fermentation media) and the initial glucose concentration 176 g/l. Control in Figure 3 represents a sample without addition of activators.
A maximum increase in ethanol concentration of 8.6 % was obtained in samples with the addition of mineral salts only. In these samples the ethanol concentration obtained in the immobilized system after 38 h was 9.70 % w/w. In contrast, mineral salts caused the lowest increase in yeast biomass growth in these systems (data not presented).
The comparison of the significant process parameters achieved in a batch SSF process with immobilized and free cells of S. cerevisiae var. ellipsoideus is presented in Table 1. The results indicate that the immobilized system was superior to the free system

Table 1: Comparison of the significant process parameters achieved after 38 h of fermentation in a batch SSF process with immobilized and free cells of S. cerevisiae var. ellipsoideus.

Type of the system	Maximum % (w/w) of ethanol	% of theoretical yield of ethanol	$Y_{P/S}$ (g/g)	P (g /(l·h))
Immobilized system without supplementation	8.90	82.41	0.51	2.34
Immobilized system with addition of minerals	9.70	90.02	0.55	2.54
Free cell system without supplementation (Mojović et al., 2006)	8.10	81.50	0.45	1.70

4. Conclusions

The immobilized system of S. *cerevisiae* var. *ellipsoideus* in a batch SSF of corn meal was found superior to the free system since higher ethanol concentration, ethanol yield and process productivity were obtained. These effects were attributed to the lower substrate inhibition and higher tolerance to ethanol of the immobilized yeast achieved in the SSF process. Additional benefits of SSF process are lower energy consumption since the second enzymatic stage of hydrolysis is carried out at lower temperature (it is 30 °C, e.g. at the temperature of fermentation) than in conventional process and a reduction of the process time. The yeast S. *cerevisiae* var. *ellipsoideus* cells entrapped in Ca-alginate showed good physical and chemical stability, and no substrate and product diffusion restrictions were noticed. The most appropriate yeast activators for the SSF process with the immobilized yeast were mineral salts ($ZnSO_4 \times 7H_2O$ - 0.3 g/l + $MgSO_4 \times 7H_2O$ - 2.0 g/l) which caused an increase in ethanol concentration for 8.60% compared to the control samples without any supplementation. The addition of mineral salts provided a high value of ethanol concentration (9.70% w/w) after 38 h of the SSF process. We are expecting additional benefits of the utilization of S. *cerevisiae* var. *ellipsoideus* immobilized system in a fed-batch or continuous fermentation mode, which is a part of our further research.

Acknowledgement

This study was supported by Ministry of Science and Technological Development of Serbia, project #TR 18002.

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