Journal of Microbiology and Biotechnology
Vol. 7. No. 6, 438-440, 1997

NOTE

Comparison of Immobilization Matrix for Ethanol Fermentation by *Zymomonas mobilis* and *Saccharomyces cerevisiae*

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A continuous fermentation system employing immobilized cells of *Zymomonas mobilis* and *Saccharomyces cerevisiae* was studied for the mass production of ethanol. Ethanol production by cells immobilized with Ca-alginate was better than those by cells immobilized with κ-carrageenan. Maximum ethanol production employing a continuous system by cells immobilized with Ca-alginate was 77.5 $g \cdot l^{-1} h^{-1}$ at a dilution rate of 1.85 h^{-1} with 82% conversion rate for *Z. mobilis* while that was 40.2 $g \cdot l^{-1} h^{-1}$ at a dilution rate of 0.92 h^{-1} with 85% conversion rate for *S. cerevisiae*. These results suggest that Ca-alginate is a better cell immobilization matrix than κ-carrageenan and that immobilized cells of *Z. mobilis* are more efficient than *S. cerevisiae* for ethanol production.

The production of ethanol by microbial fermentation as an alternate energy source has been of interest for many years because the supply of fossil fuel is limited (3, 25). Continuous production of ethanol by immobilized cells is a very efficient technology because high cell density in the fermentor can be maintained with immobilized cells (5, 14, 16, 17, 26). Entrappment of cells by various polymers such as agar, alginate, carrageenan and polyacrylamide is one of the most frequently used methods for immobilization of cells (6, 15, 22). Production of various materials including antibiotics, alcohol (2, 18), and organic acid (11) by immobilized cells has been reported. Alginate and κ-carrageenan are widely used as supports for the immobilization of living cells (5, 6, 13, 24). *Zymomonas mobilis* (7, 10, 20, 23) and *Saccharomyces cerevisiae* (4, 8, 9) are two of the most widely used ethanol producers. *Z. mobilis* has an advantage over *S. cerevisiae* in production of ethanol due to higher ethanol yield and lower biomass yield (20, 21). In this work, continuous ethanol production by *Z. mobilis* and *S. cerevisiae* cells immobilized with Ca-alginate or κ-carrageenan was compared.

Z. mobilis ATCC29191 was grown in a fermentation medium A [15% glucose, 1% yeast extract, 0.2% KH_2PO_4, 0.1% $MgSO_4 \cdot 7H_2O$, and 0.1% $(NH_4)_2SO_4$, pH 5.4] and *S. cerevisiae* IFO7056 in a fermentation medium B [10% glucose, 0.2% yeast extract, 0.1% KH_2PO_4, 0.07% $MgSO_4 \cdot 7H_2O$, 0.4% $(NH_4)_2SO_4$, and 0.1% NaCl, pH 4.5]. Cells were harvested by centrifugation at 1,800 g for 10 min and the cell pellet was resuspended in a Na-alginate solution to make 1% (w/v), 1.5% (w/v), or 1.5% (w/v) Na-alginate solution with 40 g wet weight of cell/100 ml. The mixture was passed through a syringe into 0.09 M $CaCl_2$ solution to make a bead and cured for 2 h (18). The average diameter of the bead was 3.5 mm. κ-Carrageenan was dissolved at 4% (w/v) in 0.8% (w/v) NaCl solution at 80°C and the temperature of the solution was lowered to 48°C before the same volume of cell suspension was mixed at 40 g wet weight cell/100 ml. The mixture was incubated at 10°C for 30 min and cut into sections with a dimension of 3×3×3 mm and incubated in a 0.3 M KCl solution for 1 h (12, 24). Batch fermentation was carried out in 250-ml Erlenmeyer flask containing 25 ml of medium and ethanol production was measured after 12 h. A water jacketed column (1.1 cm diameter, 38 cm) was used for continuous fermentation. The medium was pumped from the bottom of the column with various flow rates as indicated. The composition of the medium used for alginate immobilized cells was 10 or 15% glucose, 0.5% yeast extract, 0.3% $CaCl_2$, pH 5.4 and the composition of the medium for κ-carrageenan immobilized cell was 10% glucose, 0.5% yeast extract, 0.3% KCl, pH 5.4. The concentration of glucose was measured by the DNS method (19). The concentration of ethanol was measured by the dichromate oxidation method (1).

Effect of Temperature on Ethanol Production by Immobilized Cells

The optimum temperature for ethanol production by

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Key words: *Z. mobilis*, *S. cerevisiae*, immobilized cells

immobilized cells was estimated by batch fermentation. The optimum temperature for maximum ethanol production by free *Z. mobilis* and *S. cerevisiae* cells was between 30°C and 35°C but the immobilized *Z. mobilis* and *S. cerevisiae* cells showed maximum ethanol production at 30°C regardless of the kind of matrix. Ethanol production by the immobilized *Z. mobilis* cells was decreased at 35°C, which might be resulted from the loss of cell viability at high ethanol concentration in the gel matrix due to the low diffusion rate of ethanol (26). *Z. mobilis* cells immobilized with Ca-alginate showed a greater reduction in ethanol production at 35°C than the cells immobilized with κ-carrageenan, suggesting that the ethanol diffusion rate may be slower in Ca-alginate than in κ-carrageenan. However, ethanol production at 35°C by *S. cerevisiae* immobilized with Ca-alginate was not reduced dramatically. Generally, ethanol production by the immobilized *Z. mobilis* cells was higher than that of the immobilized *S. cerevisiae* cells between 25°C and 35°C.

Effect of Gel Concentration on Continuous Fermentation

Z. mobilis cells immobilized with three different concentrations of alginate at 1, 1.5, and 2% (w/v) were tested by continuous fermentation using 10% (w/v) glucose as a substrate. The optimum alginate concentration was 1.5%. The 1% gel showed lower ethanol production mainly because a large amount of cells leaked out of the matrix. The maximum ethanol productivity by *Z. mobilis* cells immobilized with 1% Ca-alginate was 65 $g \cdot l^{-1} h^{-1}$ at 1.4 h^{-1} of dilution rate, while that by *Z. mobilis* cells immobilized with 1.5% Ca-alginate was 77.5 $g \cdot l^{-1} h^{-1}$ at 1.85 h^{-1} of dilution rate. *Z. mobilis* cells immobilized with 2% Ca-alginate showed about a 50% reduction in ethanol productivity compared with 1.5% Ca-alginate and the trend was similar with *S. cerevisiae*. All the other experiments were done with 1.5% gel concentration.

Effect of Glucose Concentration on Ethanol Production

Two different concentrations, 10 and 15% (w/v) of glucose were compared with *Z. mobilis* cells immobilized with 1.5% Ca-alginate by continuous fermentation. The maximum ethanol productivity was increased from 77.5 $g \cdot l^{-1} h^{-1}$ with 10% glucose to 87 $g \cdot l^{-1} h^{-1}$ with 15% glucose. However, only 48% of glucose in 15% glucose medium was utilized while 82% of glucose in 10% glucose medium was converted to ethanol under the conditions tested. This suggests that a 10% glucose solution was better for the production of ethanol considering the fact that substrate cost is about 60~70% of ethanol production costs (26).

Effect of κ-Carrageenan on Continuous Fermentation

The optimum κ-carrageenan concentration for the immobilization of cells was 4% (w/v) (25). The continuous ethanol production by *Z. mobilis* and *S. cerevisiae* cells immobilized with 4% κ-carrageenan was compared. As with Ca-alginate, κ-carrageenan immobilized *Z. mobilis* was better than *S. cerevisiae* for continuous ethanol production.

Table 1. Comparison of ethanol productivity of *Z. mobilis* and *S. cerevisiae* immobilized with Ca-alginate or κ-carrageenan.

Immobilization system	Sugar utilization (%)	Dilution rate (h^{-1})	Maximum ethanol productivity ($g \cdot l^{-1} h^{-1}$)
Ca-alginate			
Z. mobilis	82	1.85	77.5
S. cerevisiae	85	0.92	40.2
κ-carrageenan			
Z. mobilis	70	2.1	74.5
S. cerevisiae	76	1.0	39

Ethanol Productivity

Continuous ethanol production by *Z. mobilis* and *S. cerevisiae* cells immobilized with Ca-alginate or κ-carrageenan was compared and the results are summarized in Table 1. The maximum ethanol productivity by Ca-alginate immobilized *Z. mobilis* was 77.5 $g \cdot l^{-1} h^{-1}$ while that by Ca-alginate immobilized *S. cerevisiae* was 40.2 $g \cdot l^{-1} h^{-1}$. In general, the immobilized *Z. mobilis* cells were more efficient in producing ethanol than the immobilized *S. cerevisiae* cells and Ca-alginate was slightly better than κ-carrageenan but the difference was minimal. However, Ca-alginate was a better immobilization matrix than κ-carrageenan because it is cheaper and more stable over a wider range of temperatures.

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(Received July 7, 1997)