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Ethanol Production from Lactose by Immobilized Reactor System Using a Fusant Yeast Strain of *Saccharomyces cerevisiae* and *Kluyveromyces fragilis*

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Yeast cells of a fusant strain constructed by protoplast fusion of *Saccharomyces cerevisiae* and *Kluyveromyces fragilis* were immobilized on calcium alginate beads. The increment of the ethanol tolerance of this strain to 8.0%, when compared with the parent *K. fragilis*, was confirmed. Based on the results from jar fermentation, a packed-bed reactor of the immobilized yeast cells was operated. The optimal performance of the immobilized yeast reactor for ethanol production was achieved when supplying 10% lactose (supplemented 1.0% yeast extract) at a temperature of 30°C. The maximal ethanol productivity was obtained as 13.3 g/l/hr at a dilution rate of 0.76 hr^{-1}.

KEY WORDS □ Fusant yeast, ethanol fermentation, whey fermentation, immobilized cell reactor

Whey is the liquid effluent generated by the cheese and casein manufacturing industries. Annual production of this material is estimated at 74×10^6 tons (1). Whey has a BOD_5 value of 60 to 70×10^3 mg/L, which makes disposal a serious environmental problem. Efforts to use whey in the dairy industry as a renewable resource have centered on fermenting lactose in whey to produce ethanol.

There have been two difficulties preventing large scale whey use for alcohol production. Yeast strains able to convert whey to ethanol cannot tolerate high levels of alcohol, whereas yeast strains having high ethanol tolerance cannot use the lactose in whey. Recently, several research groups (2-4) reported new hybrid strains of *Saccharomyces cerevisiae* and *Kluyveromyces* sp., constructed by protoplast fusion techniques. These fusant strains are able to use the lactose in whey efficiently, with a higher alcohol tolerance.

In preceding report (5), we described the fermentative characteristics of one of these fusant yeast strains in the production of ethanol from lactose. The fusant strain studied was developed by Farahnak *et al.* (2) from the parental strains, *Saccharomyces cerevisiae* and *Kluyveromyces fragilis*.

Here, we report the results from the operation of an immobilized yeast reactor for the production of ethanol from lactose, with comparison of the results from shaken-flask fermentation and jar fermentation (5). The fusant yeast cells used were immobilized on calcium alginate gel beads.

MATERIALS AND METHODS

Microorganisms and cultivation

Saccharomyces cerevisiae STX23-5B (Ade^- Trp^-), *Kluyveromyces fragilis* 55-55 (Met^-), and their fusant yeast strain (Fus7, Ade^- Trp^- Met^-) were kindly supplied by Dewey D.Y. Ryu, Department of Chemical Engineering, University of California, Davis, USA. These yeast strains were maintained in a YPD medium containing 1% yeast extract, 2% peptone, and 2% glucose.

Comparison of ethanol tolerance

In order to determine the ethanol tolerance of the yeast strains, 10% inoculums of the yeast culture were transferred into new YPD media, exogenously supplied with different concentrations of ethanol (5~15%), and cultivated at 30°C for 7 days with shaking at 150 rpm (6, 7). Viable yeast cells in the final culture broth were counted on YPD agar plates using the serial dilution method. The maximum ethanol tolerant concentration was estimated as that concentration which caused a drastic decrease in number of viable yeast cells.

Immobilization of fusant cells

The cells of fusant strain were immobilized on calcium alginate beads (8, 9). Fusant yeast cells grown in YPD medium at 30°C were harvested at late-logarithmic state by centrifugation. The

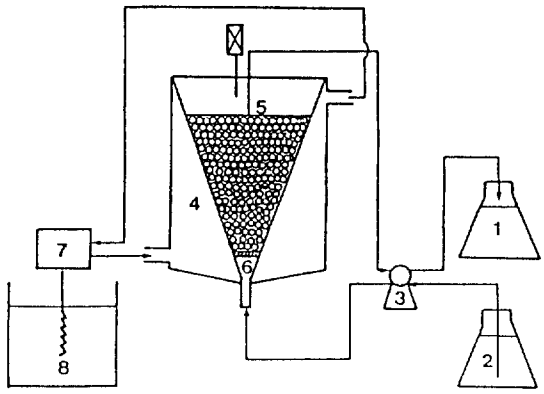

Fig. 1. *Immobilized Yeast Reactor for Ethanol Fermentation from Lactose Using A Fusant Strain.*
1. Product Reservoir; 2. Substrate Reservoir; 3. Peristaltic Pump; 4. Water Jacket; 5. Stainless Sieve; 6. Sintered Glass; 7. Water Circulator; 8. Thermostat.

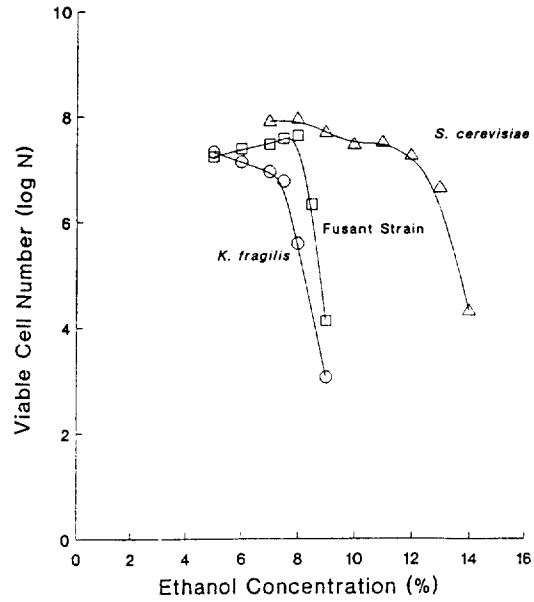

Fig. 2. *Ethanol tolerances of the fusant yeast strain and its parental strains. Kluyveromyces fragilis and Saccharomyces cerevisiae.*
Yeasts were cultivated in new YPD media exogenously supplied with different concentrations of ethanol (5~15%) at 30°C for 7 days with shaking at 150 rpm, and viable yeast cells were counted on YPD agar plates by the serial dilution method.

harvested cells were suspended in fresh YPD medium, and mixed with equal amount of sterilized 4% sodium alginate solution. Through the syringe needle (No. 22), this suspension was pushed out drop by drop into 0.05 M calcium chloride solution with stirring, to form insoluble calcium alginate beads. After hardening overnight in this solution, the beads (diameter 0.2~0.3 mm) were filtered and used as immobilized yeast cells.

Performance of immobilized yeast reactor

The immobilized fusant cells were packed in an immobilized cell reactor as shown in Fig. 1. This reactor was designed as an inverted conical shape in order to exhale easily carbon dioxide gas produced during ethanol fermentation. Outside of this reactor, water jacket was installed for the maintenance of reactor temperature. The medium containing 10% lactose and 1% yeast extract (pH 4.5) was supplied into this packed-bed reactor, which was maintained at 30°C, using peristatic pump (Model SJ-1220, Atto Corporation, Japan). The effluents of reactor were collected as samples. The residence time was calculated on the basis of void volume, substracted volume of calcium alginate beads from total reactor volume.

Analysis of ethanol concentration

The concentrations of the produced ethanol in the effluents of a packed-bed reactor were determined by the gas chromatograhic method. The Gas Chromatograph (Model 5890A) of Hewlett Packard S.A. (Meyrin, Switzerland) was used with a Porapak Q column (80~100 mesh) and a flame ionization detector. Culture samples were mixed with equal volumes of 0.25% isopropanol for use as an internal standard, then injected into the injection port at 200°C. The samples were passed through the column in 150°C oven.

RESULTS AND DISCUSSION

Comparison of the ethanol tolerance of the fusant strain with its parental strains

Since one of the aims in constructing a fusant yeast strain is to increase the ethanol tolerance during the ethanol fermentation, the ethanol tolerances of the fusant yeast strain and its parental strains, *S. cerevisiae* and *K. fragilis*, were investigated by cultivation in YPD media for 7 days with exogenously added ethanol (5~15%). As seen in Fig. 2, the fusant strain showed the maximum tolerant concentration against ethanol of 8.0%, which was slightly higher than that of *K. fragilis* (7.0%). However, this ethanol tolerance was much lower than the tolerance of *S. cerevisiae* (13.0%).

Under microscopic examination, *K. fragilis* has a long cylindrical shape, whereas *S. cerevisiae* is nearly round. The fusant yeast strain showed an intermediate morphology, having a short cylindrical form. Even though the fusant strain has

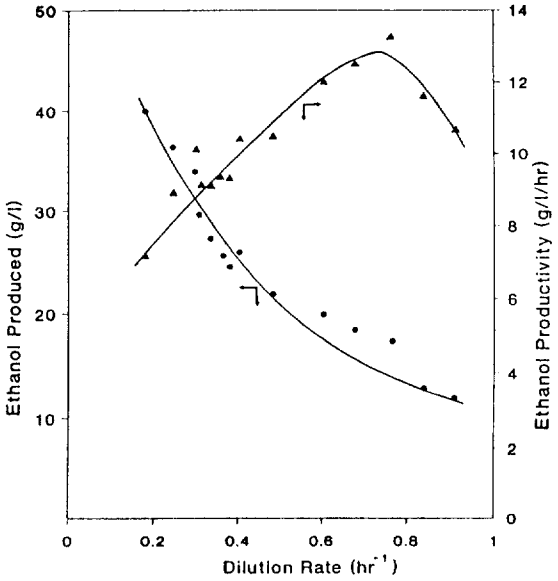

Fig. 3. *Effect of Dilution Rate on Ethanol Production from Lactose by Immobilized Fusant Yeast Strain in Packed Bed Reactor*
Medium containing 10% lactose and 1% yeast extract (pH 4.5) was supplied into packed-bed reactor, which was maintained at 30°C.

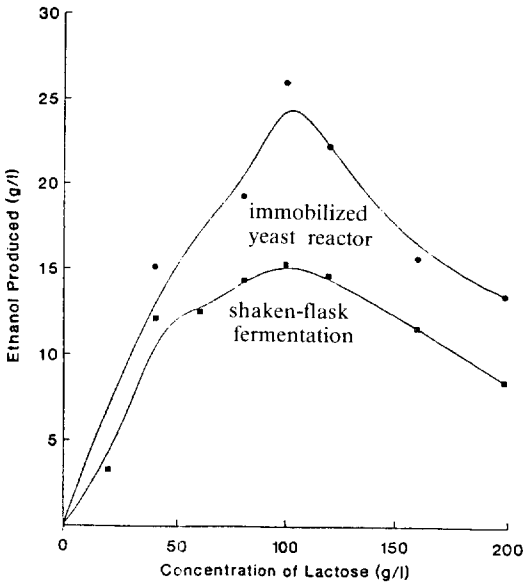

Fig. 4. *Effect of Lactose Concentration on Ethanol Production from Lactose by Immobilized Fusant Yeast Strain in Packed Bed Reactor*
Medium containing lactose and 1% yeast extract (pH 4.5) was supplied into packed-bed reactor with the dilution rate of 0.4 hr^{-1} at 30°C.

intermediate shape and auxotrophic nutrient requirements, this result implies that the ethanol tolerance of the fusant strain comes mostly from the genes of *K. fragilis* rather than the genes of *S. cerevisiae*.

Immobilization of fusant cells

The immobilized cells on insoluble matrices such as polyacrylamide, calcium alginate and κ-carrageenan were widely used in fermentation industry (10, 11). Among those matrices, Marwaha *et al.* (8, 9) reported that calcium alginate gel gave high viability and cell stability during operation of immobilized yeast reactor for ethanol production from whey by *Kluyveromyces* species. They observed the bead disruption and cell release during ethanol fermentation when κ-carrageenan was used for an immobilizing matrix. Following their reports, the fusant cells were immobilized on calcium alginate beads.

Operation of immobilized yeast reactor

In the operation of immobilized yeast reactor of fusant cells, the effect of dilution rate on ethanol production from lactose was investigated. As shown in Fig. 3, the higher dilution rate or the shorter resident time the reactor operated at, the lower concentration of ethanol was produced but the higher ethanol productivity was achieved. However, above dilution rate of 0.8 hr^{-1}, the ethanol productivity was decreased, probably due to the inhibition of ethanol production by accumulated ethanol in a bioreactor. A maximum ethanol concentration of 39.5 g/*l* (theoretical conversion yield; 77.9%) was recorded at a dilution rate of 0.18 hr^{-1}. The highest ethanol productivity was achieved as 13.3 g/l/hr at a dilution rate of 0.76 hr^{-1}. This value is similar to that from the operation of packed-column reactor for continuous ethanol production from whey lactose by the immobilized *K. marxianus* cells on calcium alginate beads (12). But the results from immobilized *K. marxianus* cells showed that the optimal dilution rate was 0.4 hr^{-1}. The differnece may be attributed to the amount of immobilized cells loaded on calcium alginate beads.

With varying the concentration of lactose in supplying media, the ethanol productivities by immobilized fusant cells were examined (Fig. 4). The same result was obtained as shaken-flask fermentation and jar fermentation of the fusant strain (5), and ethanol fermentation by the immobilized *K. marxianus* cells (8, 13). The ethanol productivity was increased with increment of lactose concentration up to 10%, but decreased when more than 10% lactose was supplied. This suggests that the osmotic tolerance, in addition to the ethanol tolerance, is important in ethanol

Table 1. *Effect of Reactor Temperature on Ethanol Production from Lactose by Immobilized Fusant Yeast Strain in Packed Bed Reactor*

Temperature (°C)	Ethanol Produced (g/l)	Ethanol Productivity (g/l/hr)
25	16.8	4.46
30	26.0	6.90
35	22.1	5.85

Medium containing 10% lactose and 1% yeast extract (pH 4.5) was supplied into packed-bed reactor with the dilution rate of 0.4 hr^{-1}.

production by the fusant strain.

The immobilized yeast reactor was also operated at different temperatures (Table 1). Same as jar fermentation of this fusant strain (5) and fermentation by the immobilized *K. marxianus* cells (8, 13), the operation at 30°C gave the best result in ethanol production from lactose.

The results obtained above will provide the basic data for the performance of the immobilized fusant yeast reactor in the production of ethanol from whey. Nevertheless, these results suggest the possibility of whey, waste from cheese-making industry, as an alternative substrate for continuous production of fuel-grade ethanol using an immobilized cell reactor system.

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초 록: ***Saccharomyces cerevisiae*와 *Kluyveromyces fragilis*의 융합 효모의 고정화 반응기 운전을 통한 유당으로부터의 에탄올 생산**

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*Saccharomyces cerevisiae*와 *Kluyveromyces fragilis*의 세포융합을 통해 얻은 융합효모를 calcium alginate bead에 고정화시킨 후 유당으로부터의 에탄올 생산을 시도하였다. 우선 융합효모의 에탄올 내성을 조사한 결과, 모 효모인 *K. fragilis* 보다 높은 8.0%에서 최대 내성을 나타내었다. 이렇게 고정화시킨 융합효모를 충전식 반응기에 충전하고 에탄올 생산을 실시하였는데, 10% 유당을 공급하고 30°C에서 운전하였을 때 가장 좋은 에탄올 생산능을 보여주었다. 특히 반응기의 희석률이 0.76 hr^{-1} 일 때 13.3 g/l/hr의 높은 에탄올 생산능을 얻을 수 있었다.