J. Gen. Appl. Microbiol., **46**, 231–234 (2000)

Short Communication

Production of ethanol using granulated yeast cells prepared by a spray dryer

Yasuyuki Isono* and Akira Hoshino

Biochemical Laboratory, Dainichiseika Color & Chemicals Mfg. Co., Ltd., Adachi-ku, Tokyo 123-8555, Japan

(Received April 21, 2000; Accepted October 13, 2000)

Key Words——ethanol; fermentation; granulate; nitrogen; spray dryer; yeast

The application of immobilized microbial cell systems to bioreactor system usually offers advantages over cell suspension systems in terms of productivity and stability of enzymatic activities (Woodward, 1985). So far, many support materials for cell immobilization, such as calcium alginate, κ-carrageenan gel, polyacrylamide, and cellulose, have been reported (Cho and Choi, 1981; Furusaki et al., 1983; Galazzo and Bailey, 1990; Wada et al., 1980).

On the other hand, the spray-drying process is widely used for drying pharmaceuticals, fine chemicals, foods, dairy products, blood plasma, numerous organic and inorganic chemicals, rubber latex, ceramic powders, detergents, and other chemicals. This process has several advantages: 1) Heat-sensitive materials can be dried under atmospheric pressure at low temperatures; 2) It allows large-scale production in continuous operation with relatively simple equipment; 3) Spray-dried products are usually homogeneous; 4) Although the operating gas temperature is usually low, the efficiency is comparable to those of direct dryers (Filkova and Mujumdar, 1987). Spray drying is also applicable to the preparation of immobilized biocatalysts. In our previous study, the alumina particles coated with yeast cells and a binder polymer using a spray dryer were prepared (Isono et al., 1995). The polymer used for the immobilizing binder in the study has a unique characteristic. Polymer is soluble in an alkaline solution, but insoluble at a pH value lower than 6.7. A novel immobilization technique has been developed to exploit this property. Thus the biocatalyst/polymer solution at high pH (adjusted with ammonia) is sprayed onto the support materials by a spray dryer. Instantaneously, the polymer forms a coat on the support with the biocatalyst because the solution pH changes to neutral range as a result of ammonia evaporation. In this paper we reported a new preparation method of granulated yeast cells that uses a spray dryer with the binder polymer and zeolite particles, and we also reported the application of granulated yeast for ethanol production.

Saccharomyces cerevisiae strain IAM 4512 was used in this study. The stock culture was maintained on agar plates supplement containing 0.3% yeast extract, 0.3% malt extract, 0.5% peptone, and 1.0% glucose at 5°C. In a preculture, *S. cerevisiae* cells were grown in shaking flasks at 30°C in a medium supplement with 0.3% yeast extract, 0.3% malt extract, 0.5% peptone, and 1.0% glucose (pH 5.5). The preculture was inoculated with one loopful of cells from the stock culture and cultivated for 24 h. Cells of exponential phase (more than 1×10^8 cells/ml) were harvested by centrifugation (3,000 rpm, 5 min) and washed twice with sterilized distilled water.

* Address reprint requests to: Dr. Yasuyuki Isono, Biochemical Laboratory, Dainichiseika Color & Chemicals Mfg. Co., Ltd., 1–9–4, Horinouchi, Adachi-ku, Tokyo 123–8555, Japan.

E-mail: isonoy@post.officenet.co.jp

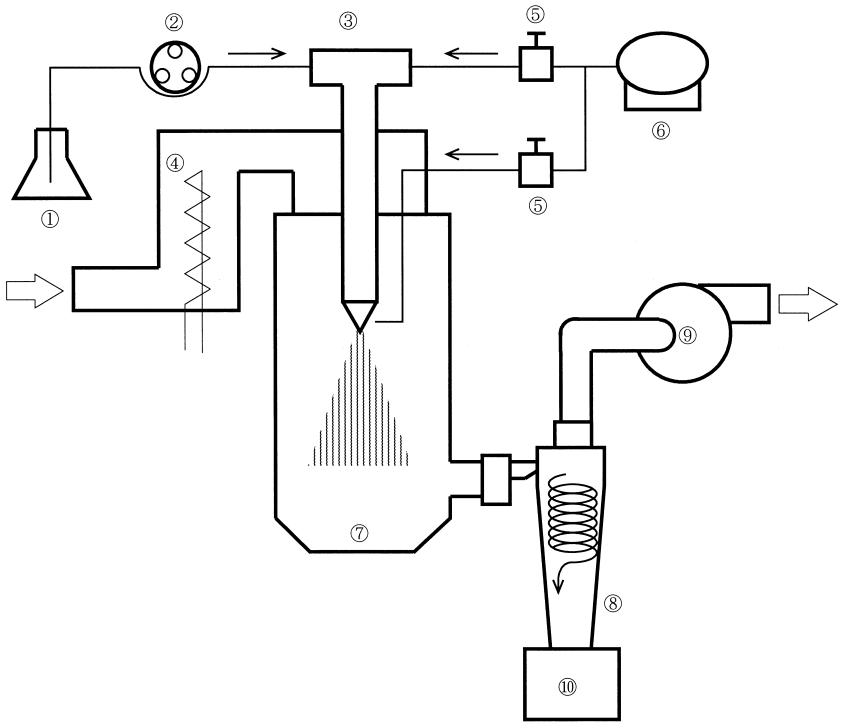

Fig. 1. Schematic diagram of the spray dryer.

1, binder reservoir; 2, feed pump; 3, atomizer; 4, heater; 5, electromagnetic valve; 6, compressor; 7, chamber; 8, classifier; 9, air exhaust blower; 10, product reservoir.

The polymer used was cellulose acetate phthalate (CAP, Daihachi Chemical Co., Ltd., Osaka, Japan). One hundred grams of the binder solution for immobilization, whose composition was water : polymer : ammonia=97 : 7 : 3, was sterilized at 121°C for 15 min, then mixed with 30 g of the harvested *S. cerevisiae* cells. Fifty grams of zeolite particles were dispersed in the mixture and granulated with a spray dryer. Figure 1 shows a schematic diagram of the dryer. The mixture stocked in the reservoir was fed into the atomizer with compressed air, then spray dried by using drying airs in which temperatures of the inlet and the outlet were adjusted to 105°C and 45°C, respectively. The pressure at the atomizing was controlled at 3.0 kg/cm^2. The average diameter of the obtained granulated yeast was measured to be 39.8 μm by a scanning electron microscope.

Batch ethanol production was carried out as follows. Immobilized cells were incubated at 35°C in 100 ml Erlenmeyer flasks containing 15 ml sterilized production media, which was composed of 0.03% yeast extract, 0.1% K_2HPO_4, 0.3% $MgSO_4$, and 1.0% sucrose. The amount of nitrogen source added to the reaction mixture corresponded to 1/30 M nitrogen. Repeated batch ethanol production was performed as follows. After the first run, which was carried out the same way as the batch operation described above, the reaction mixture was filtrated by using a membrane filter (pore size, 0.2 μm, Nihon Millipore Ltd., Tokyo, Japan). After the filtration, a recovered yeast granule was added to a fresh medium, and the second production was started. The operation was repeated five times.

Ethanol concentration was measured by using gas chromatography (Model 163, Hitachi, Tokyo, Japan) with a flame ionization detector and Chromosorb 103 (60/80 mesh) in a 3 mm×2 m glass column. The temperatures of injection and column were 220°C and 170°C, respectively. The nitrogen carrier gas flow rate was 3.0 ml/min. Concentrations of sucrose, fructose, and glucose were measured by using high-performance liquid chromatography (Model 635A, Hitachi) with a 2.6 mm×250 mm stainless-steel column packed with Lichrosorb NH_2 (Merck, NJ, USA) at ambient temperature. The mobile phase for fractionation was acetonitrile : water=80 : 20 (v/v), and its flow rate was 1.0 ml/mim. The differential refractometer (Model RI-2, Nihon Bunseki Kogyo, Tokyo, Japan) was used as a detector.

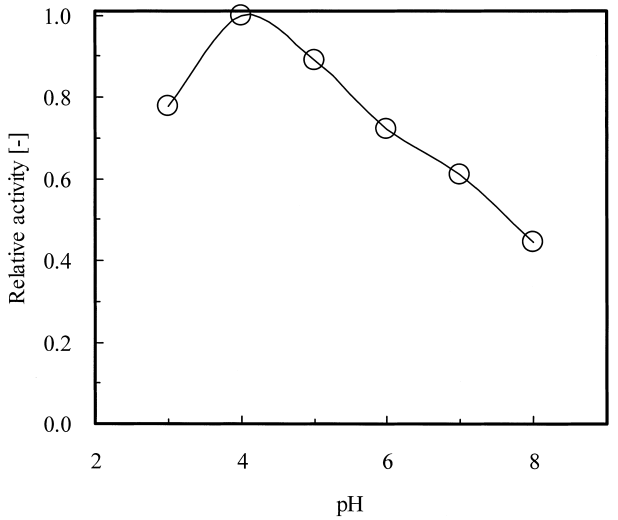

Fig. 2. Effect of medium pH on ethanol conversion by granulated *S. cerevisiae*.

Initial sucrose concentration, 100 g/L; concentration of biocatalyst, 20 g/L; reaction temperature, 35°C. The activity at the medium pH of 4.0 was taken as 1.0 of the relative activity.

The effect of medium pH on the ethanol production is shown in Fig. 2. The optimum pH for the granulated yeast was 4.0. Generally, operation of the bioreactor systems at low pH has the advantage of minimizing microbial contamination (Hatakeyama and Noda, 1984). Ethanol production using immobilized cells at low pH have been reported by Noguchi et al. (1982) (at pH 4.0) and Fukushima and Yamade (1988) (at pH 2.8–3.4). They also reported that the maintenance of the fermentation medium with lower pH was effective in preventing contamination.

An effect of the addition of nitrogen compounds in the ethanol production is summarized in Table 1. The relative activities were based on the ethanol formation rate over a 24 h reaction with no nitrogen addition. *Saccharomyces* is known to be unable to utilize nitrate. Moreover, in the media at pH values below 6.0, nitrous acid is formed, which is known to be toxic to yeast (Rose, 1987). Therefore the addition of $NaNO_3$ was found to reduce the production activity in this experiment. All amino acids tested in this study promoted the production activity. The nitrogen source is one of the important components of a fermentation broth, and several studies have been performed on the utilization of inorganic or organic nitrogen compounds by yeast (Suomalainen and Oure, 1971). The observed assimilation of other amino acids revealed that the straight-chain or aliphatic amino acid are used first, then cyclic amino acids are assimilated and proline is scarcely utilized (Rose, 1987). This tendency is consistent with the relative activities observed in this study, with the exception of phenylalanine. Moreover, the effective nitrogen compound addition to ethanol production suggested a multiplication of yeast cells. Because the granules prepared by a spray dryer have high porosity, the growing yeast cells would be entrapped in the pores of the granules and would then enhance the ethanol production.

Figure 3 shows the time course of the ethanol production. An equivalent amount of aspartic acid to 1/30 M nitrogen was added to the reaction mixture as

Table 1. Effect of the nitrogen compound addition to ethanol production from sucrose, using granulated yeast cells.

Nitrogen compound	Relative activity (%)
Inorganic	
NH_4Cl	170.3
$NaNO_3$	100.0
Amino acid	
Asn	201.6
Asp	240.6
His	123.4
Gln	171.9
Glu	228.1
Phe	192.2
Trp	132.8

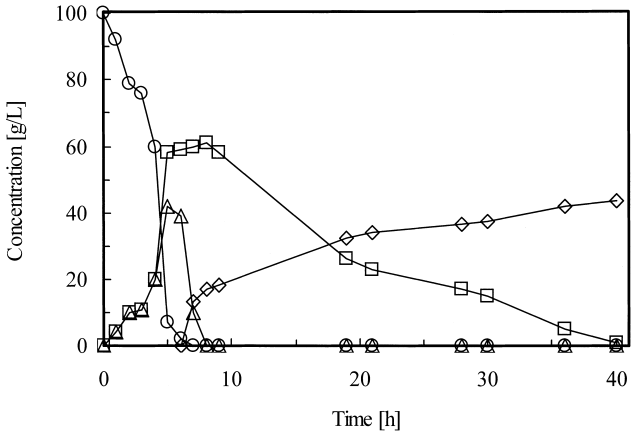

Fig. 3. Time course of ethanol production in batch fermentation by using granulated *S. cerevisiae*.

Symbols: ○, sucrose; △, glucose; □, fructose; ◇, ethanol. Aspartic acid was added and corresponded to 1/30 M nitrogen of the reaction mixture as the nitrogen source, and the pH was adjusted at 4.0. Other reaction conditions were similar to those in Fig. 2.

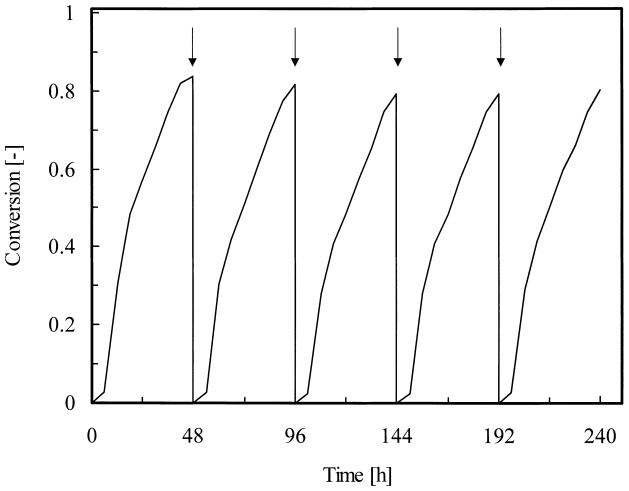

Fig. 4. Repeated batch fermentation, using granulated *S. cerevisiae*.

The arrowheads indicated the timing of the biocatalyst transfer to fresh reaction medium. The conditions of each batch reaction were similar to those in Fig. 3.

the nitrogen source, and the pH was adjusted at 4.0. Sucrose was rapidly hydrolyzed into glucose and fructose, then converted into ethanol by the granulated yeast cells. The consumption rate of glucose was higher than that of fructose. Ethanol productivity of the system was calculated to be 0.072 g/(h·g-catalyst). This value is high compared with our previous report (Isono et al., 1995) and others (Galazzo and Bailey, 1990; Kana et al., 1989).

Repeated batch production could be performed by maintaining high conversions as in Fig. 4. High conversions were maintained during the repeated batch operation, and the average conversion was calculated to be 80.7%. According to the result, the activity of the granulated yeast was regarded as stable in this experimental period. The productivity seems to be insufficient in the reaction condition; however, an improvement of productivity would be attained by using a higher concentration of substrate and introducing continuous operation. These points should be obvious through further studies.

In this study, we proposed a convenient preparation method of granulated yeast cells by using a spray dryer with the binder polymer and zeolite particles. This procedure can easily prepare porous granules with inexpensive materials in large quantities. We also indicated the high and stable activity of granulated yeast in the ethanol production. This method will also be useful for the preparation of other granular biocatalysts.

Acknowledgments

The authors wish to thank Mr. Y. Iijima, of our laboratory, for his critical reading of the manuscript.

References

Cho, G. H. and Choi, C. Y. (1981) Continuous ethanol production by immobilized yeast in a fluidized reactor. *Biotechnol. Lett.*, **3**, 667–671.

Filkova, I. and Mujumdar, A. S. (1987) Industrial spray drying system. *In* Handbook of Industrial Drying, ed. by Mujumdar, A. S., Marcel Dekker, New York, pp. 243–293.

Furusaki, S., Seki, M., and Fukumura, K. (1983) Reaction characteristics of an immobilized yeast producing ethanol. *Biotechnol. Bioeng.*, **25**, 2921–2928.

Fukushima, S. and Yamade, K. (1988) A novel process of ethanol production accompanied by extraction of sugar in cane chips. *J. Ferment. Technol.*, **66**, 423–426.

Galazzo, J. L. and Bailey, J. E. (1990) Growing *Saccharomyces cerevisiae* in calcium-alginate beads induces cell alterations which accelerate glucose conversion to ethanol. *Biotechnol. Bioeng.*, **36**, 417–426.

Hatakeyama, H. and Noda, H. (1984) F type bioreactor. *Kagaku Kogaku*, **48**, 603–605.

Isono, Y., Araya, G., and Hoshino, A. (1995) Immobilization of *Saccharomyces cerevisiae* for ethanol fermentation on γ-alumina particles using spray-dryer. *Process Biochem.*, **30**, 743–746.

Kana, K., Kanellaki, M., Papadimitriou, A., Psarianos, C., and Koutinas, A. A. (1989) Immobilization of *Saccharomyces cerevisiae* on γ-alumina pellets and its ethanol production in glucose and raisin extract fermentation. *J. Ferment. Bioeng.*, **68**, 213–215.

Noguchi, S., Nagashima, M., and Azuma, M. (1982) Continuous production of ethanol using immobilized yeast cells. *Pan-Pac. Synfuels Conf.*, **2**, 464–472.

Rose, A. H. (1987) Responses to the chemical environment. *In* The Yeast, 2nd ed., ed. by Rose, A. H. and Harrison, J. S., Academic Press, London, pp. 15–20.

Suomalainen, H. and Oure, E. (1971) Yeast nutrition and solute uptake. *In* The Yeast, 1st ed., ed. by Rose, A. H. and Harrison, J. S., Academic Press, London, pp. 3–74.

Wada, M., Kato, J., and Chibata, I. (1980) Continuous production of ethanol using immobilized growing yeast cells. *Eur. J. Appl. Microbiol. Biotechnol.*, **10**, 275–287.

Woodward, J. (1985) Immobilized Cells and Enzymes, IRL Press, Oxford, pp. 1–30.