REPEATED SIMULTANEOUS SACCHARIFICATION AND FERMENTATION OF RAW STARCH BY A COMBINATION OF A REVERSIBLY SOLUBLE-INSOLUBLE AMYLASE AND YEAST CELLS

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Key Words: Biochemical Engineering, Enzyme Engineering, Immobilized Enzyme, Amylase, Raw Starch, Reversibly Soluble-Insoluble Enzyme

Introduction

The saccharification process of raw starch is a typical key reaction essential to bioconversion of renewable resources (cassava, sorghum, potato, etc.) to useful materials by microorganisms. Recently, in order to eliminate a high-energy consuming cooking stage of native starch from brewing, some amylases capable of hydrolysing uncooked starch directly have been used in the saccharification step.¹⁾ If the relatively expensive amylases are repeatedly utilized, a significant cost reduction might be accomplished. However, in the heterogeneous reaction system, when the enzyme immobilized on an insoluble carrier is reutilized, effective use of the immobilized enzyme is hindered by severe diffusional limitation within the carrier.³⁾ To overcome the problem inherent in a reaction involving any solid substrate, we reported previously²⁾ on the preparation of a raw starch-digesting amylase (Dabiase K-27) immobilized on a reversibly soluble-insoluble (S-IS) carrier and on its application to the saccharification of raw corn starch. In the present work, we report on the repeatedly simultaneous saccharification-fermentation (SSF) of raw starch by using the reversibly S-IS Dabiase and a brewing yeast.

1. Experimental

1.1 Enzyme and substrate

The crude amylase preparation used in this study was Dabiase K-27, provided by Daikin Kogyo Co. Immobilization of Dabiase on a reversibly S-IS carrier, methacrylic acid-methylmethacrylate copolymer, Eudragit L (Higuchi Shokai Co.), was carried out as described previously.²⁾ Raw corn starch (Wako Pure

Chemical Industries Co.) was used as an insoluble substrate for both saccharification and SSF.

1.2 Yeast and culture medium

Saccharomyces cerevisiae Kyokai No. 7 was used in this study. This yeast was obtained from the National Research Institute of Brewing. The medium for SSF contained 0.3% yeast extract (Oriental Yeast Kogyo Co.), 0.3% malt extract (Difco Lab.) and 0.5% Polypepton (Daigo Eiyo Co.). The initial pH of the medium was adjusted to 5.0. Potassium metabisulfite was added to the medium as an antiseptic at a level of 0.05%. The carbon source was 2% glucose for seed culture and 10% raw corn starch for SSF.

1.3 Repeated saccharification

Raw starch of 10% concentration was hydrolysed batchwise. The immobilized Dabiase was used repeatedly as follows. Using Dabiase immobilized on Eudragit L (Dabiase-Eudragit: D-E), a hydrolysis reaction was carried out at 30°C in a glass vessel containing 0.1 M acetate buffer (pH 5.0) with stirring. Every 48 h, the pH of the reaction mixture was adjusted to 3.5 by adding gradually 2 M-acetic acid solution and then the suspension was centrifuged at 3,000 rpm for 10 min to remove the glucose produced from the D-E in an insoluble state. The precipitate of D-E containing a small amount of residual substrate was mixed with a fresh 0.1 M-acetate buffer solution (pH 5.0) for redissolving. By adding resolubilized D-E to a fresh substrate suspension, the hydrolysis reaction was again started. Thus, the same procedure was repeated for reuse of D-E in the saccharification of raw starch.

1.4 Repeated-SSF

Repeated ethanol production from raw starch was performed by adding the yeast cells to the repeated-saccharification process described above. Kyokai No. 7 cells were inoculated at a level of $3-4 \times 10^8 \text{ cm}^{-3}$

Received September 29, 1988. Correspondence concerning this article should be addressed to M. Taniguchi.

into the medium containing D-E. Nitrogen gas was sparged at 0.2 vvm through the medium to maintain anaerobic conditions. The pH of the medium was kept constant at 5.0 by adding 1 N-NaOH solution with a peristaltic pump connected to a pH controller (HBU-11, DKK Co.). The other procedures for reuse of the yeast cells as well as the immobilized Dabiase were similar to those in the repeated-saccharification process.

1.5 Other analytical methods

The number of living cells of both yeasts was counted directly with a haemocytometer, using the methylene blue stain test. Glucose and protein were assayed as described previously.²⁾ Ethanol was determined with a gas chromatograph GC-8A (Shimadzu Seisakusho Co.) equipped with a FID detector.

2. Results and Discussion

2.1 Repeated saccharification

Figure 1A shows the repeated-batch saccharification of 10% raw starch by reuse of D-E. D-E was recovered after each batch by centrifuging. D-E used in each saccharification process was 0.400 kg/m³ in terms of the concentration of protein bound to the carrier. As shown in Fig. 1A, almost complete saccharification of raw starch to glucose was observed up to the second reaction cycle. However, the amount of glucose was reduced with increasing number of reaction cycles. The quantity of glucose produced in the fifth batch reaction was about 69% that in the first one. The total amount of glucose obtained from 0.500 kg raw starch in five consecutive batches of the saccharification was 0.422 kg. Based on the amount of protein, the ratio of D-E recovered after the fifth batch cycle was very high, 98% of the initially added one. Therefore, the gradual decrease in the amount of glucose produced when the reaction was carried out repeatedly seems to be attributable to inactivation of D-E during the operations for repeated use. Apart from its inactivation, D-E was found to be reutilized almost completely in the saccharification of raw starch.

2.2 Repeated SSF

Figure 1B shows the repeated SSF of 10% raw starch in a combination of D-E and Kyokai No. 7 cells. For comparison, the time courses of ethanol concentration, which was calculated on the basis of the assumption that glucose formed in the repeated saccharification (Fig. 1A) was converted completely to ethanol, are indicated by broken lines in Fig. 1B. Through the SSF, the ratio of living yeast cells to total yeast cells was maintained at more than 85%. Little or no glucose was detected except for the initial time of every batch. Accordingly, glucose produced by D-E was converted almost completely to ethanol, and

Fig. 1. Repeated-batch saccharification of 10% raw starch (A) and repeated SSF of 10% raw starch by a combination of D-E and Kyokai No. 7 (B)

consequently ethanol alone was produced at the end of every batch of SSF. Ethanol production from raw starch was found to be limited not by the fermentation step of glucose to ethanol with the yeast cells but by the saccharification stage of raw starch with D-E. The ethanol concentration (38 kg/m³) obtained in the fifth batch culture of SSF was approximately 80% that of the first batch culture. The ethanol concentration decreased gradually as the number of reaction cycles increased. However, the degree of decrease was less than that in the saccharification process. The total ethanol produced from 0.500 kg raw starch in the repeated SSF with D-E and the brewing yeast was 0.220 kg (Fig. 1B). The value was slightly higher than the quantity (0.215 kg) estimated on the basis of the assumption described above. The increase of ethanol yield in SSF seems to be due to less inhibition of ethanol for saccharifying activity as compared with that of glucose as reported previously.⁴⁾

The results described above show that D-E was successfully reusable in SSF as well as in saccharification of raw starch. The reaction procedure using such a reversibly S-IS enzyme is expected to be widely applied to other heterogeneous reaction systems.

Nomenclature

C_e	= ethanol concentration	[kg/m ³]
C_g	= glucose concentration	[kg/m ³]
RE	= recovery of enzyme	[%]
t	= reaction time	[d]

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