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Saccharification and Alcohol Fermentation in Starch Solution of Steam-Exploded Potato

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ABSTRACT

Steam explosion pretreatment of potato for the efficient production of alcohol was experimentally studied. The amount of water-soluble starch increased with the increase of steam pressure, but the amounts of methanol-soluble material and Klason lignin remained insignificant, regardless of steam pressure. The potatoes exploded at high pressure were hydrolyzed into a low molecular liquid starch, and then easily converted into ethanol by simultaneous saccharification and fermentation using mixed microorganisms: an amylolytic microorganism, *Aspergillus awamori*, and a fermentation microorganism, *Saccharomyces cerevisiae*. The maximal ethanol concentration was 4.2 g/L in a batch culture at 15 g/L starch concentration, and 3.6 g/L in a continuous culture fed the same starch concentration. In the fed-batch culture, the maximal ethanol concentration increased more than twofold, compared to the batch culture.

Index Entries: Saccharification; alcohol fermentation; starch solution; hydrolyzed potato; steam explosion.

NOMENCLATURE

A_{GA}, amylase activity (U/mL); C_B, ethanol concentration (g/L); C_G, glucose concentration (g/L); C_S, starch concentration (g/L); C_X cell concen-

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tration (g/L); P, steam pressure (MPa); t, time (h); W_K, ratio of weight of Klason lignin to dry weight of starch solution (−); W_M, ratio of weight of methanol soluble material to dry weight of starch solution (−); W_I, ratio of weight of insoluble starch to dry weight of starch solution (−); W_W, ratio of weight of water soluble starch to dry weight of starch solution (−); Y, ethanol yield (%); η, viscosity (Pa · s); ϕ_1 ratio of decrease in starch concentration by hydrolysis to initial starch concentration (−).

INTRODUCTION

Although water pollution by toxic materials is usually kept under the allowable contaminant levels in Japan, the water pollution by organic materials does not satisfy the Japanese environmental standard in one-fourth of lakes and rivers. The main reason for this pollution is that the sewage equipment needed for the treatment of domestic wastewater is inadequate, and the number and maintenance of septic tanks remains imperfect, although most wastewater from factories and industry is treated below the detection limit value. The treatment of leftover wastes discharged from home kitchens, dining rooms, and restaurants is important, because these leftover wastes sometimes generate a highly loaded organic wastewater that causes serious water pollution. Recently, steam explosion has proven attractive as a pretreatment for the effective utilization of plant biomass *(1–3)*. Several researchers *(4,5)* reported that wood and rice straw subjected to a steam hydrolysis at high temperature and pressure, followed by a sudden reduction in pressure, were separated into cellulose, hemicellulose, and lignin by comparatively easy extraction methods, causing an enhancement of the saccharification and fermentation of holocellulose. Therefore, since the starch is converted into low molecular substances by steam explosion more easily than the cellulose, this pretreatment may also be effective for enzymatic hydrolysis of starch.

In this article, the hydrolysis of starch, which is found in comparatively large quantities in leftover wastes, and its conversion to energy, were investigated using potatoes. The chemical characteristics, saccharification, and alcohol fermentation of liquefied potatoes hydrolyzed by steam explosion were evaluated. Experiments under varying conditions of the steam explosion were carried out to clarify the effects of steam pressure on pH, viscosity, and components of the exploded starch solution. Furthermore, the time courses of saccharification of liquid starch using *Aspergillus awamori*, the effect of steam explosion on the degradation ratio of starch, and the time courses of cell concentration, starch concentration, glucose concentration, and amylase activity from a batch culture to a continuous culture, were examined experimentally. In addition, a batch cul-

ture, a continuous culture, and a fed-batch culture were studied in the simultaneous saccharification and fermentation of liquefied potatoes using mixed microorganisms, *A. awamori* and *Saccharomyces cerevisiae*, to increase the efficiency of ethanol production from the exploded potatoes.

MATERIALS AND METHODS

Starch Solution

Potato was hydrolyzed using a steam explosion method. The apparatus for the steam explosion (Japan Chemical Engineering and Machinery, Osaka, Japan) consisted of a steam generator, a high-pressure reactor, a receiver, and a condenser with a silencing action *(5)*. The capacity of the reactor was 1.2 L, the highest pressure was 5.49 MPa, and the highest temperature was 275°C. The solid and liquid products of the exploded potato were recovered in a cyclone in the bottom of the receiver, and the gaseous products passed from the top of the receiver into the condenser. Steam explosions were conducted under various steam pressures, including 0.51 MPa (151°C), 1.02 MPa (179°C), 1.52 MPa (197°C), 2.03 MPa (211°C), 2.53 MPa (223°C), and 3.04 MPa (233°C), at a steaming time of 10 min.

The viscosity of the resultant starch solution was measured by a viscometer (Viscotester VT-04, Rion, Tokyo, Japan), and the value of pH was determined using a pH meter (HM-26S, Thoa Denpa Kogyo, Tokyo, Japan). The components of the obtained starch solution, such as water-soluble starch, methanol-soluble material, insoluble starch, and Klason lignin, were extracted and weighed by the Wayman method *(4)*.

Saccharification and Alcohol Fermentation of Starch Solution

A. awamori IAM 2101 was used as an amylolytic microorganism for the saccharification, and *S. cerevisiae* Hakken 2 was used as a fermentative microorganism for the alcohol fermentation. Cells were incubated in 1 L of standard medium containing 15 g/L starch, 5 g/L polypeptone, 2 g/L yeast extract, 1 g/L $KH_2PO_4 \cdot 7H_2O$, and 0.01 g/L $MgSO_4 \cdot 7H_2O$. The incubation was carried out at pH 5.0 and 30°C for 200 h, with 100 rpm agitation.

Cell concentration was estimated by measuring cell dry wt. Starch concentration was measured by the phenol–sulfuric-acid method *(6)*. Glucose concentration was determined by the mutarotase GOD method (Glucose C-test, Wako Pure Chemical, Osaka, Japan). Ethanol concentration was measured by gas chromatography (GC-8APF, Shimadzu, Kyoto, Japan). One U of amylase activity is defined as the amount of enzyme required to produce 1 μmoL glucose/min *(7)*.

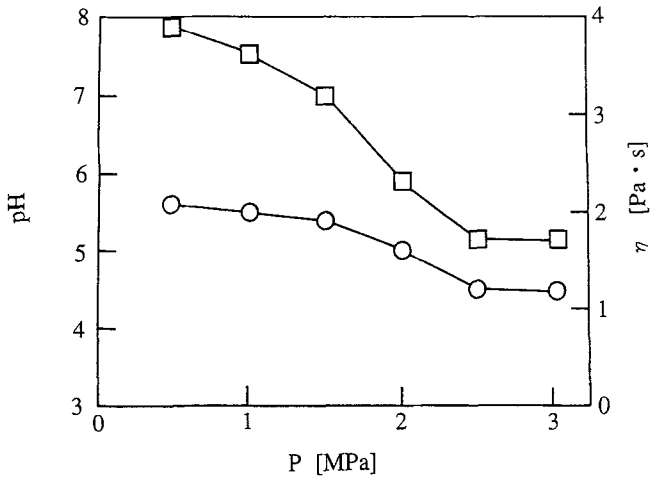

Fig. 1. Effect of steam pressure on pH and viscosity (η) in starch solution. ○, pH; □, viscosity (η).

RESULTS AND DISCUSSION

Characteristics of Starch Solution

Figure 1 shows the changes in the viscosity and pH of a starch solution during the hydrolysis of potatoes under various steam pressures. The viscosity of the exploded starch solution decreased with the increase of steam pressure, reaching 1.64 Pa·s beyond a pressure of 2.53 MPa (223°C). It suggests that the viscosity decreased because the starch solution was diluted with water from condensing steam, and because low molecular oligosaccharides were produced by the hydrolysis of starch. The pH decreased to 4.5 with the increase of steam pressure, presumably because of the formation of formic acid and levulinic acid by the starch degradation *(8,9)*.

Figure 2 shows the effect of steam pressure on the potato starch solution components after steam explosion. The ratios, W_W, W_M, W_K, and W_i, refer to the weights of water-soluble starch, methanol-soluble material, Klason lignin, and insoluble starch (with the solvents, such as water, methanol, and others) to the dry wt of the starch solution. The amount of soluble starch increased with the increase of steam pressure, reaching its maximal value, 0.43, at a steam pressure of 2.55 MPa (223°C). In contrast, the amount of insoluble starch decreased from 0.86 at lower steam pressures to 0.48 at higher steam pressures. The amount of methanol-soluble material and the amount of Klason lignin were 0.01 and 0.05, respectively, regardless of steam pressure. The amount of insoluble starch did not drop below 1.5 MPa (197°C), because the crystallinity of potato starch particles is comparatively high, and their smooth surface is resistant to hydrolysis *(10,11)*.

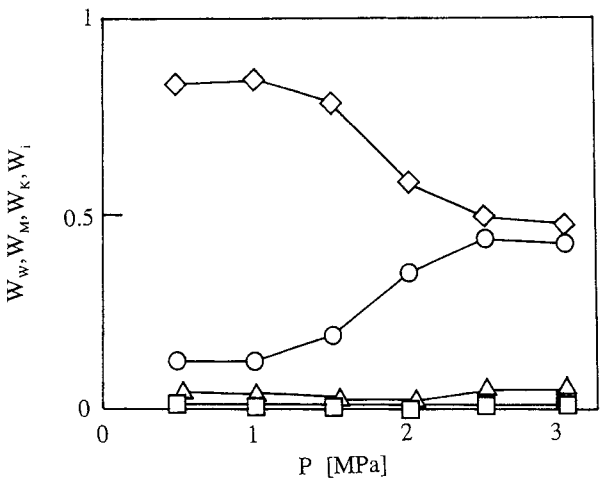

Fig. 2. Components of starch solution at different steam pressures. ○, ratio of weight of water-soluble starch to dry weight of starch solution (W_W); □, ratio of weight of methanol-soluble material to dry weight of starch solution (W_M); △, ratio of weight of Klason lignin to dry weight of starch solution (W_K); ◇, ratio of weight of insoluble starch to dry weight of starch solution (W_i).

Saccharification of Starch Solution

Figure 3 shows the batch culture by *A. awamori*; it received a steam explosion at a steam pressure of 2.53 MPa (223°C). The starch was degraded by the amylase produced by *A. awamori*, and the starch concentration decreased from 15 g/L at the start of incubation to 2 g/L at an incubation time of 80 h. Two g/L of undegraded starch remained, probably because the amylase produced by *A. awamori* could not degrade glucoside linkages, which contain phosphoric acid *(12–16)*. Fujita et al. *(17)* reported that about 90% of the starch was degraded in mold-treated starch wastewater. In this experiment, the ratio of the decrease in the starch concentration by hydrolysis to the initial starch concentration was about 87%, which very closely approached that of commercial soluble starch, 87.3%. The glucose concentration increased with the increase of incubation time, reaching 6.1 g/L at an incubation time of 40 h, because the production rate of glucose by *A. awamori* was higher than the consumption rate of glucose by the cells; it then decreased as a result of increases in glucose consumption proportional to increases in cell concentration. The amylase activity, which increased with the increase of cell concentration, resulted in a constant value similar to that of cell concentration after an incubation time of 60 h.

Figure 4 shows the time courses of starch cultures using *A. awamori* in the degradation of liquid potato exploded under various steam pressures. The experimental values, marked by the solid circles, represent the changes in starch concentration in the degradation of chopped potatoes

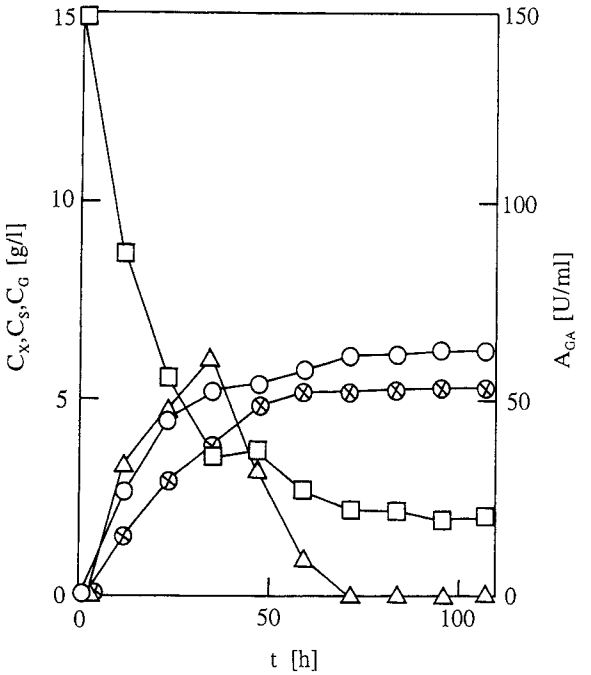

Fig. 3. Saccharification of starch solution by *A. awamori* in a batch culture. ○, cell concentration (C_X); □, starch concentration (C_S); △, glucose concentration (C_G); ⊗, amylase activity (A_{GA}).

without a steam explosion treatment; the initial starch concentration was adjusted to that of exploded potato: 15 g/L, by dilution. The time course of potato starch concentration, exploded at a steam pressure below about 1 MPa (179°C), was found to be very similar to that of untreated potato; the starch concentration decreased to 7–8 g/L, and became constant at an incubation time of about 70 h. The amount of degraded starch at a 2.03 MPa (211°C) was almost equal to the amounts obtained at 2.53 MPa (223°C) and 3.04 MPa (233°C). Beyond 2.03 MPa (211°C), the starch concentration remained constant, about 2 g/L, because they had reached their degradability limit. These results suggest that the final starch concentration at a steam pressure beyond 2 MPa (211°C) was much lower than that at a steam pressure below 1 MPa (179°C), but a further increase of steam pressure could not lower the final starch concentration.

Figure 5 shows the ratio of the decrease in the starch concentration by hydrolysis to the initial starch concentration, Φ, and the steam pressure at a steaming time of 10 min. The ratio of the decrease in the starch concentration was 0.5 at a steam pressure of 1 MPa (179°C), and then increased to 0.87 at a steam pressure of more than 2.53 MPa (223°C). From this result, it

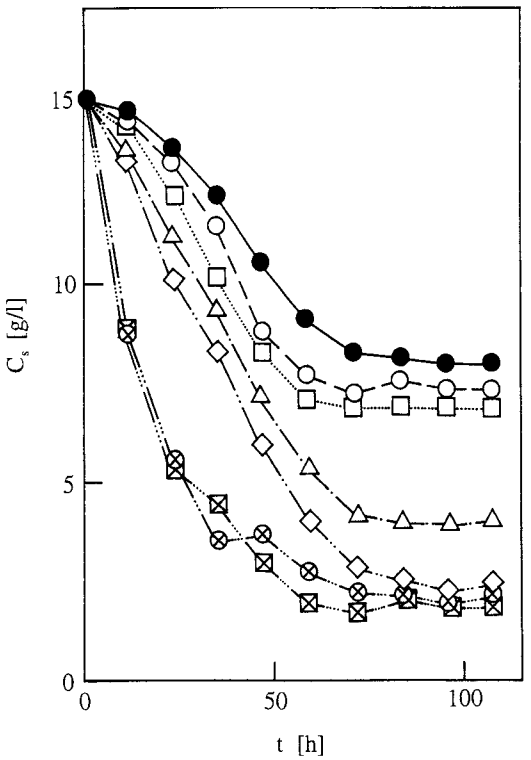

Fig. 4. Degradation of starch solutions by *A. awamori* in a batch culture. Steam pressure: ●, 0 MPa; ○, 0.51 MPa (151°C); □, 1.02 MPa (179°C); △, 1.52 MPa (197°C); ◇, 2.03 MPa (211°C); ⊗, 2.53 MPa (223°C); ⊠, 3.04 MPa (233°C).

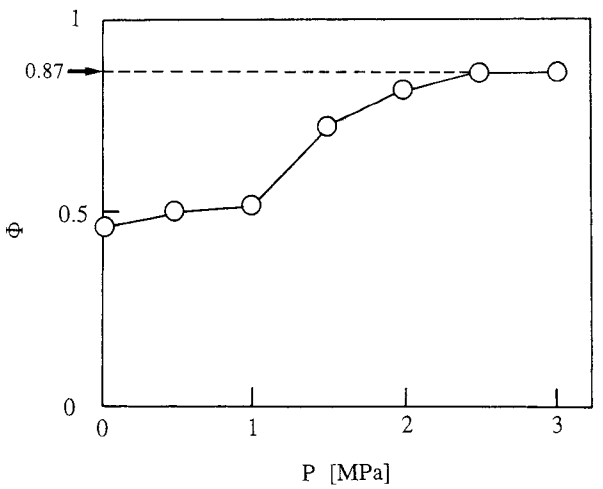

Fig. 5. Relationship between steam pressure (P) and ratio of decrease in starch concentration by hydrolysis to initial starch concentration (ϕ).

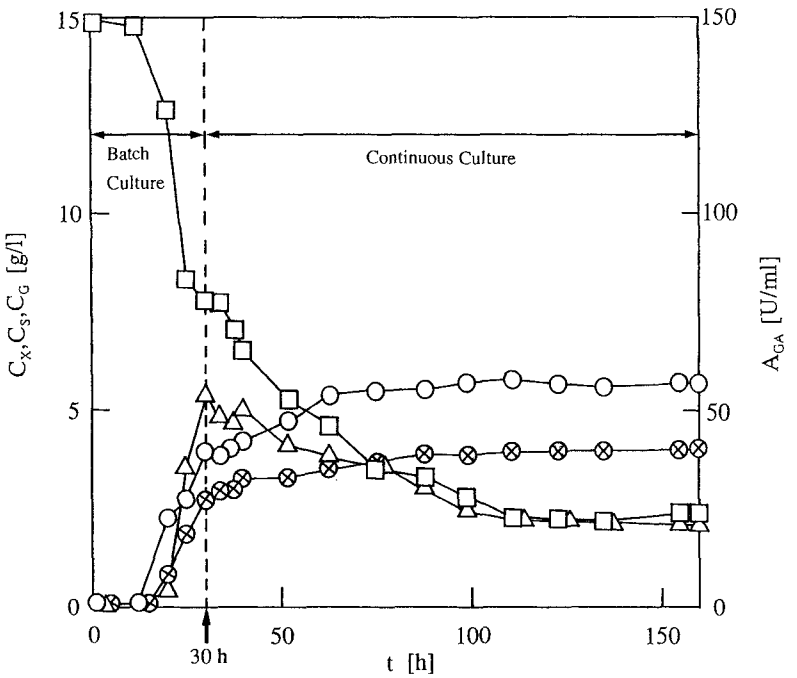

Fig. 6. Saccharification of starch solution by *A. awamori* during initial batch culture and its transition to a continuous culture. ○, cell concentration (C_X); □, starch concentration (C_S); △, glucose concentration (C_G); ⊗, amylase activity (A_{GA}).

was found that the steam explosion at 2.53 MPa (223°C) was the most effective for increasing the ratio of the decrease in the starch concentration with low energy consumption.

Figure 6 shows the dynamic behaviors of cell growth, enzyme production, and substrate consumption during initial batch culture and its transition to a continuous culture using a starch solution exploded at 2.53 MPa (223°C), and *A. awamori*. A batch culture of cells, incubated logarithmically for 30 h, was changed to a continuous culture at a dilution rate of 0.05/h by adding a 15 g/L potato starch solution continuously. In the batch culture with a comparatively low cell concentration, the starch concentration decreased rapidly, and, in contrast, the glucose concentration increased. After change to a continuous culture, the starch concentration decreased slowly to 2 g/L, and the glucose concentration decreased gradually to 2 g/L. The cell concentration and the amylase activity increased, even after the transfer, and then reached constant values at an incubation time of about 70 h after the transfer. The amylase activity and the ratio of the decrease in the starch concentration were about 40 U/mL and 85%, respectively, in a steady state, by the transfer from a logarithmic batch culture with 15 g/L of starch solution to a continuous culture using *A. awamori*.

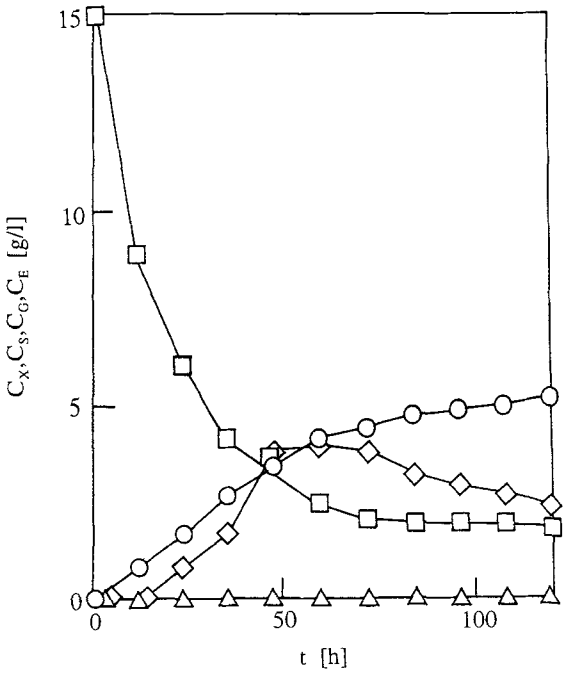

Fig. 7. Simultaneous saccharification and fermentation of starch solution by mixed microorganisms in a batch culture. ○, cell concentration (C_X); □, starch concentration (C_S); △, glucose concentration (C_G); ◇, ethanol concentration (C_E).

Alcohol Fermentation of Starch Solution

The glucose, hydrolyzed from starch by amylase, is converted into ethanol by *S. cerevisiae*. Figure 7 shows the results of simultaneous saccharification and fermentation of starch solution exploded at a steam pressure of 2.53 MPa (223°C), using *A. awamori* and *S. cerevisiae*. Since each concentration of *A. awamori* and *S. cerevisiae* could not be measured directly in the mixed culture, the total weight of the cells was measured after freeze-drying. The starch concentration was lowered from its initial concentration, 15 g/L, to about 15% of its initial value, at an incubation time of 80 h. Afterward, it became a constant value. The time courses of starch concentrations in the simultaneous saccharification and fermentation of starch, using mixed microorganisms, showed tendencies toward starch hydrolysis similar to those using *A. awamori;* the final starch concentrations were the same values: about 2 g/L. This result indicated that the ethanol produced did not inhibit the hydrolysis of starch by the amylase produced by *A. awamori*. The glucose in the simultaneous saccharification and fermentation was completely consumed, because it was converted into ethanol by *S. cerevisiae*. The ethanol concentration increased rapidly, reaching its maximal value, 4.2 g/L, at 60 h, and then decreased gradually. The decrease in

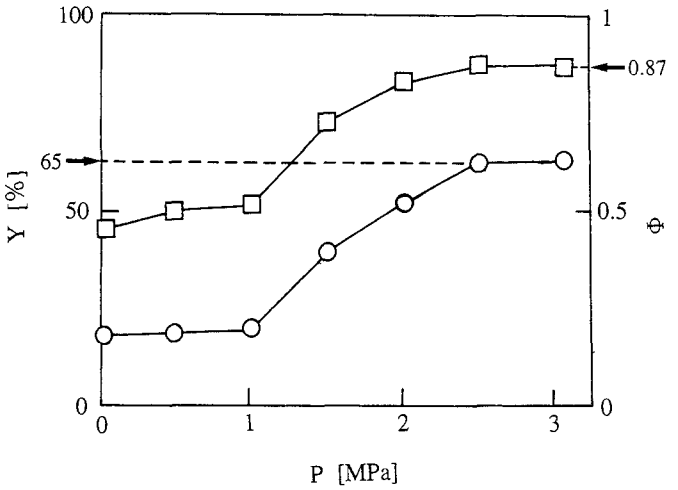

Fig. 8. Effect of steam pressure on ethanol yield (Y), and ratio of decrease in starch concentration by hydrolysis to initial starch concentration (ϕ). ○, ethanol yield (Y): □, ratio of decrease in starch concentration by hydrolysis to initial starch concentration (ϕ).

the ethanol concentration resulted in the consumption of ethanol by *A. awamori* as a carbon source. This result suggests that the simultaneous saccharification and fermentation using *A. awamori* and *S. cerevisiae* is an effective method for the conversion of starch into ethanol, and that a fed-batch culture with membrane filters for removing ethanol from the medium is the most practical for ethanol production (18,19).

Figure 8 shows the ratio of the decrease in the starch concentration and the ethanol yield under various steam pressures in the simultaneous saccharification and fermentation. Since the relationship between the ratio of the decrease in the starch concentration and the steam pressure is almost equal to that of the hydrolysis of starch by *A. awamori*, as shown in Fig. 5, it was found that neither cell growth nor ethanol production by *S. cerevisiae* affected the starch hydrolysis. The ratio of the decrease in the starch concentration and the ethanol yield increased with the increase in steam pressure, reaching their maximal values, 0.87 and 65%, at a steam pressure of 2.53 MPa (223°C).

Figure 9 shows the dynamic behaviors of cell growth, ethanol production, and substrate consumption during initial batch culture and its transition to a continuous culture in the simultaneous saccharification and fermentation of a starch solution, using mixed microorganisms, *A. awamori* and *S. cerevisiae*. The mixed microorganisms were changed from a batch culture to a continuous culture at a dilution rate of 0.05 h^{-1}, after an incubation time of 30 h, after which the mixed microorganisms grew logarithmically in the batch culture. The cell concentration, the starch concen-

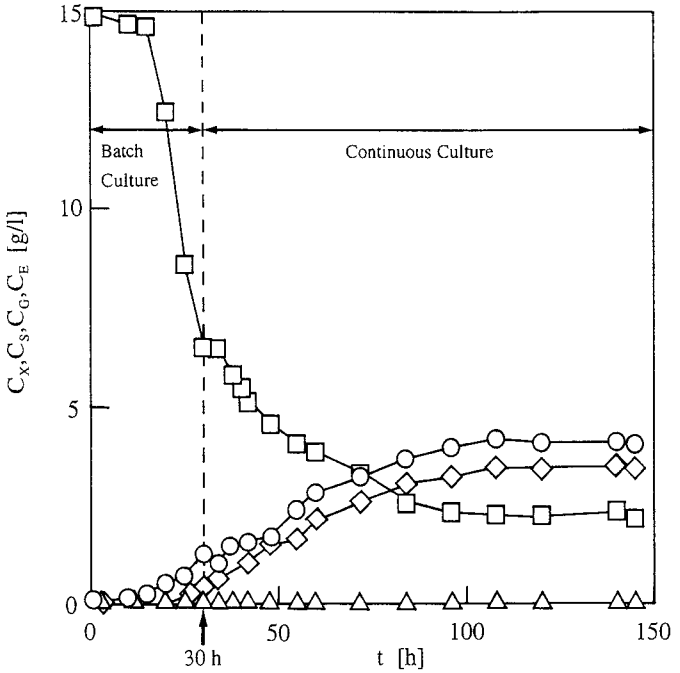

Fig. 9. Simultaneous saccharification and fermentation of starch solution by mixed microorganisms during initial batch culture and its transition to continuous culture. ○, cell concentration (C_X); □, starch concentration (C_S); △, glucose concentration (C_G); ◇, ethanol concentration (C_E).

tration, and the ethanol concentration reached their constant values, 4.2, 2.0, and 3.6 g/L, respectively, in about 80 h after the transfer. The glucose concentration was almost 0 throughout the incubation, probably because of the rapid consumption of glucose. This result shows that the ethanol production from the starch solution using *A. awamori* and *S. cerevisiae* was very effective, not only in a batch culture, but also in a continuous culture.

Figure 10 shows the dynamic behaviors of cell growth, ethanol production, and substrate consumption in a fed-batch culture of starch solution using mixed microorganisms, *A. awamori* and *S. cerevisiae*. Since the ethanol concentration reached its maximal value after 60 h in the batch culture, as shown Fig. 7, the starch concentration was adjusted to 15 g/L by adding a starch solution at 60 h and at 120 h. The starch concentration decreased with growth of mixed microorganisms, and ethanol production reached 4 g/L in the first incubation. In the second incubation, when the starch concentration was adjusted to 15 g/L by adding starch solution, the starch concentration decreased to 4.2 g/L. In the third incubation, the final starch concentration decreased to only 5.4 g/L, and its concentration gave the highest value of the three incubations. The ethanol concentration reached about 9 g/L after 200 h of incubation in the fed-

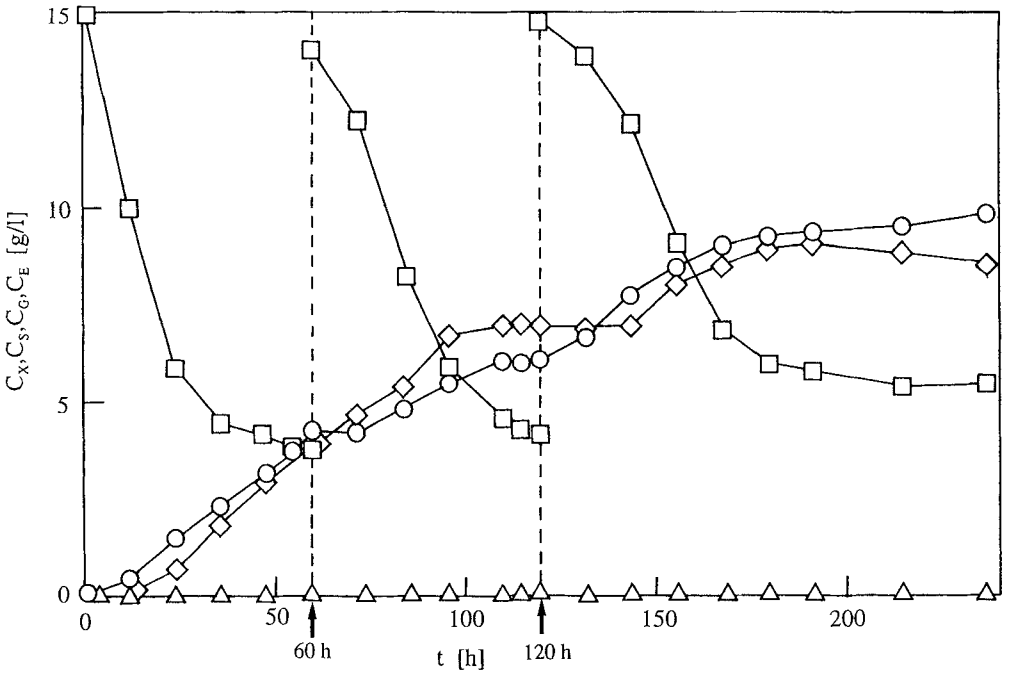

Fig. 10. Simultaneous saccharification and fermentation of starch solution by mixed microorganisms in a fed-batch culture. ○, cell concentration (C_X); □, starch concentration (C_S); △, glucose concentration (C_G); ◇, ethanol concentration (C_E).

batch culture by adding starch solution at 60 h and at 120 h. These studies were performed to demonstrate effective systems for conversion of starch into ethanol using steam explosion and simultaneous saccharification and fermentation with mixed microorganisms, and future research will be dedicated to the optimization of fed-batch culture for the mass production of ethanol from hydrolyzed starch solutions.

CONCLUSIONS

The characteristics of liquid potato hydrolyzed by steam explosion and ethanol conversion by simultaneous saccharification and fermentation system were investigated experimentally. The following findings were obtained:

1. The potato was hydrolyzed by the steam explosion, causing a significant decrease of pH and viscosity in the potato.
2. The amount of water-soluble starch in the hydrolyzed starch solution increased with the increase of steam pressure, reaching its maximal value, 0.43, at a steam pressure of 2.53 MPa (223°C).

3. About 87% of the starch solution hydrolyzed at a steam pressure of 2.53 MPa (223°C) was degraded by *A. awamori*.
4. The simultaneous saccharification and fermentation using mixed microorganisms, *A. awamori* and *S. cerevisiae*, was effective in the rapid and efficient conversion of starch into ethanol.
5. The fed-batch culture to feed starch solution consecutively could obtain a high concentration of ethanol.

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