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Short Communication

Hydrolysis of Starch by α-Amylase and Glucoamylase in Supercritical Carbon Dioxide

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The enzymes α-amylase and glucoamylase used in starch hydrolysis were found active in the supercritical carbon dioxide solvent. Higher hydrolysis of starch slurry in supercritical CO_2 was achieved by operating the reactor for the first two hours with α-amylase and to subsequent addition of glucoamylase for continued hydrolysis.

The use of enzymes as catalysts in nonaqueous media has been frequently described in the literatures (1, 10). Many enzymes show higher activity in mixtures of organic solvents than in pure water (7). Organic solvents present certain advantages such as stabilization of enzymes, and dissolution of hydrophobic compounds. An enzyme in a nonaqueous solvent may experience solvent/enzyme interactions and thus show higher activity yielding a higher rate of reaction. Other potential advantages include facilitated separation steps and enhanced enzyme specificity.

An interesting class of alternate solvents is supercritical fluids. Supercritical fluids provide a number of advantages over conventional liquid solvents. Among these advantages are high diffusivities, low viscosities and also the ability to manipulate solubilities by changing either temperature and/or pressure.

Supercritical carbon dioxide exhibits properties similar to organic solvents. Carbon dioxide has a critical temperature of 31.1°C, preventing thermal denaturation or decomposition of heat labile organics. Because of its nontoxicity, non-flammability, availability, and low cost the application of supercritical CO_2 in many physico-chemical processes has been widely investigated. However, the enzymatic reactions in supercritical CO_2 have been scarcely studied (3, 5, 6, 8, 9).

In this study the hydrolysis of starch using supercritical CO_2 was examined with an objective to explore an efficient/economic process.

MATERIALS AND METHODS

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Key words: starch hydrolysis, α-amylase, glucoamylase, supercritical carbon dioxide

Materials

Potato starch with an approximate purity of 99% and glucoamylase (6,100 U/ml) from *Asperigillus niger* were supplied by Sigma Co., and α-amylase (20,000 DU) from *Bacillus licheniformis* J-7 was purchased from Taepyeongyang Chemical Co..

Experiments

The experiment was conducted in the batch reactor system shown in Fig. 1. The reaction vessel (250 ml working volume) made of SS 316 and designed to sustain the pressure of up to 200 atm, was placed in a digital water stirring bath equipped with a temperature controller. The constant temperature inside the vessel was maintained within the accuracy of ±0.5°C.

The reaction vessel was filled with the desired amount of raw starch slurry and enzymes in a one step reaction and the air was flushed out with carbon dioxide. At

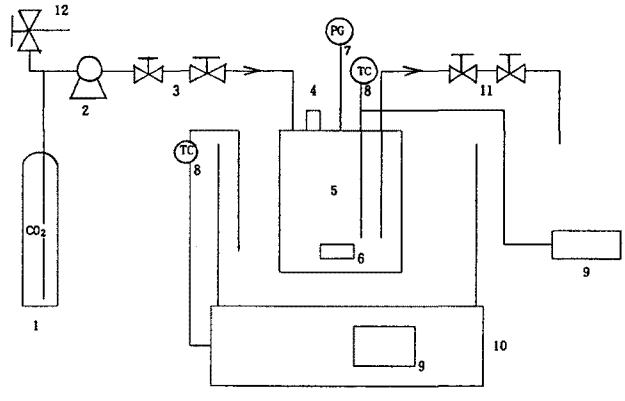

Fig. 1. Schematic diagram of apparatus.
1. CO_2, 2. Liquid Pump, 3. CO_2 Input Line, 4. Vent, 5. Reactor, 6. Magnetic Bar, 7. Pressure Indicator, 8. Thermocouple, 9. Temperature Indicator, 10. Digital Stirring Bath, 11. Sampling valve, 12. Purge valve.

a constant temperature, carbon dioxide of 99.99% purity from a dip-tube container was pumped into the reaction vessel using a high pressure liquid pump until the desired pressure was reached and the pressure was maintained with a back pressure regulator.

In the two step reaction, the reaction was carried out with α-amylase for liquefaction for two hours at specified conditions. The reaction vessel was then depressurized to an ambient condition for a successive saccharification by adding glucoamylase.

Samples were taken for analysis at each hour of a six hour operation using two sampling valves.

Analytical Methods

The reducing sugar concentrations were determined by the DNS method (4), measuring the absorbance with a spectrophotometer (Shimadzu UV-120-01) at 540nm using glucose as the standard.

RESULTS AND DISCUSSION

The conventional hydrolysis of starch is a multistage process involving the gelatinization of starch slurry by cooking it at a high temperature followed by liquefaction and saccharification by the enzymes, α-amylase and glucoamylase. In this study the experiments were conducted in the supercritical CO_2 using starch slurry without the gelatinization step.

The Effect of Pressure

Shown in Fig. 2. is the effect of pressure on the production of reducing sugar. The production of reducing sugar was increased by increasing the pressure of carbon dioxide. Higher concentrations in supercritical CO_2 at 30°C is believed due to the enhancement of solubility of the substrate.

The concentration of reducing sugar at the pressure of 80 atm was 5-fold higher than that obtained at 1 atm and this result is in accordance with the previous result (2).

The effect of temperature at an elevated pressure, 90 atm is shown in Fig. 3. The production of reducing sugar was increased by elevating the reaction temperature up to 70°C. However, at the temperature exceeding approximately 85°C, the concentration of reducing

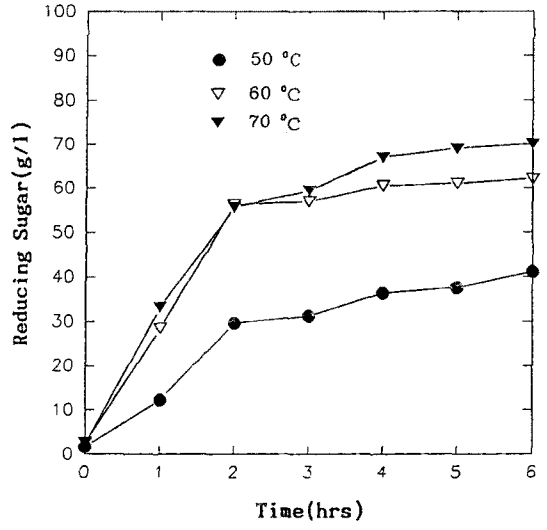

Fig. 3. The effect of temperature on the production of reducing sugar.
(100 g/l raw starch, at 90 atm, 0.5 ml α-amylase+0.5 ml glucoamylase).

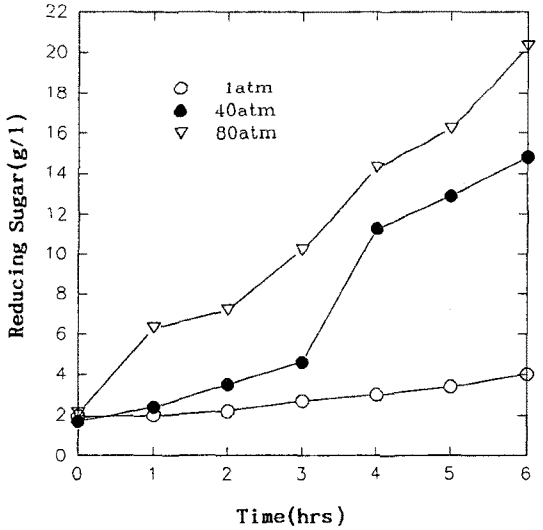

Fig. 2. The effect of pressure on the production of reducing sugar.
(100 g/l raw starch, at 30°C, 0.5 ml α-amylase+0.5 ml glucoamylase).

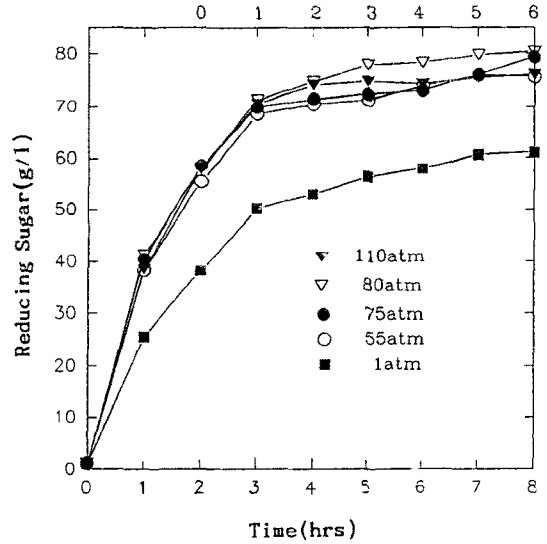

Fig. 4. The effect of pressure on the production of reducing sugar.
(100 g/l raw starch, 0.5 ml α-amylase+0.5 ml glucoamylase).

Table 1. Typical results obtained at specified conditions in the one step and two step reactions.

One-step reaction				Two-step reaction α-amylase at 70°C Glucoamylase at 50°C	
30°C		90 atm			
1 atm	4.0 g/l	50°C	41.1 g/l	1 atm	61.3 g/l
80 atm	21.5 g/l	70°C	70.1 g/l	80 atm	80.4 g/l

Enzymes: 0.5 ml α-amylase+0.5 ml glucoamylase, Substrate: 100 g/l raw starch.

sugar decreased(not shown in the figure) possibly because of reduced glucoamylase activity at the temperature above the optimum value, 60°C (11) while the α-amylase activity did not change.

Two Step Reaction

The hydrolysis reaction was carried out in two successive steps; the liquefaction of starch by α-amylase in the condition of supercritical carbon dioxide for two hours at the temperature of 70°C and the successive saccharification by adding glucoamylase at a pressure indicated and at a temperature of 50°C. The results are shown in Fig. 4 indicating a 31% increase in the concentration of reducing sugar at a pressure of 80 atm, in comparison to that obtained in the reaction carried out at ambient pressure throughout the course of the reaction. However, the reducing sugar was almost the same concentration at the pressure of 55 atm to 110 atm. In the two step reaction the effect of pressure in the range of 55 to 110 atm was not significant. It is believed that the enhanced enzyme activities at the elevated temperatures were the primary factors along with the high pressure in increasing the reaction rate, whereas the solubility of starch was rate limiting at a substantially lower temperature (Fig. 2).

Typical results obtained at specified conditions in the one step and two step reactions are summarized in Table 1.

Acknowledgement

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