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Kinetics and Rate of Enzymatic Hydrolysis of Cellulose in Supercritical Carbon Dioxide

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Abstract−Experiments were carried out on the application of supercritical fluid to the hydrolysis of cellulose by the enzyme, cellulase. The stability of cellulase was sustained at the pressures of up to 160 atm for 90 min at 50 °C in supercritical carbon dioxide. In the hydrolysis of cellulose the glucose yield was 100% at supercritical condition. Kinetic constants of hydrolysis at supercritical condition were increased as compared to those at atmospheric condition. The hydrolysis reaction was found competitively inhibited by glucose at supercritical condition.

Key words: Cellulase, Supercritical Carbon Dioxide, Kinetic Constant, Competitive Inhibition, Enzyme Stability

INTRODUCTION

Supercritical fluids serve as a particularly interesting class of solvents which may be used for enzyme-catalyzed reactions. Biochemical reactions in supercritical fluids were demonstrated first in 1985 by Randolph et al. In their pioneering work where the enzyme, alkaline phosphatase was found maintain its activity. Although the exposure of manu enzymes to supercritical fluids caused the decreases in their activities [Marty et al., 1990], some enzymes were not deactivated [Taniguchi et al., 1987]. Motivated by these observations along with favorable properties of supercritical fluids for the extensive studies on the enzymatic reactions at supercritical conditions have been carried out [Hammond et al., 1985; Nakamura et al., 1985; Chi et al., 1988; Rafi et al., 1986; Randolph et al., 1988; Lee et al., 1993].

In this study the hydrolysis of cellulose by cellulase was performed in supercritical carbon dioxide and the kinetic constants in the rate expression was evaluated.

Substantial improvement in the yield was possible in supercritical medium as compared to that obtained in the hydrolysis carried out in atmospheric condition [Jung et al., 1986; Kim et al., 1987; Hong et al., 1987].

MATERIALS AND METHODS

Cellulose (Avicel) was purchased from Fluka Co. (Switzerland) and cellulase (80,000 unit g^{-1}) from *Tricoderma reesei* were supplied by Taepyeongyang Chemical Co. (Korea). The experiments were conducted in a batch reactor system shown in elsewhere [Lee et al., 1994]. The reaction vessel (250 ml working volume) made of SS 316 and designed to sustain the pressure of up to 250 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 enzyme and Avicel with 0.1 M sodium acetate buffer (pH 4.8) and flushed out air with carbon dioxide (99.99% of purity) from a dip-tube container. The reaction vessel was enclosed and carbon dioxide was immediately pumped into the reaction vessel by using a an LDC Analytical Minipump (Gearmotor, USA) until the desired pressure was reached and the pressure was maintained with a back pressure regulator (Tescom Co., USA). The reaction mixture was agitated by a magnetic stirrer. After a designated length of time kept at a certain pressure and temperature, the sample was immediately taken out through the sampling line and then analyzed to measure the glucose concentration. The stability of enzymes in supercritical carbon dioxide was determined by incubating an enzyme in each supercritical condition. The relative enzyme activity was measured at optimum condition of atmospheric pressure. The concentration of glucose in the solution was determined by a glucose analyzer (YSI 1500, USA).

RESULTS AND DISCUSSION

1. Stability of Cellulase

Experiments were carried out to test the stability of cellulase in supercritical carbon dioxide. As shown in Fig. 1, the activity of cellulase was found sustained at the a pressure of up to 160 atm. The activity, however, decreased slightly at the a pressure of 200 atm. This is believed due to the structural change of cellulase and also to the decrease in pH caused by substantial amount of carbon dioxide fed to maintain high pressure as reported by Lee et al. [1994]. This observation is also in accordance with the experimental results of Taniguchi et al. [1987]; over 90% of the activities of enzymes tested under supercritical condition were sustained. Marty et al. [1990], however, reported the decrease in enzyme activity in some other cases because of the effect of pressure on the surface of enzyme. No change of activity was observed at the temperature of up to 50 °C which 50 °C, which was an optimum temperature for the cellulase activity at ambient pressure. At higher temperature, however, the activity rapidly decreased down to 50% at 70 °C. The cellulase activity was mostly maintained at the pressure of 120 atm

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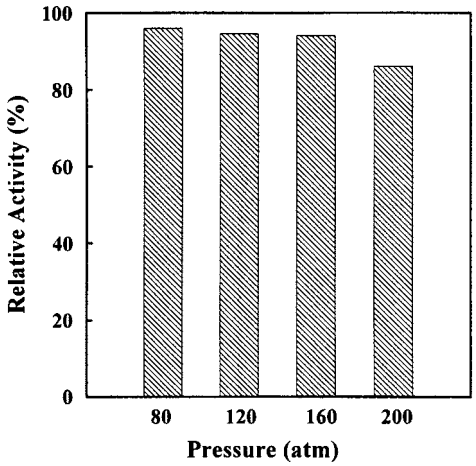

Fig. 1. Effect of pressure on cellulase stability under supercritical carbon dioxide at 50 °C for 60 min.

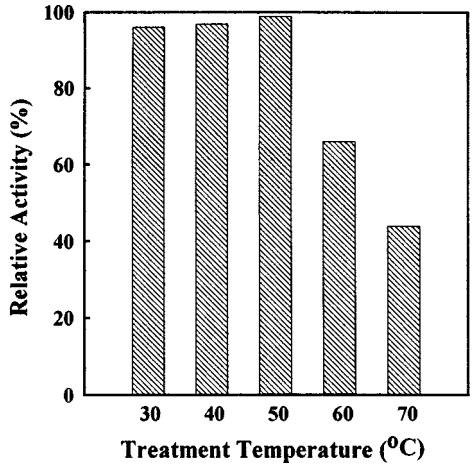

Fig. 2. Effect of treatment temperature on cellulase activity under supercritical carbon dioxide at 120 atm for 90 min.

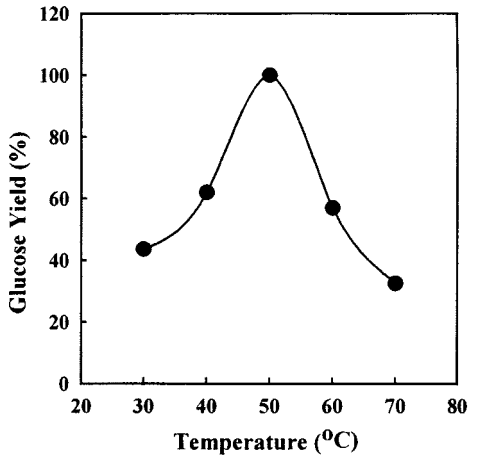

Fig. 3. Effect of temperature on Avicel hydrolysis under supercritical carbon dioxide at 120 atm for 90 min.

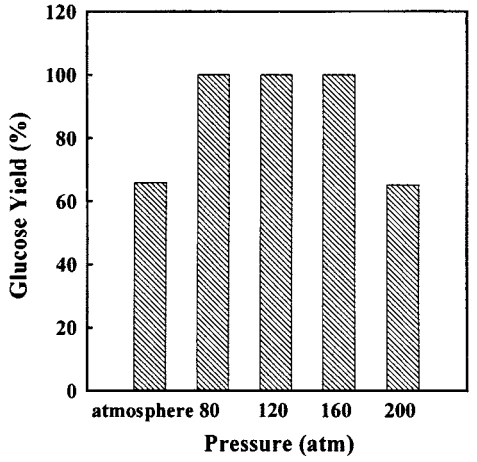

Fig. 4. Effect of pressure on Avicel hydrolysis under supercritical carbon dioxide at 50 °C for 90 min.

for 90 min and at 50 °C (Fig. 2).

2. Hydrolysis of Cellulose

Hydrolysis of cellulose (Avicel) by cellulase in supercritical carbon dioxide was performed under the conditions at which cellulase was stable sustaining its activity. In the hydrolysis the glucose yield showed its maximum (Fig. 3) at 50 °C, the temperature at which the activity of cellulase was the highest. As was in the hydrolysis at an ambient pressure, the glucose yield increased with the temperature of up to 50 °C and then decreased.

This is an expected result, as the same trend was observed in the activity of cellulase. Kamat et al. [1995] also has reported that the activities of certain enzymes tested showed the maximum values at the a temperature of around 45 °C. Since the pressure is of prime importance in the application of supercritical fluids the effects of pressure were examined. Shown in Fig. 4 are the results of hydrolysis of cellulose carried out at 50 °C and at specified pressures for 90 min. The cellulose (Avicel) of 20 g l^{-1} concentration was almost completely hydrolyzed yielding 100% of glucose, up to the pressure of 160 atm. At 200 atm, however, the yield of glucose decreased down to 65% although cellulase remained stable but with decreased activity. Marty et al. [1990] reported the effect of pressure in the structural deformation of enzyme surface resulting in the decrease in the activity. Taniguchi et al. [1987] performed the experiments on the effects in carbon dioxide medium. Experiments were carried out on the activity of nine commercial enzymes, at 30 °C, 20.3 MPa and 0.1% water+3% ethanol for 1 hr in carbon dioxide.

They found that the activities of enzymes were sustained. However, the activity of lipase decreased by 67% when the water contents content was increased by up to 50%. The rate of cellulose-cellulase hydrolysis is determined not only by the activities of cellulase in supercritical carbon dioxide medium but the mass transfer limitation because of the heterogeneous nature of solid-liquid systems; the rate is determined not only by the activity, the pressure, the temperature and the water content but the medium (solvent) and the mass transfer characteristics [Zheng et al., 1996]. Lee et al. [1994] observed that the activity of enzyme used decreased by over 30% under carbon dioxide medium as compared to that under pressurized nitrogen.

They confirmed that the decrease in pH rather than structural change of enzyme resulted in such observation. This is in accordance with the work of Dordick [1989] where both the activity and

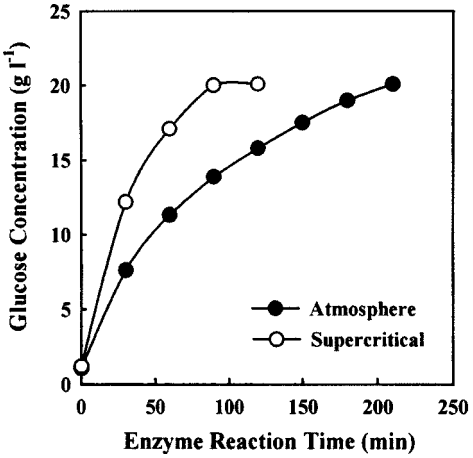

Fig. 5. Profiles of enzymatic hydrolysis of Avicel in supercritical (120 atm, 50 °C) and atmospheric conditions.

stability of enzyme were found decreased with pH change. Blanch et al. [1988] reported that the activity and stability of cholesterol oxidase were sustained at 35 °C and in the pressure range of 0.1-11.3 Mpa, while Kasche et al. [1988] observed that the activity of oligomer enzyme decreased but that of monomer enzyme was not affected in carbon dioxide medium. As shown in Fig. 5, the glucose yield at 50 °C and 120 atm reached nearly 100% for 90 min of reaction time in supercritical condition, whereas 210 min of reaction time was required at atmospheric condition. In the work of Lee et al. [1993] where the hydrolysis of starch was performed, the same trend was observed. The rate of hydrolysis is believed to be enhanced possibly due to the decrease in mass transfer resistance [Zheng and Tsao, 1996], while the activity and stability of cellulase is sustained during the hydrolysis. The increase in the rate of hydrolysis in supercritical carbon dioxide is due to decrease of mass transfer resistance between the substrate and the enzyme in the solid-liquid heterogeneous system, which also reduce reduces the inhibition of product, the glucose and due also to the change in the surface structure of cellulose which increases the accessibility of enzyme [Zheng et al., 1995], which in turn resulted in the variation of enzyme kinetic parameters. This point should further be elucidated by detailed kinetic studies.

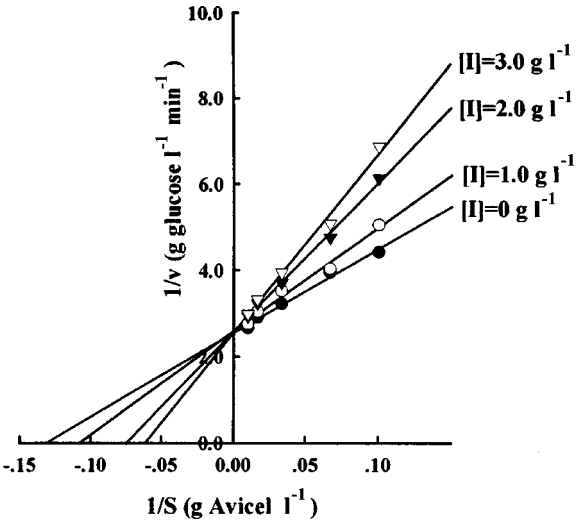

Fig. 6. A double-reciprocal plot of the initial rate subjected to product inhibition by glucose in the atmospheric condition.

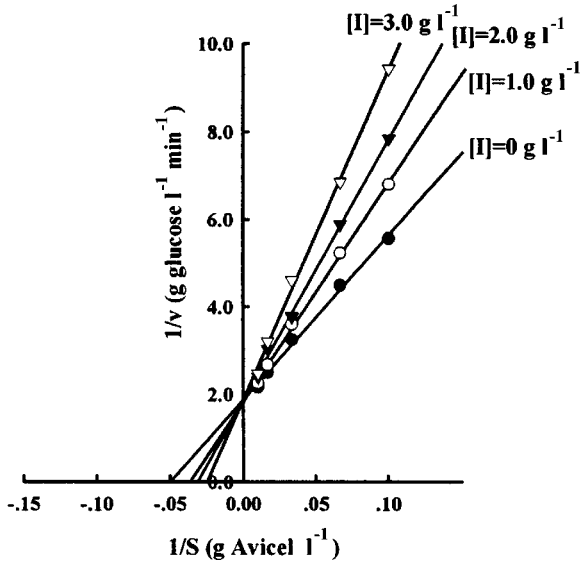

Fig. 7. A double-reciprocal plot of the initial rate subjected to product inhibition by glucose in the supercritical condition.

Table 1. Comparison of kinetic constants at atmosphere and in supercritical carbon dioxide

Condition for enzyme reaction	Kinetic constants		
	K_M (g l^{-1} min^{-1})	V_{max} (g l^{-1} min^{-1})	K_i (g l^{-1})
Atmosphere			
Supercritical CO_2			

3. Enzyme Kinetics

Fig. 6 and Fig. 7 show a double-reciprocal plots of the initial rate in the rate expression, $V=V_{max}\cdot[S]/K_M+[S]+([I]/K_i)$ subjected to product inhibition by glucose at atmosphere and in supercritical carbon dioxide, respectively. The hydrolysis reaction in both cases was inhibited competitively by glucose.

The kinetic constants of enzyme reaction at atmosphere and in supercritical carbon dioxide were calculated and compared in Table 1. The value of K_M for supercritical carbon dioxide is 2.8 times as high as that for atmospheric condition whichcondition, which is believed due to the decrease in the affinity between the substrate and the enzyme. The values of V_{max} and K_i for supercritical carbon dioxide also were higher than those for atmospheric condition, which is believed due to the decreases in mass transfer resistances of both the substrate and product. Due to higher values of V_{max} and K_i at supercritical condition as compared to those obtained at atmospheric condition, the overall hydrolysis rate are is enhanced in supercritical carbon dioxide in spite the increase in the K_M value. These results indicate that the mass transfer rather than the affinity is the rate limiting step in the heterogeneous hydrolysis reaction, leading to be athe conclusion that supercritical fluids could serve as a potential medium in achieving higher rate of cellulose hydrolysis.

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