Biotechnology and Bioprocess Engineering **2005**, 10: 52-59

Saccharification of Foodwastes Using Cellulolytic and Amylolytic Enzymes from *Trichoderma harzianum* FJ1 and Its Kinetics

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Abstract The study was targeted to saccharify foodwastes with the cellulolytic and amylolytic enzymes obtained from culture supernatant of *Trichoderma harzianum* FJ1 and analyze the kinetics of the saccharification in order to enlarge the utilization in industrial application. *T. harzianum* FJ1 highly produced various cellulolytic (filter paperase 0.9, carboxymethyl cellulase 22.0, β-glucosidase 1.2, Avicelase 0.4, xylanase 30.8, as U/mL-supernatant) and amylolytic (α-amylase 5.6, β-amylase 3.1, glucoamylase 2.6, as U/mL-supernatant) enzymes. The 23~98 g/L of reducing sugars were obtained under various experimental conditions by changing FPase to between 0.2~0.6 U/mL and foodwastes between 5~20% (w/v), with fixed conditions at 50°C, pH 5.0, and 100 rpm for 24 h. As the enzymatic hydrolysis of foodwastes were performed in a heterogeneous solid-liquid reaction system, it was significantly influenced by enzyme and substrate concentrations used, where the pH and temperature were fixed at their experimental optima of 5.0 and 50°C, respectively. An empirical model was employed to simplify the kinetics of the saccharification reaction. The reducing sugars concentration (X, g/L) in the saccharification reaction was expressed by a power curve ($X=K\cdot t^n$) for the reaction time (t), where the coefficient, K and n, were related to functions of the enzymes concentrations (E) and foodwastes concentrations (S), as follow: $K=10.894\cdot\mathrm{Ln}(E\cdot S^2)-56.768$, $n=0.0608\cdot(E/S)^{-0.2130}$. The kinetic developed to analyze the effective saccharification of foodwastes composed of complex organic compounds could adequately explain the cases under various saccharification conditions. The kinetics results would be available for reducing sugars production processes, with the reducing sugars obtained at a lower cost can be used as carbon and energy sources in various fermentation industries.

Keywords: *Trichoderma harzianum*, saccharification, lignocellulolytic enzymes, foodwastes, kinetics

INTRODUCTION

Lignocellulosic materials have an enormous potential as renewable carbon and energy sources. Therefore, their hydrolysis using multiple lignocellulolytic enzymes, such as cellulases, xylanases and amylases [3,13,25], has been extensively performed due to the cost benefit and lack of the process corrosion problem associated with acid or alkaline hydrolysis [25]. Lignocellulosic materials are usually converted to reducing sugars, like glucose and xylose, by enzymatic hydrolysis. The main potential applications of the hydrolysis of lignocellulosic materials are in the food, animal feed, textile, fuel and chemical industries, and the technology can also employed in parts of the paper and pulp, wastes management, medical/harmaceutical and pollution treatment industries *etc.* [3]. In addition, Korean foodwastes containing various lignocellulosic and amylosic materials are favorable candidates for the industrial application of fermentation if they can be hydrolyzed to lower molecular mass carbon and energy sources.

In Korea during 2001 year, foodwastes amounted to 11,237 ton/day, and were composed of vegetable (46%), grain (22%), fish and meat (16%) and fruit (16%) wastes discharged from restaurants, dinning halls, markets and households (http://www.foodwaste.or.kr). Foodwastes are the main source of decay, odor and leachate during collection and transportation due to the high volatile solids and moisture content of 85~95 and 75~85%, respectively. Most foodwastes are currently land-filled together with other wastes, resulting in various pollutant problems such as emanating odor, attracting vermin, emitting toxic gases, contaminating groundwater and wasting landfill capacity.

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This study focused on the recovery of useful resources, simultaneously satisfying the treatment of foodwastes. In the composition of foodwastes, polymers with a high reducing ability, that is, with large energy content, such as amylose, cellulose and hemicellulose were converted to glucose and xylose by enzymatic hydrolysis involving various lignocellulolytic enzymes. In the saccharification of foodwastes, a synergistic effect was observed when the enzymatic reactions of two or more enzymes were combined compared to the total activity by their individual actions [15]. Cellulases hydrolyzing cellulose especially, usually contain at least three major groups, with endoglucanase, exoglucanase, and β-glucosidase [10,14]. Also, xylanase and β-xylosidase for hemicellulose hydrolysis [26] and α-amylase, β-amylase, and glucoamylase for amylose hydrolysis [21,23] are cooperatively needed in the case of saccharification of foodwastes. The reducing sugars obtained from these hydrolyzing reactions could be utilized as carbon and energy sources in the fermentation industry, such as ethanol [25] and lactic acid production [2].

Many researchers have attempted to analyze the enzymatic hydrolysis of lignocellulosic materials. These studies have mostly been performed based on the substrate characteristics [4,5,32], product inhibition [12], deactivation of enzyme [6], adsorption of enzyme to cellulose [17-20] and the multiplicity of the enzyme components [10], and so on. However, it is difficult to sufficiently explain the enzymatic hydrolysis of many lignocellulosic materials in terms of any one of those mechanisms alone or even their combination. To simplify the complicating kinetic analysis of the hydrolyzing reaction, Wu and Ju [30] reported an empirical model equation for the relationship between the enzyme activity and reaction time, and Ooshima *et al.* [19] that between the substrate concentrations and reaction time to consider enzyme-substrate adsorption.

This study focused on development of an economical and efficient recovery method or producing reducing sugars from Korean foodwastes for use as carbon and energy sources in various fermentation industries. To improve the yield and rate of enzymatic hydrolysis of foodwastes, effective saccharification factors, such as substrate concentration, enzyme concentration, reaction time, temperature and pH, will be experimentally optimized. For the kinetics of the enzymatic hydrolysis reaction, the empirical models concerning two variables, the enzyme and foodwastes concentration, will be developed and applied in these kinetics analysis.

MATERIALS AND METHODS

Microorganism and Materials

Trichoderma harzianum FJ1, isolated from rotten wood by Kim *et al.* [11], was employed in the enzyme production cultures.

The commercial cellulosic materials, such as Avicel, α-cellulose, CMC, xylan, starch and pectin, and the lignocellulosic wastes, such as foodwastes, paper wastes, rice straw and sawdust, were used as substrates in the enzymatic hydrolysis experiments. The lignocellulosic wastes were washed 2~3 times with distilled water, dried at 80℃ for 2 day, then crushed (household electric crusher, Hanil Co., Korea) below 20 mesh and stored in a desiccator prior to use.

Enzyme Production

T. harzianum FJ1 was used for the enzyme production cultivation, according to the manner described previously [31]. The cultivations were performed using a stirred-tank bioreactor (Bio-G, 10 L, Hanil Co., Korea) containing with 5 L medium at 30°C, 200 rpm and an airflow rate of 0.6 vvm for 5 days. The pH controlled because a lower production was obtained in previous reports under such culture conditions [31]. Mandels medium was used for the cultures, and contained 2.0% (w/v) rice straw and paper wastes as the carbon source and 1.0% (w/v) peptone as the nitrogen source (initial pH 5.5). The supernatant was separated from the culture broth by centrifugation (8,000 rpm, 30 min) and filtration through a 0.45 μm cellulose acetate membrane filter (Advantec Inc., MFS, Japan). The filtrates were used in the enzymatic hydrolysis of foodwastes.

Enzyme Assay

The above filtrate was used to measure the various amylolytic and cellulolytic enzyme activities. The amylolytic enzyme activities were assayed by measuring the reducing sugars as described by Ji *et al.* [9]. The α-amylase activity was assayed by measuring the reducing sugars released using soluble starch as the substrate. The β-amylase was assayed by measuring the glucose released using soluble starch as the substrate. The glucoamylase activity was assayed by measuring the glucose released using maltose as the substrate. The reaction mixture contained 1% (w/v) soluble starch and maltose in 50 mM pH 5.0 citrate buffer and 0.2 mL of enzyme solution in a total volume of 1 mL. The reaction mixture was incubated at 50°C for 30 min. After incubation, the amount of reducing sugars and/or glucose in the supernatant was measured by the DNS method and glucose-E kit (Youngdong, Korea), respectively. One unit of α-amylase activity was defined as the amount of enzyme that liberated 1 μmol maltose per min under the standard assay conditions. One unit of β-amylase or glucoamylase activity was defined as the amount of enzyme that liberated 1 μmol glucose per min under the standard assay conditions.

The cellulolytic enzyme activities, such as filter paperase (FPase), carboxymethyl cellulase (CMCase), β-glucosidase, and Avicelase were assayed according to the method of Thomas and Bhat [28], and that of the xylanase according to a modification to the method of Gawande and Kamat [7]. One unit each of FPase, CMCase or xylanase activity was defined as the amount of enzyme required to release 1 μmol of glucose or xylose per mL·min.

One unit of β-glucosidase and Avicelase was 1 μmol of *p*-nitrophenol and cellobiose released per mL min, respectively. The reducing sugars concentration was measured according to the DNS and the Somogyi-Nelson methods, as proposed by Thomas and Bhat [28].

Effect of pH and Temperature on the Enzyme Activities

The optimum temperature for the enzyme activity was determined by assaying the enzyme at temperatures from 30 to 70°C. The thermal stability was measured by incubating the enzyme in 50 mM citrate buffer (pH 5.0) for 48 h between 40~70°C. After treatment, the enzyme solution was rapidly cooled and the residual activity determined according to above standard assay conditions.

The optimum pH was determined by monitoring the enzyme activities in the following buffer conditions: 50 mM citrate buffer for pH 3.0~5.5 and 50 mM phosphate buffer for pH 6.0~7.0. The assay conditions were those outlined above.

Saccharification of Cellulosic Materials

Commercial cellulosic materials, such as Avicel, α-cellulose, CMC, xylan, starch and pectin, and lignocellulosic materials (defined as wastes), such as foodwastes, paper wastes, rice straw and sawdust, were used as substrates in the enzymatic hydrolysis. The hydrolysis reaction mixtures were composed of 50 mL of the enzyme preparation, 50 mL of 50 mM pH 5.0 citric acid buffer and a pertinent amount of each substrate, with the reaction run in a shaking water bath at 50°C, 100 rpm for 48 h. The reducing sugars concentration of the reaction supernatant was measured by the DNS method. The saccharification ratio of pure cellulosic materials was calculated using Eq. (1) as shown below [1]. The saccharification ratio of xylan was calculated using 0.88 instead of 0.89 as the multiplying factor. Since the foodwastes were composed of various polymers, nonpolymeric organic materials, and minerals, only the cellulosic polymers producing reducing sugars in the complex were difficult to separate for the calculation of the saccharification ratio. Therefore, the saccharification ratio of the foodwastes was simply calculated, using Eq. (2) as follows:

$$\text{Sacchrification ratio of pure cellulosic materials (\%)} = \frac{\text{reducing sugars formed } (g) \times 0.89\ (0.88)}{\text{Dry substrate amount } (g)} \times 100 \quad (1)$$

$$\text{Saccharification ratio of foodwastes (\%)} = \frac{\text{reducing sugars formed } (g)}{\text{Dry substrate amoung } (g)} \times 100 \quad (2)$$

Kinetic Model of Saccharification

In this study, the reducing sugars concentration to the reaction time in the enzymatic hydrolysis of foodwastes composed of complex materials was expressed as a function of the power curve equation, which has been used by Wu and Ju [30] and Park *et al.* [20] *etc* as an empirical model.

$$X = K \cdot t^n \quad (3)$$

Where, X = reducing sugars concentration (g/L), t = reaction time (h) and K, n = constants, as determined by each different enzyme and foodwastes concentration function.

The intercept, K of the power curve model was directly proportional to the enzyme concentration (E, U-FPase/mL) and the square of the foodwastes concentration (S, g/L), which was deduced from experimental saccharification reactions by changing the enzyme and foodwastes concentrations.

$$K = f[E \cdot S^2] \quad (4)$$

The slope, n of the power curve model was also related with a direct proportion to the enzyme concentration and an inverse proportion to the substrate concentration from various experimental data.

$$n = f[E/S] \quad (5)$$

K and n were found from a best-fitting of the experimental data to the power curve model using the NLREG program (Ver. 5.3, Phillip H. Sherrod). Values of K and n in the equation were obtained in relation to the enzyme and foodwastes concentrations. The results of various saccharification reactions were predicted by the analysis.

RESULTS AND DISSCUSION

Enzymes Preparation

Fig. 1 shows the cellulolytic enzyme activities, pH and growth patterns of *T. harzianum* FJ1 in the bioreactor cultivation. The strain FJ1 exponentially grow in 2 days, and entered the secondary metabolism phase from the third day, at which point considerable cellulolytic enzymes activities appeared, suggesting that the relation between the growth phase and the enzyme production phase was similar to a type III bioprocess [22].

The enzyme preparation for the enzymatic hydrolysis used the supernatant obtained from 5 days cultivation of FJ1 in which the activities of FPase, CMCase, β-glucosidase, Avicelase, α-amylase, β-amylase, glucoamylase, and xylanase were 0.9, 22.0 1.2, 0.4, 5.6, 3.1, 2.6, and 30.8 U/mL, respectively. The multiple compositions of various cellulolytic enzymes efficiently act on the hydrolysis of cellulosic materials with complicated polymer structure. Mansfield's report [15] suggested that multiple enzymes have an advantage over the action of a single enzyme for enzymatic hydrolysis. Also, Medve *et al.* [16] observed an increased hydrolytic activity when several isoenzymes purified from the same microorganism were used in the hydrolysis of lignocellulosic materials. Con-

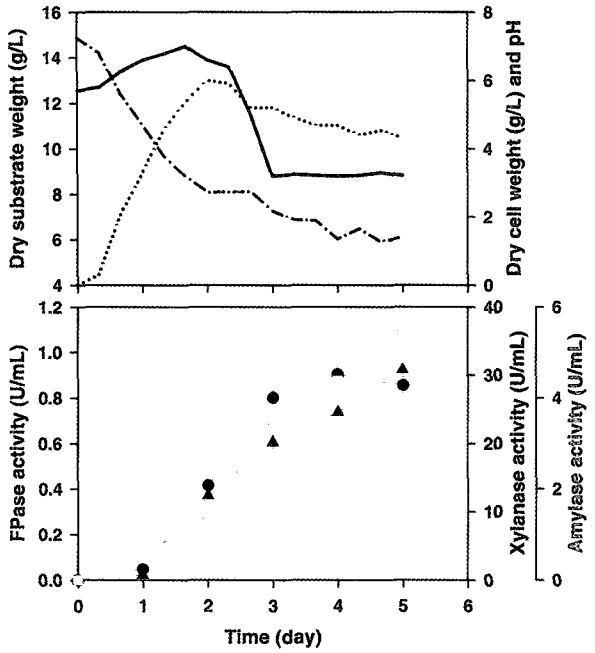

Fig. 1. Changes of dry cell weight (···), dry substrate weight (-··), pH (—) and lignocellulolytic enzymes (FPase, ●; xylanase, ▲; amylase, □) activities during the liquid cultivation of *T. harzianum* FJ1 using the bioreactor (10 L). The FJ1 was incubated in 5 L medium containing 2.0% (w/v) mixture of rice straw and paper wastes and 0.5% peptone, at 30°C, 200 rpm and air-flow 0.6 vvm. Initial pH of the medium was 6.0, and pH not controlled during cultivation.

versely, the cellulolytic enzymes composed of CMCase, β-glucosidase and Avicelase can be combined, and substituted with FPase for filter paper hydrolysis activity. The amylolytic enzymes, composed of α-amylase, β-amylase and glucoamylase, can be combined, and substituted with α-amylase for soluble starch hydrolysis activity. Therefore, α-amylase and FPase activities were monitored as the combined cellulolytic and amylolytic activities, respectively, in this study.

Effect of Temperature and pH on Amylase and FPase

The stability of enzyme activity was one of the main factors affecting the yield and rate of the emzymatic hydrolysis of lignocellulosic materials [27]. The optimum temperature and pH for amylase and FPase were around 60°C and 4.5~5.0, respectively (Fig. 2). The temperatures profile of the amylase and FPase were rather steep compared to those for the pH, showing that a difference in the proton concentration at the activate site of the enzyme had no considerably affect on the reaction mechanism. Amylase and FPase showed 90 and 57% residual activity after treatment for 48 h at 50°C, respectively, which rapidly decrease above 60°C (data not shown). The amylase and FPase produced by *T. harzianum* FJ1 showed similar thermal stability characteristics to those of mesophilic fungi, such as *Trichoderma* sp. [17] and *Aspergillus* sp. [29].

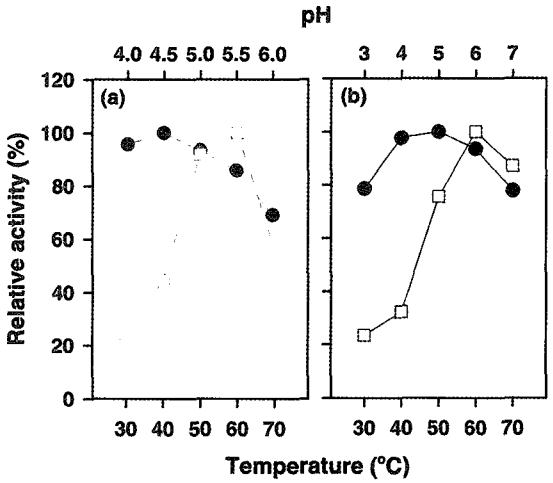

Fig. 2. Effects of pH (●) and temperature (□) on the amylase (a) and FPase (b) activities in the supernatant of *T. harzianum* FJ1 culture.

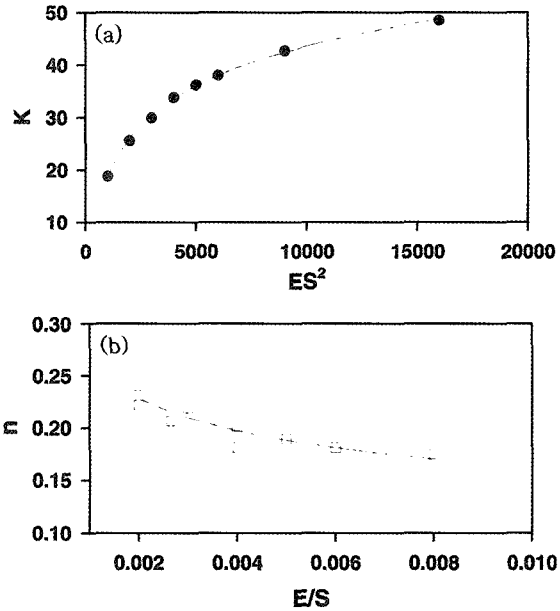

Fig. 3. The decided K (a) and n (b) values from the best fitting equations (Eqs. 6 and 7) using the saccharification data (shown as symbols) in Figs. 4 and 5.

Gan *et al.* [6] proposed that shear stress in the enzyme reaction had little effect on the reducing sugars production. Ingesson *et al.* [8] also suggested that the conversion of cellulose was more affected by the substrate concentration than the shaking regime, and that intermittent shaking during the reaction was profitable for energy savings. Therefore, the experimental optimum conditions of 50°C, pH 5.0 were tentatively inferred from above results, 100 rpm, with the exception of the enzyme and food-wastes concentrations in the enzymatic hydrolysis reaction.

Table 1. Enzymatic hydrolysis of the various cellulosic materials using the cellulolytic and amylolytic enzymes of *T. harzianum* FJ1

Substrates		Reducing sugars (g/L)	Saccharification ratio (%)
Commercial cellulolosic materials	Avicel (Fulka Co.)	18.7	33.3
	α-Cellulose (Sigma Co.)	29.2	52.0
	CMC (Junsei Co.)	21.0	37.4
	CMC (Aldrlich Co.)	26.6	47.3
	Xylan (Sigma Co., Oat spelt)	50.1	88.2
	Xylan (Sigma Co., Birchwood)	47.5	83.6
	Starch (Junsei Co.)	28.7	51.1
	Pectin (Sigma Co.)	26.8	47.7
Lignocellulolosic wastes	Foodwastes	25.1	50.2
	Paper wastes	12.8	25.6
	Rice straw	11.1	22.2
	Saw dust	2.4	4.8

The mixtures consisted of 0.4 U-FPase/mL and 5% (w/v) substrate concentration, which were incubated at 50°C, pH 5.0 with shaking 100 rpm for 1day.

Saccharification of Cellulosic Materials

To examine the hydrolyzing ability of cellulolytic multiple enzymes produced by *T. harzianum* FJ1, commercial pure cellulosic materials, such as Avicel, α-Cellulose, CMC, xylan, starch and pectin, and mixed lignocellulosic materials (as wastes), such as foodwastes, paper wastes, rice straw and sawdust, were employed as substrates for the enzyme reactions. The reducing sugars concentration and saccharification ratio according to each commercial cellulosic substrate and lignocellulosic wastes are shown in Table 1. The lignocellulolytic enzymes of strain FJ1 can hydrolyze various cellulosic and lignocellulosic substrates with different structures. The maximum reducing sugars and saccharification rate were obtained for the hydrolysis of xylan, probably due to the highest xylanase content in the enzyme preparations. Starch, with an α-1,4-glycoside band linkage, the main component of the foodwastes, was hydrolyzed by the amylolytic enzymes from same enzyme preparation. The enzymatic hydrolyses of α-cellulose and Avicel, both with crystalline structure, were also effective, indicating synergy of the CMCase, Avicelase and β-glucosidase. In addition, pectin of complex colloidal polysaccharides, with a backbone of galacturonic acid linked by α-1,4 linkages, were considerably hydrolyzed. These results suggest that cellulolytic enzymes of *T. harzianum* FJ1 can efficiently hydrolyze complex lignocellulosic wastes, such as foodwastes. Foodwastes were so efficiently converted to reducing sugars, as shown in Table 1. The reason may be due to their benign degradable structure, with a higher organic content, especially higher starch content. The other lignocellulosic wastes, such as rice straw, paper wastes and saw dust showed low reducing sugars concentration and saccharification ratio. The reason may due to very strong combination of cellulose and hemicellulose by lignin.

Sun and Cheng [25] suggested that an increase in the enzyme dosage in a saccharification process causes a cost-up, although it contributes to enhance the yield and rate of hydrolysis. In general, a dosage of about 10 U-FPase/g-cellulose is often used in laboratory studies, as this provides a hydrolysis profile with a high yield of glucose in a reasonable time, about 48~72 h, at a reasonable enzyme cost. However, this study used a relatively small enzyme dosage of 0.4 U-FPase/mL or 0.8 U-FPase/g-substrate for hydrolysis of lignocellulosic wastes, with no pretreatment, resulting in a lower saccharification cost for useful resources, such as foodwastes, which can be used as available carbon and energy sources in the fermentation industry.

Saccharification of Foodwastes

A high saccharification ratio was observed when the saccharification pH was 5.0 (data not shown), which can be explained by the fact that amylase and FPase produced by *T. harzianum* FJ1 exhibit maximal activities and stabilities around pH 5.0, as discussed previously. The optimum temperature condition for the saccharification was 50~60°C, but faster inactivation of the enzymes resulted at 60°C (data not shown). Therefore, the optimum pH and temperature conditions for the saccharification reaction of foodwastes were determined to be pH 5.0 and 50°C.

The composition of foodwastes generally changes according to season and site of origin, and a considerably different degree of hydrolysis was observed in the dried/wet state and deposit situation, as shown in Table 2.

Table 2. Comparisons of enzymatic hydrolysis of various foodwastes

Sampling site	Organic content (%)	Reducing sugars concentration (g/L)	
		Dried state	Wet state
APT	52.4 ± 5.8	17.7 ± 6.4	19.0 ± 9.2
Rest	68.2 ± 4.3	31.0 ± 8.5	35.4 ± 11.2
Caf	78.2 ± 3.5	40.8 ± 6.9	54.8 ± 5.1
Feed	51.7 ± 2.4	16.5 ± 2.5	

Organic content was measured by standard methods (USA)
ATP and Rest were collected from the storage tanks of apartments and restaurants around Gwangju city, respectively.
Caf was collected from the cafeteria at Chonnam national university.
Feed was collected from the feeding industrialization of foodwastes at Gwangju city.
The mixtures consisted of 0.4 U/mL FPase and 5% (w/v) foodwastes, which were incubated at 50°C, pH 5.0 and 100 rpm for 1 day.
The standard deviation (±) was calculated from triplicate.

Foodwastes from cafeterias, with a high organic content, showed favorable reducing sugars concentrations, whereas in the cases of APT and Feed with relatively low organic contents these values were lower. Also, foodwastes in a fresh wet state appeared to be more efficient than those swollen again after dry pretreatment in an oven. The difference probably originated from their structural twist and decrease in specific surface area due to drying, resulting in diminution of the reaction efficiency between the enzymes and substrate. In addition, when foodwastes were left at room temperature (20~25°C) for several days, the hydrolysis yield slightly decreased, but only as a neglectful effect (data not shown). With various combinations of foodwastes and enzyme concentrations, to find a more effective and economical saccharification, when 20% (w/v) fresh and wet foodwastes and 0.8 U/mL FPase, expressed as a united activity of strain FJ1, was used, a maximum of 98 g/L of reducing sugars was obtained. This result suggests that saccharified foodwastes can be efficiently utilized as carbon and energy sources in various fermentation industries, and bioconversion will simultaneously prevent the annoying environmental pollution caused by foodwastes.

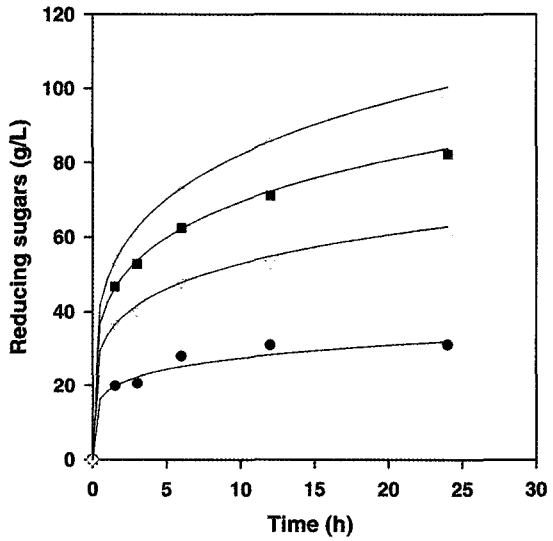

Fig. 4. Experimental data (symbols) and kinetic predictions (solid line) of reducing sugars production according to foodwastes concentrations. Symbols: ●, 50; ▽, 100; ■, 150; ◇, 200 g/L.

Kinetics of Enzymatic Hydrolysis

Many kinetic models for enzymatic hydrolysis of lingocellulosic materials, based on substrate characteristics, product inhibition, deactivation of enzyme, adsorption of enzyme on cellulose, the multiplicity of enzyme components, and so on have been developed. However, the models have difficulty sufficiently explaining, in terms of any one model only or even their combinations, the enzymatic hydrolysis of foodwastes composed of many kinds of lignocellulosic wastes. In general, reaction factors, such as temperature, pH, enzyme and substrate concentration, significantly affect the saccharification ratio of lignocellulosic materials, as described previously. The temperature and pH affected the stability of the enzymes and the hydrolysis rate, with the enzyme and substrate concentrations affecting the extent and rate of the reducing sugars produced.

In this study, to analyze the kinetics of the enzymatic hydrolysis of foodwastes, the temperature and pH were fixed at the optimum values throughout the experiments, with the enzyme and foodwastes concentrations in the kinetic model considered as important factors. The kinetic model, $X = K \cdot t^n$, where X is conversion ratio (%), t is reaction time (hr), and K and n are empirical constants was introduced. Park *et al.* [20] determined values of K and n by the functions related to enzyme concentration only. However, our experimental results showed that values of K and n were significantly correlated to enzyme and foodwastes concentration. In this kinetic model, constants, K and n were calculated as functions of the enzyme and foodwastes concentrations. The functions were deduced from best curve fitting using the experimental data from Figs. 4 and 5 for the enzymatic saccharification of foodwastes. Higher foodwastes concentrations caused

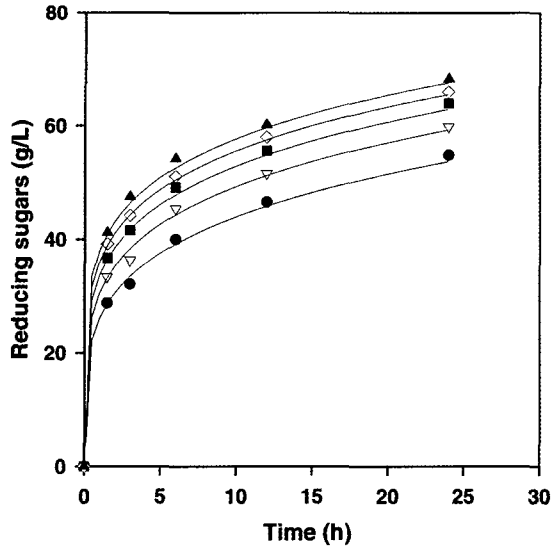

Fig. 5. Experimental data and kinetic predictions (solid line) of reducing sugars production according to the FPase concentration. Foodwastes concentrations in the reactions were 100 g/L. Symbols: ●, 0.2; ▽, 0.3; ■, 0.4; ◇, 0.5; ▲, 0.6 U/mL.

a faster initial saccharification rate and larger reducing sugars production. These two effects were reflected in the K value decided from the model (Eq. 6). The ratio of enzyme to foodwastes (E/S) concentrations was employed in deciding the n value, the dimension of the reaction rate (Eq. 7). Eqs. 6 and 7 showed high correlation coefficients of 0.9986 and 0.8364, respectively, as shown in Fig. 3, indicating that the selection of factors (E, S) and the combination between these factors were reasonable in relation to the experimental data.

$$K = 10.894 \cdot \mathrm{Ln}\,(E \cdot S^2) - 56.768 \quad (R^2 = 0.9986) \qquad (6)$$

$$n = 0.0608 \cdot (E/S)^{-0.2130} \quad (R^2 = 0.8364) \qquad (7)$$

Figs. 4 and 5 show the experimental data and the predicted results using the above models for various reaction conditions. The kinetic model could sufficiently monitor the reducing sugars production with variations in the enzyme and foodwastes concentrations. By analyzing the model, a target concentration (about 50~100 g/L) of reducing sugars for utilization as both carbon and energy sources for further value-add fermentation, such as bacterial cellulose and ethanol production, as described in our previous work [24], was obtained for various saccharification conditions. Among the many conditions, an economically favorable condition to obtain the targeted reducing sugars will be an enzyme reaction condition that uses little quantity of enzyme and that obtains much reducing sugar. It is generally recognized that the cost of the enzyme in the saccharification process of lignocellulosic materials can reach 60% of the total production cost [25].

Consequently, the kinetic developed to analyze the enzymatic hydrolysis of foodwastes composed of complex organic compounds could adequately explain the cases for the various saccharification conditions used, which would be available in reducing sugars production processes and the reducing sugars obtained at a lower cost could be used as the carbon and energy sources in various industrial fermentation processes.

Acknowledgement This research was supported by the Program for the Training of Graduate Students in Regional Innovation, conducted by the Ministry of Commerce, Industry and Energy of the Korean Government.

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[Received October 27, 2004; accepted February 4, 2005]