A Study of the Hydrolysis of Waste Paper Cellulose with a Vertically Hanging Immobilized Cellulase Reactor and the Reuse of the Immobilized Cellulase

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A commercialized cellulase from *Trichoderma reesei* has been successfully immobilized by using calcium alginate gel in our laboratory. The waste paper cellulose was hydrolyzed with a special design of the reactor to form a vertically hanging immobilized cellulase under the optimum conditions of pH 4.0 and 45 °C. Glucose, cellobiose and xylose are the major hydrolysis products. The glucose production from the hydrolysis with the vertically hanging immobilized cellulase was about 1.73-fold better than the freely suspended immobilized cellulase. The average diameter of the immobilized cellulase pellets was 4.190 ± 0.291 mm. UV light irradiation deactivates the activity of the immobilized cellulase. The advantage of the vertically hanging immobilized cellulase reactor is an easy recycle and reuse of the immobilized cellulase. Washing and soaking the recycled immobilized cellulase with distilled water for one day can restore its activity to a small extent. Overall, the application of the hanging immobilized cellulase reactor for waste paper cellulose hydrolysis is successful.

Keywords: Glucose; Waste paper; Calcium alginate; Immobilization of enzyme; Cellulase; Cellulose; Enzyme reactor.

INTRODUCTION

The invention of paper speeded the progress of human civilization and promoted the progression of science. The huge consumption of various kinds of paper such as advertising paper, packing paper, newspaper and copy paper in our daily lives indeed reflects that paper is an important material in the world and affects human life deeply. Since the raw material for making paper is the cell wall of higher grade plants, the major components of paper are cellulose, hemicellulose, and lignin.[1-3] Cellulose is a biological macromolecule constituted of thousands of glucose units, while the constituents of hemicellulose are composed of two kinds of pentose (i.e. xylose and arabinose) and three kinds of hexose (i.e. glucose, mannose and galactose). Unlike cellulose and hemicellulose, lignin is made up of aromatic compounds and can't be hydrolyzed easily. Among these three major components the plant cellulose has the largest proportion and that makes it the most abundant polysaccharide in the natural world,[4] and cellulose based wastes are worth recycling and reusing. Therefore, waste paper all around us that is essentially a product of plant cellulose is the material chosen for this study. Instead of making recycled paper from waste paper, the hydrolysis of cellulose in waste paper can produce the more useful glucose.

The hydrolysis of cellulose can be performed by either acids or bases under strong conditions. However, the microbial process or an enzymatic process favors having a high selectivity of substrate and less production of by-products under mild conditions. The hydrolytic enzyme for cellulose is called cellulase and is composed of endoglucanase, exoglucanase (or cellobio-hydrolase), and β-glucosidase.[5-6] Endoglucanase can hydrolyze the cellulose chain internally at the β-1,4-glycosidic bond. Exoglucanase can cut the non-reducing side of cellulose to cellobiose. Then cellobiose can be hydrolyzed by β-glucosidase to D-glucose. During hydrolysis a suspension of free cellulase in the pulp solution can be used to produce the glucose. However, there are several advantages of the immobilized enzyme such as an increase of enzyme activity and stability and an easy enzyme and product recovery.

In our laboratory, cellulose from acid digested waste paper has been hydrolyzed by free cellulase under four different buffering media.[6] The medium containing citric acid/citrate buffer used for glucose production is the best one; however, in consideration of production cost we decided to use H_2SO_4/NaOH as the pH controlling substance for large scale production. Moreover, we decided to use immobilized cellulase and a specially designed reactor to make the enzyme recovery, the reuse of the high cost enzyme and the product separation easier. Although there are many ways to immobi-

lize cellulose,[7-9] the material used for the immobilization of cellulase in this work is calcium alginate gel[10-13] that is formed by dripping a sodium alginate solution into a $CaCl_2$ solution. During the immobilization the cellulase contained in the sodium alginate solution can be entrapped into the calcium alginate gel. The role $CaCl_2$ played in this immobilization procedure is as a gelation agent.[13]

EXPERIMENTAL SECTION

Chemicals

The cellulase complex (EC 3.2.1.4) (Sigma, USA) used for this research work was purified from *Trichoderma reesei*. The cellulase was stored at 0-4 °C prior to use. The $CaCl_2$ (Merck, Germany) and sodium alginate (Lancaster, England) were needed to prepare the immobilized cellulase. The reagent grade NaOH (Showa, Japan) and H_2SO_4 (Showa, Japan) were used for the control of pH during the waste paper cellulose hydrolysis. The reagent grade D-glucose (Merck, Germany), D-xylose (Merck, Germany) and cellobiose (Sigma, USA) were used for the preparation of standard solutions to identify and make quantitative determination of the hydrolysis products. Waste paper was collected in the laboratory. Tap water was purified by reverse osmosis and distillation once.

The Pretreatment of Waste Paper

The waste paper was cut into small scraps with a size less than 1 cm^2. The paper scraps was put in a 250 mL Erlenmyer flask and 150 mL of 5 M H_2SO_4 solution was added into the Erlenmyer flask to digest the paper. The digestion was performed in an orbital shaker incubator (Yihder LM-530R, Taiwan) at a temperature of 40 °C and a shaking rate of 150 rpm for a period of 48 hours. After the digestion, the pH of the waste paper solution was adjusted to 7.0 with NaOH and H_2SO_4. This neutralized solution was centrifuged (Hettich 30RF, Germany) for 10 minutes at a speed of 10000 rpm to precipitate the digested waste paper. The upper aqueous solution was decanted. 100 mL of distilled water was added to the waste paper fiber and stirred to scatter them. The digested waste paper solution was centrifuged again. The procedures were repeated several times to get rid of most of the Na_2SO_4 and other soluble materials. Finally, the digested waste paper fiber was transferred into a 500 mL beaker with distilled water. An ultrasonic disruptor (Heat Systems W-385, USA) was used to further disrupt the waste paper fiber. The treatment of ultrasonic sonication was at room temperature for 30 minutes and 2 to 4 times under the following control setting: 2 s pulsar cycle; 50% duty cycle and an output control at 9. The gel-like solution was centrifuged to separate the fluid from the waste paper fiber. The fiber was rinsed into a beaker. The treated waste paper fiber was oven dried at a temperature 50 °C into a solid form. Part of the solid was ground into powder for subsequent hydrolysis reaction. The rest of the dried waste paper solid was stored in the oven at 50 °C for later use.

Immobilization of Cellulase

Several 10 mL 2% (w/v) sodium alginate solutions were prepared. 11.1 g $CaCl_2$ was dissolved in 200 mL distilled water to make a 0.5 M solution. 30.0 mg cellulase was added into the sodium alginate solution. Immobilized cellulose pellets are formed by the dripping of the cellulase containing alginate solution into the 200 mL $CaCl_2$ solution. The dripping procedure was through a silicone tube of 1.6 mm inner diameter with a peristaltic pump (Master-flex model 7524-10, USA) at a driving speed of 4 mL/min. The dripping tube is 30 cm height above the surface of the liquid to ensure a formation of spherical pellets. The $CaCl_2$ solution was stirred with a 4 cm long magnetic stirrer at a constant rate of 150 rpm in order to prevent the pellets from sticking together and to minimize the external mass transfer resistance. The pellets were stirred for 5 more minutes to improve the toughness. Then the pellets were collected by filtering and washed with distilled water. One immobilization procedure can make about 140 pellets. The diameter of pellets was measured by micrometer calipers (Mitutoyo, Japan) to a thousandth of a millimeter. After the pellet was freeze dried by a freeze dryer (Pamchum Scientific Corp FD-5030, Taiwan), the surface structure of the pellet was examined by a scanning electron microscope (SEM) (Jeol Ltd, JSM-6300, Japan).

Hydrolysis of Waste Paper Cellulose

Preparation of waste paper cellulose solution for hydrolysis

About 0.25 g pretreated waste paper solid was weighed and put into 100 mL distilled water. The flask was sterilized by a speedy autoclave (Hung Lin Medical Instruments Co, HL-340, Taiwan) for 30 minutes. The pretreated waste paper solid in solution is ultrasonically treated to dissolve until the solution becomes turbid. If there is still any waste paper solid seen in the solution, they are ground into fine powder with a glass rod. Again, the solution is ultrasonically treated until a complete dissolving of the pretreated waste paper solid. The solution is cooled down to room temperature. The pH of the solution is adjusted to the desired value with NaOH and

H_2SO_4.

Shaker-flask hydrolysis of waste paper cellulose with free cellulase

30.0 mg of free cellulase was added to the waste paper cellulose solution that was prepared according to the procedures of the previous section. The pH of the waste paper solution was adjusted to 4.8. The hydrolysis of waste paper cellulose was performed in an orbital shaker incubator at a shaking rate of 150 rpm and a temperature of 40 °C for four days. The activity of cellulase and the production of glucose were examined.

Optimum conditions for immobilized cellulase hydrolysis

The first optimum condition searched for in waste paper cellulose hydrolysis with immobilized cellulase was pH. The experiment was performed with a small scale shaker flask type reaction by using several 100 mL 0.25 g waste paper cellulose solutions prepared as described in previous section and each containing a total amount of 30.0 mg of the immobilized cellulase prepared as described above. The pH of the waste paper cellulose solutions was adjusted to the desired value and kept at the same reaction temperature. These solutions were shaken at a rate of 150 rpm for four days. The glucose production and the activity of immobilized cellulase by the assay of high-performance liquid chromatography (HPLC) were compared to determine the optimum pH. The procedure described above was followed to determine the optimum temperature; however, at this time we just kept the pH of the solution at the optimum one and varied the hydrolysis temperature.

After the determination of the optimum pH and temperature for the immobilized cellulase hydrolysis of waste paper cellulose, we also checked other factors such as UV light irradiation and the size of immobilized cellulase pellets that may affect the hydrolysis. The hydrolysis was performed in duplicate solutions and under identical pH and temperature and reacted for a period of four days. However, before the reaction start one solution was irradiated with UV light for 30 minutes and another one without any UV light irradiation. The results for carbohydrates production from both solutions were compared. Then, a smaller size of the immobilized cellulase pellet was made by using a smaller size of dripping needle. Two waste paper cellulose solutions prepared as before but one with a larger size of immobilized cellulase pellet and one with a smaller size of immobilized cellulase pellet. The two waste paper cellulose solutions containing different sizes of immobilized cellulose pellets but having the same amount of cellulose were hydrolyzed under the optimum pH and temperature and with better results for UV light irradiation testing. The effect of the size of immobilized cellulase pellet on the hydrolysis was also surveyed with respect to the glucose production and the activity of immobilized cellulase.

Stirred batch hydrolysis with immobilized cellulase

The hydrolysis of waste paper cellulose with immobilized cellulase was scaled up 10 times by using a 3L-bench scale fermentor (Bio-Top Corporation, BTF-A3L, Taiwan). The schematic assembly of the automatically controlled fermentation system is shown in Fig. 1(A). Thus, the total volume of reaction solution was 1.0 L. The experimental conditions used were the optimum pH and temperature found from the small-scale shaker flask type hydrolysis as described before with a stirring rate at 150 rpm and a reaction period of four days. The amount of waste paper cellulose and the amount of cellulase may be varied for the hydrolysis. The hydrolysis of the waste paper cellulose in this experiment is essential a stirred batch type reaction. However, the pellets of immobilized cellulase may be directly suspended in the solution or they can be put in many small net-pockets and hung on a rack. The specially designed hanging rack for hanging the net-pocketed immobilized cellulase pellets is illustrated as Fig. 1(B). The hydrolysis of waste paper cellulose performed by the hanging type immobilized cellulase can make the recycle and reuse of the immobilized cellulase very easy.

Carbohydrate Product Analysis by HPLC

The hydrolysis products in the enzymic hydrolysate are analyzed by the HPLC.[14-15] The HPLC system comprised a dual-piston solvent delivery pump (Shimadzu LC-9A, Japan), a column oven (Shimadzu CTO-6A, Japan), a refractive index detector (Shimadzu RID-6A, Japan), and a sample injection valve with a 20-μL sampling loop (Rheodyne 7125, USA). The analytical column was a cation-exchange column of 8 μm particle size and 300 × 7.8 mm dimension (Phenomenex, Rezex ROA-Organic Acid, USA). The column temperature was maintained at 40 °C. The mobile phase was 0.01 M H_2SO_4 solution running at a flow rate of 0.6 mL min^{-1}. The qualitative analysis of carbohydrate products was performed by spiking carbohydrate standards into the solutions. External standard calibration curves of various carbohydrate products were used for the quantitative analysis throughout this research.

The overall activity of immobilized cellulase can be

calculated by the following equation:[16]

$$\text{Activity of cellulase} = \frac{10^3 \bullet w}{M \bullet t \bullet C} (\text{mmol glucose hour}^{-1}\ \text{kg cellulase}^{-1}) \quad (1)$$

where w is the weight of glucose (g), M is the molecular weight of glucose (g), C is the weight of cellulase (kg) and t is the reaction time (hour). The productivity of immobilized cellulase in terms of glucose can be calculated by the following equation:[17]

$$\Delta PN = \frac{10^3 \bullet \Delta w}{M \bullet \Delta t \bullet C} (\text{mmol glucose hour}^{-1}\ \text{kg cellulase}^{-1}) \quad (2)$$

where ΔPN is the productivity number in a short time period during a reaction, Δw is the difference in weight (g) of glucose within the short time period and Δt is the time difference

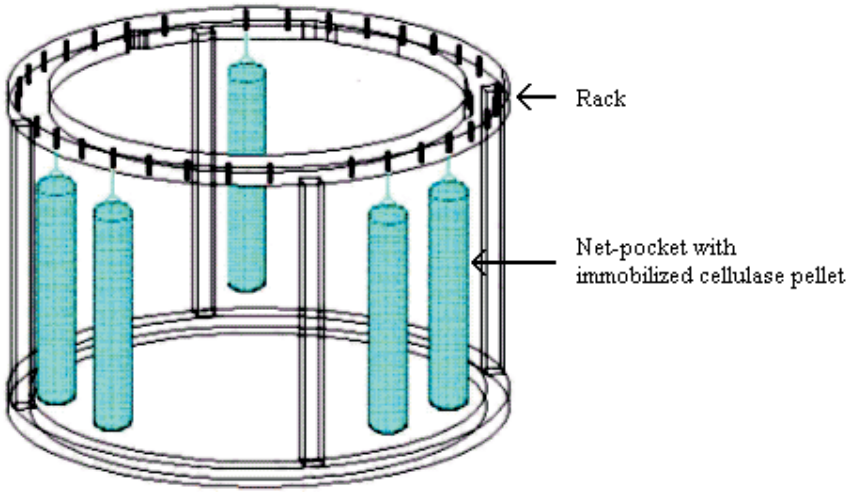

Fig. 1. (A) The schematic diagram of the hanging immobilized cellulase reactor. (B) The net-pocket filled with the immobilized cellulase pellets and hung under the rack.

(hour) of the short time interval.

Recycle and Reuse of the Immobilized Cellulase

At the end of hydrolysis the rack with the net-pocketed immobilized cellulase pellets that was still hung on the rack was taken out from the reactor and rinsed with distilled water. The hydrolysis solution was poured out from the reactor and the reactor was cleaned. 1.0 L restoring solution was added into the reactor, and the rack with the hanging immobilized cellulase was put back into the reactor. The immobilized cellulase pellets in the net-pocket and hung on the rack was soaked with stirring in the restoring solution for some period of time. The restoring solution was poured, and fresh waste paper cellulose solution was added then the hydrolysis was performed for four days under the optimal conditions as described previously.

RESULTS AND DISCUSSION

Optimum conditions for immobilized cellulase hydrolysis

The optimum pH and temperature for an immobilized enzyme may be different from that of a free enzyme.[18] Therefore, a small scale shaker-flask type reaction was used to search for the optimum pH and temperature for the immobilized cellulase. Table 1 shows the concentrations of hydrolysis products and the immobilized cellulase activity in terms of the glucose production at a fixed temperature of 40 °C but under different pH. For a 4-day reaction period the reaction performed at pH 4.0 has the largest glucose production and the largest immobilized cellulase activity. The search for optimum temperature was then performed by fixing the reaction pH at 4.0 but varying the reaction temperature from 30 °C to 70 °C, and the reaction period is also 4 days. The results in Table 2 indicate that the largest glucose production, thus the largest immobilized cellulase activity, is at the reaction with a reaction temperature 45 °C. Therefore, the experimentally determined optimum pH and temperature are at 4.0 and 45 °C respectively. The activity of immobilized cellulase at the optimum pH and temperature was also compared with the activity of free cellulase under different reaction pH and temperatures for the waste paper cellulose hydrolysis in a shaker-flask type reaction. The hydrolysis results shown in Table 3 indicate that the glucose production with immobilized cellulase indeed is superior to that with free cellulase. The relative activity of the cellulase calculated according to the total amount of carbohydrate products shows a 7-10% improvement for the hydrolysis with immobilized cellulase.

Effect of other factors on the waste paper cellulose hydrolysis

The glucose produced during the hydrolysis could be consumed by fungi for their own growth. Sterilization of the waste paper cellulose solution containing the immobilized

Table 1. The results for waste paper cellulase hydrolysis with immobilized cellulase in a shaker-flask type reaction at constant temperature and a reaction period of four days

pH	Temperature (°C)	No. of experiment	Cellobiose (ppm)	Glucose (ppm)	Xylose (ppm)	Immobilized cellulase activity (mmol glucose/hour/kg cellulase)
1.0	40	1	15.9 ± 2.0	0.2 ± 1.6	6.3 ± 3.2	0.0 ± 0.3
2.0	40	1	350.3 ± 6.0	75.4 ± 5.0	69.9 ± 1.7	13.0 ± 0.9
3.0	40	5	299.2 ± 4.6	866.2 ± 14.1	120.3 ± 1.9	158.3 ± 2.6
3.5	40	2	16.3 ± 7.1	878.9 ± 12.9	73.4 ± 9.0	168.3 ± 2.5
4.0	40	5	46.8 ± 2.2	1235.9 ± 6.2	119.1 ± 12.3	228.6 ± 1.1
4.5	40	2	37.6 ± 4.3	930.6 ± 14.7	83.0 ± 4.6	178.2 ± 2.8
5.0	40	5	76.0 ± 3.4	1131.6 ± 6.5	114.0 ± 2.8	208.2 ± 1.2
5.5	40	2	63.9 ± 2.6	918.2 ± 5.1	96.1 ± 5.0	175.8 ± 1.0
6.0	40	5	51.4 ± 1.5	1080.6 ± 3.9	124.9 ± 2.1	198.9 ± 0.7
7.0	40	1	17.3 ± 3.1	805.5 ± 3.1	151.0 ± 2.0	139.0 ± 0.5
8.0	40	1	4.6 ± 1.3	1001.3 ± 9.1	217.1 ± 2.1	172.8 ± 1.6
9.0	40	1	203.9 ± 5.5	642.5 ± 7.0	173.7 ± 15.1	110.9 ± 1.2
10.0	40	1	238.6 ± 17.0	444.1 ± 5.4	88.8 ± 4.8	76.6 ± 0.9
11.0	40	1	3.4 ± 1.1	0.0	0.0	0.0
12.0	40	1	26.6 ± 3.4	32.4 ± 2.3	10.9 ± 0.6	5.6 ± 0.4
13.0	40	1	40.1 ± 34.8	48.5 ± 9.6	4.2 ± 2.1	8.4 ± 1.7

Note: The number of measurements for the concentration of carbohydrates are five of six times (n = 5 or 6).

Table 2. The results for waste paper cellulase hydrolysis with immobilized cellulase in a shaker-flask type reaction at constant pH and a reaction period of four days

Temperature (°C)	pH	No. of Experiment	Cellobiose (ppm)	Glucose (ppm)	Xylose (ppm)	Immobilized cellulase activity (mmol glucose/hour/kg cellulase)
30	4.0	5	218.2 ± 2.9	114.2 ± 0.7	71.0 ± 1.4	21.3 ± 0.1
40	4.0	5	46.8 ± 2.2	1235.9 ± 6.2	119.1 ± 12.3	228.6 ± 1.1
45	4.0	5	58.3 ± 7.2	1642.1 ± 32.9	194.7 ± 6.4	306.7 ± 6.1
50	4.0	5	66.7 ± 5.8	1523.9 ± 9.7	164.6 ± 2.9	281.6 ± 1.4
55	4.0	5	26.6 ± 2.3	1170.2 ± 32.0	139.0 ± 4.6	218.6 ± 5.9
60	4.0	5	11.8 ± 0.9	486.6 ± 4.2	52.1 ± 0.7	92.1 ± 0.8
70	4.0	5	29.3 ± 0.9	206.7 ± 3.5	5.2 ± 0.9	40.2 ± 0.6

Note: The number of measurements for the concentration of carbohydrates are five of six times (n = 5 or 6).

Table 3. The shaker-flask type waste paper cellulose hydrolysis with immobilized cellulase or free cellulase under different reaction conditions

				Concentration of carbohydrate products			
Type of cellulase	Temperature (°C)	pH	Reaction period (day)	Cellobiose (ppm)	Glucose (ppm)	Xylose (ppm)	Relative activity* (%)
Free	40	4.8	4	83.2 ± 3.0	1803.4 ± 27.4	210.5 ± 6.5	100.0
Free	45	4.0	4	82.3 ± 2.7	1801.7 ± 10.5	205.8 ± 5.6	99.6
Immobilized	45	4.0	4	69.1 ± 3.6	1808.4 ± 14.5	208.1 ± 5.9	99.4

* The relative activity was calculated according to the total amount of carbohydrate products.
Note: The number of measurements for the concentration of carbohydrates are five times (n = 5).

cellulase can eliminate most of the effect of fungi growth. However, sterilization by high temperature and high pressure will deactivate the activity of immobilized cellulase. Therefore, UV light is used for the sterilization of the solution containing immobilized cellulase. However, the results shown in Table 4 indicate that the glucose production in the hydrolysis solution without UV light sterilization is about 90 ppm larger than that in the hydrolysis solution with UV light sterilization. The activity of immobilized cellulase after the treatment with UV light irradiation seems still deactivated slightly at an extent of about 6%. Therefore, we decided that sterilization of the immobilized cellulase solution is not necessary in later experiments.

The effect of the size of immobilized cellulase pellets for waste paper cellulose hydrolysis was tested by comparing two different kinds of pellet size. The average diameter for the two different sizes of pellet was calculated based on 50 immobilized cellulase pellets. The results in Table 4 shows that the immobilized cellulase pellet of a larger diameter for the hydrolysis produces better glucose production, and the immobilized cellulase activity is about 12% better than the small size immobilized cellulase. A larger loss of cellulase

Table 4. The effect of UV light irradiation and the size of immobilized cellulase capsule on the waste paper cellulose hydrolysis

				Concentration of carbohydrate products			
Factors	Temperature (°C)	pH	Reaction period (day)	Cellobiose (ppm)	Glucose (ppm)	Xylose (ppm)	Relative activity* (%)
With UV light irradiation	45	4.0		48.5 ± 0.5	157.5 ± 1.5	52.6 ± 0.6	89.7
Without UV light irradiation	45	4.0		51.6 ± 0.4	182.5 ± 1.5	54.1 ± 0.5	100.0
Average diameter							
4.190 ± 0.291 mm	45	4.0	4	67.2 ± 7.4	1793.3 ± 30.3	200.1 ± 7.9	100.0
2.101 ± 0.253 mm	45	4.0	4	67.0 ± 6.1	1575.1 ± 27.0	181.7 ± 5.3	88.5

* The relative activity was calculated according to the total amount of carbohydrate products.
Note: The number of measurements for the concentration of carbohydrates are five times (n = 5).

during the preparation of the smaller size immobilized cellulase pellet should be responsible for the worse glucose production. Otherwise, the larger surface area exhibited by the smaller immobilized cellulase pellet would make a better substrate binding rate for the enzyme and give better glucose production. We also assume that the mass transfer rate of substrate to the immobilized cellulase is the same for both sizes of pellet.

Bench-scale stirred batch hydrolysis with hanging immobilized cellulase

In order to improve the productivity of glucose for the immobilized cellulase hydrolysis of waste paper cellulose, a special design of the reactor was made to increase the mass transfer rate for both substrate and products. This has been accomplished by placing the immobilized cellulase pellet into a net-pocket and hanging the filled net-pocket on a rack as illustrated by Fig. 1(B). The rack is suitably fitted in the 3-L fermentor as shown in Fig. 1(A). This kind of design has the merit to make the recycling and the reuse of immobilized cellulase very easy. Thus, with this special design the hydrolysis of waste paper cellulose was performed under optimum conditions for a 4-day reaction period but varying the amount of cellulose and the immobilized cellulase to get a maximum production of glucose.

Different experiments for the glucose production along the reaction time course are shown in Fig. 2(A). The hydrolysis performed with 300 mg cellulase and 2.5 g waste paper cellulose can produce 1932.8 ± 70.0 ppm glucose with the hanging immobilized cellulase reactor that was much greater than the amount of 1577.1 ± 63.1 ppm glucose produced in a reaction with freely suspending the immobilized cellulase under exactly the same experimental conditions. As we double the amount of cellulase to 600 mg but keep using 2.5 g waste paper cellulose for the hydrolysis the amount of glucose produced around day three is about the same as that in the reaction with 300 mg cellulase and 2.5 g waste paper cellulose. However, at day four the amount of glucose produced was decreased to 1658.9 ± 15.4 ppm for reasons unknown. Since the hydrolysis requires the binding of cellulose to cellulase for glucose production, an increase of the amount of cellulase can only increase the reaction rate. This indeed is the case elucidated by the two reaction curves (one with 300 mg cellulase and 2.5 g waste paper cellulose and one with 600 mg cellulase and 2.5 g waste paper cellulose) in Fig. 2(A). Theoretically, the amount of glucose produced depends on the total amount of cellulose provided to the cellulase if we can keep a constant activity for the enzyme. The deficiency of cellulose or hemicellulose will limit the glucose production to a certain value in a hydrolysis run. In our experiments, due to the existence of materials other than the hydrolyzable cellulose and hemicellulose in the waste paper cellulose, only about 2.0 g total saccharides were produced from 2.5 g waste paper cellulose, which means about 80% yield.

As the hydrolysis proceeded by the hanging immobilized cellulase reactor with 300 mg immobilized cellulase and 5.0 g waste paper cellulose, the amount of glucose produced on the fourth day was 2721.3 ± 142.9 ppm. However, the production of total saccharides from the hydrolysis was only about 3.2 g (i.e. about 60% yield). This result indicates that an increase of the total amount of waste paper cellulose can make an increase in the glucose production. However, with a further increase of the amount of waste paper cellulose to 6.25 g, the hydrolysis shows a deactivation of the immobilized cellulase. Both the decreased yield and the deactivation of cellulase at a high level of waste paper cellulose show that substrate inhibition exists in the hydrolysis. Therefore, there is a tolerance of the amount of waste paper cellulose for the cellulase. The productivity of glucose within a short reaction time interval reflects the activity of the immobilized cellulase at that time interval.

The productivity of glucose in a reaction run indicates the overall activity of the cellulase complex, and the productivities of glucose in the time course for the five reactions are shown in Fig. 2(B). In general, the hydrolysis with low substrate concentration (i.e. 2.5 g waste paper cellulose) and a hanging immobilized cellulase reactor has the largest initial cellulase activity then it gradually decreases to about zero activity. The productivity looks like a first order kinetics. The hydrolysis with a high substrate concentration (i.e. 5.0 g and 6.25 g waste paper cellulose) has a very high initial cellulase activity; however, substrate inhibition deactivates the activity around the time of 10 hours. Although a recovery of the activity followed, the activity was then decreased just like the reaction for low substrate concentration. Doubling the amount of cellulase to 600 mg only increases the initial cellulase activity and does not help in the productivity of glucose. A significant improvement of the glucose productivity by the hanging immobilized cellulase over that of freely suspended immobilized cellulase is shown in Fig. 2(B). Probably, fixing the position of immobilized cellulase pellets can increase the binding probability of the substrate to the enzyme and can increase the mass transfer rate of both substrates and products.

Fig. 3 shows the results of xylose and cellobiose production in the time course for the five experiments as in Fig. 2. Actually, the production of xylose is by xylanase and the

production of cellobiose is due to the synergistic action of β-glucosidase in the cellulase complex. The accumulation of cellobiose reached a maximum at the beginning of the hydrolysis that indicates a lag phase for the action of β-glucosidase. The lag phase was about 10 hours for the hydrolysis with free suspension of the immobilized cellulase, and it was about 5-8 hours for the hydrolysis with the hanging immobilized cellulase. The production curves for xylose are similar to those of glucose that indicates a similarity between xylanase and cellulase. However, there is no indication of substrate inhibition for xylanase. The production of xylose was greatly enhanced by using the hanging immobilized cellulase and a

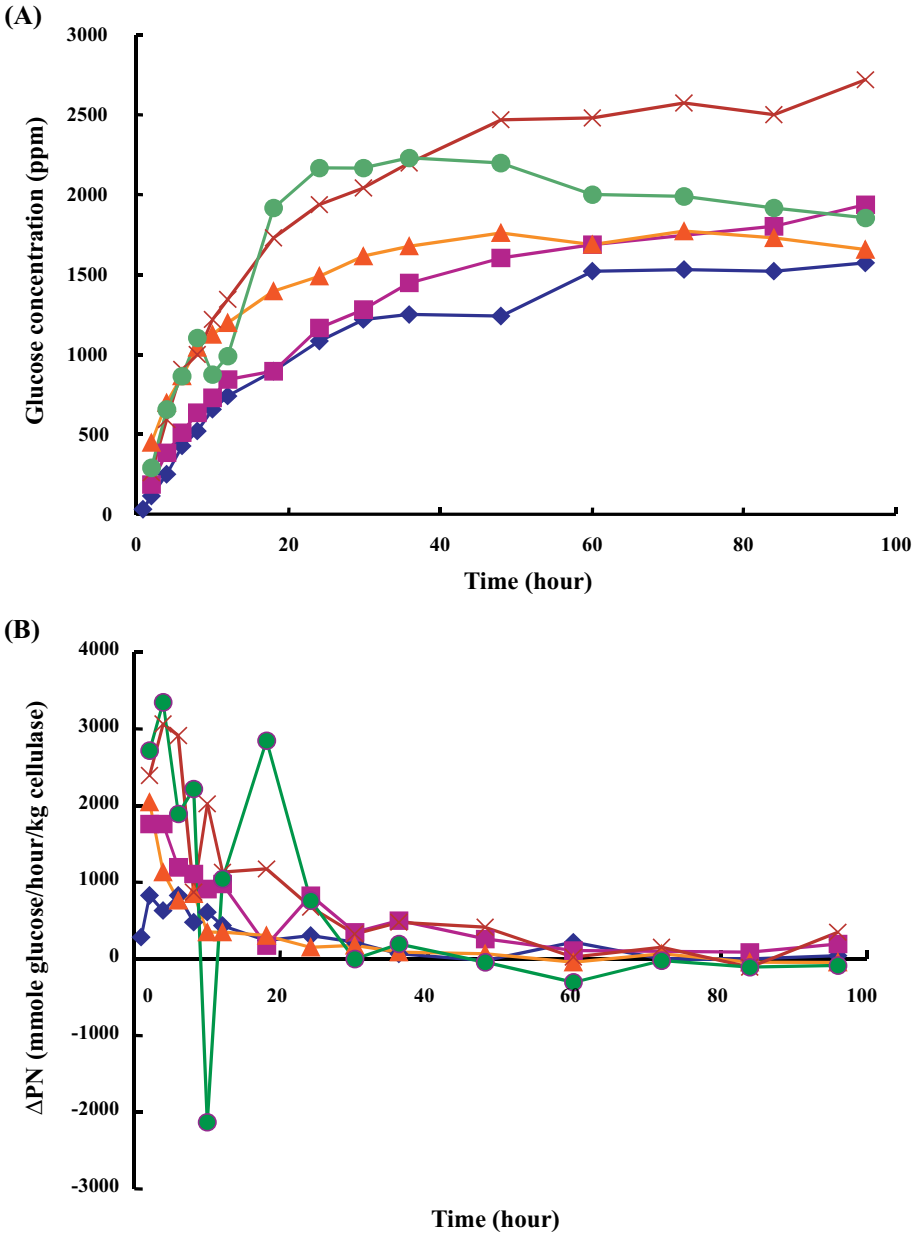

Fig. 2. The production of glucose in waste paper cellulose hydrolysis by immobilized cellulase along the reaction time course. (A) The glucose production. (B) The productivity number of glucose for immobilized cellulase during a short time period in a reaction run. (♦) Suspension of immobilized cellulase (300 mg cellulase; 2.5 g waste paper) (■) Hanging immobilized cellulase (300 mg cellulase; 2.5 g waste paper) (▲) Hanging immobilized cellulase (600 mg cellulase; 2.5 g waste paper) ( ) Hanging immobilized cellulase (300 mg cellulase; 5.0 g waste paper) (●) Hanging immobilized cellulase (300 mg cellulase; 6.25 g waste paper).

high substrate concentration.

Recycle and reuse of the hanging immobilized cellulase

We first used 1 M $CaCl_2$ solution to restore the activity of recycled immobilized cellulase; however, we did not find any glucose, cellobiose, or xylose was produced. The $CaCl_2$ solution seems not suitable for restoring the activity of immobilized cellulase. Therefore, distilled water was used to carry out the restoration of the immobilized cellulase's activity. The glucose production with the recycled immobilized cellulase reached a maximum value around the reaction time of 8 hours as indicated by Fig. 4(A). The amount of glucose produced was 12.2 ± 1.3 ppm at 8 hours. The largest amount of xylose and cellobiose produced were 23.3 ± 0.9 ppm and 17.5 ± 1.6 ppm respectively at the time 24 hours. The production of glucose did show a very small restoration of about 1% the activity of the immobilized cellulase. The amount of xylose produced shown in Fig. 4(A) was larger than that of glucose produced which indicates a better restoration of xylanase's activity. A larger accumulation of cellobiose than that of glucose also indicated that the use of water for restoring the cellulase activity is less effective for β-glucosidase in the cellulase complex. After the time of 24 hours the amounts of the three carbohydrates were gradually decreased; that is probably due to their use for fungi growth. However, the small reducing rates of the three carbohydrates show that the influence of fungi growth on the waste paper cellulose hydrolysis is small.

Another experiment was performed to test the restoration of the activity of the recycled immobilized cellulase with water for a two-day period. Results in Fig. 4(B) showed that 2.5 g of freshly prepared waste paper cellulose were reacted with the recycled cellulase under the optimum conditions for four days. The recycled cellulase was soaked in distilled water for restoring its activity for two days. Only xylose was produced during the hydrolysis and a 2.95 ± 0.47 ppm xylose were found at a reaction time of 9 hours. Then, the amount of xylose was gradually decreased. The results revealed that a longer activity recovery time does not help the restoration of the recycled cellulase activity. Therefore, a third experiment was performed by shortening the enzyme activity recovery time. After a half-day restoration for the activity of recycled immobilized cellulase with distilled water they are used to react with 2.5 g freshly prepared waste paper cellulose under optimum conditions for four days. We found that 2.17 ± 1.57 ppm cellobiose was produced at 12 hours as shown in Fig.

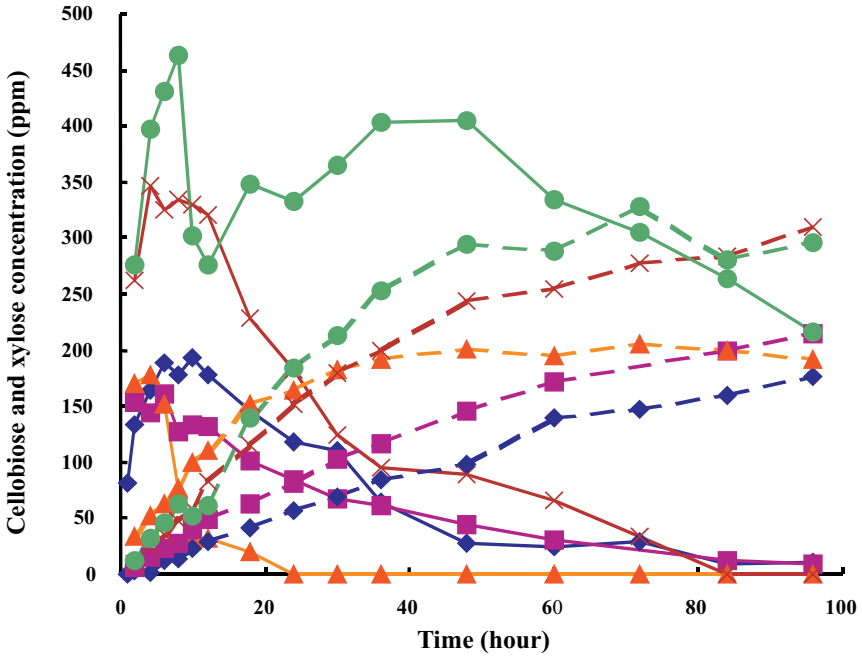

Fig. 3. The production of cellobiose (solid line) and xylose (dotted line) in waste paper cellulose hydrolysis by immobilized cellulase along the reaction time course. (♦) Suspension of immobilized cellulase (300 mg cellulase; 2.5 g waste paper) (■) Hanging immobilized cellulase (300 mg cellulase; 2.5 g waste paper) (▲) Hanging immobilized cellulase (600 mg cellulase; 2.5 g waste paper) (✕) Hanging immobilized cellulase (300 mg cellulase; 5.0 g waste paper) (●) Hanging immobilized cellulase (300 mg cellulase; 6.25 g waste paper).

4(C). The amount of cellobiose was maintained at about the same till the time of 36 hours. After 36 hours the concentration of cellobiose was decreased. Then, a little bit of glucose was produced with an amount of 0.53 ± 0.54 ppm at 48 hours. The extent of restoration of the recycled cellulase activity was not as good as that shown in Fig. 4(A). All the results indicated that the activity of the recycled immobilized cellulase can be restored to a small extent with distilled water and the suitable recovery time is one day. However, we suspect that there is a loss of the cellulase during the restoration. A fourth experiment was performed by repeating the procedures in Fig. 4(A), however, changing the amount of freshly prepared waste paper cellulose to 5.0 g. The results shown in Fig. 4(D) indicate a small extent of recovery of the activity of the recycled cellulase activity. The amount of glucose, cellobiose, and xylose produced was 3.97 ± 0.76, 3.95 ± 1.31, and 5.65 ± 0.94 ppm, respectively, at the reaction time of 24 hours. The recovery of the activity for the recycled cellulase is worse than that shown in Fig. 4(A). Nevertheless, it confirms that the suitable time needed for restoring the activity of the recycled cellulase by using distilled water is one day. Surveys on the recovery of the recycled cellulase activity and the reuse of the recycled cellulase should be studied further.

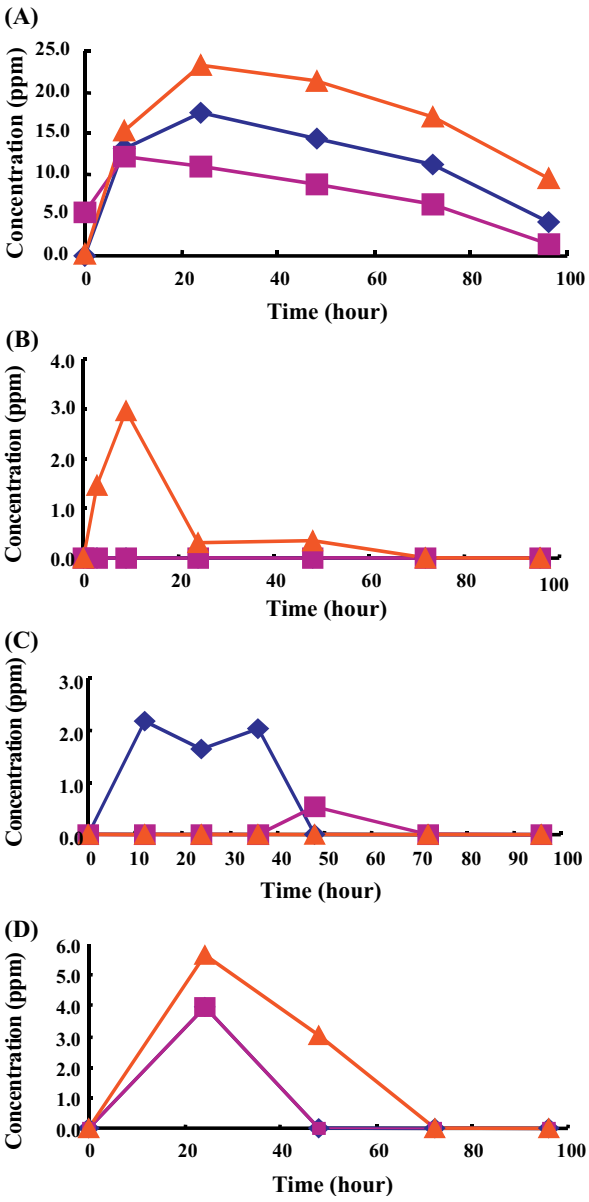

Fig. 4. The production of carbohydrates with the recycled immobilized cellulase. (♦) cellobiose, (■) glucose, (▲) xylose; (A) 2.5 g freshly prepared waste paper cellulose and 600 mg recycled cellulase soaked in distilled water for one day. (B) 2.5 g freshly prepared waste paper cellulose and 300 mg recycled cellulase soaked in distilled water for two days. (C) 2.5 g freshly prepared waste paper cellulose and 300 mg recycled cellulase soaked in distilled water for half days. (D) 5.0 g freshly prepared waste paper cellulose and 300 mg recycled cellulase soaked in distilled water for one day.

CONCLUSIONS

In our current experimental work, cellulase has been immobilized successfully by the calcium alginate gel. The immobilized cellulase can be used efficiently for the hydrolysis of waste paper cellulose to produce useful glucose. A minor amount of cellobiose and xylose were also obtained in the waste paper cellulose hydrolysis that was due to an incomplete hydrolysis of cellulose or from the hydrolysis of hemicellulose. The optimum conditions of waste paper cellulose hydrolysis by the immobilized cellulase were searched for through the shaker-flask type reaction and they were a temperature of 45 °C and a pH of 4.0. However, we also found that UV light irradiation can make a small extent of deactivation of the immobilized cellulase and the larger size immobilized cellulase pellet had a better glucose production. The glucose production from waste paper cellulose hydrolysis with immobilized cellulase in a shaker-flask type reaction performed under these optimum conditions had a much better result than the same type hydrolysis with free cellulase under the same or different conditions. The glucose production from waste paper cellulose hydrolysis with immobilized cellulase performed in a 3L-fermentor was further improved by the design of the reactor to form a hanging immobilized cellulase reactor. The amount of glucose produced by using

the hanging immobilized cellulase reactor was raised to 2721.3 ± 142.9 ppm and about 1.73-fold better than the hydrolysis with freely suspended immobilized cellulase. The hanging immobilized cellulase reactor also has the benefit for the recycling and the reusing of immobilized cellulase. The immobilized cellulase can be recovered very easily after the reaction and they can be soaked in distilled water for one day to restore activity. Although the extent of restoration of cellulase activity is small about 1% in our present result the application of a special designed hanging immobilized cellulase reactor on the waste paper cellulose hydrolysis for glucose production and the reuse of the immobilized cellulase is successful.

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