Ethanol Production from Artificial Domestic Household Waste Solubilized by Steam Explosion

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Abstract Solubilization of domestic household waste through steam explosion with subsequent ethanol production by the microbial saccharification and fermentation of the exploded product was studied. The effects of steam explosion on the changes of the density, viscosity, pH, and amounts of extractive components in artificial household waste were determined. The composition of artificial waste used was similar to leftover waste discharged from a typical home in Japan. Consecutive microbial saccharification and fermentation, and simultaneous microbial saccharification and fermentation of the steam-exploded product were attempted using *Aspergillus awamori*, *Trichoderma viride*, and *Saccharomyces cerevisiae*; the ethanol yields of each process were compared. The highest ethanol yield was obtained with simultaneous microbial saccharification and fermentation of exploded product at a steam pressure of 2 MPa and a steaming time of 3 min.

Keywords: bioconversion, bioreactors, ethanol fermentation, steam explosion, simultaneous microbial saccharification and fermentation, waste treatment

INTRODUCTION

The treatment and effective utilization of domestic or restaurant waste are important not only for the prevention of environmental pollution but also for the conversion of the waste into useful materials. Since the composition (starch, cellulose, and other materials) in domestic waste varied markedly depending on the local environment (city, town, and village) and season, *etc.*, it was difficult to select a typical waste for this study [1,2]. A model waste was created with composing materials discharged from a typical Japanese home [3,4]; this was used to develop a treatment and effective utilization process of the waste. Steam explosion has been used as a pretreatment method to facilitate the solubilization of woody, agricultural, and other wastes [5-9].

This work investigated the use of a bioreactor for converting the steam-exploded product into ethanol after the microbial saccharification of artificial waste solublized by steam explosion. The physical and chemical modification of artificial waste by steam explosion was studied. Subsequently, the consecutive microbial saccharification and fermentation, and the simultaneous microbial saccharification and fermentation were carried out using the steam-exploded product and the ethanol yields obtained in the bioreactors were compared.

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MATERIALS AND METHODS

Artificial Waste

The artificial wastes were prepared based on the composition of the leftover waste discharged from the home at Kanazawa city in Japan. Model waste A contained mainly cellulose materials and model waste B contained mainly starch materials (Table 1).

Microorganisms

Aspergillus awamori IAM 2101 and *Trichoderma viride* IFO 31327 were used as amylolytic and cellulolytic microorganisms for the microbial saccharification, and *Saccharomyces cerevisiae* Hakken No. 2 was used as a fermentative yeast for ethanol fermentation.

Table 1. Composition of model waste

Component	Composition (%w/w)	
	Model waste A	Model waste B
Potato	15	45
Rice	5	15
Pork	5	5
Sardine	5	5
Cabbage	35	15
Orange skin	20	10
Banana skin	15	5
Total	100	100

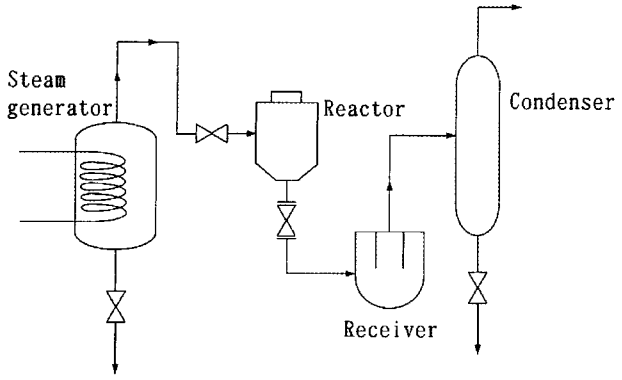

Fig. 1. Steam explosion apparatus.

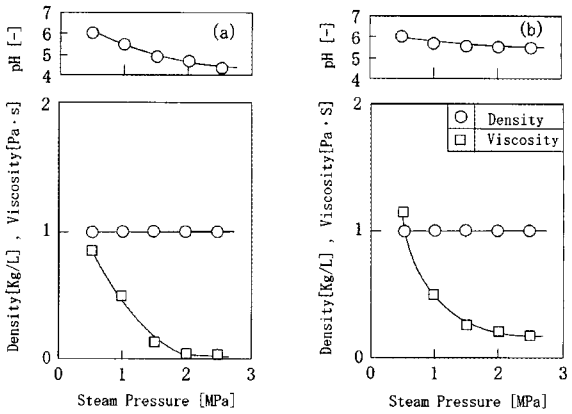

Fig. 2. Density, viscosity, and pH of exploded product under various steam pressures for a steaming time of 3 min. (a) Model waste A, (b) Model waste B.

Steam Explosion Apparatus

The steam explosion apparatus (reactor capacity 1.2 L, maximum steam pressure 5.5 MPa, maximum temperature 275°C) was made by the Japan Chemical Engineering and Machinery Co. Ltd., Osaka, Japan and consisted of a steam generator, a high-pressure reactor, a receiver, and a condenser with a silencing action as shown in Fig. 1. 100 g artificial waste was introduced into the reactor, and then steam-heated. The solid and liquid products were recovered in a cyclone at the bottom of the receiver, and the gaseous product was recovered after passing from the top of the receiver into the condenser. Steam explosions were conducted under various steam pressure and temperatures, 0.5 MPa (151°C), 1 MPa (179°C), 1.5 MPa (197°C), 2 MPa (211 °C), and 2.5 MPa (223°C), with a steaming time of 3 min.

Incubation Operation

The microbial saccharification of steam-exploded product was carried out using *Aspergillus awamori* IAM 2,101 and *Trichoderma viride* IFO 31, 327 in the reaction mixture containing 10 g/L dry exploded product in a 300 mL Erlenmeyer flask with 100 mL of 0.5 M acetate buffer (pH 5) at a rotation rate of 100 rpm and 30°C. After the microbial saccharification was carried out, the hydrolyzate of steam-exploded product was separated and then fermented by *Saccharomyces cerevisiae* Hakken No. 2 at pH 5 and 30°C.

Analysis

The density, viscosity, pH, and amounts of extractive components of exploded products were measured. The viscosity was measured using a viscometer (Viscotester VT-04, Rion Co., Ltd., Tokyo, Japan), and the pH value was determined by a pH meter (HM-26S, Thoa Denpa Kogyo Co., Ltd., Tokyo, Japan). The amounts of extractive components of exploded product, such as water soluble material (monosaccharides, oligosaccharides, and *etc.*), sulfuric acid soluble material (starch, cellulose, and *etc.*), methanol soluble lignin (low-molecular weight lignin), and Klason lignin (high-molecular weight lignin), were extracted and weighed by the Wayman method [10].

The optical density of culture was measured using a spectrophotometer (UV-120-02, Shimadzu Co., Ltd., Kyoto, Japan) at a wavelength of 660 nm. The cell concentration was estimated by measuring dry cell weight. The total sugar concentration was measured by the phenol-sulfuric-acid method [11], and the glucose concentration was determined by the mutarotase GOD method (Glucose C-test, Wako Pure Chemical, Osaka, Japan). The ethanol concentration was measured by gas chromatography (GC-8A, Shimazu Co., Ltd., Kyoto, Japan) using a Porapack Q column and a flame ionization detector.

RESULTS AND DISCUSSION

Solubilization of Imitation Waste by Steam Explosion

Figs. 2(a) and (b) show the density, viscosity, and pH of the model waste A and B exploded for 3 min under various steam pressures, respectively. The density of the model waste A and B was 1 kg/L regardless of steam pressure, and the viscosity decreased rapidly reaching almost a constant value. The viscosity decreased due to the starch material and the cellulose material being depolymerized and solubilized at a high steam pressure. Since the low viscosity solution had a high contact frequency of enzyme or cells with the substrate, it suggests that the exploded product treated beyond a steam pressure of 2 MPa is suitable for microbial incubation. The pH of the exploded product decreased with the increase of steam pressure and decreased to 4.5 and 5.5 using the model waste A and B, respectively. The pH decreased most likely because of the organic acids, such as formic acid, which were generated from the cellulose material in the model waste by the steam explosion. Therefore, the pH of the model waste A was lower than that of the model waste B because the model waste A

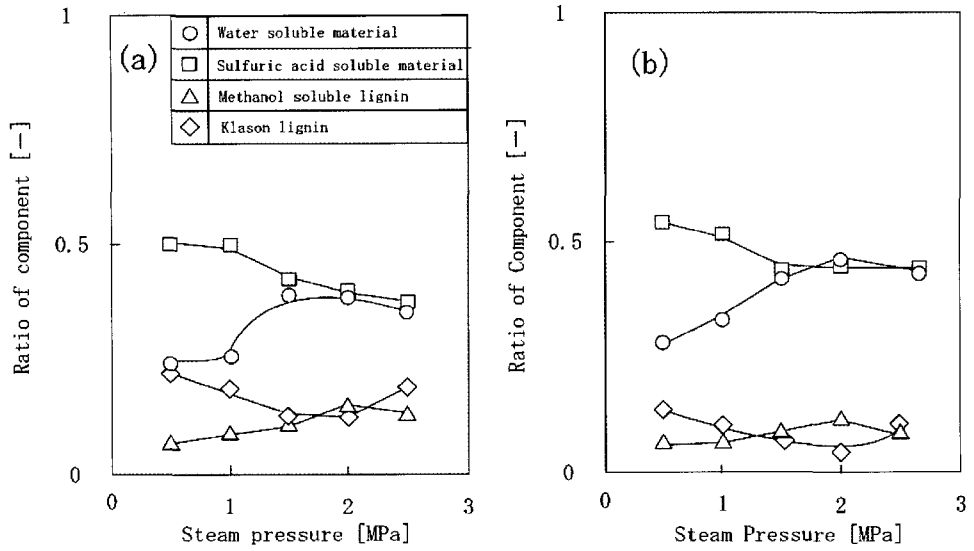

Fig. 3. Ratio of weight in extractive components to dry weight of exploded product under various steam pressures for a steaming time of 3 min. (a) Model waste A, (b) Model waste B.

contained a lot of cellulose material. Since the optimal pH for the growth of cells such *A. awamori*, *T. viride*, and *S. cerevisiae* is about 5 (data not shown), it is thought that a sample exploded beyond a steam pressure of 1 MPa is suitable for microbial saccharification and fermentation.

Figs. 3(a) and (b) show the ratios of weight in extractive components to dry weight of the model waste A and B exploded for 3 min under various steam pressures, respectively. In the case of the model waste A as shown in Fig. 3(a), the amount of water soluble material, *i.e.* a comparatively low-molecular monosaccharides, oligosaccharides, and *etc.*, increased by a steam pressure of 2 MPa because some part of the polysaccharides was hydrolyzed and converted into monosaccharides and oligosaccharides. The reason why the amount of water soluble material decreased by a steam pressure of 2 MPa depended on the fact that the monosaccharides and oligosaccharides combined with the lignin at a high steam pressure. The amount of sulfuric acid soluble material, i.e. starch, cellulose, and *etc.*, decreased by a steam pressure of 2.5 MPa. The reason why the amount of sulfuric acid soluble material decreased seems that some part of the polysaccharides was converted into a water soluble material by the hydrolysis of steam explosion. A low-molecular lignin, methanol soluble lignin, increased by a steam pressure of 2 MPa and then decreased. A high-molecular lignin, Klason lignin, decreased by a steam pressure of 2 MPa and then increased. Since in the steam explosion with a comparatively low steam pressure the high-molecular lignin was hydrolyzed into low-molecular lignin, the amount of Klason decreased below a steam pressure of 2 MPa. The increase of Klason lignin at a comparatively high steam pressure depended on the recondensation reaction of the low-molecular lignin and the combination reaction of the water soluble material and the high-molecular lignin. The exploded sample treated at a comparatively low steam pressure, *i.e.* 2 MPa, with a small amount of high-molecular lignin seems to be suitable for the microbial conversion into sugars because the high-molecular lignin inhibited the hydrolysis reaction of the cellulose by the cellulolytic microorganism. In the case of the model waste B as shown in Fig. 3(b), the tendency of changes in the amounts of extractive components was the almost same as the model waste A, but the amounts of extractive components differed from the model waste A. The difference in the amounts of extractive components between the model waste A and B was attributed to the fact that the model waste B had higher ratio of sugar component and lower ratio of lignin component compared with the model waste A.

Ethanol Production from Exploded Product by Microbial Saccharification and Fermentation

Fig. 4 shows the microbial saccharification and fermentation of 10 g/L exploded product A treated for 3 min at a steam pressure of 2.53 MPa. Two microorganisms, *i.e.* *Aspergillus awamori* that converted starch into glucose by using the amylase produced with the cell growth and *Trichoderma viride* that converted cellulose into glucose by using the cellulase produced with the cell growth, were used for the microbial saccharification of exploded product A as shown in Fig. 4(a). The sum cell concentration of the two microorganisms increased linearly by an incubation time of about 65 h and then reached a stationary phase. The total sugar concentration, *i.e.* water soluble material and sulfuric acid soluble material that can be converted into glucose by the microorganisms, decreased linearly by an incubation time of about 65 h and reached a constant value gradually. The glucose concentration increased rapidly after a lag time of about

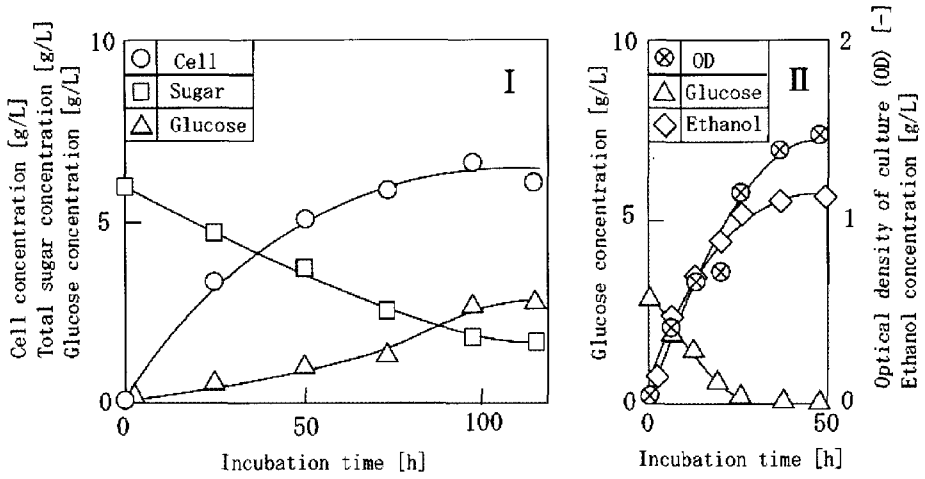

Fig. 4. Consecutive microbial saccharification and fermentation of 10 g/L exploded product A treated for a steaming time of 3 min at a steam pressure of 2.5 MPa. I: Microbial saccharifi-cation, II. Fermentation.

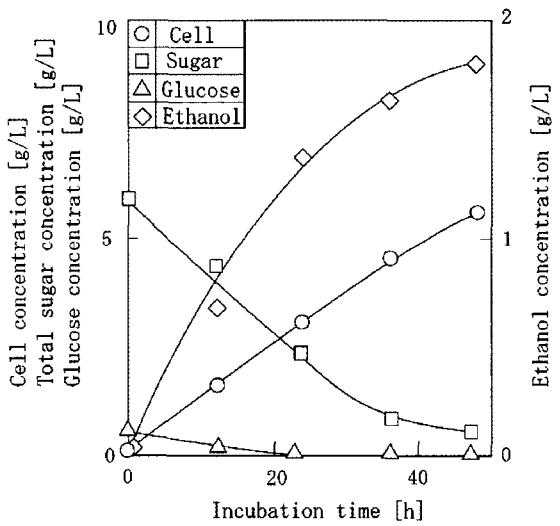

Fig. 5. Simultaneous microbial saccharfication and fermentation of 10 g/L exploded product A treated for a steaming time of 3 min at a steam pressure of 2.5 MPa.

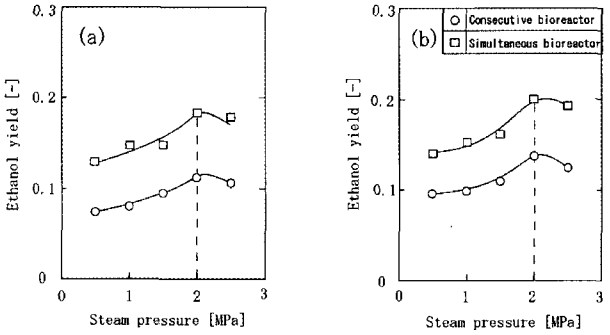

Fig. 6. Ethanol yield obtained in microbial saccharification and fermentation of 10 g/L exploded product at a steaming time of 3 min. (a) Model waste A, (b) Model waste B.

40 h and reached 2.9 g/L. Fig. 4(b) shows the ethanol fermentation of the glucose medium obtained by removing the growth cells from the culture medium after the microbial saccharification for about 120 h. *S. cerevisiae* grew with the rapid degradation of the glucose reaching a stationary phase after an incubation time of about 50 h and produced 1.1 g/L ethanol. However, since the consecutive bioreactor composed of microbial saccharifi-cation and fermentation required a long incubation time of 170 h and the glucose produced lowered the microbial saccharification action, the efficiency of ethanol decreased in the consecutive bioreactor.

Fig. 5 shows the time courses in the microbial simultaneous microbial saccharification and fermentation of 10 g/L expolded waste A. The total sugar concentration decreased significantly by the incubation time of about 35 h and reached a constant value. The respective con-centrations of the cell and the ethanol increased contrastly and reached a constant value. The reason why the glucose concentration was not observed during the incubation period depended on the high degradation rate of glucose by *S. cerevisiae* compared with the production rate of glucose by amylase and cellulase. Since 1.1 g/L ethanol was obtained in about 170 h by the consecutive microbial saccharification and fermentation and 1.8 g/L ethanol was done in about 50 h by the simultaneous microbial saccharification and fermentation, it seems that the simultaneous microbial saccharification and fermentation is a method that can be used for the efficient production of ethanol.

Figs. 6(a) and (b) show the ethanol yield obtained in the microbial saccharification and fermentation of 10 g/L exploded product A and B at a steaming time of 3 min under various steam pressures, respectively. The ethanol yield was defined as the ratio of amount of ethanol produced to the amount of exploded product. The ethanol yield of the exploded product A and B increased

with the increase of steam pressure reaching its maximum value at a steam pressure of 2 MPa and then decreased. The decrease of ethanol yield beyond a steam pressure of 2 MPa was attributed to the low susceptibility of the polysaccharide components and the enzyme synthesized by microorganisms due to the repolymerization reaction of the lignin in the steam explosion with a high pressure. Since the ethanol yield in the simultaneous microbial saccharification and fermentation was higher than that in the consecutive microbial saccharification and fermentation, it was found that the simultaneous microbial saccharification and fermentation with little inhibition of sugar produced on hydrolysis reaction was an effective method. The ethanol yield obtained from the exploded product B was higher than that from the exploded product A because the model waste B contained a lot of polysaccharide component greater than the model waste A as shown in Fig. 3. Since the highest ethanol yield was about 0.2, it seems that we can obtain 200 g ethanol from 1 kg dry artificial waste. Further study will be focused on increasing the concentration of ethanol produced by using distillation or pervaporation.

CONCLUSION

The effect of steam explosion on the solubilization of model waste and the ethanol production of exploded product by microbial saccharification and fermentation were investigated experimentally. The following findings were obtained.

(1) The viscosity of model waste increased remarkably with the increase of steam pressure, and the model waste was solubilized by the steam explosion.

(2) The amounts of extractive components in exploded product varied significantly with the steam explosion, and the steam explosion at a steam pressure of 2 MPa and a steaming time of 3 min was the most effective for the delignification of cellulose material in the model waste.

(3) The ethanol yield obtained by the simultaneous microbial saccharification and fermentation was higher than that by the consecutive microbial saccharification and fermentation, and the highest ethanol yield was provided from the exploded product at a steam pressure of 2 MPa and a steaming time of 3 min.

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