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Anaerobic Fermentation of Woody Biomass Treated by Various Methods

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Abstract Anaerobic fermentation was attempted to produce methane from the wood chip (*Eucalyptus globulus*). By the pretreatment of the wood chip using hot water with high temperature, NaOH, and steam explosion, the production of methane gas was enhanced. The pretreatment using steam explosion resulted in more amount of methane gas produced than the treatment using either hot water or 1% (w/w) NaOH with high temperature, and the steam explosion at a steam pressure of 25 atm and a steaming time of 3 min was the most effective for the methane production. The amount of methane gas produced depended on the ratio of weight of Klason lignin, a high molecular weight lignin, in the treated wood chip.

Keywords: anaerobic fermentation, methane gas, pretreatment method, steam explosion, woody biomass

INTRODUCTION

Large amounts of woody waste, *i.e.* wood chip, wood powder, bark, and others, occur in the forest industry. The methane fermentation of woody waste is one of the most attractive methods for effective utilization of waste [1-3]. In the case using wood chip as a substrate, the main source of methane production seems to be holocellulose, *i.e.* cellulose and hemicellulose. On the other hand, since lignin is hardly degraded anaerobically [4], it is not the source of methane production. Therefore, it is necessary for the efficient production of methane from wood chip to remove lignin that covered holocellulose in wood chip using a pretreatment. Various pretreatment methods, *i.e.* physical treatment, chemical treatment, and biological treatment, have been reported for this purpose. Physical treatment such as vibrating ball-mill [5,6] is able to remove a part of lignin, but it is inefficient and wastes a lot of energy. Chemical treatment with NaOH [7] or H_2O_2 [8] is effective for the degradation of lignin, producing substances that are readily fermentable; however, there are problems on the type of apparatus and procedure, and on the post-treatment. Furthermore, the biological treatment with lignin-peroxidase [9] or manganese-dependent peroxidase [10] is a low cost treatment, but it requires for a long treatment time. In the recent years, a method of decomposition involving an autohydrolysis reaction with high-temperature steam followed by a sudden reduction in pressure have been attractive as a pretreatment for the delignification of plant biomass [11-13]

In this work, various pretreatment methods, *i.e.* extraction using hot water or NaOH with high temperature, and steam explosion, were attempted for decreasing and removing lignin component in wood chip, and the optimal pretreatment method for the production of methane from wood chip was determined.

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MATERIALS AND METHODS

Sample

Eucalyptus globulus chips (25 × 10 × 2-5 mm) that are a raw material for the pulp manufacture in Japan were used as a sample of woody waste.

Extraction Using Hot Water with High Temperature

Wood chips (5 g) were added into a 300-mL conical flask containing 100 mL water and heated at 125°C for 20 min by an autoclave. The product was filtrated using glass filter (IG1, Nippon Rikagaku Kikai Co. Ltd.) and washed with 1 L distilled water, and then dried at 50°C by a heater to a constant weight.

Extraction Using NaOH with High Temperature

Wood chips (5 g) were added into a 300 mL conical flask containing 100 mL of 1%(w/w) NaOH solution and heated at 125°C for 20 min by an autoclave. The

Table 1. Chemical component of wood chips treated by various treatment methods and amount of methane gas produced from their wood chips. Dry treated sample (3.4 g) was fermented anaerobically for 14 day by using the methanogens sludge

Treatment method	Ratio of weight of component (-)			Methane gas produced (mL)
	Methanol soluble lignin	Klason lignin	Holocellulose	
Untreated	0.03	0.56	0.41	48
Extraction using hot water with high temperature	0.06	0.43	0.51	421
Extraction using 1% NaOH with high temperature	0.10	0.36	0.54	456
Steam explosion at 25 atm and 3 min	0.15	0.25	0.60	660

product was filtrated using glass filter (IG1, Nippon Rikagaku Kikai Co. Ltd.) and washed with 1%(w/w) HCl and distilled water to neutral pH, and then dried at 50°C by a heater to a constant weight.

Steam Explosion

Steam explosion apparatus (the capacity of reactor 1.2 L, the highest steam pressure 55 atm, the highest temperature 275°C) was made in 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 [14]. Wood chips (5 g) were introduced into the reactor, and then steam-heated. The product was filtrated using glass filter (IG1, Nippon Rikagaku Kikai Co. Ltd.) and washed with 1 L distilled water, and then dried at 50°C by a heater to a constant weight. Steam explosions were conducted under various steaming times, including 1, 2, 3, 5, 7, and 10 min at a steam pressure of 25 atm.

Anaerobic Fermentation

Dry wood chips treated with above methods (3.4 g) were added into a 300-mL conical flask containing 60 mL seed methanogens sludge and 240 mL inorganic solution (0.03 g/L NH_4Cl, 0.2 g/L K_2HPO_4, 0.05 g/L $MgSO_4 \cdot 7H_2O$, and 0.01 g/L yeast extract), and then incubated anaerobically at a rotation rate of 100 rpm and 37°C. The seed methanogens sludge (SS 8,000 ppm) was obtained by culturing the sludge withdrawn from an anaerobic digestion unit at a Kanazawa city wastewater treatment plant in Japan. For gas chromatographic analysis, evolved gas was drawn from the reservoir by a syringe through a silicon stopper attached to the top of the reservoir.

Analytical Methods

The amounts of the components in the wood chips treated by various treatment methods were measured using the Wayman method [15]. Dry wood chips treated (5 g) were extracted for 12 h in a Soxhlet extractor with 100 mL methanol to dissolve the methanol soluble lignin, a low molecular weight lignin. After concentration and drying of the extract, the methanol soluble lignin was weighed. The residue from the methanol extraction consisted of holocellulose and Klason lignin, a high molecular weight lignin. This residue (1 g) was added to 15 mL of 72%(w/w) sulfuric acid and left at room temperature for 4 h. This was placed in a 100 mL conical flask, washed with 560 mL distilled water, and then boiled for 4 h with reflux-cooling. After the insoluble matter was washed with hot water, it was dried at about 105°C by a heater to a constant weight, and weighed. This substance was Klason lignin. The weight of hollocelulose was calculated by subtracting the weight of Klason lignin from 1 g of the residue. In anaerobic fermentation experiments, amount of gas evolved was measured by using a TCD gas chromatography (GC-8APT, Shimadzu Co. Ltd., Kyoto, Japan).

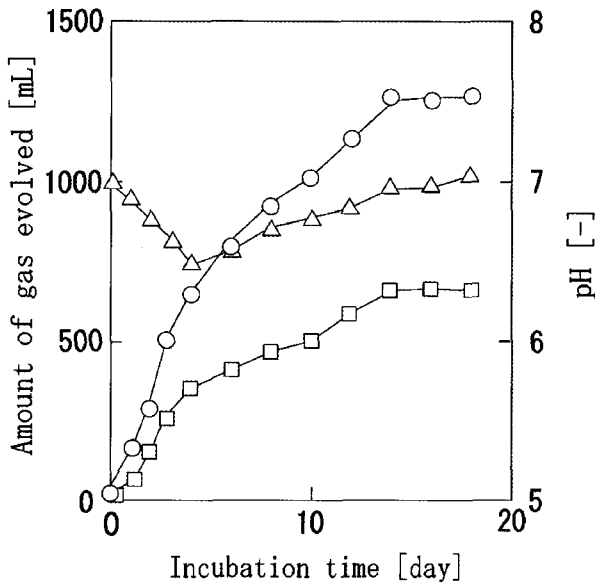

Fig. 1. Methane fermentation of steam-exploded wood chips at a steam pressure of 25 atm and a steaming time of 3 min by using methanogens sludge. Values indicate the mean of three independent experiments. Symbols: ○, total gas evolved; □, methane gas evolved; △, pH.

RESULTS AND DISCUSSION

Fig. 1 shows the time courses of total gas evolved, methane gas evolved, and pH in an anaerobic fermentation of 3.4 g dry wood chips treated at a steam pressure

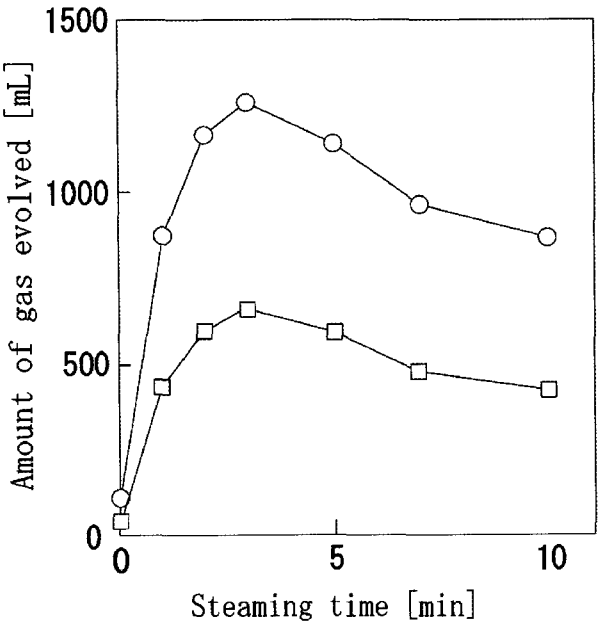

Fig. 2. Amount of gas produced from steam-exploded wood chips at a steam pressure of 25 atm. Values indicate the mean of three independent experiments. Symbols: O, total gas evolved; □, methane gas evolved.

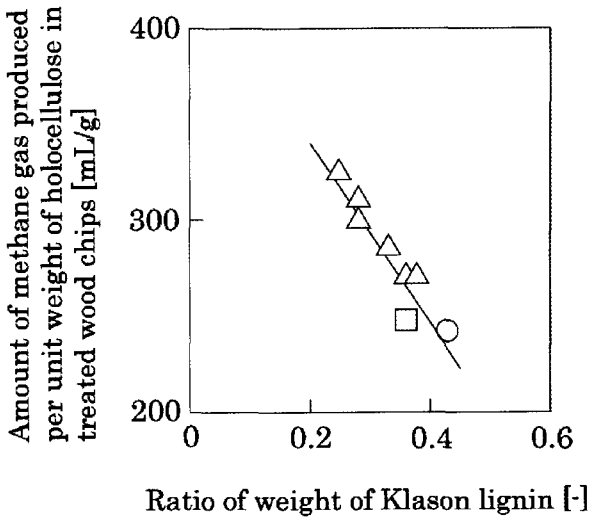

Fig. 3. Relationship between amount of methane gas produced per unit weight of hollocellulose and ratio of weight of Klason lignin in the treated wood chips. Symbols: O, extraction using hot water with high temperature; □, extraction using 1% NaOH with high temperature; △, steam explosion at 25 atm and 3 min.

of 25 atm and a steaming time of 3 min. The amounts of total gas and methane gas increased rapidly with the increase of incubation time reaching their maximum values of 1,280 and 660 mL, respectively, at an incubation time of 14 day. The value of pH decreased from its initial pH, *i.e.* 7, up to 6.5 at an incubation time of 4 h and then increased gradually reaching about 7. Organic acids, *i.e.* propionic acid, acetic acid, and formic acid, were detected in the culture at an incubation time of 4 h (data not shown). This suggests that in the early state of incubation some organic acids were produced from the wood chips treated and then converted into methane and carbon dioxide.

Table 1 shows the ratios of the dry weights of chemical components in the wood chips treated by various treatment methods to the dry weight of the wood chips treated and the amount of methane gas produced from their wood chips. The ratios of methanol soluble lignin and holocellulose in the treated wood chips were higher than those in the untreated wood chips. On the other hand, the ratio of Klason lignin in the treated wood chip was lower than that in the untreated wood chips. The amount of methane gas produced varied significantly with the treatment methods and it increased in this order of untreated, extraction using hot water with high temperature, extraction using 1% (w/w) NaOH with high temperature, and steam explosion at 25 atm and 3 min. The reason of this order seems to be attributed to the increase of amount of hollocellulose and the decrease of amount of Klason lignin in the treated wood chips. As a result, it was found that (1) the production of methane gas from the wood chips was enhanced by degrading or removing Klason lignin that inhibited the conversion of holocellulose into methane because Klason lignin, a high molecular weight lignin covered holocellulose strongly in wood chips and (2) the steam explosion method was the most effective pretreatment method for the methane fermentation of wood chips.

Fig. 2 shows the amounts of total gas evolved and methane gas evolved in the anaerobic fermentation of wood chips treated by steam explosion at 25 atm under various steaming times. The fermentation time was 14 days. The amounts of total gas evolved and methane gas evolved increased rapidly with the increase of steaming time reaching their maximum values, 1,280 and 660 mL, respectively, at a steaming time of 3 min and then decreased gradually. The reason why the amounts of the gases decreased after 3 min depended on the fact that the amount of Klason lignin increased in the treated wood chips as a result of its condensation reaction with the methanol soluble lignin, a low molecular weight lignin (data not shown).

For the amount of methane gas produced by the degradation of various organic compounds, Buswell and Sollo [16] have reported the standard equation. According to this equation, the amount of methane gas produced from 1 g hollocellulose is about 415 mL. Fig. 3 shows the relationship between the amount of methane gas produced per unit weight of hollocellulose and the ratio of amount of Klason lignin in the treated wood chips. Since the amounts of methane gas produced per unit weight of hollocellulose were 250-330 mL, it suggests that about 60-80% of hollocellulose in the treated wood chips were converted into methane gas. Kuwahara

et al. [17] investigated the anaerobic fermentation of silver fir (*Picea jezoensis* Carr.) bark powder treated by various treatments and reported that the maximum conversion ratio of hollocellulose in the bark into methane gas, *i.e.* about 50%, was obtained by the chlorite treatment with 10 g/L sodium chlorine at 100°C for 30 min. Since the chlorite treatment requires the post-treatment of sodium chlorine, it seems that the steam explosion treatment is not only an effective treatment for the methane production from woody biomass but also an environmentally friendly method. A negative correlation between the amount of Klason lignin and the amount of methane gas produced was confirmed in Fig. 3 and the following equations were obtained.

$$V_m = -449R_K + 431 \quad (1)$$

$$r = -0.94 \quad (2)$$

where V_m, R_K, and r are amount of methane gas produced per unit weight of hollocellulose, ratio of amount of Klason lignin, and correlation coefficient. From the result, it was found that for the effective conversion of woody biomass into methane it is necessary to decrease the amount of Klason lignin in the woody biomass as much as possible by using a pretreatment.

CONCLUSION

The anaerobic fermentation of wood chips treated by various methods was investigated for determining the effective pretreatment method for the methane production from woody biomass and the following findings were obtained.

1) In the methane fermentation of steam-exploded wood chips, some organic acids was produced and then converted into methane gas.

2) The steam explosion at a steam pressure of 25 atm and a steaming time of 3 min was the most effective pretreatment method for the methane production and about 80% of holocellulose in the steam-exploded wood chips was converted into methane gas.

3) A negative correlation between the amount of Klason lignin and the amount of methane gas produced was obtained in the anaerobic fermentation of treated wood chips.

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