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Hydrogen Fermentation of Cow Manure Mixed with Food Waste

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Abstract

Resource recovery and wastewater purification of agricultural waste are currently considered important ways to reduce environmental impact. In the present study, hydrogen fermentation of cow manure mixed with various defined substrates or artificial food wastes was examined by lab-scale batch mode experiments at 60°C, using hydrogen-producing bacteria naturally present in the manure. A variety of carbohydrates including cellulose and xylan were used as substrates for hydrogen production, although no hydrogen production was observed with proteinous substrates (casein, gelatin and albumin), an amino-acid mixture (Casamino acids), or lipids (palmitic acid, linoleic acid, soybean oil, and olive oil). In experiments involving artificial food wastes (boiled rice, bread, cabbage, chicken meat, fish meat, egg, mayonnaise, chocolate, and strawberry jam), the amounts of hydrogen production were positively correlated with the carbohydrate content of the foods, while no significant correlation with protein or fat content was observed. To examine the effect of hydrogen fermentation on methane fermentation, a two-step treatment (hydrogen fermentation followed by methane fermentation) was conducted using cow manure mixed with dog food. The hydrogen fermentation increased methane production two-fold as compared with the one-step treatment (methane fermentation only). In addition, the hydrogen fermentation enhanced the removal of volatile solids and biochemical oxygen demand from the mixture, suggesting that hydrogen fermentation has advantages in wastewater purification.

Discipline: Agricultural environment
Additional key words: biohydrogen, kitchen refuse, livestock manure, methane production, two-step treatment

Introduction

Resource recovery and wastewater purification of agricultural waste, such as livestock excreta, rice straw and organic waste from a food processing factory, are currently considered important ways to reduce environmental impact. Methane fermentation is a typical method used to recover energy from these wastes, and it has been put into practice. Hydrogen is a clean energy carrier which produces only electricity and water when it is used with fuel cells. Anaerobic digestion to produce hydrogen, known as hydrogen fermentation, has been studied using various organic wastes, such as sugary wastewater[11], bean curd manufacturing waste[5], a wheat starch co-product[3], palm oil mill effluent[1], food wastes[7], and cow manure[14,15]. Wastewater treated by hydrogen fermentation can be re-used as feedstock for methane fermentation, because volatile fatty acids (VFAs) produced by hydrogen fermentation serve as substrates for methane production. Therefore, a two-step treatment (hydrogen fermentation followed by methane fermentation) of organic waste has been proposed[6]. To date, two-step treatments of organic wastes such as food waste[2], household solid waste[4] and organic solid waste containing paper[12,13] have been reported.

Hydrogen fermentation of organic wastes, other than livestock manures, requires the addition of hydrogen-producing bacteria. Various kinds of bacteria including hydrogen-producing bacteria are usually contained in live-

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stock manure[9], and thus hydrogen fermentation of livestock manure does not require the addition of seed bacteria. This is an advantage of livestock manure. However livestock manure contains only a limited amount of energy available for biogas production, in contrast with other organic wastes. Therefore, the mixed treatment of livestock manure and other energy-rich wastes would be beneficial with respect to the supplies of seed bacteria and energy.

We previously reported that hydrogen could be produced from cow manure by anaerobically incubating the manure without the addition of seed bacteria[14]. Although methanogenesis was observed under mesophilic conditions (30–40°C), the activity of methanogens was suppressed under thermophilic conditions over 50°C. Two-peak temperatures for hydrogen production were observed at 60°C (main peak) and 75°C (secondary peak). Hydrogen-producing bacteria related to *Clostridium thermocellum* and *Clostridium stercorarium* were detected in the fermentation at 60°C by an analysis using 16S rDNA. The hydrogen production at 60°C peaked at day 4, and thereafter the produced hydrogen was lost to non-methanogenic hydrogen-consuming bacteria. Therefore, 4-day incubation at 60°C was the optimum condition to efficiently obtain hydrogen from the manure.

In the present study, the hydrogen fermentation of cow manure mixed with various kinds of defined substrate or artificial food waste was examined under the optimum condition. The substrate preference of bacteria contained in the manure was analyzed. In addition, to examine the effect of hydrogen fermentation on methane fermentation, the two-step treatment of cow manure mixed with dog food was conducted.

Materials and methods

1. Hydrogen fermentation of cow manure mixed with defined substrate or artificial food waste

Feces and urine of Holstein cows were collected in the cow barn of National Institute of Livestock and Grassland Science (NILGS), and stored at –20°C until use. The feces was composed of 150 g/L total solids and 128 g/L volatile solids (VS). The cow manure was prepared by mixing 0.75 g (dry matter) of the feces and 8 ml of the urine, and supplemented with 2 g of defined substrates or 3 g (dry matter) of artificial food wastes. The mixtures were diluted to 200 mL with distilled water and placed in 750 mL glass bottles. After replacement of the gas phases with nitrogen, the bottles were tightly sealed with a butyl rubber cap and incubated at 60°C for 4 days[14]. Mayonnaise and milk chocolate were purchased from Q.P. Corporation, Tokyo, Japan, and Meiji Seika Kaisha, Tokyo, Japan, respectively. Boiled rice, bread, chicken meat (deep pectoral muscle), and fish meat (bluefin, lean meat) were pre-treated with a homogenizer Polytron PT200 (Kinematica AG, Lucerne, Switzerland). The cabbage was dried at 60°C for two days and milled with a rotor mill ZM100 (Retsch, Haan, Germany).

2. Two-step treatment

Nine g (dry matter) of the feces, 24 ml of the urine, and 4.5 g (dry matter) of dog food (Vita-one, Nihon Pet Food, Tokyo, Japan) were mixed and diluted to 600 mL with distilled water. The dog food contained 58.8% carbohydrate, 22.2% protein, 8.9% fat, and 10% ash. The mixture was placed in a 1 L conical flask and anaerobically cultured at 60°C for 4 days as the first treatment. Thereafter the pH of the cultured mixture was adjusted to 7.0–7.5 with 1 N NaOH. A methanogenic sludge (40 mL) from a UASB plant in NILGS[10] was added to the mixture, and distilled water was added for a total volume of 700 ml. The mixture was further cultured at 37°C for 10 days as the second treatment.

3. Product analysis

In the experiments using the defined substrates and artificial food wastes, the biogas in the glass bottle was removed with a syringe and analyzed using a gas chromatograph Model–80C (Ohkura Electric, Saitama, Japan), equipped with two tandem columns (a Porapak Q column followed by a molecular sieve 5A column), as described previously[16]. In the experiment involving the two-step treatment, the evolved biogas was collected through a vapor trap in an Aluminized Polyethylene Bag (GL Sciences, Tokyo, Japan). The entire amount of the evolved biogas in the bag was sampled with the syringe at 24-h intervals, and the biogas volume was measured with the syringe. Biochemical oxygen demand (BOD) was measured with a BOD Trak apparatus (HACH, Colorado, USA). The data were analyzed with a simple regression model using the Excel 2003 program (Microsoft, Washington, USA).

Results and discussion

1. Hydrogen production from cow manure mixed with defined substrates or artificial food wastes

Starch and soluble sugars such as glucose and sucrose are well known to be substrates for hydrogen production in pure-culture and mixed-culture systems. However, hydrogen production from cellulose and hemicellulose has not been characterized well. It is largely unknown whether or not hydrogen is produced from a proteinous substrate and lipids in mixed-culture systems.

To investigate the substrate preference of the hydrogen-producing bacteria naturally present in cow manure, hydrogen production from various kinds of defined substrates was examined in batch mode experiments. The bacteria produced 863–1,211 mL-H_2/L-mixture from soluble sugars (xylose, glucose and sucrose) and an insoluble carbohydrate, wheat starch (Fig. 1). The bacteria were able to produce hydrogen from cellulose and xylan (a component of hemicellulose) at similar levels (843–1,331 mL-H_2/L-mixture). On the other hand, proteinous substrates (casein, albumin, gelatin, and peptone) and Casamino acids (a mixture of amino acids) were not used for hydrogen production, although they increased the production of carbon dioxide. Therefore these proteinous substrates were decomposed by the bacteria without production of hydrogen. Neither carbon dioxide nor hydrogen were produced from lipids (palmitic acid, linoleic acid, soybean oil, or olive oil), suggesting that these lipids were not decomposed. No biogas production was observed with palmitic acid, which suggests that palmitic acid might be toxic for the bacteria.

Food wastes consist of carbohydrates, protein, fat, and ash in a variety of combinations. The effect of the combination of these components on hydrogen production was analyzed using nine artificial food wastes (boiled rice, bread, cabbage, chicken meat, fish meat, egg, mayonnaise, chocolate, and strawberry jam). The nutrition facts are shown in Table 1. Hydrogen was produced from carbohydrate-rich foods (rice, bread, chocolate, and strawberry jam) at higher levels than from other protein- or fat-rich foods (Fig. 2). The coefficient of determination (R^2) for hydrogen production was 0.73 for carbohydrates and was less than 0.2 for the other nutrient contents including protein, fat, ash, and dietary fiber (Table 1). The protein content was expected to affect hydrogen production, since a nitrogen source is required for bacterial growth; however, the manure contained urine and other nutrients such as minerals and vitamins, and these would be sufficient to support the bacterial growth.

2. Two-step treatment of cow manure mixed with an artificial food waste

Figure 3 shows the time courses of biogas production in the two-step treatment of cow manure mixed with dog food. Dog food has been frequently used as a typical artificial food waste containing solid organic matter[13]. As compared to the one-step treatment (methane fermentation only), the two-step treatment continuously produced higher amounts of methane, except on days 6 and 9. The treatment time required for 50% of the total methane production to occur was 3.8 days in the two-step treatment, which was 66% of the treatment time required in the one-step treatment, 5.8 days. Generally, solubilization of solid substrate is a rate-limiting step in methane fermentation. Hydrogen fermentation would solubilize solid substrates to VFAs, and probably allowed the faster

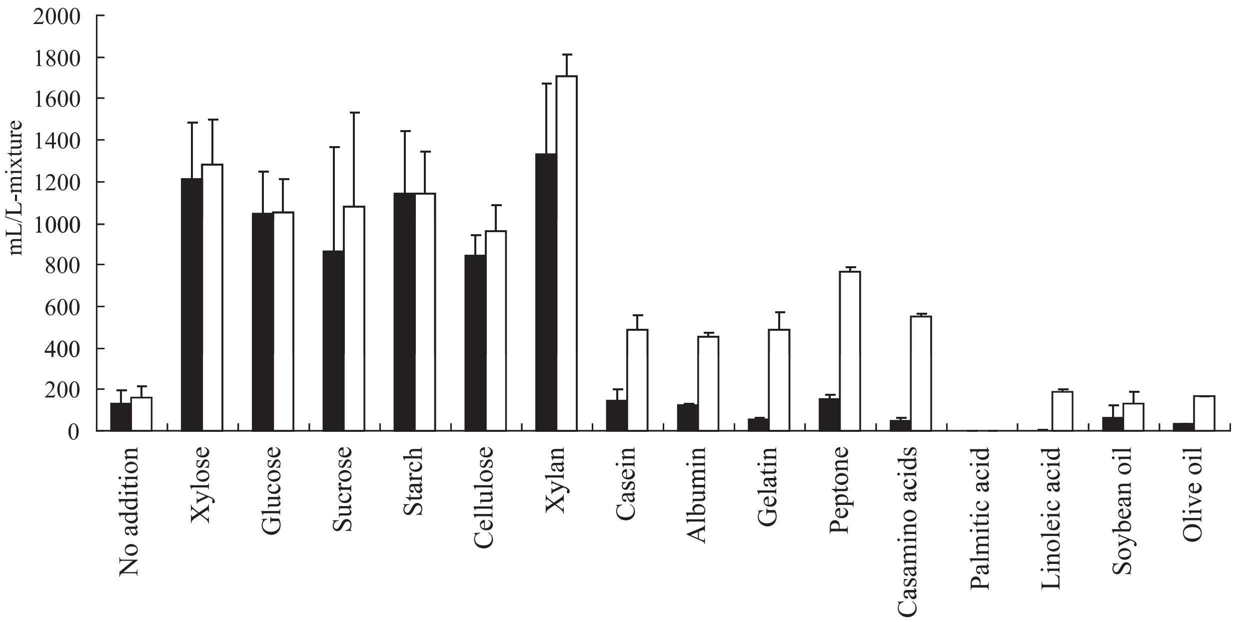

Fig. 1. Hydrogen production from cow manure mixed with defined substrates
■ : H_2, □ : CO_2.

Table 1. Nutrition facts and coefficient of determination for H_2 production

	Protein	Fat	Carbohydrates	Ash	Dietary fiber
Rice[1]	6.3	0.8	92.80	0.3	0.8
Bread[1]	15	7.1	75.3	2.6	3.8
Cabbage[1]	33.9	0.6	58.9	6.5	32.7
Chicken meat[1]	91.8	4.1	0	4.1	–
Fish meat[1]	89.2	4.1	0	5.7	–
Egg[1]	53.5	44.7	1.3	4.1	–
Mayonnaise[2]	3.3	93.6	0.8	–	–
Chocolate[2]	8.0	34.7	55.4	–	3.0
Strawberry jam[1]	1.0	0.2	98.2	0.6	2.2
R^2 for H_2 production	0.16	0.12	0.73	0.08	<0.01

Values are expressed as a percentage of dry matter.
[1]: Data from standard tables of food composition in Japan[8].
[2]: Data from suppliers.

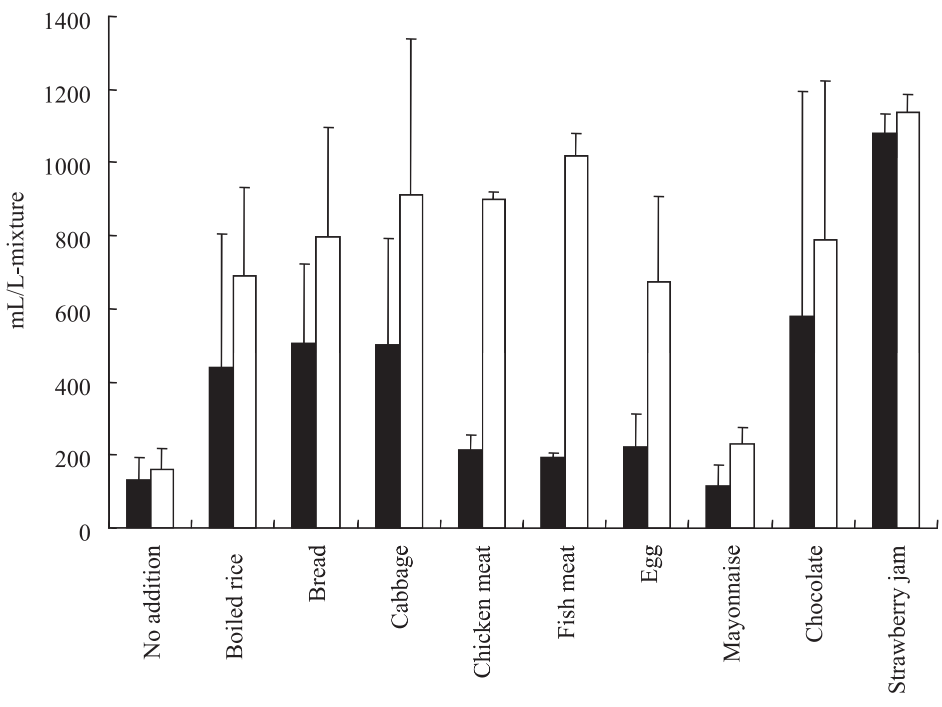

Fig. 2. Hydrogen production from cow manure mixed with artificial food wastes
■ : H_2, □ : CO_2.

production of methane. The total amount of methane production achieved by the two-step treatment was 4.2 L-CH_4/L-mixture, which was approximately two-fold higher than that achieved by the one-step treatment (Fig. 4). The ratio among the carbon dioxide, hydrogen and methane produced by the two-step treatment was 6.4 : 1.0 : 8.4. The removal of VS and BOD, indicators of organic matter, is important for wastewater purification. The two-step treatment removed 40% of VS, which was nearly double the removal rate of the one-step treatment, 19% (Fig. 5). As for BOD, the removal rate achieved by the two-step treatment was 71%, which was more than double the removal rate in the one-step treatment, 34%.

In summary, the present study showed that bacteria contained in cow manure can produce hydrogen from a variety of carbohydrates including cellulose and xylan.

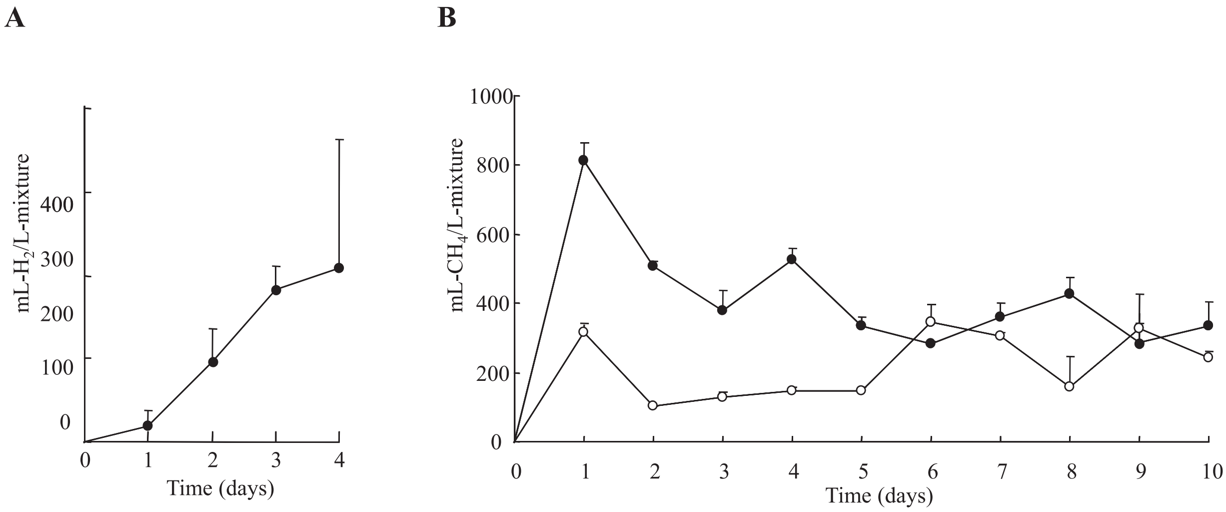

Fig. 3. Time courses of biogas production by the two-step treatment of cow manure mixed with dog food
(A): Hydrogen production in the first treatment. (B): Methane production in the second treatment. For comparison, the methane production by the one-step treatment is shown.
—○— : One-step treatment, —●— : Two-step treatment.

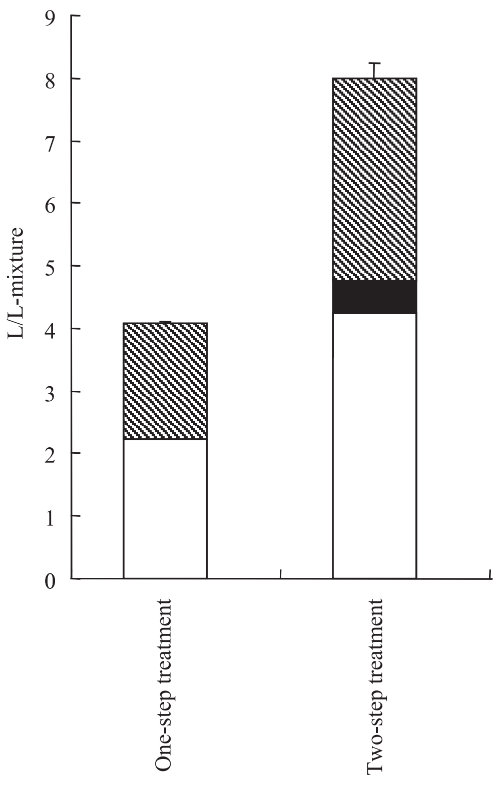

Fig. 4. Total biogas production by the one-step and two-step treatments from cow manure mixed with dog food
▧ : CO_2, ■ : H_2, □ : CH_4.

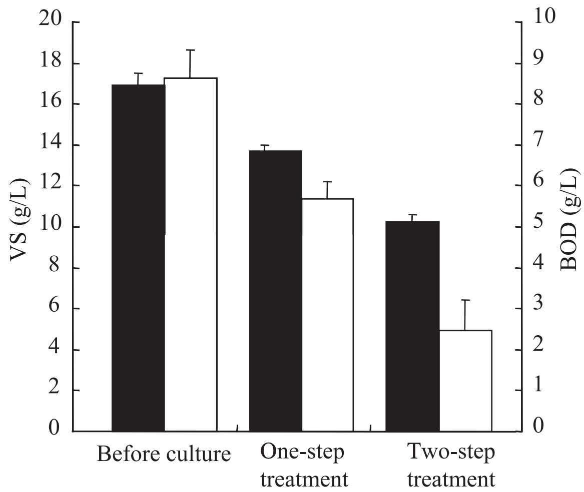

Fig. 5. VS and BOD removals by the one-step and two-step treatments of cow manure mixed with dog food
■ : VS, □ : BOD.

The bacteria preferred carbohydrate-rich food waste for hydrogen production, although the protein and fat contents did not affect hydrogen production. In the two-step treatment, hydrogen fermentation increased the amount of methane production and facilitated the production rate of methane by solubilizing solid substrates to VFAs. Moreover, hydrogen fermentation enhanced the removal of VS and BOD, indicating that the two-step treatment has advantages over one-step treatment in the purification of cow manure mixed with food waste. In practice, these properties of hydrogen fermentation might contribute to downsizing the bioreactor and shortening the total processing time.

Acknowledgments

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