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Pretreatment with rumen fluid improves methane production in the anaerobic digestion of paper sludge

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ABSTRACT

Because paper sludge discharged from the waste paper recycling process contains high levels of lignin and ash, it is not hydrolyzed effectively during anaerobic digestion. In this study, we investigated the effects of pretreatment with rumen fluid on paper sludge and on the methane fermentation process. Paper sludge was pretreated with rumen fluid at 37 °C for 6 h. Following pretreatment, 4.5% of the total solids in paper sludge were degraded and converted, and the dissolved chemical oxygen demand and volatile fatty acid concentration increased. Batch methane fermentation was conducted at 37 °C for 20 days. During methane fermentation, the degradation and hydrolysis of paper sludge were enhanced by pretreatment with rumen fluid. The amounts of total methane production from pretreated paper sludge (excluding methane generated from rumen fluid), rumen fluid and untreated paper sludge were 650.4, 819.9 and 190.8 ml, respectively. The volume of methane gas produced from pretreated paper sludge was 3.4 times larger than that from untreated paper sludge. These results indicate that pretreatment with rumen fluid enhances methane production from paper sludge.

1. Introduction

The worldwide production of paper and paperboard generates 26.2 million tons of waste per year. In Japan, approximately 60% of waste paper is recycled (Paper Recycling Promotion Center, 2010). Paper sludge, consisting of organic fibers and ash, is discharged during the production of recycled paper from waste paper. The annual yields of discharged paper sludge in Japan and the United States are 3 and 8 million tons, respectively (Wajima et al., 2006). Most discharged paper sludge is incinerated, but this process consumes large amounts of energy because paper sludge has a high moisture content and contains ash. The recovery rate of waste paper increases every year; thus, the amount of discharged paper sludge also increases. Therefore, the anaerobic digestion of paper sludge has been studied in an attempt to reduce the consumption of fossil fuel required for incineration.

Paper sludge contains lignocellulosic residues. Lignocellulosic biomass is composed primarily of three polymers (cellulose, hemicellulose and lignin). These polymers bind with each other and form a staunch structure; hence, the disintegration and hydrolysis of lignocellulosic biomass become the rate-limiting steps during anaerobic digestion (Sawatdeenarunat et al., 2015). Previous studies have indicated that physical, physico-chemical, chemical and biological pretreatments are effective for the removal of lignin, the reduction of crystallinity and the improvement of porosity (Grous et al., 1986; Lin et al., 2009, 2010; Saha et al., 2011; Wood et al., 2009). These pretreatments promote methane production, but have not been applied in large-scale fermenters due to issues such as the substantial consumption of energy, neutralization of wastewater, production of fermentation inhibiters, high cost of reagents and low pretreatment rate. Pretreatment with rumen fluid has been proposed as a novel method for efficient methane production from lignocellulosic biomass without these disadvantages.

The rumen is one of four stomach compartments in ruminants, and rumen fluid hosts a complex anaerobic microbial ecosystem consisting of 10^{10-11} cells/ml bacteria, 10^{3-5} cells/ml fungi and 10^{4-6} cells/ml protozoa. These microorganisms express a vast amount and range of enzymes to digest lignocellulosic biomass, including glycoside hydrolases and carbohydrate esterases. Therefore, the ecosystem is able to ferment lignocellulosic feed and efficiently produce volatile fatty acids (VFAs) as nutrients for ruminants (Güllert et al., 2016; Kamra, 2005; Wongwilaiwalin et al., 2013). In Japan, 1.1 million cattle are slaughtered annually, and 88,102 tons of rumen fluid is discharged from slaughterhouses. Discharged rumen fluid is treated as wastewater, which requires

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the consumption of huge amounts of energy, leading to high costs. Baba et al. (2013) investigated the high fibrolytic activity of rumen fluid and found that 6 h pretreatment with rumen fluid improved the degradability of organic fibers in wastepaper. The study demonstrated that the volume of methane gas produced from pretreated wastepaper increased by 2.6 times during anaerobic digestion. Rumen fluid pretreatment has been shown to improve biogas production from rice straw and rapeseed (*Brassica napus* L.) shoots, with the biogas volume increasing by 1.5–1.7 times (Baba et al., 2017; Zhang et al., 2016).

Previous studies have used lignocellulosic biomass that consisted mainly of cellulose and hemicellulose as a substrate for rumen fluid pretreatment. Paper sludge contains higher levels of lignin and ash than do the lignocellulosic substrates used in previous studies (Table 1) (Baba et al., 2013; Baba et al., 2017; Zhang et al., 2016). Lignin consists of three monomers (coniferyl alcohol, sinapyl alcohol and p-coumaryl alcohol). These lignin monomers form a complex three-dimensional structure through various bonds, and can resist chemical and enzymatic degradation in anaerobic digestion (Taherzadeh and Karimi, 2008). The ash in paper sludge interferes with hydrolysis and adheres to fibers in methane fermenters (Chen et al., 2012). Therefore, methane production from paper sludge is more inefficient than that from substrates that consist mainly of cellulose and hemicellulose, rather than lignin and ash. The aim of this study was therefore to investigate whether pretreatment with rumen fluid could improve methane production from paper sludge.

2. Materials and methods

2.1. Materials

Rumen fluid was collected from grass-fed lactating Holstein dairy cows via a stomach tube. The care and use of animals in this study were in accordance with the regulations of the Institutional Animal Care and Use Committee of Tohoku University.

The collected rumen fluid was immediately filtrated (1 × 1 mm mesh) to remove coarse solids. Paper sludge is discharged during the manufacture of recycled paper. Dewatered paper sludge was used as the substrate in this study. The seed sludge was collected from an anaerobic digester of waste glycerol operated at 35 °C at Tohoku University (Osaki, Japan), and 2 g/l CH_3COONa, 2 g/l glucose, 2 g/l NH_4Cl, 0.016 g/l KH_2PO_4, 0.025 g/l $CaCl_2{\cdot}2H_2O$, 0.025 g/l $MgCl_2{\cdot}6H_2O$, 0.03 g/l Fe-EDTA, 0.005 g/l $CoCl_2{\cdot}6H_2O$, 0.005 g/l $NiCl_2{\cdot}6H_2O$, 0.005 g/l $MnCl_2{\cdot}4H_2O$ and 0.1 g/l yeast extract were added for acclimation. After biogas production by the seed sludge was stopped, the seed sludge was used in the experiment. The characteristics of the rumen fluid, paper sludge and seed sludge are shown in Table 2.

2.2. Pretreatment of paper sludge with rumen fluid

Pretreatment of paper sludge with rumen fluid was conducted according to the previous report that pretreated the waste paper (Baba et al., 2013). Pretreatment of paper sludge was performed in a 500-ml reactor attached with a gas bag (working volume of 300 ml). A total of 18.4 g (9.0 g dry weight) paper sludge was added to 300 ml rumen fluid (3% w/v). Then, the mixture was purged with nitrogen gas to sustain anaerobic conditions and pretreated at 37 °C on a rotary shaker at 170 rpm for 6 h. The blank reactor contained only rumen fluid or paper sludge with deionized water instead of rumen fluid. The pretreatment of paper sludge with rumen fluid was conducted in duplicate.

Table 2
Characterization of paper sludge, rumen fluid and seed sludge.

	Paper sludge	Rumen fluid	Seed sludge
pH	–	6.8	7.7
Total COD (g/l)	–	13.0	2.2
Dissolved COD (g/l)	–	8.4	0.8
Total solid (TS) (%)	49.3	0.3	0.7
Volatile solid (%TS)	31.2	70.7	70.7
Cellulose (%TS)	8.5	32.0	22.5
Hemicellulose (%TS)	2.7	2.8	2.6
Lignin (%TS)	13.7	12.2	6.1
Crude ash (%TS)	66.3	–	–

2.3. Batch methane fermentation

Batch methane fermentation was performed in a 1000-ml reactor attached with a gas bag (working volume of 800 ml). Pretreated paper sludge and rumen fluid (300 ml) were mixed with 500 ml seed sludge. As a blank, untreated paper sludge and rumen fluid were respectively mixed with the seed sludge. Then, the mixture was purged with nitrogen gas to remove oxygen and incubated at 37 °C for 20 days. The methane yield and total volume of methane production were calculated according to Zhang et al. (2016). Chemical analyses during methane fermentation were conducted in duplicate.

2.4. Analysis

The volume of biogas produced in the gas bag was determined using a syringe. Biogas (methane and carbon dioxide) produced during pretreatment and methane fermentation was measured by gas chromatography (GC) (GC-8A; Shimadzu, Kyoto, Japan). The temperature of injection and detection was set to 100 °C, and nitrogen was used as the carrier gas. A packed column (Shincarbon-ST; Restek, Bellefonte, PA, USA) was used, and a unit equipped with a thermal conductivity detector was connected to an integrator (C-R8A; Shimadzu). Liquid samples were filtrated with a cellulose acetate membrane filter (0.45 μm pore diameter) for analysis. The dissolved chemical oxygen demand (COD) was measured using a colorimetric method using 0–1500 mg/l vials (Hach, Loveland, CO, USA). The concentration of VFAs was measured by high-performance liquid chromatography (JASCO, Tokyo, Japan) using an ion-exchange column (RSpak KC-811; Shodex, Tokyo, Japan) and an ultraviolet detector (870-UV; JASCO). The temperature of the oven was set to 60 °C. The eluent was 3 mM $HClO_4$ and the flow rate was 1.0 ml/min. The total solid (TS) and volatile solid (VS) contents were determined using the methods described by APHA (2012). The amounts of neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) in

Table 1
Components of the lignocellulosic substrates pretreated with rumen fluid used in previous studies.

	Cellulose (%TS)	Hemicellulose (%TS)	Lignin (%TS)	Crude ash (%TS)	Reference
Waste paper	71.3	18.5	8.9	–	Baba et al. (2013)
Rice straw	37.2	26.4	6.9	–	Zhang et al. (2016)
Rapeseed	21.7	3.3	3.2	–	Baba et al. (2017)
Paper sludge	8.5	2.7	13.7	66.3	This study