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Application of rumen microorganisms for enhanced anaerobic fermentation of corn stover

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Abstract

Anaerobic fermentation of corn stover by rumen microorganisms in both batch and semi-continuous cultures was studied. In the batch reactors, a high volatile solids (VS) conversion efficiency of 65–70% was achieved after 240 h incubation at 25–40 °C. The volatile fatty acids (VFA) yield ranged from 0.59 to 0.71 g g^{-1} VS utilized. Acetate, propionate and butyrate were the main aqueous products with small amounts of *i*-butyrate and valerate also being produced. Biogas was composed of methane and carbon dioxide. In the semi-continuous reactor, at loading rates of 10–30 g VS l^{-1} d^{-1} and 40 °C, a VS conversion efficiency of 65% was achieved as a solids and hydraulic retention time of 96 and 18 h, respectively. The VFA yield varied between 0.56 and 0.59 g g^{-1} VS utilized. Biogas was composed of hydrogen and carbon dioxide, but was free of methane. The experimental results demonstrate that the anaerobic fermentation of corn stover by rumen microorganisms was able to rapidly degrade the volatile solids and produce useful VFAs with high yields.

Keywords: Anaerobic fermentation; Corn stover; Rumen microorganisms

1. Introduction

Lignocellulose, a renewable biomass produced in conventional agriculture and forestry activities, has a special interest because of the large quantities and inexpensive costs [1]. In 2002, China produced 135 million metric tons of corn. After harvesting the crops, the residue is normally left in the field. This waste might be a potential resource for the production of renewable energy through chemical or biological conversion [2–5]. For example, it could be fermented to produce volatile fatty acids (VFA) with a yield of 0.50–0.55 g acid equivalent g^{-1} dry ash-free corn stover by using anaerobic cultures and dilute alkali pre-treatment [1]. Compared with chemical means, biological conversion is considered more environmentally friendly and less energy intensive [2].

However, biological conversion of lignocellulose has been hampered by its relatively refractory structure, such as the crystallinity of cellulose and the association of cellulose and hemicellulose with lignin [6–8]. Many bioconversion efforts to produce ethanol from lignocellulosic materials are non-economic, due to the requirement for expensive pre-treatment [1]. The anaerobic fermentation of waste might be a better option because it does not need any pre-treatment and the source of substrate is supplementary to agricultural production of a valuable crop. But due to the low cellulolytic activity and slow specific growth rates of the microorganisms involved, the anaerobic fermentation efficiencies of solid lignocellulosic materials are usually very low in conventional bioreactors [9]. The fermentation of lignocellulosic wastes might be improved by increasing cellulase activities in these reactors [10].

Recently, the potential applications of rumen microorganisms in artificial rumen reactors for the conversion of cellulose-rich wastes have been explored [4,5,10]. A two-stage anaerobic process has been developed [4] in which the first stage is an artificial rumen reactor where cellulose-laden wastes are degraded to VFA and carbon dioxide by rumen microorganisms. The effluent from the first reactor is converted to methane in the second methanogenic reactor [4,10]. In this closed fluid system cellulose is almost

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completely converted into biogas. Lignocellolosic wastes, such as cereal straws and domestic refuse are also able to be degraded by rumen microorganisms in a semi-continuous system [5,11].

Corn stover is an abundant agricultural residue in China. The bioconversion of this residue by anaerobic digestion from this cellulose-rich waste could provide energy and alleviate deforestation in rural areas. The available information, however, regarding the anaerobic fermentation of corn stover by rumen microorganisms is sparse. The current paper reports on this in batch and semi-continuous processes.

2. Materials and methods

2.1. Substrate

After the corn was harvested corn stover was collected from a cornfield near Hefei, China. The raw material was sun-dried for 5 days and ground to 1.0-mm particle size using a vegetable disintegrator, and later thoroughly mixed and stored in plastic bags at ambient temperatures of 15–25 °C. The chemical composition of the substrate is summarized in Table 1.

2.2. Culture and media

Rumen fluid obtained from a fistulated goat was used as a source of seed microorganisms. It was squeezed through four-layers of gauze and transferred to vials, and these vials which were purged with N_2 gas to ensure anaerobic conditions. The medium used in the experiments contained the following constituents (per liter): $NaHCO_3$, 8 g; KH_2PO_4, 1 g; K_2HPO_4, 3 g; $CaCl_2{\cdot}2H_2O$, 0.03 g; $MgCl_2{\cdot}6H_2O$, 0.08 g; NH_4Cl, 0.18 g. In addition, in order to maintain the pH above 6.0, excess calcium carbonate was added to the vials and fermenters [2].

2.3. Batch fermentation

Batch cultures were set up by adding 20 ml of rumen fluid and 80 ml of fermentation buffer to 250 ml serum bottles, and the substrate added to give concentrations of 5.0, 10.0 and 15.0 g volatile solids (VS) l^{-1}. These cultures were subsequently flushed with N_2 for 10 min in order to create anaerobic conditions, and were then sealed with *n*-butyl rubber stoppers and aluminum caps. The bottles were then incubated at 25, 30, 35 and 40 °C in a shaking incubator at 130 rpm for 240 h. Samples for gas analysis, VFAs and soluble total organic carbon (TOC) were taken at predetermined time intervals. Substrate conversion was estimated by determining the loss in weight of VS and neutral detergent fiber (NDF).

Table 1
Chemical composition of corn stover

Determination		Corn stover
Total solids (TS)	(%)	95.2 ± 3.9
Volatile solids (VS)	(% of TS)	94.3 ± 4.2
Ash	(% of TS)	5.7 ± 0.3
NDF	(% of TS)	70.8 ± 9.2
ADF	(% of TS)	45.5 ± 5.3
Hemicellulose	(% of TS)	25.3 ± 2.5
Cellulose	(% of TS)	28.4 ± 2.8
Lignin	(% of TS)	17.1 ± 2.1

Note: NDF: neutral detergent fiber; ADF: acid detergent fiber.

2.4. Semi-continuous culture system

The semi-continuous anaerobic fermentation was carried out in a 3.0 l artificial rumen reactor with a working volume of 2.0 l. The reactor was inoculated with 300 ml of strained rumen fluid, and was operated at temperatures of 35 and 40 °C and loading rates (LR) of 10, 20, and 30 g VS l^{-1} d^{-1}, respectively. Hydraulic retention time (HRT) and solids retention time (SRT) of the reactor were maintained at 18.0 ± 0.5 h and 96.0 ± 4.0 h, respectively. This was achieved by supplying fresh fermentation medium via a peristaltic pump and removing liquid effluent through a filter unit of 30 μm pore size. The difference between the rate of buffer supplied and filtered-effluent removed caused an increase in the reactor working volume. This increase was removed daily as homogeneous reactor-contents so as to give the desired SRT [10]. The corn stover digester feed was added daily after removing 25% of the homogenous reactor contents were removed once a day just before addition of substrate. The reactor contents were mixed for a period of 30 s every 5 min by a magnetic stirrer. A schematic diagram of the reactor is shown in Fig. 1.

All experiments continued until a reactor steady state had been reached, as indicated by constant effluent TOC (±5%) and VFA concentration (±5%) on a daily basis. The extent

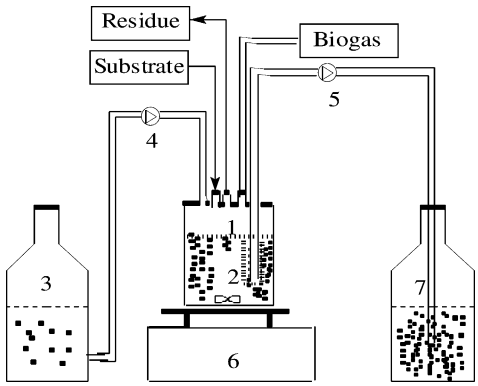

Fig. 1. Schematic diagram of the semi-continuous reactor: (1) acidogenic reactor; (2) 30 μm pore size filter; (3) fermentation medium reservoir; (4) fermentation supply pump; (5) filtered effluent removal pump; (6) magnetic stirrer; (7) filtered effluent.