Enhancing the Anaerobic Digestion of Corn Stalks Using Composite Microbial Pretreatment

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A composite microbial system (XDC-2) was used to pretreat and hydrolyze corn stalk to enhance anaerobic digestion. The results of pretreatment indicated that sCOD concentrations of hydrolysate were highest (8,233 mg/l) at the fifth day. XDC-2 efficiently degraded the corn stalk by nearly 45%, decreasing the cellulose content by 22.7% and the hemicellulose content by 74.1%. Total levels of volatile products peaked on the fifth day. The six major compounds present were ethanol (0.29 g/l), acetic acid (0.55 g/l), 1,2-ethanediol (0.49 g/l), propionic acid (0.15 g/l), butyric acid (0.22 g/l), and glycerine (2.48 g/l). The results of anaerobic digestion showed that corn stalks treated by XDC-2 produced 68.3% more total biogas and 87.9% more total methane than untreated controls. The technical digestion time for the treated corn stalks was 35.7% shorter than without treatment. The composite microbial system pretreatment could be a cost-effective and environmentally friendly microbial method for efficient biological conversion of corn stalk into bioenergy.

Keywords: Composite microbial system, biogas, pretreatment, hydrolysate, anaerobic digestion

In recent years, energy resources and environmental protection have become important global concerns. In developing countries, there has been increasing interest in developing technologies to harness and utilize renewable energy resources such as biomass [21]. Lignocellulosic biomass is one of the most abundant resources in the world; it is renewable and can be degraded by microorganisms [13]. Agricultural wastes such as rice straw, wheat straw, and corn stalks are important sources of lignocellulosic biomass, and China is among the countries with the highest field crop straw production in the world. More than 600 million tons of crop straw are produced annually in China [14]. The production of biogas through anaerobic digestion offers significant advantages over other forms of agricultural waste treatment [22].

A lot of literature is written about different pretreatment methods to enhance the digestibility of lignocellulosic biomass, because pretreatment is the rate-limiting step. Physical (mechanical comminution and hydrothermolysis) and chemical (acid pretreatment, alkaline pretreatment, and oxidative delignification) pretreatments have been proven to effectively enhance anaerobic digestion. However, physical and chemical pretreatment methods require significant energy use and are not environmentally friendly. Biological pretreatment offers some conceptually important advantages, such as low chemical and energy uses, but very few controllable and sufficiently rapid systems have been found [2].

Natural lignocellulosic biomass is difficult for microorganisms to degrade because of the combination of cellulose, hemicellulose, and lignin. Although microbial decomposition of lignocelluloses has been studied extensively, most of these studies used a pure culture of microorganisms [7]. In our laboratory, some composite microbial systems (MC1, XDC-2, and SD-Y) with efficient and stable cellulose degradation characteristics have been developed. MC1 can degrade rice straw by 60% within four days at 50°C [4, 9]; XDC-2 can degrade rice straw by 50% within nine days at 35°C [7]; SD-Y can degrade switchgrass by nearly 70% within four days, decreasing the cellulose content by 67.3% and the hemicellulose content by 73.5% [25]. However, these systems have not yet been tested for performance in biogas production. Therefore, in order to develop a novel microbial pretreatment method for biogas production, we used XDC-2 to pretreat and hydrolyze corn stalks in this study and analyzed the effect on biogas production.

The objectives of this study were: (1) to investigate the effect of microbial pretreatment on biogas production, and determine the optimal time of pretreatment; and (2) to analyze the changes in the main chemical components and microbial community during pretreatment.
Material and Methods

Preparation of Materials and Medium for Pretreatment by Composite Microbial System XDC-2

Corn stalk: Corn stalks were harvested and dried naturally at the China Agricultural University (Haidian District, Beijing City, China). The characteristics of the corn stalk tissue used in this study are shown in Table 1. Before use, corn stalks were chopped into 10-20 mm pieces using pruning shears and dried again at 80°C for 48 h prior to use.

Bacterial community: XDC-2, which is capable of effectively degrading cellulose, was developed and characterized in our laboratory [7]. The system was cultured in peptone cellulose solution (PCS) containing 1% (w/v) corn stalk for three days at 5°C and stored at -20°C in 20% glycerol. The previous microbial composition results, which were analyzed by clone library and sequencing, indicated that the 26 different clones assembled into three phyla: Clostridiales, Proteobacteria, and Bacteriodetes. Among the 26 clones, 46.2% clones belonged to the cluster Clostridiales, and were related to the genera Clostridium, Sporomusa, Oscillibacter, and Psychrosinus; 38.4% belonged to the cluster Proteobacteria, and were related to the genera Devosia, Pseudomonas, Escherichia, and Alcaligenes [7].

Medium: The peptone cellulose solution (PCS) was composed of 2 g of peptone, 1 g of yeast extract, 2 g of CaCO₃, 5 g of NaCl, and 11 of H₂O (pH 8.0). The medium was autoclaved at 121°C for 20 min.

Pretreatment Assays by Composite Microbial System XDC-2

The main purpose of pretreatment with the composite microbial system XDC-2 is to make cellulose and hemicellulose available and digestible for downstream processes. Several 1,000 ml Erlenmeyer flasks (stoppered with aluminum foil) containing 500 ml of autoclaved PCS medium and 1% (w/v) corn stalk as the single carbon source were prepared. After inoculation with the activation culture of the preserved inoculums (seed volume of 5%), the medium was cultured under static conditions at 35°C. To determine the optimal length of time for pretreatment, fermentation was allowed to continue for 16 days, and samples were taken to obtain correlative numerical data on the characteristics of the corn stalk.

Table 1. Characteristics of corn stalk used in the experiments.

<table>
<thead>
<tr>
<th>Characteristics</th>
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<tbody>
<tr>
<td>TS (%)</td>
<td>94.91 ± 0.8</td>
<td>C/N</td>
<td>38.04</td>
</tr>
<tr>
<td>VS (TS%)</td>
<td>95.08 ± 1.2</td>
<td>Lignin (TS%)</td>
<td>7.70 ± 0.8</td>
</tr>
<tr>
<td>TC (TS%)</td>
<td>41.84 ± 1.9</td>
<td>Cellulose (TS%)</td>
<td>33.35 ± 1.6</td>
</tr>
<tr>
<td>TN (TS%)</td>
<td>1.10 ± 0.2</td>
<td>Hemicellulose (TS%)</td>
<td>28.74 ± 2.1</td>
</tr>
</tbody>
</table>

*Values are the means ± SD (n ≥ 3).

The pH of the hydrolysate during the pretreatment was also determined on days 0, 1, 2, 3, 5, 7, 10, 13, and 16 using a pH meter (Model B-212, Horiba, Inc., Japan).

The optimal length of time for pretreatment was determined by analyzing the changes in the main chemical components of the corn stalk.

Anaerobic Digestion

The results of pretreatment indicated that the optimal length of time for pretreatment is five days. Therefore, the samples (untreated corn stalks and 500 ml of hydrolysate treated for five days by XDC-2 with corn stalks) were digested in batch anaerobic digesters. The volume of each anaerobic digester was 1 l, with a working volume of 750 ml. Each digester was seeded with the anaerobic sludge taken from a mesophilic anaerobic digester from the Deqinyuan Biogas Plant (Beijing, China). The sludge contained 57.2 g/l total solids (TS), 31.5 g/l volatile solids (VS), and 39.6 g/l mixed liquor suspended solids (MLSS). The anaerobic sludge MLSS was seeded in each digester at 15 g/l [27]. Ammonia chloride (NH₄Cl) was added to each digester to adjust the carbon/nitrogen ratio (C/N) to 25, which is believed to be optimal for anaerobic bacteria growth [27]. The anaerobic digestion experiment was repeated three times at mesophilic temperature (35°C).

Analyses of Volatile Products of the Hydrolysate by GC–MS

On days 2, 5, 7, 10, and 16, samples obtained from the hydrolysate were filtered through an aperture of 0.22 µm and analyzed using GC–MS (model QP-2010, Shimadzu, Japan) on-line with a capillary column, CP-Chirasil-Dex CB (25×0.25 mm). The analytical conditions were as follows: column temperatures: 60°C (for 1 min) → 100°C, 7°C/min → 195°C (for 2 min), 18°C/min; injector temperature: 190°C; ion source temperature: 200°C; carrier gas: He (60 kPa); rate of flow: 34 ml/min; splitter ratio: 1/20; voltage of detector: 0.7 kV; sample volume: 1 µl.

The GC–MS peaks were qualitatively analyzed using the NIST database.

Biogas Analyses

Biogas volume was monitored every day using the water displacement method, and the corresponding cumulative biogas volume was calculated. The measured volume was then converted to a volume of gas at standard temperature and pressure using the ideal gas law. The methane content of the biogas was analyzed every day using a biogas analyzer (Biogas Check, Geotech, Britain).

Chemical Composition Analyses

The total carbon (TC) and total nitrogen (TN) of the corn stalks were determined by the TC analyzer (Skalar Primacssle, The Netherlands). The TS, VS, and MLSS of the corn stalks, anaerobic sludge, and...