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Molecular characterization of bacterial and archaeal communities in a full-scale anaerobic reactor treating corn straw

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HIGHLIGHTS

- The anaerobic degradation pattern of corn straw for biogas production was investigated.
- Besides volatile acids, aromatic compounds are intermediates in anaerobic degradation.
- Hydrolytic and fermentative microorganisms dominate the bacterial community.
- High proportion of syntrophic propionate and aromatic acids degrading bacteria were detected.
- Hydrogenotrophic methanogens were more dominant than aceticlastic methanogens.

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ABSTRACT

A 16S rRNA gene-based method was used to characterize the structure of bacterial and archaeal communities in a full-scale, anaerobic reactor treating corn straw. Degradability experiment indicated biogas slurry had high microbial activity, the TS removal rate was 53% and the specific methanogenic activity was 86 mL CH_4 g VSS^{-1} d^{-1}. During anaerobic degradation of corn straw, volatile acids and aromatic compounds (*p*-cresol, phenylpropionate, phenol and benzoate) were detected as transient intermediates. Phylogenetic analysis revealed bacterial community exhibited high diversity, 69 bacterial phylotypes in 13 phyla were identified. *Firmicutes* (48.3%), *Chloroflexi* (20.1%), *Actinobacteria* (9.1%), *Bacteroidetes* (7.7%), and *Proteobacteria* (7.2%) represented the most abundant bacterial phyla. Hydrolytic and fermentative bacteria were major bacterial populations. Moreover, a relatively high proportion of syntrophic propionate and aromatic compounds degrading bacteria were detected. In the archaeal clone library, 11 archaeal phylotypes affiliated with two phyla of *Crenarchaeota* (10%) and *Euryarchaeota* (90%) were identified.

1. Introduction

Crop residues, such as corn straw, wheat straw, and rice straw are abundant and important renewable biomass resources. Anaerobic digestion is a promising alternative for the treatment of organic wastewater and solid waste, as the process combines waste treatment and energy recovery. Currently, anaerobic digestion has been widely applied for the treatment of most of biodegradable waste, e.g., municipal sewage sludge, kitchen waste and animal manures. Anaerobic digestion of straw has been studied for nearly a century (Acharya, 1935). However, the implementation of full scale biogas plants used straw as the sole feedstock has not yet been demonstrated. The major reason is that straw consists mainly of cellulose, hemicellulose and lignin, which are extremely recalcitrant to degradation. Numerous lab-scale experiments have focused on the pretreatment of lignocellulose to break down the structural integrity of lignocellulosic biomass and to enhance enzymatic action on cellulose, determination of optimal parameters of anaerobic straw digestion, and co-digestion with other wastes (Dinuccio et al., 2010; Zhong et al., 2011, 2012). Whereas, the knowledge of the microbial community involved in anaerobic digestion system for the treatment of crop residues is still limited.

Anaerobic bioconversion of lignocellulosic biomass to methane needs cooperation of complex microbial populations, including hydrolytic (celluloytic), saccharolytic, homoacetogenic, syntrophic hydrogen-producing bacteria and methanogenic archaea. The anaerobic decomposition of crop residues occurs naturally, the process and the functional groups of microorganisms participated in anaerobic degradation of soil organic matter have been revealed, especially on methanogens in anoxic rice fields (Großkopf et al.,

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1998; Lu et al., 2000) and straw-rotting soils (Conrad et al., 2012). Furthermore, several artificial microbial systems treating lignocellulosic biomass have also been investigated, such as an anaerobic mixed fermentation reactor fed with maize silage, green rye and liquid manure (Kröber et al., 2009), an anaerobic microbial system decomposing poplar wood chips (van der Lelie et al., 2012). However, no studies have characterized the microbial community in a pilot-scale or full-scale biogas reactor with corn straw as the sole feedstock. In this study, the structure of bacterial and archaeal communities in a full-scale biogas reactor treating corn straw was investigated by 16S rRNA sequencing analysis. In addition, the characteristics of anaerobic corn straw degradation and intermediates production were analyzed.

2. Methods

2.1. Source of biogas slurry

Anaerobic biogas slurry was taken from a full-scale mesophilic (35 °C), anaerobic completely stirred tank reactor (CSTR) (volume, 500 L) constructed in china. The reactor was fed with untreated corn straw as the sole feedstock for seven months, and with cow manure as the original inoculum. Corn straw contained 94.4% total solids (TS), of which 36.8%, 27.7% and 8.0% were cellulose, hemicellulose and lignin, respectively. The reactor was operated at an organic loading rate (OLR) of 1.2 kg TS m^{-3} d^{-1} and hydraulic retention time (HRT) of 40 days. The volatile suspended solids (VSS) of the biogas slurry was 6682 mg L^{-1}. The fermentation pH was about 7.0. Volatile fatty acids (VFA) concentration in the biogas slurry was 590 mg L^{-1} acetate, 374 mg L^{-1} propionate, 130 mg L^{-1} *n*-butyrate and 50 mg L^{-1} *i*-butyrate at the time for investigating the microbial communities, respectively. The biogas slurry was washed and suspended with phosphate buffer (10 mM, pH 7.2) for several times. The suspension solution was centrifuged at 5000 rpm for 10 min, and the pellets were collected and used for DNA extraction.

2.2. Degradability experiment

To investigate the degradability of corn straw and methanogenic activity of biogas slurry, batch experiments were carried out in 60 mL (liquid volume, 20 mL) serum vials at 37 °C with anaerobic biogas slurry as the inoculum without shaking. The medium used for cultivation was prepared as described previously (Sekiguchi et al., 2000). A total volume of 100 mL of biogas slurry was washed and suspended with medium for several times. The suspension solution was centrifuged at 5000 rpm for 10 min, and the pellets were collected, and resuspended in aliquots in 20 mL of medium without substrate. The cell suspension was then inoculated into bottles containing medium supplemented with corn straw (TS, 1%) as sole carbon source. Naturally harvested and sun-dried corn stalks were chopped into 1–2 cm pieces using pruning shears and dried at 105 °C for 48 h prior to use. In order to investigate the accumulation of metabolic intermediates, 5 mM 2-bromoethanesulfonate (BES), a specific inhibitor of methanogenesis, was added. The incubation and inhibition experiments were performed in duplicate. Concentrations of fermentation products in batch experiments were represented as the mean of duplicate experiments. The TS of corn straw was measured before and after the degradation assay. Productions of methane, volatile acids and aromatic compounds were measured periodically.

2.3. Analytical methods

The concentrations of the aromatic compounds were analyzed by high pressure liquid chromatography (HPLC) using a zorbax SB-C18 reverse-phase column (5 μm 0.5 × 150 mm, Agilent Technologies Inc, Shanghai, China) held at 25 °C and a UV detector set at 210 and 220 nm. The mobile phase was a 70:30 (v/v) mixture of methanol and 1% aqueous acetic acid at a flow rate of 0.8 mL/min. VFA, methane and hydrogen were measured as described previously (Yuan et al., 2011).

2.4. DNA extraction, PCR amplification and construction of 16S rRNA gene library

Genomic DNA was extracted based on the indirect extraction method (Gabor et al., 2006). PCR amplification, cloning, and sequencing procedures for constructing 16S rRNA gene clone libraries were performed as previously reported (Sekiguchi et al., 1998) with slight modifications. For construction of the 16S rRNA gene clone library, the following primer set for PCR amplification of bacterial and archaeal 16S rRNA genes was used: EUB8F (5′-AGAGTTTGATCMTGGCTCAG-3′; positions 8–27 in the *Escherichia coli* gene), and the reverse primer UNIV1492R (5′-TACGGYTACCTTGTTACGACTT-3′; positions 1492–1513 in *E. coli*) for the domain *Bacteria*, and ARC109F (5′-ACKGCTCAGTAACACGT-3′; positions 109–125 in *E. coli*) and UNIV1492R for the domain *Archaea*, respectively. The amplification conditions were: denaturing step of 95 °C for 9 min and 20 cycles of denaturation at 95 °C for 50 s, annealing at 50 °C for 1 min, and extension at 72 °C for 2 min; the final step was followed by post extension at 72 °C for 10 min. The PCR products were purified with a TIAN quick MiDi purification kit (Tiangen Inc., Beijing, China) and resuspended in nuclease-free water. Purified bacterial and archaeal PCR products were ligated into a pGEM-T easy vector with the TA cloning kit (Promega Inc., Beijing, China) following the manufacturer's instructions and transformed into *E. coli* DH5α competent cells, respectively. White colonies were selected to conduct colony PCR with the vector-specific primers M13F and M13R. Approximately 200 bacterial clones and 100 archaeal clones were randomly picked and screened by comparing restriction fragment length polymorphism (RFLP) patterns with *Hae*III and *Hha*I restriction endonucleases. Clones were grouped according to RFLP banding patterns, and scanning image analyses were performed manually. Then unique phylotypes were identified. Chimeric sequences were identified using the CHIMERA CHECK program of RDP (Cole et al., 2003) and excluded from subsequent analysis. Multiple alignments of the sequences from this study and reference sequences were performed using CLUSTAL X (Thompson et al., 1997). Phylotype was defined as a group of cloned sequences with >97% identity. The phylogenetic tree was constructed by the neighbor-joining method implemented in the MEGA5 computer software program. The confidence values of branches in the phylogenetic tree were determined using bootstrap analysis based on 1000 resamplings. The relative abundance of each phylotype in the library was calculated by dividing the number of cloned belonging to this phylotype by total number of cloned in each library.

2.5. Nucleotide sequence accession numbers

The GenBank/EMBL/DDBJ accession number of the 16S rRNA gene sequence of bacterial and archaeal clones are AB780888 to AB780956 and AB780957 to AB780967, respectively.

3. Results and discussion

3.1. Methanogenic activity of biogas slurry

To assess the methanogenic activity of biogas slurry, batch experiments were performed with corn straw (TS, 1%) as sole