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Research paper

Responses of anaerobic microorganisms to different culture conditions and corresponding effects on biogas production and solid digestate quality

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ABSTRACT

Microbial communities of anaerobic digestion have been intensively investigated in the past decades. Majority of these studies focused on correlating microbial diversity with biogas production. The relationship between microbial communities and compositional changes of the solid digestate (AD fiber) has not been comprehensively studied to date. Therefore, the objective of this study was to understand the responses of microbial communities to different operational conditions of anaerobic co-digestion and their influences on biogas production and solid digestate quality. Two temperatures and three manure-to-food waste ratios were investigated by a completely randomized design. Molecular analyses demonstrate that both temperature and manure-to-food waste ratio greatly influenced the bacterial communities, while archaeal communities were mainly influenced by temperature. The digestion performance showed that biogas productivity increased with the increase of supplemental food wastes, and there were no significant differences on carbohydrate contents among different digestions. The statistical analyses conclude that microbes changed their community configuration under different conditions to enhance digestion performance for biogas and homogenized solid digestate production.

1. Introduction

Anaerobic digestion (AD) is one of the oldest biotechnologies that mankind has practiced to treat organic wastes for several centuries. A complex anaerobic microbial consortium converts organic matter in the wastes into methane biogas - a carbon neutral and renewable energy source, and correspondingly alleviates the odor and pathogen problems. The classic AD systems often used animal manure or sewage sludge as feedstock to provide nutrients and inoculate anaerobic microorganisms [1]. However, due to the structural and nutritional limitation of manure and sludge, single-sourced AD systems have been described as “not energy efficient nor cost effective” [2]. Co-digestion of multiple feedstocks was hence introduced to enhance AD performance of biogas production and total solids (TS) reduction [3–5]. In addition, the overall performance of an AD system depends on not only the composition of feedstock, but also operational parameters such as temperature [6]. Conventional operational temperatures range from mesophilic (30–38 °C) to thermophilic (49–57 °C), and it has been proven that

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operational temperature is one of the most important determinants of the microbial community structure in an AD system [6,7].

Numerous studies have been conducted on the microbiology of anaerobic co-digestion system to correlate biogas production with microbial diversity [8–12]. However, the relationship between microbial communities and compositional changes of the solid digestate (AD fiber) has not been widely reported [13]. Several recent studies have discovered that solid digestate has a similar cellulose conversion potential with other energy crops and residues such as switchgrass and corn stover, and it can be used as a cellulosic feedstock for biorefining of fuel and chemical production [14–18]. Therefore, a clear understanding on the relationship between mixed feedstock, microbial communities, biogas production, and solid digestate quality should be achieved in order to advance AD technology into a pretreatment unit operation for the next-generation fuel and chemical biorefining.

The objective of this study was to delineate the responses of microbial communities to changes in substrate composition and reaction temperature of anaerobic co-digestion. Dairy manure was mixed with food waste as the substrates to feed anaerobic digesters. The 16S rRNA gene-based 454 pyrosequencing, Terminal Restriction Fragment Length Polymorphism (T-RFLP) and clone library were used to investigate the communities. Microbial communities was also statistically correlated with performance parameters such as TS reduction, biogas production, and AD fiber quality (cellulose, xylan, and lignin).

2. Materials and methods

2.1. Feedstock

Fresh dairy manure was collected from the Michigan State University dairy farm (42°41′53.80″N, 84°29′8.63″W), and stored at −20 °C prior to use. Dairy cows were fed on an alfalfa and corn silage blend diet formulated according to the standard Total Mixed Rations (TMRs) [19]. Food waste collected from cafeterias on campus was homogenized using a commercial immersion blender (Waring WSB70, Waring, Stamford, CT) and stored at −20 °C prior to use.

2.2. Anaerobic digestion systems

A completely stirred tank reactor (CSTR) was used as the anaerobic digester in this study. Three different weight ratios of dairy manure to food waste were used as feeds for the anaerobic digesters: 100:0, 90:10 and 80:20 (based on dry weight). Each digester contained 5% TS. Two culture temperatures of 35 and 50 °C were tested. The hydraulic retention time (HRT) was 20 days. A completely randomized design (CRD) was applied on both factors of manure-to-food waste ratio and temperature. Six treatments with replicates were cultured on New Brunswick shakers (Eppendorf, Enfield, CT) at 150 rpm for 4 full HRTs (80 days). All digesters had a working volume of 0.50 L with 0.25 L headspace. The digesters were first purged with nitrogen gas for 30 s and then sealed with rubber septum caps. Daily biogas accumulation was measured using a water displacement system. Biogas sample from the digesters was collected for gas composition analysis. All digesters were fed every other day with 50 mL of afore mentioned feed. Feed was prepared a few days before the feeding according to the manure-to-food waste ratios, and stored at 4 °C. Prior to the feeding, an equal volume (50 mL) of digestate was removed from the digesters as the digestate samples: 40 mL of the digestate samples were stored at −20 °C for TS, cellulose, xylan, and lignin analyses. 10 mL of the digestate samples were stored at −80 °C for microbial community analysis. The pH of all digesters was controlled above 6.70 by dosing 20% sodium hydroxide (NaOH) at the start-up stage of digestion (first one to two weeks). The operations of sampling, feeding, and pH adjustment were carried on using a Simplicity 888 automatic anaerobic chamber (PLAS Lab, Lansing, MI) purged with a medical grade specialty gas (85% nitrogen, 10% hydrogen and 5% carbon dioxide).

2.3. Analytical methods

Methane and carbon dioxide content were quantified using a SRI 8610c gas chromatograph (Torrance, CA). The system was equipped with a thermal conductivity detector. The detector was kept at 150 °C during the analysis. Hydrogen and helium were carrier gases, and maintained at 21 psi. The biogas sample volume was 100 μL, and the syringe was purged three times before sample injection. Fiber composition of the digestate was analyzed according to the National Renewable Energy Laboratory (NREL) Analytical Procedure (LAP) [20]. The free sugars and starch was analyzed using a commercial starch assay kit (Catalog No. SA20. Sigma–Aldrich Co. LLC, St. Louis, MO).

2.4. Bacterial community analysis

A Power-Soil DNA isolation kit (MO BIO Laboratories, Carlsbad, CA) was utilized to extract community genomic DNA from digestate samples, and a NanoDrop™ Lite spectrophotometer (Thermo Fisher Scientific Inc., Waltham, MA) was applied to quantify the DNA extraction. Polymerase Chain Reactions (PCR) were conducted to amplify the bacterial 16S rRNA gene sequences using a forward primer 357F (5′-CCTACGGGAGGCAGCAG-3′) and a reverse primer 926R (5′-CCGTCAATTCMTTTRAGT-3′) which targeted on the hypervariable V3–V5 region of rRNA genes [21–23]. A 454 “A” adapter and unique barcode sequences were incorporated in the reverse primer, and a “B” adapter was incorporated in the forward primer. A 15 μL reaction solution contained 0.33 μM primer, 0.125 U μL^{-1} high fidelity Taq polymerase (Life Technologies™, Grand Island, NY), 1X Taq reaction buffer, 0.2 mM of each dNTP, 2 mM $MgSO_4$, 0.1 mg mL^{-1} BSA, and 10 ng DNA template. The reaction solution was mixed with DNase and RNase free water for PCR reaction. The amplification included an initial denaturing step at 95 °C for 5 min, followed by 30 cycles of 3 temperature steps (denaturing at 95 °C for 45 s, annealing at 50 °C for 45 s, and elongation at 72 °C for 90 s), and a final extension at 72 °C for 5 min. The PCR products were purified using QiaQuick PCR Product Purification kit (Qiagen, Valencia, CA). Purified amplicons were diluted to 0.5 ng dsDNA μL^{-1} and sequenced using a Roche 454 GSFLX Titanium Sequencer at the Research Technology Support Facility of Michigan State University. All bacterial 16S rRNA amplicon sequences were trimmed, screened and analyzed using Ribosomal Database Project (RDP) Pyrosequencing Pipeline Initial Process tools with a minimum sequence length of 300 bp and no ambiguous bases [24]. Chimeras were identified using USEARCH implemented UCHIME algorithm in reference mode with Silva Gold Alignment database [25]. Sequences were assigned with genus names at 80% confidence level by RDP Multi-Classifier and clustered at 97% similarity by Complete Linkage Clustering [13].

2.5. Archaeal community analysis

DNA extracts from the previous step were also used for archaeal community analysis. The archaeal communities were examined using 16S rRNA gene-based Terminal Restriction Fragment Length Polymorphism (T-RFLP). The 16S rRNA gene was amplified with archaeal domain-specific primers 344 aF-FAM (FAM-5′-CGGGGYGCASCAGGCGCGAA-3′) and 1119aR (5′-