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Molecular characterization of anaerobic digester microbial communities identifies microorganisms that correlate to reactor performance

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HIGHLIGHTS

- Digesters with the highest methane accumulation were efficient at catabolizing VFA.
- The abundance of individual organisms correlated to reactor performance.
- The same microorganisms dominated the reactors regardless of input material.
- Both hydrogenotrophic and acetoclastic methanogens were detected.
- Hydrogenotrophic methanogenesis was the dominant methane-producing pathway.

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ABSTRACT

A time-course analysis was conducted of thermophilic anaerobic digestion of dairy manure and wheat distillery thin stillage. Sequencing of chaperonin targets provided a phylogenetic survey of both bacteria and archaea in the digestate, along with an appraisal of the diversity of the reactor microbiome. A total of 1129 bacterial operational taxonomic units (OTU) were detected in the reactors, with OTU related to *Clostridium* becoming numerically dominant by day 7, and *Acetivibrio*-related OTU by day 35. Archaeal communities were less diverse, with 19 OTU detected representing both acetoclastic and hydrogenotrophic methanogens. Regardless of input material, the same organisms came to dominate the reactors, reflecting strong selective pressures present in the digesters. Principal coordinate analysis of the microbial communities showed that the bacterial communities clustered based on factors other than input material. Bacterial and archaeal OTU were identified with significant correlations to performance parameters, suggesting important roles in the methane production pathway.

1. Introduction

If sufficiently optimized, anaerobic digestion (AD) of agricultural waste is an efficient waste disposal system and a source of renewable energy. The process is dependent on the metabolic activity of a complex microbial consortium to convert the input material, which may consist of agricultural, animal or food processing waste, into methane gas. Optimizing the operation conditions to favor the growth and metabolic activity of organisms that break down the organic input material to produce specific end products is desirable; however, a lack of detailed understanding of these microbial communities has hindered progress in this regard (Dar et al., 2008). The current practice of modifying organic loading rates or altering the pH of the digestate has yielded mixed results (Werner et al., 2011; Westerholm, 2012). Moreover, a lack of tools for directly monitoring the composition of the digester microbiome further complicates the situation with drops in methane production and spikes in volatile fatty acid (VFA) accumulation often going unexplained (Ward et al., 2008).

Although most AD is maintained at mesophilic temperatures, thermophilic conditions provide the most thorough breakdown of the organic inputs. When sufficiently optimized thermophilic AD is more efficient and requires shorter hydraulic retention times; however, the thermophilic microbial community has been shown to be less diverse, more unstable, and more sensitive to fluctuations in operational parameters (Weiland, 2010). Grain ethanol distillery waste products, such as those generated from corn, wheat, and barley, can be converted to methane under thermophilic conditions, which provides a source of relatively stable and nutrient rich organic material that might otherwise be a waste

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product (Ziganshin et al., 2011). The distillation process during ethanol production consumes essentially all of the available six carbon sugars in the stillage, converting them to ethanol using yeast fermentation. The remaining stillage waste material contains predominantly five carbon sugars, complex carbohydrates such as cellulose, lipids and proteins (Mustafa et al., 2000). Studies examining the biogas potential of this substrate have shown the process to be energy efficient in terms of carbon balance, especially when the energy produced from the digester is used to offset energy expenditures during ethanol production (Agler et al., 2008; Eskicioglu et al., 2011; Schaefer and Sung, 2008). Recently, studies have examined the co-digestion of stillage waste with manure as a way to boost methane production as well as increase the stability and consistency of the AD process (Westerholm, 2012).

Previous studies examining the composition and dynamics of the bacterial communities associated with thermophilic AD reactors have left many unanswered questions. While microbial communities appear to undergo large shifts in species diversity over the short term, they show surprising robustness and consistency over the long term, even after changes in operating parameters or exposure to toxins (Schauer-Gimenez et al., 2010; Werner et al., 2011). Many of these studies have been unable to show a consistent relationship between microbial composition and digester performance, in particular methane production and volatile solids consumption (Krause et al., 2008; Liu et al., 2009; Wang et al., 2009). More recently, molecular characterization of digester communities combined with quantitative PCR assays have successfully correlated specific microorganisms to digester performance parameters including methane production and volatile fatty acid catabolization (Lv et al., 2013).

During AD, organic material is converted to methane by a microbial consortium consisting of both bacteria and methanogenic archaea. Anaerobic bacteria initially degrade the substrate by hydrolysis and acidogenesis. The end products of this initial breakdown are CO_2, H_2 and VFA including acetate, propionate, butyrate, formate, succinate and lactate. Acetogenic bacteria further oxidize the VFA, generating acetate, CO_2 and H_2. The final stage, methanogenesis, is achieved through the metabolic activity of acetoclastic or hydrogenotrophic methanogens. Acetate can be converted to CH_4 directly by acetoclastic methanogens, of which *Methanosarcina* and *Methanosaeta* are the most frequently described (Demirel and Scherer, 2008). Alternatively, acetate can be oxidized to CO_2 and H_2 by bacteria in a syntrophic association with hydrogenotrophic methanogens (e.g. *Methanothermobacter*, *Methanoculleus*) (Demirel and Scherer, 2008). To achieve optimal methane production in this system, balance must be maintained between bacterial and archaeal metabolic activity. An increase in metabolic intermediates can be inhibitory to other critical organisms in the consortium, and result in reduced reactor performance or a complete collapse of methanogenesis. A better understanding of the specific microorganisms that are essential at each stage of methanogenesis and their interaction with each other is critical for optimizing reactor design and operation as well as troubleshooting issues with regard to reactor performance.

Molecular methods, including universal target amplification combined with pyrosequencing and quantitative PCR, allow analysis of the microbial community at a resolution that can distinguish between closely related species, and at a depth that permits detailed examination of community structure parameters such as richness and diversity. A protein coding gene, chaperonin 60 (*cpn*60) is universally conserved among eukaryotes, bacteria and some archaea and, while there are exceptions, is more commonly present as a single copy gene, allowing for accurate quantification of organisms (Hill et al., 2004). Type I chaperonins (*cpn60*) are present in bacteria and some archaea, have been shown to provide greater resolution between closely related organisms compared to 16S rRNA-encoding genes, and have recently been proposed as a suitable molecular barcode for bacteria using the International Barcode of Life criteria (Links et al., 2012). Type II chaperonins, or thermosomes, are present in archaea and the eukaryotic cytosol and universal primers have recently been developed for amplifying this target from mixed microbial communities (Chaban and Hill, 2012). A database of reference type I and II chaperonin sequences (www.cpndb.ca) provides a breadth of reference sequences on par with that available for 16S rRNA-encoding sequences (Hill et al., 2004). These tools have been exploited to examine microbial communities from a variety of environments (Chaban and Hill, 2012; Dumonceaux et al., 2006), but no previous studies have examined both type I and type II chaperonins in mixed bacterial/archaeal communities such as those associated with AD.

A time-course analysis was performed of bacterial and archaeal communities within thermophilic digesters processing wheat ethanol stillage and dairy cattle manure, and molecular methods were used to quantify and monitor organisms critical in the methanogenesis pathway. Ecological parameters of the microbial communities were examined (evenness, richness, and diversity), as these have been shown previously to affect reactor performance, especially as it relates to reactor variability (Schauer-Gimenez et al., 2010; Werner et al., 2011). The information gained by characterizing the microbiome of both high- and low-performing digesters will help to identify a target microbial population and composition associated with maximum reactor performance. The data can also be used to inform reactor design and dictate the operational parameters for introducing and recycling microorganisms during digestion.

2. Methods

2.1. Input materials

Wheat grain distillery thin stillage was obtained from Terra Grains Inc. (Moose Jaw, SK, Canada), a facility producing ethanol from dry-ground wheat grain. Manure was collected from dairy cattle (University of Saskatchewan, Saskatoon, SK, Canada). The starter inoculum (INC) was generated by incubating dairy cattle manure anaerobically at 55 °C for 2 weeks prior to beginning the trial. Total (TS) and volatile (VS) solids for each of the input materials were determined using standard protocols (American Public Health Association, 1995). Values for %VS and %TS of the input material are listed in Supplemental Table S1.

2.2. Bench-scale AD

Bench-scale thermophilic reactors digesting inoculum (INC), dairy manure (MAN), wheat grain distillery thin stillage waste (TST) or thin stillage combined with dairy cattle manure (TSM) were set up in 1 L glass bottles and sealed with air-tight lids fitted with silicone septae. Dairy manure or thin stillage were added individually or mixed in a 1:1 ratio of stillage:manure based on VS content. The stillage or stillage/manure combinations were then mixed with a starter inoculum in a 1:1 ratio based on VS content. The total volume of material in each digester was adjusted to 300 mL and ~5% TS with sterile water. All input combinations were run in duplicate. The initial pH of the digestate mixtures ranged from 7.4 to 9.3. Bottles were sealed and flushed with N_2 gas at 82.74 kPa (12 psi) for 5 min using an outlet needle before being bled down to 3.45 kPa (0.5 psi), and incubated at 55 °C for 48 days in an anaerobic chamber with an atmosphere of 85% N_2, 10% H_2 and 5% CO_2.