Bioresource Technology 157 (2014) 127–133

Contents lists available at ScienceDirect

Bioresource Technology

journal homepage: www.elsevier.com/locate/biortech

Microbial community composition is consistent across anaerobic digesters processing wheat-based fuel ethanol waste streams

Jennifer Town [a,b], Holly Annand [c], Dyan Pratt [c], Tim Dumonceaux [a,b,*], Terrance Fonstad [c]

[a] Agriculture and Agri-Food Canada, 107 Science Place, Saskatoon, SK S7N 0X2, Canada
[b] Department of Veterinary Microbiology, WCVM, University of Saskatchewan, 52 Campus Dr., Saskatoon, SK S7N 5B4, Canada
[c] Department of Civil and Geological Engineering, University of Saskatchewan, 57 Campus Dr., Saskatoon, SK S7N 5A9, Canada

HIGHLIGHTS

- Manure amendment increased the rate of methane accumulation.
- Manure amendment of thin stillage had a synergistic effect on methane accumulation.
- Bacterial communities had similar structure and composition across digester inputs.
- All reactors were populated by both acetoclastic and hydrogenotrophic methanogens.

ARTICLE INFO

Article history:
Received 13 December 2013
Received in revised form 14 January 2014
Accepted 19 January 2014
Available online 27 January 2014

Keywords:
Thermophilic anaerobic digestion
Wheat ethanol byproducts
Thin stillage
Whole stillage
Quantitative PCR

ABSTRACT

Biochemical methane potential (BMP) assays were conducted on byproducts from dry-grind wheat-based ethanol plants amended with feedlot manure at two input ratios. Whole stillage (WST), thin stillage (TST) and wet cake (WCK) were tested alone and with 1:1 and 2:1 ratios (VS basis) of byproduct:feedlot manure in bench-scale batch reactors. The addition of manure increased both the rate and consistency of methane production in triplicate reactors. In addition, digesters co-digesting thin stillage and cattle manure at 1:1 and 2:1 stillage:manure produced 125% and 119% expected methane based on the biomethane potential of each substrate digested individually. Bacterial community analysis using universal target amplification and pyrosequencing indicated there was a numerically dominant core of 42 bacteria that was universally present in the reactors regardless of input material. A smaller-scale analysis of the archaeal community showed that both hydrogenotrophic and acetoclastic methanogens were present in significant quantities.

1. Introduction

Global bioethanol production has increased in recent years, due to environmental pressures, and is the most common renewable biofuel for motor vehicles (Sarkar et al., 2012). In Canada, fuel ethanol production is dominated by the fermentation and distillation of starchy grains like corn and wheat. For every liter of ethanol produced via grain fermentation, between 8 and 15 L of byproduct effluent is generated and must be disposed of (Saha et al., 2005). In a plant producing corn-based ethanol, downstream processing of these waste streams consumed 46.8% of the plant's total energy needs (Eskicioglu et al., 2011). Disposal of these waste streams can be major economic limitation to ethanol production, negatively impacting the financial feasibility and energy balance of ethanol facilities. Bottlenecks in downstream waste processing can also disrupt system balances and delay further ethanol production. The whole stillage waste generated during ethanol production can be further separated into its liquid (thin stillage) and solid (wetcake) components using centrifugation. Research into disposal methods for this waste stream have primarily focused on processing thin stillage due to its high chemical oxygen demand (COD) and environmental impact on both soil and waterways.

Anaerobic digestion of ethanol byproducts could potentially provide a disposal method for bioethanol waste, while returning both heat and electricity to the process. The concept of a closed loop biorefinery system, where the byproduct of one entity becomes input for the next, links these processes with the overall system operating in concert. The result would be a decrease in the carbon footprint of the bioethanol facility, and an improved net energy balance for bioethanol production. The engineering and

* Corresponding author at: Agriculture and Agri-Food Canada, 107 Science Place, Saskatoon, SK S7N 0X2, Canada. Tel.: +1 306 385 9450.
E-mail address: tim.dumonceaux@agr.gc.ca (T. Dumonceaux).

http://dx.doi.org/10.1016/j.biortech.2014.01.074
0960-8524/

economic challenges of integrating ethanol production and anaerobic digestion have already been examined for corn stover and sugarcane (Liska et al., 2009; Rabelo et al., 2011) but the methane generating potential of wheat-based ethanol byproducts has not been widely published.

Co-locating an ethanol plant and an anaerobic digester at a beef feedlot could provide even more economic and environmental advantages. Manure is a widely used feedstock for anaerobic digestion because it decreases the volume of greenhouse gas emissions released during normal manure storage (Møller et al., 2004). Manure itself is a good substrate for co-digestion with other organic material because it can adjust the carbon-to-nitrogen (C:N) ratio of feedstock, provide buffering capacity, and supply essential nutrients that improve methane yields (Labatut and Scott, 2008; Ward et al., 2008). The biogas potential of manure is highly variable and it depends on the type of animal, the animal's feed, climate conditions, the type of bedding used, and the storage conditions of manure before anaerobic digestion occurs (Møller et al., 2004).

Co-digestion of feedlot manure with ethanol byproducts has been shown previously to increase both methane yield and process stability during the digestion of agricultural and ethanol production wastes (Westerholm et al., 2012; Ye et al., 2013). The increased richness of microorganisms and nutrients achieved using manure amendment has also been shown to increase the stability of the process while improving the ability of the community to respond to operational changes and toxin exposure (Schauer-Gimenez et al., 2010; Werner et al., 2011).

A more thorough examination of the biomethane potential (BMP) of these substrates, both singly and in combination, will help to determine the economic feasibility of this biorefinery model. This also provides an opportunity to characterize the microbial communities present in thermophilic digesters processing ethanol byproduct waste. Current knowledge gaps in this area, particularly with regards to the digestion of wheat-based ethanol byproducts, make it difficult to ensure optimal reactor design and operational conditions to achieve the maximum methane potential for these substrates. Next generation sequencing technologies provides an opportunity to characterize the bacterial and archaeal communities of these digesters and identify attributes of both community structure and composition that contribute to methane production in this system.

2. Methods

2.1. Input materials

Ethanol byproducts were sampled from Terra Grain Fuels (Moose Jaw, SK, Canada), a dry-grind wheat-based ethanol plant. Samples were collected and then stored at 4 °C until needed. Manure samples were collected from an Alberta beef feedlot for the 1:1 trial and from a Saskatchewan beef feedlot for the 2:1 trial and stored at 4 °C until required. Seed inoculum was obtained from a HiMark Biogas anaerobic digester (Vegreville, AB, Canada), operating primarily on feedlot manure, stored at −20 °C, and used for both manure amendment trials. Prior to the start of each trial, the inoculum was thawed and incubated in a sealed bench-scale reactor containing an N_2 atmosphere at 55 ± 2 °C for 5 and 7 days, respectively. TS and VS were determined by standard methods (APHA, 1995) with a modified incubation temperature of 70 °C during TS determination to prevent loss of volatile solids (Angelidaki, 2009). TS, VS and VS/TS ratio for ethanol byproducts, manures and inoculum used in each experiment are outlined in Supplemental Table S1.

2.2. Biochemical methane potential (BMP) assay

Two BMP experiments were performed to determine the ultimate methane yield and methane production rate that could be achieved from ethanol byproducts receiving two different ratios of feedlot manure. BMP assays were performed under thermophilic (55 ± 2 °C) conditions as described previously (Angelidaki, 2009; Owen et al., 1979). Ethanol byproduct was combined with feedlot manure in a 1:1 or 2:1 ratio of byproduct:manure based on VS content. This mixture was then combined 1:1 with inoculum. The volume of input material was adjusted to 5% TS in 300 ml with sterile water. Each input combination was incubated in triplicate 1 L bench-scale reactors sealed with screw caps fitted with rubber septae. Samples were taken to measure actual TS, VS and pH of each prepared mixture (Table 1). The headspace of the sealed bottles was flushed with N_2 gas for 5 min at room temperature, bled down to 3.45 kPa (0.5 psi) and incubated at 55 ± 2 °C. Biogas accumulation was assayed using a pressure transducer equipped with a 25G sampling needle and pressure readings were converted to biogas volumes at standard temperature and pressure. Gas samples were taken using a 20 ml syringe equipped with a stopcock and 25G needle, transferred to a dehumidified, evacuated 5 ml vial, and stored at 4 °C until analysis. After sampling, the bottles were vented down to 3.45 kPa (0.5 psi), swirled gently, and returned to the incubator. The experiment concluded when the daily biogas production volume dropped below 1% of the total accumulated biogas for each trial; day 38 for trial 1 and day 42 for trial 2.

2.3. Biogas composition

Biogas samples were analyzed using gas chromatography (Angelidaki, 2009). The relative percentages of CH_4, CO_2, H_2, N_2 and O_2 were determined using a Varion model 450-GC with front and middle TCD detectors (CP-2003, Agilent, Santa Clara, CA, USA). Injector, oven and detector temperatures were 100, 50 and 150 °C, respectively. The front column was a Hayesep Q 80/100 CP81069 (1 m × 3.175 mm) using argon make up gas flowing at 20 ml/min. The middle column was a Molsieve 5A 80/100 CP81025 (1 m × 3.175 mm) using helium make up gas flowing at 20 ml/min. The standard gas used for calibrating the GC was composed of H_2(0.5%), CH_4(40%), N_2(1%), O_2(5%), CO_2(bal%).

Biogas yields were corrected to account for endogenous metabolism of the inoculum by subtracting the average biogas produced in the INC control reactors and reported as CH_4/g VS added (Angelidaki, 2009). Expected biogas yields (B_0) for manure amended reactors were calculated by adding the proportional biogas production from the ethanol byproduct (EB) and manure (MAN) mono-digestions (Ye et al., 2013):

$$\text{Trial1}: \quad B_{0_{\text{Expected}}} = \frac{1}{2}B_{0_{\text{EB,mono}}} + \frac{1}{2}B_{0_{\text{MAN,mono}}}$$

$$\text{Trial2}: \quad B_{0_{\text{Expected}}} = \frac{2}{3}B_{0_{\text{EB,mono}}} + \frac{1}{3}B_{0_{\text{MAN,mono}}}$$

In addition to calculating the mean accumulated methane yield (B_0) across replicates in each trial, data points from the methane production profiles were plotted according to the following equation to determine the methane production rate (k) and regression analysis was used to describe the fit of the data to first-order rate kinetics (Angelidaki, 2009):

$$ln\frac{(B_0 - B)}{B_0} = -kt$$

Comparison of average methane accumulation between input combinations was done using Student's t-test ($p < 0.05$).