Biomass and Bioenergy 119 (2018) 90–96

Contents lists available at ScienceDirect

Biomass and Bioenergy

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

Research paper

Anaerobic co-digestion of grass and forbs – Influence of cattle manure or grass based inoculum

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ARTICLE INFO

Keywords:
Anaerobic co-digestion
Biogas
Forbs
Inoculum source
Multi-species grassland

ABSTRACT

Anaerobic co-digestion of agricultural by-products or wastes with complementarity characteristics is commonly used to enhance methane yield. This study firstly explores the possibility of co-digesting grass and forb species (white clover, chicory and plantain) differing in nutrient composition in enhancing methane yield. This was examined with two inocula (a cattle manure-based inoculum and a grass-based inoculum) in a batch assay. Results showed that co-digesting grass and forbs synergistically enhanced methane yield potential on average by 31 L kg^{-1} volatile solids (+11%) and reduced lag phase time by 0.8 day in the grass-based inoculum, but not in the cattle manure-based inoculum. Mixtures containing plantain showed more consistent synergistic effect than chicory. Synergistic effects were attributed to more balanced nutrient composition (especially C/N ratio) in grass-forb mixtures. We demonstrate that anaerobic co-digestion of grass and forbs is feasible for enhancing methane yield, which promotes the utilization of multi-species grasslands for bioenergy production.

1. Introduction

Biogas produced through anaerobic digestion of agricultural by-products, wastes or energy crops plays an important role in providing renewable bioenergy [1,2]. Biogas production can be enhanced through mixing two or more substrates with complementary characteristics because the anaerobic co-digestion process facilitates a better nutritional balance (e.g. N) and/or decreases the probability of inhibition (e.g. ammonia), leading to improved efficiency of microorganisms involved in anaerobic digestion [3,4].

Animal manure is a commonly used substrate co-digested with agricultural residues and energy crops to enhance biogas production and improve fertiliser values of digestates [5,6]. That is because animal manure contains higher nutrient concentrations (e.g. N) can facilitate the degradation of nutrient-poor agricultural residues. However, the production and pre-storage of animal manure are often associated with high risks of environmental pollution, such as greenhouse gas emissions (e.g. methane) and nutrient losses (e.g. ammonia) [7,8]. Hence, search for alternative and sustainable feedstock to animal manure has been put on the research agenda.

There is an increasing interest in using grassland biomass for bioenergy production in Europe and North America [9,10]. Current studies have been mainly focusing on different grass species or varieties [11,12]. Yet, the reported biogas production from grass species is generally low and varies widely among various energy crops [1,10], probably because grasses alone contain relatively lower and unbalanced nutrients.

Recent studies show that some deep-rooting forbs species (e.g. chicory) are rich in some macro- and micro-nutrients (potassium, sulphur, zinc and boron) due to their uptake from deeper soil layers [13], while legume species (e.g. white clover) are well known to be rich in nitrogen (N) in plant tissues. These forbs have recently shown notable biogas yields [14,15]. Hence, co-digesting grass and forbs with complementary nutrient composition could have a higher probability of synergistically enhancing methane production (i.e. higher methane production in mixtures than the sum of methane production from individual species digested separately). In addition, this positive effect would be more likely to occur in nutrient-poor inoculums produced from grass species than nutrient-rich inoculums from animal manure. That is because grass-forb mixtures provide nutrient composition that is more balanced than with single species, thereby compensating the possible nutrient limitation for microbial growth in nutrient-poor inoculum.

This study is to examine (1) how co-digesting grass and forbs in different substrate composition influences methane production potential and other kinetic parameters either synergistically, antagonistically, or neutrally; and (2) whether these effects depend on inoculum source. Two sources of inocula were used: one was produced based on

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https://doi.org/10.1016/j.biombioe.2018.09.009
Received 21 March 2018; Received in revised form 16 August 2018; Accepted 19 September 2018

commonly used cattle manure and agricultural residues (hereafter called as “Manure inoculum”); the other was produced using organically managed grass ley (“Grass inoculum”). To address these questions, the batch test was employed because it enables us to characterise the kinetics of specific substrates under relative stable conditions (e.g. constant temperature) during anaerobic digestion. We hypothesized that synergistic effects occur in the Grass inoculum, but neutral effects occur in the Manure inoculum.

2. Materials and methods

2.1. Substrates

The substrates were from an existing field experiment established in spring 2014 at the Foulumgaard Experimental Station, Aarhus University, Denmark (56°29′44 N, 9°34′3 E). The experimental site had a mean annual rainfall of 770 mm and mean annual temperature of 7.7 °C. The soil is classified as a Typical Hapludult with 6.4% clay, 8.5% silt, 44% fine sand, 39% coarse sand and 2% organic matter. The experiment followed organically managed practices and hence no fertiliser and pesticide were applied. To address the objectives of this study, pure stands of ryegrass (*Lolium perenne* L.), white clover (*Trifolium repens* L.), chicory (*Cichorium intybus* L.) and plantain (*Plantago lanceolata* L.) were selected. Each pure stand had two field replicates. Plants were harvested four times (May, July, August and October, respectively) by cutting the herbage at 7 cm stubble height using a Haldrup plot harvester (Haldrup C-85, Denmark). In August 2016, the harvested samples from each of the four pure stands were mixed across replicates. A subsample (ca. 1 kg) was taken from each plant species, with unsown species removed. The subsample was then chopped into 1–2 cm size manually and stored at −20 °C until further analysis.

2.2. Inoculum preparation

Two sources of inocula were tested in this study, and prepared at the full-scale biogas plant in Research Centre Foulum, Aarhus University, Denmark. One is the Manure inoculum, collected from a full-scale thermophilic 1200 m^3 reactor (53 °C), and fed by 74% cattle manure, 8% deep litter, 8% maize, 8% grass and 2% silage for more than one year. This full-scale thermophilic digester was controlled at organic loading rate (OLR) of approximately 9 kgVS m^{-3} d^{-1} and HRT was 14 days. The other is the Grass inoculum, collected from a 100 L thermophilic pilot reactor which fed by 100% grass ley (tall fescue) for more than 3 months. The OLR and hydraulic retention time (HRT) for this pilot-scale reactor was 3 kgVS m^{-3} d^{-1} and 20 days, respectively. The grass ley was established in spring in 2014 and managed with a two-cut strategy without fertiliser and pesticides. In October 2016, the grass ley was harvested and ensiled. The two inocula were filtered using manual sieve and stored for 2 weeks at 53 °C to minimise the residual biogas production and adapted them to batch conditions.

2.3. Anaerobic batch experiment

The methane potential of the substrates was analysed by a biomethane potential (BMP) test according to the approach described by Møller et al. [16]. Samples from the four species were used to construct 13 substrate compositions, comprising four single species, and nine mixed substrates varying with the forb species and mixture ratios of plant species on a basis of volatile solid (VS) (Table 1). Substrate composition represents biomass proportions of different species commonly observed in agricultural grasslands with different management practices.

The batch experiment was arranged in a factorial design with the 13 substrates and the two inocula as two factors. In addition, two blank controls with only the inoculum (Manure or Grass) were included. All the 28 treatments were examined in triplicate. The batch experiment was conducted at thermophilic conditions (53 ± 1 °C) for 96 days. In brief, 5 g VS (about 220–250 g) of inoculum was added in each 500 mL infusion bottle, followed by adding 5 g (VS) substrate, resulting in a ratio of 1:1 ($VS_{inoculum}$: $VS_{substrate}$). The biogas volume was measured nine times at days 2, 5, 7, 12, 16, 26, 37, 61, 96, respectively, by inserting a needle connected to a tube with inlet to a column filled with acidified water (pH < 2) through the butyl rubber. The biogas produced was calculated by recording the volume of water displaced until the pressure between column and bottle headspace was equal. Biogas volume was normalised to standard conditions (0 °C and 1.013 bar). Methane produced from each sample was calculated by multiplying biogas volume with methane fraction, and corrected by subtracting the volume of methane produced from the control.

To assess whether inhibition may occur in the batch experiment, the digestates of four single species in both inocula and two controls at the last measurement (Day 96) were characterised for its VS, pH, volatile fatty acids (VFA) and total ammonia N (TAN).

Table 1
Species composition of the 13 substrates used for the batch experiment.

Substrates	Substrate composition (% of VS)			
	Ryegrass (G)	White clover (W)	Chicory (C)	Plantain (P)
100G	100			
100W		100		
100C			100	
100P				100
30G-50W-20C	30	50	20	
50G-30W-20C	50	30	20	
30G-30W-40C	30	30	40	
30G-50W-20P	30	50		20
50G-30W-20P	50	30		20
30G-30W-40P	30	30		40
30G-50W-10C-10P	30	50	10	10
50G-30W-10C-10P	50	30	10	10
30G-30W-20C-20P	30	30	20	20

G, ryegrass; W, white clover; C, chicory; P, plantain.

2.4. Analytical methods

Subsamples of four plant species and two inocula were dried at 60 °C for 48 h, and then ground < 0.5 mm (CT 193 Cyclotec™ Sample Mill, Denmark). The total solid (TS) and VS were analysed using the procedure by APHA [17]. Total carbon (C) and N concentrations were analysed using a LECO CNS-1000 analyser (LECO Corp. St. Joseph, MI). The analysis of macro- and micro-nutrient concentrations was performed with ICP-MS (Inductively Couple Plasma-Mass Spectrometry) using a NexION 300 (PerkinElmer, USA). The acid detergent fibre (ADF), neutral detergent fibre (NDF) and acid detergent lignin (ADL) of plant samples were determined using the Van Soest method [18]. Cellulose was calculated as the difference between ADF and ADL, and hemicellulose as the difference between NDF and ADF. Crude protein (CP) was calculated by multiplying N concentration with 6.25. All samples were analysed in duplicates, and the averaged values were presented.

Biogas composition was analysed by using gas chromatography (7890A, Agilent Technologies, USA) equipped with a thermal conductivity detector (TCD) and helium as the carrier gas. For digestates at the end of the experiment, pH value was measured using a Portamess 911 pH meter (Knick, Berlin, Germany). Dissolved VFA were determined using a gas chromatograph (Agilent technologies, CA 95051, USA) equipped with a flame ionization detector (FID) and helium as the carrier gas. The TAN was determined using photometric kits (Spectroquant® Test Kits, Merck, Germany). All samples were analysed