Renewable Energy 63 (2014) 524–530

Contents lists available at ScienceDirect

Renewable Energy

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

Grass for biogas production: The impact of silage fermentation characteristics on methane yield in two contrasting biomethane potential test systems

J. McEniry [a], E. Allen [b], J.D. Murphy [b], P. O'Kiely [a,*]

[a] Animal & Grassland Research and Innovation Centre, Teagasc, Grange, Dunsany, Co. Meath, Ireland
[b] Bioenergy and Biofuels Research Group, Environmental Research Institute, University College Cork, Ireland

ARTICLE INFO

Article history:
Received 14 September 2012
Accepted 5 September 2013
Available online 29 October 2013

Keywords:
Grass silage
Additive
Preservation
Aerobic stability
Anaerobic digestion

ABSTRACT

Grassland biomass is likely to be harvested and stored as silage to ensure a predictable quality and a constant supply of feedstock to an anaerobic digestion facility. Grass (*Phleum pratense* L. var. Erecta) was ensiled following the application of one of six contrasting additive treatments or a 6 h wilt treatment to investigate the effects of contrasting silage fermentation characteristics on CH_4 yield. In general, silage fermentation characteristics had relatively little effect on specific CH_4 yield (from 344 to 383 Nl CH_4 kg^{-1} volatile solids). However, the high concentrations of fermentation products such as ethanol and butyric acid following clostridial and heterofermentative lactic acid bacterial fermentations resulted in a numerically higher specific CH_4 yield. While the latter fermentation products of undesirable microbial activity have the potential to enhance specific CH_4 yield, the numerically higher specific CH_4 yield may not compensate for the associated total solids and energy losses during ensiling.

1. Introduction

Grassland biomass can be an excellent feedstock for biogas production and will likely be the dominant feedstock for on-farm anaerobic digestion in temperate Northwest Europe [1,2]. In order to ensure a predictable quality and a constant supply of grass to an anaerobic digestion facility, it is most likely to be harvested and stored as silage [1]. The main objective of ensilage is the efficient preservation of the energy content of a crop and this is achieved by the combination of an anaerobic environment and the bacterial fermentation of sugar. The lactic acid produced in the latter process lowers the pH and prevents the proliferation of spoilage microorganisms [3].

However, fermentation under farm conditions is not a controlled process and silage fermentation characteristics will depend on the nutrients fermented and the microorganisms responsible [4]. Silage which has undergone a desirable fermentation is generally characterised by a low pH, high lactic acid content and low concentrations of butyric acid and ammonia-N [5,6]. Furthermore, the ensiled energy is almost completely recoverable in a closed lactic acid dominant fermentation [7]. In contrast, and despite the negligible loss of energy, the production of ethanol by yeast during fermentation is undesirable because no acidification occurs [8]. Similarly, under sub-optimal ensiling conditions a secondary clostridial fermentation may lead to considerable total solids (TS) and energy losses due to extensive production of CO_2 and H_2 from the fermentation of lactate and hexose sugars [3].

A range of fermentation products formed during ensiling can influence specific CH_4 yield. For example, the specific CH_4 yield of some silages has been reported to be higher than for the original parent material due to the proportionately greater loss of TS than energy during the formation of fermentation products such as ethanol and 1,2-propanediol [9–11]. Similarly, a more heterofermentative lactic acid bacteria (LAB) fermentation with higher concentrations of acetic acid has been reported to enhance CH_4 production [12,13]. However, in all these cases, the potential losses occurring during fermentation must also be taken into account in order to make a more complete assessment of the overall effects of silage fermentation.

However, in general, only a limited number of studies [10,12,14] have provided information on the impact of grass silage fermentation characteristics on CH_4 production. Thus, the objective of this study was to investigate the effects of contrasting grass silage

* Corresponding author. Tel.: +353 46 9061100; fax: +353 46 9026154.
E-mail addresses: josephmceniry@gmail.com (J. McEniry), padraig.okiely@teagasc.ie (P. O'Kiely).

0960-1481/$ – see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.renene.2013.09.052

fermentation characteristics on CH_4 yield. Methane production was determined in two contrasting biomethane potential (BMP) test systems.

2. Materials and methods

2.1. Approach

Grass was ensiled following the application of one of six different additive treatments or a 6 h wilt treatment to generate seven silages with markedly contrasting fermentation characteristics. The effect of grass silage fermentation characteristics on specific CH_4 yield (i.e. normalised litre per kilogram volatile solids; Nl CH_4 kg^{-1} VS) was subsequently determined using two contrasting BMP test systems. Silage aerobic stability and the impact of aerobic deterioration on specific CH_4 yield were also determined, since these potentially impact on the efficiency of CH_4 production and are affected by the silage fermentation process.

2.2. Harvest and ensiling

Timothy (*Phleum pratense* L. var. Erecta) was grown in four field plots (each 20 m^2) at Grange (53° 52′ N, 06° 66′ W) under an inorganic fertiliser N input of 125 kg N ha^{-1} and harvested on 24 May 2010. The herbage from each plot was harvested using a Haldrup forage plot harvester (J. Haldrup, Løgstor, Denmark) to an average 6 cm stubble height and passed through a precision-chop harvester (MEX V1, Pottinger; nominal chop-length of 19 mm) immediately prior to ensiling.

Prior to filling laboratory silos, seven randomly selected samples of chopped herbage (each 8 kg) from each plot were assigned to each of the following treatments: (1) Control (i.e. no additive applied), (2) Formic acid based additive (Add SafeR®, 70 g ammonia and 640 g formic acid per 1 kg additive; Trouw Nutrition, UK), 5 L t^{-1}, (3) Sucrose, 10 kg t^{-1}, (4) Calcium carbonate ($CaCO_3$; Sigma, Dublin, Ireland), 10 kg t^{-1}, (5) Homofermentative LAB inoculant (Ecosyl 100®, *Lactobacillus plantarum* MTD1, 1×10^6 colony forming units g^{-1} herbage; Ecosyl Products Ltd., North Yorkshire, U.K.) plus sucrose (20 kg t^{-1}) and $CaCO_3$ (4 kg t^{-1}), (6) Heterofermentative LAB inoculant (Pioneer 11A44®, *Lactobacillus buchneri*, 1×10^5 colony forming units g^{-1} herbage; Southern Farm and Fuel Supplies, Cork, Ireland) plus sucrose (20 kg t^{-1}) and $CaCO_3$ (4 kg t^{-1}) and (7) 6 h wilting period. Herbage for the 6 h wilt treatment was wilted outdoors on sheets of polythene with frequent manual tedding. There was no rainfall during harvesting or wilting.

A constant weight (5 kg) of each herbage was then ensiled in laboratory silos [15] for 110 days. No effluent was produced during storage. Representative samples of the herbage pre- and post-ensiling were stored at −18 °C prior to chemical analyses and determination of specific CH_4 yield (silage samples only).

2.3. Aerobic stability

After each of the silages had been weighed and sampled on day 110, the remainder of the silage was used to assess aerobic stability and deterioration [16]. Briefly, each silage was placed in a polythene-lined polystyrene box within an insulated room (4.35 m × 3.66 m × 2.80 m) where the ambient temperature was held at 20 ± 1 °C. Thermocouples were placed in the middle of the silage in each box and the temperature was recorded every hour (for 192 h) by a data logger (SQ ELTEK 80 T, Eurolec Instrumentation Ltd., Dundalk, Ireland). Uninsulated plastic containers of water (4 × 3.8 L) stored near the silage acted as reference temperatures to which all silage temperatures were compared. The main indices of aerobic stability and deterioration were expressed as (a) the interval in hours until the temperature increased more than 2 °C above the reference temperature (b) maximum temperature rise and (c) the accumulated temperature rise (°C) up to 192 h of aerobiosis. The similar water content of the six unwilted silage treatments would confer similar specific heat characteristics on them, so that recording changes in their temperatures during exposure to air should reflect their relative heat production. The lower water content of the Wilt 6 h treatment necessitates some caution when comparing its aerobic stability or deterioration index values to the other treatments.

A representative sample of each silage was taken after 8 days (i.e. 192 h) exposure to air and samples were stored at −18 °C prior to chemical analyses and determination of specific CH_4 yield (using the micro-BMP system only).

2.4. Chemical analysis

Representative herbage samples pre- and post-ensiling were dried at 98 and 85 °C, respectively, for 16 h in an oven with forced air circulation to estimate TS content, and the values for silage samples were corrected for the loss of volatiles [17]. Replicate samples were also dried at 40 °C for 48 h before being milled (Wiley mill; 1 mm screen). Dried, milled samples were used for the determination of *in vitro* total solids digestibility and neutral detergent fiber, acid detergent fiber, crude protein, ash and water soluble carbohydrate concentrations and buffering capacity (herbage pre-ensiling only) as previously described by King et al. [18]. Herbage VS concentration was subsequently determined (VS = TS − ash). Using silage samples taken prior to drying, the pH was determined from an aqueous extract using a handheld pH meter (R 315 pH, Reagecon Diagnostics Ltd., Dublin, Ireland). Further silage juice was extracted for the analysis of lactic acid, volatile fatty acids (i.e. acetic acid, propionic acid and butyric acid), ethanol and ammonia-N as previously described by McEniry et al. [19].

The pH and TS concentration of the silages after 8 days exposure to air and the TS (85 °C for 16 h) and VS content of the sludge inoculum used in subsequent BMP tests were also determined using methods described above.

2.5. BMP test systems

Two contrasting BMP test systems were used to investigate the effects of silage fermentation characteristics on specific CH_4 yield as outlined below. The impact of exposure of silage to air on specific CH_4 yield was determined in the micro-BMP system only.

2.5.1. Micro-BMP

Dried, milled samples were used to determine the specific CH_4 yield of each silage in 160 ml micro-BMP tests, in accordance with VDI 4630 [20] and as described previously by McEniry et al. [2]. Drying the silage samples facilitated their preservation and the processing of a relatively large representative sample of undried herbage to provide a smaller representative sub-sample for analyses [21]. Briefly, inoculum and substrate were added to 160 ml incubation bottles at a VS inoculum to substrate ratio of 2:1 and at a final VS concentration of 10 g kg^{-1}. The inoculum (pH = 7.98; 4 g TS kg^{-1}, 2 g VS kg^{-1}) was obtained from a farm digester treating cattle manure (Agri-Food and Biosciences Institute, Hillsborough, Northern Ireland). Micro- and macro- mineral solutions were added to ensure that nutrient conditions were not limiting [2] and distilled water was added to each bottle to adjust the final volume to 70 ml.

In order to determine the CH_4 yield of the inoculum, six replicate bottles with no substrate (i.e. blanks) were incubated under