Renewable Energy 127 (2018) 474–480

Contents lists available at ScienceDirect

Renewable Energy

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

Synergies from co-digesting grass or clover silages with cattle slurry in *in vitro* batch anaerobic digestion

H. Himanshu [a, b, c], J.D. Murphy [b, c], J. Grant [d], P. O'Kiely [a, *]

[a] Animal & Grassland Research and Innovation Centre, Teagasc, Grange, Dunsany, Co., Meath, Ireland
[b] Science Foundation Ireland (SFI) MaREI Centre, Environmental Research Institute, University College Cork, Cork, Ireland
[c] School of Engineering, University College Cork, Cork, Ireland
[d] Teagasc Food Research Centre, Ashtown, Dublin 15, Ireland

ARTICLE INFO

Article history:
Available online 2 May 2018

Keywords:
Co-digestion
Synergy
Silage
Slurry

ABSTRACT

Co-digestion of forage silage with cattle slurry can greatly extend the stability of methanogenesis as compared to mono-digestion of the silage. Biogas and methane yields of the mixtures of perennial ryegrass silage (grass harvested at two growth stages i.e. stem elongation vs. floral development) with cattle slurry and of red clover silage (clover harvested at two growth stages i.e. mid-vegetative vs. early seed-pod development) with cattle slurry were measured, and synergistic effects were investigated. Silage and slurry were incubated as sole substrates or as part of binary mixtures (forage silage:cattle slurry ratios of 1:0, 0.75:0.25, 0.5:0.5, 0.25:0.75 and 0:1 on a volatile solid basis). The maximum measured synergistic effects for perennial ryegrass silages with cattle slurry and red clover silages with cattle slurry were observed at 0.75:0.25 and 0.5:0.5 (forage silge:cattle slurry), respectively. The forage silage:cattle slurry ratio to produce the maximum synergistic effects differed with the forage species ensiled and its growth stage when harvested.

1. Introduction

In Ireland over 90% of the agricultural land is under grassland [1]. Mean yields of biomass from this grassland are relatively high, and the potential exists to greatly increase yields so they remain in excess of current or expected livestock requirements [2]. The 7 million cattle [3] currently utilising this grassland spend about one-third of each year indoors and therefore produce a substantial amount of manure primarily in the form of slurry. Although the latter is used as a fertiliser and soil conditioner, this important function would not be compromised by its use for methanogenesis prior to landspreading.

Perennial grasses and forage legumes are commonly conserved by ensiling in northern Europe, with perennial ryegrass (*Lolium perenne* L.) and red clover (*Trifolium pratense* L.) being widely used examples of these grassland herbages. In both cases, but particularly with grass, growth stage at harvesting will significantly alter the herbages chemical composition and thus impact on the relative ease with which microbial enzymes can hydrolyse its fibre components during anaerobic digestion [4–7]. Ultimately this will strongly influence the rate and extent of methanogenesis that will occur [8,9].

Although the lower total solids (TS) and volatile solids (VS) concentrations of cattle slurry compared to grass or legume silages result in reduced methane output when expressed on a feedstock fresh weight basis, forages such as grass silage are prone to process imbalance when mono-digested over an extended duration at significant organic loading rates [10]. Complementarity between the chemical and microbiological compositions of silages made from forages (e.g. high carbon to nitrogen (C/N) ratio; borderline concentrations of some minerals or trace elements; marginal buffering capacity) and cattle slurry (e.g. elevated NH_3 concentration; rich source of some minerals or trace elements; stabilising buffering capacity; source of some micro-organisms beneficial to anaerobic digestion) can greatly enhance the longevity of stable and productive methanogenesis when these feedstocks are co-digested. Furthermore, some of these balancing effects have the potential to result in a synergistic outcome with reduced risk of factors such as pH instability, NH_3 inhibition and limiting C/N ratios. The synergistic effects have been reported for grass with sewage sludge [11], municipal solid waste with cow manure [12]

* Corresponding author.
E-mail address: padraig.okiely@teagasc.ie (P. O'Kiely).

https://doi.org/10.1016/j.renene.2018.04.086
0960-1481/

and solid slaughterhouse wastes with agri-residue [13]. Such synergistic effects are most often associated with co-digestion of feedstocks of quite contrasting C/N ratio [14]. However, information on synergistic effects when forage silage is co-digested with cattle slurry is limited [15].

The innovation in this paper is that it is the first to study biogas and methane yields arising from the co-digestion of perennial ryegrass silage (harvested at two growth stages, PRG1 and PRG2) or red clover (harvested at two growth stages, RC1 and RC2) with cattle slurry and to assess synergistic effects. This involved digestion of forage silage with cattle slurry in binary mixture ratios of 1:0, 0.75:0.25, 0.5:0.5, 0.25:0.75 and 0:1 (VS basis).

2. Materials and methods

2.1. Feedstocks

Six field plots of perennial ryegrass (PRG; *Lolium perenne* L., var. Gandalf) and of red clover (RC; *Trifolium pratense* L., var. Merviot) were grown at Teagasc Grange (53°30′N, 6°40′W, 83 m above sea level), and three plots per species were harvested at each of two dates in the primary growth (11 May and 6 July) as reported by King et al. [16]. The growth stages of PRG were 2.4 and 3.8 [17] and of RC were 3.1 and 7.4 [18] (see footnotes in Table 1 for the explanation of growth stages). The silages from these forage samples were prepared in laboratory silos without field-wilting or application of additives, for a period of 100 d at 15 °C, as described in McEniry et al. [5]. The silage samples were dried at 40 °C for 48 h in an oven with forced air circulation and then milled (Wiley mill; 1 mm pore screen). These dried, milled samples were used for the biomethane potential (BMP) assay and feedstock chemical analysis.

The cattle slurry sample was collected from an underground tank in a roofed slatted-floor cattle building at Teagasc Grange. It was produced by beef cows consuming grass silage *ad libitum* and consisted of faeces and urine. The collected cattle slurry was thoroughly mixed and stored at −20 °C until it was assessed in the BMP assay.

The inoculum was obtained from an on-farm anaerobic digestion (AD) reactor digesting cattle slurry and grass silage at the Agri-Food and Biosciences Institute in Hillsborough, Co. Down, Northern Ireland. This was de-gassed in an incubator for 5 d at 38 °C. The inoculum was then mixed with a wooden spatula and, under a continuous flow of CO_2, and filtered through a 2 mm pore sieve.

2.2. Feedstock chemical analysis

The TS and VS of forage silage, cattle slurry and inoculum were measured according to Standard Methods 2540 G [19]. The chemical characteristics i.e. acid detergent fibre (ADF), acid detergent lignin (ADL) and neutral detergent fibre (NDF; assayed with heat-stable amylase and sodium sulphite) of the dried milled forage samples were based on the analytical method of Van Soest [20]. In brief, ADF, ADL and NDF were determined using the filter-bag technique [21,22] with an ANKOM fibre analyser (ANKOM Technology, Fairport, NY, USA). The methods used to determine other chemical parameters e.g. total solids digestibility, crude protein, water soluble carbohydrate and silage fermentation characteristics have been described in detail by McEniry et al. [5]. The C/N ratio of forage silage and cattle slurry was determined using a LECO CN 2000 (Leco Corporation, St. Joseph, MI, USA). The TS, VS and other chemical properties of forage silage, cattle slurry and inoculum are presented in Table 1.

2.3. Batch digestion test and biomethane potential assay

The three experimental replicate samples of each of the four dried milled forage silage were individually weighed into forage silage:cattle slurry ratios (VS basis) of 1:0, 0.75:0.25, 0.5:0.5, 0.25:0.75 and 0:1. The methane yield of each of these sole or combined substrate samples was determined in triplicate (i.e. analytical replicates) 160 ml incubation bottles as previously described in McEniry and O'Kiely [23] with a few minor adjustments. This method follows the VDI 4630 guideline [24]. The inoculum and substrate were added to the incubation bottles at a 2:1 VS inoculum-to-substrate gravimetric ratio to provide an organic loading of 10 g VS kg^{-1} total medium. Micro- ($MgSO_4.7H_2O$, 5 mg L^{-1}; H_3BO_3, 0.3 mg L^{-1}; $ZnCl_2$, 0.1 mg L^{-1}; $NiCl_2.6H_2O$, 0.75 mg L^{-1}; $MnCl_2.4H_2O$, 1 mg L^{-1}; $CuCl_2.2H_2O$, 0.1 mg L^{-1}; $CoCl_2.6H_2O$, 1.5 mg L^{-1}; $Na_2SeO_3.5H_2O$, 0.02 mg L^{-1}; $Al_2(SO_4)_3.18H_2O$, 0.1 mg L^{-1}; $(NH_4)6Mo_7O_{24}.4H_2O$, 0.1 mg L^{-1}) and macro- (NH_4HCO_3, 0.4 g L^{-1}; $KHCO_3$, 0.4 g L^{-1}; $NaHCO_3$, 0.4 g L^{-1}) mineral solutions were also added to ensure that mineral nutrient conditions were not limiting [23]. The final total medium volume of each bottle was adjusted to 70 ml using distilled water, leaving a headspace of 90 ml in each bottle. The 90 ml headspace, during regular overhead pressure measurement and release, limits the build-up of access pressure and therefore helps the rubber seals prevent the gas escaping [25]. Six blank replicates (i.e. without forage silage or cattle slurry) and six positive control replicates (cellulose, Sigma, 22184) were also prepared. All bottles were flushed with N_2 gas for 1 min, with gas flow rate of *ca.* 4 L min^{-1} to achieve anaerobic conditions in the headspace [24] and sealed with butyl rubber stoppers and aluminium crimp caps. Bottles were incubated at 38 °C for 45 d and mixed daily by manual swirling. The headspace pressure was recorded on days 3, 6, 10, 12, 15, 19, 26, 35 and 45 of the batch digestion using a detachable pressure

Table 1
Chemical properties of perennial ryegrass (PRG) and red clover (RC) silages, cattle slurry and inoculum. All units in g kg^{-1} TS unless indicated otherwise.

	Growth stage[a,b]		TS (g kg^{-1})	VS	Hemicellulose (NDF[1]-ADF[1])	Cellulose (ADF[1]-ADL[1])	ADL[1]	C/N (g g^{-1})	TSD[1]	CP[1]	WSC[1]	LA[1]	AA[1]	PA[1]	BA[1]	Eth[1]	NH_3-N[1] (g kg^{-1}N)
PRG	PRG1	2.2	185	901	140	246	14	14.1	820	208	12.6	138.1	21.9	2.3	1.7	33.2	105.3
	PRG2	3.8	345	936	247	336	40	23.2	628	88	47.5	47.2	2.2	0.9	4.1	4.6	62.2
RC	RC1	1.0	164	892	60	242	28	12.2	717	255	9.4	29.4	41.6	5.1	3.7	40.7	131.7
	RC2	7.0	232	893	84	269	64	32.3	617	138	19.0	89.5	4.9	1.1	3.9	5.5	45.4
Cattle slurry	–	–	116	783	–	–	–	8.7	–	–	–	–	–	–	–	–	–
Inoculum	–	–	50	677	–	–	–	–	–	–	–	–	–	–	–	–	–

[a] Growth stage of PRG was determined according to Moore et al. [17] where stage 2.0–2.9 = elongation - stem elongation and stage 3.0–3.9 = reproductive - floral development; growths stage of red clover was determined according to Ohlsson and Wedin [18] where stage 1.0 = mid-vegetative stage and 7.0 = early seed-pod development. TS: Total solids; VS: Volatile solids; TSD: Total solids digestibility; NDF: Neutral detergent fibre; ADF: Acid detergent fibre; ADL: Acid detergent lignin; C/N: Carbon to nitrogen mass ratio; CP: Crude protein; WSC: Water-soluble carbohydrates; LA: Lactic acid; AA: Acetic acid; PA: Propionic acid; BA: Butyric acid; Eth: Ethanol and NH_3-N: Ammonia-nitrogen.

[b] From McEniry et al. [5].