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Investigation of effect of particle size and rumen fluid addition on specific methane yields of high lignocellulose grass silage

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HIGHLIGHTS

- Treatments to stimulate hydrolysis were assessed on a high fibre grass silage.
- Particle size reduction and rumen fluid addition were assessed by methane yields.
- Batch tests did not reveal the impact of these variables reporting similar yields.
- Operation of continuous digestion of grass >3 cm was mechanically problematic.
- The best case was <1 cm silage with rumen fluid addition yielding 371 L CH_4 kg^{-1} VS.

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ABSTRACT

This work examines the digestion of advanced growth stage grass silage. Two variables were investigated: particle size (greater than 3 cm and less than 1 cm) and rumen fluid addition. Batch studies indicated particle size and rumen fluid addition had little effect on specific methane yields (SMYs). In continuous digestion of 3 cm silage the SMY was 342 and 343 L CH_4 kg^{-1} VS, respectively, with and without rumen fluid addition. However, digester operation was significantly affected through silage floating on the liquor surface and its entanglement in the mixing system. Digestion of 1 cm silage with no rumen fluid addition struggled; volatile fatty acid concentrations rose and SMYs dropped. The best case was 1 cm silage with rumen fluid addition, offering higher SMYs of 371 L CH_4 kg^{-1} VS and stable operation throughout. Thus, physical and biological treatments benefited continuous digestion of high fibre grass silage.

1. Introduction

To meet the mandatory EU transport targets set under the Renewable Energy Directive (EC, 2009), a number of digestible feedstocks have been identified for gaseous biofuel production in Ireland including food waste (Browne and Murphy, 2013), green seaweed and slurry (Allen et al., 2014). Grass silage, a substantial crop resource, has also been recognised for its potential contribution (McEniry et al., 2013). It has been reported that digesting grass silage and dairy slurry on a 1:1 volatile solids (VS) basis can achieve over 10% renewable energy supply in transport (RES-T) using just 1.1% of grassland in the country (Wall et al., 2013). However, grass is not a homogenous feedstock and its chemical characteristics can vary significantly (McEniry and O'Kiely, 2013). Grass silage harvested at an advanced growth stage will typically have higher lignocellulosic content and lower dry solids digestibility (DSD). Optimising the digestion of this type of crop can potentially improve the knowledge employed by farmers and developers in tailoring the design of their technologies and maximising biogas production. Two treatments are investigated in this work to improve the digestibility of low DSD grass silage: particle size reduction and rumen fluid addition.

Limited literature is available on the optimum particle size of grass silage for anaerobic digestion. Previous batch digestion tests suggested that a particle size of approximately 1 cm may be optimum (Kaparaju et al., 2002). Other crop substrates such as maize, sorghum, forage rye, winter rye and triticale have been examined for the effect of particle size in batch trials, using both fresh and ensiled substrates (Herrmann et al., 2012a). Shorter chopping lengths were shown to increase the availability of fermentable

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substrates, and hence were recommended in maximising methane yields. Intensive chopping (to a particle size below 7–8 mm) was not recommended for such substrates as the potential energy output gain did not merit the associated additional energy input costs (Herrmann et al., 2012b). A range of particle sizes for grass digestion are discussed in the scientific literature. For batch biomethane potential (BMP) assays, particle sizes of 1 cm have been widely reported (Lehtomäki et al., 2008; Xie et al., 2011). Previous studies at continuous scale have investigated macerated grass silage with particle size of approximately 1 cm (Wall et al., 2014b), however longer particle lengths of 2–3 cm have also been reported in co-digestion of grass silage with cow manure (Jagadabhi et al., 2008; Lehtomäki et al., 2007).

Rumen fluid, containing archaea, bacteria, protists and fungi (Yue et al., 2013), is found in the first compartment of a ruminant's stomach (reticulo-rumen) and possesses high cellulosic-degrading properties (Gijzen et al., 1990; Hu et al., 2004). Rumen fluid has been sought to potentially enhance the digestion of lignocellulosic biomass by hydrolysing the linkages between cellulose, hemicellulose and lignin (Yue et al., 2013). A pH range of 6.8–7.3 has been suggested as the optimum range for rumen microorganisms to hydrolyse such structures (Hu et al., 2004) that can constitute up to 75% of grass silage dry matter (Xie et al., 2011). Hydrolysis is the rate-limiting step of the anaerobic digestion process for these lignocellulosic materials (Lynd et al., 2002).

Rumen fluid has primarily been used as an inoculum source and is widely indicated in literature to have a beneficial impact. It has been reported that rumen fluid can potentially breakdown cellulose structures in such materials faster than other inoculum sources (Yue et al., 2013). In semi-continuous digestion of corn stover, the use of strained rumen fluid inoculum effected rapid destruction of VS and generated a high production of volatile fatty acids (VFAs) (Hu and Yu, 2005). A study comparing two inoculum sources, digester sludge and rumen fluid, for the digestion of aquatic plants indicated that rumen fluid increased product formation rate in terms of g chemical oxygen demand (COD) per g total solids (Yue et al., 2012). The digestion of bagasse and maize bran with strained rumen fluid exhibited VFA production within 3 days, indicating effective hydrolytic conversion to acids (Kivaisi and Eliapenda, 1995). However according to Sawatdeenarunat et al. (2015) the use of rumen fluid as an inoculum source has restrictions as it would be difficult to direct such large quantities to full-scale commercial biogas plants.

The use of rumen fluid as a co-substrate has also been investigated. Co-digestion of palm oil mill effluent with small quantities of rumen fluid (5–10% by volume) in semi-continuous reactors was examined at different hydraulic retention times (HRTs) and organic loading rates (OLRs) (Alrawi et al., 2011). High COD removal efficiencies and high methane content were observed in co-digestion. Studies on the influence of rumen fluid in treating municipal solid waste also highlighted that higher proportions of rumen fluid gave higher destruction rates of organic matter (Lopes et al., 2004). Continuous systems operating with rumen fluid were also shown to be more efficient than batch cultures due to a more stable pH environment.

The objective of this study was to examine the effect of physical (particle size reduction) and biological (rumen fluid addition) treatments, to stimulate hydrolysis, on the digestion of grass silage with low DSD for the production of biomethane.

2. Methods

2.1. Grass silage

The grass silage, a first-cut perennial ryegrass *(Lolium perenne)*, was harvested on June 24th at an advanced growth stage (grass was stemmy, had fully headed-out and was after flowering) when it had a relatively high lignified fibre content. The dry solids (DS) and volatile solids content of the grass silage was 217 g kg^{-1} and 907 g VS kg^{-1} DS, respectively. The measured neutral detergent fibre (NDF) was 716 g kg^{-1} DS while the DSD was low at 555 g kg^{-1}. The grass silage represented a much less digestible crop substrate for anaerobic digestion than examined in previous grass silage digestion studies where the DSD was higher at 653 g kg^{-1} (Wall et al., 2014a,b). Once harvested, the grass was initially wilted for 48 h and subsequently baled and stretch-wrapped in polyethylene film. For storage and handling purposes the silage was then subdivided into smaller rectangular bales of approximately 25 kg, again wrapped in stretch film, and stored at ambient room temperature (18–20 °C). To represent the different particle sizes the grass silage was comminuted by two methods. The "<1 cm" particle size was achieved using a heavy duty mincer (Buffalo Heavy Duty Mincer, 250 kg h^{-1}) which macerated the grass silage. To achieve the ">3 cm" particle size the grass silage was chopped with a scissors by hand. This meant that the silage, with two different methods of chopping, not only varied in particle size but also differed in the extent of physical shredding, tearing and disruption. Subsamples of the two grass silages were stored at −20 °C until required for experimental use.

2.2. Rumen fluid

To collect a sufficient quantity of rumen fluid, six fattened beef heifers were offered hay (made from stemmy grass) ad libitum as their sole dietary ingredient for 7–10 days prior to collection. Post-mortem, rumen contents were retrieved, mixed to ensure a homogenous sample and squeezed through muslin cloth and a large sieve to leave only the strained rumen fluid. Approximately 70 L of this liquor was decanted into 50 mL and 4 mL vials and immediately frozen in liquid N and stored at −20 °C until required. The pooled rumen fluid had a pH of 7.22 and a DS and VS content of 22 g kg^{-1} and 636 g VS kg^{-1} DS, respectively. At both batch and continuous scale, rumen fluid additions were made at a rate of 50 mL per kg grass silage added. This was based on the grass silage (217 g DS kg^{-1}) being able to retain the liquid without excess seeping out and removing soluble substrates with it. The frozen rumen fluid was thawed and heated to approximately 39 °C immediately prior to application. It has been shown (Prates et al., 2010) that freezing small volumes of rumen fluid in liquid N, and implementing a quick thawing process, provided a negligible effect on the microbial diversity.

2.3. Biomethane potential (BMP) assay

The Bioprocess™ automatic methane potential test system (AMPTS) was used to carry out BMP assays in triplicate on the selected substrates, as well as a cellulose standard (Sigma Aldrich, CAS Number: 9004-34-6) and an inoculum control. Each bottle had a 400 mL working volume with 250 mL of headspace. The bottle contents were individually mixed by stirrers at 30 rpm and operated every other minute. Temperature for all bottles was held constant at 37 °C by means of a large heated water bath. A calculated quantity of each substrate and inoculum was initially added to the bottles corresponding to a 2:1 inoculum-to-substrate ratio which is recommended to overcome any problems with process inhibition (Chynoweth et al., 1993). Distilled water was added to bring the content level in the bottle to 400 mL and the headspace was flushed with nitrogen prior to start-up to ensure anaerobic conditions. A 3 M sodium hydroxide (NaOH) solution was used to remove carbon dioxide and other trace gases from the biogas produced. The resultant methane was sent to a flow measurement device which measured gas through water displacement. Pressure