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Research paper

Anaerobic mono-digestion of lucerne, grass and forbs – Influence of species and cutting frequency

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ABSTRACT

In the present study, biogas potentials of multispecies swards including grass, lucerne, caraway, ribwort plantain and chicory from two- and four-cut regimes (Mix-2 and Mix-4) for mono-digestion applying batch and continuous modes under lab-scale conditions were investigated. The gas yields in terms of volatile solids (VS) loaded from Mix-2 and Mix-4 were compared with pure stand lucerne from the four cuts regime (Lu-4). The batch test results indicate that methane yield on a VS basis was highest from Mix-4 (295 $L\,kg^{-1}$), followed by Mix-2 (281 $L\,kg^{-1}$) and Lu-4 (255 $L\,kg^{-1}$). The results were confirmed with continuous experiments, during which the reactor digesting Mix-4 was stable throughout the experiment with low ammonia and volatile fatty acid (VFA) concentration. Meanwhile, mono-digestion of Lu-4 led to elevated VFA levels, even at a comparatively low organic loading rate of 1.76 $g\,L^{-1}\,d^{-1}$ but it was not possible to ascertain whether this was due to organic overload alone or if high ammonia levels during Lu-4 digestion were contributing to the reduced performance. It was found that four cuts per year was suitable for a lab-scale mono-digestion system as the substrate was less fibrous and has lower dry matter content, which minimize blockage during feeding and digestate unloading. Micronutrient concentrations, including cobalt, nickel and molybdenum decreased over time during the continuous experiments and were critically lower than the optimum concentration required by methanogens, particularly in Mix-4, but the gas yields of the reactor treating this substrate showed no decrease over time.

1. Introduction

In stockless organic plant production systems, it is a challenge to maintain adequate soil fertility, with stagnating or declining crop yields and weed problems as the consequences. In order to counteract this, it is necessary to incorporate perennial forage legumes, such as lucerne or clover, with the ability to fix nitrogen from the atmosphere into the crop rotation [1,2]. Inclusion of perennial legumes may improves the soil fertility of arable cropping systems that rely on internal nutrient cycling [3]. Nevertheless, grass-legume swards are characterized with a limited number of plant species. Increasing plant diversity is often beneficial to increase productivity and stability of the plant production systems [4]. Thus, there is a growing interest to explore the potential of multispecies swards to promote biomass yield and increase nutritive value by inclusion of forage forbs [4,5].

Recent studies have identified the positive influence of including forage forbs such as chicory, *Cichorium intybus* L.; caraway, *Carum carvi* L. and ribwort plantain, *Plantago lanceolata* L in the grass-legumes swards [3,4]. These forbs are characterized by deep-roots and have the ability to utilize the nutrient in deep soil layers when grown with grass-legume mixtures, causing improvement of the mineral nutrition [4]. Including forbs in grass-legume swards also enhances floristic diversity, which is beneficial for bees and pollinators [6]. Moreover, it was proved that incorporation of forbs such as plantain in grass-clover mixtures increases biomass yield [3,5], thus holding large potential for bioenergy production.

The use of different types of grasses as substrates for biogas production has been widely investigated [7–9], yet, the influence of integrating new and uncommon forage forbs in grass-legume swards on digestibility and methane production characteristics within mono-anaerobic digestion is still scarce. Anaerobic digestion (AD) may in this case serve a double purpose, namely production of energy in the form of methane and the possibility of using the digestate as a fertilizer or for producing biochar to be applied to annual crops in the crop rotation

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Abbreviations

AD	Anaerobic digestion
TS	Total solid
VS	Volatile solid
TAN	Total ammonium nitrogen
TN	Total nitrogen
VFA	Volatile fatty acids
FM	Fresh matter
DM	Dry matter
OLR	Organic loading rate
HRT	Hydraulic retention time
Lu-4	Lucerne silage from four cuts
Mix-2	Lucerne-forbs-grass silage from two cuts
Mix-4	Lucerne-forbs-grass silage from four cuts
$D_{Lu\text{-}4}$	Digester fed with Lu-4
$D_{Mix\text{-}2}$	Digester fed with Mix-2
$D_{Mix\text{-}4}$	Digester fed with Mix-4
NDF	Neutral detergent fiber
ADF	Acid detergent fiber
ADL	Acid detergent lignin

[10–12].

Methane yield from grassland biomass may vary depending on plant species, cutting period and management intensity [13]. Plant species may differ in terms of chemical compositions and hence could possibly influence methane yield [13]. Yet, the results of batch tests performed by Wahid et al. [9] indicated a contrasting observation, as specific methane yield of different pure stand forbs were comparable. Prochnow et al. [13], mentioned that results of test series with pure stand grass species demonstrated that grass species are mainly influenced on area-specific methane yield rather than grass-specific methane yield. However, grassland consists of a mixture of plant species, not only pure stands of single grass species. The question arises if multispecies plants may affects specific methane yields and this is indeed conceivable [13].

Management intensity of grassland such as cutting frequency may affect methane yields [13]. Traditionally, grass-legume mixtures are harvested four times during the year under temperate conditions in order to optimize feed quality. However, for methane production, previous studies with forbs have indicated that two harvests per year significantly decrease harvest costs without compromising methane yields [9] although grass-lucerne-forbs mixtures cut twice per year may have higher dry matter and fiber fraction compared to the mixture cuts four times yearly, since the time for plant growth is prolonged.

Grass-lucerne-forbs mixture is a seasonal biomass and must be preserved as biogas plants need to be fed continuously. Ensiling is a preferred preservation method that can preserve the biomass energy with minimal loss if good ensiling management is applied. The ensiling process has been reported to have positive effects on methane yields [14]. However, it has been suggested by Kreuger et al. [15] that perceived yield increases can often be explained by measurement errors due to volatilization of compounds during VS determination, and the authors found no increase in the methane yields of a variety of biomasses following correction for loss of volatiles.

This study is a continuation from previous work [9], following our preliminary investigation on the biogas potential of pure stand forbs and grass-clover mixture at different cutting regimes. In the present study, in addition to examining pure stand lucerne, a multispecies mixture consisting of lucerne, caraway, chicory, ribwort plantain and perennial ryegrass at different cutting regimes were investigated for mono-digestion in batch and continuous modes at mesophilic (35 °C) and thermophilic temperatures (52 °C) respectively. The AD of the silages were performed without addition of manure, tailored to the characteristics of stockless organic production systems. Without adding manure, the diminution of nutrients required by anaerobic microbes over time might be a concern, thus, macro- and micronutrients concentration of raw and digested materials were also analyzed.

2. Material and methods

2.1. Substrates

The plant material came from an experiment established in spring 2014 at the Foulumgaard Experimental Station, Aarhus University, Denmark (56°29′44 N, 9°34′3 E, elevation *ca.* 50 m) with different grassland mixtures and two cutting regimes (two and four cuts per year) in a split-plot design with four replicates. Only the last annual cut was used for this experiment. No fertilizer was applied in this experiment. To address the objectives of this study, the mixture consisting of perennial ryegrass, *Lolium perenne* L.; lucerne, *Medicargo sativa* L.; chicory, *Cichorium intybus* L.; caraway, *Carum carvi* L. and ribwort plantain, *Plantago lanceolata* L. was selected under two- and four-cut regimes and monoculture lucerne under four-cut regime. Plant material for this experiment was harvested on the 6th of October to a stubble height of 7 cm following a regrowth period since last harvest of 92 and 49 days in the two- and four-cut regimes, respectively. The foliar material from a four field replicates were mixed, chopped to approximately 5 cm and 5-kg portions were vacuum packed in polyamide/polyethylene bags immediately. The bags were stored at room temperature for three months for the material to ferment to a silage. In lucerne pure stand, the dry matter harvest yield was 1.1 Mg ha^{-1} (SE = 0.09), in the two- and four-cut regimes of the mixture, the yields were 4.0 (SE = 0.22) and 1.1 (SE = 0.06) Mg ha^{-1}, respectively. The botanical composition of the mixture under the two-cut regime was 49% lucerne, 33% chicory, 9% plantain, 3% grass, 1% caraway and 5% unsown species on a dry matter basis. Under four-cut regime, the dry matter mass fractions were 34% lucerne, 23% chicory, 19% plantain, 12% grass, 1% caraway and 11% unsown species.

2.2. Gas production

2.2.1. Biochemical methane potential

The batch test was conducted at mesophilic temperature (35 °C) to determine the ultimate methane yield. Inoculum used for the batch test was collected from a mesophilic post digester at the full-scale biogas plant in Research Center Foulum, Aarhus University, Denmark. The digester was mainly treating animal manure and agricultural residues such as cattle and pig manure, wheat straw and grass. The inoculum was filtered using a manual sieve with 2 mm mesh size and pre-incubated at 35 °C for 14 days to deplete the residual biodegradable organic material (degasification) [16]. The average total solids (TS) and VS of the inoculum were 3.0% and 2.1% mass fractions respectively.

The batch test was prepared according to the procedure described by Møller et al. [17]. Approximately 200 g of inoculum was added to each 500 mL infusion bottle, followed by the addition of substrate with a mass ratio of 1:1 ($VS_{inoculum}$: $VS_{substrate}$) in three replicates. A control with only inoculum was included. The bottles were incubated at 35 °C for 90 days. The measurement of biogas volume was achieved by inserting a needle connected to a tube with inlet to a column filled with acidified water (pH < 2) through the butyl rubber bottle cap. The biogas produced was calculated by the water displaced until the two pressures (column and headspace in bottles) were equal. Biogas compositions were analyzed using gas chromatography (7890 A, Agilent Technologies, USA). Methane produced from each sample was corrected by subtracting the volume of methane produced from the inoculum control. The resulting specific methane yields were normalized