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Chemical characteristics of biomass from nature conservation management for methane production

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HIGHLIGHTS

- Biomass from nature conservation management was studied for methane production.
- Ensiling did not enhance methane potential compared with hay of the same feedstock.
- Daily methane yield and its total potential depended on functional groups.
- BMP was higher in grasses and sedges/rushes with lower K, Mg and lignin content.
- Methane was released quicker by legumes and other forbs with higher N and P content.

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ABSTRACT

The aim of the current study was to assess the biochemical methane potential (BMP) of different functional groups harvested from different semi-natural grassland types that are valuable for nature conservation purposes. Ensiling of particular biomass did not significantly influence its methane yield, however, the ranking of functional groups by their methane yield varied during the experiment. During the first days of the experiment, methane was released most rapidly by legumes and other forbs with higher N and P contents. At the end of the BMP experiment the quantity of methane produced was higher in grasses and sedges/rushes with lower K, Mg and lignin content. Hence, measurement of feedstock chemical composition is an essential input to develop suitable technology for anaerobic digestion of late harvested biomass from semi-natural grasslands.

1. Introduction

Recently much attention has been paid to the bioenergy potential of unused herbaceous biomass from extensively managed semi-natural grasslands (Amon et al., 2007; Heinsoo et al., 2010; Tonn et al., 2010; Hensgen et al., 2012; Herrmann et al., 2013; Melts et al., 2013). The potential of biomass from these grasslands for bioenergy production depends on local conditions. While Steubing et al. (2010) found that biomass from extensive meadows and mountain pastures in Switzerland had only limited bioenergy potential, in contrast it was also demonstrated that the energy output from semi-natural grasslands is comparable with the local most popular herbaceous bioenergy crop (*Phalaris arundinacea*) in Scandinavian and Baltic regions (Heinsoo et al., 2011). Such diversity of opinions can be explained by the variability of biomass quantity by grassland types (Heinsoo et al., 2010; Tonn et al., 2010).

While we assume that the energy input requirement for pretreatment for different bioenergy conversion methods of biomass from semi-natural grasslands is equal, the energy output during methane production will be less than 60% of that we can get from combustion (Melts et al., 2013). However, direct combustion is not always a suitable method for semi-natural grassland biomass due to its high N concentrations causing NO_x emissions, and high ash, K and Cl concentrations leading to particle emissions, fouling and corrosion (Tonn et al., 2010). Hence, in some cases biogas production is suggested as a reasonable utilization option for semi-natural grassland biomass (Amon et al., 2007; Herrmann et al., 2013). Recent reports have demonstrated that the feedstock-specific biogas yield depends on the type of semi-natural grassland (Herrmann et al., 2013; Melts et al., 2013). These results can be explained by the different proportion of functional groups, and hence the chemical composition in semi-natural grassland biomass (Hensgen et al., 2012; Melts et al., 2014). Mixed-species feedstock

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with higher grass content tends to have higher sugar content which is more easily digestible than forb-dominated feedstock, thereby resulting in higher saccharification yields (Garlock et al., 2012). Although biomass with a high ratio of sedges/rushes is suggested to be less suitable as a feedstock for biogas production (Herrmann et al., 2013; Melts et al., 2013), a species of this group from South America (*Schoenoplectus californicus* ssp. *Tatora*) has been found to be a promising co-substrate for biogas production (Alvarez and Lidén, 2008). Ensiling of energy crops has been demonstrated as a feasible method for preserving methane yield (Pakarinen et al., 2008) and silage additives are usually recommended for adequate preservation of grassland biomass from landscape management (Herrmann et al., 2013).

The main aim of this study was to assess the biochemical methane potential of different functional groups harvested from three types of semi-natural grassland that have value for nature conservation in the Scandinavian and Baltic regions. The objectives of the current study were to identify: (i) how silage sample particle size affects methane yield, (ii) whether the methane yield of hay is comparable to the methane yield of silage, (iii) whether BMP or methane dynamics is dependant on functional group, (iv) which chemical elements in studied feedstock have an impact on methane yield.

2. Methods

2.1. Study sites and fieldwork

The study was carried out on the central mainland of Estonia. Two Northern boreal alluvial meadows (NATURA 2000 habitat code 6450), two Fennoscandian lowland species-rich dry to mesic grasslands (*6270) and two Fennoscandian wooded meadows (*6530) were visited for biomass sampling. For site selection, the database of semi-natural grasslands from the Estonian Seminatural Community Conservation Association's was used. According to its data all the selected meadows had been managed without any seeding or additional fertilization and mowed once per year at least over the preceding three years.

Fieldwork was undertaken between July 5 and July 8, 2011. The aboveground biomass of different functional groups: grasses (*Poaceae*), sedges/rushes (*Cyperaceae*/*Juncaceae*), legumes (*Fabaceae*) and non-leguminous broadleaved forbs (hereafter called as "other forbs") from every meadow was harvested manually with scissors at ground level and stored in mini-grip bags. For further studies the biomass of each functional group from both sites of each grassland type was mixed together and transported to the lab. For hay feedstock around 50 g of biomass per functional group and grassland type was dried at 60 °C in an oven and stored afterwards in a dry dark room at room temperature.

2.2. Feedstock pre-treatment

Most of the collected biomass from each functional group of each grassland type studied was used to imitate silage processing on a small scale. For this purpose the biomass was chopped using a commercial universal food chopper (Robert Bosch Hausgeräte GmbH, Germany) for about 30 s to achieve a particle size ranging from 12 to 30 mm. About 300 g of raw chopped biomass was inoculated and mixed with commercially available biological additive Sil-All (Alltech Inc., Nicholasville, KY, USA) at the recommended rate of 0.02 g kg^{-1} fresh biomass. Sil-All additive contained the bacteria (*Lactobacillus plantarum* (2.50×10^{10} CFU g^{-1}), *Pediococcus acidilactici* (1.95×10^{11} CFU g^{-1}), *Pediococcus pentosaceus* (5.00×10^{10} CFU g^{-1}) and *Lactobacillus salivarius* (5.00×10^{9} CFU g^{-1})), enzymes (α-amylase (9000 BAU g^{-1}), cellulase (150 CMC g^{-1}), β-glucanase (2500 IU g^{-1}), xylanase (3750 IU g^{-1}), dextrose, sipernat, sodium aluminosilicate (2%) and ponceau red (up to 50 g). After this procedure the biomass was mixed and divided into three equal subsamples (100 g each) which were vacuum-sealed into 195 × 290 mm plastic bags by vacuum packaging machine (Laica S.p.A, Italy). These plastic bags were stored at 15 °C for 109 days in a dark room to provide our experiment with ensilaged biomass.

2.3. Chemical analyses

From both hay and ensiled biomass samples some material was used for chemical analyses in the Laboratory of Plant Biochemistry of the Estonian University of Life Science. The contents of cellulose, hemicellulose, lignin and the concentrations of calcium (Ca), magnesium (Mg), nitrogen (N), phosphorus (P) and potassium (K) were measured. Analyses of the organic compounds, N and K were carried out according to standardized methods (AOAC, 1990). For Kjeldahl Digest determination of Ca, and Titan Yellow Mg determination, a Fiastar 5000 was used (AN 5260 and ASTN90/92, respectively). The content of P was determined by Kjeldahl Digest AN 5242 stannous Chloride method. Total solids (TS) and volatile solids (VS) amounts in the biomass samples were determined according to standardized method (APHA, 1998) after drying the biomass and inoculum at 105 °C for overnight (TS) or after incineration at 525 °C for 2 h (VS).

2.4. Biochemical methane potential

Biochemical methane potential (BMP) of herbaceous biomass was measured in a batch experiment with three replicates of mixed biomass from each functional group and grassland type in the Laboratory of Bio- and Environmental Chemistry in the Estonian University of Life Sciences. All measurements were carried out simultaneously. Before the BMP experiment the samples of openly stored biomass ("hay") and half of the ensilage biomass ("silage") were dried and milled to achieve particles of about 1 mm length for a better contact between the biomass and inoculum. The remaining silage samples were used in the experiment without drying, but cutting this material manually to particles ranging from 1 to10 mm length, to evaluate the effect of silage pre-treatment ("raw silage").

Before the experiment the inoculum, that had been obtained from the mesophilic anaerobic reactor of Paljassaare Wastewater Treatment Plant in Tallinn, was sieved (1 mm) and preincubated at a temperature of between 36 and 37 °C for three days and its characteristics (20 g TS (total solid) kg^{-1} WW (wet weight) and 533 g VS (volatile solids) kg^{-1} TS) were identified. About 0.28 g VS of feedstock was added to the mixture of water (50 ml) and inoculum (150 ml) into serum bottles (575 ml) at a substrate and inoculum ratio of 1:6 in terms of VS added. Such a high inoculum ratio was used to prevent the accumulation of volatile fatty acids and acidic conditions (Angelidaki et al., 2009) and to harmonize the experiment's results with the database of methanogenic potential of crops and wastes of the same lab (Luna del Risco and Dobourguier, 2010). Before closing the bottles with rubber stoppers and aluminium cap, the bottles were flushed with a mix of N_2 (20%) and CO_2 (80%) for ten minutes. The bottles were incubated at 36 °C for 45 days in Memmert isothermal chambers. During this time the volume of gas produced was measured ten times (2nd, 4th, 7th, 10th, 14th, 18th, 25th, 30th, 38th and 45th day). Gas composition was analyzed with a gas chromatograph (Micro GC (Varian CP-4900)). Before each measurement the pressure in each bottle was noted and after each measurement manual mixing of the substrate was carried out. Methane yield was expressed as norm m^3 (273 K and 1013 mbar) per kg of VS (kg^{-1} VS).