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Research paper

Combined extrusion and alkali pretreatment improves grass storage towards fermentation and anaerobic digestion

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A B S T R A C T

Grass is an abundant feedstock which is commonly used as animal fodder. It can also be utilized for bio-production, however its availability is generally not consistent through time. Storage is therefore an economic necessity to ensure continuous, steady supply for bioprocessing. Extrusion tests were performed, before or after $Ca(OH)_2$ addition (75, 100 or 200 g kg^{-1} total solid (TS) of grass), with the objective to achieve 3 months storability without major carbon loss. The performances were determined by the changes in biomass characterisation, methane production through anaerobic digestion and carboxylate production through fermentation, in three storage conditions: fresh (no storage), and afterensiling or wilting for 3 months. For wilting, $Ca(OH)_2$ addition of 100 and 200 g kg^{-1} TS before extrusion, and 200 g kg^{-1} TS after extrusion significantly preserved the biomass. For ensiling, the biomass were well preserved. Ensiling with $Ca(OH)_2$ addition of 100 g kg^{-1} TS before extrusion was found the optimum preservation method in this study, with methane production of 237 ± 10 (test) vs 265 ± 29 (initial control) cm^3 g^{-1} $VS_{initial}$ CH_4, and carboxylate production of 124 ± 8 (test) vs 109 ± 4 (initial control) mg g^{-1} $VS_{initial}$ Carbon. Especially when storage conditions were sub-optimal (wilting), addition of 100 g kg^{-1} TS $Ca(OH)_2$ before extrusion outperformed the non-treated, wilted condition to a large extent (178 ± 18 vs 50 ± 8 cm^3 g^{-1} $VS_{initial}$ CH_4 and 86 ± 17 vs 18 ± 4 mg g^{-1} $VS_{initial}$ Carbon). Overall, combined extrusion and alkali pretreatment improves grass storability.

1. Introduction

Lignocellulosic biomass can be a renewable resource for biogas and biochemicals such as carboxylic and hydroxycarboxylic acids [1]. Grass, as one of the most abundant plant families on Earth, has become an interesting and fitting target for this purpose. It is a readily available waste stream with low economic value and limited applications. In Flanders alone (north part of Belgium), grasslands represent an estimated 42×10^6 kg of available biomass annually [2]. Despite the advantages, the supply of the biomass occurs decentralized and production is not consistent throughout the year, with most of them harvested only twice a year. A preservation method would play an important role to ensure a continuous and thus more economic supply towards the bioindustry, including the possibility to store grass locally until needed for centrally processing.

In the past, several methods have been investigated including ensiling, drying and pelletizing, use of chemicals such as acid and alkali and among others on farm storage systems [3]. The most common approach to store grass is ensiling which is nowadays commonly assisted by biological additives such as lactic acid bacteria [4]. During ensiling, lactic acid produced lowers the pH to around 4 and inhibits the growth of other microorganisms, hence preserving the biomass [5]. If optimal conditions for ensilage are not met, e.g. there is a low density of biomass or a high buffer capacity present, microbial degradation may occur in the silage due to bacterial and fungal growth. A high moisture content of biomass induces a secondary *Clostridia* based fermentation, producing butyric acid, which leads to a poor quality ensilage [6]. Key parameters that have to be taken into consideration for ensiling include dry matter content, buffering capacity, water soluble carbohydrates and water activity [7]. Next to ensiling, harvested biomass can be left in open-air condition to wilt for a short period to reduce the moisture content of biomass in order to improve storage. However, if wilting is

Abbreviations: TS, total solid; VS, volatile solid; sCOD, soluble chemical oxygen demand; mg g^{-1} $VS_{initial}$ Carbon, milligram per gram initial volatile solid of Carbon (e.g. 1 mol of acetic acid is 60 g and contains 2 mol of carbon molecules, $2 \times 12.01 = 24.02$ g Carbon of acetic acid); $VS_{initial}$, initial volatile solid of grass before pretreatment and storage

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[1] Dr Way Cern Khor sadly passed away during the review phase of this study. His co-authors commemorate his passion and enthousiasm.

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prolonged, biomass can be degraded biologically. Rigdon et al. [8] found that biomass which is stored uncovered for 6 months led to a dramatic decrease in total solid content from 0.88 to 0.60 g g^{-1} sorghum.

Herrmann et al. [9] investigated the effect of particle size reduction on ensiling for biogas production and found that short chopping length (6–33 mm) gives a maximum increase in methane yield afterwards. Extrusion also leads to particle size reduction by shear, which may lead to an improved biodegradability and easier growth of microorganisms beneficial for biomass conservation such as lactic acid bacteria, hence improving the efficiency of ensiling for storage purpose [10]. It is currently a promising pretreatment method for lignocellulosic biomass due to its versatility and ease of process modification, the continuous process operation and ease of scaling up, and lower energy consumption compared to other comminution technologies [11].

Next to physical methods such as extrusion, acid and alkali addition have also been widely studied for their applications in biomass treatment [12]. Sulphuric acid and calcium hydroxide have both been tested on perennial grass as on-farm pretreatment for storability and both showed promising results, acid treatment gave a conversion of cellulose to ethanol of 0.16–0.83 mol mol^{-1} ethanol over cellulose, while alkali treatment yielded 0.18–0.55 mol mol^{-1} ethanol over cellulose [13]. Sodium hydroxide is also often used as a chemical pretreatment [14]. Calcium hydroxide would be considerably cheaper, but is more difficult for application due to it being solid and having a low water solubility (1.6 kg m^{-3} at 293 K and 0.7 kg m^{-3} at 373 K) [15].

Considering the above, the objective of this study was to investigate the possibility of combining extrusion and $Ca(OH)_2$ pretreatment to improve storability and availability for biodegradation after storage of the lignocellulosic biomass (grass). Three $Ca(OH)_2$ concentrations (i) 75, (ii) 100 or (iii) 200 g kg^{-1} total solid of grass $Ca(OH)_2$ were added either (i) before or (ii) after extrusion, and these were compared to a set of controls with no treatment and extrusion-treated only biomass. The biomass were scored under three storage conditions, namely (i) fresh, (ii) ensiling and (iii) wilting. The (i) biomass compositions, (ii) methane yield and (iii) carboxylate production were recorded initially and after 3 months of storage to compare the effectiveness of the pretreatment.

2. Material and methods

2.1. Materials

Lignocellulosic biomass – grass (from landscape, West Flanders, extensive management, harvested on September 2014) was harvested using a flail mower and kindly provided by Inagro (West Flanders, Belgium; Latitude: 50.901,545; Longitude: 3.124,464). The grass was coarsely sieved to remove fine particles and the length distributions of grass were 2–5 cm. The grass was stored in vacuum bags at 4 °C until usage.

2.2. Process overview

In this study, the grass was subjected to a series of process steps including pretreatment, storage, anaerobic digestion and fermentation. The overall process scheme is presented in Fig. 1, and each of the processes are described in detail in the following sections.

2.3. Pretreatment

The pilot scale twin-screw extruder (MSZ B 22e, 2 × 11 kW) was kindly supplied by Lehmann-maschinenbau (Pohl, Germany). The grass was split into two batches, one batch was not extruded, and the other batch was extruded at rotation speed of 6.28 rad s^{-1}, with or without alkali addition. 5 kg of grass was used in each treatment to ensure that the extrusion chamber was filled and biomass was extruded. The mass flow of grass through the extruder was approximately 0.2 kg s^{-1}. Three concentrations of $Ca(OH)_2$, (i) 75, (ii) 100, and (iii) 200 g kg^{-1} total solid $Ca(OH)_2$ of grass, were applied as dry solid on grass and mixed thoroughly by manual operation, either (i) before or (ii) after extrusion. After pretreatment, treated and non-treated grass were further split into three sub-batches. One batch was incubated for 24 h to allow for hydrolysis before anaerobic digestion and fermentation (for fresh condition). The other two sub-batches were used for storage test.

2.4. Storage

The two sub-batches of grass were stored at room temperature (average 18 ± 3 °C) for 3 months (90 days) for storability test. Two storage conditions were used – (i) ensiling and (ii) wilting. For ensiling, 5 g of grass were stored in 8 cm^3 glass vials to ensure a minimum density of 600 kg m^{-3} fresh material (180 kg m^{-3} dry matter, which was well within the recommended range of 140–260 kg m^{-3} dry matter required for ensiling in the study of Honig et al. [16]). The glass vial was sealed with butyl rubber to minimize oxygen penetration. While for wilting, 5 g of grass were stored in 100 cm^3, non-gas-tight, transparent plastic containers, which was 50 kg m^{-3} fresh material. Weights of grass were measured gravimetrically before and after storage to determine the weight losses, this was expressed as volatile solid loss, in g g^{-1} $VS_{initial}$. For each combination of pretreatment and storage, a triplicate test was performed. As 24 conditions (resulting from combination of pretreatment and storage method, including non-treated grass) with 3 replicates were tested, an overall total of 72 tests were performed.

2.5. Biochemical methane potential (BMP) test

BMP test was performed to study the effect of the pretreatment and storage on methane yield from the substrates. The performance was scored in three conditions: fresh (24 h after pretreatment or non-treated), and after ensiling or wilting for 3 months. The inoculum (total solid 58.2 ± 3.2 g kg^{-1} inoculum, volatile solid 38.8 ± 3.4 g kg^{-1} inoculum) was obtained from a 200 m^3 co-digestion plant digesting cow manure and grass, assuring the inoculum was well adapted for lignocellulosic substrates. The batch assays were set up according to Khor et al. [17]. Glass bottles of 500 cm^3 were filled with 300 cm^3 of inoculum, and a corresponding amount of lignocellulosic biomass to achieve a loading ratio below 0.5 g g^{-1} VS inoculum over VS substrate. Negative controls (only inoculum and water, without feedstock) were also included to take into account the biogas production from the VS of inoculum itself. The biogas produced was collected in graduated glass cylinders filled with an acidified barrier solution (sulphuric acid at pH 2). The BMP test was carried out at 37 °C and lasted 30 days to evaluate the pretreatment efficiency. Both gas production and gas composition were measured two to three times weekly. The methane production was always compared to the initial biomass (before pretreatment and storage) to take into account VS loss, and it was expressed as cm^3 g^{-1} $VS_{initial}$. The anaerobic digestion test was terminated after 30 days as the daily biogas production rate was less than volume fraction of 1% of the total biogas produced, and to allow comparison between the tests before and after storage.

2.6. Fermentation test

Batch fermentation was performed simultaneously to investigate the fermentability of the treated biomass to carboxylates. The performance was scored in three conditions: fresh (24 h after pretreatment), and after ensiling or wilting for 3 months. Fermentation was carried out in 20 cm^3 serum bottles for 8 days, for each of the pretreatment and storage replica. Each serum bottle was filled with 1 g of substrate, 9 cm^3 of M9 medium and 1 cm^3 of rumen fluid. Compositions of M9 medium were 8.5 kg m^{-3} Na_2HPO_4, 3.0 kg m^{-3} KH_2PO_4, 0.5 kg m^{-3} NaCl, 1.0 kg m^{-3} NH_4Cl, 0.24 kg m^{-3} $MgSO_4$, 0.011 kg m^{-3} $CaCl_2$. Negative