BIOMASS AND BIOENERGY XXX (2013) 1–10

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Ensiling as biological pretreatment of grass (*Festulolium* Hykor): The effect of composition, dry matter, and inocula on cellulose convertibility

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ARTICLE INFO

Article history:
Received 27 December 2012
Received in revised form
7 July 2013
Accepted 2 August 2013
Available online xxx

Keywords:
Ensiling
Grass
Biomass characterisation
Enzymatic hydrolysis
Cellulose convertibility

ABSTRACT

Grass biomass is a prospective type of lignocellulosic biomass for bioenergy and fuel production, but the low dry matter in grass at harvest calls for new pretreatment strategies for cellulosic conversion. In this study, ensiling was tested as a biological pretreatment method of the high yielding grass variety *Festulolium* Hykor. The biomass was harvested in four cuts over a growing season. Three important factors of ensiling: biomass composition, dry matter (DM) at ensiling, and inoculation of lactic acid bacteria, were assessed in relation to subsequent enzymatic cellulose hydrolysis. The organic acid profile after ensiling was dependant on the composition of the grass and the DM, rather than on the inocula. High levels of organic acids, notably lactic acid, produced during ensiling improved enzymatic cellulose convertibility in the grass biomass. Ensiling of less mature grass gave higher convertibility. Low DM at ensiling (<25%) resulted in the highest cellulose convertibilities, which ranged from 32 to 70% of the available cellulose in the four cuts after ensiling. The study confirms that ensiling can enhance cellulose convertibility of green biomass, and provides new insight to ensiling as a biological pretreatment method for green biomass conversion.

1. Introduction

Grassland biomass may become an important low cost lignocellulosic raw material for fuels and chemicals in the future, as grassland covers about 69% of the world's agricultural area [1,2]. Additionally, grassland biomass may add significant ecological value, including protection against soil erosion and habitat creation [2]. Cultivation of temperate grass allows for several harvests (2–4 cuts) during a season contributing to the high yield. It is well known that the

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http://dx.doi.org/10.1016/j.biombioe.2013.08.015

chemical composition of grass changes between cuts over the season and with the stage of maturity at harvest [3,4]. This aspect has not been thoroughly examined in relation to processing of grass biomass for biorefining, but is important to take into account when assessing grass biomass as a feedstock for biofuels or biochemicals, since changes in composition may affect the processing and product yields to a high extent.

Another important aspect of a low cost lignocellulosic biomass supply is efficient storage and pre-processing methods [5]. The fact that grass is harvested at low dry matter (DM) typically of 18–20% DM makes dry storage at >90% DM troublesome. Instead, through ensiling, grass can be stored at lower DM (20–50%). Ensiling is the classical method of forage crop preservation optimised throughout the past two centuries to provide nutrient rich animal feed all year round [6]. Ensiling encompasses moist solid state anaerobic fermentation by lactic acid bacteria (LAB). The ensiling involves production of organic acids and a decrease in pH that consequently prevents growth of fungi, yeasts and bacteria which may otherwise decompose the carbohydrate structure in the biomass [7]. Three main factors influence the outcome of ensiling: (i) Biomass composition; (ii) biomass DM at ensiling, and (iii) the microbial community responsible for the fermentation [7].

Silage, the resulting biomass product of ensiling, has gained increased focus as a biomass feedstock for biofuel production in recent years [8]. The method poses several potential advantages as opposed to dry storage. The main advantages include (i) less dependence on dry weather conditions prior to harvest, hence, better harvest-timing, (ii) reduced biomass losses during harvest due to less handling steps and no loss from dust formation, (iii) no need for energy intensive drying, and (iv) possibilities of combined storage and pretreatment [9,10]. Combination of storage and pretreatment at ambient temperature and pressure holds considerable potential cost and energy savings compared to common and more severe pretreatments of chemical or physiochemical means [9].

Already 50 years ago Dewar et al. (1963) [11] showed that during ensiling, hemicellulose from perennial rye was hydrolysed initially by enzymes extracted from the grass and during longer storage (7–28 days) by means of acid hydrolysis at pH 4. These changes in biomass composition suggest that ensiling may be utilised as a biological pretreatment method for cellulosic biofuel and biochemicals production.

Four studies on ensiling as a biological pretreatment have reported results of cellulose conversion through enzymatic hydrolysis, all with the aim of producing energy carriers of either ethanol or biogas and the studies have consistently been reporting improved enzymatic saccharification for the ensiled biomass [9,12–14].

It is an obvious tenet that the grass biomass composition, DM, and type of inoculum will influence the ensiling process as well as the silage quality, which in turn may affect the subsequent enzymatic cellulose convertibility. Nevertheless, in the currently available studies, the biomass and the conditions of ensiling have varied considerably, making it difficult to derive consistent rules for optimal ensiling for lignocellulose pretreatment. The objective of this study was to investigate the relations of three factors; biomass composition, initial DM, and addition of LAB inocula, upon enzymatic saccharification of cellulose after ensiling, using *Festulolium* Hykor as the grassland biomass. *Festulolium* Hykor is a crossbreed of the temperate grasses tall fescue (*Festuca arundinacea*) and perennial rye (*Lolium perenne*) developed by DLF TRIFOLIUM for high yield potential (18 tonne/ha) and high persistency throughout the season. However, the possible influence of the differences in the grass biomass composition of *Festulolium* Hykor across different harvests during a season, i.e. different cuts, on ensiling and silage quality has not been investigated.

2. Materials and methods

2.1. Raw material

The four cuts of the grass biomass, *Festulolium* Hykor (DLF TRIFOLIUM, Denmark), were harvested over the season 2011 (1st cut: 01.06.2011, 2nd cut: 06.07.2011,3rd cut: 20.09.2011,4th cut: 01.11.2011) from a DLF TRIFOLIUM demo plot, sized 1.5 × 8 m and located in southern Zealand, Denmark (55° 20′ N, 12° 23′ E), with a HALDRUP F-55 harvester (Inotec Engineering GmbH). The grass was collected right after harvest, cut to 2–5 cm pieces and split into four portions. Three of the portions were dried to different DM concentrations by means of different drying times at 25–30 °C (drying time ranged from 2 to 48 h). DM content was monitored by use of a halogen DM analyser (Mettler Toledo HR83 Halogen) and exact measurements where done according to the standard procedure developed by the National Renewable Energy Laboratory (NREL) in the US [15]. The last portion of each cut was dried at 60 °C and stored as hay for raw material comparison in compositional analysis and enzyme hydrolysis (see below).

2.2. LAB inocula

The commercially available inocula LACTISIL Grass plus (GP) and LACTISIL CCM (CCM) (Chr. Hansen, Hørsholm, Denmark) were in freeze dried form, prepared individually in a 0.05 g DM/L water suspension, and added to the grass samples for ensiling at a level equalling 4.0 mg DM inocula/kg fresh grass as according to [13].

GP consists of the lactic acid bacteria (LAB) *Pediococcus pentosaceus* and *Lactobacillus plantarum*, which are both homofermentative. CCM consists of pure *Lactobacillus buchneri* which is heterofermentative. Each grass sample was mixed carefully and thoroughly with each inoculum solution in a large plastic tray and samples were taken for final DM measurements prior to each ensiling.

2.3. Ensiling

The ensiling was carried out using a vacuum based plastic bag system according to [16]. A Variovac EK10 vacuum packaging machine (Variovac Nordic A/S, DK-7100 Vejle, Denmark) and 35 × 45 cm vacuum bags were used to pack approx. 100 g DM grass for each treatment.

Please cite this article in press as: Ambye-Jensen M, et al., Ensiling as biological pretreatment of grass (*Festulolium* Hykor): The effect of composition, dry matter, and inocula on cellulose convertibility, Biomass and Bioenergy (2013), http://dx.doi.org/10.1016/j.biombioe.2013.08.015