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Production of sugars from grass silage after steam explosion or soaking in aqueous ammonia

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ABSTRACT

Grass is an underutilized potential feedstock for lignocellulosic sugar production for biorefinery applications and can be stabilized by ensiling for year-round supply. This study compared soaking in aqueous ammonia and steam explosion with dilute acid as pretreatments for enzymatic saccharification of grass silage. Both treatments led to high hydrolysability of the silage carbohydrates. An ammonia loading of 10% per DM was sufficient in an overnight soaking at 90 °C whereas the maximum yield from steam explosion treatment was obtained with 1% acid loading at 190 °C for 10 min. The soluble carbohydrates of silage had to be removed by washing before pretreatment as otherwise severe degradation of sugars was observed. The use of an acid catalyst only had a small effect for total yield in steam explosion, but increased monomerization and degradation of hemicellulosic sugars during pretreatment. Considering the surplus potential of grass production in Europe, grass silage was found to be a very prominent feedstock for lignocellulosic sugar production.

1. Introduction

Efficient utilization of agricultural biomass is a cornerstone of bioeconomy, aiming at sustainable use of renewable feedstocks for the production of biofuels and chemicals. Due to structural changes in the dairy and meat production sector, a surplus potential of grass crops has emerged, estimated at a magnitude of 20 Mton (DM) annually in Europe (Grass, 2004; Mandl, 2010). This has led to the concept of green biorefineries, most often focusing on the soluble proteins and carbohydrates (Andersen and Kiel, 2000). However, utilization of the fiber fraction as a 2nd generation biorefinery raw material has received less attention compared to abundant research on agricultural residues such as straw (Sieker et al., 2011; Thomsen and Haugaard-Nielsen, 2008). In comparison to the estimated 258 Mt annual crop residues in Europe (Scarlat et al., 2010), the 20 Mt surplus potential of grass production is substantial, with particularly competitive crop yields in the northern regions of the globe.

Grasses and forage legumes provide an abundant, nutrient rich and environmentally favorable crop, primarily cultivated for ruminant feed. With a high productivity it is a particularly competitive crop in humid temperate areas such as Northwestern Europe. Typical cultivated forage species in Europe include ryegrass (*Lolium perenne*), timothy (*Phleum pratense*) and meadow fescue (*Festuca pratensis*), and legumes such as red clover (*Trifolium pratense*) (Hopkins, 2000).

In feed production forage is stabilized primarily by ensiling after harvest, and silage could thus provide a year round feedstock supply also for biorefineries, utilizing ensiling processes already in place. Ensiling refers to preservation without drying by lowering the pH of fresh biomass via fermentation to around 4.0, where microbial growth is inhibited. With grasses this may be promoted by using additives, such as enzymes, microbes and organic acids (McDonald et al., 1991; Seppälä et al., 2016).

The fiber content of silage depends on plant species and stage of plant maturity at harvest and ranges from approximately 40% to 55% of dry matter (Huhtanen et al., 2006; Van Soest, 1991), including cellulose, hemicelluloses and lignin. Due to the high fiber content the use of ensiled grass as feed is restricted to ruminants and horses, whereas monogastric animals such as pigs and poultry are not able to degrade cellulose and thus silage cannot be utilized as part of their diet. Processing of the fiber fraction into soluble sugars would allow wider utilization of silage as a feedstock for fermentative production of renewable chemicals as well as advanced feed products such as single cell protein, reducing dependence on imported protein feed.

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As with virtually any lignocellulosic biomass, a pretreatment is required for efficient enzymatic saccharification of ensiled grass carbohydrates. The aim of this study was to investigate the suitability of two different pretreatments, soaking in aqueous ammonia and dilute acid steam explosion, to achieve maximal hydrolysis yields from grass silage. These pretreatments have frequently been applied on agricultural residues and energy crops such as elephant grass and Bermuda grass (Eliana et al., 2014; Gao et al., 2014; Kim et al., 2003; Li and Kim, 2011; Sipponen et al., 2014). Savoie et al. (1998) applied ammonia fiber explosion (AFEX) for pretreatment of forages, where concentrated ammonia at elevated temperature and pressure is rapidly released, leading to physical degradation and chemical modification of the fiber fraction. Oxidative pretreatments have also been studied for forages (Sieker et al., 2011; Thomsen and Haugaard-Nielsen, 2008).

Steam explosion and dilute acid treatments are commonly utilized for biomasses such as wheat straw for the production of lignocellulosic ethanol and chemicals (Gao et al., 2014; Horn et al., 2011; Larsen et al., 2012; Sipponen et al., 2014). The harsh conditions of steam explosion dissolve hemicelluloses and part of the released sugars are degraded to furans and organic acids, which act as inhibitors in fermentation of the produced sugars. Optimal conditions for cellulose hydrolysis are higher than the optimum for hemicellulosic sugar recovery (Horn et al., 2011) and therefore a compromise must be found between hydrolysability and sugar degradation.

The present study compares soaking in aqueous ammonia and steam explosion with dilute acid as pretreatments for subsequent enzymatic saccharification of the fiber fraction of silage. Ammonia soaking is a simple process and can be performed with low pressure requirements and could thus be considered affordable as a localized process at the site of silage production, *i.e.* on a farm. Steam explosion is a high pressure process, which could be better suited for a centralized refining concept. The dependence of total sugar yields on process conditions is presented and the formation of microbial inhibitors during steam explosion is studied.

2. Materials and methods

2.1. Silage

The grass silage used was produced at farm scale using a precision chopper and a formic acid based additive applied at a rate of 5 kg per ton (AIV2 Plus, Eastman Chemical Company, Finland), and stored in a horizontal silo covered with plastic at the experimental farm of Natural Resources Institute Finland, Jokioinen, Finland. The ensiled grass was a mixture of timothy and meadow fescue, which were harvested in June 2015. The silage was removed from the silo after ca. 8 month storage time after which it was stored frozen.

2.2. Pre-washing of silage

To recover water-soluble carbohydrates prior to pretreatments, silage was pre-washed with water (60 °C) for 20 min. The amount of water used in the washing was 10 times the amount of dry matter. The washing water was separated by spin-drying the wet silage in a bolt cloth bag (50 μm mesh size) and analyzed for dry matter content and composition. The silage was washed in two separate batches. Prior to the second washing batch, soluble sugars of the silage had been partially consumed by microbial activity and thus the reported soluble sugar composition and yield is based on the first washing batch, while the washed solids composition is the average of the two batches.

Table 1
Design of steam explosion experiments.

Exp number	Run Order	Temperature (°C)	Time (min)	Acid (%)
1	1	170	5	0
2	3	190	5	0
3	6	170	15	0
4	12	190	15	0
5	9	170	5	2
6	5	190	5	2
7	17	170	15	2
8	7	190	15	2
9	2	170	10	1
10	4	190	10	1
11	8	180	5	1
12	10	180	15	1
13	13	180	10	0
14	14	180	10	2
15	11	180	10	1
16	16	180	10	1
17	15	180	10	1

2.3. Silage composition analysis

Polysaccharide and lignin composition for washed silage was determined according to the method by NREL (Sluiter et al., 2011). Extractives were determined gravimetrically from air-dried and milled silage by extracting with heptane in a Soxhlet apparatus for 5 h. Polysacchardies were hydrolysed with sulphuric acid and monomeric sugars were determined by HPAEC with pulse amperometric detection (Dionex ICS 3000 equipped with CarboPac PA1 column). The polysaccharide content in the samples was calculated from the corresponding monosaccharides using an anhydro correction factor of 0.88 for pentoses and 0.9 for hexoses. Acid-soluble lignin in the hydrolysate was determined from absorbances at 215 and 280 nm using an equation described by Goldschmid (1971). Klason lignin content was determined gravimetrically and corrected for protein and ash. Elemental analysis for solid samples was carried out for both Klason lignin and the whole material using FLASH 2000 series analyzer (Thermo Scientific) to determine the nitrogen content. Protein contents were determined according to nitrogen content in dried samples by multiplying the nitrogen content with 6.25. Protein solubilization was calculated from protein content in dried silage samples before and after washing. Ash contents of the whole material and Klason lignin were measured gravimetrically after combustion of the samples at 550 °C for 23 h. The amount of soluble sugars in unwashed silage was defined from the sugar yield in pre-washing.

2.4. Ammonia treatment

In the ammonia treatment, silage was incubated in closed 250 ml glass bottles (Schott) placed in a convection oven at variable temperatures and ammonia concentrations. NH_3 loadings of 1–33% per DM were screened in preliminary experiments and loadings of 10–15% were used in the final experiments. Unwashed or washed silage was mixed with aqueous ammonia at 20% DM with total reaction mass of 50 g in closed 250 ml containers and incubated for 20 h at temperatures between 45 and 90 °C.

2.5. Steam explosion

A series of steam explosion experiments was carried out using pre-washed silage. The experiment conditions followed a three-level fractional face centered composite design (Table 1) produced using Modde Pro 11.0.2 software (MKS Umetrics) with acid loading (0–2% per DM), temperature (170–190 °C) and time (5–15 min) as variables. The thermal severity of steam explosion was described with the severity factor Log(R_0), which was calculated as the