Pretreatments and enzymatic hydrolysis of *Miscanthus x giganteus* for oligosaccharides production: delignification degree and characterisation of the hydrolysis products

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Background and Objectives

Miscanthus x giganteus is a perennial grass which grows rapidly and gives high yields of biomass per hectare. It can be grown in poor quality soil and is non invasive. Due to its high cellulose and hemicellulose content, it has attracted considerable attention in Europe and U.S. as a possible energy crop, for example to produce bioethanol.
Enzymatic hydrolysis of *Miscanthus* to fermentable sugars for ethanol production has recently been studied. However, there is no information of enzymatic hydrolysis of this crop to produce oligosaccharides. These have recently gotten attention for their health benefits or as a raw material for the synthesis of pharmaceutical and other chemical products.
The aim of the present study is to compare two low cost delignification methods (formic/acetic acid[1] and soaking in aqueous ammonia) on *Miscanthus x giganteus* and to assess the suitability to produce cellobiose and other oligosaccharides after enzymatic hydrolysis.

Materials and Methods

Compositional analysis

❖ *Miscanthus x giganteus* comes from a crop cultivated in spring 2007, harvested and air dried in spring 2009, Belgium (Tournai). The dry matter content was of 93%.

❖ The NREL analytical procedures were used to determine total solids, extractives, protein and ash contents.

❖ Lignin content was compared by two methods: the acid detergent lignin method (ADL)[2] and the Klason lignin procedure[3].

❖ Structural carbohydrates were determined by acid hydrolysis and alditol acetate derivatisation for GC analysis.

Pretreatments of *Miscanthus*

❖ **Formic acid /Acetic acid/Water**

Presoaking: 50°C for 30 min in formic acid /acetic acid/water (30/50/20%). Liquid/solid ratio: 24/1.

Soaking: 107°C for 1 and 3 hours and 90°C for 2 hours in formic acid /acetic acid/water (30/50/20%). Liquid/solid ratio: 24/1. Agitation: 450 rpm.

❖ **Soaking in aqueous ammonia (SSA)**

Presoaking: 20°C for 30 min in aqueous ammonia (25%). Liquid/solid ratio: 12/1.
Soaking: 60°C for 12h in aqueous ammonia (25%). Liquid/solid ratio: 12/1

Enzymatic hydrolysis Pretreated material (50 g dry matter /L) was suspended in citrate buffer (0.05 M, pH 4.8) at 50°C for 24 hours. Celluclast 1.5L: 0.4 FPU/g dry matter.

Hydrolysis product analysis High-performance anion exchange chromatography with pulsed amperometric detection (HPAEC-PAD). Columns: PA-10 and PA-100.

Results

Compositional analysis

Lignin

Method	Lignin (% of total dry material)
Acid detergent lignin	12.9 ± 0.5
Lignin	
Acid insoluble (Klason)	23.0 ± 0.7
Acid soluble	1.5 ± 0.2

Lignin concentrations in raw material determined by both methods were different; Klason lignin value (24.5%) was greater than the acid detergent lignin (ADL) concentration (12.9%). Possible reasons:
➢ the high content of free phenolic hydroxyl groups in grass lignins is thought to account for their high solubility in alkali[4].
➢In several tropical forages species the neutral detergent extraction solubilizes a lignin-carbohydrate complex[5].
➢Acid soluble lignin fraction is lost in one of the steps of the ADL procedure[4].
➢It appears that Klason lignin is a more accurate estimate of cell-wall lignin content for grasses.

Total composition

Component	% of total dry material
Water extractives	3.7 ± 0.1
Ethanol extractives	2.7 ± 0.1
Protein	1.7 ± 0.1
Lignin ▪Acid insoluble(Klason) ▪Acid soluble	23.0 ± 0.7 1.5 ± 0.2
Polysaccharides	67.4
Ash	2.4 ± 0.1
Total	102.4

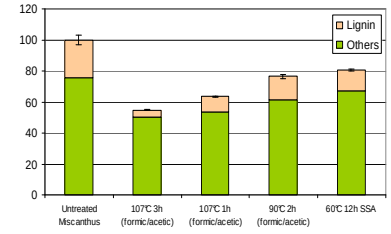
Monosaccharide composition

Component	% of total dry material
Glucose	48.4 ± 4.8
Xylose	15.7 ± 1.1
Arabinose	1.9 ± 0.1
Galactose	1.2 ± 0.2
Mannose	0.2 ± 0.2
Total	67.4

• Structural carbohydrates represented the largest fraction (67.5%). The most abundant monosaccharide was glucose (48.4%), representative of cellulose. Xylose was the second most important monosaccharide.

• A high lignin content (24.5%) was found; a pretreatment is necessary before performing enzymatic hydrolysis.

Pretreatments of *Miscanthus*

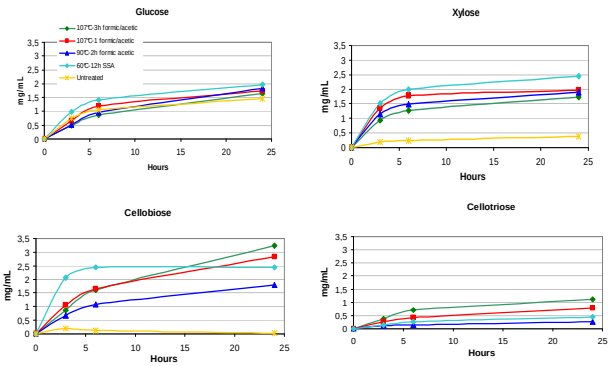


Delignification	81%	59%	38%	39%

• Pretreatment by the formic/acid mixture (107°C, 3h) resulted in the highest deliginification rate (81%), but also in an important loss of polysaccharides.

• Formic/acid method (90°C, 2h) was similar to the SSA in delignification. However, SSA resulted in a lower solubilization of the polysaccharides.

Enzymatic hydrolysis



• Hydrolysis of untreated *Miscanthus* resulted in very small production of cellobiose.
• Formic/acetic acid and SSA pretreatments successfully allowed the hydrolysis of *Miscanthus*. The major hydrolysis products found were glucose, xylose, cellobiose and cellotriose. Xylobiose, cellotetraose and cellopentoses were found in very small quantities (les than 0.5 mg/mL)
• Pretreatments by the formic/acid mixture (107°C, 3h and 1h) and SSA resulted in the highest cellobiose production.

Conclusions

❖ Formic/acetic acid and SSA pretreatments successfully allowed the delignification of *Miscanthus*.

❖ HPAEC-PAD successfully characterized the hydrolysis products, monosaccharides and oligosaccharides, from *Miscanthus* hydrolysis.

❖ Delignification of *Miscanthus* was important in order to produce cellobiose.

❖The suitability of pretreated *Miscanthus x giganteus* to produce cellobiose after enzymatic hydrolysis was demonstrated.

References
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