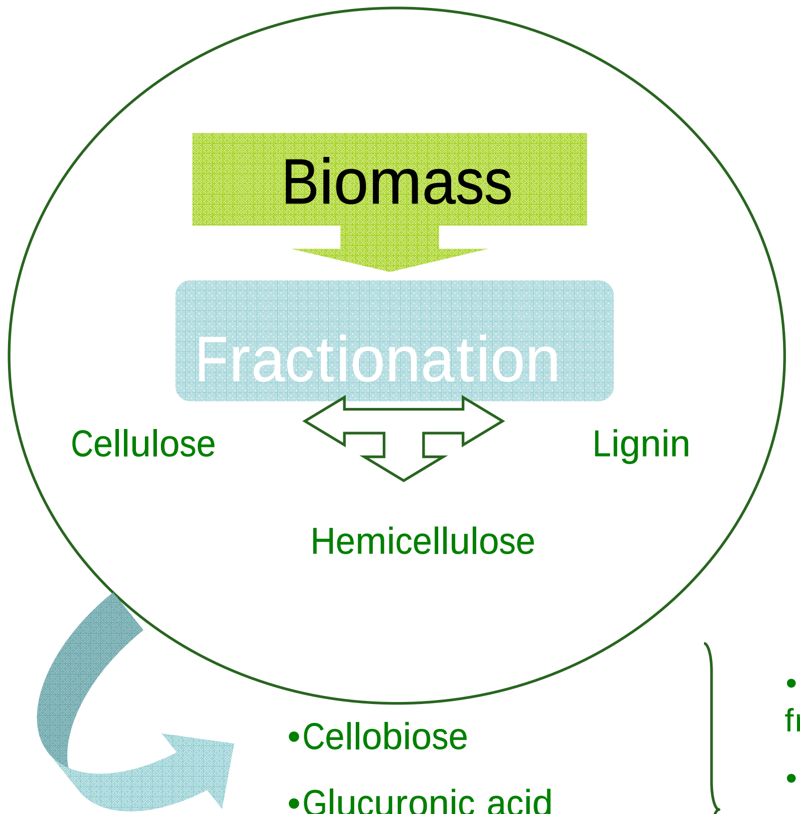
Pretreatments and enzymatic hydrolysis of *Miscanthus x giganteus* for oligosaccharides production: delignification degree and characterisation of the hydrolysis products

***Caroline Vanderghem**, Nicolas Jacquet, Christophe Blecker , Michel Paquot*

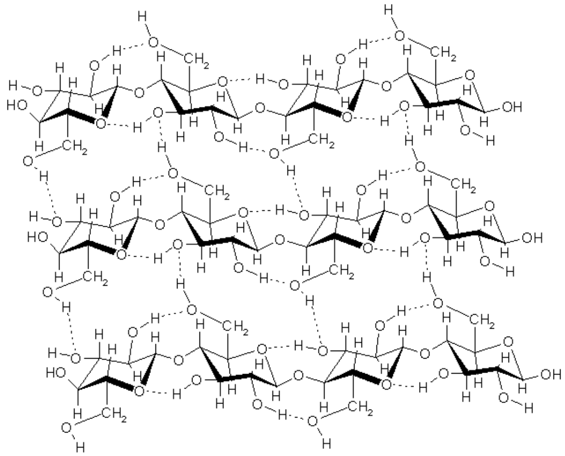
Department of Industrial Biological Chemistry, Gembloux Agro-Bio Tech, Liège University, Belgium

<u>Mission of TECHNOSE</u>: "Extraction of valuable components from lignocellulosic biomass"

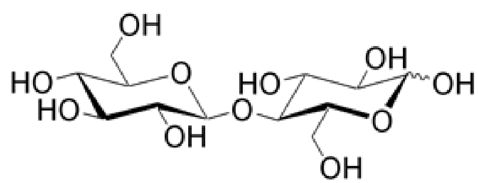


- Cellobiose
- Glucuronic acid
- Arabinose
- ...

- High-value chemicals and fragances
- Flavoring agents
- Food related products
- Nutraceuticals
- Biosurfactants
- ...



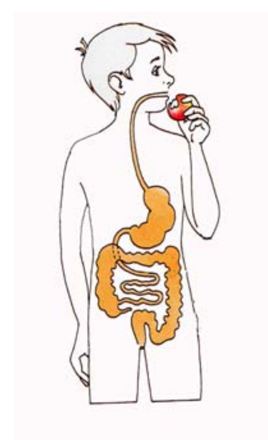
Cellulose



Cellobiose

•In an acylated form: it is suited to thickening or structuring a water-immiscible liquid in a cosmetic formulations

(Franklin et al., 2002)



•Prebiotic

(Nakamura et al., 2004)

Background and Objectives

❖ The aim of the present study was:

1) to compare two low cost delignification methods (formic/acetic acid and soaking in aqueous ammonia) on *Miscanthus x giganteus*

2) to assess the suitability to produce cellobiose and other oligosaccharides after enzymatic hydrolysis of the pretreated material

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.

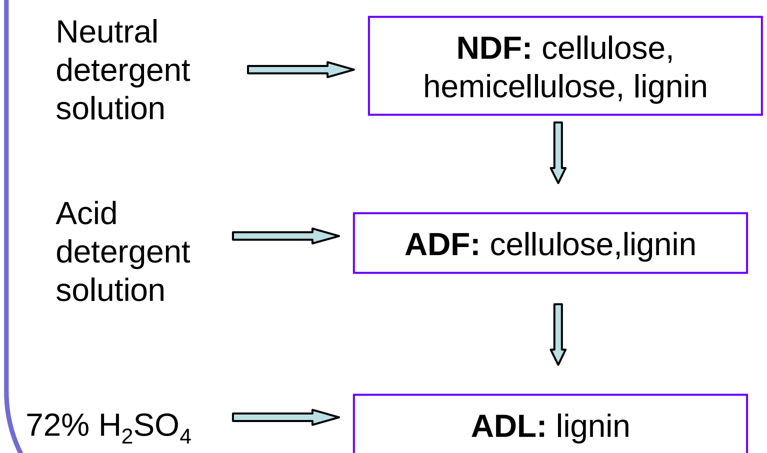
❖ Structural carbohydrates were determined by acid hydrolysis and alditol acetate derivatisation for GC analysis.

Materials and Methods

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

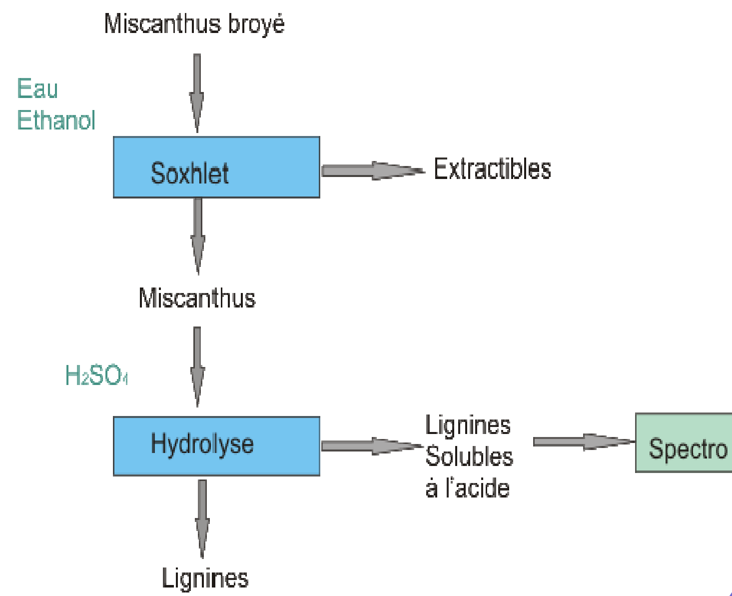
❖ Acid detergent lignin (Van Soest procedure)

Subsequents steps of chemical treatments to solubilize ''non-fibre'' components and final determination of the residue obtained

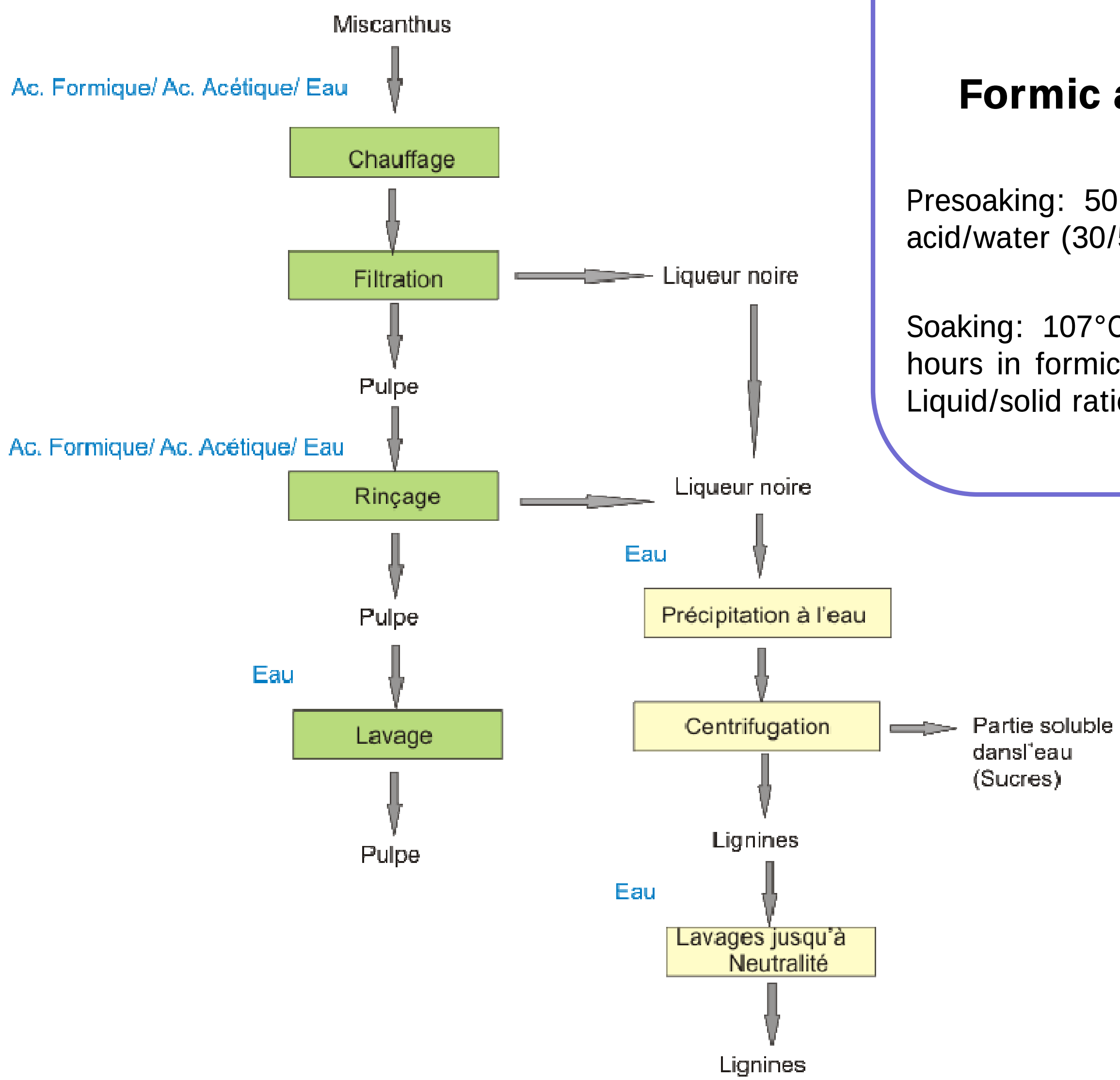


❖ Klason lignin

Strong acid hydrolysis and determination of two fractions: acid soluble lignin and acid insoluble lignin



Materials and Methods



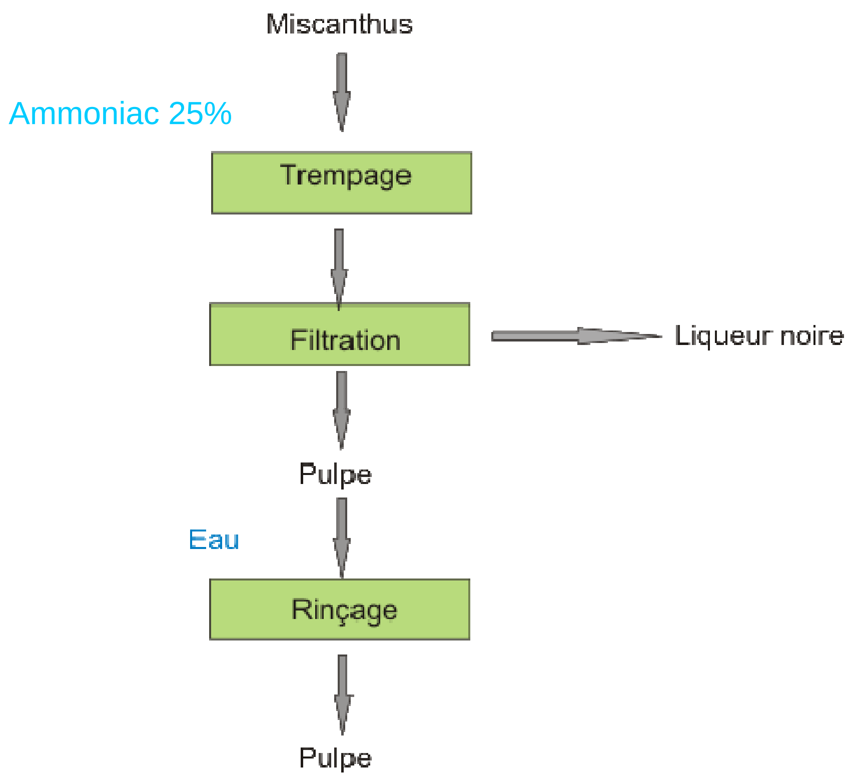
Pretreatments of *Miscanthus*

Formic acid / Acetic acid / Water

Presoaking: 50°C for 30 min in formic acid /acetic acid/water (30/50/20%). Liquid/solid ratio: 12/1.

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: 12/1. Agitation: 450 rpm.

Materials and Methods



Pretreatments of *Miscanthus*

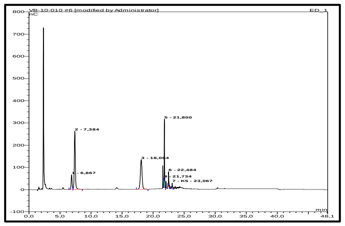
Soaking in aqueous ammonia (SSA)

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

Materials and Methods

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. Loading of 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

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:

➢In several tropical forages species the neutral detergent extraction solubilizes a lignin-carbohydrate complex *(Lowry et al., 2002)*

➢Acid soluble lignin fraction is lost in one of the steps of the ADL procedure *(Jung et al., 1997)*

➢It appears that **Klason lignin** is a more accurate estimate of cell-wall lignin content for grasses.

Results

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 (Kalson) ▪Acid soluble	 23.0 ± 0.7 1.5 ± 0.2
Polysaccharides	67.4
Ash	2.4 ± 0.1
Total	102.4

Monosaccharide composition

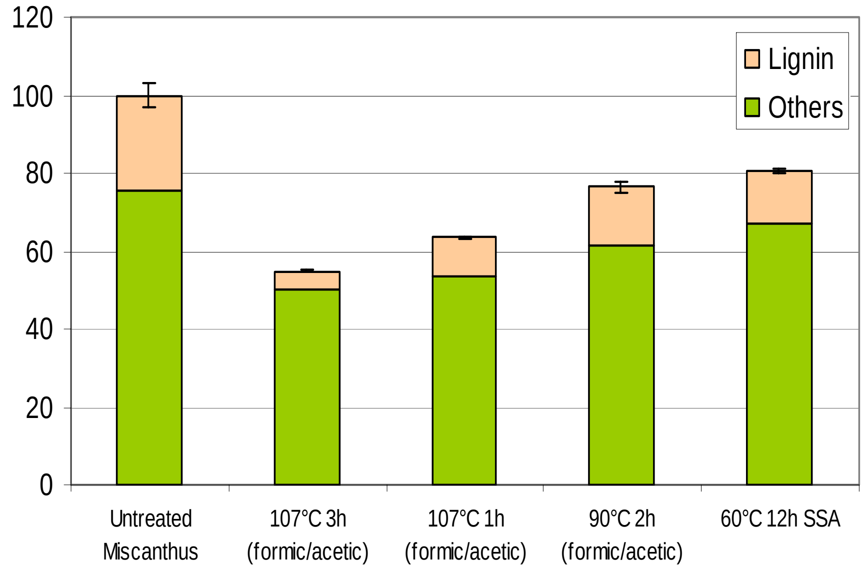
Component	% of total dry material
Glucose	48.4 ± 4.8
Xylose	15.7 ± 1.1
Arabinose	1.9 ± 0.1
Galactose	1.2 ± 0.2
Mannose	0.2 ± 0.2
Total	67.4

- 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.

Results

Delignification of *Miscanthus*



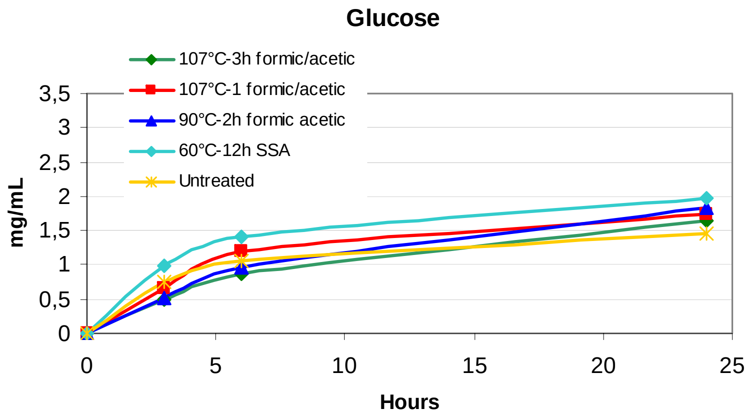
Delignification	81%	59%	38%	39%

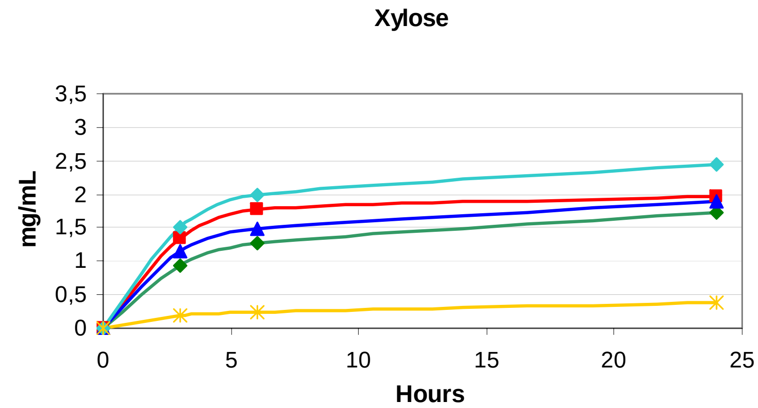
- Pretreatment by the formic/acid mixture (107°C, 3h) resulted in the highest deliginification rate (81%), but also in an important loss of polysaccharides.

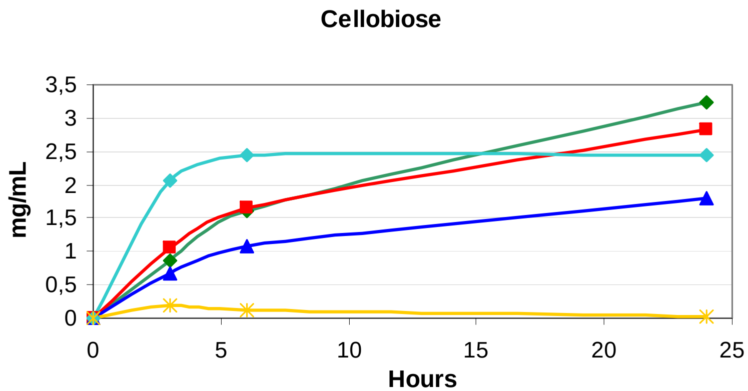
- Formic/acid method (90°C, 2h) was similar to the SSA in delignification. However, SSA resulted in a lower solubilization of the polysaccharides.

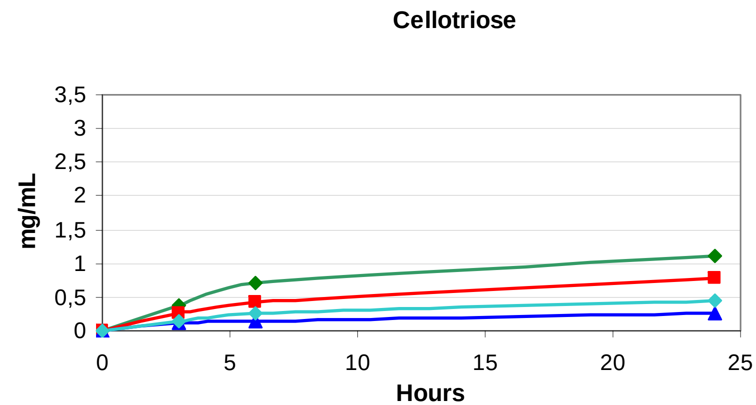
Results

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.

Thank you!