# SUNLIBB

## Sustainable Liquid Biofuels from Biomass Biorefining

Grant Agreement no. 251132

**Collaborative Project**
**EU 7th Framework Programme**
**ENERGY**

Project duration: 1st October 2010 – 30th September 2014

## Deliverable 3.3

**"Detailed characterisation of Miscanthus matrix polysaccharides"**

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Workpackage: **3**
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Dissemination Level: **PU**

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**SUNLIBB deliverables**

| **Del No:** D3.3 | **Deliverable Name:** Detailed characterisation of Miscanthus matrix polysaccharides | | | |
|---|---|---|---|---|
| **WP3** | **Lead partner:** P9 UCAM | **Dissemination level:** PU | **Delivery date :** month 24 September 2012 | **Actual delivery date:** 31/10/12 |

D3.3 was completed in Project Month 25, as part of Milestone 9 (Matrix polysaccharide structure and composition defined in maize and Miscanthus).

**Objective:**

Branched xylan is the main component of the cell wall matrix embedding the cellulose fibres. Glucuronoarabinoxylan (GAX) with arabinosyl and methyglucuronic acid branches on the xylose backbone is found in grasses (such as maize and Miscanthus). For this deliverable we aimed to identify the detailed structure of GAX in Miscanthus.

**Results:**

To do this, we fingerprinted the xylan from Miscanthus stem tissue. We used AIR (Alcohol Insoluble Residue) treated with 4M NaOH prior to different enzymatic hydrolysis. The hydrolysates were then reductively aminated with a fluorophore and analysed on DASH (DNA sequencer Assisted Saccharide High throughput analysis, Li *et al.* 2013). The traces generated exhibit qualitative similarities (*i.e.* similar oligosaccharide profile) between Miscanthus and maize but also some minor quantitative differences between the two. These data support the report by Kulkarni *et al*, (2012), that GAXs of different grasses have a comparable structure.

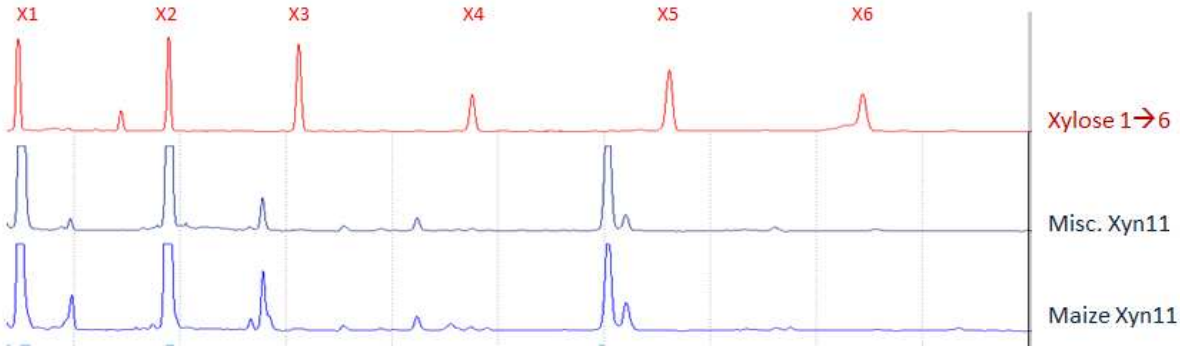


Fig1 : DASH traces of Miscanthus and maize AIR digested by Glycoside Hydrolase from Family 11 (GH11).

For the detailed structural characterisation of the oligosaccharide fragments, we employed HILIC (Hydrophilic Interactions Liquid Chromatography) coupled off-line to MALDI-ToF/ToF-MS/MS. HILIC allows the separation of structural isomers while detailed structural information can be obtained with high energy MALDI-CID. With this approach, the most abundant GH10 and GH11 hydrolysis products of Miscanthus AIR extracted from stems were identified, and are summarised in table 1, below.

Fig 2: *Miscanthus sinensis* GH10 and GH11 oligosaccharide products

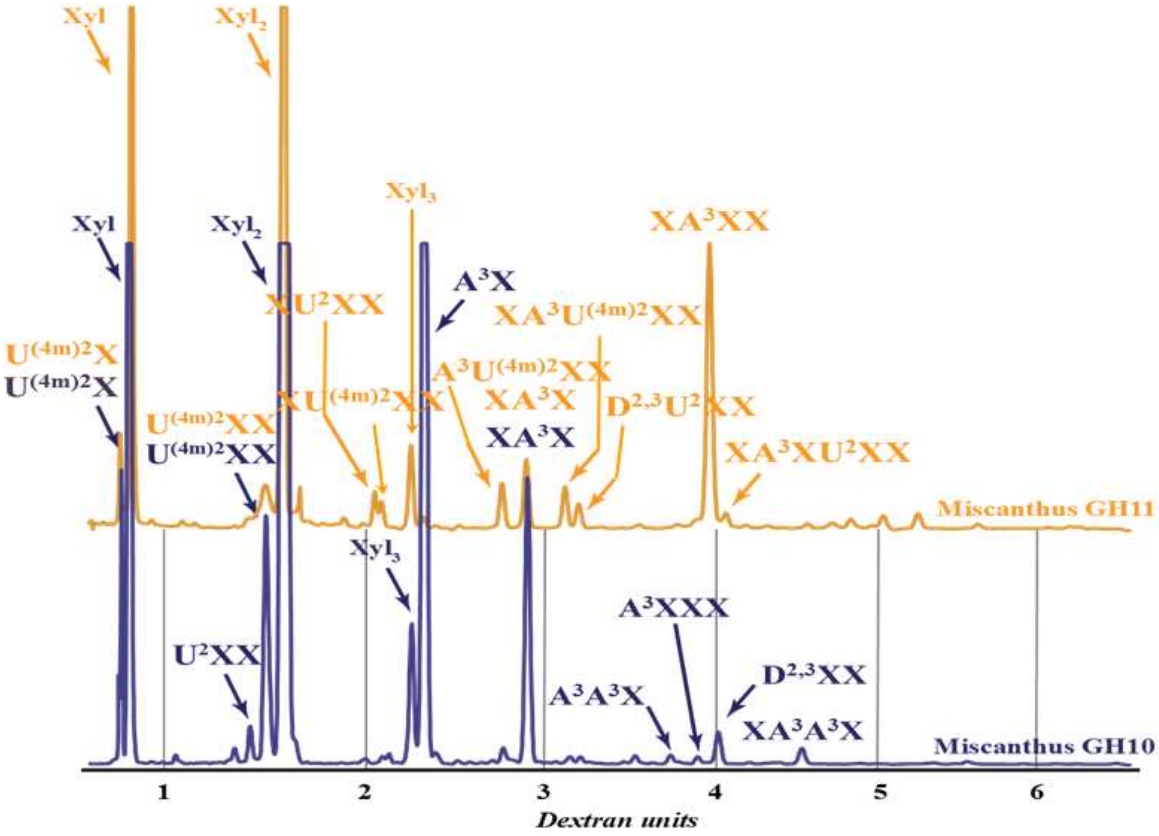


Table 1: *Miscanthus sinensis* GH10 and GH11 oligosaccharide products. Oligosaccharides identified by HILIC-MALDI-TOF/TOF-MS/MS produced from xylanase 10 digestion of GAX from Miscanthus stems. Oligosaccharides with glucuronic acid in place of 4-O-methyl glucuronic acid are also present in low quantities. Single letter code according to Faure *et al*. 2009.

| | GH10 | GH11 |
|---|---|---|
| Oligosaccharide name | Dextran position | Dextran position |
| $U^{2}X$ | N/A | |
| $U^{(4m)2}X$ | 0.79 | 0.79 |
| X | 0.87 | 0.87 |
| $U^{2}XX$ | 1.44 | |
| $U^{(4m)2}XX$ | 1.51 | 1.51 |
| $X_2$ | 1.6 | 1.6 |
| $XU^{2}XX$ | | 2.03 |
| $XU^{(4m)2}XX$ | | 2.07 |
| $X_3$ | 2.24 | 2.24 |
| $A^{3}X$ | 2.31 | |
| $A^{3}U^{(4m)2}XX$ | | 2.75 |
| $XA^{3}X$ | 2.89 | 2.89 |
| $XA^{3}U^{(4m)2}XX$ | | 3.11 |
| $D^{2,3}X$ | 3.15 | |
| $D^{2,3}U^{2}XX$ | | 3.19 |
| $A^{3}A^{3}X$ | 3.73 | |
| $A^{3}XXX$ | 3.89 | |
| $XA^{3}XX$ | | 3.96 |
| $D^{2,3}XX$ | 4.01 | |
| $XA^{3}XU^{2}XX$ | | 4.05 |
| $XA^{3}A^{3}X$ | 4.52 | |

**Conclusion:**

We have now described the main oligosaccharides composing the Miscanthus arabinoglucuronoxylan matrix. There was a surprisingly large amount of MeGlcA and GlcA decorations. We also discovered the more complex side chains of Xyl linked to alpha1,3 arabinofuranose. Surprisingly, we did not see any 2-linked arabinofuranose. The establishment of the xylan composition is a major step towards producing a detailed model of the overall structure of the grass cell wall.

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Li X, Rubstov D, Faria-Blanc N, Mortimer JC, Turner SR, Krogh KB, Johansen KS, Dupree P. (2013) *Biotech Biofuels* 6(1):94