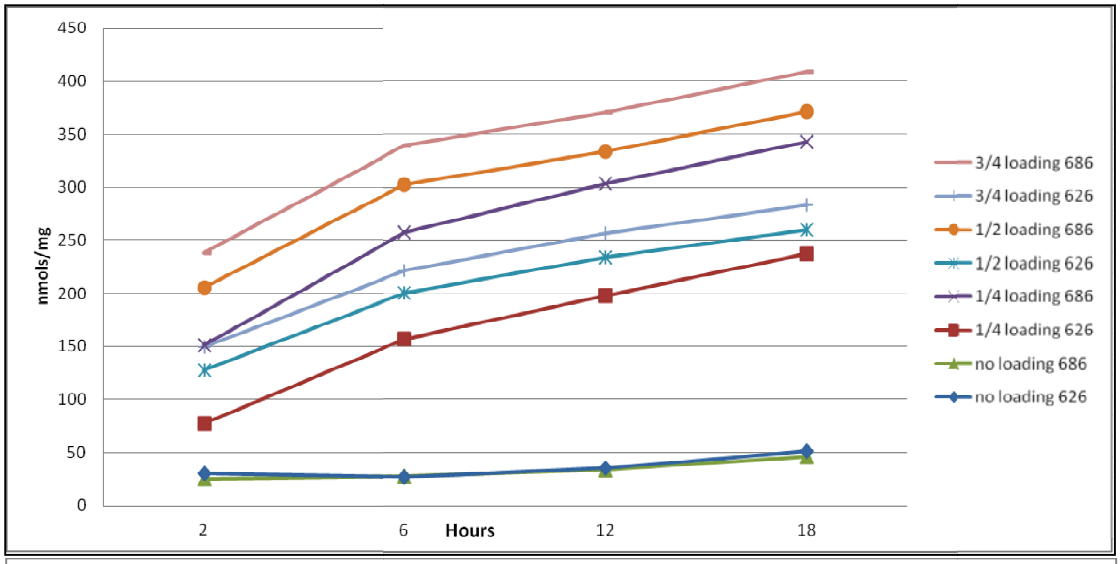
Joint Deliverable 5.1: HT saccharification assay established in Brazil

The purpose of this deliverable is to set up a common methodology for the evaluation of saccharification as a mayor trait to phenotype populations in order to breed specifically for industrial applications of biomass. The experience of Partner 1 in development this methodology would be useful to speed up and work synergistically with the Brazilian counterpart [1]. This deliverable should have been completed by month 12, but the delay in the release of funds necessary to purchase the relevant equipment produced a delay in the development of the Platform in Brazil.
Professor Igor Polikarpov visited the HT platform in York and in coordination with Dr. Leonardo Gomez designed a project that was further defined during the collaboration around the characterization of novel biomass crops by Marisa Lima at the University of York (paper submitted and [2]).
The platform is based on a Tecan Evo 200 liquid handling station and has been designed for high throughput saccharification assays as well as for HT cloning of potential glycosyl hydrolases. This platform will be deployed during August 2013 for the determination of the saccharification potential in a sugar cane mapping population. Please see letter from Professor Polikarpov included with the present document.

Joint Deliverable 5.2: HT saccharification assay for maize, miscanthus and sugar cane validated

The validation of the HT saccharification assay was performed during the first reporting period. The objective of this validation is to establish the conditions of pretreatment, enzyme loading and hydrolysis for exposing the genotype- determined differences in the susceptibility to enzymatic digestion by cellulases.
To establish the optimal conditions we use increasing concentration of enzyme at different incubation times for each crop. Figure 1 shows the kinetics of the release of reducing sugars from two different maize genotypes at different enzyme loadings and incubation times. The enzyme loadings of : 10, 3.3, 1.65. and 1.2 FPU/g of biomass were used. The optimal conditions were established at 1.2 FPU/g for maize and 3.3 FPU/g for miscanthus. The optimal hydrolysis time for both crops is 8 h in order to expose differences between genotypes.
Once the conditions of saccharification are established, the following step is to determine the pretreatment to be applied to obtain the maximal differences between genotypes. Figure 2 shows the effect of acid, alkaline and water pretreatment on the saccharification of 20 miscanthus genotypes selected from the SUNLIBB population generated at Wagenigen. The results clearly show that the pretreatment with water at 90 °C for 40 min allows a substantial hydrolysis of the biomass while showing clear differences in saccharification potential between lines. In conclusion, hot water pretreatments were selected for screening both, maize and miscanthus.
The saccharification assay was also validated for sugarcane. This assay was applied to bagasse, stems and straw. Please find attached one example of this as a manuscript submitted to Biotechnology for Biofuels and under revision at present.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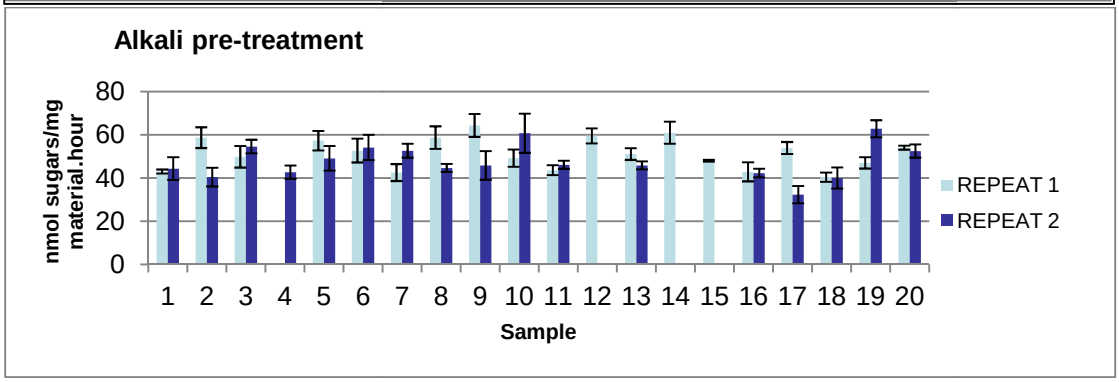
Alkali pre-treatment
nmol sugars/mg material.hour
80
60
40
20
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20
Sample
REPEAT 1
REPEAT 2