Transfer of a miniaturized method for high-throughput screening of biomass pretreatment and saccharification and application on poplar and miscanthus clones

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In order to meet increasing demand for second-generation biofuels, exploring large sets of lignocellulosic biomass candidates appears to be one of the key levers in bio refinery. Indeed, assessment of saccharification potential of different variants provided from breeding programs on promising biomasses such as poplar and miscanthus can lead to the identification of less recalcitrant and more economic candidates.

Based on recently described high-throughput screening methods [1], we have developed our own miniaturized assay to explore the saccharification potential of 10 *Populus deltoides x trichocarpa* genotypes grown under short (SRC) and very short (vSRC) rotation coppice conditions. For this purpose, we have used a high-throughput automate system based on metal reactors and 96-well microplate devices. The system allows to perform hygrothermal pretreatment (180°C, 40 mn) on 5 mg biomass followed by enzymatic saccharification using Cellic CTec2 at high loading levels. This experimental set-up has also been evaluated and shown very good results with regards to precision of biomass dispensing, microplate sealing and repeatability performance. Our approach is based on the comparison of the sugar release variability after saccharification of untreated and pretreated samples.

Interestingly, our results indicate clearly that pretreatment step is very useful for lignocellulosic biomass screening purpose since more variability in glucose release is observed when saccharification is preceded by hot water treatment. Indeed, glucose yields ranged between 15% and 30% without pretreatment while they were between 57% and 88% after pretreatment. Our results indicate that SRC and vSRC poplars behave differently: vSRC samples were more digestible than SRC one's before pretreatment while no significant difference was observed after pretreatment. Seven years old miscanthus from three species follow the same trend with glucose yields ranging between 20% and 71% without pretreatment and between 40% and 92% after hot water treatment.

This original approach makes it possible to screen lignocellulosic biomass while evaluating the impact on saccharification potential of several factors such as the biomass particle size, the pretreatment duration and severity, and the enzyme loading.

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Reference

[1] M.J. Selig, M.P. Tucker, R.W. Sykes, K.L. Reichel, R. Brunecky, M.E. Himmel, M.F. Davis and S. R. Decker. Lignocellulose recalcitrance screening by integrated high-throughput hydrothermal pretreatment and enzymatic saccharification. Industrial Biotechnology 6 (2010), 104-111.