

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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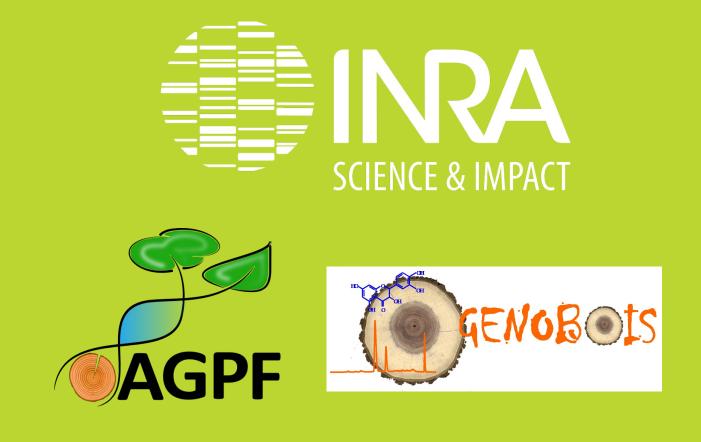
Nassim Belmokhtar, Vincent Segura, Brigitte Chabbert, Nathalie Boizot, Kévin Ader, et al.. Transfer of a miniaturized method for high-throughput screeninig of biomass pretreatment and saccharification and application on poplar and miscanthus clones. Exploring lignocellulosic biomass! Explorons la biomasse lignocellulosique!, Jun 2016, Reims, France., 2016. hal-02793860

HAL Id: hal-02793860 https://hal.inrae.fr/hal-02793860

Submitted on 5 Jun 2020

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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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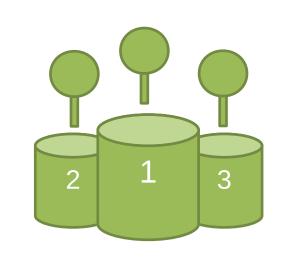
Introduction

In order to meet increasing demand for second-generation biofuels, exploring large sets of lignocellulosic biomasses appears to be one of the key levers to improve bio refinery yields.

Poplar and miscanthus breeding programs have provided large sets of varietal outputs, whose saccharification potential assessment could lead to identify the best biofuel-oriented varieties.



Assessment of saccharificatio n potential



2nd generation bioethanol production



Enzymatic hydrolysis without

pretreatment

GOPOD assay

(Megazyme®)

HPLC

Plant breeding programs

High saccharification yielded varieties

Experiments

Objectives of this study:

L- Transfer into breeding programs and adaptation of a high-throughput screening method developed at NREL (USA)1

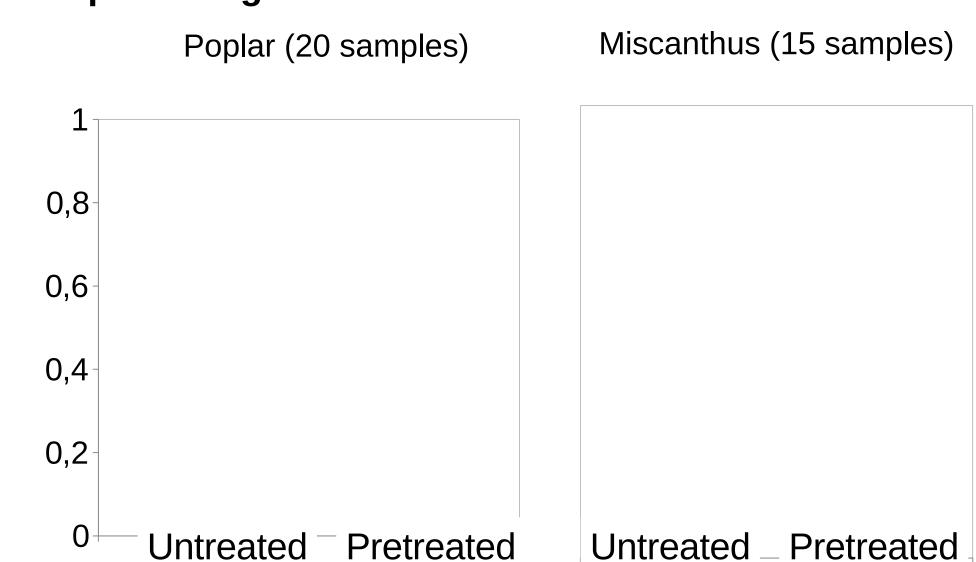
Cellkraft®

2- Demonstration, on poplar and miscanthus biomass, of the importance of pretreatment step in screening process

Steam Lignocellulosic biomass (180° C) from short rotation coppice (SRC) and very **Enzymatic hydrolysis** short rotation coppice (50° C, 70 mg.g-1 Dry Hastelloy 96-well microplate (SBS-type): (vSRC) poplars and Matter of *Cellic Ctec-2*) Thermal and corrosion resistance miscanthus clones 4 steam ports surrounding each well Glucose **Stainless** Stackable in Hastelloy holder quantification pretreatment reactor **5 mg** of biomass per well (1,67 % w/v) Fully automated station for PARR Instrument® Reaction volume: 340 µL per well biomass grinding (A) and (NREL, Denver, USA) precise dispensing (B) (Xyloforest project) **Steam generator**

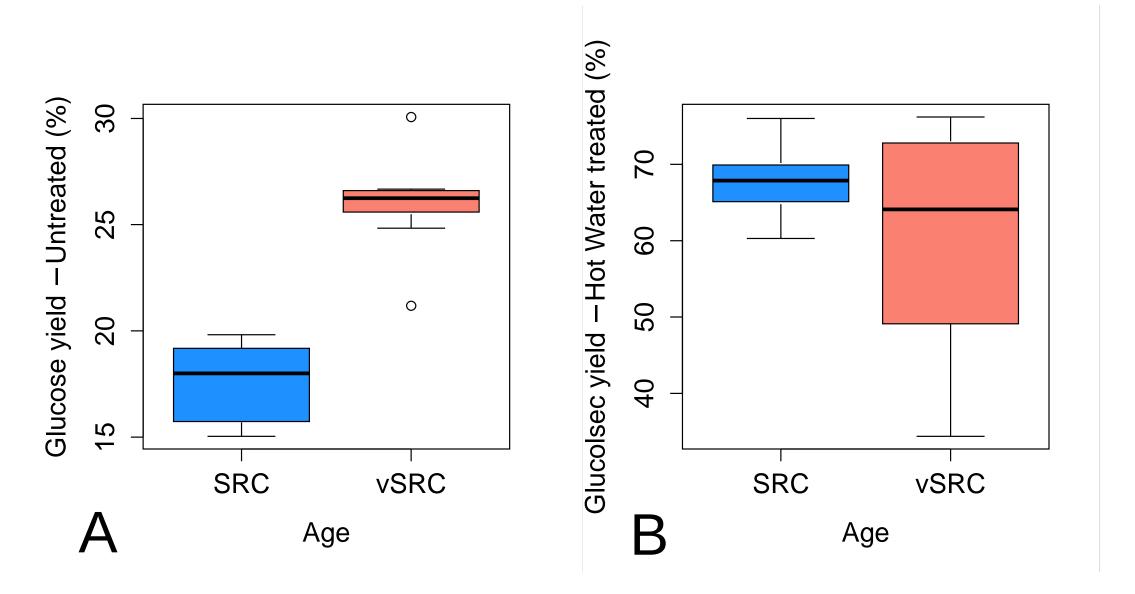
Results

1- Impact of pretreatment on the variability of glucose release percentage



- Hot water pretreatment in 96-well microplates increases significantly saccharification yield for both plants
- More variability in glucose release is observed after hot water pretreatment mainly for poplar clones

2- High-throughput screening of 10 SRC and 10 vSRC poplar varieties



- vSRC poplars release higher glucose amounts than SRC ones when saccharified without pretreatment (A)
- SRC and vSRC poplars show similar enzymatic glucose yield after hot water pretreatment (B)
- More variability in glucose release is observed for SRC poplar without pretreatment (A) in contrast to vSRC ones which express more variability after hot pretreatment (B)

Conclusions

- Based on the comparison of the sugar release of poplar and miscanthus biomasses, our approach allowed us to stress the importance of a pretreatment step before enzymatic hydrolysis to magnify the expression of the genetic variability. Furthermore, this approach revealed different responses to saccharification of SRC and vSRC poplar biomasses without or after a pretreatment.
- Our developed high-throughput method is well adapted to screen, with high accuracy, a large set of samples from different lignocellulosic biomasses to evaluate their potential for saccharification.

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.

Acknowledgments:

This work was funded by *BPIFrance* in the R&D project *Futurol* Innovative equipments was funded by *Xyloforest*, an Equipex Project (ANR) (INRA Aquitaine)





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