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Evaluation of high throughput screening methods in picking up differences between cultivars of lignocellulosic biomass for ethanol production

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ABSTRACT

We present a unique evaluation of three advanced high throughput pretreatment and enzymatic hydrolysis systems (HTPH-systems) for screening of lignocellulosic biomass for enzymatic saccharification. Straw from 20 cultivars of winter wheat from two sites in Denmark was hydrothermally pretreated and enzymatically processed in each of the separately engineered HTPH-systems at 1) University of California, Riverside, 2) National Renewable Energy Laboratory (NREL), Colorado, and 3) University of Copenhagen (CPH). All three systems were able to detect significant differences between the cultivars in the release of fermentable sugars, with average cellulose conversions of 57%, 64%, and 71% from Riverside, NREL and CPH, respectively. The best correlation of glucose yields was found between the Riverside and NREL systems ($R^2 = 0.2139$), and the best correlation for xylose yields was found between Riverside and CPH ($R^2 = 0.4269$). All three systems identified Flair as the highest yielding cultivar and Dinosor, Glasgow, and Robigus as low yielding cultivars. Despite different conditions in the three HTPH-systems, the approach of microscale screening for phenotypically less recalcitrant feedstock seems sufficiently robust to be used as a generic analytical platform.

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1. Introduction

The development of crops specifically bred for ethanol production would help overcome a major obstacle in biofuel production, namely feedstock recalcitrance. Utilizing less recalcitrant plants opens up the possibility of combined economic benefits of higher yields at milder pretreatment conditions and lower enzyme dosages. However, implementing genetic selection programs for reduced recalcitrance and understanding the associated genetic modifications requires methods to evaluate large populations for their digestibility. In response to the need for screening methods to breed less recalcitrant feedstock for cellulosic ethanol production, several research institutions recently engineered high-throughput pretreatment and enzymatic hydrolysis systems (HTPH-systems). In this case, high throughput systems are considered systems that have been miniaturized and automate from a larger-scale assay using custom-designed laboratory hardware and/or a rapid assay for sugar determination at the end of hydrolysis, as to handle large sample sets with minimum labour.

To the best of our knowledge, few automated HTPH-systems exist around the world; examples are described by Studer et al. [1], Selig et al. [2], Santoro et al. [3], and Zhang et al. [4]. Three of these platforms are based on metal reactors in a 96-well microplate format that are capable of withstanding temperatures and pressure needed for hydrothermal pretreatment [5]. These three HTPH-systems are located at the University of California, Riverside [1], the National Renewable Energy Laboratory (NREL), Colorado [2], and the University of Copenhagen (CPH), Denmark. As only limited experience has been obtained with this type of biomass screening, evaluating the robustness of screening methods in picking up differences between cultivars are urgently needed.

To detect differences between phenotypes, the HTPH-platforms must be so accurate that the analytical variation is small in comparison with natural variation between phenotypes. The HTPH-platform from Riverside was initially capable of detecting difference in enzymatic saccharification greater than 10%, with a standard deviation of the laboratory method (i.e., standard deviation when the same sample is repeated) of 4.1% total sugar yield for poplar material [1]. When processing different winter wheat straw cultivars in the Riverside system, the standard deviation of the laboratory method of total sugar conversion was reduced to 3.0% and the system proved capable of detecting naturally existing variation in cultivars that significantly affected saccharification [6]. Selig et al. [2] reported standard deviations of the laboratory method for poplar control plates of 6%–8.5% after pretreatment and enzymatic saccharification in the NREL system, while the CPH platform achieved a standard deviation of the laboratory method of 8.7% with a plate of standard wheat straw (unpublished data). However, even though the repeatability of the HTPH-systems appears to be good, the question still remains whether it is the same properties of the straw that we are measuring with the three methods and whether cultivar differences would be the same. In short, how do the results from the HTPH-systems correlate?

Several authors have described an array of factors influencing the HTPH axiom "you get what you screen for" pointing to the importance of sample heterogeneity, size reduction, distribution, pretreatment chemistry and severity as well as enzyme activity [7]. Previous studies have also shown that interactions exist between enzyme loading and wheat straw cultivars [8], thus it is unknown if cultivars will behave similarly in the various HTPH-systems. Small technical differences in the HTPH-systems, such as size reductions or heating and cooling techniques, might lead to different results and a lack of correlation between the HTPH systems. Therefore, the scope of this paper is not to achieve exactly the same yields in all HTPH-systems, but rather to see if each of the HTPH-systems in question point to the same cultivars as more or less recalcitrant, despite methodological and technical differences.

The aim of this study was to evaluate three HTPH systems on their ability to measure sugar (i.e., glucose and xylose) release from different cultivars of winter wheat straw and determine the correlation between the systems. This will indicate how much the conclusions of such microscale screening methods can support selection and comparison of cultivars.

2. Materials and methods

2.1. Wheat straw

Winter wheat straw was sampled at two sites in Denmark, where field experiments comparing cultivars were conducted in two completely randomized blocks at each site. At full maturity, wheat straw was harvested and approximately 80 g dm (dry matter) of straw was sampled as representative of each block. Straw collection was done the same day at the two sites. Growing conditions were kept similar at the two sites, thus straws represented the natural variation (in climate, soil type etc.) in the biomass feedstock in Denmark. Cultivars were Northern European breeds: Abika, Ambition, Audi, Dinosor, Flair, Florett, Glasgow, Hattrick, Inspiration, Jenga, Oakley, Opus, Penso, Potenzial, Robigus, Samyl, Skalmeje, Smuggler, Tommi, and Tuscan. One sample was lost during harvest; thus total sample set was 79 samples. The straw was collected as air dried (approx. 7% moisture) in the field, milled to <1 mm pieces on a cyclone mill (President, Holbæk, Denmark), and stored at ambient temperature until any further analysis.

2.2. HTPH-systems

The conditions of processing were for all three HTPH-systems based on previous knowledge for near-optimal hydrothermal pretreatment of wheat straw, and high enzyme loading was applied to be sure that inhibition of enzymes by compounds released in pretreatment and hydrolysis did not interfere with enzyme action [9,10]. No prior treatment of the air-dried, milled samples was done at Riverside or NREL before hand-weighing (Riverside) or robotically dispensing (NREL) the samples to the 96-well plates, whereas CPH included automated grinding and dispensing.

2.2.1. Riverside

The analysis was performed as described in Lindedam et al. [6] on the system described by Studer et al. [1]. Briefly, 1% dm

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