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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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solids was pretreated in triplicate in a 96-cup reactor plate in a steam chamber for 17.6 min at 180 °C, with heat up and cool down time of approx. 40 s and 10 s (to reach 120 °C), respectively [1]. Hydrolysis was started by loading 40 FPU g^{-1} of dm of Celluclast 1.5L:Novozym188 mixture (5:1 w/w). This roughly equals an enzyme loading of total enzyme protein on dry biomass at 83 mg g^{-1} of dm. Both enzymes were supplied by Novozymes (Bagsværd, Denmark). Hydrolysis was conducted for 72 h, 50 °C, and sugars were detected on HPLC as described in the previous publication [6]. For the Riverside method, enzyme-only blanks were used to test that soluble sugar in the enzyme solution did not interfere with glucose and xylose peaks.

2.2.2. NREL

The analysis was slightly modified from the method described by Selig et al. [2]. Briefly, 2% dm solids (5.0 mg ± 0.3 mg in 250 μL de-ionized H_2O) was pretreated in triplicate in a 96-well plate in a steam chamber for 17.5 min at 180 °C, with heat up and cool down time of approx. 52 s and 1.5 min (to reach 120 °C), respectively [2]. Hydrolysis was started by loading total enzyme protein on dry biomass at 70 mg g^{-1} of Cellic® CTec2 (Novozymes, Bagsværd, Denmark). After enzymatic hydrolysis at 50 °C for 70 h, the release of glucose and xylose was measured by a glucose oxidase/peroxidase assay and a xylose dehydrogenase assay, respectively (Megazyme International Ireland, Wicklow, Ireland). For the NREL method, glucose (and any xylose) in the enzyme mix was accounted for with enzyme-only blanks in every plate.

2.2.3. CPH

Though the system has been preliminarily described [4], publication of in-depth system validations is in progress. A hybrid protocol was followed; mixing conditions to which the system was previously tested and conditions to resemble the Riverside and NREL HTPH-systems. In short, the platform consisted of a custom-designed robot (Labman Automation Ltd.; United Kingdom) performing automated grinding, feeding, and weighing, much as described in Santoro et al. [3]. Including automated dispensing meant grinding the biomass to a fine powder with the majority of particles sizes below 250 μm. Triplicate measurements of 6% dm solids were weighed into custom-built, un-coated 96-well aluminium plates. One plate at a time was pretreated on a custom-made aluminium heating block in a closed chamber for 10 min at 190 °C. Ramping to target temperature took approx. 6 min, and cooling to 120 °C was achieved within approx. 1 min by a closed water flow system. Once cooled to room temperature, hydrolysis was conducted at an enzyme loading of total enzyme protein on cellulose in dry biomass at 70 mg g^{-1} of Cellic® CTec2 (Novozymes, Bagsværd, Denmark) at 50 °C for 72 h. The release of glucose and xylose was measured using an Ultimate 3000 HPLC (Dionex, Germering, Germany) equipped with refractive index detector (Shodex, Japan). The separation was performed in a Phenomenex Rezex ROA column at 80 °C with 5 mol m^{-3} H_2SO_4 as eluent at a flow rate of 0.6 ml min^{-1}. The values for xylose when measured on the HPLC contained also the amounts of galactose and mannose. The release of glucose was adjusted by subtracting the calculated amount of glucose added with the enzyme solution.

2.3. Compositional analysis

Compositional analysis of the 79 samples was performed by the two-step acid hydrolysis of carbohydrates according to the procedure published by NREL [11], with results previously published in Lindedam et al. [6]. The analysis was performed on an HPLC for which the values for xylose contained also the amounts of galactose and mannose, and the arabinose sugars were added in calculations of total hemicellulose.

2.4. Statistical analysis

Results from HPLC measurements of glucose (+cellobiose) and xylose (+arabinose) released from combined pretreatment and enzymatic hydrolysis are presented in gram per gram dry matter of unpretreated wheat straw (g g^{-1} of dm) by the following calculation (Eq. (1)):

$$\text{g (x) g}^{-1}\text{ dm} = \frac{C_x(\text{g L}^{-1}) * \text{reaction volume (L)}}{\text{biomass (g dm)}} \quad (1)$$

where x denotes glucose (C6), xylose (C5) or glucose plus xylose (TS for total sugar), C_x is the concentration of x measured by HPLC, corrected for the total reaction volume in the wells and the amount of dry matter biomass weighed into each well. Results from the glucose oxidase/peroxidase and xylose dehydrogenase assays were normalized to standard switchgrass material from the BioEnergy Science Center (BESC) and presented in gram per gram dry matter of wheat straw (g g^{-1} of dm). The normalization accounted for minor procedural "drift" from day-to-day by normalizing all the results to a standard reference martial for each plate.

Conversion of cellulose or xylan was calculated as the amount of glucose and xylose released from pretreatment and hydrolysis as a percentage of the maximum theoretical release possible; based on the concentration of hydrated substrate determined by compositional analysis and corrected for solid loading in hydrolysis.

The standard deviation of the laboratory method (SDL) was based on the laboratory triplicates in each HTPH-system and used to compare the systems accuracy and reproducibility (Eq. (2)):

$$\text{SDL} = \sqrt{\frac{\sum_{i=1}^{n}\sum_{j=1}^{m}\left(X_{ij} - \overline{X}_j\right)^2}{n * m - 1}} \quad (2)$$

where i is the individual laboratory replicate out of n replications ($n = 3$) and j is the individual sample out of m samples ($m = 79$).

Comparison of straw from wheat cultivars used Tukey simultaneous tests to fit general linear models (proc GLM) at a 95% confidence level [12], where a lines statement was included for pairwise comparisons of mean values.

3. Results and discussion

3.1. Comparison of yields and conversions

Table 1 summarizes the sugar (i.e., glucose and xylose) yields from the three HTPH-systems. The Riverside platform

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Table 1 – Maximum, minimum, and average sugar values released from 79 wheat straw samples in three HTPH systems.

	Max g g^{-1} of dm			Min g g^{-1} of dm			Average g g^{-1} of dm (RSD%)		
	Glu	Xyl	TS	Glu	Xyl	TS	Glu	Xyl	TS
Riverside	0.25	0.20	0.44	0.19	0.16	0.35	0.22 (5.8)	0.18 (5.2)	0.39 (5.1)
NREL	0.29	0.23	0.52	0.20	0.18	0.38	0.23 (8.0)	0.20 (6.1)	0.43 (6.7)
CPH	0.37	0.22	0.59	0.26	0.15	0.42	0.31 (6.5)	0.19 (6.5)	0.50 (6.2)

Glucose (Glu), xylose (Xyl) or total sugar (TS) values with relative standard deviations (RSD) in parenthesis from the average values of triplicates measurements of 79 wheat straw samples in each HTPH-system at Riverside, NREL, and Copenhagen.

generally gave lower values than NREL, which again was lower than results from the CPH platform.

Fig. 1 shows biomass conversions in each HTPH-system, and Fig. 2 shows the actual total sugar yields of the 79 samples from each system. Riverside converted on average 57% and 74% of the glucan and xylan, respectively, while NREL converted 64% and 88% of the glucan and xylan and CPH converted 71% and 76% of the glucan and xylan (Fig. 1). Remarkably, for CPH-system, the high glucan conversion was accompanied by a similar degree of xylan conversion, whereas for the other two systems xylan conversions exceeded glucan conversions. This was most likely related to the use of a higher pretreatment temperature in CPH leading to both more accessible cellulose, but also a higher level of xylose degradation to e.g. furfural giving a relatively low xylan conversion. In part, the better cellulose accessibility could also be explained by higher solubilization of hemicellulose [13]. We have speculated if the uncoated plates in CPH-system could have caused aluminium catalysed degradation of xylose during pretreatment [14], though it is unlikely that only xylose, and not glucose should be affected by the Lewis acid degradation, thus accounting for the observed low xylan conversion in CPH.

When using the HTPH-systems to search for feedstocks of higher digestibility, it is critical to know if the process conditions reduce the sensitivity of digestion assays by pushing all sample digestion yields closer to the theoretical maximum, or if the yields are still a function of structural variations [15,16].

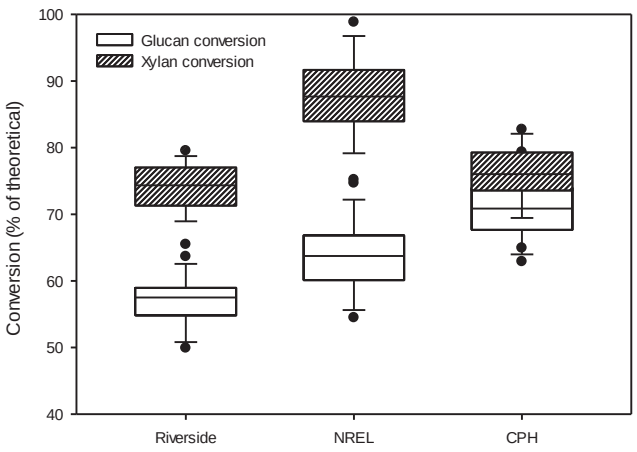

Fig. 1 – Boxplot of the glucan and xylan conversion in three HTPH-systems: Riverside, NREL and CPH. Plot shows the median value (line), the 25th and 75th percentile (lower and upper limit of vertical box), 10th and 90th percentile (whiskers) and the 5th and 95th percentile (dots) for 79 samples in each system.

In other words: for the purpose of using the HTPH-systems for screening conversions close to 100% should be warranted, as detection of differences in accessibility will be concealed. For this reason, one could argue that a system like NREL producing xylan conversions as high as nearly 100% of available substrate is "too good". However, with cellulose being the major player in commercial ethanol production and the NREL system only getting approx. 65% of the cellulose conversion, we still expected sample differences to be related to substrate accessibility. Additionally, a similar trendline throughout the samples in total sugar yields from all three systems (Fig. 2), though their conversions ranged from the low 50s to the low 90s (Fig. 1), suggests that the digestibility we observed with this kind of screening was a reflection of chemical composition and structures – unaffected by HTPH process differences.

Though the log severity of pretreatment in all three systems was approx. 3.6 [17], major process differences were introduced in the steps before and after pretreatment: For instance, ball milling can significantly increase the digestibility of the biomass [18], and more comminution in the CPH system might also be part of the overall higher total sugar yield (Fig. 2) and the high glucan conversion compared to NREL (Fig. 1), which used the same enzyme preparation. NREL and Riverside processing was largely similar throughout, except for the enzymes used. Hence, the comparatively low glucan conversions (Fig. 1) and low total sugar yields (Fig. 2) in Riverside are very likely due to the use of less efficient enzymes.

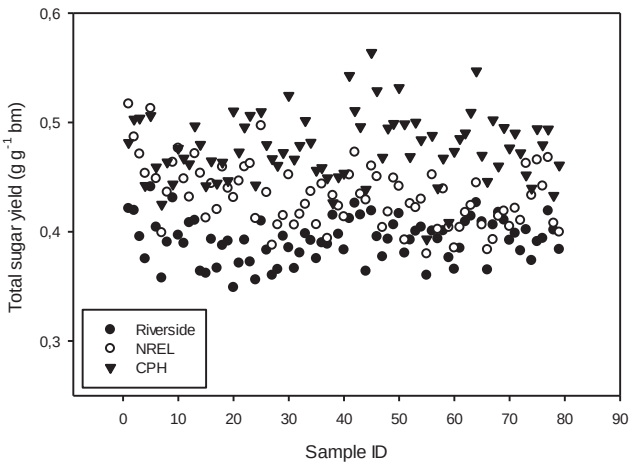

Fig. 2 – Total sugar yield expressed in grams per gram dry matter of 79 wheat straw samples in each of three HTPH-systems: Riverside, NREL, and CPH.

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3.2. Correlations between HTPH-systems and their accuracy

Correlations between the three HTPH-systems for all sugars are listed in Table 2 and examples of the best glucose and xylose correlations are displayed in Fig. 3. Generally, better correlations existed for xylose measurements than glucose measurements because the systems had less variation in measuring xylan conversion. Xylan release also relied less on enzyme formulation than glucan breakdown. Assessing the correlations between the HTPH-systems should consider the narrow span of average values in which the variations are to be detected (Fig. 3), and take into account that the correlations between triplicate measurements in the individual methods are not much better (Riverside total sugar correlations of triplicates are $R^2 = 0.35–0.42$, NREL total sugar correlations of triplicates are $R^2 = 0.17–0.26$, and CPH total sugar correlations of triplicates are $R^2 = 0.15–0.20$). The correlations between glucose and xylose yields in each system are $R^2 = 0.458$, 0.609, and 0.679 for Riverside, NREL and CPH, respectively.

The standard deviation of laboratory results (SDL) for total sugar yields was lowest in Riverside, highest in CPH, and intermediate at NREL (Table 3). The total variation in the data caused by the system can be evaluated by the absolute value of the variation coefficient (CV) given in parenthesis, which is the SDL divided by the average TS yield (Table 1) in percentage. These CV represent the entire process from grinding/dispensing through digestion as well as sugar quantification by HPLC (Riverside and CPH) or monosaccharide assays (NREL). When measuring genetically identical samples in their HTPH system, Santoro et al. [3] found CVs of 5%–7% (Arabidopsis and corn stover), while a real plant population of Arabidopsis gave a CV of 11.5% [3]. A CV of 7% was reported for glucan conversion performed with AFEX pretreated corn stover in microplate methodology [19]. The CVs observed in this study are acceptable within the frames of coefficients of variability, which generally should be less than 15% for inter-assay CVs expressing plate-to-plate consistency, and less than 10% for intra-assay CVs expressing the individual CVs for all replicates over multiple plates. The larger variation in CPH system was most likely introduced in the pretreatment step, as the triplicate metal well-plates were heated separately. The CPH and Riverside system can only pretreat one 96 well plate metal reactor at a time, whereas the NREL system can steam-treat up to 20 metal 96 well-plates at once. Seeing the high reproducibility of the Riverside system, despite the single plate heating system, we speculate if steam used to heat free-standing metal cups (Riverside) might be a more reproducible method of pretreatment compared to electrical heating of a metal well-plate (CPH). Thermal distribution tests were presented in the original articles for Riverside [1] and NREL [2], and no heat sinks were reported. For the NREL system oxidation-reduction assays were used for sugar analysis of poplar with convincing correlations with glucose and xylose detection by HPLC of $R^2 = 0.980$ and $R^2 = 0.996$ [1]. However, using the assays to determine sugar concentrations over 1.5 mg mL^{-1} seemed to have larger variability [1] and correlating HPLC data with glucose oxidase/peroxidase on the CHP dataset with sugar concentrations of 10–20 mg mL^{-1} resulted in a correlation coefficient of $R^2 = 0.75$ (data not shown).

We concluded that stronger correlations between the HTPH-systems are not possible unless better SDL and CVs can

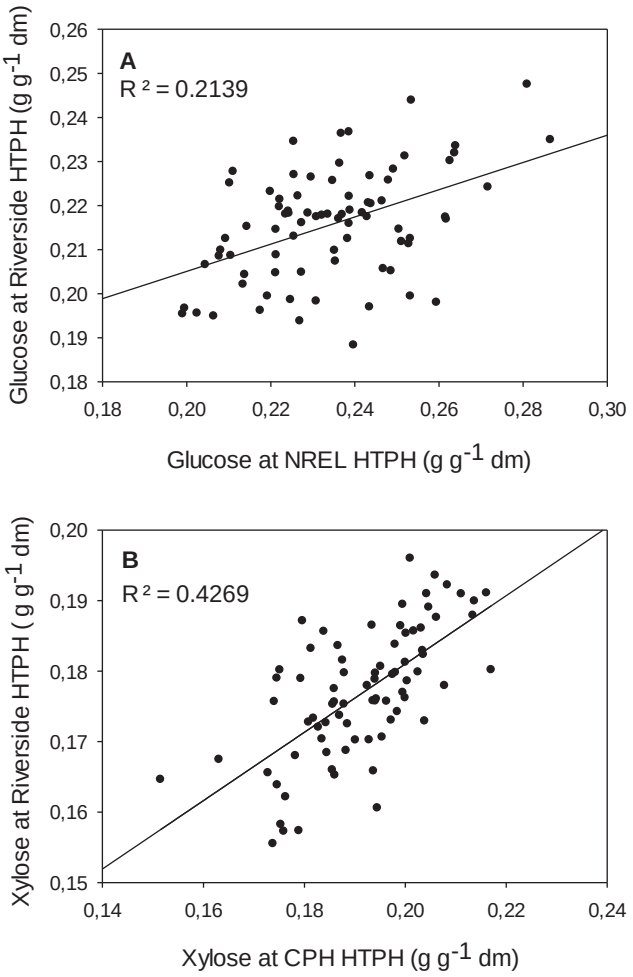

Fig. 3 – A) Linear correlation fitted to plot of glucose (g g^{-1} of dm) measured with NREL versus Riverside HTPH-systems and B) Linear correlation fitted to plot of xylose (g g^{-1} of dm) measured with CPH versus Riverside HTPH-systems.

Table 2 – R^2 values for linear correlations between pairwise comparison of three HTPH-systems measuring yields of glucose, xylose, or their total (TS) expressed in grams per gram dry matter.

	Riverside-NREL	Riverside-CPH	NREL-CPH
Glucose	0.2139	0.0885	0.1314
Xylose	0.281	0.4269	0.2644
TS	0.2901	0.2197	0.1904

Table 3 – Standard deviation of laboratory results (SDL) of total sugar in g g^{-1} of dm and variation coefficient in percentage (CV%) of three HTPH-systems.

		Riverside	NREL	CPH
SDL (CV%)	TS	0.0129 (3.3)	0.0224 (5.1)	0.0363 (7.3)

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Table 4 – P-values for test of effect of cultivar and site in 711 observations (79 samples * 3 HTPH-systems * 3 laboratory replicates), sorted by HTPH-system.

		Pr > F		
		Riverside	NREL	CPH
Cultivar	Glucose	<0.0001***	<0.0001***	<0.0001***
	Xylose	<0.0001***	<0.0001***	<0.0001***
	TS	<0.0001***	<0.0001***	<0.0001***
Site	Glucose	0.3189	<0.0001***	0.0609
	Xylose	<0.0001***	0.1673	0.1090
	TS	0.0008***	<0.0001***	0.0666

Degrees of freedom are 19 for cultivar and 1 for site. Values marked with * is significant at $P \leq 0.05$, ** at $P \leq 0.01$ and *** at $P \leq 0.001$.

be achieved. However, such an optimization of the systems does not seem necessary, when judging the individual systems reproducibility. If optimization was chosen, possible reproducibility bottlenecks could be the plate-to-plate variation in the pretreatment step for CPH and the assay reliability of the colorimetric sugar determination assay for NREL.

3.3. *Can cultivar differences be detected by HTPH-systems?*

The statistical analysis revealed that all three HTPH-systems detected cultivars to have a significant effect on the release of both glucose and xylose, and thus their total (Table 4). The effect of site was significant only for xylose in Riverside and glucose in NREL, and their corresponding total sugars, while the effect of site was non-significant in CPH.

Table 5 shows the mean values for total sugar of the 20 cultivars used in the experiment. The values were tested pairwise for significant differences, consequently listing which cultivars were identified as higher or lower digestible phenotypes by each HTPH-system. Regardless of the HTPH-system, Flair was the best cultivar in terms of sugar yields. Concurrence could be extracted from the three HTPH-systems, with some of the highest yielding cultivars being Flair, Ambition, Jenga, Smuggler, (Abika, Audi) and the poorest yielding cultivars being Dinosor, Glasgow, Robigus, Tuscan, and Skalmeje. Although the HTPH-platforms differed not only in engineering, but also regarding enzyme blends, solids loading, pretreatment temperature, and method for product quantification, all three systems picked up "the same" differences between cultivars. Keeping in mind that the cultivars in this study represent a very narrow variation (no large geographical, climatic or annual variation) the relative performance between cultivars seem to be determined rather robustly.

As an example of whether or not microscale measurements can be extrapolated to large scale, we point out that 3 of the cultivars have been pretreated in a pilot-scale ethanol plant in bales of 500 kg and the observed ranking placed Ambition and Smuggler as less recalcitrant then Skalmeje [8]. All 3 HTPH-systems displayed the same ranking (Table 5). Glasgow and Robigus are by all systems placed in the bottom of the list (Table 5). Interestingly, these cultivars are particularly known as having short straws, good lodging resistance, and are together with Oakley and Skalmeje resistant to the attacks of the insect *Sitodiplosis mosellana* by production of a toxin. We speculate that parameters associated with the physical strength of the straw and potentially toxin residues may translate into a less digestible material for the enzymes to convert.

It deserves to be noted that the relative placing of a few cultivars varied considerably between the systems, most clearly seen for Inspiration ranking second best at Riverside, in the middle at NREL, and in bottom half at CPH (Table 5).

Table 5 – Mean values of total sugar released (g g^{-1} of dm) for 20 cultivars in Riverside, NREL and CPH HTPH-systems.

Riverside							NREL						CPH					
Mean TS	Cultivar	Significans					Mean TS	Cultivar	Significans				Mean TS	Cultivar	Significans			
0.424	Flair	A					0.479	Flair	A				0.532	Flair	A			
0.413	Inspiration	A	B				0.459	Ambition	A	B			0.507	Jenga	A	B		
0.412	Ambition	A	B				0.455	Abika	A	B			0.504	Audi	A	B		
0.406	Smuggler	A	B	C			0.451	Samyl	A	B	C		0.500	Ambition	A	B	C	
0.402	Jenga	A	B	C	D		0.450	Smuggler	A	B	C		0.496	Abika	A	B	C	
0.399	Audi	A	B	C	D	E	0.448	Audi	A	B	C		0.493	Penso	A	B	C	
0.398	Florett	A	B	C	D	E	0.447	Jenga	A	B	C		0.484	Florett	A	B	C	D
0.398	Penso	A	B	C	D	E	0.446	Potenzial	A	B	C		0.480	Hattrick	A	B	C	D
0.397	Abika	A	B	C	D	E	0.435	Inspiration	A	B	C		0.477	Oakley	A	B	C	D
0.395	Opus	A	B	C	D	E	0.435	Florett	A	B	C		0.476	Samyl	A	B	C	D
0.394	Samyl	A	B	C	D	E	0.432	Penso	A	B	C		0.475	Opus		B	C	D
0.392	Tommi	A	B	C	D	E	0.432	Dinosor	A	B	C		0.475	Tuscan		B	C	D
0.391	Potenzial	A	B	C	D	E	0.424	Opus	A	B	C		0.474	Potenzial		B	C	D
0.389	Hattrick	A	B	C	D	E	0.419	Tommi	A	B	C		0.469	Inspiration		B	C	D
0.386	Skalmeje		B	C	D	E	0.415	Skalmeje		B	C		0.463	Smuggler		B	C	D
0.382	Oakley		B	C	D	E	0.413	Hattrick		B	C		0.453	Skalmeje		B	C	D
0.370	Tuscan			C	D	E	0.413	Oakley		B	C		0.451	Glasgow		B	C	D
0.368	Robigus				D	E	0.408	Robigus		B	C		0.441	Dinosor			C	D
0.365	Dinosor				D	E	0.407	Tuscan		B	C		0.435	Tommi				D
0.365	Glasgow					E	0.394	Glasgow			C		0.433	Robigus				D

Values with same letter are not significantly different at significance level 0.05.

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Judging by the SDLs (Table 3) Riverside was the most robust screening method for picking up cultivar differences, than NREL, finally CPH. However, we conclude that microscale screening for genetic selection in wheat straw was not only possible with the present HTPH-systems, but also to some extent similar regardless of which system was used. This lends credibility to the massive efforts put into downscaling systems for biomass screening – though the strategies for germplasm selection must still be aware that different conditions can be adapted for different screening purposes.

4. Conclusions

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 ($R^2 = 0.4269$). Even though technical differences existed between the three HTPH-systems, the results unanimously display the cultivar Flair to be the superior feedstock out of 20 cultivars. Though interchangeably, groupings of high- or low yielding cultivars were the same in all HTPH-systems. We conclude that microscale screening for genetic selection in wheat straw is not only possible with the present HTPH-systems, but can also be performed with such accuracy that it can be used as a generic analytical platform applying the same technical principles of the systems used.

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