Supplemental information for:

Producing concentrated solutions of monosaccharides using biphasic CO₂-H₂O mixtures

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S1 High-solids enzymatic hydrolysis reaction system

A custom high-solids enzymatic hydrolysis reaction system was built for this study. This setup consists of a set of rollers that can roll up to 12 "rotating drum" reactors. A picture of this setup is available in Figure S1.



Figure S1: Picture of the custom-built high-solids enzymatic hydrolysis reaction system. The set of motorized rollers is shown with 6 reactors on the back row and one on the front row. In the background, the incubator in which the whole setup is placed for temperature control purposes, is shown.

S2 Characterization of sampling error

Given that a single pretreatment run could only process 60 g of initial dry solids, multiple pretreatment runs are required to run multiple high-solids enzymatic hydrolysis reactions (which require 45 g of initial dry solids each). Therefore, we attempted to determine whether sampling the same reactor three times introduced more error than sampling a single reactor three times. We compared the yields and their 95% confidence intervals

(based on triplicate sampling) obtained for the high-solids enzymatic hydrolysis of unpretreated corn stover in three separate reactors. The corn stover was obtained in 2009 from the National Renewable Energy Laboratory (NREL). Corn stover was chosen as a substrate because of it offered high yields (close to 30%) even when unpretreated. This allowed us to run these experiments without pretreating biomass. Its composition was given in a previous study¹.



Figure S2: Glucan and hemicellulose sugar yields obtained as a function of enzymatic hydrolysis time for unpretreated corn stover at varying initial solid contents. Reactors 1, 2 and 3 refer to three separate reactors that were loaded with identical reaction mixtures and sampled at the same time. Error bars represent 95% confidence intervals.

As the yields and their respective 95% confidence intervals demonstrate in Figure S2, the error resulting from sampling a given reactor is comparable to that of the error between two separate reactors. Indeed, except for a single point (hemicellulose sugar yields, 30 wt% solids, 24 hr) all average reactor yields fall within each other's confidence intervals. In addition, for over half the data points, the error resulting from sampling exceeds the differences between the average reactor yields. The implication of these results is that sampling a given reactor three times leads to just as accurate an estimate of the uncertainty involved in high-solids enzymatic hydrolysis as sampling three separate reactors. This allowed us to use one reactor and only 45 g of initial dry solids of pretreated biomass per experiment, reducing the amount of pretreatment runs necessary to complete this study.

References

1. J. S. Luterbacher, J. W. Tester, and L. P. Walker, *Biotechnol. Bioeng.*, 2010, **107**, 451-460.