

Supplemental Info for AEILPT of biomass

Biomass pretreatment protocol

Biomass was switchgrass supplied by Daniel Putman, University of California - Davis. The switchgrass (20-40 mesh) was dried in a convection oven at 40°C for 24 hours before use. 1-Butyl-3-methylimidazolium chloride ($\geq 98\%$, CAS: [79917-90-1](#)) was purchased from Sigma-Aldrich and heated to 80°C to allow handling. Amberlyst 15DRY (moisture $\leq 1.5\%$, CAS: 39389-20-3) was purchased from Sigma-Aldrich and used as received.

The parameters used for the experiments are as follows unless otherwise noted in the text: 5g of switchgrass was placed in 95 g of [C₄C₁im]Cl and heated to 160°C in a Parr reactor for 90 minutes with stirring at 100 rpm. The reaction was swiftly cooled in 2 minutes to 80-90°C using the internal cooling of the Parr reactor. The reactor was opened and 1 g of Amberlyst catalyst was added. The reaction was then heated to 100 °C and kept at temperature with the reaction time varied, and stirring of 100 rpm. After reaction time was completed, the vessel was cooled to 80 °C and 300 ml of H₂O added whilst stirring vigorously, to precipitate the biomass. It was apparent that the resulting mixture contained coarse solids, which settled to the bottom of the container, and finer solid matter that remained suspended in solution. The mixture was filtered through 100- μ m nylon mesh, yielding a filter cake of coarse material. This filter cake was thoroughly washed in copious amounts of water to remove residual ionic liquid. The filtrate contained the fine suspended solids. The filtrate was centrifuged at 14,000 x g for 30 minutes to separate the fine materials from the liquid portion. The liquid was decanted and 500 ml of liquid added. This mixture was then centrifuged again and the process repeated 5 times.

Carbohydrate and lignin analysis

The carbohydrate composition of biomass samples was determined according to standard NREL protocol (Sluiter et al. 2006). Monomeric sugars in the supernatant were measured with a Agilent HPLC equipped with a Bio-Rad Aminex HPX-87H column (Richmond, CA) at a rate of 0.6 mL of 5 mM H₂SO₄ per min at 60°C. Solids remaining after two-stage acid hydrolysis were held at 105°C overnight. The weight of the dried solids corresponds to the amount of acid-insoluble lignin and ash in the sample. The weight of the ash only fraction was then determined by heating the solids to 575°C for 24 hours.

In vitro Enzymatic Assays

To produce CX2, comp cells prepared from freshly acquired MG1655 cells were transformed with (pCellulase or pXylanase). Cells harboring each plasmid were grown overnight in LB supplemented with 100 μ g/ml carbenicillin. They were diluted 1:20 into fresh LB with 100 μ g/ml carbenicillin and production of the cellulase and xylanase were induced at OD₆₀₀ = 0.7 with 1 mM IPTG. After the

overnight induction the culture media containing each enzyme was sterile filtered with a 0.2 micron filter. Equal parts of the xylanase containing media and cellulase containing media were combine to formed cellulase cocktail CX2. For hydrolysis assays, pretreated switchgrass was rinsed twice with water, once with ethanol and then two more times with sterile distilled water. This biomass was added wet to undiluted CX2 to a final concentration of 1% switchgrass (w/v). Enzymatic hydrolysis was carried out at 37° C with 250 RPM agitation. Samples were withdrawn at the indicated times and frozen for subsequent analysis.

To measure the hydrolysis kinetics, the soluble reducing sugar equivalents in each sample were measured using the 3,5-dinitrosalicylic acid (DNS) assay. 1% DNS assay solution was prepared with 1% DNS, 0.05% sodium sulfite, and 1% sodium hydroxide. To a 2 ml Eppendorf tube, 0.5 ml of the DNS assay solution and 0.5 ml of the sugar solution were added. Samples were heated to 90°C for eight minutes quenched with 167 µl 40% potassium sodium tartrate and then cooled to room temperature. The absorbance at 575 nm is then proportional to the concentration of reducing sugar, which was calculated with a standard curve created with known amounts of glucose. The final concentration of solublized sugars after 24 hours was determined after oligosaccharides in the samples were converted to monosaccharides by acidifying to 4% H₂SO₄ and then autoclaving at 140° C for one hour. The sugar concentration was measured with HPLC as described above.

For cellulase loading assays, the washed sterilized biomass from above was resuspended in fresh LB to a final concentration of 1% switchgrass (w/v). Then 10 fold dilutions of sterile filtered Celluclast cellulase from *Trichoderma* were added starting from 1% v/v (700U/g biomass) to 0.0001% v/v (0.07U/g biomass). Hydrolysis was carried out at 37°C with 250 RPM agitation. Samples were withdrawn at the indicated times and frozen for subsequent analysis. Oligosaccharides were converted to monomers and sugar concentrations were measured with HPLC as above.

Each data point shown is the average of two replicates performed with switchgrass from two different pretreatment runs. Values within each replicate typically differed by less than 25%.

Total sugar yield was calculated as:

$$\begin{aligned} &(\text{Saccharification yield of coarse fraction}) * (\text{Weight of coarse fraction recovered}) + \\ &(\text{Saccharification yield of fine fraction}) * (\text{Weight of fine fraction recovered}) \end{aligned}$$

Growth Assays

For these experiments, samples of *E. coli* MG1655 freshly acquired from the ATCC were used. These cells were transformed with either pCellulose or pXylan and then grown up overnight at 37°C in LB with 100 µg/ml carbenicillin. The next day, cells with each plasmid alone were used for seeding the monocultures while strains were combined to seed the coculture. These inoculums were diluted 1:20 into fresh MMM

supplemented with 100 µg/ml carbenicillin and 1% pretreated switchgrass (w/v) and incubated at 37°C. Growth was measured by counting the increase in the number of viable cells in each culture. Every 24 hours, samples were removed from each culture, diluted 1:10,000 and then plated on LB. Data is average of three independent replicates.

Fatty acid production from consolidated bioprocessing

Plasmid pHatty was produced in two steps. First, *tesA* was amplified from the *E. coli* chromosome without the N-terminal periplasmic leader sequence and then cloned into the Bgl-Brick site in BglBrick plasmid pBB-A5K under the control of the *lacUV5* promoter with Gibson cloning. Then, *fadR* was then amplified from the *E. coli* chromosome and placed under the control of the kanamycin resistance promoter with Gibson cloning to generate pHatty.

pHatty was cotransformed with either pXylan or pCellulose into an MG1655 strain in which *fadE* was deleted using the method described in Datsenko and Wanner. The cotransformants were grown up separately at 37°C in glycerol-free terrific broth (TB) supplemented with 50 µg/ml kanamycin and 100 µg/ml carbenicillin overnight. The next day, both cultures were combined and then diluted 1:20 into 1 ml fresh MMM cultures supplemented with 10% LB, 50 µg /ml kanamycin, 100 µg/ml carbenicillin and 1% pretreated switchgrass and then incubated at 37°C. After 4 hours, fatty acid production was induced by the addition of 1 mM IPTG and fatty acid production was allowed to proceed for 1 week. Free fatty acid titers were measured as previously described. Data is average of three independent replicates

Fatty acid production from simultaneous saccharification and fermentation

MG1655 cells transformed with pHatty were grown overnight at 37°C in MMM + 10% LB supplemented with 1% glucose and 50 µg/ml kanamycin. The following day, cells were diluted 1:20 into 1 ml fresh MMM supplemented with 1% pretreated switchgrass, 50 µg /ml kanamycin and 1 µl (0.7 U) Celluclast *Trichoderma* cellulase from Sigma and incubated at 37°C. After 8 hours, free fatty acid production was induced with 1 mM IPTG. Fatty acid production proceeded for 72 hours and then free fatty acid was quantified as described previously. Data is average of three independent replicates

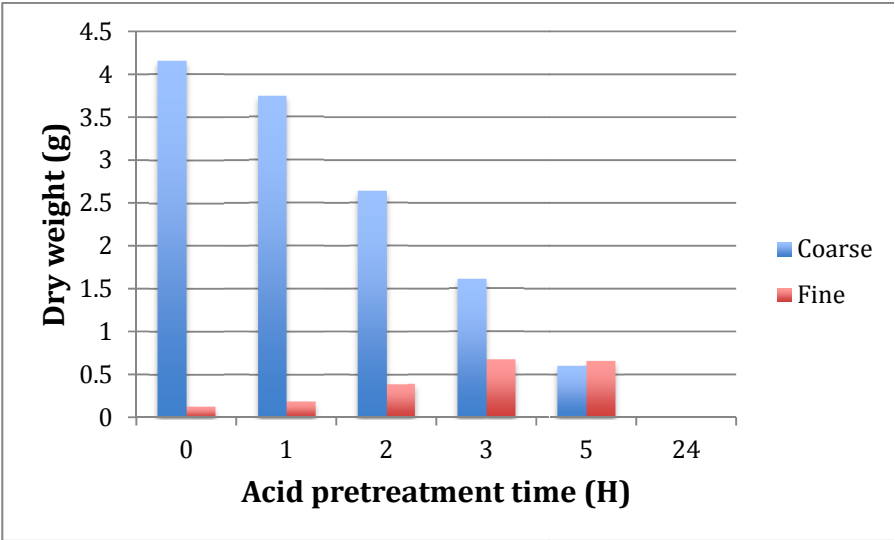


Fig. S2 Cellulytic *E. coli*. Cellulose is hydrolyzed by cellulase *cel* to cellobiose, which is hydrolyzed to glucose monomers by β -glucosidase *cel3A*. Glucose can then be imported into the cell and enter central carbon metabolism.

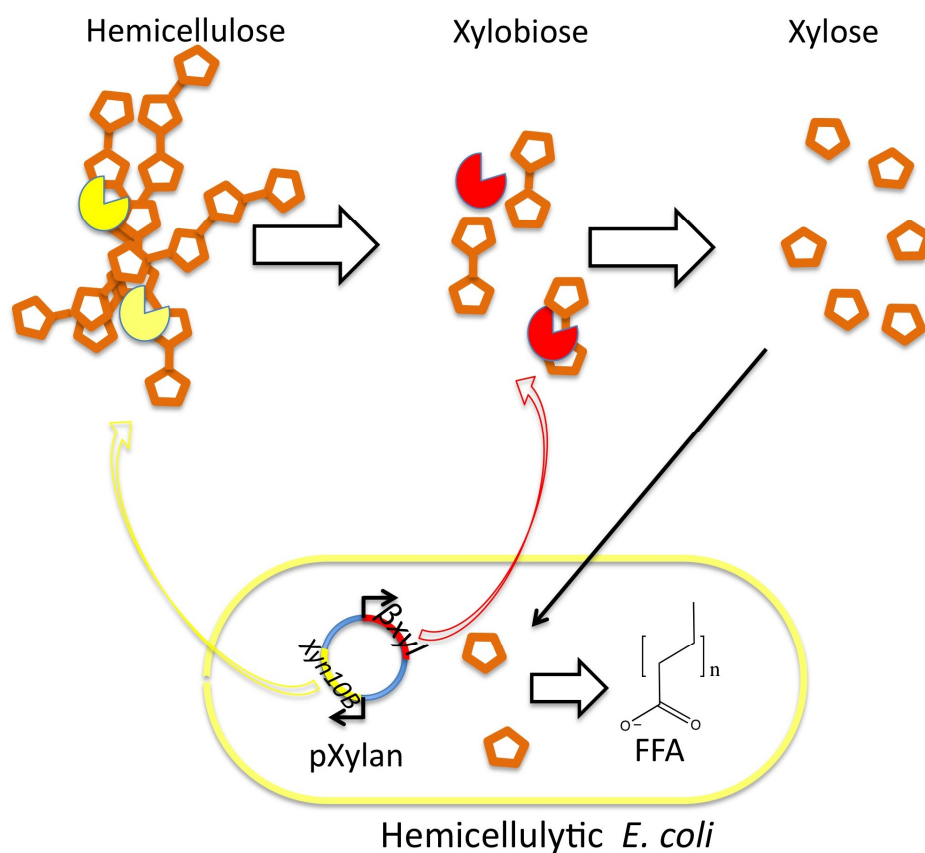


Fig. S3 Hemicellulytic *E. coli*. Xylan is hydrolyzed by xylanase *Xyn10B* to xylobiose, which is hydrolyzed to xylose monomers by β -xylobiosidase *gly43F*. Xylose can then be imported into the cell and enter central carbon metabolism.

References

- Sluiter A, Hames B, Ruiz R, Scarlata C, Sluiter J, Templeton D, Crocker D. 2006. Determination of structural carbohydrates and lignin in biomass. <http://devafdc.nrel.gov/pdfs/9572.pdf>. Laboratory Analytic Procedure LAP-002.