Green Chemistry

RSC Publishing

PAPER

View Article Online
View Journal

Acid enhanced ionic liquid pretreatment of biomass†

Cite this: DOI: 10.1039/c3gc37086k

Dan Groff,[a] Anthe George,[a,b] Ning Sun,[a] Noppadon Sathitsuksanoh,[a] Gregory Bokinsky,[a] Blake A. Simmons,[a,b] Bradley M. Holmes*[a,b] and Jay D. Keasling*[a,c]

Received 20th December 2012,
Accepted 7th March 2013

DOI: 10.1039/c3gc37086k

www.rsc.org/greenchem

Acid enhanced ionic liquid pretreatment is a promising method for boosting the yield of sugars produced from purified cellulose. Acid enhancement significantly increases the yields of sugars from the lignocellulosic feedstock switchgrass. The addition of Amberlyst 15, a protic acid resin, to the ionic liquid pretreatment of switchgrass boosted the yield of sugar up to ten times, decreased the amount of cellulase required for saccharification and increased the biocatalytic conversion of switchgrass into free fatty acids 10-fold to 22% of the theoretical yield.

Every day, the United States consumes around 20 million barrels of petroleum for transportation fuels, heating, and as the primary feedstock for the chemical industry.[1] Our heavy reliance on fossil fuels is poised to create a number of problems including economic instability and anthropogenic climate change.[2] This has led to growing interest in developing alternative sources of carbon for use as a fuel and chemical feedstock. For instance, the supply of lignocellulosic biomass, the most abundant renewable organic feedstock on the planet, is projected to be at least 1.3 billion tons in the US alone, providing a feedstock with the potential to displace approximately 25% of the fossil fuels currently being used for transportation fuels.[2]

Biomass is primarily composed of three types of polymers: cellulose, which is composed of β (1–4) linked glucose chains that hydrogen bond to form stiff crystalline rods; hemicellulose, which is composed primarily of xylan and wraps around cellulose fibrils; and lignin, a polyphenylpropanoid that cross-links hemicellulose.[3] The most significant obstacle to the use of the sugar present in cellulose and hemicellulose is the recalcitrance of lignocellulose to depolymerization.[2,4] Pretreatment (PT) or dissolution with ionic liquids (ILs), such as 1-ethyl-3-methylimidazolium acetate ([C_2mim][OAc]) or 1-butyl-3-methylimidazolium chloride ([C_4mim]Cl,[5] is a promising method that facilitates the downstream conversion of biomass into fermentable sugars with hydrolytic cellulose cocktails.[6] Heating cellulosic biomass dissolved in IL is believed to enhance enzymatic saccharification in two ways: it removes lignin[7] and it breaks down the crystalline lattice of the cellulose to produce amorphous cellulose (Fig. 1).[4b] Certain ionic liquids are also attractive as pretreatment solvents because they have a low vapor pressure and are miscible with water. Compared to aqueous solutions of other solvents commonly used for pretreatment including ammonia and sulphuric acid, [C_4mim]Cl is less toxic to animals.[8]

One of the major drawbacks to this type of biomass pretreatment is the price of ILs: [C_2mim][OAc] is the most effective IL for pretreatment found to date,[9] but at a cost of $50/kg would represent approximately half the cost of converting cellulosic biomass to ethanol.[10] In contrast, [C_4mim]Cl costs approximately 1/60th of [C_2mim][OAc],[11] but it is not as

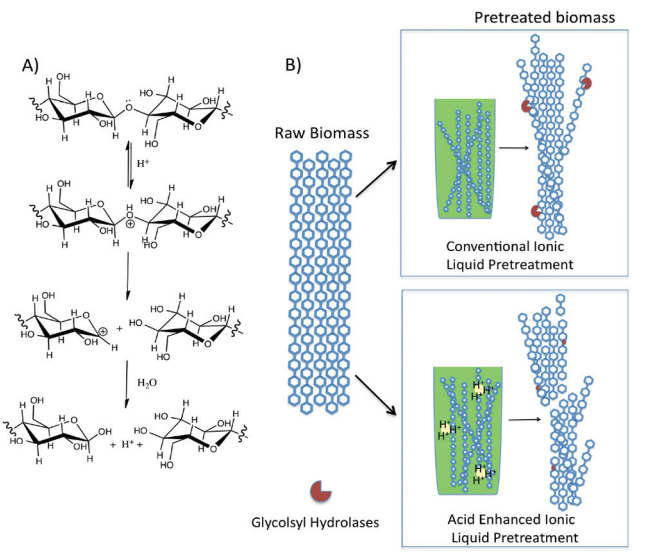

Fig. 1 Acid enhanced ionic liquid pretreatment. (A) Acid catalyzes hydrolysis of adjacent glucose monomers within an individual cellulose strand. (B) Biomass treated this way is more suitable for biocatalytic conversion into fuels and other chemicals.

[a]Joint BioEnergy Institute, Emeryville, California, USA. E-mail: jdkeasling@lbl.gov, bradley.m.holmes@gmail.com
[b]Sandia National Laboratories, P.O. Box 969, Livermore, California, USA
[c]Department of Bioengineering and Department of Chemical & Biomolecular Engineering, University of California, Berkeley, California 94720, USA

†Electronic supplementary information (ESI) available. See DOI: 10.1039/c3gc37086k

effective as a pretreatment solvent.[12] Even when produced at scale, there will still be significant differences in the cost of producing different ionic liquids. This will have a significant impact on the economic viability of downstream renewable chemicals produced. In addition, it should be possible to recycle and reuse ionic liquids, which will offset their high prices and lead to substantially greener processes than those which employ non-recyclable solvents. Demonstrated methods for IL recycling include nanofiltration,[13] vacuum distillation,[14] and supercritical CO_2 extraction.[15] Recycled [C_4mim][Cl] was reused five times while maintaining its activity as a biomass pretreatment solvent.[14] Furthermore, for a combined IL/catalyst system, it was possible to reuse both the catalyst and ionic liquid at least four times without a drop in the performance of either component.[16]

A general method to improve the performance of cheaper ILs will be an important development for the adoption of IL pretreatment. One strategy that has been demonstrated with purified cellulose is the addition of a solid protic-acid resin such as Amberlyst 15, which catalyzes the hydrolysis of the ether linkages between adjacent glucose molecules in cellulose, reducing the chain length of the cellulose polymers (Fig. 1).[17] In previous studies, this pretreatment resulted in partial depolymerization and an enhancement of approximately 15% to the downstream enzymatic saccharification and total sugar yields with α-cellulose, a highly refined version of cellulose.[17,18] While these foundational studies with α-cellulose are exciting we were curious whether these results could be extended to industrially relevant feedstocks.[19] Here we hypothesize that IL–Amberlyst treatment of more complex biomass would result in greater improvements because this material is naturally more recalcitrant to enzymatic hydrolysis.[18] Maximizing the conversion of these complex cellulose substrates into sugar will be key in developing greener alternatives to petroleum feedstocks. In addition Amberlyst 15 is less toxic than other acid catalysts used for pretreatment such as HCl or H_2SO_4.

In this study, switchgrass, a perennial crop with strong potential as a renewable feedstock, was selected as the lignocellulosic substrate.[19] The IL–Amberlyst 15 pretreatment was performed in two steps. First, biomass was added to [C_4mim]-Cl and heated at 160 °C for solubilization/delignification. Next, the temperature was reduced to 100 °C, and the solid acid catalyst Amberlyst 15 added. After the acid-catalyzed hydrolysis, biomass was then precipitated by the addition of DI H_2O (Fig. 2A). Two biomass fractions were obtained, a coarse fraction retained by a 20 m filter and a much finer fraction that precipitated in the filtrate. It was found that the amount of material in both fractions was a function of the length of the acid treatment at 100 °C, with a maximum yield of the fine fraction obtained for incubations of three hours (Fig. S1†). For the 2- and 3-hour acid incubations, the content of this fraction was 50% richer in glucan than the starting material (Fig. 2B). The fine fraction may be a mixture of oligosaccharides that were hydrolyzed from cellulose in the coarse and dissolved portions of switchgrass, which precipitated upon addition of antisolvent.[17] Concomitant with the higher levels of carbohydrate in the finer material, the remaining coarse fraction was enriched in lignin.

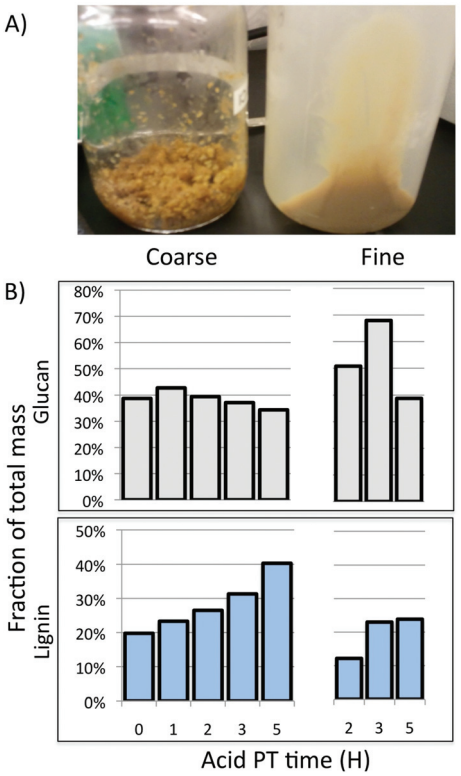

Fig. 2 Biomass recovered from AEILPT. (A) Coarse fraction (left) and fine fraction (right) which accumulates in presence of acid catalyst. (B) Fractional composition of each sample. The top graph shows the glucan content, and the bottom shows lignin content; the course fraction is shown on the left and the fine is on the right. (Not enough of the fine fraction was obtained from the 0 and 1 hour acid treatments for compositional analysis.)

To test whether the changes in the biomass that occurred during incubation with the acid catalyst facilitated downstream enzymatic digestion, each fraction was hydrolyzed separately using CX2, a synthetic binary cellulase cocktail secreted by *E. coli* containing one cellulase and one xylanase that contained around 415 carboxymethylcellulase units per liter (Fig. 3A).[5] An enhancement of enzymatic digestion was observed for all IL–Amberlyst samples treated for greater than 1 hour, with the finer fractions hydrolyzing more effectively than the coarser fractions. Switchgrass pretreated an hour or less with acid was resistant to hydrolysis by enzyme mixture CX2, and only 0.9% of biomass was converted to soluble sugars in these samples (Fig. 2B). Sugar yield was found to vary with the length of time the biomass was incubated with the Amberlyst resin, with an optimum of 3 hours when hydrolysed with CX2. The soluble glucan increased 16-fold and the total sugar yield increased nearly 10 times over [C_4mim]Cl pretreatment alone, with 7.6% of the initial weight as sugars being released. This is similar to yields with [C_2mim][OAc] treated switchgrass, which released 8.1% of its dry weight as sugars (Fig. 3B).

The use of cellulolytic enzymes contributes significantly to the cost of producing chemicals from lignocellulosic

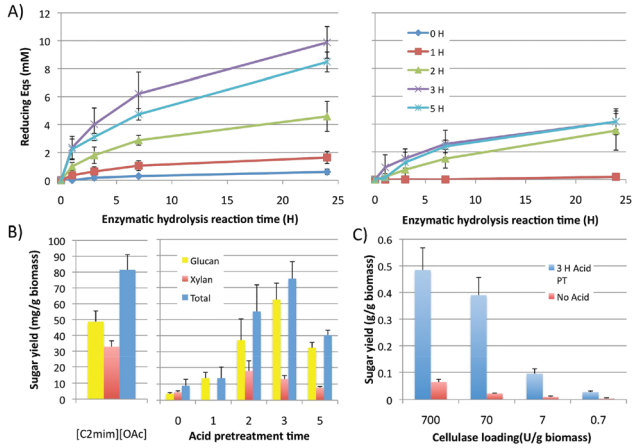

Fig. 3 Acid enhanced pretreatment of biomass improves enzymatic hydrolysis. (A) Saccharification of 1% w/w switchgrass pretreated in [C_4mim]Cl and acid for times ranging from 0 to 5 hours with cellulase cocktail CX2. At the indicated times, samples were removed and soluble sugars were assayed using DNS. The left graph show hydrolysis of the fine fraction, and the coarse fraction is on the right. (B) Total sugar yield per gram switchgrass after 24 hours from reactions shown in (A). (C) The combined fractions from the 3 hour (blue) and the 0 hour (red) acid pretreatment were saccharified with dilutions of Celluclast cellulase cocktail for 24 hours and the soluble sugar in each sample was measured.

biomass.[20] The previous experiment showed that biomass dissolved in IL and treated with an acid catalyst was significantly more amenable to enzymatic hydrolysis, and so less cellulase would be required to achieve the same rate and yield of saccharification. To quantify this, the fine and coarse fractions of switchgrass were combined and hydrolysed with Celluclast, a commercial cellulase cocktail. Four enzyme loadings from 700 U g^{-1} biomass to 0.7 U g^{-1} biomass were investigated (Fig. 3C). Samples incubated in IL with Amberlyst 15 yielded approximately seven fold more sugars than biomass pretreated with no acid saccharified with the same enzyme loadings. Incubation with the acid catalyst reduced enzyme requirements by 99%, with samples treated with acid requiring only 7 U g^{-1} cellulase to release 10% of their mass as sugar while untreated samples digested with 700 U g^{-1} yielded only 7% sugar. With all samples, this complex cellulase mixture was more efficient than CX2 alone, when saccharifying with similar cellulase loadings.

Acid enhancement appears beneficial for simple *in vitro* hydrolysis of biomass, but conversion of the released sugar into more useful chemicals is equally important. To demonstrate that biomass pretreated this way also increases the yields of downstream products, it was used as the substrate for consolidated bioprocessing (CBP), a process in which the organism(s) producing enzymes for lignocellulose depolymerization also convert the released sugars into some desired product.[6,21] This has the potential to substantially reduce biofuel costs as no separate saccharification step is necessary, and no additional enzymes are required. A recently developed strain of *E. coli*, which expresses four proteins that enable it to depolymerize and consume sugars from complex biomass, was used as a biocatalyst for CBP.[6] Initially these cellulolytic *E. coli* were grown in Mops minimal medium (MMM) with IL-pretreated switchgrass as the sole carbon source, so that production of free sugars from switchgrass is required for cell growth. Both the xylan and cellulose are more readily converted into monosaccharides from biomass processed using acid-enhanced IL pretreatment (AEILPT) compared to regular IL pretreatment, as can be seen from the growth of monocultures capable of consuming one fraction or the other. In a coculture of *E. coli* strains engineered with cellulose- or hemicellulose-degrading enzymes, the acid treated biomass was a much better substrate for growth, with a 1% switchgrass solution supporting the growth of 95.8×10^7 CFU mL^{-1} (Fig. 4B), 12 times more than the number of cells (7.8×10^7 CFU mL^{-1}) that grew on the conventionally treated biomass (Fig. 4A). Such a significant increase in cell growth on AEILPT biomass is not surprising considering the increased saccharification (Fig. 3A and 3B) in which the CX2 cellulase cocktail was also used.

To show that these cells could convert the liberated sugar into more valuable chemicals *via* CBP, free-fatty acids (FFA) were chosen as the target product because they are used by the pharmaceutical, cosmetics, and biofuel industries.[22] The cellulase and xylanase plasmids were cotransformed with plasmid pHatty, which harbors the thioesterase *tesA* and the fatty-acid-responsive transcription factor *fadR*, which in combination were shown to produce near theoretical yields of FFAs.[23] Yields were maximized by using *E. coli* cells with fatty acid β-oxidation eliminated through deletion of *fadE*, the second gene in this pathway. These strains were used to convert 1% switchgrass to FFA in MMM (Fig. S2 and S3†). The conversion of biomass pretreated with only [C_4mim]Cl was 6 mg L^{-1}, or a yield of 0.24% (Fig. 4C). Enhancing biomass pretreatment with

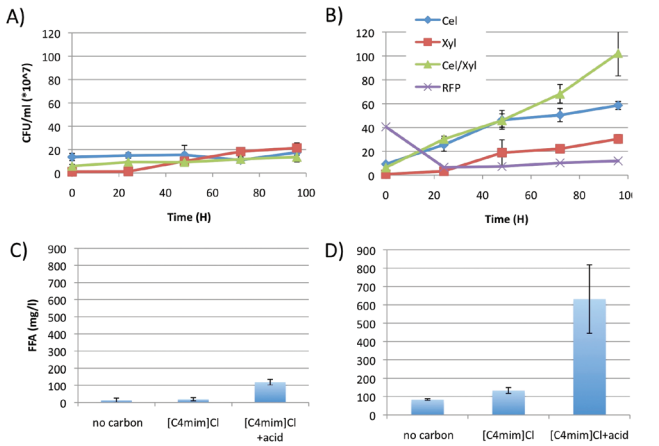

Fig. 4 Utilization of AEILPT switchgrass as carbon source for growth and production of chemicals. All experiments were performed in MMM + 1% pretreated swtichgrass. (A) and (B) Growth of cellulytic *E. coli* transformed with pCellulose, which conferred ability to grow on cellulose (Cel), or pXylan which conferred the ability to grow on xylan (Xyl); Cel/Xyl is a co-culture with both strains and RFP is the negative control which expresses Red fluorescent protein. (A) Growth on biomass from the regular pretreatment. (B) Growth on biomass from 3 H acid treatment. (C) FFA production through CBP. Cells cotranformed with fatty acid producing plasmid pHatty and pCellulose or pXylan were mixed to form a co-culture capable of converting both cellulose and xylan to FFA. (D) FFA production with SSF. *E. coli* transformed with pHatty was used to produce fatty acids in switchgrass/MMM supplemented with 70 U cellulase g^{-1} biomass.

acid increased conversion 18 times to 107 mg L^{-1} FFA, a yield of 4.3%, an increase similar to the growth assays above.

Although CBP is a promising route for low-cost biomaterial production, the yields are currently too low for industrial applications. Simultaneous saccharification and fermentation (SSF) is a related mode of biorefinery operation in which cellulases are added from an externally produced source.[24] This removes the additional burden of producing cellulase from an organism already producing the enzymatic pathway to convert sugar into fuel. To determine whether AEILPT would also increase the titers of biofuel produced during SSF, *E. coli* cells transformed with only pHatty were used to produce FFA from pretreated switchgrass. This strain was grown in MMM supplemented with 1% pretreated switchgrass and Celluclast cellulase cocktail at 70 U g^{-1} biomass (Fig. 4D). Standard pretreatment with $[C_4mim]Cl$ and no acid lead to FFA production of 49 mg L^{-1} from biomass, a 2.0% yield. From biomass pretreated with acid and $[C_4mim]Cl$, titers increased to 548 mg L^{-1}, nearly 22% of theoretical yield. It is worth noting that Celluclast functions optimally at pH 5.0 and 50 °C, and a cellulase blend that functioned better at temperatures and pHs compatible with *E. coli* growth would potentially improve yields even more.

These studies demonstrate that incorporating acid catalyzed hydrolysis into IL pretreatment significantly enhances the performance of a relatively poor, but inexpensive, pretreatment solvent. Acid enhanced pretreatment lead to higher yields of sugar from the model lignocellulosic crop, switchgrass, and reduced the cellulase loading necessary for saccharification. Enhanced saccharification leads to higher bioconversion using both simultaneous saccharification and fermentation and consolidated bioprocessing. This general strategy should work with other ILs with strongly acidic anions such as halides, phosphonates, and sulfonates. The use of inexpensive ILs for pretreatment, a decrease in the cellulase loading required for saccharification, and higher bioconversion yields will be important for development of lignocellulosic material as a replacement feedstock for petroleum.

Acknowledgements

This work conducted by the Joint BioEnergy Institute was supported by the Office of Science, Office of Biological and Environmental Research, of the U.S. Department of Energy under Contract No. DE-AC02-05CH11231.

References

1 S. Davis, S. Diegel and R. Boundy, *Transportation Energy Data Book*, ORNL, Oak Ridge, 2011, vol. 30.
2 M. E. Himmel, S.-Y. Ding, D. K. Johnson, W. S. Adney, M. R. Nimlos, J. W. Brady and T. D. Foust, *Science*, 2007, **315**, 804–807.
3 D. Groff, P. I. Benke, T. S. Batth, G. Bokinsky, C. J. Petzold, P. D. Adams and J. D. Keasling, *Appl. Environ. Microbiol.*, 2012, **78**, 2221–2229.
4 (*a*) Y. Nishiyama, P. Langan and H. Chanzy, *J. Am. Chem. Soc.*, 2002, **124**, 9074–9082; (*b*) G. Cheng, P. Varanasi, C. Li, H. Liu, Y. B. Melnichenko, B. A. Simmons, M. S. Kent and S. Singh, *Biomacromolecules*, 2011, **12**, 933–941.
5 M. E. Zakrzewska, E. Bogel-Lukasik and R. Bogel-Lukasik, *Energy Fuels*, 2010, **24**, 737–745.
6 G. Bokinsky, P. P. Peralta-Yahya, A. George, B. M. Holmes, E. J. Steen, J. Dietrich, T. Soon Lee, D. Tullman-Ercek, C. A. Voigt, B. A. Simmons and J. D. Keasling, *Proc. Natl. Acad. Sci. U. S. A.*, 2011, **108**, 19949–19954.
7 C. Li, B. Knierim, C. Manisseri, R. Arora, H. V. Scheller, M. Auer, K. P. Vogel, B. A. Simmons and S. Singh, *Bioresour. Technol.*, 2010, **101**, 4900–4906.
8 T. D. Landry, K. Brooks, D. Poche and M. Woolhiser, *Bull. Environ. Contam. Toxicol.*, 2005, **74**, 559–565.
9 M. Zavrel, D. Bross, M. Funke, J. Buchs and A. C. Spiess, *Bioresour. Technol.*, 2009, **100**, 2580–2587.
10 D. Klein-Marcuschamer, B. A. Simmons and H. W. Blanch, *Biofuels, Bioproducts Biorefining*, 2011, **5**, 562–569.
11 H. Tadesse and R. Luque, *Energy Environ. Sci.*, 2011, **4**, 3913–3929.
12 A. Sant'Ana da Silva, S. H. Lee, T. Endo and E. P. Bon, *Bioresour. Technol.*, 2011, **102**, 10505–10509.
13 J. Fernández, R. Bartel, U. Bottin-Weber, S. Stolte and J. Thoming, *J. Membr. Sci. Technol.*, 2011, **4**, S-4.
14 P. Lozano, B. Bernal, I. Recio and M. P. Belleville, *Green Chem.*, 2012, **14**, 2631–2637.
15 X. Peng, Y. Hu, Y. Liu, C. Jin and H. Lin, *J. Nat. Gas Chem.*, 2010, **19**, 81–85.
16 H. Yu, J. Hu, J. Fan and J. Chang, *Ind. Eng. Chem. Res.*, 2012, **51**, 3452–3457.
17 R. Rinaldi, R. Palkovits and F. Schuth, *Angew. Chem., Int. Ed.*, 2008, **47**, 8047–8050.
18 R. Rinaldi, P. Engel, J. Buchs, A. C. Spiess and F. Schuth, *ChemSusChem*, 2010, **3**, 1151–1153.
19 M. R. Schmer, K. P. Vogel, R. B. Mitchell and R. K. Perrin, *Proc. Natl. Acad. Sci. U. S. A.*, 2008, **105**, 464–469.
20 D. Klein-Marcuschamer, P. Oleskowicz-Popiel, B. A. Simmons and H. W. Blanch, *Biotechnol. Bioeng.*, 2012, **109**, 1083–1087.
21 L. R. Lynd, P. J. Weimer, W. H. van Zyl and I. S. Pretorius, *Microbiol. Mol. Biol. Rev.*, 2002, **66**, 506–577.
22 E. J. Steen, Y. Kang, G. Bokinsky, Z. Hu, A. Schirmer, A. McClure, S. B. Del Cardayre and J. D. Keasling, *Nature*, 2010, **463**, 559–562.
23 F. Zhang, J. M. Carothers and J. D. Keasling, *Nat. Biotechnol.*, 2012, **30**, 354–359.
24 G. P. Philippidis, T. K. Smith and C. E. Wyman, *Biotechnol. Bioeng.*, 1993, **41**, 846–853.

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 (≥ 98 %, CAS: 79917-90-1) was purchased from Sigma-Aldrich and heated to 80°C to allow handling. Amberlyst 15DRY (moisture ≤ 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 $[C_4C_1im]Cl$ 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 Amberylst 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 H_2O 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-µm 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 H_2SO_4 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 µg/ml carbenicillin. They were diluted 1:20 into fresh LB with 100 µg/ml carbenicillin and production of the cellulase and xylanase were induced at OD_{600} = 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 Eppindorf 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% H_2SO_4 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:

(Saccharification yield of course fraction)*(Weight of coarse fraction recovered)+

(Saccharification yield of fine fraction)*(Weight of fine fraction recovered)

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 Datsanko 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

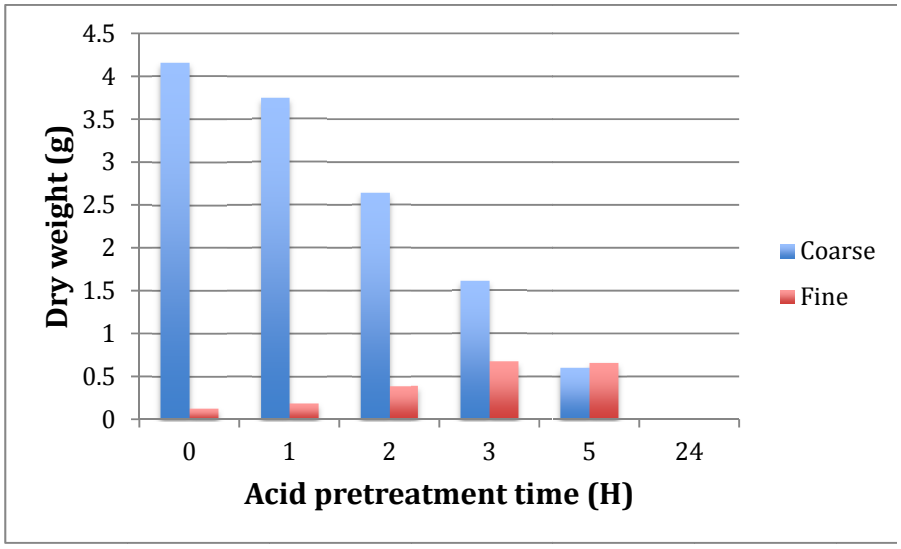

Fig. S1 Dry weight recovered in each fraction after acid enhanced ionic liquid pretreatment

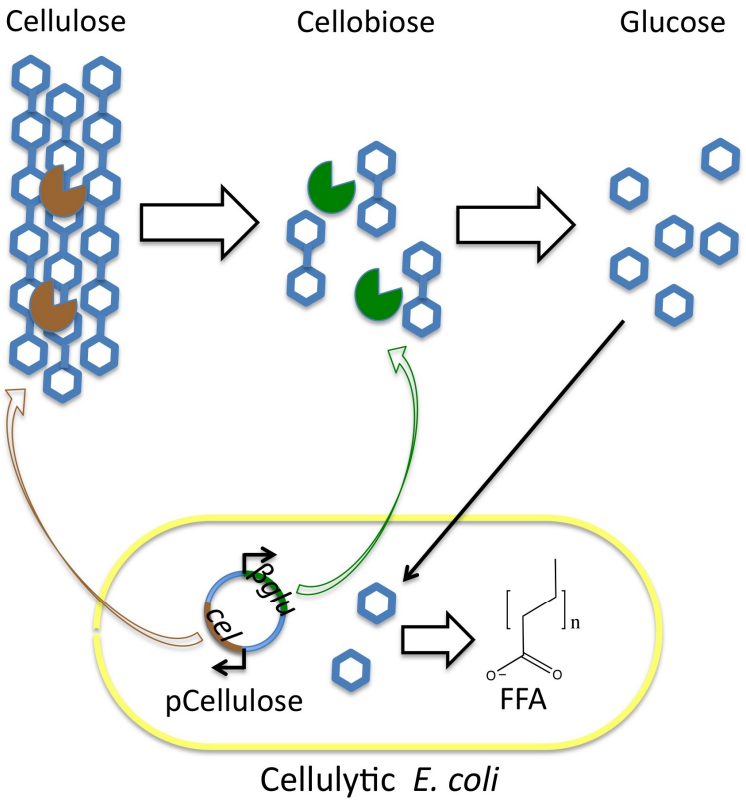

Fig. S2 Cellulytic *E. coli*. Cellulose is hydrolyzed by cellulase *cel* to cellobiose, which is hydrolyzed to glucose monomers by β-glucosidase *cel3A*. Glucose can then be imported into the cell and enter central carbon metabolism.

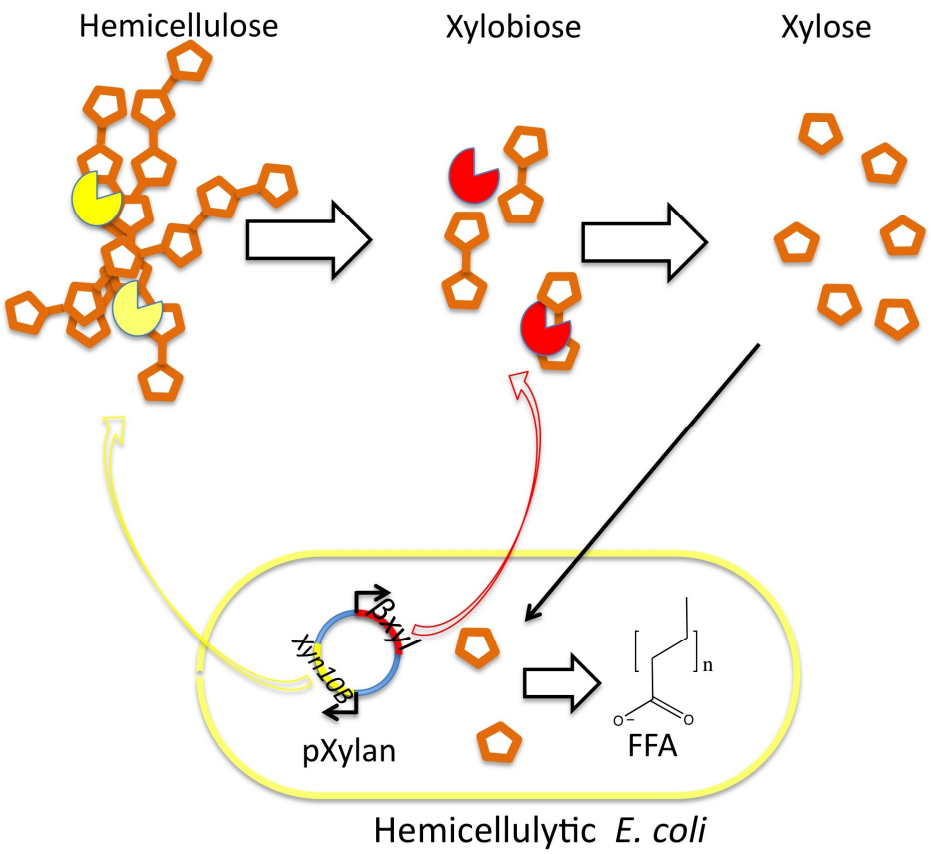

Fig. S3 Hemicellulytic *E. coli*. Xylan is hydrolyzed by xylanase *Xyn10B* to xylobiose, which is hydrolyzed to xylose monomers by β-xylobiosidase *gly43F*. Xylose can then be imported into the cell and enter central carbon metabolism.

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.