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Cellulose solvent- and organic solvent-based lignocellulose fractionation enabled efficient sugar release from a variety of lignocellulosic feedstocks

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HIGHLIGHTS

- COSLIF can effectively pretreat numerous feedstocks.
- Glucan digestibilities of most feedstocks were ~93% at a low cellulase loading.
- COSLIF could be regarded as feedstock-independent biomass pretreatment.
- Feedstock-independent fractionation would be vital to success of biorefineries.

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ABSTRACT

Developing feedstock-independent biomass pretreatment would be vital to second generation biorefineries that would fully utilize diverse non-food lignocellulosic biomass resources, decrease transportation costs of low energy density feedstock, and conserve natural biodiversity. Cellulose solvent- and organic solvent-based lignocellulose fractionation (COSLIF) was applied to a variety of feedstocks, including Miscanthus, poplar, their mixture, bagasse, wheat straw, and rice straw. Although non-pretreated biomass samples exhibited a large variation in enzymatic digestibility, the COSLIF-pretreated biomass samples exhibited similar high enzymatic glucan digestibilities and fast hydrolysis rates. Glucan digestibilities of most pretreated feedstocks were ~93% at five filter paper units per gram of glucan. The overall glucose and xylose yields for the Miscanthus:poplar mixture at a weight ratio of 1:2 were 93% and 85%, respectively. These results suggested that COSLIF could be regarded as a feedstock-independent pretreatment suitable for processing diverse feedstocks by adjusting pretreatment residence time only.

1. Introduction

The production of biofuels and value-added biochemicals from renewable abundant non-food lignocellulosic biomass would bring benefits to the environment, rural economy, and national security. Additionally, it would create a large number of new biomanufacturing jobs, which cannot be outsourced, because of high transportation costs for lower energy density biomass feedstocks as compared to crude oil, coal, and corn kernels (Judd et al., 2012; Zhang, 2011). The largest technical and economical obstacle to second generation biorefineries is cost-effective release of fermentable sugars from lignocellulosic biomass (Lynd et al., 2008; Rollin et al., 2011; Zhang, 2011).

Miscanthus x giganteus (briefly called Miscanthus) and *Populus nigra x Populus maximowiczii* (hybrid poplar) are regarded as promising bioenergy crops because they have high productivities and low requirements for plantation. Miscanthus is a perennial C4 grass, featuring a long production lifetime (e.g., 10–15 years) (Wang et al., 2010). Extensive trials in Europe result in an average biomass productivity, more than 30 dry metric tons per hectare per year with minimal agricultural inputs, much higher than an average yield of 10–15 tons per hectare per year of switchgrass (Heaton et al., 2004; Khanna et al., 2008; Miguez et al., 2009; Somerville et al., 2010). Poplar and their hybrids are fast-growing and short-rotation woody crops, which can be grown in marginal lands with a mean above-ground biomass productivity of ~14 dry metric tons per hectare per year (Sannigrahi et al., 2010). Since hybrid poplar has a wide spatial distribution in North America and Canada, it can be grown close to biorefineries. Moreover, woody biomass, such as poplar, has several advantages compared to agricultural

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residues and bioenergy grass crops, such as high polysaccharide contents (i.e., 40–50% glucan and 20–30% xylan) (Moxley and Zhang, 2007) and higher mass density, rendering lower transportation cost (Balan et al., 2009).

Numerous pretreatment technologies, such as dilute acid, steam explosion, ammonia fiber explosion (AFEX), aqueous ammonia recycle percolation (ARP), and lime, have shown to be effective to pretreat herbaceous biomass (i.e., corn stover and switchgrass) (Mosier et al., 2005; Rollin et al., 2011; Wyman et al., 2005, 2009). However, most pretreatments are ineffective for woody biomass. For example, enzymatic glucan digestibilities of dilute acid-, AFEX-, and ARP-pretreated poplar were 47%, 39%, and 36%, respectively, at an enzyme loading of 15 filter paper units (FPUs) of cellulase per gram of glucan (Balan et al., 2009; Wyman et al., 2009). These low enzymatic digestibilities may be due to more recalcitrant structure and higher lignin contents. With consideration of diverse feedstocks in different regions and a large variety in feedstock quality due to growth conditions, harvesting seasons, and storage conditions, developing feedstock-independent pretreatment without significant changes in pretreatment conditions would be of importance to implement large-scale second generation biorefineries. Additionally, the utilization of mixed feedstocks in biorefineries would decrease feedstock logistic hurdles and maintain biodiversity.

Cellulose solvent- and organic solvent-based lignocellulose fractionation (COSLIF) has been developed to fractionate lignocellulose by using a combination of a concentrated phosphoric acid as a cellulose solvent and an organic solvent (e.g., acetone or ethanol) under modest reaction conditions (Rollin et al., 2011; Zhang et al., 2007). COSLIF has been demonstrated to efficiently pretreat several feedstocks, such as bamboo (Sathitsuksanoh et al., 2010), common reed (Li et al., 2009; Sathitsuksanoh et al., 2009), hemp hurd (Moxley et al., 2008), corn stover (Zhu et al., 2009), bermudagrass (Li et al., 2009), switchgrass (Sathitsuksanoh et al., 2011), gamagrass (Ge et al., 2012), giant reed, elephant grass, and sugarcane (Ge et al., 2011). Because concentrated phosphoric acid as a cellulose solvent can dissolve cellulose fibers, resulting in effective disruption of highly ordered hydrogen bonding network of crystalline cellulose (Conte et al., 2009; Sathitsuksanoh et al., 2011) and drastic increases in cellulose accessibility to cellulase (CAC) (Rollin et al., 2011; Zhu et al., 2009).

The goal of this study was to examine pretreatment efficiency of COSLIF on Miscanthus, hybrid poplar, and their mixtures at various mass ratios, bagasse, wheat straw, and rice straw by adjusting the pretreatment time at the same temperature and the same biomass to phosphoric acid ratio.

2. Methods

2.1. Chemicals and materials

All chemicals were reagent grade and purchased from Sigma–Aldrich (St. Louis, MO), unless otherwise noted. Phosphoric acid (85% w/w) and ethanol (95% v/v) were purchased from Fisher Scientific (Houston, TX). Microcrystalline cellulose, Avicel PH105 (20 μm) was obtained from FMC Corp (Philadelphia, PA). Regenerated amorphous cellulose (RAC) was prepared through a series of steps: Avicel slurrying in water, cellulose dissolution in concentrated phosphoric acid, and cellulose regeneration in water (Rollin et al., 2011). The *Trichoderma reesei* cellulase (Novozyme® 50013) and β-glucosidase (Novozyme® 50010) were gifted by Novozymes North America (Franklinton, NC). They had activities of 84 filter paper units (FPUs) of cellulase per mL and 270 units of β-glucosidase per mL. Corn stover, hybrid poplar, wheat straw, and alamo switchgrass (*Panicum virgatum*) were procured from the National Renewable Energy Laboratory (Boulder, CO). *M. giganteus* sample was procured from University of Illinois (Urbana, IL). Industrial hemp stalks, provided by the Equator Group (Los Angeles, CA), were grown in Canada. The hemp hurds were obtained after manual removal of the fiber of the industrial hemp stems (Moxley et al., 2008). Common reed (*Phragmites australis*) was obtained from the U.S. Army Edgewood Chemical Biological Center (Aberdeen, MD) (Sathitsuksanoh et al., 2009). Bamboo, rice straw, and bagasse samples were procured from the Industrial Technology Research Institute (Taiwan). The moso bamboo was grown in Taiwan and the full-size culm with around a half- to one-year age was harvested and then dried naturally (Sathitsuksanoh et al., 2010). All naturally-dried biomass samples were milled into small particles by a Pallmann counter-rotating knife ring flaker (Clifton, NJ). The resulting particulates with nominal sizes of 40–60 mesh (250–400 μm) were used for all pretreatment experiments. All milled lignocellulosic samples were kept at −20 °C until pretreatment.

2.2. Carbohydrate and lignin assays

The carbohydrate composition of biomass and residual biomass after hydrolysis was determined with a modified quantitative saccharification (QS) procedure (Moxley and Zhang, 2007). In the modified QS, secondary hydrolysis was conducted in the presence of 1% (w/w) sulfuric acid at 121 °C for 1 h to more accurately determine the quantities of sugars susceptible to acid degradation (e.g., xylan). After $CaCO_3$ neutralization and centrifugation, monomeric sugars in the supernatant were measured with a Shimadzu HPLC equipped with a Bio-Rad Aminex HPX-87P column (Richmond, CA) at a rate of 0.6 mL of deionized water per min at 60 °C (Moxley and Zhang, 2007). The standard NREL biomass protocol was used to measure lignin and ash (Sluiter et al., 2008). In brief, 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 h. Percent acid-soluble lignin in the sample was determined by measuring the UV absorption of the acid hydrolysis supernatant at 320 nm. All carbohydrate and lignin assays were conducted in triplicate.

2.3. COSLIF procedure

The COSLIF was prepared as described previously (Rollin et al., 2011; Sathitsuksanoh et al., 2009). In short, approximately 1.05 g of naturally-dry biomass with a moisture content of approximately 5% was mixed with 8 mL of 85% (w/w) H_3PO_4 in a 50 mL plastic centrifuge tube at 50 °C and 1 atm for 60 min, unless otherwise noted. The pretreatment was ceased by adding 20 mL of 95% (v/v) ethanol and then mixed well. Solid–liquid separation was conducted in a swing bucket centrifuge at 4500 rpm at room temperature for 10 min. After the supernatant was removed, the pellets were suspended in 40 mL of 95% (v/v) ethanol. After centrifugation, the solid pellets were washed by 40 mL of deionized water two times. After centrifugation, the remaining solid pellets were neutralized by 2 M sodium carbonate. The pretreated wet biomass was stored in the presence of 0.1% (w/v) NaN_3 at 4 °C prior to enzymatic hydrolysis.

2.4. Enzymatic hydrolysis

The COSLIF-pretreated samples were diluted to 10 g glucan per liter in a 50 mM sodium citrate buffer (pH 4.8) supplemented with 0.1% (w/v) NaN_3, which prevented the growth of microorganisms. COSLIF-pretreated samples were completely suspended in a rotary shaker at 250 rpm at 50 °C. The enzyme loadings were 5 FPUs per

Table 1
Compositional analysis of new tested feedstocks.

Compositions (wt.%)	Miscanthus	Hybrid poplar	Bagasse	Wheat straw	Rice straw
Before pretreatment					
Glucan	41.00 ± 0.09	40.13 ± 0.43	31.81 ± 0.53	32.36 ± 0.13	27.93 ± 0.20
Xylan	18.42 ± 0.05	11.95 ± 0.13	13.28 ± 0.13	18.23 ± 0.09	13.86 ± 0.14
Galactan	ND	1.26 ± 0.01	0.218 ± 0.001	ND	1.99 ± 0.13
Arabinan	2.08 ± 0.01	1.12 ± 0.20	2.50 ± 0.10	2.676 ± 0.001	2.86 ± 0.02
Mannan	ND	3.33 ± 0.01	1.38 ± 0.09	ND	ND
Lignin	23.10 ± 0.20	28.1 ± 2.11	14.96 ± 0.09	17.72 ± 1.37	24.53 ± 0.10
After pretreatment					
Glucan	55.54 ± 1.20	58.79 ± 0.84	43.50 ± 2.02	51.20 ± 0.35	39.41 ± 1.72
XMG[a]	12.39 ± 0.85	5.70 ± 0.21	8.18 ± 0.48	6.97 ± 0.24	7.05 ± 0.34
Lignin	26.24 ± 0.01	23.59 ± 0.03	20.81 ± 0.02	16.62 ± 0.03	16.61 ± 0.01

ND indicates not detected.
[a] XGM, xylan, mannan, and galactan combined.

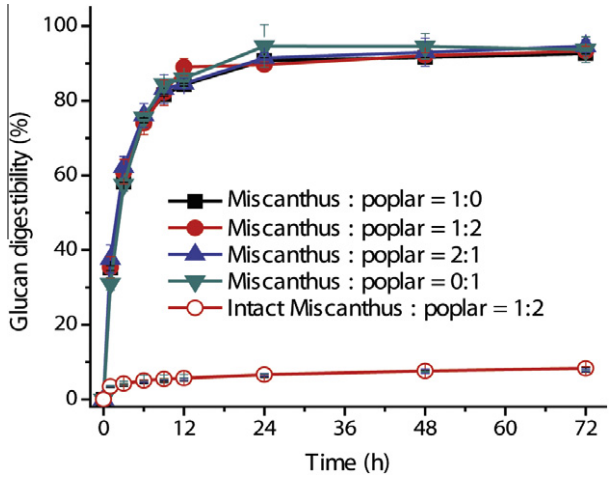

Fig. 1. Enzymatic hydrolysis profiles of COSLIF-pretreated biomass mixtures at the enzyme loading of 5 FPUs of cellulase and 10 units of β-glucosidase per gram of glucan at 50 °C. Enzymatic hydrolysis of non-pretreated biomass at 15 FPUs of cellulase per gram of glucan exhibited similar hydrolysis profiles. For simplification, the hydrolysis profile of a Miscanthus: poplar ratio of 1:2 was shown only.

gram of glucan and 10 units of β-glucosidase per gram of glucan. Eight hundred microliters of well-mixed hydrolysate were removed, followed by immediate centrifugation at 13,000 rpm for 5 min. Exactly 500 μL of the supernatant was transferred to another micro-centrifuge tube and stayed at room temperature for 30 min, to allow the conversion of all cellobiose to glucose. The

Table 2
Total surface accessibility to cellulase (TSAC), cellulose accessibility to cellulase (CAC), and glucan digestibility after 72 h under 5 FPUs of cellulase and 10 units of β-glucosidase per gram of glucan.

Substrate	TSAC (m^2/g biomass)	CAC (m^2/g biomass)	Glucan digestibility (%)
Intact Miscanthus	0.18 ± 0.01	0.087 ± 0.001	7.9 ± 0.6
Intact poplar	0.23 ± 0.01	0.14 ± 0.01	7.8 ± 0.0
Miscanthus:poplar = 1:0	20.7 ± 1.2	18.9 ± 1.7	92.6 ± 0.0
Miscanthus:poplar = 1:2	16.8 ± 2.2	15.0 ± 1.2	93.3 ± 1.3
Miscanthus:poplar = 2:1	17.1 ± 1.3	15.7 ± 1.1	92.6 ± 1.7
Miscanthus:poplar = 0:1	18.2 ± 1.1	17.4 ± 0.9	93.7 ± 3.4

supernatant was then acidified by adding 30 μL of 10% (w/w) sulfuric acid, followed by freezing overnight. The frozen samples were thawed, mixed well, and then centrifuged at 13,000 rpm for 5 min, to remove any precipitated solid sediments. The soluble glucose and xylose in the enzymatic hydrolysate were measured by HPLC equipped with a Bio-Rad HPX-87H column at a rate of 0.6 mL of 0.1% v/v sulfuric acid per min at 60 °C (Zhang et al., 2007). Galactose and mannose co-eluted with xylose. After 72 h hydrolysis, the remaining hydrolysate was transferred to a 50 mL centrifuge tube, centrifuged at 4500 rpm for 15 min, and soluble sugar content was determined using the same procedure as other hydrolysate samples, as described above. After all remaining hydrolysate was decanted, and the pellets were resuspended in 20 mL of water and centrifuged to remove residual soluble sugars from the pellets. The sugar content of the washed pellets was determined by modified QS as described above. Enzymatic glucan digestibility after 72 h was calculated using the ratio of soluble

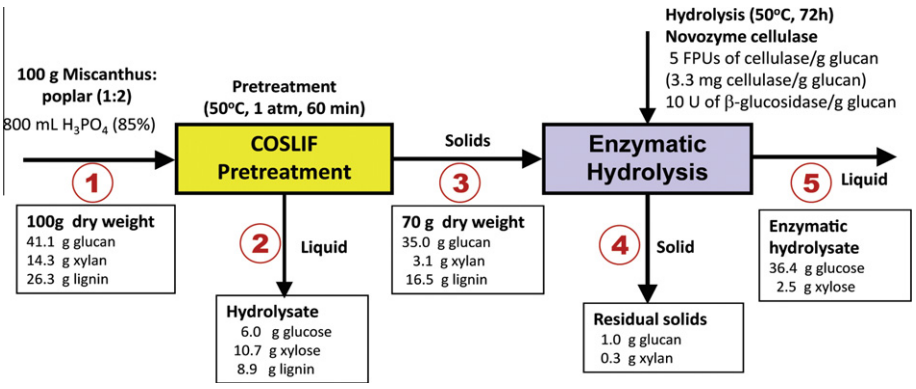

Fig. 2. Mass balance of the biomass mixture at a Miscanthus:poplar = 1:2 pretreated by COSLIF followed by enzymatic hydrolysis by 5 FPUs of cellulase per gram of glucan.

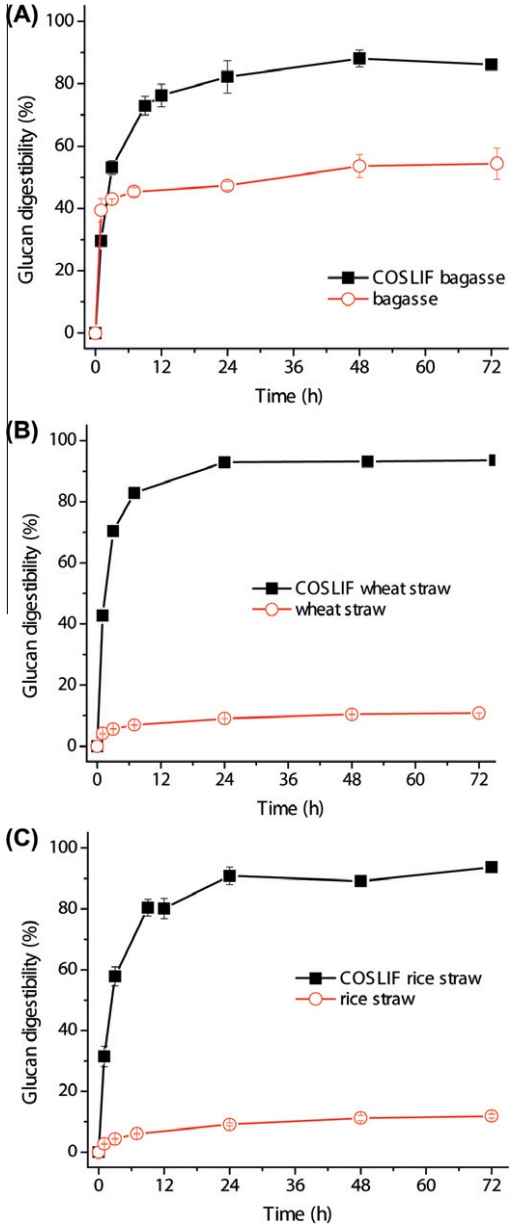

Fig. 3. Enzymatic hydrolysis profiles of COSLIF-pretreated bagasse (A), wheat straw (B), and rice straw (C), COSLIF pretreatment conditions were 50 °C, atmospheric pressure, and pretreatment temperature of 45 min for wheat straw as well as 30 min for bagasse and rice straw.

glucose in the supernatant to the sum of this soluble glucose and the glucose equivalent of the residual glucan (Rollin et al., 2011; Zhang et al., 2007).

2.5. Other assays

The total substrate accessibility to cellulase (TSAC), cellulose accessibility to cellulase (CAC), and non-cellulose accessibility to cellulase (NCAC) were determined based on the maximum adsorption capacity of the TGC protein containing a green fluorescent protein and a family 3 cellulose-binding module in the presence or absence of bovine serum albumin (Rollin et al., 2011; Zhu et al., 2009). TGC fusion protein was produced in *Escherichia coli* BL21 (pNT02), purified by adsorption onto regenerated amorphous cellulose (RAC), and desorbed with ethylene glycol (EG) (Hong et al., 2008). EG was then removed through dialysis in a 50 mM sodium citrate buffer (pH 6.0) and the TGC solution was concentrated using the Millipore 10,000 Da molecular weight cut-off centrifugal ultrafilter columns (Billerica, MA).

3. Results and discussion

Previous COSLIF studies suggested that (i) phosphoric acid only above a critical concentration (83%) can efficiently disrupt recalcitrant lignocellulose structures (Moxley et al., 2008); (ii) the best pretreatment judged based on a maximal sugar release: a combinatorial result of a maximal retention of solid cellulose and a maximal enzymatic cellulose hydrolysis (Sathitsuksanoh et al., 2009); (iii) enzymatic hydrolysis of pretreated biomass can be conducted at five filter paper units per glucan (Sathitsuksanoh et al., 2010), a third of typical enzyme loading for most pretreated biomass (Balan et al., 2009; Wyman et al., 2009); and (iv) naturally dry biomass with low moisture contents can be pretreated by concentrated phosphoric acid directly (Sathitsuksanoh et al., 2009). Also concentrated phosphoric acid has dual functions: a cellulose solvent for disrupting recalcitrant biomass structure and an acid for depolymerizing polysaccharides and even degrading sugars. It was found that the first function was dominant at low temperatures (e.g., $\leqslant$50 °C). In contrast, its second function became stronger when reaction temperature increased. Therefore, the optimal COSLIF pretreatment temperature was around 50 °C (Sathitsuksanoh et al., 2009) while pretreatment time could change depending on biomass type.

The carbohydrate and lignin compositions of Miscanthus and poplar samples are shown in Table 1. Two feedstocks have comparable overall carbohydrate contents but differ in carbohydrate compositions. For example, Miscanthus did not contain detectable mannan while poplar contained 3.33 wt.% mannan. Also, poplar contained a lignin content of 28 wt.%, higher than Miscanthus (i.e., 23 wt.%). In this study, Miscanthus and poplar samples were mixed at four ratios, i.e., 1:0, 1:2, 2:1, and 0:1. Non-pretreated biomass samples regardless of their ratios showed similar hydrolysis profile with a glucan digestibility of 8% after 72 h of enzymatic hydrolysis at an enzyme loading of 15 FPUs per gram of glucan. Non-pretreated Miscanthus: poplar at a ratio of 1:2 was shown as a representative (Fig. 1). COSLIF-pretreated biomass mixture samples at four ratios were hydrolyzed at the enzyme loading of 5 FPUs of cellulase and 10 units of β-glucosidase per gram of glucan (Fig. 1). It was found that the optimal reaction time for poplar and Miscanthus was 60 min at 50 °C. All four COSLIF-pretreated biomass mixtures had similar hydrolysis profiles (Fig. 1). The pretreated biomass mixtures were hydrolyzed fast, and 50% of substrates were hydrolyzed after 3 h. The glucan digestibilities were ~90% after 24 h and ~93% after 72 h, suggesting efficient enzymatic hydrolysis of COSLIF-pretreated biomass regardless of their ratios at a low enzyme loading. Different lignin contents in Miscanthus and poplar did not show significant influences on digestibility, in agreement with previous discovery that decreasing lignin content in feedstock was not important for enhanced glucan digestibility when cellulose accessibility to cellulase was increased greatly by using the cellulose solvent (Rollin et al., 2011).

Mass balance on the basis of 100 g of dry biomass at Miscanthus: poplar = 1:2, including COSLIF pretreatment followed by enzymatic hydrolysis, is shown in Fig. 2. After COSLIF, 6.0 g of soluble glucose equivalent and 10.7 g of soluble xylose equivalent were removed. The reactive cellulose material was hydrolyzed by the commercial fungal cellulase containing hemicellulase activity, releasing 36.4 g of soluble glucose and 2.5 g of soluble xylose equivalent. The overall glucose and xylose yields were 92.8% and 84.7%, respectively.

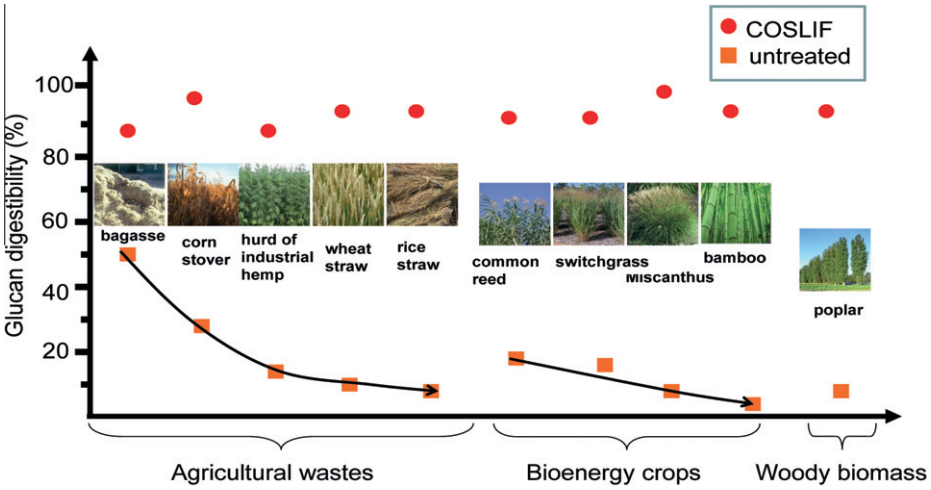

Fig. 4. COSLIF appeared to be a feedstock-independent technology. All biomass feedstocks were pretreated by COSLIF at 50 °C and atmospheric pressure with a reaction time of 30 min for bagasse, corn stover, and rice straw; of 45 min for wheat straw, switchgrass, and hurd of industrial hemp; and of 60 min for common reed, Miscanthus, bamboo, and poplar.

High glucan digestibility of pretreated biomass was attributed to drastic changes in supramolecular structure of biomass before and after COSLIF pretreatment, examined by scanning electron microscope (Moxley et al., 2008; Zhu et al., 2009) (data not shown). After COSLIF, highly ordered hydrogen bonding network of crystalline cellulose fibers was disrupted, resulting in a drastic increase in CAC. Total substrate accessibility to cellulase (TSAC) increased from 0.21 (i.e., 0.18∗1/3 + 0.23∗2/3) to 16.8 m^2 per gram of biomass at Miscanthus: poplar = 1:2 (Table 2). The CAC values of intact Miscanthus and poplar were 0.09 and 0.14 m^2 per gram of biomass, respectively. After COSLIF, the CAC value of pretreated biomass at Miscanthus: poplar = 1:2 was 14.99 m^2 per gram of biomass. COSLIF enhanced CAC by ~125 fold, resulting in highly reactive cellulosic materials suitable for enzymatic cellulose hydrolysis at a low enzyme loading.

In addition to Miscanthus, poplar, and their mixtures, COSLIF was applied to three other feedstocks: bagasse, wheat straw, and rice straw. Their carbohydrate and lignin contents before and after COSLIF are shown in Table 1. These intact feedstocks exhibited different enzymatic hydrolysis profiles, indicating their different degrees of recalcitrance. Non-pretreated rice straw and wheat straw had low glucan digestibilities (<10%) after 72 h at 15 FPUs of cellulase per gram of glucan (Fig. 3A and B). In contrast, non-pretreated bagasse had a very high glucan digestibility of ~47% (Fig. 3C). High digestibility of bagasse may be due to leaching that removed as much as sucrose from freshly-harvested sugar cane, where leaching, drying, followed by milling may disrupt biomass fiber more efficiently than other non-pretreated feedstocks. It was found that the optimal pretreatment times for bagasse, wheat straw, and rice straw were 30, 45, and 30 min, respectively, shorter than those of Miscanthus and poplar. Regardless of large differences in enzymatic glucan digestibility of non-pretreated biomass, the three COSLIF-pretreated biomass samples showed similar hydrolysis profiles and comparatively high glucan digestibilities, i.e., 85–90% after 24 h enzymatic hydrolysis (Fig. 3). These results suggested that COSLIF converted different recalcitrant biomass feedstocks to the same substrate reactivity because pretreated biomass through dissolution of the cellulose solvent and regeneration had similar substrate properties.

Lignocellulosic biomass feedstocks could be classified to agricultural wastes, bioenergy crops, and woody biomass (Fig. 4). Different species of non-pretreated biomass feedstocks showed a large variation of their glucan digestibilities at 15 FPUs of cellulase per gram of glucan. Agricultural wastes showed a decreasing order in the recalcitrance to enzymatic hydrolysis: bagasse (47%) > corn stover (23%) > hurd of industrial hemp (14%) > wheat straw (11%) > rice straw (10%). Compared to agricultural wastes, bioenergy crops had lower enzymatic glucan digestibilities in a descending order of common reed (19%) > switchgrass (17%) > Miscanthus (8%) > bamboo (3%). Non-pretreated poplar had a glucan digestibility of ~7%. Although different feedstocks had different glucan digestibilities, reflecting their different recalcitrant degrees, all of the COSLIF-pretreated biomass feedstocks had similar high digestibilities (>87%) after 72 h at an enzyme loading of 5 FPUs of cellulase per gram of glucan. Clearly, concentrated phosphoric acid as a good cellulose solvent effectively enabled the dissolution of cellulose fibers, greatly increased substrate accessibility, and mitigated the disparity of biomass recalcitrance for different feedstock. Therefore, COSLIF could be regarded as a "nearly" feedstock-independent pretreatment.

Typical COSLIF pretreatment conditions were 50 °C and atmospheric pressure with a pretreatment time from 30 to 60 min – depending on the type of feedstocks. Although different intact feedstocks showed great variations in enzymatic digestibility (Fig. 4), suggesting their different recalcitrant structures resistant to hydrolytic enzymes, the use of concentrated phosphoric acid at 50 °C can efficiently dissolve them so to erase their inherent structure difference and result in amorphous biomass with similar high-accessibility (Table 2) (Rollin et al., 2011; Sathitsuksanoh et al., 2011). As a result, COSLIF-pretreated biomass feedstocks exhibited similar enzymatic glucan digestibility regardless of their sources (Fig. 4). When concentrated phosphoric acid is used as the cellulose solvent, it should be used at 50 °C or lower for avoiding extensive hydrolysis of polymeric carbohydrates and sugar degradation. Under these conditions concentrated phosphoric acid mainly works as a cellulose solvent to dissolve cellulose rather than as an acid (e.g., fuming HCl or concentrated sulfuric acid) to hydrolyze cellulose and hemicellulose to oligomeric and monomeric sugars.

4. Conclusions

COSLIF effectively pretreated a variety of feedstocks from herbaceous to wood because the cellulose solvent (concentrated phosphoric acid) under low temperature can dissolve biomass

regardless of their significantly different structures and compositions and generate highly reactive amorphous cellulose. The pretreated biomass feedstocks yielded high enzymatic glucan digestibilities, which were attributed to high substrate accessibility to cellulase. Feedstock-independent pretreatment could be vital to biorefineries that would fully utilize different local biomass resources and maintain natural biodiversity.

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