Case studies on sugar production from underutilized woody biomass using sulfite chemistry

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ABSTRACT: We examined two case studies to demonstrate the advantages of sulfite chemistry for pretreating underutilized woody biomass to produce sugars through enzymatic saccharification. In the first case study, we evaluated knot rejects from a magnesium-based sulfite mill for direct enzymatic sugar production. We found that the sulfite mill rejects are an excellent feedstock for sugar production. In the second study, we presented SPORL (sulfite pretreatment to overcome the recalcitrance of lignocelluloses), a sulfite pretreatment process based on modified sulfite pulping for robust bioconversion of softwood forest residues. Sulfite pulping technology is well developed, with proven commercial scalability, and sulfite pretreatment is a strong contender for commercial adoption.

Application: Mills can consider sulfite chemistry, which has the advantage of high-yield sugar production from woody biomass through enzymatic saccharification.

Producing sugars from underutilized woody biomass can be a potential revenue stream for pulp mills without competing with feedstock for pulp production. To efficiently release sugar from woody biomass through enzymatic saccharification, a pretreatment step is required to remove the strong recalcitrance of wood polymer matrix to biological deconstruction [1]. Several chemical-including pulping processes have been studied for pretreating woody biomass [2-6]. However, limited successes were achieved in terms of good sugar yield. Sulfite chemistry has several unique characteristics that are considered disadvantages for pulping; for example, depolymerization of hemicelluloses often results in pulps with low strength and yield [7]. Furthermore, acidic or bisulfite pulping requires low temperature and prolonged time for delignification to avoid lignin condensation at low pH. However, these disadvantages can be beneficial

for pretreating woody biomass for sugar production using enzymes. Unlike pulping, where the goal is to achieve as much as delignification as possible while preserving hemicelluloses, pretreating biomass for sugar production does not need to achieve complete delignification but requires significant dissolution of hemicelluloses [8] to produce a porous substrate to improve cellulose accessibility to cellulase. The dissolution of hemicelluloses can also fractionate hemicelluloses into the form of monomeric sugars, which is very desirable for biomass biorefining. The ability of delignification by sulfite under acidic conditions can facilitate hemicellulose dissolution at high temperatures to reduce reaction time while partially solubilizing and sulfonating lignin. **Table I** lists the utility of the characteristics of sulfite chemistry for enzymatic saccharification of woody biomass by comparing with their effects on wood pulping [9-13].

Sulfite Chemistry Characteristics	Pulping	Saccharification
Depolymerizing hemicelluloses	Reduces pulp strength and yield	Improves hemicellulose fractionation and fiber porosity to improve cellulose accessibility to cellulase
Degrading cellulose	Reduces pulp degree of polymerization (DP) and strength	Facilitates cellulose saccharification
Sulfonation of lignin	1. Improves delignification 2. Lignosulfonate as a coproduct	1. Facilitates partial delignification at low pH and high temperatures to reduce reaction time 2. Reduces nonproductive binding of cellulase to lignin [10,12] 3. Enhances cellulose saccharification [11,13] 4. Lignosulfonate as a coproduct [9]

I. Sulfite chemistry characteristics on wood enzymatic saccharification and in comparison with their effects on wood pulping.

We have demonstrated the robust performance of sulfite pretreatment to overcome the recalcitrance of lignocelluloses (SPORL), based on modified sulfite pulping for ethanol production from a variety of woods including hybrid poplar and softwoods [9,14-17]. All these studies used pulp mill wood chips (i.e., competing feedstock with lumber and fiber productions). In this study we will demonstrate sulfite chemistry for high yield sugar production from two underutilized feedstocks, sulfite mill rejects and Douglas-fir harvest forest residue. Case study 1 was a study of glucose production from magnesium sulfite pulp mill rejects, and case study 2 was a study of high titer sugar production from Douglas-fir harvest forest residue by SPORL.

A few studies have demonstrated that sulfite mill rejects are highly digestible for sugar production [18-20]. The main characteristic of the present sulfite mill rejects was from magnesium sulfite pulping of softwood, different from ammonia sulfite pulping in previous studies. The metal base may affect enzyme activities for sugar production, which warrants the present study. Softwood forest residues are available in large quantities in the United States, but are highly recalcitrant to enzymatic saccharification due to high lignin content. Few studies reported sugar production from softwood forest residue. Our previous study was conducted at a laboratory scale of 150 g ovendry (o.d.) forest residue [21]. We will demonstrate sulfite pretreatment at a pilot scale and using a sulfite solution prepared according to pulp mill practice; that is, bubbling sulfur dioxide (SO_2) into a hydroxide solution instead of using commercial sodium bisulfite with sulfuric acids to adjust pH reported in all our previous studies [9,14-17,21].

In view of the mature technology for sulfite pulping, this study has practical importance, especially considering colocating sugar production on kraft pulp mills for recovery chemicals as well as making use of underutilized woody biomass at pulp mills.

MATERIALS AND METHODS
Case study 1: Sulfite mill rejects

Sulfite mill rejects were obtained from Cosmo Specialty Fibers Inc. (Cosmopolis, WA, USA). The mill produces high-grade dissolving pulp from softwood using magnesium sulfite with magnesium recovery. The rejects were unbleached reject knots with a typical particle size of 2 in. The collected rejects had a moisture content of approximately 70% and were shipped to the USDA Forest Service, Forest Products Laboratory (FPL), in Madison, WI, USA. Burning these rejects at the mill did not produce much heat due to the high moisture content (private communication with two sulfite mills). The as-received rejects were then directly disk milled in a 12-in. laboratory disk refiner (Andritz Sprout–Bauer Atmospheric Refiner; Springfield, OH, USA) using two disks with plate pattern DB2-505 at a disk plate gap of 1 mm, approximately 10 times larger than that used for typical mechanical pulping. The energy consumption for refining was minimal at approximately 100 W-h/kg because of the large plate gap used.

### Case study 2: Douglas-fir harvest forest residue

The Douglas-fir forest residue was from a regeneration harvest Douglas-fir stand in Lane County, OR, USA, and owned by Weyerhaeuser Co. Forest residue was chosen because of its lower cost than wood, and competing for feedstock with pulp and lumber production can be avoided. A horizontal drum fixed-hammer grinder (Model 4710B, Peterson Pacific Corp.; Eugene, OR, USA) equipped with a combination of 76- and 102-mm grates was used to grind road piles of the residue **(Fig. 1).** The ground residue was shipped to Weyerhaeuser Co. at Federal Way, WA, USA, by truck. The moisture content of the residue measured at arrival was 43.9%. A gyratory screen (Black-Clawson; Middleton, OH, USA) equipped with a 44.5-mm (1.75-in.) diameter round-hole punched-plate top deck was used to remove oversized particles and a 3.2-mm (1/8-in.) clear-opening woven wire bottom screen (6 wires/in. mesh) to remove fines. The oversize fraction was further hammer milled, which resulted in near zero oversized particles and 14.9% fines from the 9.8% original screen oversize fractions. The total rejection of fines was 9.0%. Fractionation through screening was found to selectively remove bark and ash [22,23]. The accept forest residue labeled as FS-10 was then air-dried to a moisture content of 15% before being shipped to the FPL.

A sulfite pretreatment (SPORL) was applied to 61.75 kg FS-10 of 81.4% moisture using a pilot-scale rotating digester of 390 L [24]. A dilute sulfite solution was prepared by bubbling 3.3 kg SO_2 at a gauge pressure of 34.5 kPa into a 139-L solution containing 1.25 kg (95% purity) calcium hydroxide. The resultant total SO, and calcium bisulfite charge on o.d. weight FS-10 was 6.6 wt% and 6.46 wt%, respectively. The FS-10 was steamed after loading into the digester to result in a final pretreatment liquor-to-o.d. wood ratio of 3.55:1 (L/kg). This gave an equivalent true combined SO, concentration in the cooking liquor of 1.15 wt% and true free SO, concentration of 0.68 wt%. These SO, loadings are significantly lower than the approximately 8 wt% total SO, (at liquor-to-wood ratio of 4:1) typically used in sulfite pulp mills, or a reduction of 80%.

To accommodate facility limitations at sulfite mills, the pretreatment temperature was conducted to 145°C, slightly higher than typical sulfite pulping temperature. It took approximately 37 min for the 390-L digester to be heated to T = 145°C using a steam jacket. The temperature was maintained for another 240 min to result in an effective pretreatment duration, t^{T145}, approximately within the calculated time of 225–270 min based on optimal pretreatment condition of T = 180°C for t^{T180} = 25–30 min [9], as in Eq. (1):

$$t^{T145} = \exp\left[\frac{E}{R}\left(\frac{1}{T_{145}} - \frac{1}{T_{180}}\right)\right] \cdot t^{T180} \qquad (1)$$

where E = 100,000 J/mole is activation energy and R = 8.314 J/mole/K is the universal gas constant. Eq. (1) follows the rough rule of thumb of chemical kinetics; that is, for every
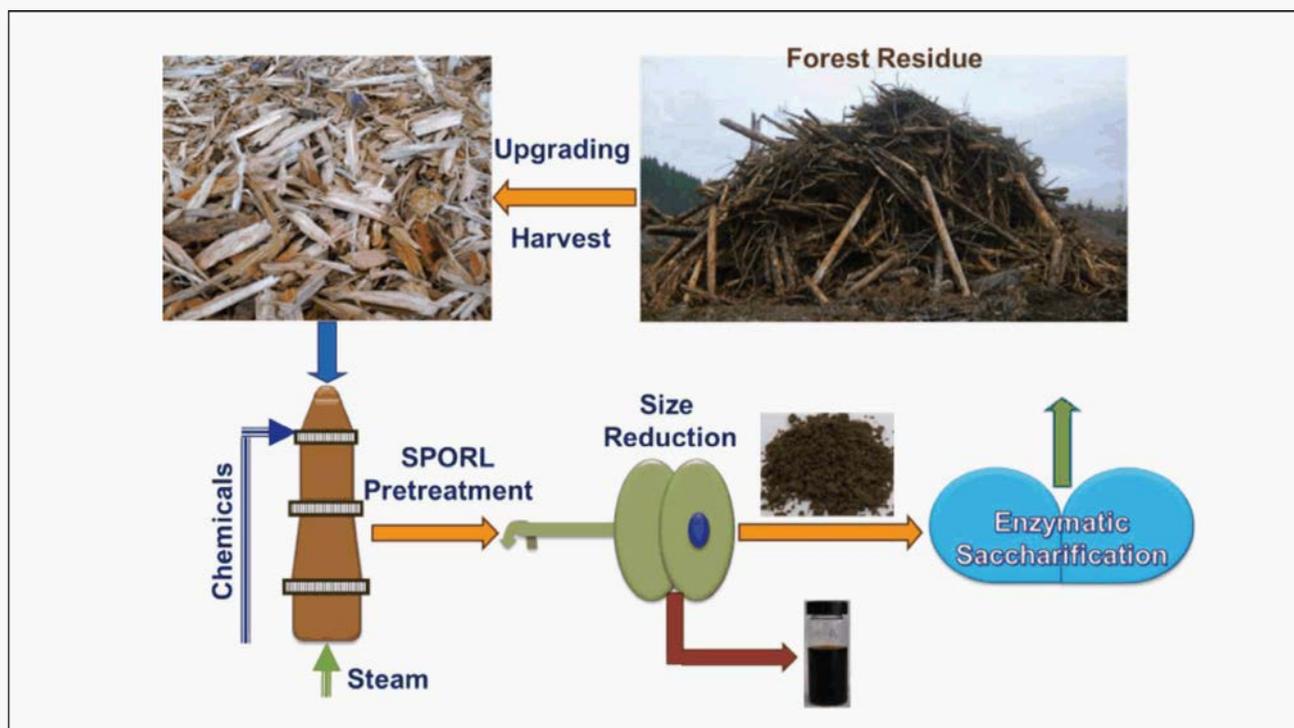
1. *Schematic flow diagram of the experiments.*

10°C decrease in reaction temperature, the rate decreases by one-half. Thus, based on this rule of thumb or Eq. (l), the pretreatment time can be reduced to approximately 120 min when a pretreatment time of 155°C is used. The method was shown to perform well for scale-up from 0.15 kg at 180°C to 2 kg at 165°C in a previous laboratory study [9].

At the end of pretreatment, the digester was discharged into a blow tank. Volatiles, which included SO_2, were vented to a wet scrubber. The freely drainable portion of the spent liquor was collected and then neutralized using lime and proportionally fed with the collected wet solids to the disk refiner described previously to produce FS-10 whole slurry using a disk plate gap of 1 mm (Fig. 1). The solids were separated from the liquor using a screen box. The polysaccharides were therefore recovered from the disk-milled unwashed wet solids and separated from the spent liquor. A fraction of wet solids was washed for enzymatic saccharification and yield determination.

Chemicals and cellulase
Commercial cellulase Cellic CTec3 (CTec3) and hemicellulase Cellic® HTec2 (HTec2) were provided by Novozymes North America (Franklinton, NC, USA). It was found that HTec2 has significant cellulase activities in addition to hemicellulase activities. Sodium acetate, acetic acid, sulfuric acid, and sodium bisulfite (all ACS reagent grade) were used as received from Sigma-Aldrich (St. Louis, MI, USA). Calcium hydroxide (95% purity) was purchased from Alfa Aesar (Ward Hill, MA, USA). Sulfur dioxide anhydrous gas was from Airgas USA (Madison, WI).

Enzymatic saccharification
The refined magnesium sulfite mill rejects were enzymatically saccharified at 5% (w/v) solids loading. The pretreated and washed Douglas-fir forest residue was saccharified at three solids loadings of 5%, 10%, and 15% (w/v). The unwashed pretreated wet solids were also saccharified, but at total solids loading of 15 wt% and 18.5 wt% to access potentially achievable sugar titer. Acetate buffer of 50 mmol/L. and pH 5.5 was used to conduct the enzymatic hydrolysis. Based on our previous studies [21,25], using pH 5.5, which is higher than what is commonly used (pH 4.8-5.0), can reduce nonproductive cellulase binding to lignin to enhance saccharification. Various cellulase (CTec3) loadings, including supplementation of hemicellulase (HTec2), were evaluated. Solid suspensions were incubated in flasks and agitated with a shaker (Model 4450, Thermo Fisher Scientific; Waltham, MA, USA) at 50°C and 200 rpm. Duplicate hydrolysis runs were conducted. The reported results are averages of the duplicate experiments. Glucose in the hydrolysate was determined using a commercial biochemistry analyzer (YSI 2700S, YSI Inc.; Yellow Springs, OH, USA). The terminal hydrolysates were also analyzed using high performance liquid chromatography (HPLC) for mannan and xylan conversion determination [26].

## RESULTS AND DISCUSSION
Case study 1
Chemical composition analysis indicated that the refined magnesium sulfite mill rejects had a very high carbohydrate content of over 65% **(Table II),** which was highly enriched

Klason Lignin, %	Ash, %	Arabinan, %	Galactan, %	Glucan, %	Xylan, %	Mannan, %	Total Carbohydrate, %
27.7	1.2	ND	0.2	61.3	1.3	2.3	65.1

II. Chemical composition of the as-received sulfite mill rejects.
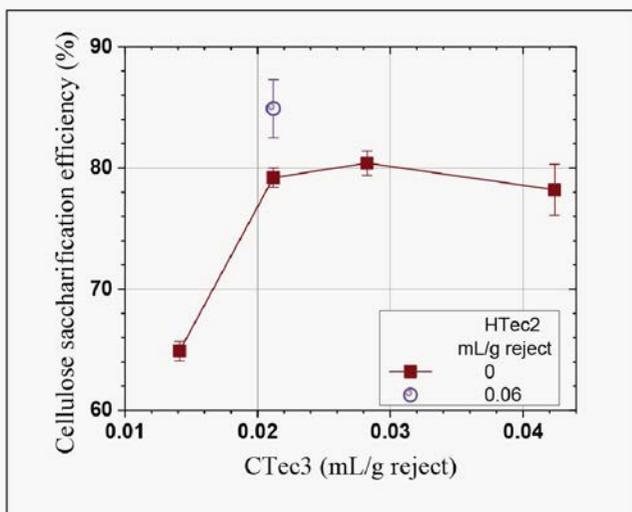
2. Effect of enzyme loading on terminal cellulose saccharification efficiency.
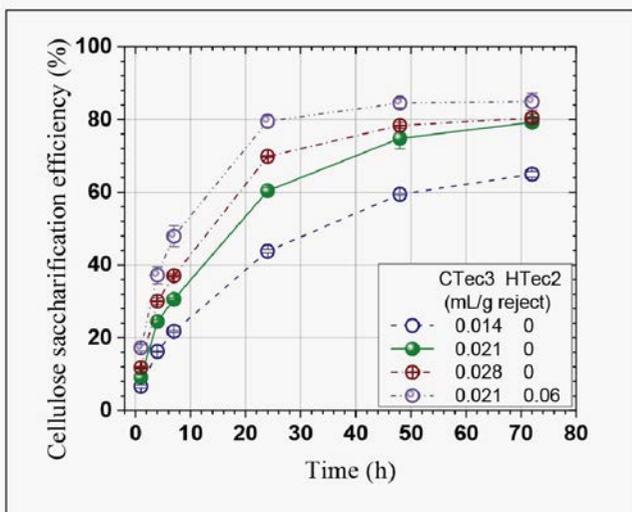
3. Time-dependent cellulose saccharification efficiency at different enzyme loadings.

with glucan that can be enzymatically hydrolyzed into glucose. Enzymatic saccharification of the refined rejects produced maximal cellulose saccharification efficiency of 80% **(Fig. 2)** at a CTec3 loading as low as 0.021 mL/g of rejects. In this case, saccharification efficiency is defined as the percentage of glucan in the solids that are enzymatically saccharified into glucose. Increasing the CTec3 loading only increased the initial rate of cellulose saccharification, but had a negligible effect on the terminal cellulose saccharification

efficiency **(Fig. 2 and Fig. 3).** Supplementation of HTec2 at a very high dosage of 0.06 mL/g of reject did improve the cellulose saccharification efficiency after taking into account the cellulase activity in the hemicellulase (Fig. 2). HPLC analysis indicated that approximately 85% of the xylan and mannan in the rejects were saccharified into respective monomeric sugars with the addition of hemicellulase. The low cellulase loading and enriched glucan content made the sulfite mill rejects very suitable for sugar production, especially considering that the rejects are readily available and are typically burned for heat in boilers. By using the cellulose conversion data at 21 mL CTec3/kg of rejects without hemicellulase application **(Figs. 2 and 3),** 490 kg of glucose can be produced from 1 metric ton (o.d.) rejects. This glucose yield is similar to the yields from rejects generated by ammonia sulfite pulping of softwoods, which was reported in a previous study **[18];** the current results suggest that the magnesium base of sulfite pulping did not affect enzymatic hydrolysis.

Case study 2

SPORL pretreatment of Douglas-fir forest residue FS-10 resulted in a washed solids yield of approximately 58%. The chemical compositions of the washed treated solids are listed in **Table III** along with those of the untreated FS-10 and treated unwashed wet solids. Component removals were calculated using the yield and composition of the washed solids. The lower sulfite loading and relatively higher pretreatment temperature than pulping resulted in a partial wood chip delignification of approximately 45% (Table III). Partial delignification is not an issue because complete removal of lignin is not necessary for cellulose saccharification **[8].** However, over 90% of the galactan, xylan, and mannan were dissolved. SPORL pretreatment significantly enriched glucan content to 57% in the washed solids due to the removal of lignin and hemicelluloses. The pretreatment at 145°C resulted in low sugar degradation to fermentation inhibitors in the pretreatment spent liquor **(Table IV)** as expected **[27,28].** The 5-hydroxylmethylfurfural (HMF) and furfural concentrations were 0.35 and 1.13 g/L, respectively. Low concentrations of these inhibitors are important to facilitate the downstream conversion of the pretreated residues by fermentation at high solids loadings.

The washed solids were found to be easily digestible. Enzymatic glucose concentration reached 90 g/L after 48 h of saccharification at a washed solids loading of 15%, which was equivalent to 95% cellulose saccharification **(Fig. 4).** It ap-

| Sample Label	Klason Lignin	Arabinan	Galactan	Glucan	Xylan	Mannan	Yield, %
Untreated FS-10	29.30	1.04	2.00	40.97	5.70	9.67	100.0
Washed treated FS-10 component removed, %	28.1; 44.6	ND; NA	0.23; 93.3	57.34; 19.1	0.80; 91.9	1.26; 92.4	57.8; NA
Unwashed treated FS-10	25.0	0.36	1.26	44.07	2.34	5.14	89.2

III. FS-10 cell wall chemical composition (%) before and after the SPORL pretreatment.

Arabinose	Galactose	Glucose	Mannose	Xylose	HMF	Furfural
1.47	5.88	10.48	21.5	10.2	0.35	1.13

IV. Sugar and furan titers (g/L) in the FS-10 spent liquor from SPORL pretreatment at 145°C.

Unwashed Pretreated FS-10 solid, %	CTec3 on Solid, % wt/wt	HTec3 on Solid, % wt/wt	Glucose in Hydrolysate, g/L	Cellulose Saccharification, %
14.9	4.0	0.4	79.7	93.8
18.5	5.0	0.5	97.9	92.5

V. High-solid enzymatic hydrolysis of FS-10 pretreated at 145°C.
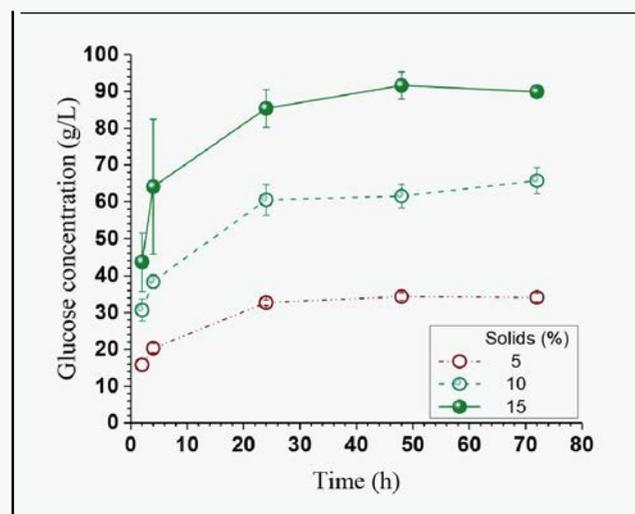
4. Time-dependent enzymatic glucose concentration of washed pretreated FS-10 at different solids loadings with CTec3 dosage: 15 FPU or 0.069 mL/g glucan.

peared that the enzymatic solids loading did not substantially affect the cellulose saccharification for the range of solids loading studied.

Lignin sulfonation is a key characteristic of the SPORL process. The dissolved lignin, lignosulfonate (Ls), was found to have similar dispersion properties to a commercial LS [9]. Furthermore, LS has less affinity to cellulase, and thereby results in negligible nonproductive binding to the cellulase due to its strong hydrophilicity [10]. As a result, the unwashed solids that contained two-thirds of the spent liquor were found to be even easier to digest than the washed solids because of the LS present in the spent liquor. This suggested that

LS can enhance enzymatic saccharification [11]. As shown in **Table V,** cellulose saccharification efficiencies were over 90% at relatively low CTec3 loadings with glucose titers over 80 g/L at a total solids loading of 15%. At total solids loading of 18.5%, the glucose titer was approximately 100 g/L. This suggested that substrate washing can be eliminated. Furthermore, pretreatment spent liquor that contains soluble LS and hemicellulose sugars can be combined with the solid fraction in saccharification, an advantage of sulfite chemistry for sugar production.

An overall mass balance was conducted based on the polysaccharide recovery (as monomeric sugars) from the unwashed wet solids and the remaining spent liquor for the enzymatic hydrolysis conducted at 14.9% total solids (**Table V**). As shown in **Fig. 5,** the total glucose yield was approximately 93%. Mannan and xylan recoveries (as monomeric sugar) were both approximately at 50%. This translated to overall major carbohydrates (glucan, mannan, and xylan) recovery of approximately 80%. Approximately 45% of the lignin was solubilized as LS, which had a similar degree of sulfonation as commercial LS products [24].

CONCLUSIONS

Sulfite pulping chemistry has several advantages for sugar production from underutilized recalcitrant woody biomass, as demonstrated by the good sugar production from sulfite mill rejects along with the robust performance of the SPORL process for converting softwood forest residue. SPORL also offers a solution to using low-grade feedstock without competing for wood for pulp production. The well-developed sulfite pulping technology, proven commercial scalability, existing sulfite pulping infrastructure, and the human capital
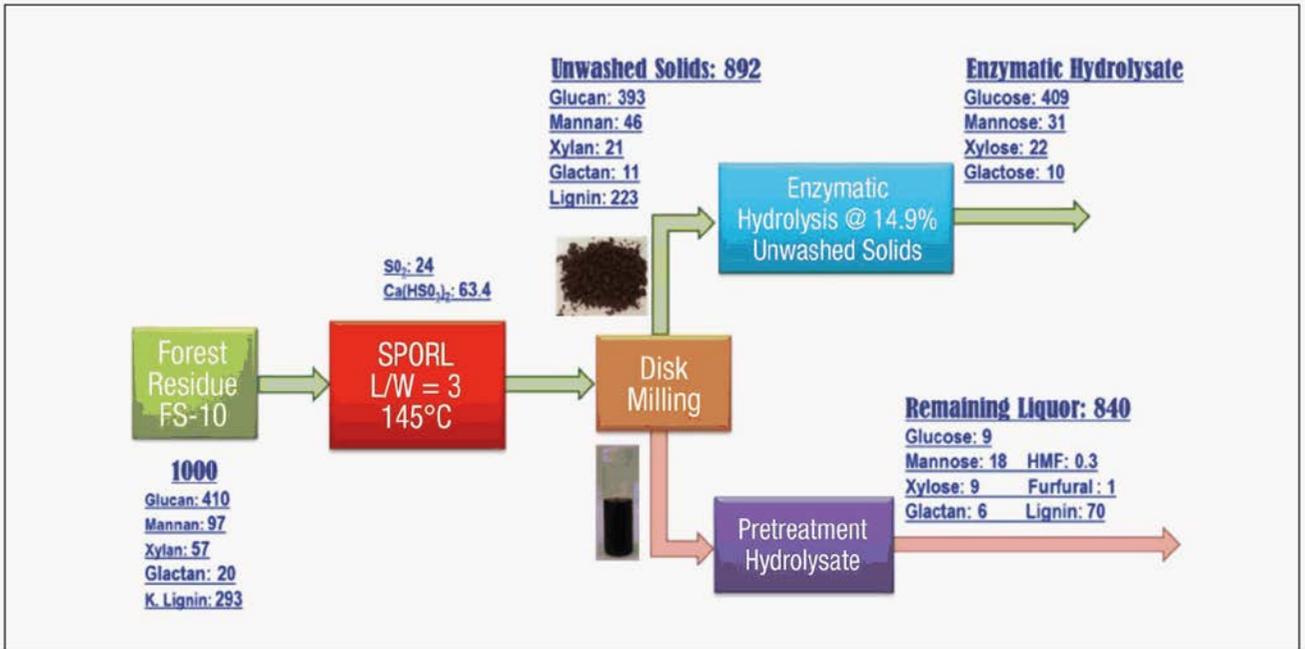
5. *Overall mass balance of SPORL pretreatment at 145°C of FS-10 based on saccharification efficiency of unwashed solids at 14.9% total solids. All units are in kg or metric ton.*

ABOUT THE AUTHORS

Producing sugar or biofuel from underutilized woody biomass can produce another revenue stream for pulp mills. This work was conducted as part of our biorefinery research program at the Forest Products Laboratory.

The biggest challenge was to deal with contaminants such as ashes in forest residues. We found that simple physical fractionation to discard the very fine fractions can remove most contaminants. Our work showed that sulfite chemistry is ideal for sugar production through enzymatic hydrolysis. The soluble lignin can be directly marketed.

The application for mills is that sulfite mill sludge can be directly used for high-yield sugar production through enzymatic saccharification. As our next step, we are looking for mill interest and collaboration.

Zhu is a research general engineer and Gleisner and Gilles are physical science technicians at the USDA Forest Service, Forest Products Laboratory, Madison, WI, USA. Chandra is an assistant professor,

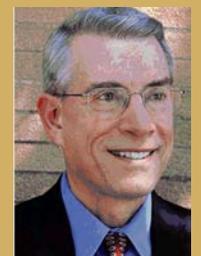*Zhu* *Gleisner* *Gilles* *Chandra*

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in the pulp and paper industry make sulfite pretreatment such as SPORL a strong contender for commercial adoption.

The SPORL process not only can use significantly less SO, loading than sulfite pulping (reduced by 80%), it also can reduce cooking time when a slightly higher cooking temperature of 155°C is used based on the rough rule of thumb of reaction kinetics, which yet need to be demonstrated in future study. The lignosulfonate produced from SPORL had good dispersion properties and is directly marketable as a revenue stream to improve the economics of the forest biorefinery. Economic analysis needs to be conducted in the future. **TJ**

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