CLEAN SUGAR AND LIGNIN PRETREATMENT TECHNOLOGY

Authors	ORGANIZATION
Johnway Gao	Weyerhaeuser
Amar Neogi	Weyerhaeuser

This report has been modified from the original report. Proprietary information has been redacted. The original report has been submitted to the USDA-NIFA.

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Any opinions, findings, conclusions, or recommendations expressed in this publication are those of the author(s) and do not necessarily reflect the view of the U.S. Department of Agriculture.

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LIST OF ACRONYMS

APU	Akita Prefectural University
ATR	Attenuated total reflectance
BDT	Bone dried ton
BTU	British thermal unit
CO_2	Carbon dioxide
EIA	Energy Information Administration
FS	Feedstock sample
IR	Infrared
kWh	Kilowatt per hour
LCA	Life cycle assessment
LLC	Limited liability company
MJ	Megajoule
MT	Metric ton
MW	Milled wood
OD	Oven dried
T%	Transmittance
TEA	Techno-economic analysis
WDM	Wet-disk milling
(X_{gm})	Geometric mean dimension

EXECUTIVE SUMMARY

The large-scale tandem milling of Douglas-fir wood has been completed. The energy consumption for steps from wood chipping to fine wood milling and to sugar hydrolysate production are estimated and used for the techno-economic analysis (TEA) of low sulfur green fuel pellet and sugar production. Based on the current market price for wood fuel pellets and sugar, the TEA indicates that the economics look very plausible for a clean lignin fuel pellet and lignocellulosic sugar production by wood milling as a pretreatment technology.

The potential global warming gas emission is minimal for this pretreatment technology. No chemical is used in the pretreatment. No water is needed in wood milling pretreatment technology. Wood milling could become a unique depots sized technology for biomass pretreatment to produce lignin and simple sugars.

The clean sugar and lignin pretreatment technology by wood milling can produce excellent hydrolysate with almost no impact on yeast biocatalyst growth and with enhancement on isobutanol production, all without any delay in isobutanol production. The results in this report indicate that clean sugar from milled wood can be widely used in various bioconversion processes for high value bio-based product production.

INTRODUCTION

A majority of current pretreatment technologies use chemical or thermal treatments to alter the lignocellulosic structure and enhance saccharification. Many of these methods have been proven to be effective with high yield. But the disadvantage of these methods involve the cost of chemicals, chemical recycling, or capital to achieve efficient scale; environmental consequence of the chemical use; and contamination of both the pretreated feedstock or lignin co-products. Evidence exists in the literature that milling of lignocellulosic materials to micron- or nano-scale can produce good hydrolysis performance without the use of any chemicals in the pretreatment. To effectively utilize this approach to mechanical pretreatment, energy efficient methods of milling are needed. Recent advances in ball mill designs have yielded commercial equipment that potentially can mill wood in the sub-micron size range with low energy input. This technique has been investigated in preliminary trials and found to produce adequate sugar yields in an un-optimized condition. A preliminary techno-economic analysis estimates nearly a 40% savings in total pretreatment costs compared to the current bisulfite pretreatment processes. This technology also demonstrates a strong potential for cost effectiveness at small scales, thereby facilitating deployment in depot settings.

TASK 1: WOOD MILLING TECHNOLOGY REVIEW

In the current literature for lignocellulosic sugar conversion from biomass, quantitative analyses showed that in some instances, wet-disk milling (WDM) can achieve a high cellulose saccharification yield of 78.5% from milled rice straw with an energy consumption of 5.4 MJ/kg biomass (Hideno et al., 2009). In some other instances, WDM can only achieve a 44.7% of cellulose saccharification yield when bagasse is milled, and a 59.5% yield when sugarcane straw is milled (da Silva et al., 2010). Resized biomass particles of 2 mm in size were used and fed into the WDM milling machine with a 20-40 micron gap between the upper and lower grinders, where the biomass to water ratio is about 1 to 20. The water need is large for WDM. The energy consumption of the WDM process is high for lignocellulosic sugar conversion from biomass without co-product credit.

In 2008, a tandem ring milling machine was introduced by Takahashi et al. (2008). In the past few years, Dr. Hideaki Mori and Dr. Takehiko Takahashi and their teams at Akita Prefectural University (APU) accomplished groundbreaking work around this tandem milling technology. The milling is a dry milling process that does not require water. Japanese cedar of less than 20% moisture was tested in the milling machine. A mean wood particle size of about 30 microns was achieved in 60 minutes with 71% cellulose saccharification yield. The initial goal for economical energy milling was targeted at around 0.9 MJ/kg wood (Mori et al., 2012). In the NARA 2nd trimester, with the large scale tandem milling trials reviewed by APU, it was estimated that the targeted energy consumption of Douglas-fir milling would be around 2 to 5 MJ/kg wood predicted from the cedar wood milling (Mori, December 3, 2013, APU project communication). The large-scale tandem milling energy on Douglas-fir was completed in June 2014.

The wood milling process has four steps, including (1) Wood residuals chipping; (2) Chip size reduction before drying; (3) Chip drying to reduce chip moisture from 50% to 10%; and (4) Wood milling. The first three steps are well known technologies, and the equipment is commercially available. The last step of wood milling at large-scale had been tested, with detail discussions in the later sections of this report.

TASK 2: LCA OF WOOD MILLING

In the Pacific Northwest, hydroelectricity will be used as the wood chipping and milling energy. However, wood chip drying can use either natural gas or wood residuals as the energy source, which is evaluated for its life cycle assessment (LCA).

The LCA on natural gas was reported by ICF International (2012) that 78.5 g CO_2e/MJ will be generated at natural gas upstream processing and end combustion for heating energy generation. Thus, the chip drying energy of 0.8278 kWh/OD kg wood (see tandem wood milling section) translates into an emission of 234 g CO_2e/kg OD wood, summarized in Table CSL-2.1.

If wood residuals are used as fuel for wood drying, the report by Bowyer (2012) shows that the emission of 24.6 g CO_2e/kWh or 6.8 g CO_2e/MJ will be generated at wood pellet combustion for heating energy generation. Thus, the chip drying energy of 0.8278 kWh/OD kg wood translates into an emission of 20.4 g CO_2e/kg OD wood as shown in Table CSL-2.2.

Table CSL-2.1. Carbon dioxide equivalent emission at natural gas production and its conversion to wood chip drying by natural gas

Emission Amount	kg CO_2e /MWh	(g CO_2e /MJ)	(g CO_2e /OD kg wood drying)
CO_2e*	282	78.5	234

*Sum of upstream and end combustion emission for natural gas production

Table CSL-2.2. Carbon dioxide equivalent emission at wood chip drying using residual wood as fuel

Emission Amount	kg CO_2e /MWh	(g CO_2e /MJ)	(g CO_2e /OD kg wood drying)
CO_2e*	24.6	6.8	20.4

TASK 3: TANDEM WOOD MILLING

The wood milling process has four steps before the milled wood powder can be enzymatically hydrolyzed for clean sugar and lignin production.

These four steps include:
(1) Wood residuals chipping
(2) Chip size reduction before drying
(3) Chip drying to reduce chip moisture from 50% to 10%
(4) Wood milling

WOOD RESIDUALS CHIPPING

Commercial chipping machines are available for wood residual chipping operations. Table CSL-3.1 shows the scale and energy requirement for wood residual chipping.

Table CSL-3.1. Specific energy consumption by a wood drum chipper

Items	Output Size (mm)	Specific Energy Consumption (kWh/OD kg wood)	Scale (metric ton/hr)	Commercial Product
Wood chipping	38	0.0098	27.2	Yes†

†Parameters provided by Zhengzhou Tianyuan Environmental Protection Machinery Co., Ltd

CHIP SIZE REDUCTION

Two technologies are reviewed for chip size reduction: one of which is developed into a wood resizing machine, Crumbler™ M24 by Forest Concepts LLC, and the other is a commercially available sawdust making machine. In the tandem wood milling process, wood chips with 2 mm mean size are required.

CRUMBLER™ M24 MACHINE

On September 24, 2013, Douglas-fir FS-10 feedstock chips with a geometric mean dimension (X_{gm}) of 13.42 mm and a moisture content of 10% were first resized into small chips with X_{gm} of 1.29 mm in the Crumbler™ M24 machine at Forest Concepts LLC (Auburn, WA). The energy consumption was recorded via LabView® software Universal. Power.Meter.0.2.5 and analyzed via an Excel® based program Comminution Energy Rev16.5. Figure CSL-3.1 shows the FS-10 before and after resizing. Table CSL-2.3 shows the specific energy consumption E (MJ/OD Mg or tonne) to resize FS-10 chips to 1.29 mm X_{gm}, which were used in small scale milling trials at Akita Prefectural University.

Figure CSL-3.1. Raw Douglas-fir FS-10 chips with 13.42 mm X_{gm} and finished feedstocks with 2 mm X_{gm}

The calculation of specific energy to convert raw FS-10 wood chips through a cascading multi-stage process involves measurement of the energy consumed during subsequent passes and applying that energy to the diminishing mass of material to be reprocessed as shown in Table CSL-3.2. The 13.2 mm chips were first resized down to about 5 mm before the chips were reduced to 2.21 mm (X_{gm}).

From the far right column in Table CSL-3.2, it can be seen that the first pass of the raw wood chips through the Crumbler™ M24 with 4.8 mm cutters was 27 MJ/OD Mg (7.5 kWh/OD tonne). Processing all the material through recirculation until essentially all of it passed the no. 10 screen (X_{gm} = 1.29 mm) consumed 162 MJ/OD Mg or 0.162 MJ/OD kg (45 kWh/tonne). Note that these values would have been considerably lower had the chips been processed at moisture levels above the fiber saturation point. It would be expected that a minor increase in target screen size to 2.4 mm would have required fewer passes through the Crumbler™ machine and would have still yielded a geometric mean particle size of less than 2 mm. It is estimated that increasing the screen to 2.4 from 1.88 mm would reduce the specific energy to approximately 130 MJ/OD Mg or 0.130 MJ/OD kg (36 kWh/tonne).

SAWDUST MACHINE

The sawdust machine is commercially available for the production of 2 mm size chips, without requiring two steps in the size reduction process. Table CSL-3.3 shows the scale and energy requirement for wood residual chipping. This sawdust machine does not require initial wood chipping to 5-13 mm size.

Table CSL-3.2. Comminution energy results of raw Douglas-fir FS-10 feedstock chips with 13.42 X_{gm} to finished feedstocks with 1.29 mm X_{gm}

Line	Sample ID	Description	GMD (X_{gm}) mm	Comminution Ratio	% Retained from prior run	% Original Mass	Specific E (MJ/OD Mg)	
							Run	Cumulative, Original mass basis
1	2013.09.23.001	Raw Chips	13.4	-	-	100	-	-
2	2013.09.23.001. A-C	1st Pass 4.8mm	5.66	2.4	-	100	27	27
3	2013.09.23.001. A-C.A	2ndPass 4.8mm	4.12	1.4	-	100	16	43
4	2013.09.23.001. A-C.B	1st Pass 1.6mm	2.21	1.9	-	100	67	110
5	2013.09.23.002	2ndPass 1.6mm	2.21		73	73	37	137
6	2013.09.23.003	3rd Pass 1.6mm	2.21		70	51	26	150
7	2013.09.23.004	4th Pass 1.6mm	2.21		62	32	20	157
8	2013.09.23.005	5th Pass 1.6mm	2.21		58	18	17	160
9	2013.09.23.006	6th Pass 1.6mm	2.21		56	10	14	161
10	2013.09.23.007	Final Product	1.29		53	5	12	162

Table CSL-3.3. Specific energy consumption by a sawdust machine

Items	Output Size (mm)	Specific Energy Consumption (kWh/OD kg wood)	Scale (metric ton/hr)	Commercial Product
Size reduction by sawdust machine	2	0.0505	2	Yes†

†Parameters provided by Zhengzhou Tianyuan Environmental Protection Machinery Co., Ltd

WOOD CHIPS DRYING

Tandem wood milling or wood milling to fine wood particles requires wood chip moisture of about 10-12% or less to be efficient. A wood chip drum dryer with natural gas burner to provide heat energy for wood drying is commercially available. Table CSL-3.4 shows the actual energy needs to dry the wood chips from 50% moisture to 10% moisture. The specific energy requirement is 1375 Btu/lb water (Savovic Z, TSI, Lynwood, WA, March 2014, personal communication).

Table CSL-3.4. Specific energy consumption by a sawdust machine

Items	Output Size (mm)	Specific Energy Consumption (kWh/OD kg wood)	Scale (metric ton/hr)	Commercial Product
Chip drying	NA	0.8278	8	Yes‡

‡Parameters provided by Zhengzhou Tianyuan Environmental Protection Machinery Co., Ltd. The specific energy includes 0.0382 kWh/OD kg as the mechanical energy for the rotary drum dryer.

TANDEM WOOD MILLING

LARGE SCALE TANDEM MILLING MACHINE

On October 21-25, 2013. We (Johnway Gao and Mike Wolcott) visited the [text redacted] tandem ring milling technology [text redacted]. We evaluated the energy consumption of the 1st built tandem ring milling machine of one metric ton (MT)/hr, which is under optimization in design and performance.

The 1 MT/hr tandem machine milling (Version HV-70 model) requires original twin 6-meter-long tandem milling chambers, which were equipped with two motors, each of 100 kW actual running power. The 6-meter chamber tandem ring milling machine in Figure CSL-3.2 was 1st built in 2011 and tested for its structural stability. It was found that the 6-meter machine was not quite stable in operation, in part from excessive sympathetic vibration. Subsequently, an improved chamber version, Improved HV-70 in Figure CSL-3.3 with twin 3-meter chambers with 0.5 MT/hr capacity, was built in 2012 to replace the 6-meter chambers. The Improved HV-70 was proven to be stable during operation. Large-scale optimization in power, wood chip loading (productivity) and performance have not been completed yet but is ongoing with their current program. At low solid loading of Japanese cedar chips (apparent density = 0.1 kg/L) at 144 kg/hr and 240 kg/hr, the energy consumption could be estimated as 2.5 and 1.4 MJ/kg OD wood, under an assumption that half of the equipped motor power for the 6-meter milling machine could be needed for the 3-meter Improved HV-70 tandem ring milling machine determined in a discussion on October 25, 2013. The best enzymatic hydrolysis yields were 62.5% and 37.6%, respectively. Upon large-scale optimization, it is plausible that lower energy consumption will be sufficient to achieve a good enzymatic hydrolysis yield. At the current set up, the large-scale tandem mill is still equipped with 2x 100 kWh twin motors.

At Akita Prefectural University, the plan of large-scale design for the tandem ring milling machine will eventually achieve the goal of processing capacity of 1-5 metric ton/hr. From October 2013 to December 2014, trials will be conducted around (1) the Improved HV-70 model (3-meter model); (2) a new TR-3000 model (100 kg/hr, under construction now and to be completed around September or October, 2014); (3) large scale prototype design/production/test. It should be noted that large-scale tests or trials require more than 100 kg OD chips of 2 mm X_{gm} size.

[Figure CSL-3.2 redacted]
[Figure CSL-3.3 redacted]

SMALL SCALE TANDEM MILLING TRIALS

Douglas-fir FS-10 feedstocks of 10 kg with 1.29 X_{gm} were sent to Akita Prefectural University for milling trials while we were there for the onsite visit. Small-scale milling screening trials were conducted in the small-scale tandem ring milling machine (1.6 kg capacity), which is shown in Figure CSL-3.4a and Figure CSL-3.4b. This small machine has much higher energy consumption as compared to the 0.5 MT/hr machine due to the small machine's heavy frame design vs. its small capacity. However, the small trials could help researchers understand the particle size distribution vs. milling time and lignocellulosic conversion yield to sugar. Various milling trials under different times of 30, 60, 80 and 100 minutes were conducted. Figure CSL-3.5 shows the milled FS-10 samples under various milling times.

[Figure CSL-3.4a redacted]
[Figure CSL-3.4b redacted]

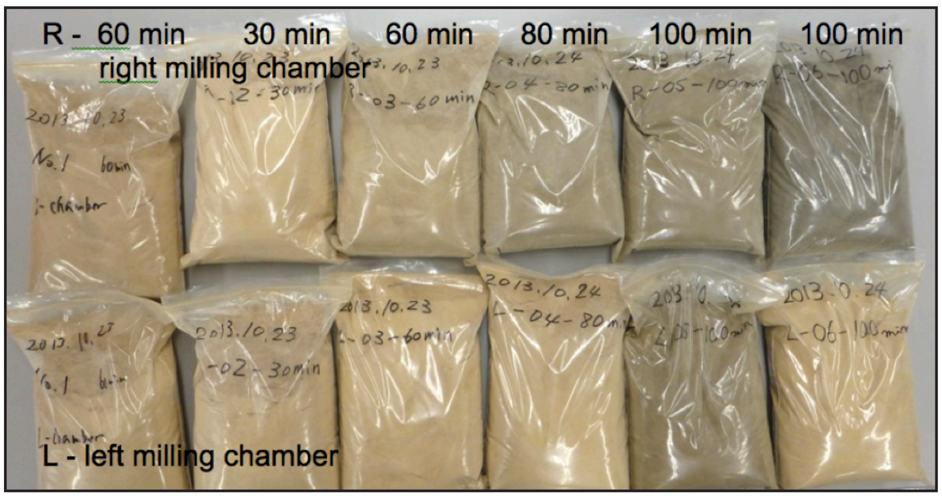

Figure CSL-3.5. Milled wood powders of FS-10 chips, under various milling time, 30, 60, 80, and 100 minutes. L-from left milling chamber; R- from right milling chamber. Lighter color of milled wood in L-chamber indicates milling temperature was cooler than that in the R-chamber (onsite photo during our visit to Akita Prefectural University)

DOUGLAS-FIR TANDEM MILLING ENERGY TEST

In the NARA 2nd trimester, APU reviewed the large-scale tandem milling design and Japanese cedar mill trial results. It is estimated that the targeted energy consumption of Douglas-fir milling would be around 2 to 5 MJ/kg wood, predicted from the cedar wood milling (Mori, December 3, 2013, APU project communication). Dry Douglas-fir FS-01 chips of 2250 lbs were sent to APU on March, 2014.

In the NARA 3rd trimester, APU tested a few large-scale tandem milling trials with the HV-70Av machine on Douglas-fir FS-10. The FS-10 chips were first re-chipped to a mean size of 2 mm before the tandem milling. The HV-70Av is the short version of HV-70 model and it has a length of 3 meters, equipped with twin chambers with its original twin motors, 100 kW each. The actual power consumption varies depending on wood loading. Various amounts of Douglas-fir FS-01 2 mm wood chips were milled in the tandem mill chamber. The milling results are shown in Table CSL-3.5, provided by Akita Prefectural University. The chip amount in each chamber was loaded with 12, 24, 36, and 48 kg, respectively in each trial, which requires different specific energy consumption as shown in the table. The tandem milling time was 30 minutes. The milled wood sample was enzymatically hydrolyzed in 2% solid to show the saccharification yield of milled wood, completed by APU. The tandem-milled FS-10 samples, about 7-8 kg each, were sent back to Weyerhaeuser for characterization and evaluation of high solid hydrolysis. Results for the received samples are shown in Figure CSL-3.6.

With the tested energy consumption on larger scale tandem milling of 19% and 28% volumetric load range, the total wood milling energy requirement is summarized in Table CSL-3.6.

Table CSL-3.5. Tandem milling energy consumption and saccharification yield of milled wood*

Trials	Chamber Volumetric Occupancy of Input Materials (%)	Energy Consumption (kWh/OD kg wood)	Saccharification Yield of Milled Wood (%)
1 – 12 kg	9	3.716	67.4
2 – 24 kg	19	2.075	63.3
3 – 36 kg	28	1.363	48.5
4 – 48 kg	37	1.037	39.5

*Numbers were originated from the report chart and its table (Mori H, project communications on June 13, 2014 and on July 8, 2014).

Table CSL-3.6. Wood milling energy requirement in each size reduction step with tested fine milling

Steps	Output Size (mm)	Specific Energy Consumption (kWh/OD kg wood)	Scale (metric ton/hr)	Commercial Product
1. Wood chipping	38	0.0098	27.2	Yes†
2. Size reduction	2	0.0505	2	Yes†
3. Chip drying	NA	0.8278	8	Yes ‡
4. Fine milling*	0.030-0.050	1.3632-2.0748 (tested)	0.048-0.072	Not yet
Total Energy (kWh/OD kg)	2.2459-2.9574			

†Parameters provided by Zhengzhou Tianyuan Environmental Protection Machinery Co., Ltd
‡Parameters provided by Zhengzhou Meichang Mining Machinery Co., Ltd. The specific energy includes 0.0382 kWh/OD kg as the mechanical energy for the rotary drum dryer.
*The fine milling of Douglas-fir FS-01 was tested in Akita Prefectural University.

Table CSL-3.7. Specifications of an impact milling machine

MODEL	TYMF500	TYMF800	TYMF900	TYMF6R
Blade (Pcs)	36	72	96	6
Motor power (kw)	18.5	30	55	30
Sieving machine (kw)	1.1	3	5.5	3
Max feeding size (mm)	10	15	20	5
Rotating speed (r/min)	3200	3200	2900	380
Final size (mesh)	10-300	10-300	10-300	60-300
Output capacity (t/h)	0.05-0.7	0.05-1	0.08-1.5	0.1-0.4
Dimension (m)	6x1.5x3.5	6x1.8x3.5	7x2.2x4	5x2.5x4

Figure CSL-3.6. Large scale tandem-milled FS-01 samples by the Improved HV-70Av tandem mill; ~7-8 kg of each sample received by Weyerhaeuser

SUPERFINE WOOD POWDER PRODUCTION BY IMPACT MILLING

Small scale Douglas-fir FS-10 milling trials were also completed with impact milling equipment manufactured by Zhengzhou Tianyuan Environmental Protection Machinery Co., Ltd, China. Table CSL-3.7 lists the specifications of a few impact mills. TYMF6R mill was used for the milling trial, which had an estimated output of 50 kg/hr at 300-400 mesh. Chip amount of 100 kg of chips were needed for each milling test due to the machine size. The total energy consumption is 0.74 kWh/OD kg when the chip moisture is 11%.

Figure CSL-3.7 shows the superfine wood milling machine and its chamber configuration. The mechanism is that after materials are fed into milling chamber, the materials are pulverized under the shearing force and the extrusion force generated between grinding roller and grinding ring. The classifiers on the top of these impact mills collect qualified powders. Milled FS-10 samples from two replicate trials, TY1 and TY2 by the TYMF6R milling machine were received, as shown in Figure CSL-3.8.

Figure CSL-3.7. (a) Impact milling chamber; (b) Impact milling machine; (c) Integrated multiple impact milling unit

Figure CSL-3.8. Milled FS-10 samples by impact milling equipment

SMALL-SCALE CLEAN SUGAR AND LIGNIN PRODUCTION

To provide Gevo, Inc. with a hydrolysate of milled FS-10 feedstocks and the NARA Co-Product team with lignin rich residuals from the hydrolyzed milled FS-10 powder, larger scale and higher solid hydrolyses at 15% consistence with 5.5% CTec3/HTec3 enzyme product were conducted in 1000 ml shake flasks with 700 ml working volume. After hydrolysis, a simple vacuum filtration was used to separate hydrolysate from the lignin rich solid residuals. The hydrolysate has a sugar titer of about 7.5% total sugar, which was further concentrated to 10% total sugar titer with a vacuum evaporator at 61-63°C. The generated 5-liter hydrolysate, as shown in Figure CSL-3.9, had been sent to Gevo, Inc. for the preliminary isobutanol fermentation evaluation. More hydrolysate of 1.8 liters (Figure CSL-3.10; un-concentrated) had been sent to Gevo, Inc. on March 25, 2014.

The hydrolysis-generated 1.1 OD kg equivalent lignin residuals (3x washed by deionized water at equal wet weight each time), as shown in Figure CSL-3.11, had been sent to the NARA Co-Product team on November 21, 2013 for characterization and application analyses. In addition, fermentation by regular ethanol producing yeast was also conducted on milled wood hydrolysate without separation of hydrolyzed wood residuals from the hydrolysate by filtration. The resulting fermented milled residuals of 0.75 kg OD, as shown in Figure CSL-3.12, was also produced and sent to the NARA Co-Product team on January 30, 2014 for evaluation.

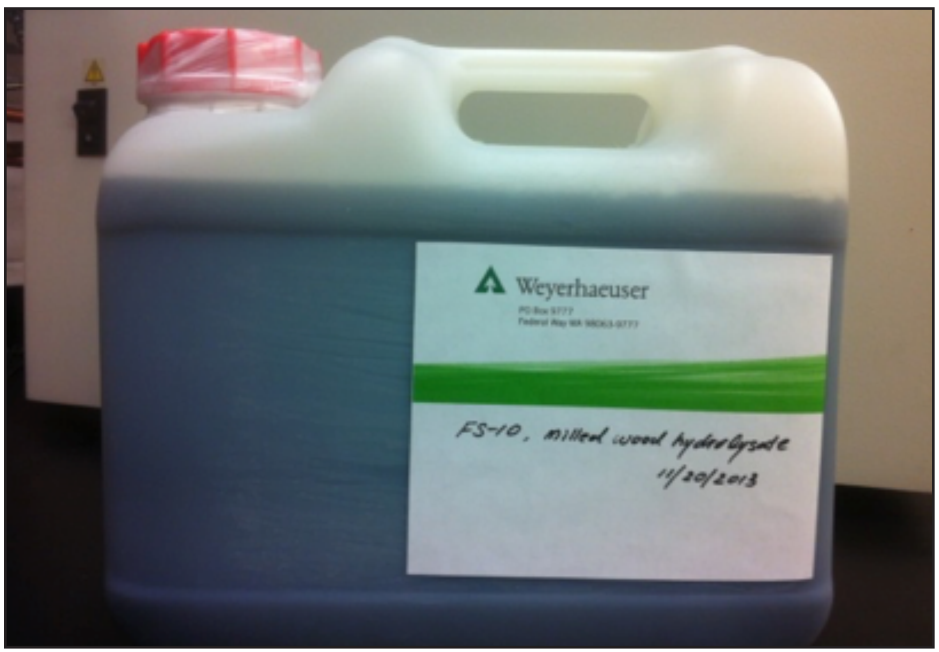

Figure CSL-3.9. Hydrolysate of 5 liters with 10% total sugar concentration, sent to Gevo, Inc. on Nov. 21, 2013

Figure CSL-3.10. More hydrolysate of 1.8 liters, sent to Gevo, Inc. on March 25, 2014 for the GIFT™ fermentation system testing

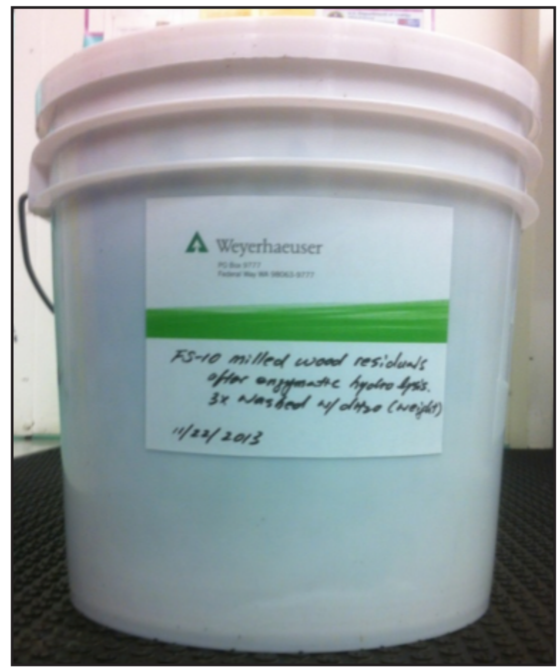

Figure CSL-3.11. Lignin rich residuals of 1.1 OD kg equivalent, sent to the NARA Co-Product team on Nov. 21, 2013

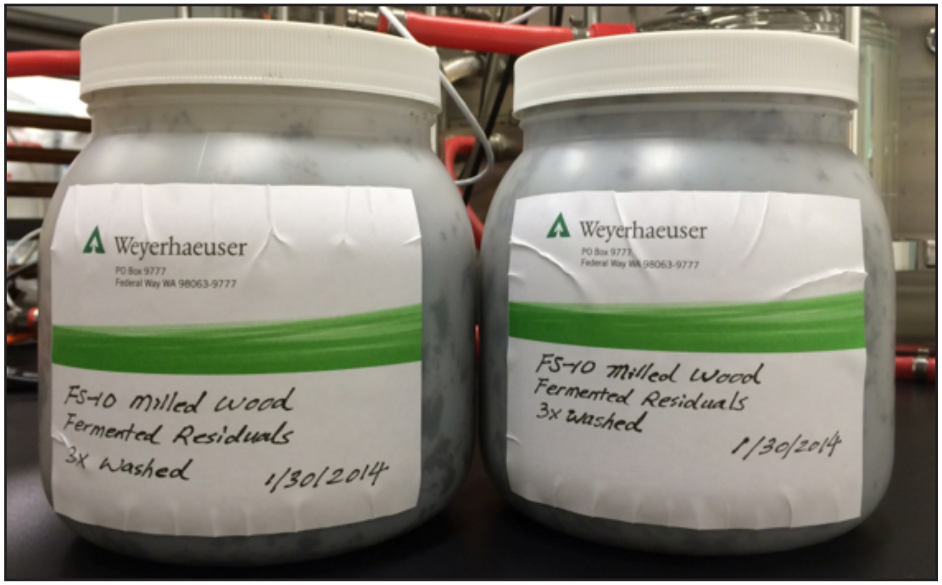

Figure CSL-3.12. Lignin rich residuals of 0.75 OD kg equivalent, sent to the NARA Co-Product team on Jan 30, 2014

CLEAN SUGAR SAMPLE FERMENTATION BY GEVO, INC.

With the small scale clean sugar sample of hydrolyzed FS-10 milled wood (MW), Gevo, Inc. has completed the initial growth and fermentation tests with isobutanol-producing yeast in the shake flask tests. The results showed that, without any lag phase, the 100% milled wood hydrolysate (FS-10 MW) could achieve 80% growth in 24 hours and produce 20% more isobutanol than the positive control (FS-10 MW Mock) at 48 hours. Gevo, Inc. indicated that this is one of the best results at 100% hydrolysate for isobutanol fermentation. The results provided by Gevo, Inc. are shown in the Figures CSL-3.13 and CSL-3.14 (milled wood project communication on March 26, 2014).

Further, the concentrated hydrolysate from milled wood hydrolysis was tested from duplicate vessels in the production phase using Gevo's GIFT ™ fermentation system for isobutanol fermentation. The results are shown in Figures CSL-3.15a, CSL-3.15b and CSL-3.15c (milled wood project communication on June 2, 2014). Within the 24-hour comparison, the total isobutanol titer using the 100% FS-10 milled wood hydrolysate is better than the mock media at mid fermentation (Figure CSL-3.15a). With higher isobutanol titer, the average specific isobutanol productivity (g isobutanol/g cells/hour) in Figure CSL-3.15b and the volumetric productivity (g isobutanol/liter of media/hour) in Figure CSL-3.15c are higher for the milled wood hydrolysate than that of the mock media.

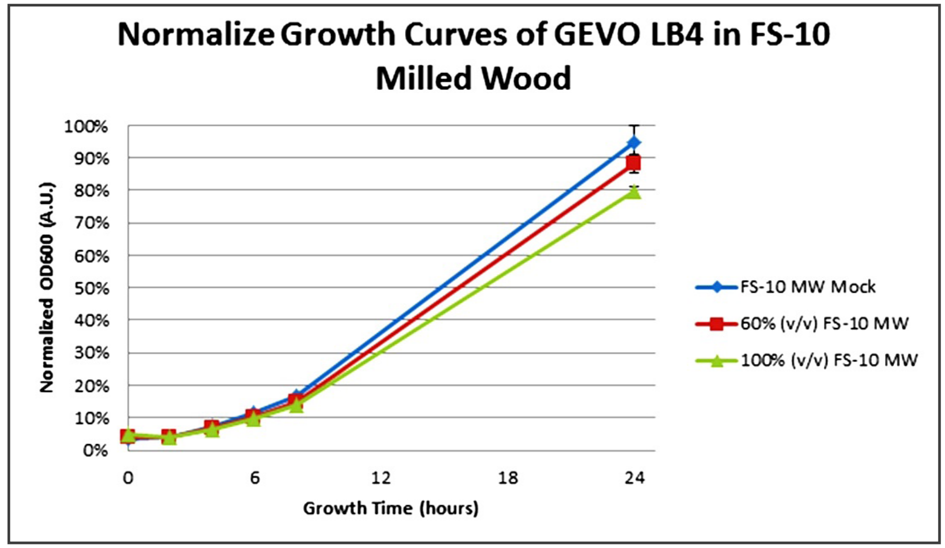

Figure CSL-3.13. Normalized growth curves by Gevo, Inc., performed on FS-10 milled wood (MW) hydrolysate. "Mock" stands for an artificial medium with the same sugar concentration prepared from pure sugars (results provided by Gevo, Inc. in the milled wood project communication on March 26, 2014)

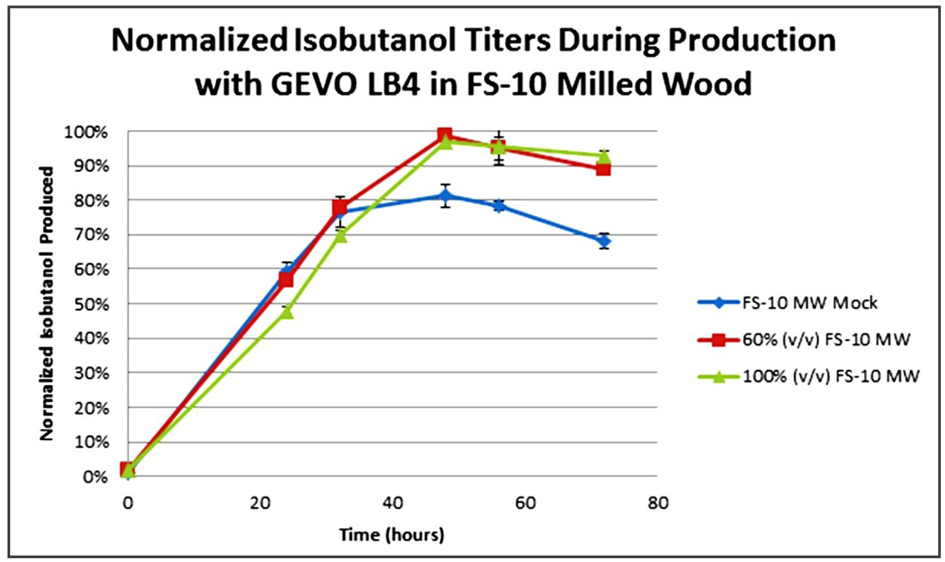

Figure CSL-3.14. Normalized growth curves by Gevo, Inc., performed on FS-10 milled wood (MW) hydrolysate. "Mock" stands for an artificial medium with the same sugar concentration prepared from pure sugars (results provided by Gevo, Inc. in the milled wood project communication on March 26, 2014)

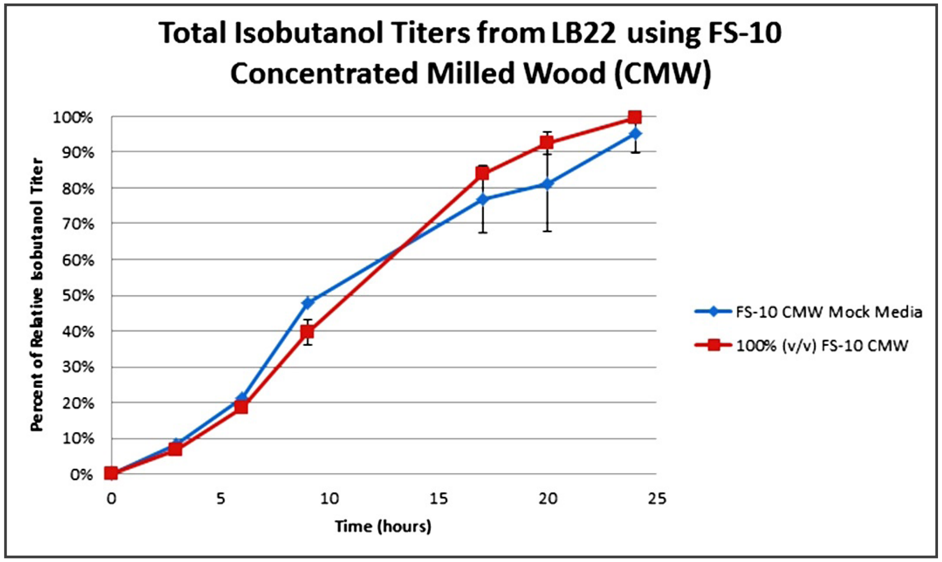

Figure CSL-3.15a. Percent of relative isobutanol titer on concentrated FS-10 milled wood (MW) hydrolysate in Gevo's GIFT™ system. "Mock" stands for an artificial medium with the same sugar concentration prepared from pure sugars (results provided by Gevo, Inc in the milled wood project communication on June 2, 2014)

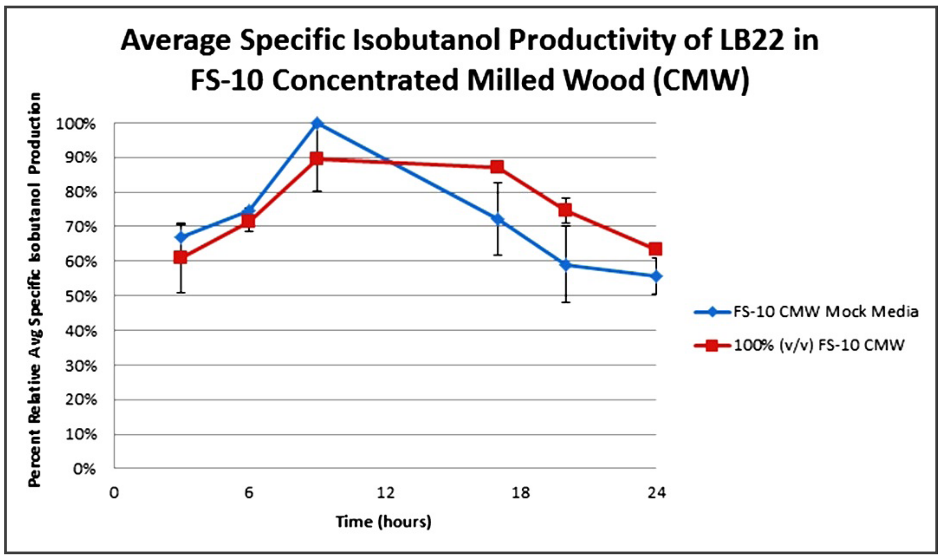

Figure CSL-3.15b. Percent of average specific isobutanol productivity (g/g cells/hour) on concentrated FS-10 milled wood (MW) hydrolysate in Gevo's GIFT™ system. "Mock" stands for an artificial medium with the same sugar concentration prepared from pure sugars (results provided by Gevo, Inc. in the milled wood project communication on June 2, 2014)

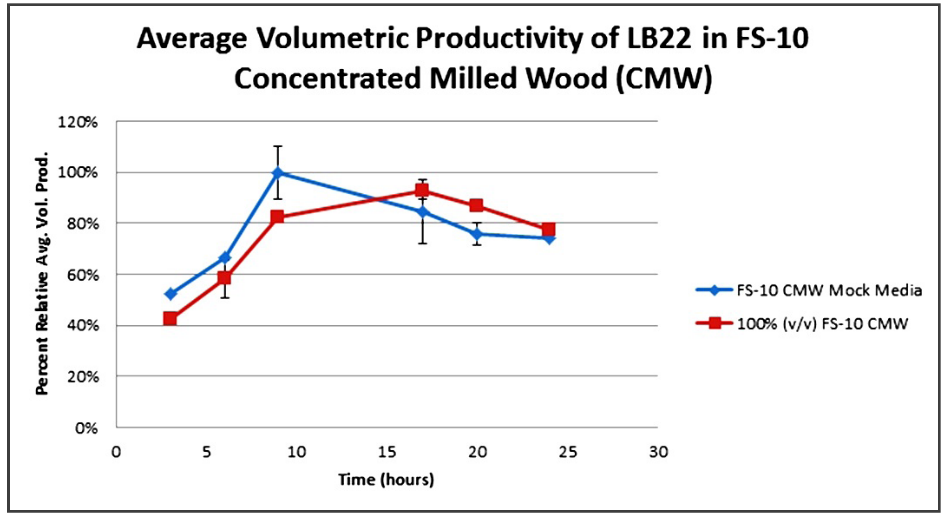

Figure CSL-3.15c. Percent of average volumetric isobutanol productivity (g/L media/hour) on concentrated FS-10 milled wood (MW) hydrolysate in Gevo's GIFT™ system. "Mock" stands for an artificial medium with the same sugar concentration prepared from pure sugars (results provided by Gevo, Inc. in the milled wood project communication on June 2, 2014)

OPTIMIZATION OF MILLED WOOD HYDROLYSIS

PARTICLE DISTRIBUTION UNDER VARIOUS MILLING TIME

Small-scale tandem milling produced various FS-10 milled wood samples as shown in Figure CSL-3.16. There is some particle size variation in milling between the left chamber and the right chamber. The left chamber was cooled to a lower temperature than the right chamber due to a serial cooling connection (left chamber first, and then to right chamber) rather than a parallel cooling connection. Within 30 minutes of milling, the mean particle diameters of wood powder were 32-38 microns in the left chamber and 34-43 microns in the right chamber, respectively. Within 60 minutes of milling, the mean particle diameters of wood powder was 29-34 microns in the left chamber and 41-44 microns in the right chamber, respectively. Longer milling of 80-100 minutes seems to cause wood particle agglomerations even after sonication prior to particle size analysis and counting.

The crystallinity of the FS-10 milled Douglas-fir wood was measured by X-ray diffraction by Akita Prefectural University. The results are shown in Figure CSL-3.17. After 60 milling time, the milled wood crystallinity shows no further or significant reduction, which means 60 minutes of milling is sufficient.

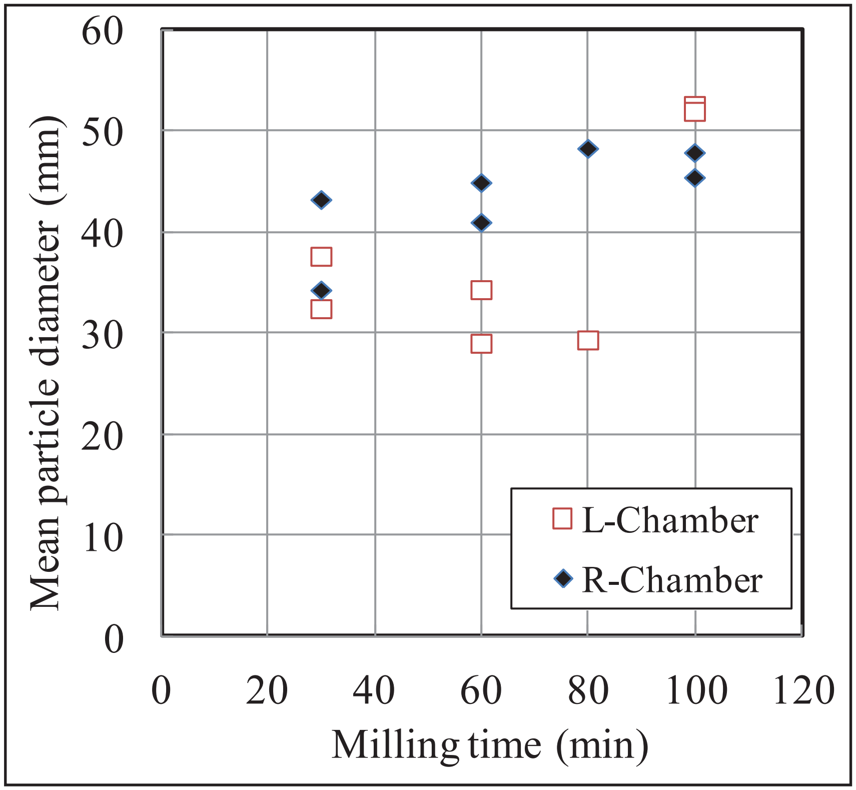

Figure CSL-3.16. Mean particle diameter of milled FS-10 under various milling times

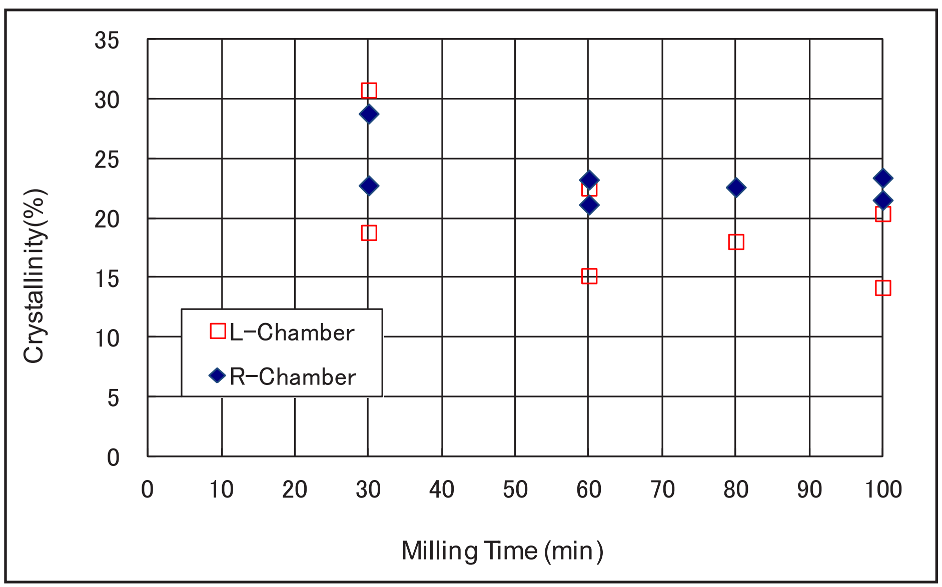

Figure CSL-3.17. Cellulose crystallinity of milled FS-10 under various milling times

PARTICLE DISTRIBUTION COMPARISON BETWEEN TANDEM MILLING AND IMPACT MILLING

The particle size distributions of the milled Douglas-fir FS-10 samples were analyzed in a Beckman Coulter Counter, Model MultisizerTM 4 (Beckman Coulter, Inc., Fullerton, CA). The Coulter Counter is equipped with an aperture tube 280 μm, capable of measuring particle sizes from 5.6 to 224 μm that are within the size range of the milled wood particles. A milled wood sample was first suspended at 1% suspension in sterile deionized water for overnight, and subsequently sonicated for 5 minutes in a sonication water batch to adequately separate any particle aggregates. A 0.100-ml particle suspension of each sample was added to and suspended in 0.45 μm pre-filtered 250 ml of Coulter Counter electrolyte with a constant agitation speed of 100 rpm. More than 240,000 particles in four tests from each sample were counted and analyzed for size distribution.

The comparison results of particle size distributions, d10, d50 and d90 by cumulative particle differential volume are shown in Table CSL-3.8 and in Figure CSL-3.18. The results show that the d10, d50, and d90 of the continuous impact milling (TY1 and TY2) are smaller than those from the tandem milling.

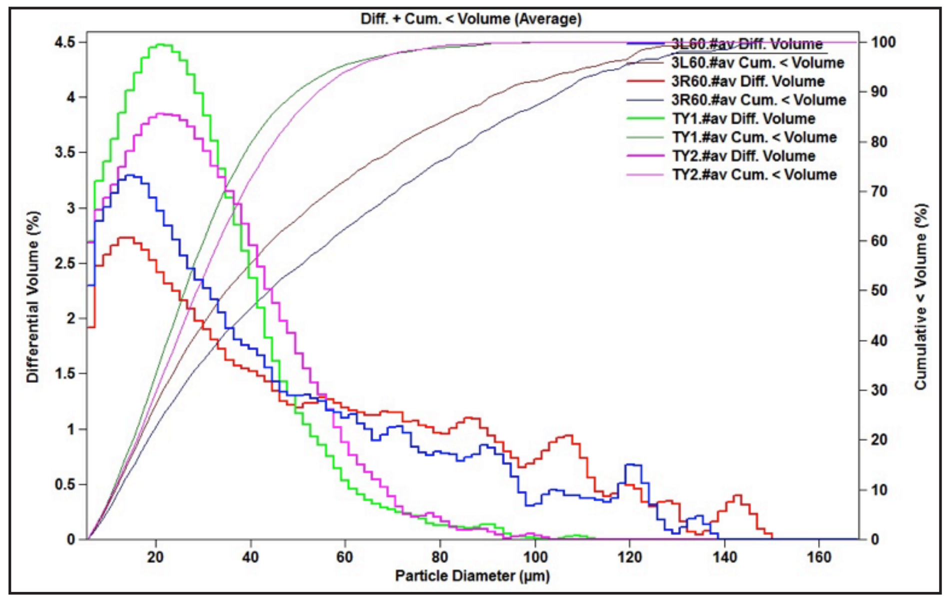

Figure CSL-3.18. Particle size distribution plots by cumulative particle differential volume. L60 and R60: 60 min milling trials in tandem mill in left or right chamber, respectively; TY1 and TY2: two continuous milling trials in impact mill

Table CSL-3.8. Particle size distribution by cumulative particle differential volume

Sample ID.	Milling Mode	Milling Time (min)	d10 (µm)	d50 (µm)	d90 (µm)
1L30	batch, tandem mill	30	12.30	38.50	93.80
2R30	batch, tandem mill	30	10.96	33.64	89.24
3L60	batch, tandem mill	60	11.42	35.06	93.18
4R60	batch, tandem mill	60	12.33	43.70	105.10
7L80	batch, tandem mill	80	11.02	34.27	93.07
8R80	batch, tandem mill	80	11.98	43.93	108.40
11L100	batch, tandem mill	100	12.15	37.76	80.21
12R100	batch, tandem mill	100	13.41	50.56	111.84
TY1	Continuous, impact mill	NA	10.77	26.13	49.90
TY2	Continuous, impact mill	NA	11.11	28.75	54.42

PARTICLE IMAGING BY SCANNING ELECTRON MICROSCOPE

Besides particle size distribution analysis, particle morphologies were imaged using a scanning electron microscope (SEM). Figures CSL-3.19a (tandem-milled sample, left chamber), CSL-3.19b (tandem-milled sample, right chamber), and CSL-3.19c (continuously impact-milled) show that the continuous impact-milled FS-10 wood particles have distinct and different particle morphology from the batch tandem-milled FS-10 wood particles. The batch tandem-milled wood particles are smoothly edged or roundish while the continuous impact-milled wood particles are flaky. The flaky morphology of the continuous impact-milled wood particles probably have much higher cellulose crystallinity, which can be further analyzed by Infrared spectroscopy and enzymatic hydrolysis of the milled wood particle samples.

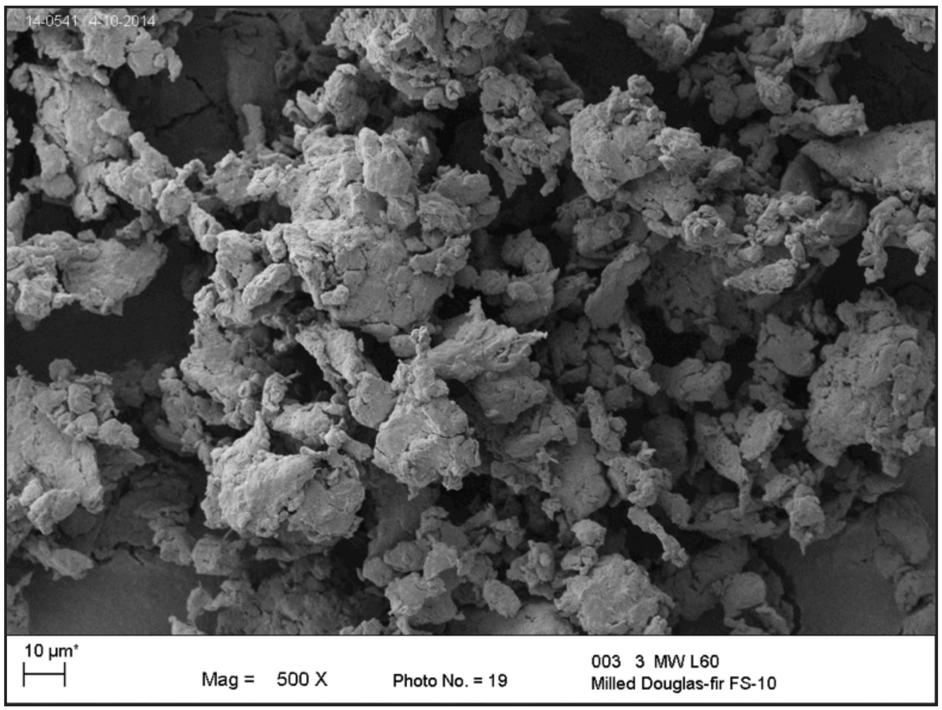

Figure CSL-3.19a. SEM particle image of left chamber 60-minute tandem-milled FS-10 particles

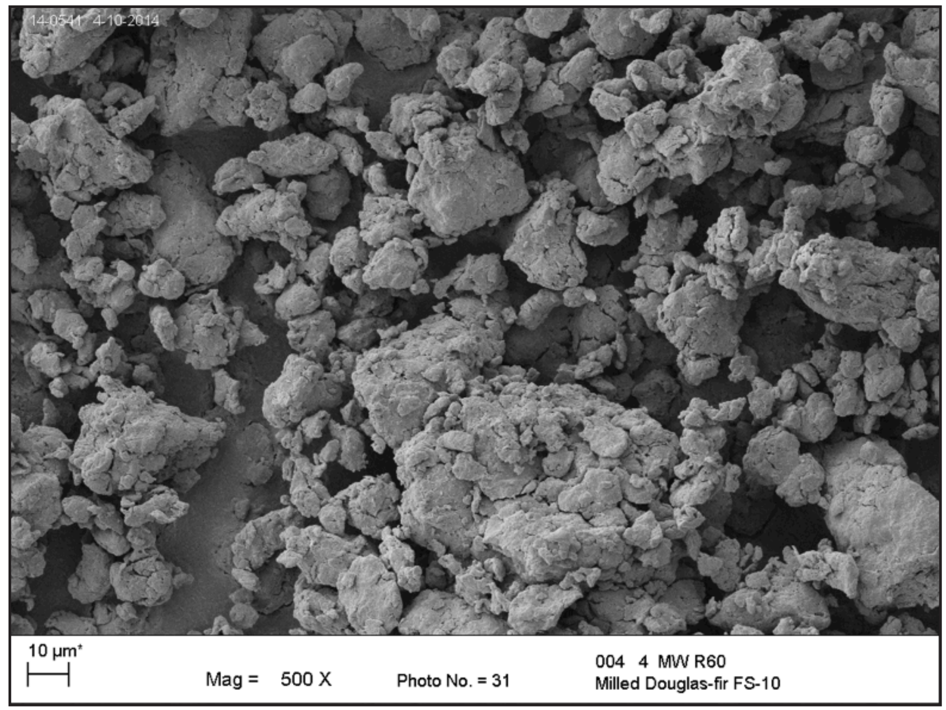

Figure CSL-3.19b. SEM particle image of right chamber 60-minute tandem-milled FS-10 particles

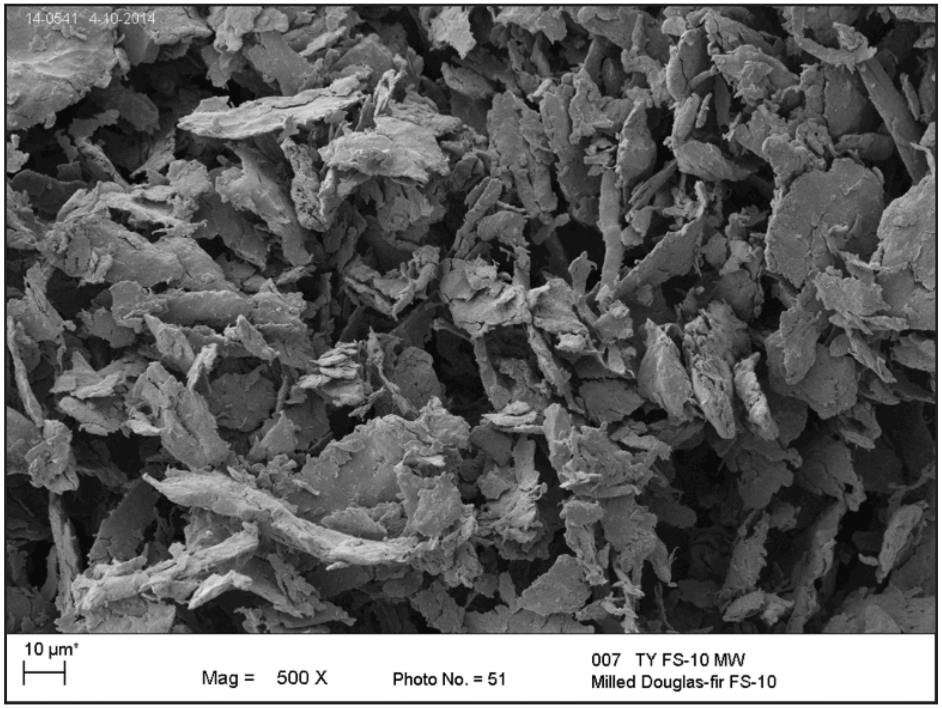

Figure CSL-3.19c. SEM particle image of continuously impact-milled FS-10 particles

CRYSTALLINITY ANALYSIS BY INFRARED SPECTROSCOPY

An infrared (IR) spectroscopy with a diamond ATR (attenuated total reflectance) accessory was used to compare the crystallinity of the tandem-milled FS-10 sample and the impact-milled FS-10 sample against the non-milled FS-10 chips. The IR spectra are shown in Figures CSL-3.20a and CSL-3.20b. By comparison, it is noted in the IR spectra that there is significant transmittance (T%) difference at the IR wave number at, for example, 1316 (1/cm), between the milled FS-10 and the un-milled 2 mm FS-10 chips. Nelson and O'Connor used index values at an unambiguous wave number 1372 (1/cm) vs. at 893 (1/cm) to assess the cellulose I and II crystallinity (Nelson and O'Connor, 1964). The difference wave number was caused probably by the material differences between FS-10 lignocellulosic materials and the cellulose I/cellulose II.

The changes in crystallinity before and after tandem and impact milling of FS-10 chips are estimated by the IR absorbance ratio of 1316 (1/cm) to 897 (1/cm). The results in Table CSL-3.9 show that the IR absorbance ratios of the tandem-milled FS-10 samples drop significantly from 1.120 (non-milled FS-10 chips) to 0.180 and 0.168 by the 60-minute left chamber and the right chamber tandem milling, respectively. However, the ratio of the continuously impact-milled FS-10 sample only drops to 0.458, which is smaller than the non-milled chips but larger than the tandem-milled samples. Thus, it is estimated that the continuously impact-milled sample has higher crystallinity than the tandem-milled FS-10 samples.

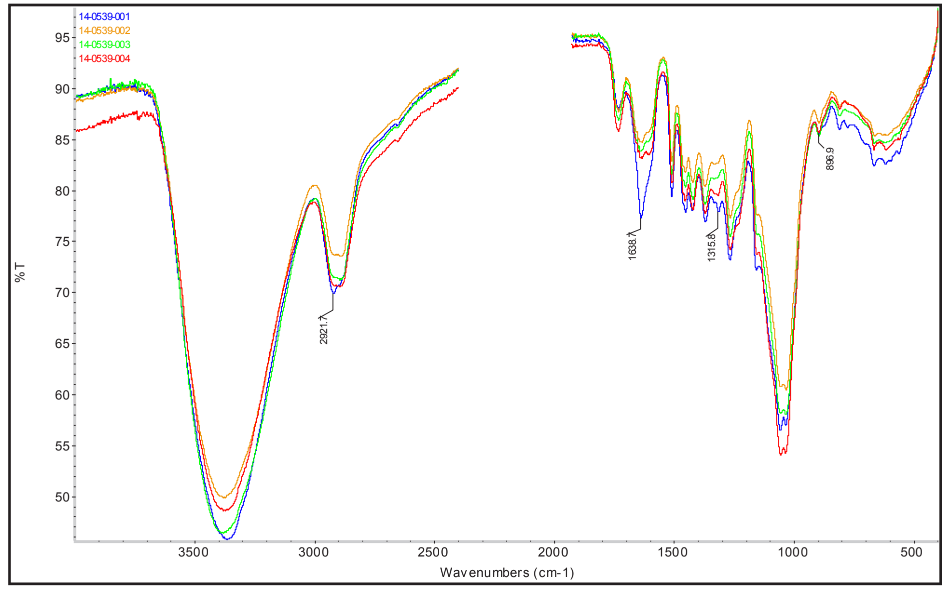

Figure CSL-3.20a. Full IR spectra of 2 mm FS-10 chips (14-0539-001), left chamber 60-minute tandem-milled FS-10 sample (14-0539-002), right chamber 60-minute tandem-milled FS-10 sample (14-0539-003), continuously impact-milled FS-10 sample (14-0539-004)

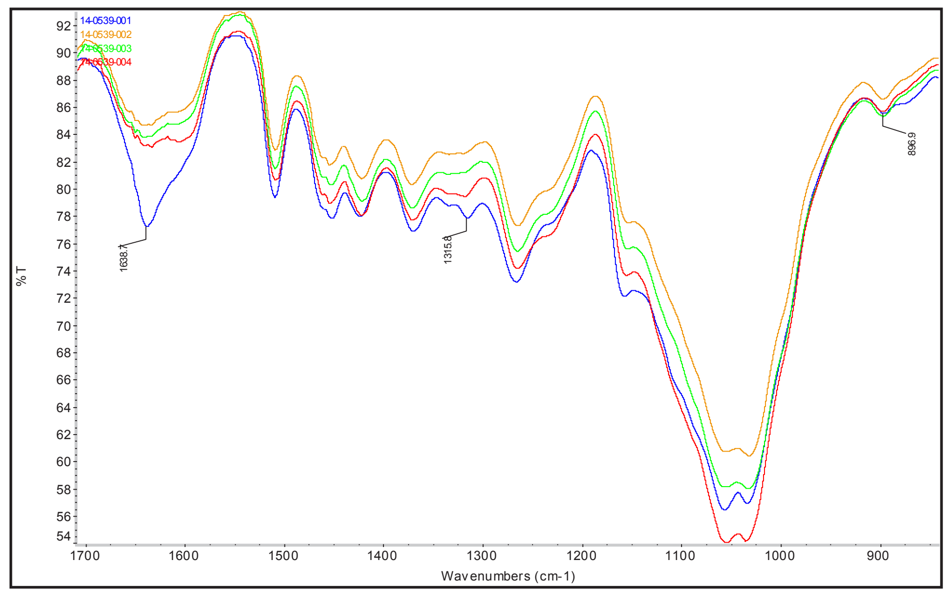

Figure CSL-3.20b. Partial IR spectra of 2 mm FS-10 chips (14-0539-001), left chamber 60-minute tandem-milled FS-10 sample (14-0539-002), right chamber 60-minute tandem-milled FS-10 sample (14-0539-003), continuously impact-milled FS-10 sample (14-0539-004)

Table CSL-3.9. Infrared absorbance ratio of 1316 (1/cm) to 897 (1/cm) of FS-10 samples

Sample	Sample ID	Infrared Ratio* a_{1316}/a_{897}
2 mm Chips	14-0539-001	1.120
60-min tandem-milled, left chamber	14-0539-002	0.180
60-min tandem-milled, right chamber	14-0539-003	0.168
Continuously impact-milled	14-0539-004	0.458

*The ratio is calculated by the net absorbance peak height at 1316 cm-1 divided by the height at 897 cm-1.

COMPOSITION OF MILLED WOOD

All the small scale tandem-milled FS-10 milled wood sugar compositions were analyzed and are shown in Table CSL-3.10. Results show that there is small sugar loss after the milling process. Some samples were analyzed for lignin and the lignin composition shows some proportional increase, due to small sugar loss in the milled samples, as shown in Table CSL-3.11.

The large-scale tandem-milled FS-01 milled wood sugar compositions were analyzed and are shown in Table CSL-3.12. The large-scale tandem mill is the improved HV-70Av with revised tandem milling rings. Results show that there is very small variation of sugar composition among the milled samples.

Table CSL-3.10. FS-10 milled wood carbohydrate composition under various milling conditions

FS-10 Sample ID	Milling Time (min)	Total Polymer Sugar (%)	Loss (%)
2 mm chips	0	60.40	NA
1.L30	30	59.85	0.91
2.R30	30	60.28	0.20
3.L60	60	59.10	2.15
4.R60	60	58.48	3.18
7.L80	80	58.99	2.33
8.R80	80	58.41	3.29
11.L100	100	58.85	2.57
12.R100	100	58.92	2.45

Table CSL-3.11. FS-10 milled wood lignin composition under various milling conditions

FS-10 Samples	Milling Time (min)	Total Lignin (%)	Increase (%)
2 mm chips	0	27.34	NA
L/R 60	60	27.96	2.2
L/R 100	100	28.23	3.3

Note: L-left milling chamber; R-right milling chamber; L/R-left and right data average

Table CSL-3.12. FS-01 milled wood carbohydrate composition under various milling volume conditions

FS-01 Sample ID	Chamber No.	Chamber Volume Load (%)	Total Polymer Sugar (%)	Ash (%)
FS-01 chips	NA	NA	62.5	0.20
1 – 12 kg EW	E&W	9	63.2	0.33
2 – 24 kg E	E	19	63.2	0.26
3 – 36 kg E	E	28	63.3	NT
3 – 36 kg W	W	28	63.1	0.31
4 – 48 kg E	E	37	63.8	NT
4 – 48 kg W	W	37	62.8	NT

HYDROLYSIS TEST AND OPTIMIZATION OF TANDEM-MILLED WOOD

Enzymatic hydrolysis of milled FS-10 was first tested by Akita Prefectural University. The hydrolysis was conducted at a low solid of 2% consistency with 5% Meicelase enzyme product (solid enzyme product) on wood powder. The hydrolysis results at 48 hours are shown in Figure CSL-3.21 that is provided by Akita Prefectural University. The low solid loading at 2% consistency achieves saccharification efficiencies or yields of holocellulose (cellulose and hemicellulose), 67% by 30 minute milling and 71-81% by 60 minute milling, respectively, as shown in Figure CSL-3.21.

The hydrolysis of higher solid loading at 10% consistency was conducted at the Weyerhaeuser Technology Center. The hydrolyses achieved saccharification efficiencies of holocellulose, 53-56% by 30 minute milling and 56-72% by 60 minute milling, as shown in Figure CSL-3.22. Milling longer at 80-100 minutes only shows slight yield increase and the total yield ranges from 60-75%, as compared to 60 minute milling. The yields are based on the initial FS-10 feedstock composition.

The small scale FS-10 milled Douglas-fir was also hydrolyzed at 20.6% consistency by 6% CTec2 and 0.6% HTec2 enzyme dose on milled wood, achieving hydrolysis yield at 58% at 4 day hydrolysis with total sugar titer of 7.7 %(wt/wt) shown in Figure CSL-3.23. Hydrolysate fermentation by ethanol yeast can achieve 4.2% (wt/wt) ethanol at 24-hr fermentation. More hydrolysis optimization will be conducted in NARA's 4th trimester.

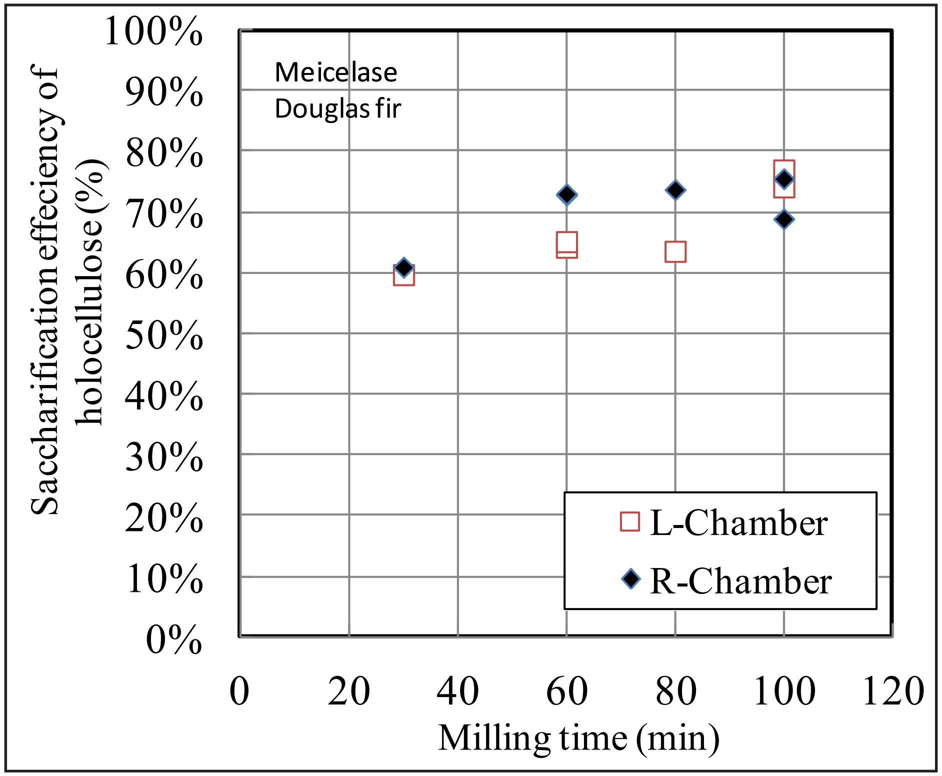

Figure CSL-3.21. Enzymatic hydrolysis efficiency or yield of low solid concentration at 2% consistency with 5% Meicelase enzyme product on wood powder. Hydrolysis time = 2 days

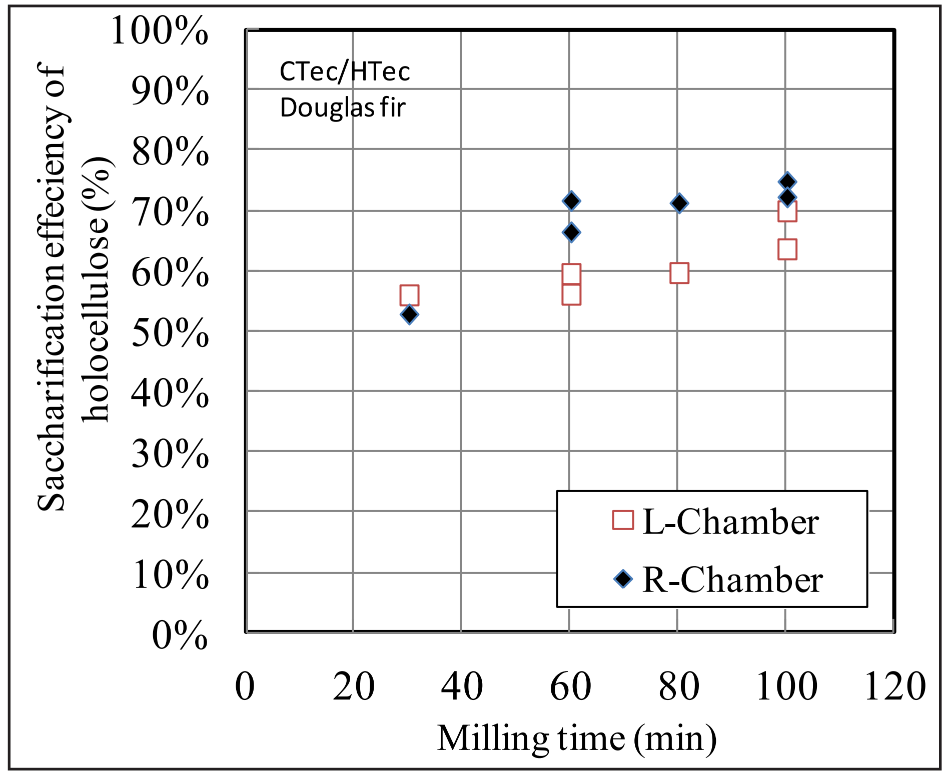

Figure CSL-3.22. Enzymatic hydrolysis efficiency or yield of higher solid concentration at 10% consistency with 4.4% CTec3/HTec3 enzyme product on wood powder. Hydrolysis time = 4 days

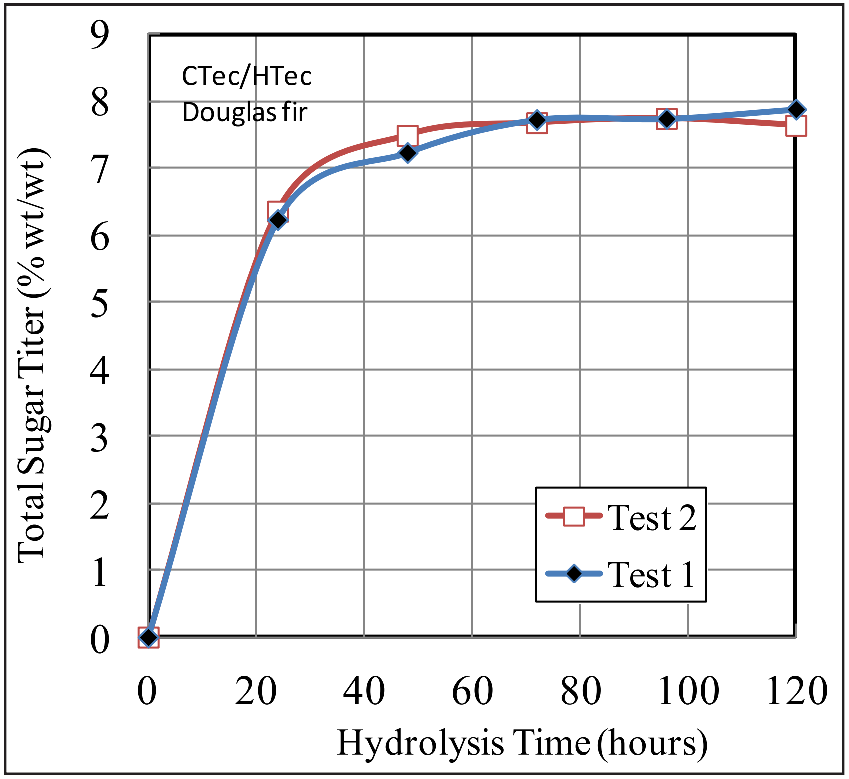

Figure CSL-3.23. The small scale milled Douglas-fir FS-10 hydrolysis sugar titer profile. Conditions: milled wood at 20.6% (wt/wt) from 60-minute milling sample; milled wood sugar composition at 58.48%; enzyme dose at 6% CTec2 and 0.6% HTec2 enzyme product on milled wood; pH at ~5.0-5.2; temperature at 50°C; shaking at 200 rpm

Enzyme dosage effects on hydrolyzing tandem-milled FS-10 was also studied. Table CSL-3.13 shows the saccharification yields of various milled wood loading under various enzyme dosages. CTec3 and HTec3 enzyme products were used as percent of enzyme products on OD milled wood weight. At 96-hour hydrolysis tests, at both 10% and 19% solid loading, 3.5% total enzyme dose on milled wood can achieve similar saccharification yields of 57.6 % and 54.6%, respectively, with only about 3% drop when the solid increases from 10% to 19%. With 5.5% total enzyme dose on milled wood, the hydrolysis yields at 96 hours are 62.3%, 57.1% and 60.0%, respectively for solid loads of 10%, 19% and 21%, without significant differences. At 6.6% enzyme dose, the 21% solid hydrolysis had a hydrolysis yield of 59.7%. Therefore, the enzyme dose at about 5.5% is considered suitable for the hydrolysis of high solid range around 19-21%. Figure CSL-3.24 shows the plots of the enzyme dose vs. milled wood saccharification yield.

At high solid hydrolysis at 19% or above with low enzyme dosage, such as 1.5% and 3.5% enzyme on solid, it should be noted that the initial hydrolysis became gelatinized around 24 hours and difficult for mixing. The hydrolysis became much more liquefied around 72 hours. Therefore, it would be beneficial to use fed-batch hydrolysis to lower the initial viscosity of the high solid hydrolysis.

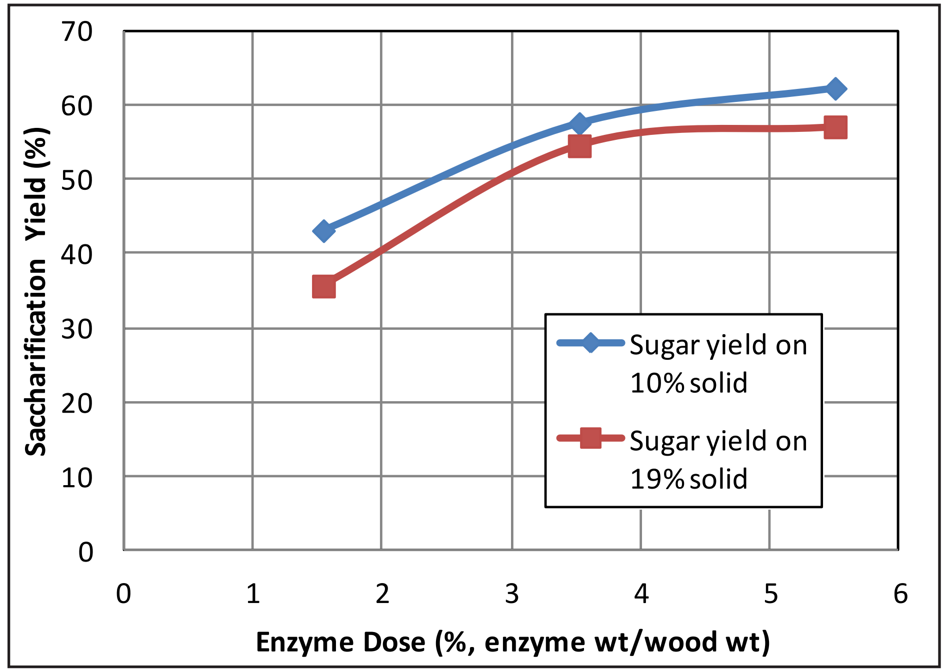

Figure CSL-3.24. The tandem-milled wood saccharification yields on various enzyme dosages

FED-BATCH HYDROLYSIS WITH HIGH SOLID LOADING

To achieve higher sugar titer in the milled wood hydrolysis, a higher solid loading is required. In light of the high viscosity at high solid loading, a fed batch approach can be used. This test used an initial solid loading of 15%. After 48-hour enzymatic hydrolysis, additional 10% solid was added along with proper amount of enzyme loading on solid. In this test, a total of 6.6% enzyme on solid was used since a total of 25% solid was used. The sugar titer is shown in Figure CSL-3.25. With the initial 15% solid load, the sugar titer achieved 5.4% at 48-hour hydrolysis. After additional 10% solid addition, the hydrolysis could reach 9.0% at 96-hour hydrolysis. The sugar yield at 96-hour hydrolysis is 55.5%. After that, the sugar titer leveled off at 10.1% with a sugar yield of 62% at 168 hours.

HYDROLYSIS OF LARGE-SCALE TANDEM-MILLED FS-01

The large-scale tandem-milled FS-01 wood samples were hydrolyzed at high solid contents of 19% and 17.5%. The results are shown in Table CSL-3.14. With good loading of wood in the tandem milling, the sugar yields are 42.2% and 49.1%, respectively for the 24-kg and 36-kg milling trials.

Table CSL-3.13. Enzymatic hydrolysis yields of various tandem-milled FS-10 samples at different hydrolysis solids with various enzyme dosages. Note that HTec3 is 10% of the added CTec3 on loaded milled wood samples

Test	Solid Load (%)	CTec3 (wt/wt %)	HTec3 (wt/wt %)	72-hr Hydrolysis Yield (%)	72-hr Sugar Titer (%)	96-hr Hydrolysis Yield (%)	96-hr Sugar Titer (%)
1	10	1.4	0.14	42.2	2.7	43.1	2.8
2	10	3.2	0.32	56.2	3.7	57.6	3.7
3	10	5.0	0.50	60.4	3.9	62.3	4.1
4	19	1.4	0.14	34.1	4.3	35.7	4.5
5	19	3.2	0.32	49.3	6.1	54.6	6.8
6	19	5.0	0.50	55.5	6.8	57.1	7.0
7	21	5.0	0.50	56.9	7.9	60.0	8.4
8	21	6.0	0.60	56.7	7.8	59.7	8.2

COMPOSITION OF HYDROLYZED RESIDUALS

After enzymatic hydrolysis and fermentation of the small scale tandem-milled FS-10, the residuals of hydrolysis and fermentation were analyzed for sugar and lignin content after the residuals were washed three times with deionized water to remove any monomeric sugars. The remaining polymer sugar in the residuals are shown in Table CSL-3.15. The results show that the milled wood residuals after enzymatic hydrolysis or hydrolysis and fermentation had significant increased lignin content to about 47-54% in comparison to its original lignin content of about 28%. At enzyme dose of 5.5-6.6%, the original milled-wood polymer sugar reduced to 30-37% from its original 59%. Due to high lignin content in the residuals, the heating value is very high, ranging from 9220- to 9460 Btu/lb dry residual solids.

HYDROLYSIS TEST OF IMPACT-MILLED WOOD

Two trials of continuous impact-milled FS-10 samples TY1 and TY2 were hydrolyzed at 10 and 15% solid loading with 5.5% total enzyme on solid. The results in Table CSL-3.16 show that the hydrolysis yields are very low, less than 15%, in both milled FS-10 samples, although the mean particle sizes are smaller in the impact-milled particles than the tandem-milled particles as previously shown in Table CSL-3.8. In addition, the crystallinity analysis previously shown in Table CSL-3.9 indicates that the impact-milled FS-10 sample has much higher crystallinity than the tandem-milled FS-10 samples. The higher crystallinity of the continuously impact-milled FS-10 explains the much lower saccharification yields.

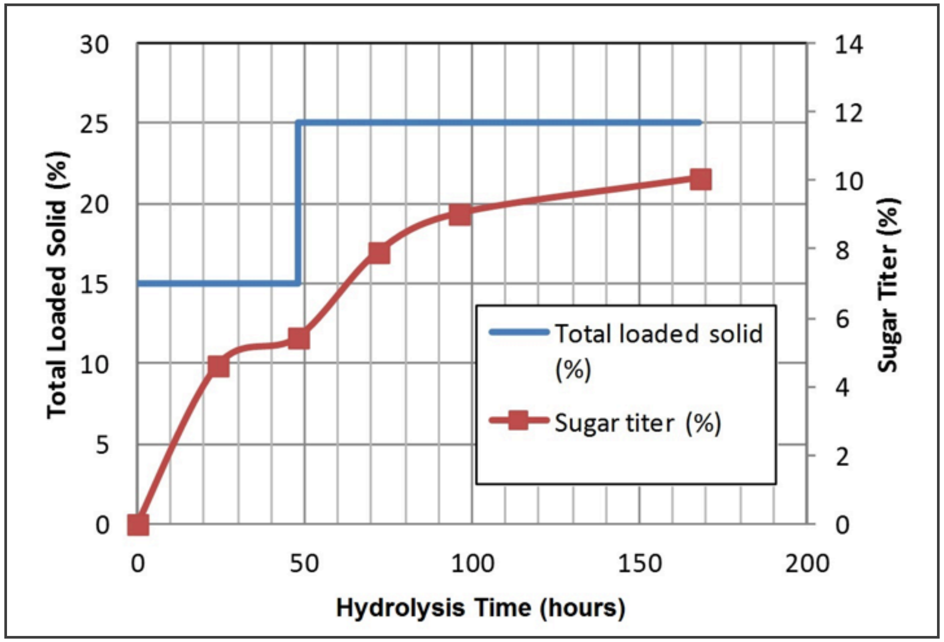

Figure CSL-3.25. Sugar titer profile of fed-batch hydrolysis of 25% tandem-milled FS-10

Table CSL-3.14. Enzymatic hydrolysis yields of various tandem-milled FS-01 samples at different hydrolysis solids with various enzyme dosages. Note that HTec3 is 10% of the added CTec3 on loaded milled wood samples

Test	Solid Load (%)	CTec3 (wt/wt %)	HTec3 (wt/wt %)	72-hr Hydrolysis Yield (%)	72-hr Sugar Titer (%)	96-hr Hydrolysis Yield (%)	96-hr Sugar Titer (%)
1. 12 kg EW	19.3	5.0	0.50	59.4	7.9	60.7	8.1
2. 24 kg E	17.5	5.0	0.50	47.0	5.8	49.1	6.0
3. 36 kg W	17.5	5.0	0.50	40.3	5.0	42.2	5.2
4. 48 kg W	17.5	5.0	0.50	36.0	4.4	36.8	4.5

Table CSL-3.15. Residual composition of enzymatically hydrolyzed and yeast-fermented of tandem-milled FS-10 wood under various solid loading and enzyme doses in hydrolysis

Test	Solid Load (%)	Total Enzyme Dose (%) on Wood	Process	Polymer Sugar in Residuals (%)	Total Lignin in Residuals (%)	Btu of Residuals (btu/lb)	Sulfur in Residuals (%)
5	19	3.5	hydrolysis	38.8	46.5	9220	NT
6	19	5.5	hydrolysis	36.6	48.2	9255	NT
7	21	5.5	hydrolysis	36.8	47.6	9160	NT
8	25	6.6	fed-batch hydrolysis	36.0	47.5	9235	NT
9	10	5.5	hydrolysis & fermentation	34.9	50.9	9280	0.055
10	20	6.6	hydrolysis & fermentation	30.4	54.1	9460	0.045

NT: not tested.

Table CSL-3.16. Enzymatic hydrolysis yields of two impact-milled FS-10 samples at two hydrolysis solids with 5.0% CTec3 and 0.5% HTec3 on the solid

Test	Solid Load (%)	CTec3 (wt/wt %)	HTec3 (wt/wt %)	72-hr Hydrolysis Yield (%)	72-hr Sugar Titer (%)
1-TY1	10	5.0	0.50	14.6	0.9
2-TY1	15	5.0	0.50	13.3	1.7
3. TY2	10	5.0	0.50	12.8	0.8

TASK 4: TECHNO-ECONOMIC ANALYSIS (TEA) FOR CLEAN SUGAR AND LIGNIN PRODUCTION

The actual energy consumption for tandem wood milling are at 1.3632 and 2.0748 kWh/OD kg wood, respectively for high solid milled wood saccharification yields of 42.2% and 49.1%. The total energy consumption from wood chipping to hydrolysate production are 2.2459 and 2.9574 kWh/OD kg wood, including both electricity and natural gas. These numbers are used for the techno-economic analysis (TEA) and evaluation for clean sugar and lignin production from Douglas-fir residual chips.

With the NARA depot concept, a depot sized sugar and lignin fuel pellet plant is assumed to process annual softwood wood residuals of 222,000 bdt/year. The techno-economic analysis is conducted for this depot size plant. Natural gas is used for wood drying. The plant parameters are summarized in Table CSL-4.1, and the commodities cost or product sale price information is shown in Table CSL-4.2.

Table CSL-4.1. Sugar production cost under various assumptions

Items	Amount
Biomass Input (bdt/yr))	222,000
Operation days (/yr)	330
Plant staff (FTE)	42
Sugar yield from biomass (%)	42.2%
Sugar output on dry basis (bdt/yr)	62,866
Lignin fuel pellet on dry basis (bdt/yr)	165,415
Total Capital	$10,122,140
Installed Capital	$20,244,280
With 20% Contingency	$24,293,135
With 20% OSBL	$29,151,763
With 10% owner's cost	$32,066,939
Annual Capital Recovery Rate (%)	12%
Annual Capital Recovery ($/yr)	$3,848,033
Annual Operation and Labor ($/yr)	$5,045,612
Annual Feedstock Cost ($/yr)	$14,430,000
Grant Annual Expense ($/yr)	$23,323,645

Table CSL-4.2. Commodities cost information for the TEA

Items	Cost/Sale Price ($)
Biomass cost at depot door ($/bdt)	65
Electricity cost ($/kWh)	0.0401
Natural gas cost ($/MMBtu)	4.5
High lignin fuel pellet bulk price ($/lb)	0.1066
Sugar price (dry basis equivalent) ($/lb)	0.1696

A cost stack bar chart is shown in Figure CSL-4.1 for the sugar cost with lignin fuel pellet as co-product. The total plant cost is $32.1 million for the production of 62,866 bdt sugar/year and 165,415 bdt lignin fuel pellet/year, with an annual expense of $23.3 million. The lignin fuel pellet has a good credit of $0.2060 per lb of sugar produced.

The sugar product cost is very competitive at $0.1165/lb when the feedstock price is at $65/bdt. At the current market sugar price of $0.1696/lb, the net profit would be $0.0529/lb sugar or a total profit of $6,656,387/yr, indicating a good TEA case.

The feedstock cost is very significant in sugar production, and the production cost can be reduced to $0. 0902/lb if the feedstock price can be reduced from $65/bdt to $50/bdt. In certain locations, if the feedstock cost is $40/bdt, the sugar production cost can be as low as $0.0725/lb. At a higher tandem milling consumption of 2.0748 kWh/OD kg to achieve a higher saccharification yield of 49.1%, the sugar cost can be as low as $0.1223/lb sugar when the feedstock cost is only $40/bdt. Various cases of cost analyses are summarized in Table CSL-3.3.

Further, the low sulfur lignin may partially replace the commercial bunker oil in industrial boiler systems, but more work is needed in this area. The bunker oil usage worldwide is about 200,000 barrels per day or 73,000,000 barrels per year (International Energy Statistics, Bunker Fuels, 2012, EIA, http://www.eia.gov). The low sulfur green lignin fuel could potentially penetrate into the bunker oil market and provide a sustainable solution for the low sulfur lignin co-product. The partial replacement of bunker fuel by 10% and 30% lignin fuel would need, respectively, about 2.3 and 7.0 million metric tons of lignin residuals per year.

Table CSL-4.3. Sugar production cost under various assumptions

Case No.	Feedstock Cost ($/bdt)	Tandem Milling Energy (kWh/OD kg wood)	Sugar Yield (%)	Sugar Production Cost ($/lb)
1	65	1.3632	42.2	0.1165
2	50	1.3632	42.2	0.0902
3	40	1.3632	42.2	0.0725
4	65	2.0748	49.1	0.1602
5	50	2.0748	49.1	0.1375
6	40	2.0748	49.1	0.1223

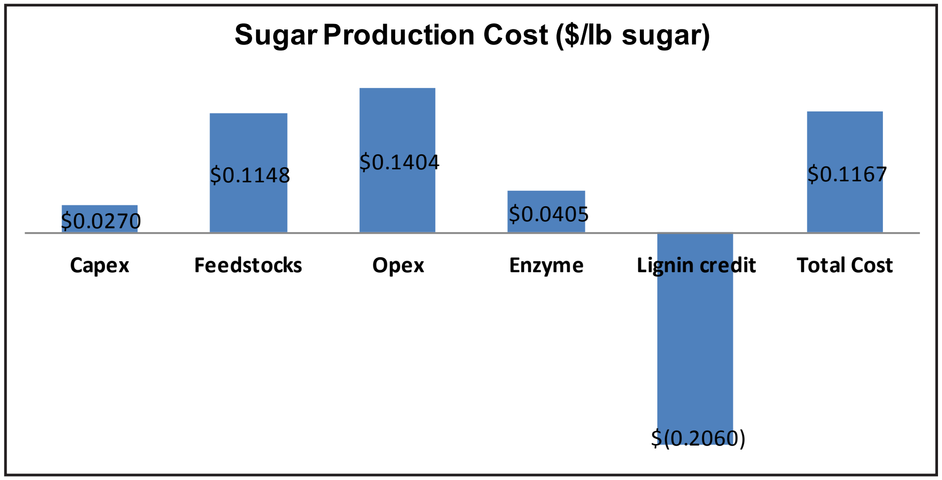

Figure CSL-4.1. Sugar production cost stack bar chart. Analysis conditions: feedstock cost at $65/ton; plant size 222,000 bdt wood/year; sugar yield from milled wood 42.2%; high lignin fuel pellet selling price at $0.1066/lb; NW electricity cost at $0.0401/kWh; installed capital with additional 20% contingency cost, 20% OSBL cost and 10% owner's cost; annual capital recovery at 12%; sugar and lignin plant staffed with 42 FTE; wood drying by natural gas price at $4.50/MMBtu; operated for 330 days/year

CONCLUSIONS

The large-scale tandem milling of Douglas-fir wood has been completed. The energy consumptions of steps from wood chipping to fine wood milling and to sugar hydrolysate production are estimated and used for the techno-economics analysis (TEA) of low sulfur green fuel pellet and sugar production. Based on the current market price of wood fuel pellet and sugar, the TEA indicates that the economics look very plausible for clean lignin fuel pellet and lignocellulosic sugar production by wood milling as a pretreatment technology.

The potential global warming gas emission is minimal in this pretreatment technology. No chemical is used in the pretreatment. No water is needed in wood milling pretreatment technology. Wood milling could become a unique depot size technology for biomass pretreatment and for sugar and lignin fuel production.

The clean sugar and lignin pretreatment technology by wood milling can produce excellent hydrolysate with almost no impact on isobutanol organism growth and with enhancement on isobutanol production, all without any delay in isobutanol production. This indicates that clean sugar from milled wood can be widely used in various and other bioconversion processes for high value bio-based product production.

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NARA OUTPUTS

RESEARCH PRESENTATIONS

Poster Presentation at NARA Annual Meeting in Corvallis, OR in September 2013: Mild Bisulfite Pretreatment, by Dwight Anderson and Johnway Gao

Gao, Johnway; Anderson, Dwight; "Optimization of Mild Bisulfite Pretreatment on Softwood," Harvesting Clean Energy Conference, Helena, Montana, February 4-6, 2014.

Gao, Johnway; Anderson, Dwight; "Optimization of Mild Bisulfite Pretreatment on Pacific Northwest Softwood," Northwest Wood-Based Biofuels and Co-Products Conference, Seattle, Washington, April 28-30, 2014.

SAMPLES

- In September 2013, 100 kg FS-10 chips were reduced to 2 mm mean size and this size reduction energy consumption was tested in Forest Concepts LLC, Auburn, WA.
- In October 2013, 10 kg FS-10 2 mm size chips were milled into powder by tandem milling machine in Akita Prefectural University
- In November 2013, 5 liters of milled wood hydrolysate was sent to Gevo, Inc. for fermentation test.
- In November 2013, 1.1 kg (oven dry based) of un-fermented hydrolyzed FS-10 milled wood residuals were sent to the NARA Co-Product team for lignin evaluation.
- In November 2013, 100 kg FS-10 chips were shipped to Zhengzhou Tianyuan Environmental Protection Machinery Co., Ltd for some wood milling tests. Samples were back and to be report in the NARA 3rd trimester report.
- In January 2014, 0.75 kg (oven dry based) of fermented hydrolyzed FS-10 milled wood residuals were sent to the NARA Co-Product team for lignin evaluation.
- In March 2014, additional 1.8 liters of milled wood hydrolysate was sent to Gevo, Inc for GIFT™ test.
- In March 2014, 2250 lbs. of FS-10 chips were shipped to Akita Prefectural University for tandem milling optimization studies in the improved HV-10 tandem milling machine.

NARA OUTCOMES

This work established a proof-of concept that wood-milling technology can be used as a pretreatment option for the conversion of forest residuals into alcohols. Based on these results, this work is being further extended at the Composite Materials and Engineering Center (CMEC) at Washington State University under the guidance of Jinwu Wang and Michael Wolcott. Their intent is to design a sugar depot model using milling technology.

FUTURE DEVELOPMENT

The targeted energy consumption by wood milling, which is being optimized, is used for the techno-economic analysis (TEA) for clean sugar and lignin production from wood residual chips. Based on the TEA, if the targeted energy consumption of wood milling is met or achieved within the range of 0.56-0.84 kWh/OD kg wood at a sugar conversion yield of about 60%, the economics by wood milling as a pretreatment technology looks very plausible from clean sugar and lignin fuel.

RECOMMENDATIONS

- Wait for the large scale milling energy consumption results to further calibrate the techno-economics analysis results on sugar production cost.
- Need to characterize and understand more on energy-efficient milling to achieve high yield milling (saccharification) by understanding more in wood particle characterization.
- Need to optimize enzymatic hydrolysis on milled wood.
- Need to understand market feasibility on wood pellet, since the sugar production cost is largely dependent upon the lignin credit (high Btu lignin-rich wood pellet).

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APPENDIX

[Material Redacted]