Lignin-Derived Chemicals from Pulping Liquors

Cooperative Research and Development Final Report

CRADA Number: CRD-17-00714

NREL Technical Contact: Brenna Black

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Technical Report
NREL/TP-2800-80388
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Cooperative Research and Development Final Report

Report Date: May 27, 2021

In accordance with requirements set forth in the terms of the CRADA agreement, this document is the CRADA final report, including a list of subject inventions, to be forwarded to the DOE Office of Scientific and Technical Information as part of the commitment to the public to demonstrate results of federally funded research.

Parties to the Agreement: Sustainable Fiber Technologies

CRADA Number: CRD-17-00714

CRADA Title: Lignin-Derived Chemicals from Pulping Liquors

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Sponsoring DOE Program Office(s):

Office of Energy Efficiency and Renewable Energy (EERE) Bioenergy Technologies Office (BETO)

Joint Work Statement Funding Table showing DOE commitment:

Estimated Costs	NREL Shared Resources a/k/a Government In-Kind
Year 1 Year 2	$324,252 $173,853
TOTALS	$498,105

Executive Summary of CRADA Work:

National Renewable Energy Laboratory (NREL) has developed a robust portfolio for lignin valorization using a "biological funneling" approach, which adopts a microbial process to convert the inherent heterogeneity of lignin-rich streams into single high-value products (Linger *et al.* 2014, 12013; Beckham *et al.* 2016, 40; and Favaro *et al.* 2019, 208). In parallel, Sustainable Fiber

Technologies (SFT) has developed a proprietary, high-pH process to convert non-woody biomass into valuable pulp for myriad biomaterial applications. Therefore, by combining the expertise of both SFT and NREL, this project aimed to valorize co-products from an industrial pulping process of non-woody feedstocks. A detailed analytical characterization of an SFT pulping stream was used to aid the development of a bench-scale separations strategy to isolate a mixture of low molecular weight compounds, and ultimately to aid in bioreactor cultivations used to produce polyhydroxyalkanoate (PHA) biopolymers at titers, rates, and yields necessary for moving towards commercialization.

Summary of Research Results:

The overall goal of this project was to valorize lignin-rich streams from an industrial pulping process of non-woody feedstocks. Specifically, NREL and SFT propose to work together on a collaborative Technology Commercialization Fund project with the following specific aims and major deliverables:

1. Produce batches of 10-100 L of pulping liquor from non-woody feedstocks of commercial interest
2. Characterize SFT lignin streams using cutting-edge, comprehensive analytical approaches including two dimensional heteronuclear single quantum correlation nuclear magnetic resonance (2D HSQC NMR), gel permeation chromatography (GPC), and liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS)
3. Conduct bench-scale molecular weight (MW) fractionation experiments to isolate low MW aromatic and aliphatic compounds for valorization
4. Conduct bioreactor experiments to produce muconate via an engineered microbe and scale to 10 L for best cultivation conditions
5. Isolate and purify muconic acid from the lignin-derived streams

Following adjustments to individual tasks, this project was a successful initial effort that showed exciting potential for commercialization. Merging expertise between NREL and SFT resulted scalable bioreactor production of 40% PHA from bioavailable carbon in the pulping liquor co-product. PHA was collected and isolated to 97% purity, which was suitable for downstream processing as a sustainable replacement for fossil fuel-based thermoplastics. Analytical involvement in the earlier tasks established that the SFT pulping liquor co-product had an optimal composition to produce PHA rather than the initial target of muconic acid. Therefore, the latter tasks were edited accordingly to target the production of PHA. Overall, the swift initial success of valorizing the pulping liquor co-products was attributed to proficiency and experience of the associated teams, however, further biocatalytic optimization and scale-up aligned with techno-economic analysis are suggested for further advancements towards commercialization.

Task 1 – SFT to produce 10 L batches of pulping liquor from a non-woody feedstock of commercial interest; deliver to NREL

Task 1 was successfully completed during the outlined CRADA period of performance.

During the initial efforts of this project, SFT produced 10 L of wheat straw, switchgrass, and an undisclosed biomass pulping liquor via their Phoenix Process™. The pulping liquor was shipped

and successfully received by NREL. Two chest freezers were also purchased and installed for the purpose of long-term storage of this material and any future material received from SFT. It was decided that all alkaline pulping liquor produced by SFT and shipped to NREL was to be frozen to prevent chemical and/or biological changes of the material. Frozen storage was preferred over the addition of a biocide, as biocide would prevent proper characterization and have a profound negative effect on downstream separations and biological upgrading efforts.

Task 2 –NREL to report a full characterization of at least two SFT lignin streams using comprehensive analytical approaches

Task 2 was successfully completed during the outlined CRADA period of performance.

The three alkaline pulping liquors received from SFT in 10 L batches for Task 1 (Wheat straw, WSL; undisclosed biomass, UHP; and switchgrass, SWG) were comprehensively analyzed for their potential in upstream separations and biological upgrading. Compositional, gel-permeation chromatography (GPC), liquid chromatography with UV (LC-UV) and mass spectrometry (LC-MS) detection, gas chromatography (GC-MS), and near magnetic resonance (NMR) analyses were performed.

Initially, compositional analysis was performed to determine fraction insoluble solids (used for mass balance determination), solids, and liquor content (Sluiter, Hames *et al*. 2008 and Salvachúa *et al*. 2020, 290). From this data, it was notable that the lignin content from the solids was quite low compared to the lignin content from the liquors (**Figure 1**). This indicated that more of the lignin was soluble in the SFT pulping liquors, likely as a result of the Phoenix Process™ that was employed. The main constituent in the solids was ash, whereas the liquors contained a greater variety of compounds ranging from lignin, to sugars (**Figure 2**), and acids. All three aforementioned components from the liquors can be potentially utilized in some capacity during biological upgrading.

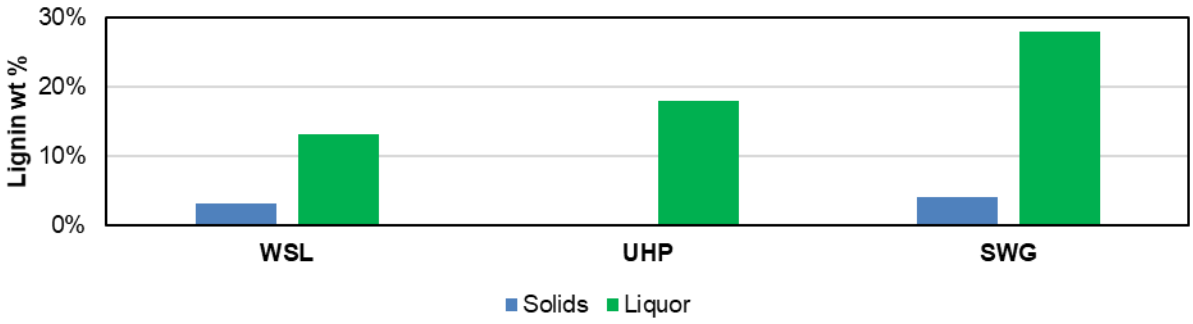

Figure 1. Lignin composition resulting from compositional analysis of the solids and liquor fractions of three SFT pulping liquors. WSL, wheat straw; UHP, undisclosed biomass; SWG, switchgrass.

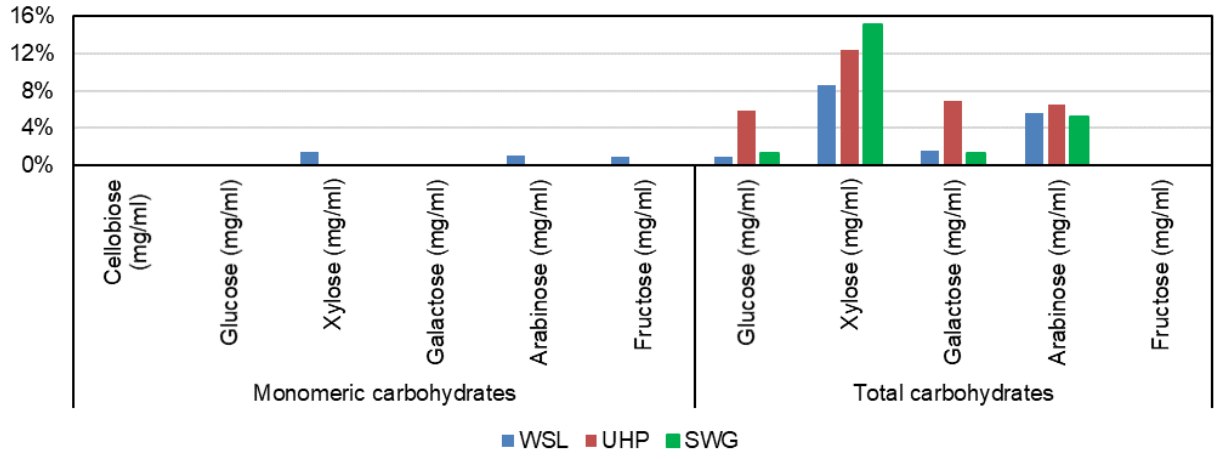

Figure 2. Sugars composition, both monomeric and total carbohydrates, resulting from compositional analysis of the liquor fraction of three SFT pulping liquors. WSL, wheat straw; UHP, undisclosed biomass; SWG, switchgrass.

Traditional organic mobile phase GPC was performed to determine the overall molecular weight distribution of the lignin in the SFT pulping liquors (**Figure 3**). From the resulting chromatograms, the wheat straw and UHP samples contained less monomeric lignin (represented at approximately150-200 Mw) than the switchgrass when comparing the overall lignin molecular weight spread of each sample. The wheat straw contained higher molecular weight lignin compounds overall compared with the other pulping liquors.

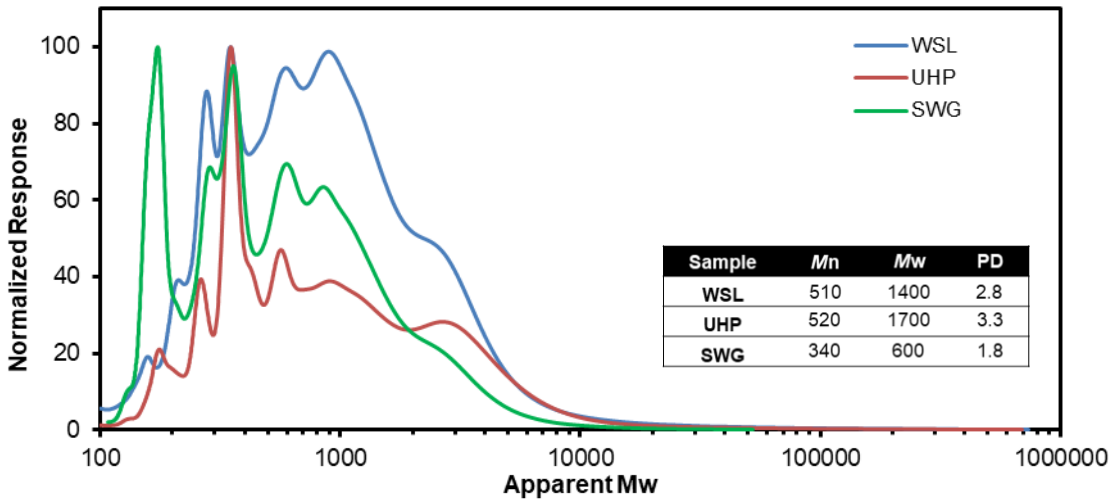

Figure 3. LC-UV$_{280nm}$ chromatogram of lignin molecular weight distribution by GPC separation. WSL, wheat straw; UHP, undisclosed biomass; SWG, switchgrass. *M*n, number average molecular weight; *M*w, weight average molecular weight; PD, polydispersity.

LC-MS/MS (ion trap) was used to initially manually identify monomeric lignin species present from the three pulping liquors. Once the lignin monomers were identified they were then quantified by additional LC-MS/MS (triple quadrupole with multiple reaction monitoring) experiments using authentic standards to determine detector response. The resulting lignin monomers present at or above quantifiable concentrations are presented in **Figure 4**. Lignin monomers identified in the alkaline pulping liquors were: 4-hydroxybenzoic acid, 4-hydroxybenzaldehyde, vanillic acid, syringic acid, vanillin, *p*-coumaric acid, acetovanillone, syringaldehyde, acetosyringone, phenol, and ferulic acid. Lignin monomers are directly biologically upgradable to high value compounds such as muconic acid.

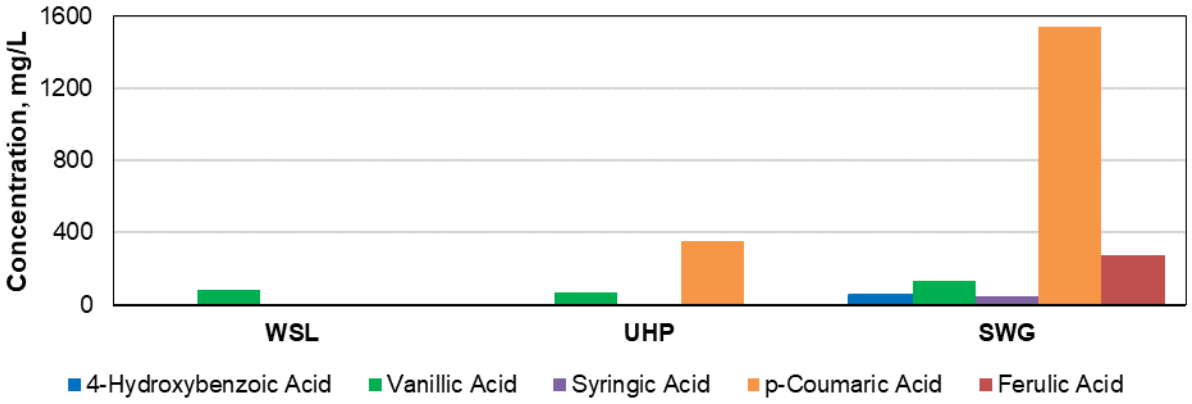

Figure 4. LC-MS/MS quantitation of lignin monomers identified in alkaline pulping liquors. WSL, wheat straw; UHP, undisclosed biomass; SWG, switchgrass.

Acids such as oxalic, malic, succinic, glycolic, lactic, formic, and acetic were also quantified (**Figure 5**) from the SFT pulping liquors by LC-UV_{210nm} to further understand the lower molecular weight fraction profile of the samples. Acids can be utilized as a biological carbon source in upgrading steps, but are not directly convertible to high value products such as muconic acid.

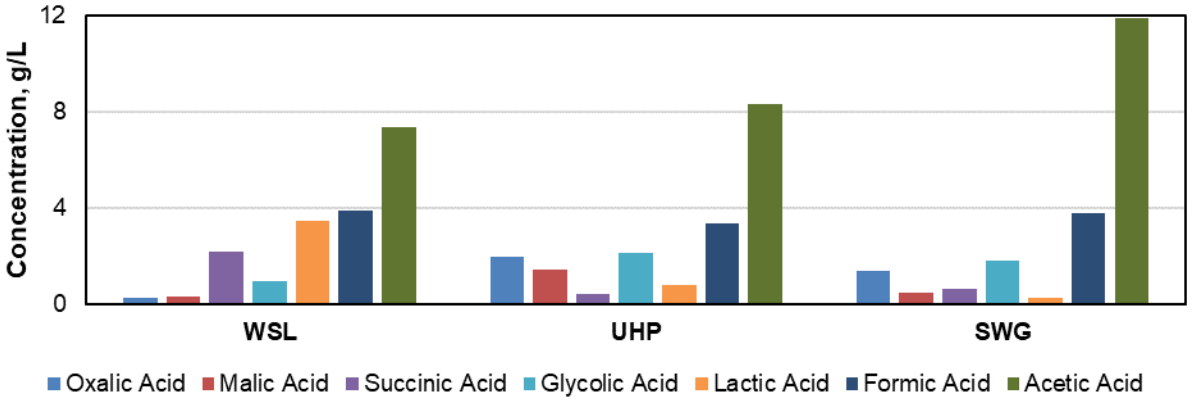

Figure 5. LC-UV quantitation of aliphatic acids identified from the alkaline pulping liquors. WSL, wheat straw; UHP, undisclosed biomass; SWG, switchgrass.

Various NMR analyses were also performed on the SFT pulping liquors, including ^{13}C and ^{1}H-^{13}C HSQC. From the ^{13}C spectra (**Figure 6**), which allows for the detection of carbon functional groups that are not presented in other NMR techniques, aldehyde and carboxylic acid groups were present in all three samples. Peaks outside of these functional group regions were too low in S/N to be properly assigned.

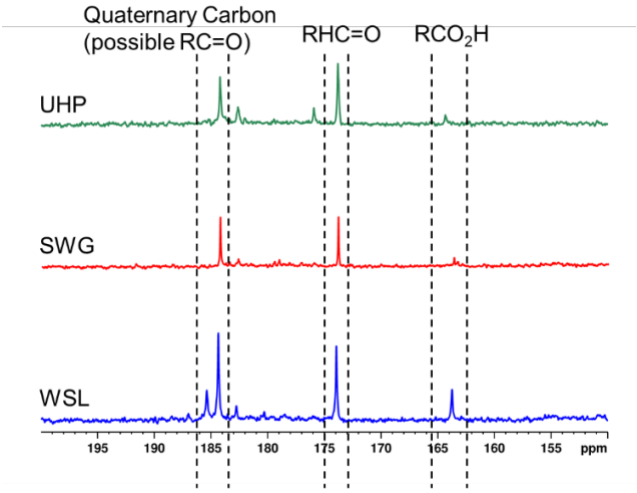

Figure 6. ^{13}C NMR spectra produced from the analysis of the alkaline pulping liquors. WSL, wheat straw; UHP, undisclosed biomass; SWG, switchgrass.

From the ^{1}H-^{13}C HSQC analysis, two spectra can be presented: the aromatic/aldehyde region and the aliphatic region. The aromatic/aldehyde region is presented in **Figure 7**, indicating that of the three SFT alkaline pulping liquors, SWG had the greater concentration of aromatic compounds. WSL was also observed to have an aldehyde region with HSQC NMR. Data from the ^{13}C NMR analysis corroborates this, additionally, an aldehyde region (**Figure 6**) was also detected in the UHP and SWG SFT samples but at a lesser concentration. Additionally, a poplar enzyme extracted lignin sample was also presented as a comparison in **Figure 7** to show usual lignin features detected by HSQC NMR.

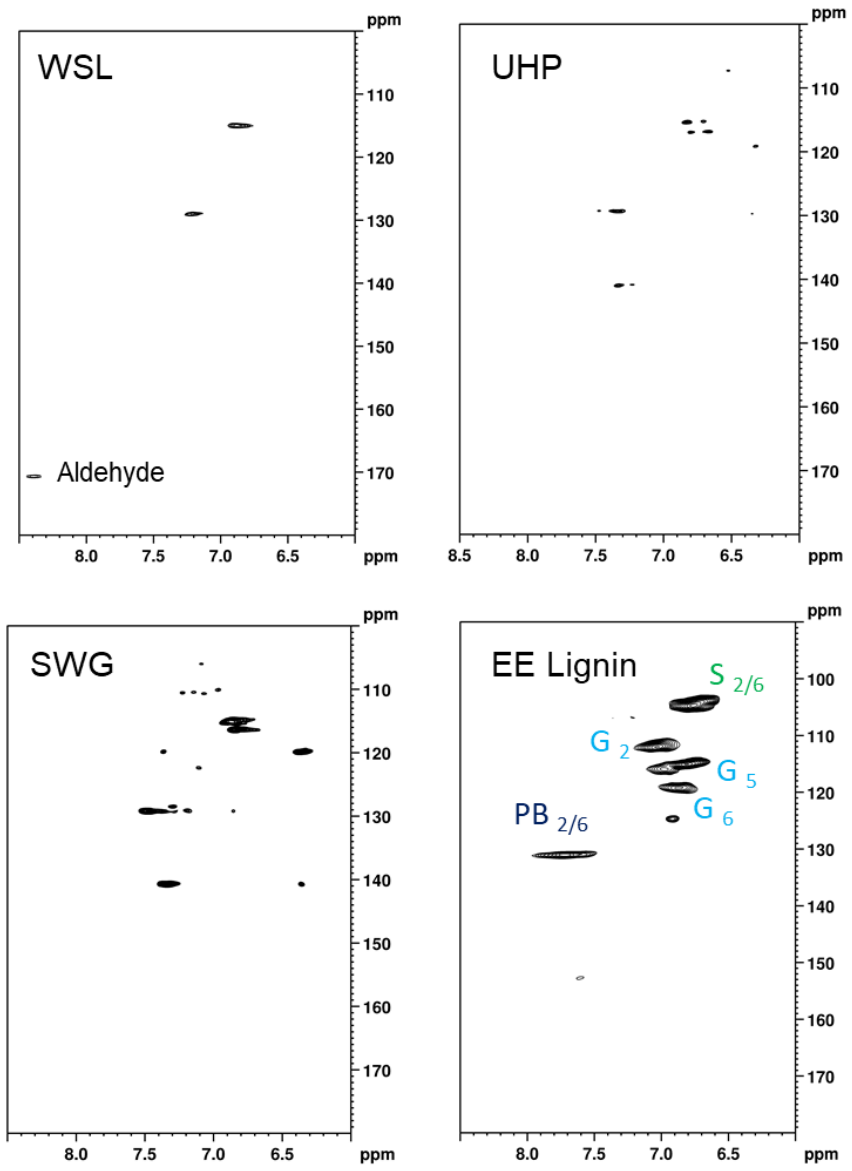

Figure 7. ^{1}H-^{13}C HSQC NMR spectra presenting the aromatic and aldehyde regions produced from the analysis of the alkaline pulping liquors. WSL, wheat straw; UHP, undisclosed biomass; SWG, switchgrass; EE Lignin, poplar enzyme extracted lignin presented for comparison.

The aliphatic region of the ^{1}H-^{13}C HSQC analysis is presented in **Figure 8**, indicated that each of the three SFT alkaline pulping liquors contain acetate, as highlighted with a red square. High acetate levels are confirmed by LC-UV aliphatic acid quantitation. Carbohydrate-type compounds are highlighted with a blue box. A poplar enzyme extracted lignin sample was also presented as a comparison in **Figure 8** to show usual lignin features detected by HSQC NMR.

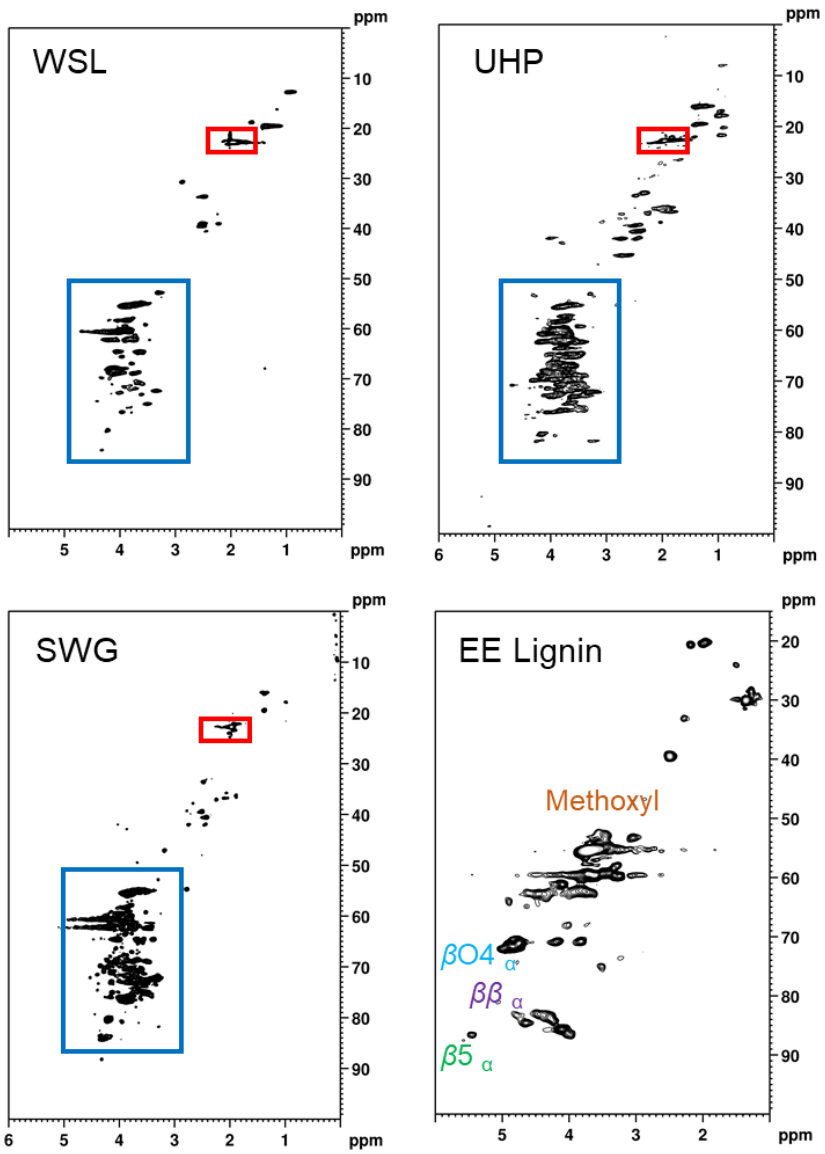

Figure 8. ^{1}H-^{13}C HSQC NMR spectra presenting the aliphatic region produced from the analysis of the alkaline pulping liquors. WSL, wheat straw; UHP, undisclosed biomass; SWG, switchgrass; EE Lignin, poplar enzyme extracted lignin presented for comparison. Acetate is highlighted by a red square; carbohydrate-type compounds are highlighted by a blue box.

Ultimately, for efficient separations and biological upgrading to muconic acid, an abundance of aromatic monomers is necessary. Surprisingly, from the resulting data, none of the three alkaline pulping liquors analyzed consisted of a high lignin monomer composition. Since aromatic monomers are directly biologically converted to muconic acid this will significantly reduce the amount of high value product formed, regardless of the feedstock selected. The switchgrass feedstock contained the highest total aromatic monomer concentration at roughly 2 g/L, and with an expected 75% conversion to muconate, this does not yield appreciable amounts for worthwhile upgrading. Additionally, the wheat straw liquor is the most economically viable for SFT to produce, and this stream contained much less total aromatic monomers than the SWG at 83 mg/L. However, it was noted that regardless of the feedstock, the alkaline liquor streams

comprised high aliphatic acids concentration. Aliphatic acids, as well as aromatic monomers, can be biologically funneled to polyhydroxyalkanoates (PHA), which are a biodegradable substitute for conventional non-degradable plastics (Sluiter, Hyman *et al.* 2008).

Task 3 – Employ bench-scale molecular weight (MW) fractionation experiments to isolate low MW aromatic and aliphatic compounds for valorization; Demonstrate 80% recovery of low MW compounds from lignin

Task 3 was successfully completed during the outlined CRADA period of performance.

The filtration of wheat straw lignin with ceramic membranes appears promising for the isolation of low molecular weight (LMW) lignin derived compounds (both aliphatic and aromatic compounds). In particular, a ceramic nanofiltration membrane system, as described below, was used to recover 82% of LMW compounds from the SFT provided wheat straw liquors.

Initially two ceramic nanofiltration membranes were screened by determining the flux of wheat straw liquor (WSL) at a pressure of 200 psig. The system used for flux analysis is shown in **Figure 9A**. Before nanofiltration, the raw WSL was filtered through a cheese-cloth and 400 micron S.S. screen filter via gravity filtration. During filtration, the permeate mass was recorded as a function of time in order to calculate the WSL flux in LMH (liters per meter squared per hour). As the feed becomes concentrated on the retentate side of the membrane, the flux declines as shown in **Figure 9B**. The flux decline is likely due to membrane fouling and the increased osmotic pressure of the feed as the feed volume is reduced. The ceramic membrane A3T09G (450 Da nominal MWCO - TiO_2 selective layer) was selected for further used to meet the requirements of the current task because of the 3-fold higher initial flux over the A3S1G membrane. Differences in membrane performance is likely due to fabrication and materials used within the selective layer material (TiO_2 vs. SiO_2) of the membrane.

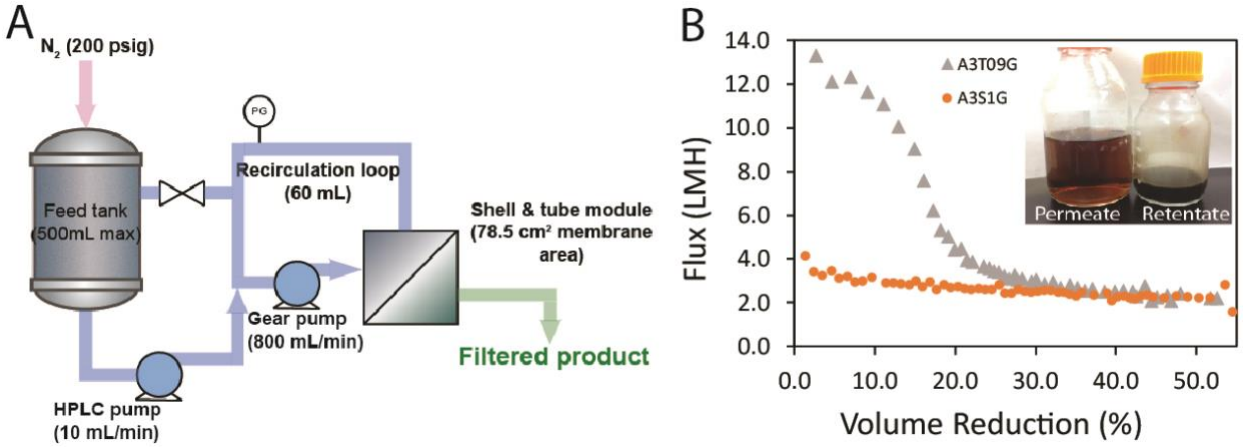

Figure 9. A) A schematic of the cross-flow filtration system and module (Inopor, Germany). To maintain back pressure to the recirculation loop, a HPLC was installed to pump feed material into the loop. Two Inopor nanofiltration membranes were screened for wheat straw lignin filtration - A3T096 has a TiO_2 selective layer and A3S1G has a SiO_2 selective layer. B) The wheat straw lignin flux declines as the volume of the feed material decreases. Membrane A3T09G has a 3-fold higher initial flux and is recommend for future work based on its higher performance. The membranes were cleaned with a solution of 1% Liquinox and 1% NaOH after each use.

The WSL composition and carbon analysis were used to calculate the final recovery of LMW compounds from the feed. The WSL contains 18.3 g/L of aliphatic acids such as acetic acid (7.3 g/L), formic acid (3.9 g/L), and succinic acid (2.1 g/L). Using elemental analysis, the feed WSL was determined to contain 14.5 g/L of carbon and following 46% of the feed material can be assigned to low molecular weight aliphatic and aromatic compounds. The aim of the filtration was to recover LMW compounds in the permeate stream (a photo of the permeate is shown in the inset of **Figure 9B)**. GPC was used to analyze the molecular weight distribution of the feed, permeate, and retentate streams (**Figure 10**). Carbon analysis (**Table 1 and 2**) was then used to determine the carbon content in the permeate and retentate streams and to confirm that at least 80% of the low molecular weight compounds were recovered in the permeate stream. Based on the dry weight and carbon content, 82% of the LMW lignin was collected in the permeate stream after filtration, therefore meeting the goal of this task.

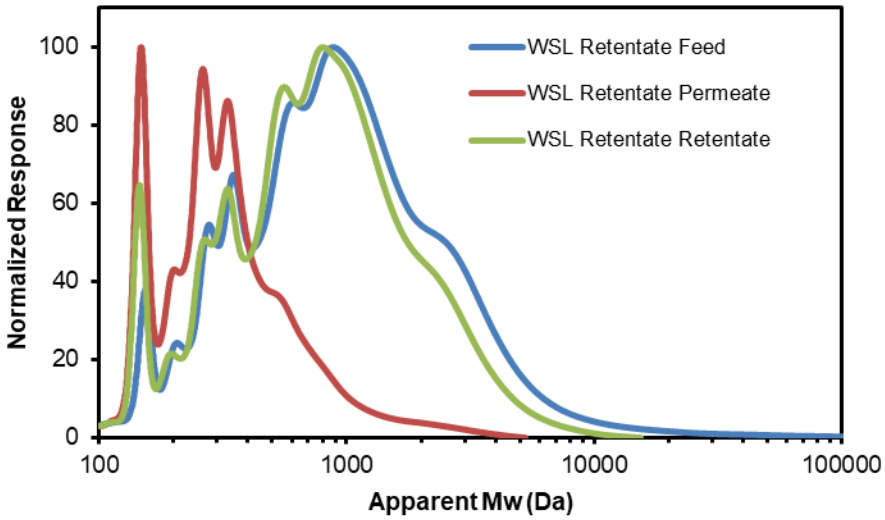

Figure 10. GPC chromatographs showing the apparent molecular weight distribution of wheat straw lignin (WSL) provided by SFT (Feed) and permeate and retentate after nanofiltration. The weighted average molecular weight (M_w) of WSL permeate is 470 Da (PD of 1.5) – a significant decrease compared to both the feed and retentate weighted average molecular weight (M_w of 1700 Da, PD of 3.5).

Table 1: C, H, N analysis of dried sample, as a percentage of dried weight

WSL	*% C*	*% H*	*% N*
Feed	31.6	4.1	0.45
Permeate	23.1	3.9	0.062
Retentate	36.1	4.3	0.66

Table 2: C, H, N on a volume basis

WSL	*C (g/L)*	*H (g/L)*	*N (g/L)*
Feed	14.5	1.9	0.21
Permeate	8.4	3.0	0.02
Retentate	27.5	1.6	0.5

Task 4 – SFT to produce 100 L batch of pulping liquor from a non-woody feedstock of commercial interest; Deliver to NREL

Task 4 was successfully completed during the outlined CRADA period of performance.

In order to continue onto the biological upgrading portion of this project, SFT produced 100 L of wheat straw pulping liquor via their Phoenix Process™. The wheat straw liquor was shipped and successfully received by NREL. The wheat pulping liquor was selected from previous characterization and separation results showing that it is a favorable stream for biological upgrading. Additionally, the wheat pulping liquor is the main waste stream produced by SFT, and of commercial interest to valorize.

Task 5 – Conduct 1 L scale bioreactor experiments to produce muconate via an engineered microbe and scale to 10 L for optimal cultivation conditions. Produce at least 75% yield muconic acid from lignin-derived monomers.

Task 5 was changed to reflect specific findings from Task 2 and successfully completed during the outlined CRADA period of performance.

In Task 2, the 10 L batch selection of SFT pulping liquors were characterized and the results showed less aromatic monomer concentration than expected which would directly affect the amount of muconic acid produced via biological conversion. Since the aim of this CRADA was to ultimately valorize waste streams from an industrial pulping process of non-woody feedstocks, PHA as an alternative target molecule was selected. Both aliphatic acids, which were found to be abundant in the pulping liquor streams, and aromatic monomers can be funneled into medium chain length PHAs (*mcl*-PHA). PHA can be used as a biodegradable substitute for conventional non-degradable plastics (Sluiter, Hyman *et al*. 2008). Therefore, Task 5 goal was changed to reflect this: **Produce polyhydroxyalkanoates (PHA) in shake flasks via an engineered microbe. Produce at least 15% yield of PHA from bioavailable carbon.**

To complete the current task, the performance of engineered *Pseudomonas putida* KT2440 was evaluated at two concentrations of the SFT WSL stream (45 and 85%) in minimal M9 media with a low nitrogen (2 mM ammonium sulfate). Seed cultures were prepared in LB media, incubated for 18 h at 30ºC with 225 rpm agitation, and inoculated in the lignin-containing cultures at an initial optical density at 600 nm (OD600) of 0.2. These cultivations were conducted in 250 mL shake flasks with 50 mL of media and incubated for 75 h at 30ºC with 225 rpm agitation.

The growth lag in 85% SFT WSL pulping liquor was considerably longer than that observed in 45% WSL liquor (**Figure 11A**), which indicates the potential toxicity of the former. Likely due

to this lag and an incomplete carbon utilization, the production of *mcl*-PHAs at 75 h was higher in the 45% SFT WSL than the 85% SFT WSL, reaching a titer of 150 mg/L and a yield of 23% (**Figure 11B,C**). Regarding the composition of the *mcl*-PHAs, the major product was the hydroxyacyl methyl ester (HAME) 3- hydroxydecanoate (C10) (**Figure 11D**). These results show that engineered *P. putida* KT2440 can tolerate the SFT WSL stream and convert the bioavailable carbon to value-added compounds such as *mcl*-PHAs. Therefore, a *mcl*-PHA yield (g *mcl*-PHA/g cell dry weight, CDW) of 23% from a diluted SFT WSL stream was achieved, meeting the goal of this task.

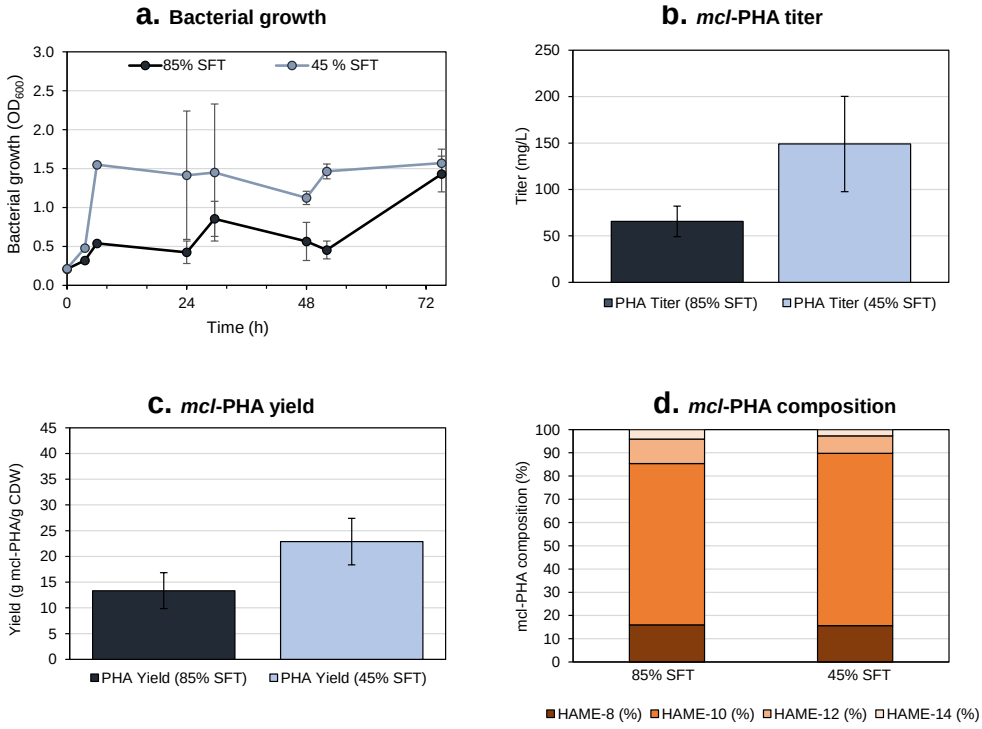

Figure 11. Engineered *P. putida* KT2440 performance in SFT-lignin streams at concentrations of 45% and 85%. A) Bacterial growth (OD600), B) *mcl*-PHA titers (mg/L), C) *mcl*-PHA yields (g *mcl*-PHA/g CDW), D) *mcl*-PHA composition.

Task 6 – Isolate and purify muconic acid from the lignin-derived streams at a minimum purity of 99.8%

Task 6 was changed to reflect specific findings from Task 2 and successfully completed during the outlined CRADA period of performance.

Similar to Task 5, the goal of Task 6 was changed to reflect the specific findings of available carbon type in the SFT pulping liquors, as characterized in Task 2. In continuation with the changes made to task 5, the goal for task 6 was also altered: **Transfer the production of polyhydroxyalkanoates (PHA) to 0.5-L-scale bioreactor experiments. Maintain at least 15% yield of PHA from bioavailable carbon. Employ bench-scale separations to obtain polyhydroxyalkanoates from the lignin-derived stream a purity of >97%.** This last goal of the project was designed to adopt the original end goals of the project and apply them to a new high-value target molecule better suited to valorize the SFT WSL pulping liquor.

Therefore, to complete the current task, the performance of engineered *Pseudomonas putida* KT2440 was evaluated in 50% SFT lignin liquor plus minimal M9 media and low nitrogen concentration (1 mM ammonium sulfate). Seed cultures were prepared in shake flasks in LB media, incubated for 18 h at 30ºC and 225 rpm, and inoculated in the lignin-containing 0.5 L bioreactors at an initial optical density at 600 nm (OD600) of 0.2. The bioreactors contained 450 mL of media and were maintained at 30ºC and pH 7 (via NaOH addition) for 72 h. The agitation initiated at 350 rpm and the dissolved oxygen was maintained at 30% by agitation.

Engineered *P. putida* KT2440 grew successfully in 50% SFT WSL pulping liquor (**Figure 12A**) and the *mcl*-PHA titer reached 220 mg/L at 72 h (**Figure 12B**). The *mcl*-PHA yield (g PHA/ g CDW) was 40% (**Figure 12C**), which is considerably higher than that obtained in Task 5 under shake flask conditions (23%). Oxygen concentration can have an important effect in *mcl*-PHA production, and it is likely to be higher in bioreactors than shake flasks. In addition, the concentration of nitrogen was decreased to 1 mM in this experiment (compared to 2 mM in the shake flask experiment), which can also have an important effect on carbon funneling towards the target product instead of cell biomass. Concerning the composition of the *mcl*-PHAs, the major detected product was the hydroxyacyl methyl ester (HAME) 3- hydroxydecanoate (C10) (**Figure 12D**), as similarly observed in Task 5. These results show that engineered *P. putida* KT2440 can accumulate *mcl*-PHAs at high yields. In addition, it is predicted that both titers and yields can be improved via bioprocess and further strain development. Therefore, a *mcl*-PHA yield (g *mcl*-PHA/g cell dry weight, CDW) of 40% from 50% SFT pulping liquor was achieved, meeting the first goal of this task.

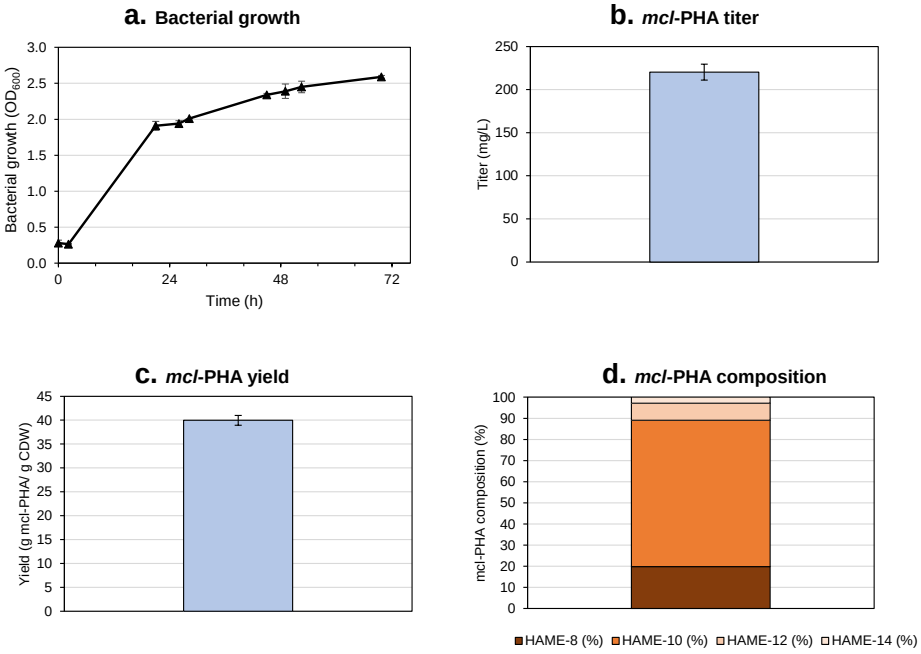

Figure 12. Engineered *P. putida* KT2440 performance in SFT WSL stream at an initial concentration of 50%. A) Bacterial growth (OD600), B) *mcl*-PHA titer (mg/L), C) *mcl*-PHA yield (g *mcl*-PHA/g CDW), and D) *mcl*-PHA composition.

In addition to achieving 40% yield of PHA from 50% SFT WSL in bioreactors, PHAs were isolated to demonstrate a final product at a purity necessary for biodegradable thermoplastic applications. Therefore, an aliquot of the dried cell mass (**Figure 13A**) containing 40% mcl-PHAs (g mcl-PHA/g cell dry weight, CDW) was used. To purify the *mcl*-PHAs, the dried cells were first exposed to an acid solution for the removal of proteins (0.1 M H_2SO_4 at 40℃ for 1 hour), with the undissolved solids are shown in **Figure 13B**. The non-PHA material was then solubilized with sodium hydroxide (pH 10) for 10 mins and then household bleach (~6% sodium hypochlorite) was added to the sample with a sample to bleach volume ratio of 1:1. The sample was then centrifuged at 15,000 rpm for 15 min and the pellet was resuspended in water (**Figure 13C**). The *mcl*-PHA was then dried in vacuum oven at 60℃ (**Figure 13D**).

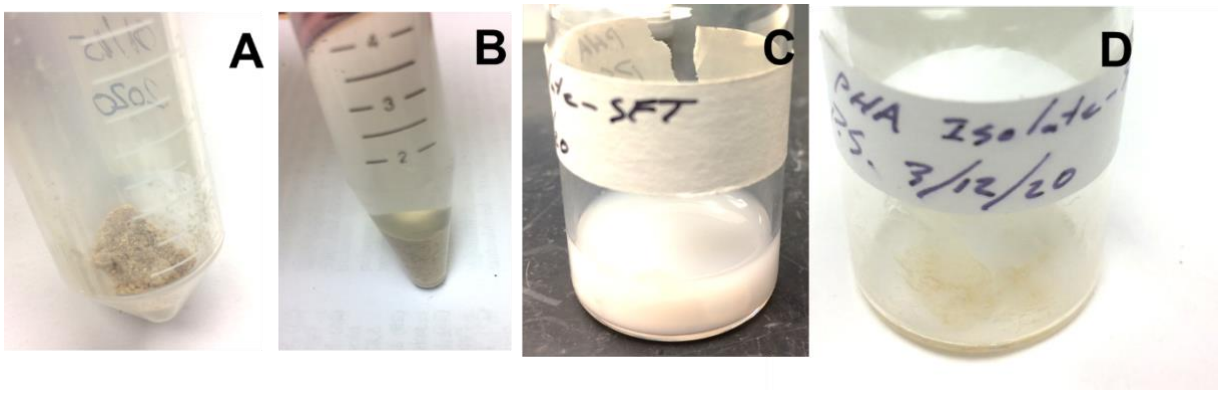

Figure 13. A) Dried cell mass of *P. putida* KT2440 with accumulated *mcl*-PHAs from growth on 50% SFT WSL pulping liquor (189 mg. B) Cell mass after acid treatment. C) PHA solution after base treatment and bleaching. D) Dried PHA sample (40 mg).

The composition of the dried isolated PHA is shown in **Figure 14** as determined by a standard derivatization method and GC-MS analysis. The major detected product was the hydroxyacyl methyl ester (HAME) 3-hydroxydecanoate (C10) (66 wt. %), followed by 3-hydroxydodecanoate (C12) (9 wt. %). It was noted that the major detected product in the dried cell mass before isolation was also HAME-10 (69 wt. %), indicating that the separations procedure maintained the PHA composition. Medium chain length PHAs were isolated at a purity of 96.5% (g *mcl*-PHA, total g) from dried *Pseudomonas putida* KT2440 cells that were grown on 50% SFT lignin liquor, thus completing the final goal for this task. Future separation work is suggested to focus on the scale-up of the separation process and to determine the maximum yield and overall economics of the process.

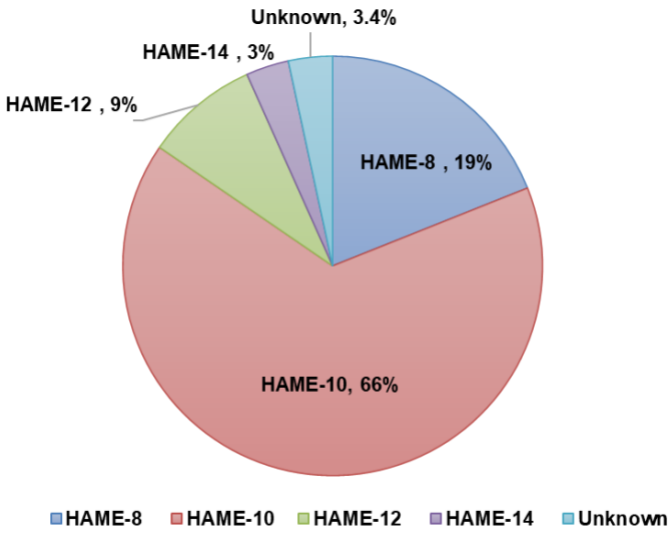

Figure 14. The composition of isolated mcl-PHA

Conclusion:

Through this work, NREL has utilized established techniques to guide the valorization of a predominant SFT pulping liquor co-product. Valuable compositional data gained from analytical analyses of SFT liquors was used to select a suitable high-value target molecule for carbon upgrading. Biological conversion of SFT pulping liquors garnered an unexpectedly high yield of product in both an initial shake-flask and final bioreactor systems with minimal optimization. Thus, demonstrating the potential for biological conversion of the direct SFT feedstocks from the separations and refining operations upstream. Additionally, product isolation was achieved at a purity necessary for biodegradable thermoplastic applications. Further optimization and scale-up with associated techno-economic analysis, are suggested as future steps for maximum product yield from biological conversions and separation processes.

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Subject Inventions Listing:

None

ROI #:

None