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Statistical Design of Experiments for Purification of Vanillin from Commercial Lignin using Ion Exchange

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INTRODUCTION

Lignin comprises a heterogenous group of organic polymers present in cell walls of all vascular plants. As the largest renewable source of aromatic carbon on earth, lignin also represents a valuable raw material for biorefining.[1] In nature, lignin is synthesized from three phenylpropanoid monomers resulting in a complex, polydisperse mixture.[2,3] These monolignols vary in methoxy substitution at the 3- and 5-positions of the phenol, and their ratios depend on plant taxonomy.[4,5] The β-O-4 ether linkage between monomers accounts for approximately 50% of the total linkages in softwood lignin and 65% of total linkages in hardwood lignin.[6,7] In order to valorize this abundant natural product, there have been many studies that have focused on lignin depolymerization to produce low molecular weight aromatic compounds such as phenols, phenyl alcohols and benzaldehydes.[8,9]

Due to their reactive chemical handles, benzaldehydes represent valuable feedstocks for chemical elaboration. Lignin oxidation to produce vanillin (4-hydroxy-3-methoxybenzaldehyde), has been widely studied,[10-13] and the bio-derived vanillin market within the food/beverage, fragrance, and pharmaceutical industries was approximated at $150M in 2018.[14] Reported vanillin yields depend on lignin type, ranging from 1-8% for kraft lignin,[15,16] 1-15% for organosolv lignin,[15,17] and 7-12% for lignosulfonate.[18,19] Vanillin yields from softwood lignin are typically higher than those from hardwood lignin.[12] To achieve high purity vanillin from lignin, purification steps such as distillation and multistage crystallization are required,[20,21] and these methods are not always economically viable.[12]

Recently, our group has used vanillin and related derivatives to synthesize protic and aprotic benzylammonium ionic liquids in good to excellent yields.[8,22] To prepare these bio-based ionic liquids, commercially available aromatic aldehydes (vanillin, syringaldehyde, furfural, *p*-anisaldehyde) were first subjected to reductive amination.[23] After additional synthetic steps,[8,22] the ionic liquid products demonstrated the ability to dissolve cellulose,[8] and/or effectively pretreat biomass for enzymatic hydrolysis to fermentable sugars. [8,22]

Chromatographic methods to recover vanillin and other benzaldehydes from depolymerized lignin have recently been reported. For example, Gomes *et al.* applied adsorption chromatography to fractionate vanillin, acetovanillone, and vanillic acid after lignin depolymerization.[9] Similarly, Mota *et al.* investigated the downstream processing of oxidized industrial kraft liquor, and achieved an increased concentration of vanillin and syringaldehyde.[24] Both studies relied upon SP700 resin, a porous polystyrene resin. To develop a more sustainable route to lignin-based ionic liquids, we sought to investigate oxidation of commercially available lignin, chromatographic purification of vanillin, and *in situ* conversion of depolymerized lignin mixtures into aminophenols.

BioChoice®, a commercially-available pine lignin was investigated in this study. Approximately 60 ton/day of BioChoice® lignin is produced and sold by Domtar Paper Company in Plymouth, North Carolina. Characterization of BioChoice® lignin was performed by Hu *et al.*, and it was found to be comparable to indulin AT, another pine kraft lignin.[25] Although the production processes for the two lignin sources differ, only subtle differences in chemical structure was observed by NMR.[25] To our knowledge, the only other study of BioChoice® lignin was performed by Jiang *et. al.* showing that polydispersity was reduced after sequential solvent extraction.[26] In the latter study, lignin was not depolymerized prior to fractionation.

In this study, a series of lignin depolymerization experiments were conducted using Box-Behnken statistical design to maximize vanillin yield.[27] Optimal oxidation conditions were selected based on the Response Surface Method.[28] Vanillin was separated by extraction followed by chromatographic fractionation. Two resins were compared for the fractionation process - Diaion® HP-20 polystyrene, and AG® 1-X8 anion exchange with a molecular weight exclusion of 1kD (**Figure 1**). The ion exchange method demonstrated superior purification results for vanillin recovery; the vanillin content reached approximately 55 wt%. Chromatographic fractions from both resin types were quantified by GC-MS and further characterized by GPC, FT-IR and NMR. Lastly, pooled fractions from the ion exchange resin, containing 46 wt% vanillin, were reacted using reductive amination (**Scheme 1**). Facile acid-base work up resulted in the desired product, 4-((diethylamino)methyl)-2-methoxyphenol (**1**), at 75% yield and 94 wt% purity. In addition to their utility in making bio-based ionic liquids, aminophenols have been reported as emergency antidotes for cyanide and hydrogen sulfide poisoning,[29,30] as well as chemical intermediates, e.g. for the synthesis of isoquinolone drugs.

More recently, an *in situ* synthesis of benzazepines with antibiotic properties have recently been performed on depolymerized lignin.[31]

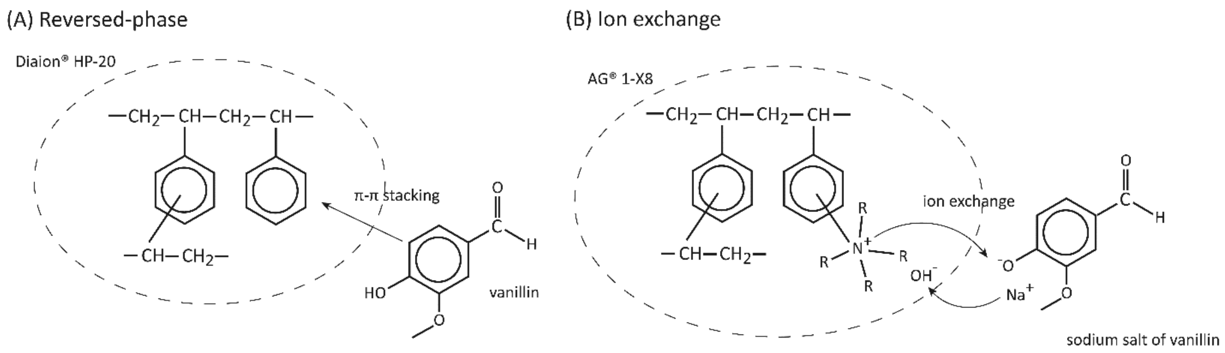
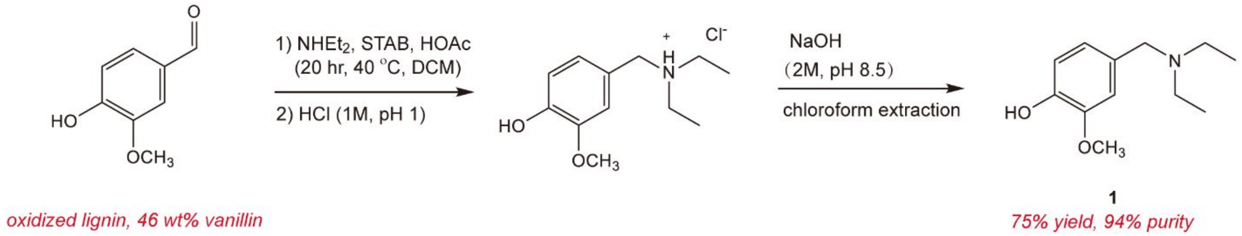


Figure 1. Mechanism of vanillin separation for (A) Diaion® HP-20 reversed phase polystyrene resin and (B) AG® 1-X8 anion exchange resin.



Scheme 1: Reductive amination of vanillin as a 46 wt% mixture obtained from anion exchange chromatography of oxidized lignin. Acid-base workup of 4-((diethylamino)methyl)-2-methoxyphenol product (**1**) allowed for phase-separations of its HCl salt and free base forms to reach a final purity of 94 wt%. STAB = sodium triacetoxyborohydride

EXPERIMENTAL SECTION

Oxidative depolymerization of lignin

BioChoice® kraft lignin was obtained from the Domtar Paper Co, LLC (Fort Mill, SC). Before experiments, lignin was oven dried overnight at 75 °C. The dried lignin was used directly for depolymerization in a 600 mL pressure reactor (Parr Instrument Co.). In each experiment, 1.40 g of dried lignin was loaded into the reactor vessel along with 70 mL of 2 M NaOH solution. The reactor was sealed, and then sparged 5x with molecular oxygen (99% purity) before charging at specified pressure. The reactor was heated to the target temperature over 15-20 min while stirring speed was maintained at 250 rpm. Reaction times were recorded after reaching target temperature.

As benzaldehydes are fairly reactive species, the vanillin yield was thought to be determined by the temperature used in the reaction, the pressure of the initially charged O_2, and the reaction time. Seven preliminary experiments were conducted at 180-220 °C for 1-3 h with 1-2 atm of initial O_2 pressure, and 1.8 - 5.2 wt% vanillin yields were obtained based on the dried weight of lignin (data not shown). In order to optimize vanillin yield, a response surface method was used to determine the optimal temperature, reaction time, and O_2 pressure. Next, a Box-Behnken design (JMP Pro 14, SAS Institute, Inc., Cary, N.C.) was used to optimize these process parameters to maximize vanillin yield and extracted lignin mass. Based on the preliminary data, three levels for the independent variables were chosen from 180 -220 °C, 1 – 3 h, and 1 - 2 atm of initial oxygen pressure (**Table 1**). Two sets of 14 experiments were performed (n = 28 in total) on independent variables of temperature (X_1), reaction time (X_2), and initial O_2 pressure (X_3) (**Table S1**). Two center points were used to determine the experimental error and reproducibility of the data. Low and high levels of the independent variable were coded as −1 and +1. The experimental sequence was then randomized to minimize the effects of uncontrolled errors. The response variable for this study were vanillin yield (mass %) and extracted lignin (mass %). The response from the independent variables was then used to develop an empirical model corresponding to the response variable ($\hat{Y}$) *via* a second-degree polynomial equation as given in Equation 1:

$$\hat{Y} = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 + \beta_{23} X_2 X_3 + \beta_{11} X_1^2 + \beta_{22} X_2^2 + \beta_{33} X_3^2 \qquad \text{(Eq. 1)}$$

where $\hat{Y}$ is the response, β_0 is the intercept, whereas β_1,β_2,$\beta_3$and β_{12},β_{13},β_{23} and β_{11},β_{22},β_{33} are linear, interaction, and quadratic effect regression coefficients, respectively. The experimental results were fitted to a polynomial model to optimize the reaction parameters for the production of vanillin and extracted lignin using a desirability approach.

Table 1. Experimental factors and their coded levels of independent variables in the Box-Behnken design.

Factors	Code	Coded variable levels		
		-1	0	1
Temperature (°C)	X_1	180	200	220
Reaction time (hr)	X_2	1	2	3
Initial O_2 pressure (atm)	X_3	1	2	3

Separation, quantification and characterization of vanillin from depolymerized lignin

Two chromatography methods, AG® 1-X8 anion exchange (BioRad) and Diaion® HP-20 (Supelco) were compared to purify vanillin from oxidized lignin. While HP-20 required methanol and acetone, anion exchange was achieved with 1 M NaCl as the mobile phase. Details of post-oxidation lignin extractions and resin purifications, including schematics can be found in the **SI Section II**. Briefly, each resin type was placed into separate 350 mL fritted Buchner funnels, and sequentially washed with 200 mL of solvent. For HP-20, the sequential solvent washes were: 20% aqueous methanol (2 x 100 mL), 40% aqueous methanol (2 x 100 mL), 60% aqueous methanol (2 x 100 mL), 80% aqueous methanol (2 x 100 mL), 100% methanol (2 x 100 mL) and 100% acetone (2 x 100 mL). For anion-exchange, the sequential solvent washes were 5 x 200 mL 1M NaCl followed by 200 mL MeOH. Each fraction was collected and dried *in vacuo*. The dried product of each fraction was weighed and then dissolved in acetone for quantitative GC-MS and GPC analysis. Vanillin was quantified in all fractions using GC-MS, and the full procedure for quantitative analysis of vanillin yield is provided in **SI Section I**. Spectroscopic analysis methods, including GPC, IR and NMR were also used to characterize selected depolymerized lignin fractions and are detailed in **SI Section III**, respectively.

In situ reductive amination of vanillin in oxidized lignin mixture

The purified lignin fractions obtained from AG® 1-X8 anion exchange method were pooled (71 mg of mixture, 46.4 wt% vanillin; 32.9 mg (0.216 mmol) vanillin) and subjected to a synthetic procedure modified from our previous work.[22] The lignin mixture was placed in a 7 mL scintillation vial, dissolved with 3 mL of dichloromethane (DCM), and then mixed with 250 μL of diethylamine (177 mg, 2.4 mmol). The mixture was stirred at room temperature for 30 min, and then mixed with 160 mg (0.755 mmol) of sodium triacetoxyborohydride and 50 μL (52.5 mg, 0.873 mmol) of acetic acid. The vial was then tightly sealed and heated to 40 °C for 20 h. The reaction mixture, containing DCM solvent and desired product (4-((diethylamino)methyl)-2-methoxyphenol), was washed with 2 × 5 mL of diluted HCl (pH 1). The DCM phase contained unreacted acetovanillone, and was discarded. The aqueous phase contained the desired product as its HCl salt. The acidic aqueous phase was further extracted with 2 × 5 mL of diethyl ether, which was also discarded. The pH of the aqueous phase was adjusted to pH 8.5 with 2 M NaOH and the desired product was extracted as its free base with 6 × 5 mL of $CHCl_3$. After solvent removal *in vacuo*, 34 mg (0.162 mmol) of 4-((diethylamino)methyl)-2-methoxyphenol was obtained as a light brown oil, representing a yield of 74.9% yield (**Scheme 1**). The extracted final product was characterized by ^{1}H NMR and GC-MS, and the latter allowed for a purity calculation of 94.3 wt% (**Figure S10-S13**).

RESULTS AND DISCUSSION

Development of the model equation

Table S1 shows the vanillin yields from oxidative depolymerization of lignin obtained from Box-Behnken designed experiments. The results showed vanillin yields varied in the range of 1.8 - 7.5%, which are consistent with 1-10% vanillin yields for alkaline lignin oxidation reported in the literature.[12,15,32-34] Vanillin yield is greatly affected by the lignin source and alkaline oxidation of kraft lignin typically yields less than 10% vanillin.[10,12] The extracted lignin column of **Table S1** shows the mass percentage of lignin extracted with EtOAc at pH 1.5. In a control experiment, untreated lignin provided only 48.6 wt% of extract, and 0% of vanillin yield. The model-recommended optimum levels for the vanillin yield were temperature at 197 ℃, reaction time for 2.4 h, and initial O_2 pressure of 2.2 atm, corresponding to a predicted vanillin yield of 5.3% at the 95% confidence interval ([4.1,6.4]). Similarly, under the optimum reaction parameters, the predicted extracted lignin yield was 77.9% at the 95% confidence interval ([70.6,85.1]). The quadratic regression models for vanillin yield and extracted lignin are shown as Equations 2 and 3 below:

$$\hat{Y}_{Vanillin} = 5.18 - 0.21\,X_1 + 0.29\,X_2 + 0.26\,X_3 - 0.5\,X_1^2 - 0.24\,X_2^2 - 1.26\,X_3^2 + 0.64\,X_1X_2 + 0.49\,X_1X_3 + 0.62\,X_2X_3 \quad \text{(Eq. 2)}$$

$$\hat{Y}_{Extracted\ lignin} = 76.8 - 8.84\,X_1 + 0.89\,X_2 - 2.94\,X_3 - 4.39\,X_1^2 + 0.09\,X_2^2 + 2.04\,X_3^2 + 0.01\,X_1X_2 - 5.9\,X_1X_3 - 2.5\,X_2X_3 \quad \text{(Eq. 3)}$$

where, X_1, X_2, and X_3 are temperature, reaction time, and initial O_2 pressure, respectively. The coefficients with one factor (X_1, X_2, X_3) represent the linear effects, while the coefficients with second order term (X_1^2, X_2^2, X_3^2) and two-factor term (X_1X_2, X_1X_3, X_2X_3) represent the quadratic effect and interaction effects, respectively.

Another lignin depolymerization experiment was conducted under the aforementioned optimal conditions (197 ℃, 2.4 h, and initial O_2 pressure of 2.2 atm). The GC-MS result showed the vanillin yield in the depolymerized products reached 5.3% with a lignin extraction of 77.1%. Thus, the results aligned very well with the model's prediction. While vanillin was the major monophenolic compound in the product mixture, acetovanillone was also detected, (3.5% yield based on lignin dry weight). Acetovanillone is a known product of lignin depolymerization.[35] It is noteworthy that vanillic acid was not detected in the product mixture, suggesting that the optimized conditions did not lead to over-oxidation of vanillin. Information regarding the effects of reaction temperature, time and oxygen pressure could be gleaned from the comparison of the model's predicted vanillin yields and extracted lignin masses with experimentally obtained values.

Effect of reaction parameters on vanillin yield and extracted lignin.

A model was fit to the experimental data in order to find the optimized reaction conditions to maximize vanillin yield and estimate the significance of each parameter (temperature, time, and O_2 pressure) towards vanillin yield and extracted lignin mass.

Reaction temperature: The combined effects of temperature with reaction time and initial O_2 pressure on vanillin yield are shown in **Figures 2 A and B**. Response surface plots illustrate that vanillin yield increased with decreasing temperature, with highest yield obtained at 197 °C. Additionally, results from ANOVA (**Table S2**) indicate that both linear and interaction effect of temperature with reaction time and initial O_2 pressure were not significant ($p > 0.05$). **Figures 3**

A and B illustrate the effect of temperature coupled with interaction of reaction time and initial O_2 pressure on extracted lignin (%). Results from ANOVA (**Table S3**) illustrate that in the design specified levels, temperature had a significant effect ($p = 0.0002$) on extracted lignin. Additionally, the interaction effect of temperature and initial O_2 pressure was also significant ($p = 0.0379$) while the interaction effect of temperature and time was not significant on extracted lignin mass.

Reaction time: **Figures 2 A and C** illustrate the combined effects of reaction time with temperature and initial O_2 pressure on vanillin yield. The response surface plots demonstrate that changes in reaction time does not have a significant impact on vanillin yield. Similarly, the interaction effect of reaction time with temperature and initial O_2 pressure were not significant ($p > 0.05$) with respect to the vanillin yield within the design specified range. The combined effect of reaction time with temperature and initial O_2 pressure on extracted lignin is illustrated in **Figures 3 A and C**. Results from ANOVA (**Table S3**) illustrate that in the designed specified levels, reaction time had no significant effect on extracted lignin, which is also corroborated from the 3D response surface plots.

Initial O_2 pressure: The linear and interaction effect of initial O_2 pressure with temperature and reaction time is shown in **Figure 2 B and C**. Response surface plots demonstrate that vanillin yield increased with an increase in initial O_2 pressure reaching its maximum at 2.2 atm with subsequent reduction in yield at higher pressures. **Figure 3 B and C** illustrate the combined effect of initial O_2 pressure, with reaction time and temperature, on extracted lignin. Results from ANOVA (**Table S3**) illustrate that linear effect of initial O_2 pressure and interaction effect with reaction time has no significant impact on extracted lignin. However, interaction effect of initial O_2 pressure with temperature has significant effect on extracted lignin ($p = 0.0379$).

Taken together, results from the Box-Behnken designed experiments illustrate that the specified levels for temperature, reaction time and initial O_2 pressure had no significant effect on vanillin yield. However, temperature and interaction effect of initial O_2 pressure with temperature had significant impact on extracted lignin.

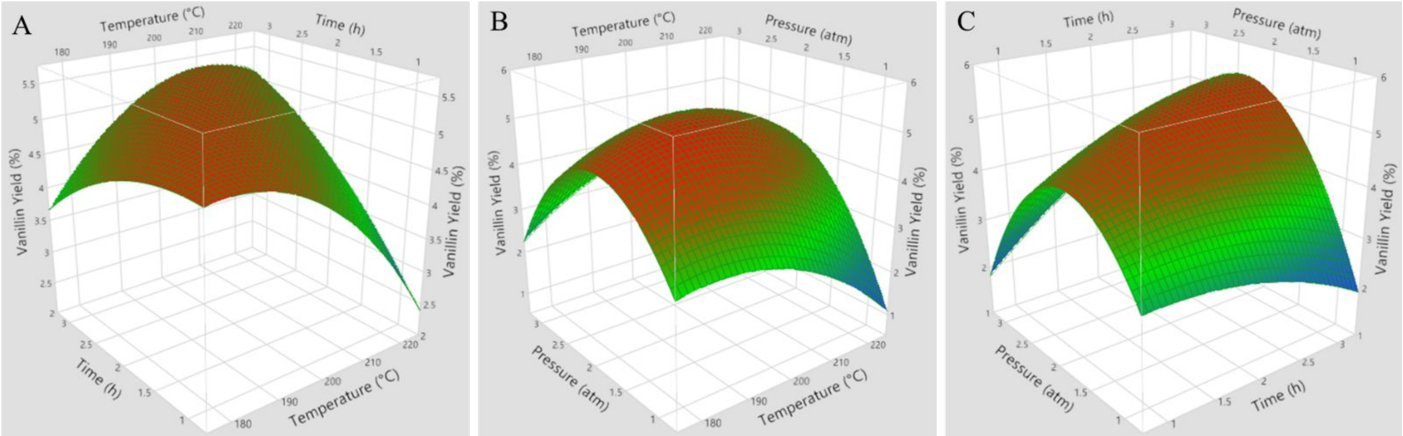


Figure 2. Surface response plots for vanillin yield showing the effects of A) reaction time and temperature, B) initial O_2 pressure and temperature and C) initial O_2 pressure and reaction time.

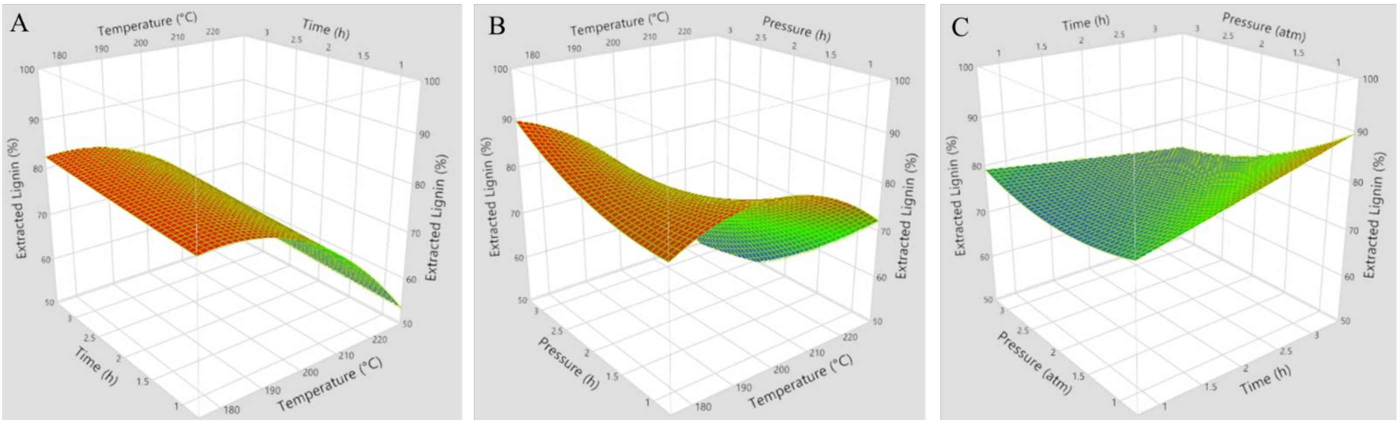


Figure 3. Surface response plots for extracted lignin showing the effects of A) reaction time and temperature, B) initial O_2 pressure and temperature and C) initial O_2 pressure and reaction time.

Vanillin purification using chromatographic resin.

Diaion® HP-20 resin is a polystyrene/divinylbenzene matrix selected to exploit π-π interactions for aromatic compounds (**Figure 1A**). **Table 2** shows the mass of lignin and vanillin purity in each fraction from the HP-20 purification. Because HP-20 is aromatic reversed-phase adsorbent resin, washing with increasing concentrations of aqueous methanol was used elute to the vanillin analyte. As the methanol concentration increased, more bulk lignin was desorbed from the resin, but the same trend did not occur with vanillin. Vanillin showed a trend of increasing desorption from the HP-20 resin as the methanol concentration increased from 20-60%. These results suggest that depolymerized lignin has a greater affinity for resin than vanillin, likely due to greater amount of aromatic ring interaction between the resin and the larger lignin fragments. The highest purity of vanillin (16.1 wt%) as well as largest amount of vanillin (9.84 mg) was achieved in the 60% methanol fraction, suggesting this solvent mixture is most suitable for the desorption of vanillin from HP-20 resins. The method showed a high vanillin recovery (89%), indicating most vanillin can be desorbed from the resin. The 60% methanol fraction was dried *in vacuo* and used for FT-IR, and NMR analysis. This fraction will hereafter be referred as the HP-20 purified fraction.

Table 2. Fraction mass and vanillin purity obtained by HP-20 purification using different elution solvents. The total mass of vanillin loaded onto the resin was 29.7 mg.

Solvent Fraction	**Fraction mass (mg)**	**Vanillin mass (mg)**	**Vanillin purity (%)**
20% MeOH	15	0.4	2.7
40% MeOH	36	2.33	6.5
60% MeOH	**61**	**9.84**	**16.1**
80% MeOH	83	9.04	10.9
100 MeOH	87	4.19	4.8
Acetone	82	0.73	0.8
Total	364	26.53	-

To improve the vanillin recovery, another type of resin separation was attempted. AG® 1-X8 anion exchange resin consists of styrene divinylbenzene copolymer lattice containing quaternary ammonium cations attached to hydroxide anions. According to the manufacturer, the resin has a 100-fold higher selectivity for a phenolate anion as compared to a hydroxide anion. Therefore, we hypothesized that the vanillate anion would exchange with the hydroxide anion and adsorb onto the resin (**Figure 1B**). Phenolates can then be desorbed with an excess of another anion such as chloride, which has 22-fold greater affinity than hydroxide. Because the resin is 8% crosslinked, it also serves as a size exclusion resin for the adsorption of molecules smaller than 1 kD.

The results of anion exchange vanillin purification are shown in **Table 3**. Because the pK_a of the phenolic proton of vanillin is 7.4,[36] it exists primarily in the anionic (e.g. vanillate) form under the pH 8 conditions used. Thus, vanillate can be adsorbed onto the resin and subsequent desorbed with 1 M NaCl. As shown in **Table 3**, vanillin was gradually desorbed from the resin while washing with 1 M NaCl solution. The first 200 mL NaCl solution resulted in desorption of a large amount of lignin from the resin, albeit only 26% pure vanillin. After the first 200 mL elution, the fraction mass decreased gradually. However, higher purity vanillin (45%-55%) was obtained in NaCl fractions 2-5. The final methanol wash, which disrupts non-ionic interactions (e.g. Van der Waals forces) with the resin, resulted in the lowest vanillin mass and purity. These results suggest that the initial 200 mL wash with 1M NaCl caused a faster desorption of phenolic lignin other than vanillin, and in subsequent washes the desorption rates of impurities and vanillin were similar to one another. Because of the decreased amount of lignin remaining on the resin, the mass transfer of impurities and vanillin decreased with additional washing steps. The methanol wash was proven to be unnecessary due to its low vanillin yield. The anion exchange method also showed a total vanillin recovery of 82%. Further washing with NaCl might increase the total recovery of vanillin, however, it would be difficult to desorb all the vanillin on the resin due to decreased mass transfer. The second fraction of NaCl washing, which had a vanillin purity of 54.8%, was dried under vacuum and used for FT-IR, and NMR analysis. This fraction will hereafter be referred to as the anion exchange purified fraction.

Anion exchange chromatography was found to be more effective than reversed phase (HP-20), and thus we decided to substitute artificial sea water as the mobile phase. Unfortunately, artificial seawater was slightly inferior to 1 M NaCl (**Table S5**) and both the dried mass of each fraction and vanillin purity decreased. This result is likely due to the lower ionic strength of sea water (total salt concentration < 0.6 M), and therefore concentration of, or salt addition to, seawater could potentially improve its solvent properties for anion exchange chromatography of lignin.

Table 3. Fraction mass and vanillin purity in the fractions obtained by anion exchange purification. The total mass of vanillin loaded onto the resin was 37 mg.

Fraction	**Mass (mg)**	**Vanillin mass (mg)**	**Vanillin purity (%)**
NaCl wash fraction 1	31	8.07	26.0
NaCl wash fraction 2	**15**	**8.22**	**54.8**
NaCl wash fraction 3	16	7.13	44.6
NaCl wash fraction 4	7	3.20	45.7
NaCl wash fraction 5	6	3.36	56.0
MeOH wash	10	0.30	3.0
Total	85	30.28	-

GPC analysis

GPC estimates molecular weight (MW) distribution of a sample mixture, with compounds of higher MW eluting faster and thus displaying shorter retention times. To obtain a relationship between the retention time and MW, polystyrene and vanillin standards were analyzed (**Figure S4, Table S4**). The estimated MW shown in **Table S4** is based solely on the MWs of polystyrene standards, and does not exactly represent the average MW of lignin due to unknown differences in polymer branching. However, GPC data using polystyrene standards provides valuable information on MW changes during depolymerization and fractionation.[37]

Figure 4 shows the results of GPC analysis for the fractioned lignin obtained by the two purification methods. It can be seen that the original kraft lignin mainly consists of compounds with MWs > 50 kDa and compounds with MWs in the range of 152 – 9000 Da (**Figure 4A**). However, after the oxidative depolymerization process, the MWs in the depolymerized lignin became smaller and all peaks have shifted to the right. A reduced signal was observed in the region of MWs > 50 kDa, while the signal in the region of 152 – 2500 Da increased significantly. Particularly, a peak was detected at approximately 18.5 min in the depolymerized lignin samples, which matched the retention time of the vanillin standard (**Figure S4B**).

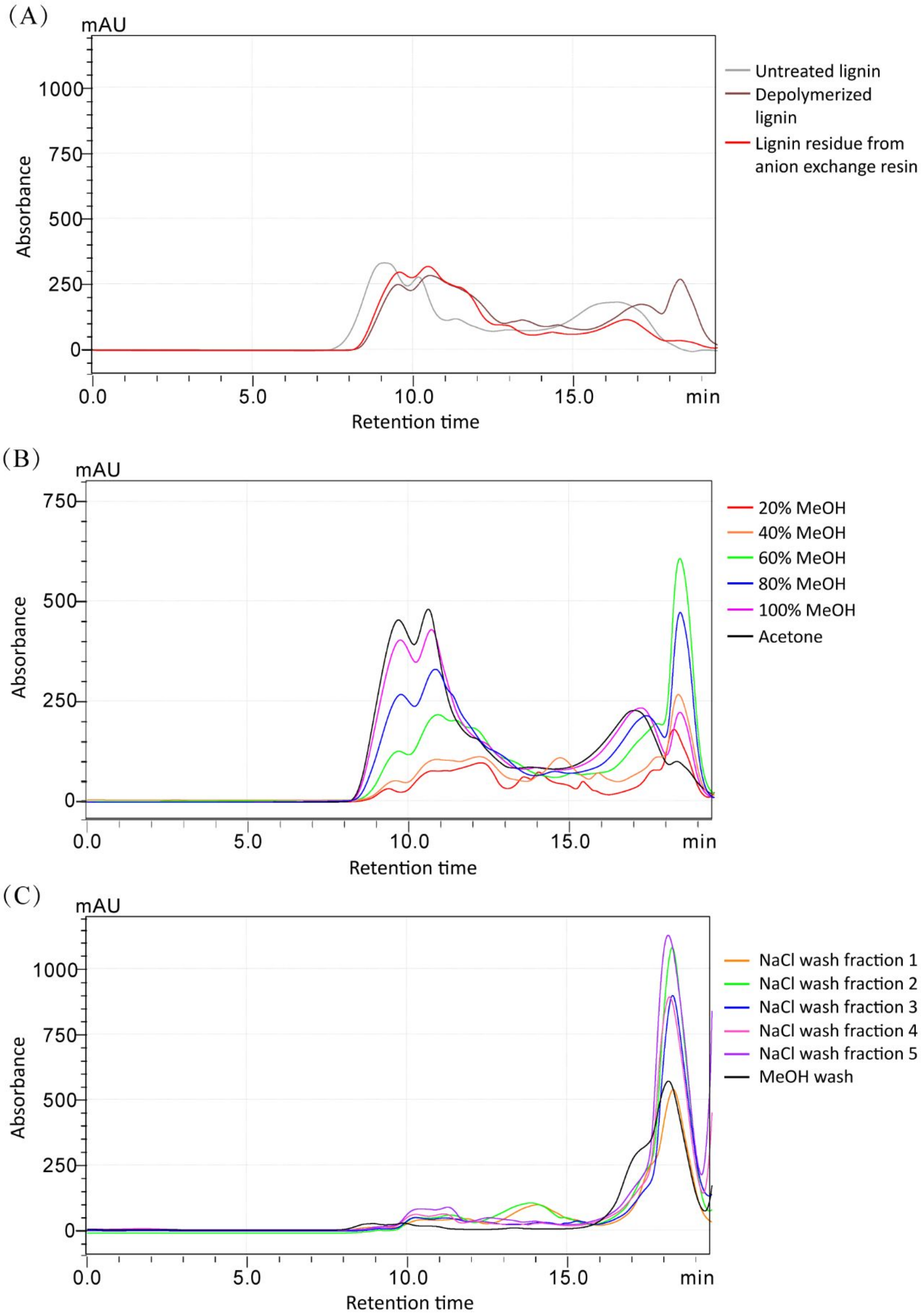

(A)
mAU
1000
750
500
250
0
Absorbance
0.0
5.0
10.0
15.0
min
Retention time
Untreated lignin
Depolymerized lignin
Lignin residue from anion exchange resin
(B)
mAU
750
500
250
0
Absorbance
0.0
5.0
10.0
15.0
min
Retention time
20% MeOH
40% MeOH
60% MeOH
80% MeOH
100% MeOH
Acetone
(C)
mAU
1000
750
500
250
0
Absorbance
0.0
5.0
10.0
15.0
min
Retention time
NaCl wash fraction 1
NaCl wash fraction 2
NaCl wash fraction 3
NaCl wash fraction 4
NaCl wash fraction 5
MeOH wash

Figure 4. GPC analysis at 280 nm for (A) untreated kraft lignin, oxidatively depolymerized lignin, and lignin residue that was not retained by AG® 1-X8 resin purification. (B) fractions generated by HP-20 resin purification, and (C) fractions generated during by anion exchange resin purification.

The chromatograms of the purified fractions were consistent with the results from GC-MS analysis. Fractions with a higher vanillin purity also showed a stronger signal in the low MW region. For the HP-20 purification, the strongest signal at 18.5 min was obtained from the 60% methanol fraction (**Figure 4B**). Further increasing the concentration of methanol caused the greater desorption of high MW compounds, leading to lower purity vanillin. Because high MW compounds were detected in all HP-20 factions, coupling size exclusion separation or membrane separation with HP-20 might be needed to increase vanillin purity.

Anion exchange resin effectively adsorbed the smaller MW compounds. In **Figure 4A**, the chromatogram of the lignin residue fraction not retained by anion exchange resin shows a significantly smaller peak in the region of 152 – 2500 Da. All lignin fractions generated from NaCl elution had lower amounts of high MW compounds (**Figure 4C**). This is likely due to the size exclusion capacity of the anion exchange resin, leading to the higher purity vanillin fractions.

FT-IR analysis. The FT-IR spectra of the resin purified fractions, as well as the untreated kraft lignin, depolymerized lignin, and vanillin standard are shown in **Figure 5**. A clear structural change resulted from alkaline oxidation. IR spectra for the depolymerized lignin and resin purified fractions show increased C=O stretching of carbonyl/carboxyl group,[38] C=O stretching of aldehyde group,[39,40] and C-C aryl-aldehyde stretching,[41] at frequencies of 1700, 1663, and 1170 cm^{-1} respectively. On the other hand, signals at 1080 and 1030 cm^{-1} showed a decrease in absorbance for the depolymerized lignin and resin purified fractions, indicating decreased C-O aryl-ether stretching.[39,41-43] This evidence supports β-O-4 bond breaking and subsequent formation of carbonyl compounds during oxidation.

In particular, the anion exchange purified fraction showed an increase in frequencies at 1663 and 632 cm^{-1}, and a significant decrease in frequencies at 1700 cm^{-1} compared to other samples. This result suggests the anion exchange purified fraction was enriched in vanillin as compared to sundry carbonyl containing lignin fragments, consistent with GC-MS analysis.

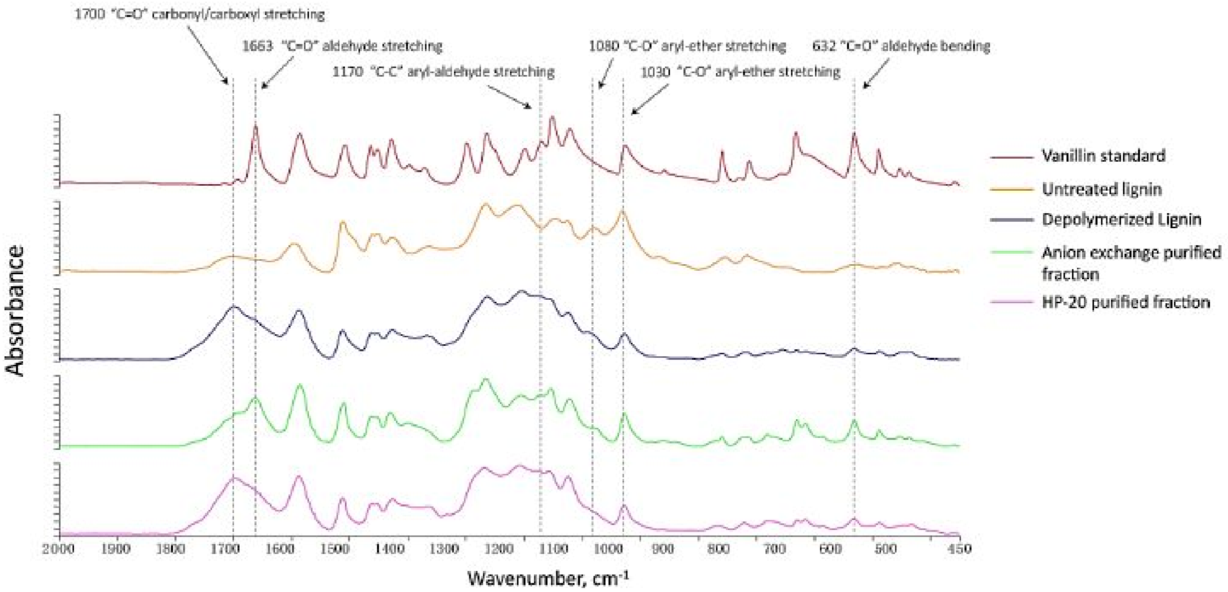


Figure 5. FT-IR spectra of untreated lignin, depolymerized lignin, and resin purified fractions.

NMR analysis. ^{1}H and ^{13}C NMR analysis of the specified HP-20 and anion exchange purified fractions showed clear presence of vanillin (**Figure S5-S8**). In the ^{1}H spectra, singlets for 1 H (δ = 9.83 ppm) and 3 H (δ = 3.94 ppm) were observed for aldehyde and methoxy resonances, respectively, in fractions from both resin types. Three protons were observed on the aromatic ring at chemical shifts of δ = 7.01, 7.50 and 7.56 ppm, consistent with the complete assignment of vanillin. In ^{13}C spectra, the chemical shift of the aldehyde carbon atom was observed at δ = 191.1 ppm. The carbon atom of the methoxy group was present at δ = 56.3 ppm. Another four carbon atoms on the aromatic rings were observed at δ = 110.9, 115.9, 127.0 and 130.7 ppm.

The quaternary carbons on the 3- and 4-positions of the aromatic ring were detected in the HP-20 purified fraction at δ = 149.0 and 153.6 ppm, respectively. In general, the ^{1}H and ^{13}C NMR spectra of the fractions were consistent with vanillin spectra reported in the literature,[44] confirming the presence of vanillin in fractions from both types of resins. When comparing both ^{1}H and ^{13}C spectra for the individual resin fractions, it was observed that the HP-20 purified fraction showed more impurities than the anion exchange purified fraction, corroborating the GC, GPC and IR analysis.

2D-HSQC analysis was conducted on untreated and depolymerized lignin, and shows ^{1}H-^{13}C correlations used to identify structural changes resulting from lignin depolymerization. The results are presented in **Figure S9**. **Figures S9A and S9C** show the aliphatic regions of the lignin samples. The spectra clearly demonstrate the presence of linkages commonly found in softwood lignin, including β-O-4 (δ ^{1}H/^{13}C = 4.79/71.2 ppm, 4.32/84.0 ppm, 3.19-3.77/60.0 ppm), β-5 (δ ^{1}H/^{13}C = 5.49/86.6 ppm, 3.49/53.2 ppm, 3.22-3.82/62.8 ppm), and β-β (δ ^{1}H/^{13}C = 4.63/84.9 ppm, 3.08/53.6 ppm, 3.75/71.0 ppm, 4.16/71.0 ppm) linkages.[1,45-47] These bonds were not found in the depolymerized lignin (**Figure S9C**), indicating deconstruction during oxidation. As shown in **Figures S9B and S9D** (aromatic regions), the untreated lignin showed strong aromatic signals of guaiacyl and *p*-hydroxyphenyl units, however these signals were greatly reduced in the depolymerized lignin. In addition, certain sp^2 C-H couplings are only observed in the depolymerized lignin, diagnostic of vanillin, acetovanillone, *p*-hydroxybenzoic acid, *p*-coumaric acid and ferulic acid.[46] Although the HSQC results show the presences of *p*-hydroxybenzoic acid, *p*-coumaric acid and ferulic acid, these compounds were not detected by GC-MS, suggesting these compounds might not exist as monophenolic compounds in any appreciable concentrations.

***In situ* aminophenol synthesis and purification.** Because benzaldehydes undergo reductive amination to form benzylamines, it was hypothesized that benzaldehyde containing lignin mixtures could be further purified by reductive amination followed by a simple acid/base work up.[23] To test our hypothesis, reductive amination of a mixture containing vanillin was conducted with excess diethylamine and sodium triacetoxyborohydride (**Scheme 1**) by modifying previously reported methods.[22] As shown in **Figure 6**, HPLC shows that reductive amination of a depolymerized lignin mixture containing 46 wt% vanillin, followed by acid/base work up, is an effective method for synthesizing and separating 4-((diethylamino)methyl)-2-methoxyphenol from major impurities, such as acetovanillone, and minor impurities such as oxidized lignin fragments. These results prove that the modest vanillin purity, obtained by anion exchange chromatography of oxidized lignin, is suitable for reductive amination to yield a highly pure aminophenol.

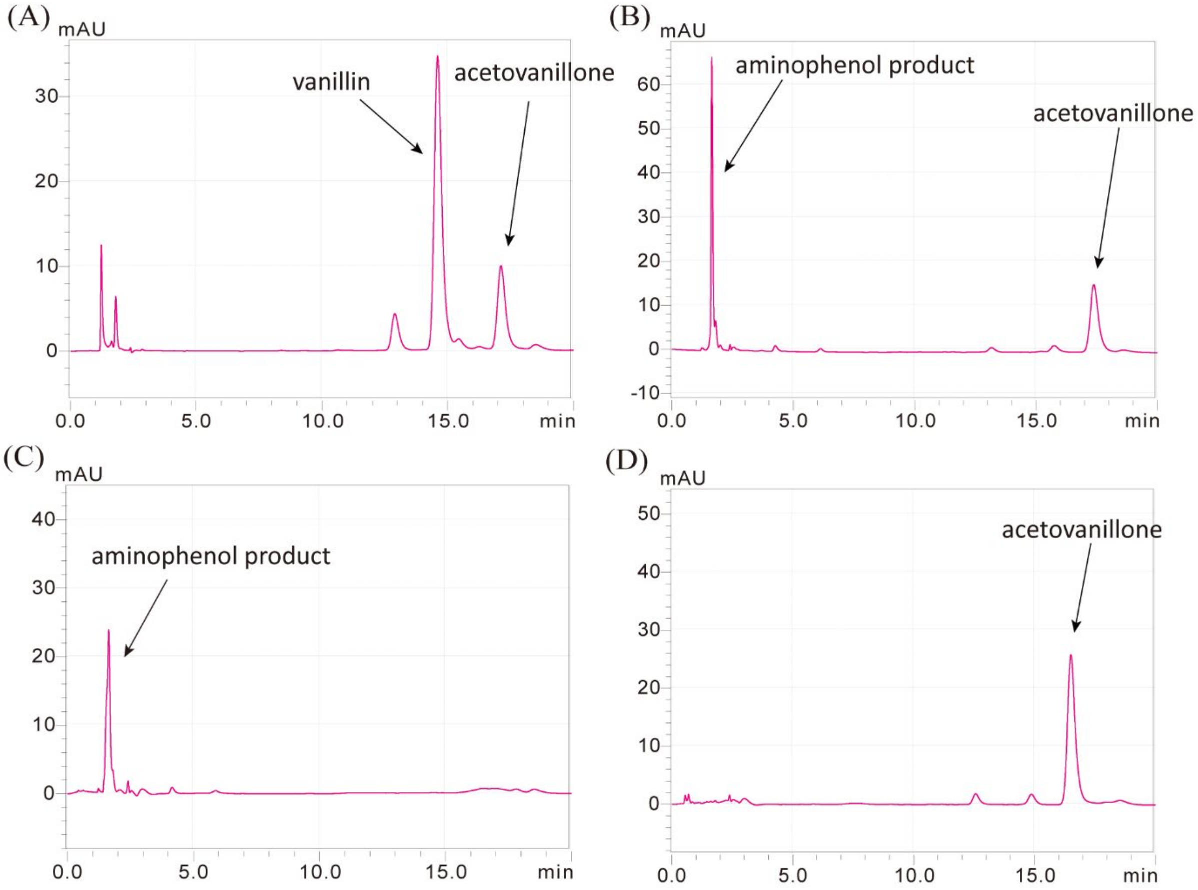


Figure 6. HPLC chromatogram of reductive amination reaction performed on 46 wt% vanillin-containing depolymerized lignin mixture. (A) reaction mixture at 0 hr. (B) reaction mixture at 20 hr. (C) aqueous phase after HCl extraction, containing aminophenol product. (D)

dichloromethane phase after HCl extraction, containing unreacted acetovanillone. Details of HPLC analysis are given in **SI Section IV.**

CONLUSIONS

By optimizing reaction conditions, oxidative depolymerization of BioChoice® commercial lignin showed vanillin yields of approximately 5 wt%, which aligns with literature values for kraft softwood. As a statistical tool, the Box-Behnken experimental design strategy showed that interaction effects of initial O_2 pressure and temperature had significant effect on the extracted lignin response variable. The only major monophenolic compounds observed in the depolymerized product mixtures were vanillin and acetovanillone. Because no vanillic acid was detected, the optimized reaction conditions preserved the desired benzaldehyde product. Solvent extraction coupled with chromatographic methods showed promising results for vanillin purification from depolymerized lignin. Using AG® 1-X8 anion exchange resin and 1M NaCl mobile phase, a maximum vanillin purity of 55 wt% was achieved. Methanol elution from HP-20 resin resulted in high vanillin recovery (89%), and future studies using ethanol and isopropanol are warranted. Analysis with GC-MS, GPC, FT-IR and NMR showed vanillin as the major product in the purified stream. Though not optimized in our study, sea water also showed promise as a mobile phase for anion exchange separations of depolymerized lignin.

The modest vanillin purities obtained by anion exchange were further enhanced by reductive amination and subsequent acid-base work up, leading to the isolation of 4-((diethylamino)methyl)-2-methoxyphenol with 94.3 wt% purity. It is predicted that the methods described herein can be applied to other lignocellulosic feedstocks, to isolate and functionalize additional platform chemicals. We are currently investigating the antibiotic properties of quaternary benzalkonium salts prepared from aminophenols, as they could potentially eradicate microbes and/or viruses from hard non-porous surfaces.[48]

CONFLICTS OF INTEREST

The authors state that there are no conflicts to declare.

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U.S. Government retains a nonexclusive, paid-up, irrevocable, worldwide license to publish or reproduce the published form of this work or allow others to do so, for U.S. Government purposes. The authors would also like to thank Domtar Paper Co, LLC for providing the BioChoice® lignin, NSF ATE Project 1601636 (awarded to AMS), NSF MRI Grant CHE-1624377 (Awarded to DNB), and Dr. Steve Bertz for helpful discussions.

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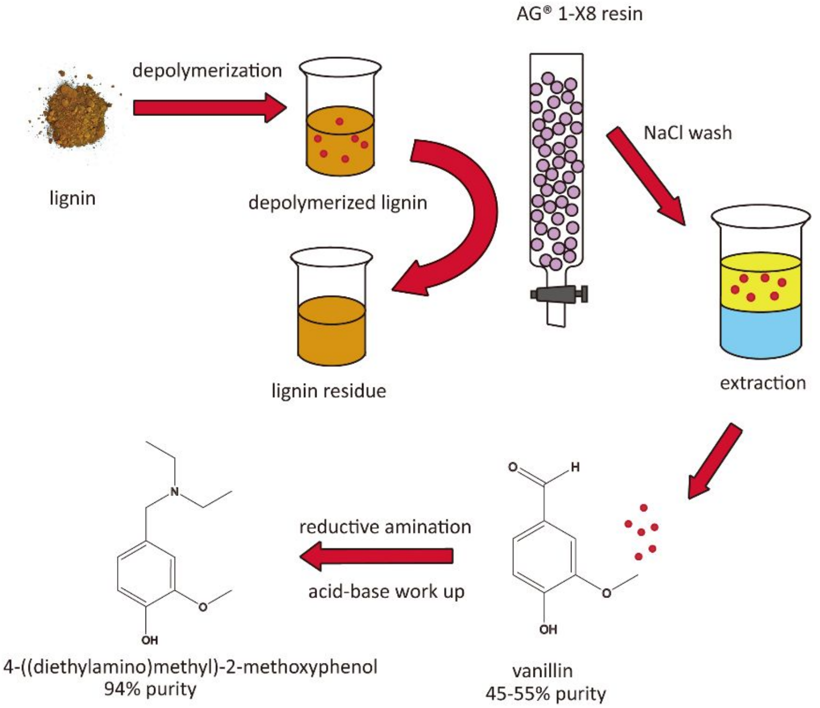
Table of Contents Graphic:



Production and purification of vanillin from lignin and conversion to an intermediate of ionic liquid and/or pharmaceutical synthesis.