

Optimization of Formic Acid/Acetic Acid for Lignin Extraction in Talahib (*Saccharum spontaneum L.*) and the Analysis of the Adsorbent Properties of Lignin Towards Cu(II) and Pb(II) in Aqueous Solution

Ma. Rickamae P. Datiles and Kevinilo P. Marquez*

Institute of Chemistry, University of the Philippines Los Baños, Brgy. Batong Malake, Los Baños, Laguna 4031

*Author to whom correspondence should be addressed; email: kgperez3@up.edu.ph

ABSTRACT

In this paper, Talahib (*Saccharum spontaneum L.*), was subjected to lignin extraction using Formic Acid/Acetic Acid Pulping. The effect of the formic acid/acetic acid/water ratio and the reaction time to the lignin yield was determined. It was found that increasing the concentration of formic acid decreased the lignin yield due to the formation of formate esters, which in turn decreased its reactivity to condense with other materials in the solution. In terms of reaction time, highest yield was observed at lowest cooking time due to the absence of recondensation reactions. Then, the pulp was subjected to further delignification using peroxyacid treatment, which aided in the isolation of any residual lignin left. The isolated lignin was characterized using UV-Vis and ATR-FTIR spectroscopy. Analysis of the adsorbent capability of lignin towards Cu(II) and Pb(II) showed % equilibrium removal amounts of 14.149 ± 0.227 % and 11.384 ± 0.572 %, respectively, both with an equilibrium contact time of 20 minutes. Through kinetic analysis, it was observed that both adsorption processes follow a pseudo-second order model, which means that the adsorption rate depends on the concentrations of both the metal cation and the lignin adsorbent. It also showed that the substrate uptake was greater with Cu(II) (0.330 ± 0.0082 mg/g lignin) compared to Pb(II) (0.262 ± 0.0159 mg/g lignin).

Keywords: *Acetic Acid, Adsorption, Formic Acid, Lignin*

INTRODUCTION

The general theme on the study of lignocellulosic biomass is based on its abundance and low value as a starting material. Lignin is one of the most abundant renewable resource (Watkins et al, 2015) derived from agricultural wastes, energy crops, and wood wastes (Zeng et al, 2014) and is currently being explored as a potential source of chemicals and biomaterials (Watkins et al, 2015). It is mainly made up of (1) cellulose, which is the most abundant (40-45% of the

lignocellulose mass), exhibiting rigidity, as well as insolubility, and resistance against solvents (Anwar, Gulfranz and Irshad, 2014); (2) hemicellulose, which consists of heteropolysaccharides characterized by its random amorphous structure of little strength (Joshi et al, 2011) that can be easily hydrolyzed by dilute acid or base; and (3) lignin, which is a complex compound responsible in linking cellulose and hemicellulose together (Jingjing, 2011).

Lignin is often characterized by its three basic phenylpropanoid units namely, p-coumaryl, coniferyl, and sinapyl alcohol (Figure 1), which can be differentiated by the number of methoxyl side groups present (Marriot et al., 2015). Considering its structure, specifically the hydrophilic hydroxyl and carboxyl group, lignin is capable of binding positively charged metal to form soluble complex (Ge and Li, 2018).

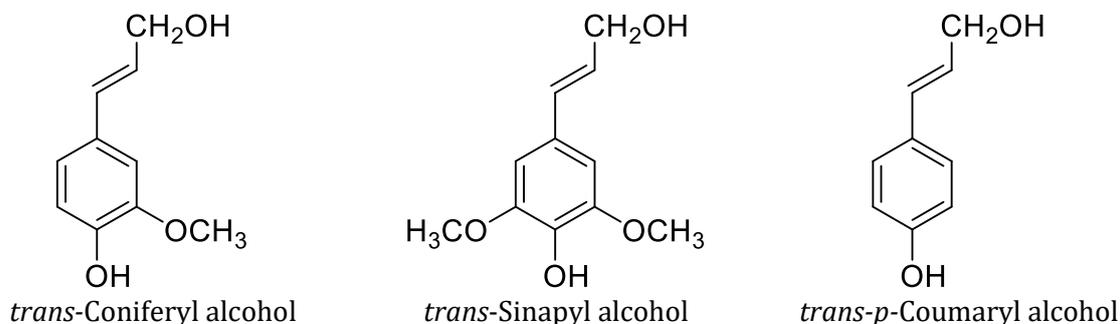


Figure 1. Structural units of lignin (Watkins et al, 2015)

Lignin can be isolated from non-wood fibrous raw materials such as talahib (*Saccharum spontaneum*) through Formic Acid/Acetic Acid (FA/AA) treatment. In acetic acid cooking, the resulting pulp has an acceptable mechanical property, which retains a large part of silica derivatives in the unbleached pulp. Moreover, compared to Kraft pulping, FA/AA treatment is more ideal as the former produces products that have detrimental effect on the environment. Moreover, FA/AA process produces valuable by-products from hemicelluloses and sulfur-free lignin fragments, which are hard to recover. Aside from this, lignin yield can also be controlled and optimized through the fractionation of formic acid, acetic acid, and water, as well as the reaction time (Jahan et al, 2014). In terms of reaction time, optimized time should be determined as to maximize the dissolution of lignin to the acetic acid and to avoid side reactions due to prolonged interaction with the solvent. In terms of the fractionation of formic acid, acetic acid, and water, it is expected that increase in these ratios will result to an increase the lignin yield.

According to Su et al. (2009), many bodies of water in the Philippines have a high concentration of heavy metals such as chromium, lead, and cadmium brought about by water-intensive industries including food and dairy, textile, and pulp/paper industries, which lack proper waste management facility to treat this hazardous waste. Commonly, high concentration of heavy metals is reduced through precipitation, membrane filtration, ion-exchange, and adsorption. Among all of these, adsorption is often used as it is considered to be most efficient treatment, using activated carbon as an adsorbent (Fu and Wang, 2011). However, if a low-cost and non-conventional adsorption of heavy metals is considered, utilization of lignin is the most economical and convenient option considering that it can be isolated from agricultural wastes and grasses, which are often discarded and thrown away. Moreover, it has a property to bind with heavy metals such as lead, mercury, cadmium, chromium, arsenic, zinc, copper, and nickel, isolating it from different bodies of water (Ge and Li, 2018).

It is the objectives of this study to determine the effect of the ratio of formic acid, acetic acid, and water, as well as the reaction time on the lignin yield from talahib (*Saccharum spontaneum*), characterize the lignin using UV-Vis and ATR-FTIR, and determine the adsorption property of the extracted lignin on heavy metals such as lead and copper.

METHODS

Sample Preparation. The lignin source, *Saccharum spontaneum* or talahib was collected from the local field in Bulacan, specifically from a vacant lot along the main road on McArthur highway at around October when the rainy season was approaching. It was then washed, sun-dried, and cut into smaller pieces to remove foreign materials and moisture and to increase the surface area.

Reagents. Formic acid (FA), acetic acid (AA), hydrogen peroxide, and distilled water were bought from Chemline Scientific (Quezon City, Philippines).

FA/AA treatment was the first done to extract lignin, which utilized 80% of organic acid composed of formic acid and acetic acid for pulping. The solution was prepared by mixing the required volume of formic acid, acetic acid, and water at a large beaker using a stirring rod.

The resulting pulp was then subjected to further delignification using the peroxyformic acid/ peroxyacetic acid treatment. The solution was prepared by mixing 8 mL of 20% H₂O₂ to 80% FA/AA mixture in a large beaker using a stirring rod.

Formic Acid/Acetic Acid/Water Pulping and Peroxyformic Acid/ Peroxyacetic Acid Treatment. The method used for the isolation of lignin was the procedure suggested by Nuruddin, et al. (2011) wherein the chopped raw material was subjected to two delignification procedures, which includes an additional treatment with FA/AA/water solution and treatment with peroxyformic acid/ peroxyacetic acid solution.

Five grams of chopped biomass was weighed and placed in a 250-mL round-bottom flask. It was then mixed with the required amount of freshly prepared FA/AA/water solution with liquor to solid ratio of 8:1. The resulting mixture was refluxed on a hot plate under the following conditions:

- a) FA/AA/water ratio of 30:50:20, 40:40:20, and 50:30:20;
- b) Reaction time of 2, 3, and 4 hours at boiling temperature

The resulting pulp was removed from the spent liquor through filtration using cheesecloth.

Isolation of Lignin. After pulping and delignification, the resulting spent liquor was heated at 105°C. The concentrated liquor was then added in distilled water, which was five times the volume of the remaining liquor to precipitate the lignin. The latter was isolated through evaporation of the solvent system.

Characterization of Lignin. Isolated lignin from *Saccharum spontaneum* was characterized using Ultraviolet (UV) Spectroscopy (Thermo Scientific Multiskan GO Microplate) and Fourier Transform Infrared Spectroscopy (FTIR) (Shimadzu IRSpirit Fourier Transform Infrared (FTIR) Spectrophotometer). For UV spectrophotometric method, the isolated lignin was dissolved in 96:4 dioxane-water solution and subjected to UV analysis for the determination of the presence of characteristic peaks.

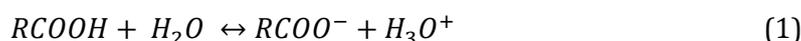
The characteristic wavelengths of lignin are around 200-230 nm and 260-280 nm brought about by the aliphatic and aromatic units. For FTIR spectrophotometric method, the lignin type was determined by the functional groups present such as the methoxyl, carbonyl, and hydroxyl groups. The typical bands are around 1500-1600 cm⁻¹ and 1470-1460 cm⁻¹

Analysis on the Adsorption Capability of the Isolated Lignin. Prior to the analysis of the adsorbing capability of the isolated lignin, standard curves for the determination of the concentration of metal ions removed were firstly derived. A stock solution of 10 mg-L⁻¹ lead and copper solutions were prepared and diluted to 1.0, 3.0, 5.0 and 7.0 mg-L⁻¹ concentrations.

For the kinetic experiment, 0.125 g of lignin was thoroughly mixed in 50 mL of 0.01 M of sodium nitrate (NaNO₃) electrolyte solution in a 100-mL Erlenmeyer flask. The suspension was magnetically and vigorously stirred to hydrate the lignin. The metal ions were then added individually under magnetic stirring. Small sample volumes were collected at different time intervals, filtered, and subjected to UV-Vis spectrophotometry at 253 nm and 260 nm for the Cu(II) and Pb(II) solutions, respectively. For both substrates, the adsorption rate kinetics were analyzed using both the pseudo-first order and pseudo-second order analysis, to determine the dependence of the reaction rate to the concentration of the heavy metal substrate (Cu and Pb) and adsorbent (lignin).

RESULTS AND DISCUSSION

Formic Acid/Acetic Acid/Water Pulping. For herbaceous biomass such as talahib (*Saccharum spontaneum*), the FA/AA/water method is often used for the isolation of lignin. The acids hydrolyze and dissolve the biomass affording a black liquor (Vanderghem et al., 2012). In this method, the chemical reaction involved was the dissociation of organic acids in the solution, forming solvated protons, which facilitated the breakdown of plant matter in an organic acid environment when water was added. For the mixture, the formic acid present serves as the proton supplier while the acetic acid serves as the lignin solvent (Equation 1).



In a concentrated organic acid solution, molecules are closely linked to each other by hydrogen bonds; therefore, protons are not readily available. The addition of water breaks the links between the molecules of organic acid to favor the ionization and dissociation of these acids and to supply the necessary proton in the reaction (Jahan et al., 2014). The FA/AA/water solution cleaved the linkages between the lignocellulosic components, subsequently dissolving the lignin in acetic acid, which served as the dissolution solvent. The formic acid thus served as a catalyst for the cleavage of the lignin-carbohydrate complexes (Lam et al, 2001).

The delignification process at varying cooking time and FA/AA ratio was investigated to optimize the lignin yield (Table 1).

Table 1. % yield of lignin at varying solution composition and reaction time.

Ratio of FA/AA/Water	Reaction Time, hr		
	1.00	2.00	3.00
	Lignin Yield, %	Lignin Yield, %	Lignin Yield, %
30:50:20	55.75 ± 0.059	52.03 ± 0.026	49.29 ± 0.020
40:40:20	52.04 ± 0.038	50.00 ± 0.055	47.92 ± 0.026
50:30:20	49.29 ± 0.054	47.16 ± 0.025	45.92 ± 0.015

Results show that the highest average lignin yields occur at both minimum reaction time and minimum formic acid concentration in agreement with the results of Vanderghem et al. (2012) and Jahan et al. (2014), which showed that increasing the reaction time decreases the concentration of monomeric compounds, due to enhanced side reactions such as pyrolytic and recondensation reactions. In the experiment conducted by Vanderghem et al., *Miscanthus* sp. was subjected to 30:50:20 ratio of FA/AA/water at varying reaction time of 1, 2, and 3 hours. This resulted to a maximum yield of 96.7% at 1.00 hr reaction time while minimum yield of 54.1% at 3.00 hr reaction time. A similar trend was observed in the study of Jahan et al (2014) where mustard stalk, mustard branches, and lentil stalks were subjected to 30:50:20 % by volume ratio of FA/AA/water at varying reaction time of 2, 3, and 4 hours, isolating maximum lignin yield at 80.73%, 67.4%, and 79.1% from 1-hour cooking time.

To assess the effect of the varied parameters on the yield of lignin, a two-way ANOVA was performed (Table 2).

Table 2. Two-Way ANOVA analysis of data

Source of Variation	SS	df	MS	F	P-Value	F _{crit}
Ratio	118.5464727	2	59.27323635	39417.14	1.68 x10 ⁻³³	3.554557
Reaction Time	88.29686029	2	44.14843014	29359.03	2.38 x10 ⁻³²	3.554557
Interaction	5.216060472	4	1.304015118	867.1797	2.59 x10 ⁻²⁰	2.927744
Within	0.027067369	18	0.001503743			
TOTAL	212.0864608	26				

Results show that the F-value generated from the ratio was 39417.14, which is greater than the corresponding F_{crit} (3.554557), indicating that the lignin yield at different solution composition is significantly different. This means that the change in the % yield was significant as the ratio of the organic acids in the extracting solution was varied. Similar results were obtained from the reaction time where the F-value generated (29359.03) is greater than the corresponding F_{crit} (3.554557) indicating that the changes in % yield was significant as reaction time was varied. The model terms are deemed significant due to the small P-values.

Increasing the amount of formic acid in the pulping mixture promotes the formation of hydroxyl moieties into formate esters (Figure 2) making it more electron-withdrawing. As a consequence, the reactivity of the dissolved lignin is reduced, making it harder to condense with other lignin fragment in the solution (van Heiningen et al, 2017), which may result to a low cooking or liquor yield.

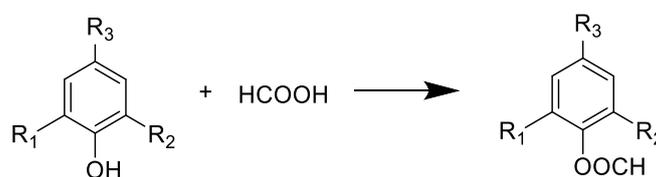


Figure 2. Formation of formate esters of the hydroxyl groups in the lignin

Varying the ratio of the organic acid and reaction time for pulping showed that the lignin yield (%) decreased as the cooking time increased from 1 hour to 3 hours. In some studies, the yield increased with the reaction time until it reached a maximum value and then decreased dramatically due to recondensation reactions occurring at longer reaction times.

The residual lignin during the initial pulping through the peroxyformic acid/ peroxyacetic acid was isolated (Table 3) affording additional yields brought about by the additional delignification process.

Table 3. Additional % lignin yield after further delignification

Ratio of Peroxyformic/Peroxyacetic Acid/Water	Reaction Time, hr		
	1.00	2.00	3.00
	Lignin Yield, %	Lignin Yield, %	Lignin Yield, %
30:50:20	9.18	9.20	9.15
40:40:20	9.98	9.77	9.75
50:30:20	10.45	10.36	10.43

Table 4. Effect of further delignification conditions on the additional lignin yield*

Ratio of Peroxyformic/Peroxyacetic Acid/Water	Additional Percent Lignin Yield, %			
	Trial 1	Trial 2	Average	Standard Deviation
30:50:20	10.42	10.34	10.38	0.056
40:40:20	9.58	9.52	9.55	0.038
50:30:20	8.97	8.95	8.96	0.013

*pulps in this experiment were isolated under the same initial conditions: (40:40:20 FA/AA/water at 1.00 hr reaction time) before further delignification

Table 5. One-way ANOVA for the effect of varying conditions of further delignification*

Sources of Variation	SS	Df	MS	F	P-Value	F _{crit}
Between groups	2.034596	2	1.017298	649.67	0.000111	9.552094
Within groups	0.004698	3	0.001566			
TOTAL	2.039294	5				

*pulps in this experiment were isolated under the same initial conditions: (40:40:20 FA/AA/water at 1.00 hr reaction time) before further delignification

A general increase in the additional yield at higher concentrations of peroxyformic acid (Table 3) was observed, although the data is also influenced by the conditions of the initial pulping. To verify this observation, pulps isolated under the same initial pulping condition (40:40:20 FA/AA/water at 1.00 hr reaction time) were subjected to further delignification using the 3 different peroxyformic/ peroxyacetic acid solutions (Table 4) and subjected to one-way ANOVA (Table 5). Result shows that the F-value generated between the group (649.67) is greater than the corresponding F_{crit} (9.552094) indicating that the additional lignin yield varies significantly with varying peroxyformic/ peroxyacetic acid ratio. The model term is also significant based on the calculated P-value. This suggests that the effects of the further delignification on the yield of the extraction is the same as that of the initial reaction.

Considerations for the choice of extraction method are: (1) the cost of the extracting agents and equipment required, and (2) the yield of the extraction. In a similar study by Marquez and Ramos (2019), alkali extraction with aqueous NaOH was employed. The overall cost was significantly lower due to the lower cost of the materials used, but the yield for the alkali extraction was 15.19% on the average. The extraction method in this study is significantly more costly, but the 45.9% to 55.8% average yield is significantly higher, and the cost can be optimized since the yield varies depending on the composition of the acidic extract and cooking time, although it is still having a higher average cost compared to the alkaline extraction method.

The physical and chemical properties of extracted lignin vary depending on its biological source and extraction method. In the experiment conducted by Watkins and her colleagues (2015), non-wood biomass such as Alfa fibers, pine straw, wheat straw, and Flax fibers resulted to lignin content ranging from 34% to 14.88% after subjecting to FA/AA pulping. FTIR analysis showed that the extracted lignin from all of the non-wood sources contain phenol, which underwent esterification due to the reaction with formic acid. It also showed absorbances due to the syringyl and guaiacyl, which are confirmatory characteristics of lignin. The thermal properties (DSC and TGA) showed high measurements of enthalpy suggesting that lignin isolated from non-wood sources are more stable and can be used as flame retardant material (Watkins et al., 2015).

Similar characteristic functional groups such as oxidized phenolic, guaiacyl, and syringyl units were also observed in the study by Simon and her colleagues (2013) in wood biomass (Beech

trees) subjected to FA/AA pulping. However, compared to non-wood biomass, lignin yield was higher, ranging from 80.5% to 90.7% depending on the conditions employed.

In terms of thermal stability, both wood and non-wood biomass exhibit a quite similar range of temperature by which maximum rate of weight loss occur. For non-wood biomass, it occurs at around 330°C while for wood biomass, it occurs at around of 325-350°C depending on the density. However, in terms of temperature at full degradation, non-wood biomass extended up to more than 800°C while wood biomass only extends until 600°C depending on the density. Thus, lignin from non-wood biomass is most suitable for as a flame-retardant material compared to wood biomass (Popescu et al., 2011).

Similar mechanism is also employed to alkaline extraction wherein lignin and hemicellulose are dissolved in the given medium. Moreover, since it has lower activation energy for delignification of herbaceous species compared to woods, just like FA/AA pulping, it is more suitable for the extraction of lignocellulose on non-wood biomass. However, for this method, since milder conditions are observed compared to acid fractionation, reaction time is quite longer, as well as limited, considering that several alkaline chemicals are converted to irrecoverable salts (Oriez et al., 2020).

The lignin isolated in this method is insoluble in neutral and acidic conditions due to the ionization of its carboxylic and phenolic group. Moreover, since it is quite close to the structure of native lignin just like FA/AA lignin, it is advantageous to several chemical activation and valorization pathways unlike Kraft lignin (Kraft pulping) and lignosulfonates (sulfite pulping), which contains high sulfonic acid functional groups (Oriez et al., 2020). However, compared to FA/AA pulping, for herbaceous biomass such as the Talahib, 15.9% maximum yield was observed within the employed conditions in the experiment. Thus, the former is more suitable for extraction.

Characterization of Isolated Lignin. The isolated lignin from FA/AA pulping was characterized using UV-Vis spectroscopy and ATR-FTIR spectroscopy. The isolated lignin was dissolved in 96:4 dioxane-water solution by volume prior to UV-Vis spectrophotometry. Analysis showed an absorption maximum wavelength at around 298 nm, corresponding to the phenolic groups in lignin (Figure 3).

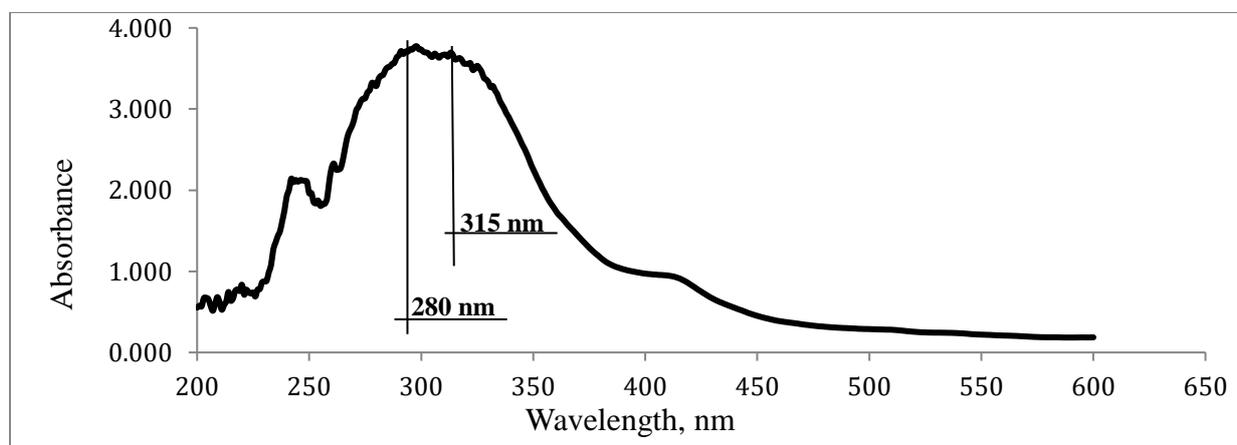


Figure 3. UV-Visible spectrum for the isolated lignin

Almost all samples displayed 2 peaks at around 280-285 and 315-320, which correspond to the non-conjugated phenolic groups and conjugated phenolic groups (p-coumaric and ferulic acids) present in its structure. The first peak (around 282 nm) was due to the presence of conjugated bonds and aromatic rings, which are capable of electronic transition from π to π^* excited state due to the presence of pi electrons in the double bond, corresponding to a narrow energy gap (compared to σ - σ^* transition) at around 220-700 nm. This is a similar case for the second peak

(around 320 nm), which is mainly due to the C=O groups conjugated to the aromatic ring (Lu et al., 2017).

FTIR analysis of extracted lignin revealed that almost all samples displayed bands at 3500-3000, 1700 – 1725 cm^{-1} , 1600 – 1625 cm^{-1} and 1500 – 1525 cm^{-1} , 1300 – 1325 cm^{-1} and 1200 cm^{-1} (Figure 4). However, for organosolv lignin such as the isolated lignin in Talahib, bands at 1716 – 1711 cm^{-1} , 1600 and 1500 cm^{-1} , 1300 cm^{-1} and 1200 cm^{-1} are expected due to the esterification of phenol and alcohol of the propane chain, presence of aromatic compounds such as phenolic hydroxyl group, presence of syringyl unit, and presence of guaiacyl unit, respectively (Watkins et al., 2015).

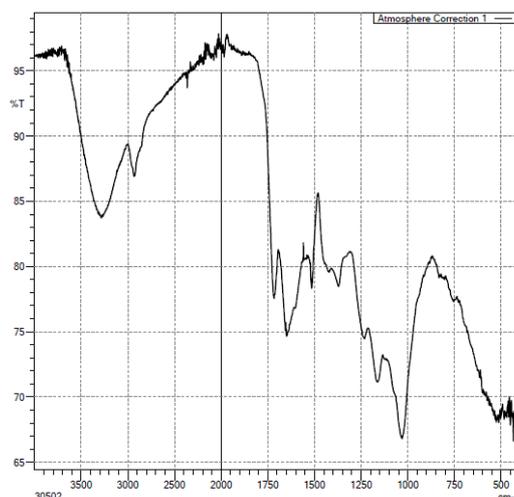


Figure 4. FTIR spectrum of Organosolv lignin extracted from Talahib (*Saccharum spontaneum*)

Analysis on the Metal Adsorption of Lignin. Lignin is a natural amorphous cross-linked resin that has an aromatic three-dimensional polymer structure containing a number of functional groups such as phenolic, hydroxyl, carboxyl, benzyl alcohol, methoxyl, and aldehyde groups, making it potentially useful as an adsorbent material for removal of heavy metals from water.

Table 6 shows the data for the calibration curves of the prepared Cu(II) and Pb(II) solutions, where absorbance values were measured at 253 nm and 260 nm, respectively. Values of the correlation coefficients show that both standard curves follow the Beer-Lambert Law.

Table 6. Calibration Curve Data for Aqueous Standard Solutions of Cu(II) and Pb(II)

Parameters	Slope (L mg^{-1})	y-intercept	R^2
Cu(II)	0.145406854	-0.009224143	0.9993
Pb(II)	0.013581464	-8.56698×10^{-6}	0.9992

Results of kinetic experiments are shown in Figures 5 and 6. The adsorption equilibria for Cu(II) and Pb(II) were achieved at a minimum solid-solution contact time of 20 mins. To evaluate the differences in the adsorption kinetic rates, the pseudo first-order and pseudo-second order kinetic models were tested for fitness to the results. The rate law for a pseudo-second order kinetic model best described the experimental data with the correlation coefficients (R^2) of 0.9505 and 0.9452 for Cu(II); 0.9634 and 0.9591 for Pb(II).

The determination of the adsorption rate and percent substrate removal were also performed. Addition of NaNO_3 in the mixture ensures a practically constant solution ionic strength. From the absorbance values collected every after 5 mins within the 40-min reaction time, it was observed that the equilibrium % removal is achieved at the 20-min mark (Figure 5). It can also be observed that the % removal for Cu(II) is generally higher than that of Pb(II) (Table 7).

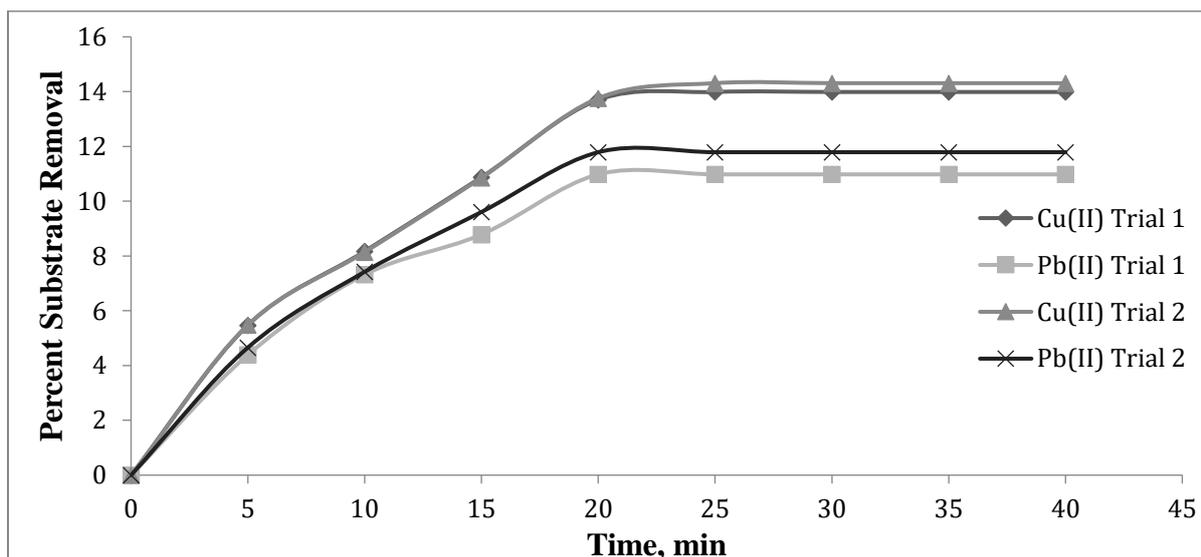


Figure 5. Graph of the Percent Removal Rate for Cu(II) and Pb(II)

Table 7. Data for the % Removal of Both Substrates

Substrate	% Removal		
	Trial 1	Trial 2	Average
Cu(II)	13.988	14.309	14.149 ± 0.227
Pb(II)	10.979	11.789	11.384 ± 0.572

To explore this trend further, kinetic analysis was performed to determine the dependence of the adsorption process to the concentrations of both the adsorbent (lignin) and the substrates Cu(II) and Pb(II). The effect of contact time on the adsorption was then determined using a linearized pseudo-first order (Equation 2) and pseudo-second order (Equation 3) kinetic equations

$$\log(q_{eq} - q_t) = (q_{eq}) - \frac{K_{pf}t}{2,303} \quad (2)$$

q_{eq} (mg g⁻¹) - substrate uptake per gram of lignin at equilibrium

q_t (mg g⁻¹) - substrate uptake per gram of lignin at a certain time

K_{pf} (min⁻¹) - rate constant of the pseudo-first order rate equation

t (min) - contact time for the adsorption

$$\frac{1}{q_t} = \frac{q_{eq}^2}{K_{ps}} - \frac{t}{q_{eq}} \quad (3)$$

K_{ps} (g mol⁻¹min⁻¹) - rate constant of the second-order adsorption

The plot of the effect of contact time on the adsorption process shows the time where equilibrium is achieved (maximum value) and how fast can the removal happen. The first step was to determine which kinetic model best describes the adsorption process. Using the data for the contact time and adsorbate uptake, or the amount of heavy metal substrate removed in solution (presumed to be adsorbed by lignin), the correlation coefficients for both models were calculated. Results show (Table 8) the the pseudo-second order model is the better choice for the kinetic model.

Table 8. Correlation Coefficients for the Determination of the Adsorption Kinetics

Substrate	R ² , pseudo-first order	R ² , pseudo-second order
Cu(II)	0.945177589	0.950496864
Pb(II)	0.959143394	0.963379977

A pseudo-second order mechanism suggests that the reaction rate is dependent both on the concentrations of the substrate and the adsorbent sites. Since the lignin adsorbent exists as a granular material suspended in solution, there will only be a limited number of heavy metal adsorbates that could adsorb in its surface. Thus, increasing the amount of lignin in solution would increase the rate of adsorption. However, other factors might come into play, like the solubility of lignin in the aqueous matrix, pH, temperature, among others.

Table 9 shows the pseudo-second order parameters for the adsorption of both substrates. Results show that the amount of Cu(II) adsorbed in lignin at equilibrium is greater than that of Pb(II). The greater size of the Pb(II) means that lignin has less available adsorption sites for the cation compared to the smaller Cu(II), which explains the lower equilibrium substrate uptake of Pb(II) despite reaching the same equilibrium contact time of 20 minutes.

Table 9. Pseudo-Second Order Kinetic Parameters for the Adsorption Process

Substrate	v_0 , mg g ⁻¹ min ⁻¹	k, g mg ⁻¹ min ⁻¹	q _e , mg g ⁻¹
Cu(II)	0.055 ± 0.0014	0.507 ± 0.0381	0.330 ± 0.0082
Pb(II)	0.053 ± 0.0012	0.776 ± 0.0767	0.262 ± 0.0159

CONCLUSIONS

The study was able to optimize several variables of FA/AA pulping such as the ratio of formic acid and acetic acid, and reaction for an effective extraction and isolation of lignin from *Saccharum spontaneum*, which will be useful for the absorption of heavy metals such as lead and copper.

In the process of extraction, it was observed that as the concentration of formic acid increased, the amount of the lignin isolated decreased due to the formation of formate esters of the aromatic ring, reducing its reactivity to condense with other soluble lignin fragment. Similar observations also resulted from the increase in cooking time due to the occurrence of recondensation at prolonged contact. Maximum percent yield was observed in 30:50:20 FA/AA/water solution with 1 hour cooking time (55.766%) while minimum percent yield was observed in 50:30:20 FA/AA/water solution with 3-hour cooking time (45.909%).

UV spectrophotometric method confirmed qualitatively the chemical structure of the lignin due to the observed wavelengths (282 nm and 320 nm) characteristic of conjugated and non-conjugated units of the lignin. FTIR bands at 1700 cm⁻¹ range, 1500 – 1600 cm⁻¹, 1300 cm⁻¹ and 1200 cm⁻¹ were attributed to the formate ester and unconjugated carbonyl stretching, aromatic phenolic and hydroxyl group, and syringyl and guaiacyl groups, respectively.

The isolated lignin from FA/AA pulping was shown to adsorb heavy metals such as lead and copper. The percentage removal for Cu(II) are generally higher (14.15%) than for Pb(II) (11.38%). The effects of other parameters, such as pH and temperature, can be investigated to explore the specific nature of the interactions between the adsorbent and adsorbate. Functionalization of the isolated lignin can also be explored as to increase its adsorption capability. Other heavy metals, as well as organic solutes, can also be tested as substrates to increase the knowledge in the adsorption capability of lignin.

For the extraction process, it is recommended that the resulting liquor from pulping be isolated using more thorough methods of filtration or isolation. For the optimized reaction time, on the other hand, a lower minimum cooking time can be explored as to determine whether maximum time before the recondensation reactions.

REFERENCES

Anwar Z, Gulfraz M, Irshad M. Agro-industrial lignocellulosic biomass a key to unlock the future bio-energy: a brief review. *J Radiat Res Appl Sci*. 2014 Feb; 7(2):163-173. <https://doi.org/10.1016/j.jrras.2014.02.003>

Fu F, Wang Q. Removal of heavy metal ions from wastewaters: a review. *J Environ Manage*. 2011 Mar; 92(3):407-418. <https://doi.org/10.1016/j.jenvman.2010.11.011>

Ge Y, Li Z. Application of lignin and its derivatives in adsorption of heavy metal ions in water: a review. *ACS Sustain Chem Eng*. 2018 Mar; 6(5):7181-7192. <https://doi.org/10.1021/acssuschemeng.8b01345>

Jahan MS, Rumeen JN, Rahman MM, Quaiyyum A. Formic acid/acetic acid/water pulping of agricultural wastes. *Cellulose Chem Technol*. 2014; 48(1-2):111-118. Retrieved from: [https://cellulosechemtechnol.ro/pdf/CCT1-2\(2014\)/p.111-118.pdf](https://cellulosechemtechnol.ro/pdf/CCT1-2(2014)/p.111-118.pdf)

Jingjing L. Isolation of lignin from wood [dissertation]. [Imatra]: Saimaa University of Applied Sciences; 2011. 60 p. Retrieved from: <https://core.ac.uk/download/pdf/38048748.pdf>

Joshi B, Bhatt MR, Sharma D, Joshi J, Malla R, Sreerama L. Lignocellulosic ethanol production: current practices and recent developments. *Biotechnol Mol Biol Rev*. 2011 Nov; 6(8):172-182. <https://academicjournals.org/journal/BMBR/article-full-text-pdf/8D9C02C11840.pdf>

Lam HQ, Bigot YL, Delmas M, Avignon, et G. Formic acid pulping of rice straw. *Ind Crops Prod*. 2001 Jul; 14(1):65-71. [https://doi.org/10.1016/S0926-6690\(00\)00089-3](https://doi.org/10.1016/S0926-6690(00)00089-3)

Lu Y, Lu YC, Hu HQ, Xie FJ, Wei XY, Fan X. Structural characterization of lignin and its degradation products with spectroscopic methods. *J Spectrosc*. 2017 Nov; 2017:8951658. <https://doi.org/10.1155/2017/8951658>

Marquez KP, Ramos RMCR. Alkali lignin from Talahib grass (*Saccharum spontaneum L.*) as an adsorbent for chromium (III) and phenolphthalein: analysis of the adsorption kinetics and mechanism. *KIMIKA*. 2019 Dec; 30(2):17-26. <https://doi.org/10.26534/kimika.v30i2.17-26>

Marriott PE, Gomez LD, McQueen-Mason SJ. Unlocking the potential of lignocellulosic biomass through plant science. *New Phytol*. 2015 Oct; 209(4):1366-1381. <https://doi.org/10.1111/nph.13684>

Nuruddin M, Chowdhury A, Haque SA, Rahman M, Farhad SF, Sarwarjahan M, Quaiyyum A. Extraction and characterization of cellulose microfibrils from agricultural wastes in an integrated biorefinery initiative. *Cellul Chem Technol*. 2011 May; 45: 347-354.

Oriez V, Peydecastaing J, Pontalier PY. Lignocellulosic biomass mild alkaline fractionation and resulting extract purification processes: conditions, yields, and purities. *Clean Technol*. 2020 Feb; 2(1):91-115. <https://doi.org/10.3390/cleantechnol2010007>

Popescu MC, Popescu CM, Lisa G, Sakata Y. Evaluation of morphological and chemical aspects of different wood species by spectroscopy and thermal methods. *J Mol Struct.* 2011 Mar; 988(1-3):65–72. <https://doi.org/10.1016/j.molstruc.2010.12.004>

Simon M, Brostaux Y, Vanderghem C, Jourez B, Paquot M, Richel A. Optimization of a formic/acetic acid delignification treatment on beech wood and its influence on the structural characteristics of the extracted lignins. *J Chem Technol Biotechnol.* 2013 May; 89(1): 128–136. <https://doi.org/10.1002/jctb.4123>

Su GS, Martillano KJ, Alcantara TP, Ragragio E, De Jesus J, Hallare A, Ramos G. Assessing heavy metals in the waters, fish and macroinvertebrates in Manila Bay, Philippines. *J Appl Sci Environ Sanitation.* 2009 Nov; 4(3):187-195. Retrieved from: https://www.researchgate.net/profile/Glenn-Sia-Su/publication/44024637_Assessing_heavy_metals_in_the_waters_fish_and_macroinvertebrates_in_Manila_bay_Philippines/links/0f317533f70a9579bb000000/Assessing-heavy-metals-in-the-waters-fish-and-macroinvertebrates-in-Manila-bay-Philippines.pdf

Vanderghem C, Brostaux Y, Jacquet N, Blecker C, Paquot M. Optimization of formic/acetic acid delignification of *Miscanthus×giganteus* for enzymatic hydrolysis using response surface methodology. *Ind Crops Prod.* 2012 Jan; 35(1):280–286. <https://doi.org/10.1016/j.indcrop.2011.07.014>

Van Heiningen A, Yasukawa Y, Dido K, Francis R. Minimizing precipitated lignin formation and maximizing monosugar concentration by formic acid reinforced hydrolysis of hardwood chips, In: Ruiz H, Hedegaard T, Trajano H, editors. *Hydrothermal processing in biorefineries*. Springer Cham; 2017. https://doi.org/10.1007/978-3-319-56457-9_18

Watkins D, Nuruddin MD, Hosur M, Tcherbi-Narteh A, Jeelani S. Extraction and characterization of lignin from different biomass resources. *J Mater Res Technol.* 2015 Jan; 4(1):26-32. <https://doi.org/10.1016/j.jmrt.2014.10.009>

Zeng J, Tong Z, Wang L, Zhu JY, Ingram, L. Isolation and structural characterization of sugarcane bagasse lignin after dilute phosphoric acid plus steam explosion pretreatment and its effect on cellulose hydrolysis. *Bioresour Technol.* 2014 Feb; 154:274–281. <https://doi.org/10.1016/j.biortech.2013.12.072>