



An- Najah National University

Faculty of Engineering

Chemical Engineering Department

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Lignin Isolation from Wood by Organosolv Method

Prepared by:

Batool Abu-Ayyash

Farehan Marshoud

Sarah Albakry

Submitted to:

Dr. Majd Shhadi

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Abstract

The growing awareness of the environmental concerns in the subsequent decade has brought attention to the importance of having efficient and renewable processes. Lignin represents the second most abundant natural polymer on earth and might play an important role as an alternative in petrochemical industry. Also, it may be considered as an aromatic renewable source. Kraft, liginosulfonate and Organosolv are the main three methods are usually used for lignin isolation from wood.

In this project, lignin from sawdust was isolated by using Organosolv method. Two different mixtures; ethanol-water (50 vol %) with 1.0 wt. % H_2SO_4 as a catalyst, and formic acid-acetic acid (85 vol. %) were investigated. Furthermore, the effect of the reaction time on the lignin yield using 50 vol. % ethanol-water solution was also conducted in this current study. Also, the amount of total lignin in wood sample was measured using Klason method for calculating the acid insoluble residue (AIR) in addition to UV-spectrophotometer for acid soluble lignin (ASL) amount.

According to the results, the percentage of lignin in the wood sample was 30 wt. %. The research has also shown that the yield of lignin using ethanol-water mixture obviously increased with increasing the reaction time which accounted the maximum for 16.87 wt. % of the total amount of lignin present after 5 hours of heating process. For a certain amount of wood sample, it was found that the lignin yield using formic acid-acetic acid mixture was higher than the lignin yield obtained from ethanol-water mixture. Other factors such as safety, environmental issues and economic must be considered in the final selection of method recommended for lignin extraction from wood.

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CHAPTER 1

INTRODUCTION

Petroleum is one of the most important natural resources and a raw material for the blend of different chemicals, a series of issues has arisen, for example; decreased petroleum reserves in the world, emissions of the toxic substances from the final product, and the problem of global warming requires severe reductions in the use of fossil fuel. These issues prompted the developing interest for cleaner and renewable sources of chemicals for fuels and products. So wood biomass could stand as prominent source for producing renewable energy to reduce production cost and toxic emissions and other applications of wood (Sun and Ren, 2010, Koumba-Yoya and Stevanovic, 2017, Welker, Balasubramanian, Petti, Rai, Bolt and Mendu, 2015).

Previous studies were found that the alternative feedstocks for biofuel and biochemical generation such as lignocellulosic material could reduce CO₂ emissions enormously. Wood is essentially composed of about 45 wt. % cellulose, 25 wt. % hemicelluloses, 25 wt. % lignin, and extractives. Cellulose becomes an important raw material for the pulp and paper as well hemicellulose show high potential for utilization in biomedical field (Welker, Balasubramanian, Petti, Rai, Bolt and Mendu, 2015, Li, 2011, Arhat, Venditti, Ubbe, Aha, Becquart and Ayoub, 2017).

Lignin is derived from the Latin term lignum, which means wood. The nature of wood was recognized by Anselme Payen (1838) and referred to a carbon rich substance as the “encrusting material” which embedded cellulose in the wood. Then in 1865 the encrusting material was defined as lignin by Schulze and lignin has been described as a random. There are many functions of lignin including provides structure of plant cell wall, resistance to chemical and biological degradation. Finally, it plays an essential function in a plant’s natural defense against degradation by hindering penetration of destructive enzymes through the cell wall. Although lignin is important to trees, but in most chemical papermaking fibers it is undesirable and is removed by pulping and bleaching processes (Sjöström, 1993, Nitsos, Stoklosa, Karnaouri, Vörös, Lange, Hodge, Crestini, Rova and Christakopoulos, 2016, Watkins, Nuruddin, Hosur, Tcherbi-Narteh and Jeelani, 2015).

Lignin, making up to 10–25 wt. % of wood component, is the second most abundant natural polymer after cellulose that expected to play an important role in the near future as a raw material for the production of bio-products. Owing to the large lignin rate in the plant cell wall lignin may-be the largest renewable source of aromatic compounds (Nitsos, Stoklosa, Karnaouri, Vörös, Lange, Hodge, Crestini, Rova and Christakopoulos, 2016, Watkins, Nuruddin, Hosur, Tcherbi-Narteh and Jeelani, 2015).

There are several direct and indirect methods for determination the total amount of lignin in wood. Klason method which is the direct method that measure acid-insoluble lignin whereas UV spectrophotometer used as indirect method and determine the concentration and purity of lignin (Li, 2011).

The complex structure of the lignin network is the major reason for making the isolation of unaltered lignin for structural and/or compositional analysis a challenging task. Many processes are developed and used in the extraction and isolation of lignin from other constituents of plant material for paper and other products. These processes include Kraft pulping process which treated wood chips with a mixture of sodium sulfide (Na_2S) and sodium hydroxide (NaOH), at raised temperature. In addition, lignosulfonates are one of the traditional forms of lignin that has been used for the high sulfonic acid functional groups which gives them great binding and emulsifying properties. Finally, Organosolv lignin extraction process, consists of solubilization and extraction of lignin with an organic solvent such as ethanol, formic acid and acetic acid (Koumba-Yoya and Stevanovic, 2017, Sjöström, 1993, Watkins, Nuruddin, Hosur, Tcherbi-Narteh and Jeelani, 2015).

Objectives

The main objective of this project is to isolate lignin from sawdust by Organosolv method by using two different mixtures; ethanol-water (50 vol %) and formic acid-acetic acid (85 vol %) Furthermore, the effect of the reaction time on the lignin yield using 50 vol. % ethanol-water solution is conducted. To analyze lignin, Klason method for acid insoluble residue (AIR) and UV-spectrophotometer for acid soluble lignin (ASL) are used to determine the total lignin represents in wood.

CHAPTER 2

2. LITIRITURE REVIW

2.1.Wood

Wood is one of the most abundant resources for renewable energy and industrial product. Is also one of the most complex materials, composed of polymers of lignin and carbohydrates that are physically and chemically bound together. Wood is essentially composed of about 45 wt. % cellulose, 25 wt. % hemicelluloses, 25 wt. % lignin, and extractives (Novaes, Kirst, Chiang, Winter-Sederoff and Sederoff, 2010). Wood species can be divided into two groups: (1) Hardwood which is wood that comes from deciduous trees, lose their leaves every autumn. Hardwood trees generally have leaves. (2) Softwood which is wood from coniferous trees, evergreens .Softwood trees generally have needles and cones (Houck and Eagle, 1998). Their relative composition varies greatly as shown in Table 1. Table 2 shows many differences between these two groups.

Table 1: Main compositions of Hardwood and Softwood (Novaes, Kirst, Chiang, Winter-Sederoff and Sederoff, 2010).

Constituent (wt. %)	Hardwood	Softwood
Cellulose	40-44	40-44
Hemicellulose	15-35	20-32
Lignin	18-25	25-35

Table 2 : The main differences between hardwood and softwood.

	Hardwood	Softwood	Ref.
Examples	Examples of hardwood trees include Oak, maple, hickory and birch.	Examples of softwood trees include Pine, fir, spruce and juniper.	(Houck and Eagle, 1998)
Fire Resistance	In general, hardwood provides longer burning fires than softwood.		(Houck and Eagle, 1998)
Density	Most hardwoods have a higher density than most softwood.	Most softwood has a lower density than most hardwoods.	(Houck and Eagle, 1998)
Cost	Hardwood is typically more expensive than softwood.		<i>(Hardwood vs Softwood - Difference and Comparison / Diffen, 2011)</i>
Uses	Used in high-quality furniture, decks, and flooring.	Used in building components (e.g., windows, doors), furniture, medium-density fiberboard (MDF), paper, Christmas trees, and much more.	<i>(Hardwood vs Softwood - Difference and Comparison / Diffen, 2011)</i>

2.2.Cellulose

Cellulose is the main constituent of wood carbohydrates. It is a polysaccharide consisting of glucose units. Also, its molecule is linear, and it easily forms hydrogen bonds with neighboring molecules. Cellulose is insoluble in water, dilute acidic solutions, and dilute

alkaline solutions at normal temperatures. There are some applications for cellulose such as it becomes an important raw material for the pulp and paper, fibrous chemical industries and textile. Cellulose is the first most abundant natural polymer that expected to play an important role in the near future (Sun and Ren, 2010, Jingjing, 2011, Chen, 2014). The structure of cellulose molecule is shown in Figure 1.

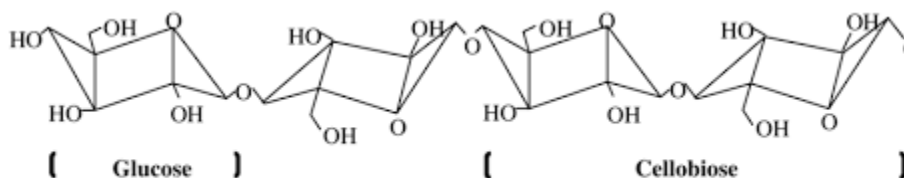


Figure 1: Molecular chain structure of cellulose (Li, 2011).

2.3. Hemicellulose

Hemicellulose belongs to a group of heterogeneous polysaccharides which are formed through biosynthetic routes different from that of cellulose. Hemicellulose has an important role to form the bonds between the fibers that give the paper fiber network its strength. Hemicellulose has a random, amorphous structure with little strength. It is easily hydrolyzed by dilute acid or base compared with cellulose which is crystalline, strong, and resistant to hydrolysis. Hemicelluloses show high potential for utilization in remarkable applications such as biomedical field such as wound dressing and immune system activation against cancer cells, hemicellulose acetate was used as an internal plasticizer for cellulose acetate films and hemicellulose-based binders were used for paper coatings (Jingjing, 2011, Arhat, Venditti, Ubbe, Aha, Becquart and Ayoub, 2017).

2.4. Lignin

Lignin is a complex chemical compound which may exist in wood as branched-chain polymer molecules. Also, it is insoluble in water stable in nature and acts as glue that connects cellulose and hemi-cellulose, giving stiffness to the plant, making the cell wall hydrophobic (organic attracting) and protecting it against microbial degradation. It is

concentrated mainly in the region of the middle lamella (a thin, sticky membrane between plant cells that cements the cell walls together and characterized by its relatively high level of pectin) (Watkins, Nuruddin, Hosur, Tcherbi-Narteh and Jeelani, 2015). As it is illustrated in the Figure 2, lignocellulosic matrix is a complex structure in which the cellulose is surrounded by a monolayer of hemicellulose and embedded in a matrix of hemicellulose and lignin. Furthermore, lignin specifically creates a barrier to enzymatic attack while the highly crystalline structure of cellulose is insoluble in water, then the hemicellulose and lignin create a protective sheath around the cellulose. Also, Lignin is an amorphous polymer with a chemical structure that distinctly differs from the other macromolecular constituents of wood (Li, 2011).

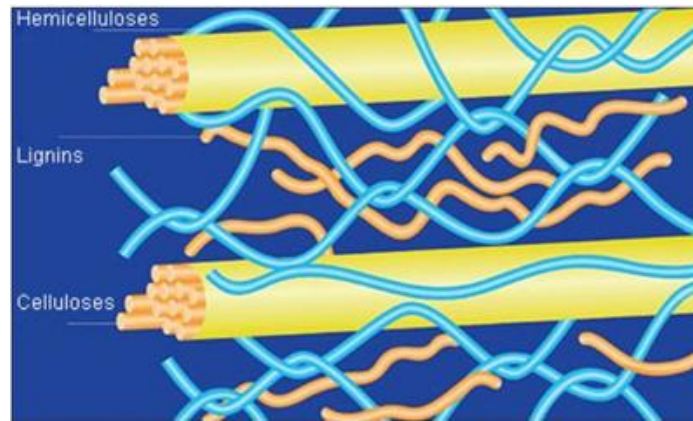


Figure 2: The position of lignin within lignocellulosic matrix (Li, 2011).

The lignin polymer molecule is made up by enzymatic polymerization of three phenylpropane monomers, guaiacyl (G) from precursor coniferyl alcohol, sinapyl (S) from sinapyl alcohol, and *p*-hydroxyphenyl propane (*p*-H) units from precursor *p*-coumaryl alcohol as shown in Figure 3. the resulting structure is a unique and very complex macromolecule that contains a number of different types of linkages (Sjostrom, 2013). The dominant linkage is the β -aryl ether (β -O-4) representing 45-85% of linkages in lignin. Figure 4 shows the Common linkages between the phenylpropane units which depend on the type of wood material, Hardwood and softwood Lignin classification is traditionally done according to the precursors of the polymer. G lignin is typical of softwood species,

with the remainder consisting mainly of p-H units. In contrast, generally GS lignins found in hardwood species, are mainly composed of G and S units in varying ratios (Li, 2011).

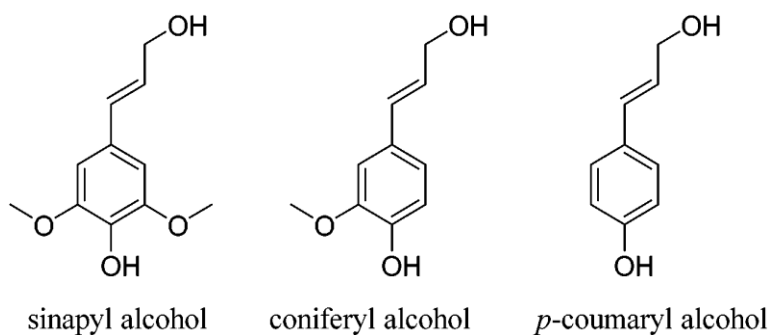


Figure 3: Dominant building blocks of the three dimensional polymer lignin (Harmsen, Huijgen, Bermudez and Bakker, 2010).

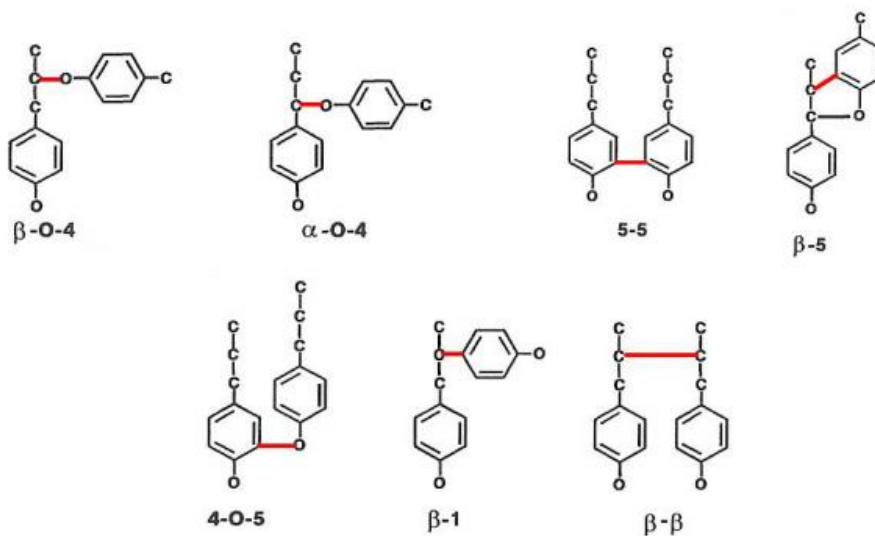


Figure 4: Common linkages between the phenylpropane units (Sjostrom, 2013).

2.5. Lignin Isolation Methods from Wood

Many isolation processes are developed to extract and isolate lignin from associated wood components to produce a suitable pulp for the manufacture of paper and other related products (Li, 2011). These processes do the extraction by

1. Physical pretreatment which involves breakdown of biomass size and crystallinity by milling or grinding (Brodeur, Badal, Collier, Ramachandran and Ramakrishnan, 2011).
2. Chemical pretreatment that are purely initiated by chemical reactions for disruption of the biomass structure such as alkaline pretreatment (involves the use of bases like sodium, potassium, calcium, and ammonium hydroxide), acid pretreatment (involves the use of concentrated and diluted acids) for the pretreatment of lignocellulosic biomass and other methods such as wet oxidation and green solvents (Harmsen, Huijgen, Bermudez and Bakker, 2010, Brodeur, Badal, Collier, Ramachandran and Ramakrishnan, 2011).
3. Biochemical pre-treatments which involves the use of microorganisms (mainly fungi) to degrade lignin and hemicellulose but leave the cellulose intact (Brodeur, Badal, Collier, Ramachandran and Ramakrishnan, 2011).

Currently, different extraction processes shown in Figure 5 which classified into two main categories, sulfur and sulfur-free processes, respectively, are used worldwide (Laurichesse and Avérous, 2014).

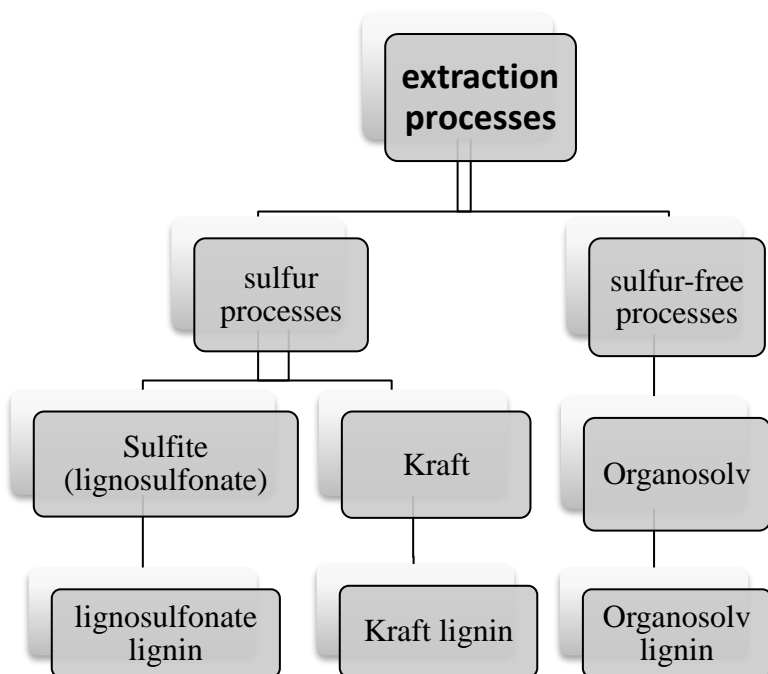


Figure 5: different extraction processes to separate lignin from lignocellulosic biomass and the corresponding production of technical lignins (Laurichesse and Avérous, 2014).

2.5.1. Kraft Lignin Process

Kraft process is the dominant process in pulp and paper production. It is used sodium hydroxide and sodium sulfide to do the pulping process. Kraft pulp is the most productive of the other pulp, accounting for two-thirds of the world's production. The Kraft process has special advantages compared to other pulp processes because of the ability of Kraft process to handle about all species of wood, the high strength of Kraft pulp, and high chemical recovery efficiency which is about 97%.

The Kraft process has three main functions which included co-generating steam and power, minimizing the environmental impact of waste material (black liquor) from the pulping process, and recycling pulping chemicals, NaOH and Na₂S (Tran and Vakkilainen, 2008).

There are two main steps for producing Kraft lignin; begin with pulping process to produce black liquor and then separating Kraft lignin from black liquor the following sections outline these two steps:

❖ Kraft pulping process to produce black liquor

The Kraft process fits almost all kinds of wood. The presence of resins, waxes, or fats in the wood does not impede the pulping process, because the chemical liquor used is alkaline which is a solution of sodium hydroxide NaOH and sodium sulfide Na₂S (Sun and Ren, 2010, Champion, 1961). Lignin is bonded with cellulose fibers in the wood and therefore the Sulfate pulping is used to break these bonds.

The pulping process is made by inserting the wood chips onto the batch digesters and adding the liquor to it which is a solution of sodium hydroxide and sodium sulfide, (Benjamin, Douglass, Hansen, Major, Navarre and Yerger, 1969). The steam is used to heat up to about 180 °C and to keep this temperature inside the digesters during the pulping process at pressures up to 130 psi, with a digestion time of 2–4 h (These conditions can vary depending on the type of wood used). During the process, small amounts of sulfide react with lignin in wood chips. Causes a release number of volatile compounds which

contain methyl mercaptan and dimethyl sulfide, this gives the distinctive odors of kraft mills (Benjamin, Douglass, Hansen, Major, Navarre and Yerger, 1969).

When the cook is finished inside the digester, there is residual internal pressure in the digester used to discharge the pulp into a blow tank as shown in Figure 6. Inside the blow tank, steam and gases released are ventilated and removed using direct or indirect contact condensers, thus the condensable vapors removed also heat is recovered. As for noncondensable gases, they are absorbed in condensing water. The pulp is diluted into the blow tank and then pumped into multistage drum filters then washing with water is carried out to remove spent pulping chemicals. The liquor during the washing process is exposed to the atmosphere and there is some loss of volatile gases and vapors. The resulting filtrate is black liquor (Benjamin, Douglass, Hansen, Major, Navarre and Yerger, 1969).

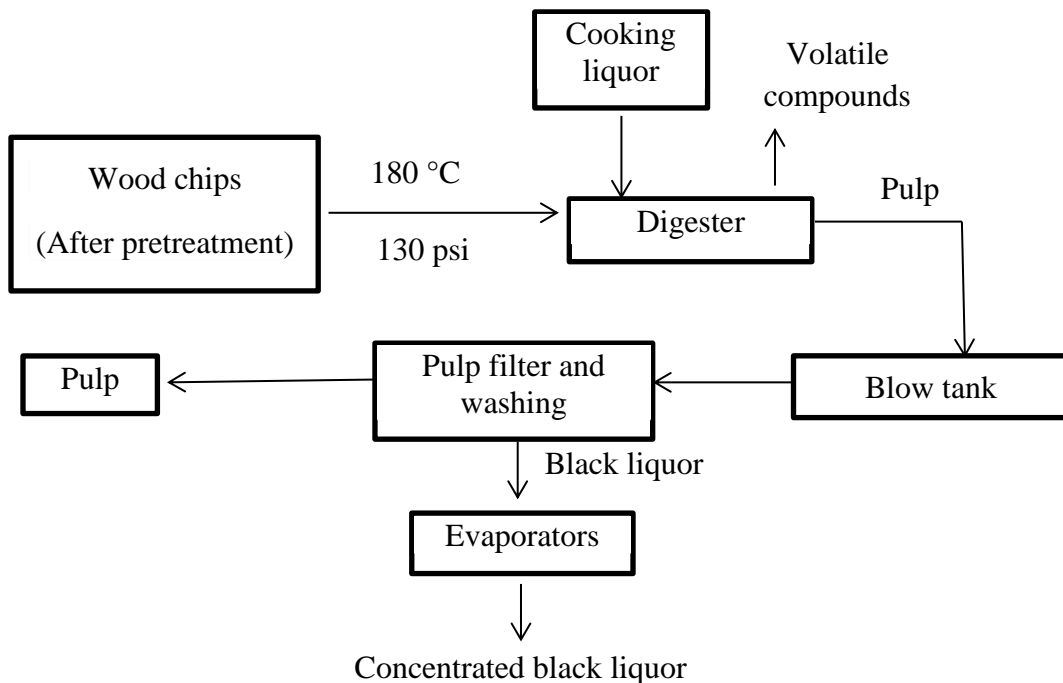


Figure 6: Main unit processes of manufacturing of black liquor (Benjamin, Douglass, Hansen, Major, Navarre and Yerger, 1969).

Sulfite process has been the most abundant type of industrial lignin commercially available throughout the history. At the beginning it was developed depending on using the calcium bisulfite

(an inexpensive pulping chemical). It has many advantages such as the high brightness of the pulp, the ease of bleaching and the cheapness of chemicals that are used in the process. Despite its benefits, it has some disadvantages; the most important one is that the cellulose fibers typically are weaker than those produced by the Kraft process. Besides, there is a limited number of wood species that can be applied to this process (Kjellin and Johansson, 2010). sulfite (lignosulfonate) lignin is obtained by using sulfurous acid and/or a sulfite salt containing magnesium, calcium, sodium, or ammonium (Aro and Fatehi, 2017). The product is a mixture of sulfonated lignin, sugars, sugar acids, resins and inorganic compounds (Calvo-Flores and Dobado, 2010). The structure of lignosulfonate is shown in Figure 7 below.

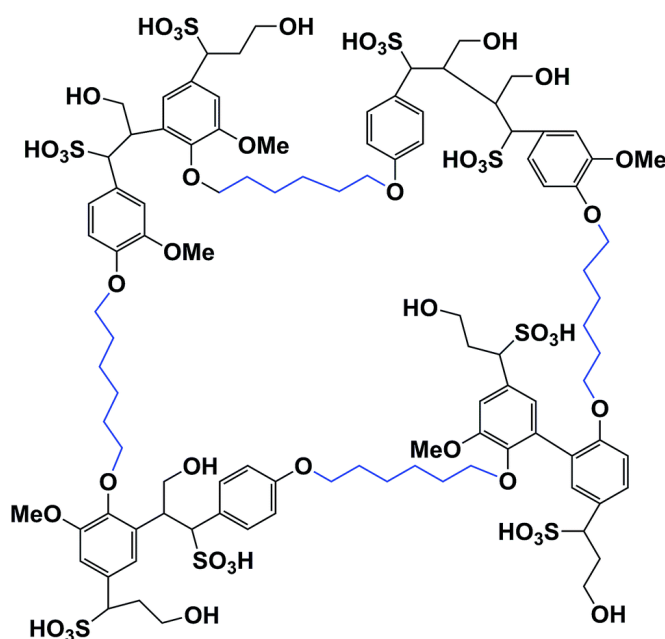


Figure 7: The structure of lignosulfonate (Hong, Li, Zeng, Zhang, Peng and Qiu, 2015).

Lignosulfonates have many favorable properties comparing with Kraft lignins including higher molecular weights of about (6940 g/mol) while Kraft lignins about (4430 g/mol) and a relatively higher sulfur content of about 5% compared to Kraft lignins (about 1–2%) (Ek, Gellerstedt and Henriksson, 2009, Brudin and Schoenmakers, 2010). Also, They are soluble in weak acids and bases but almost completely insoluble in the common organic solvents such as acetone and ethyl acetate (Lo, 1970).

There are two main steps for producing lignosulfonates; begin with Pulping process to produce spent liquor and then separating lignosulfonates from spent liquor.

Pulping process to produce spent liquor

To isolate lignosulfonate, the first step is the Sulfite pulping which is an acid-base process that is used for dissolving the lignin bonding material from wood chips. A part of the sulfurous-acid base is converted to a bisulfite to buffer the cooking action. Figure 8 below shows a summary for the pulping process (Benjamin, Douglass, Hansen, Major, Navarre and Yerger, 1969).

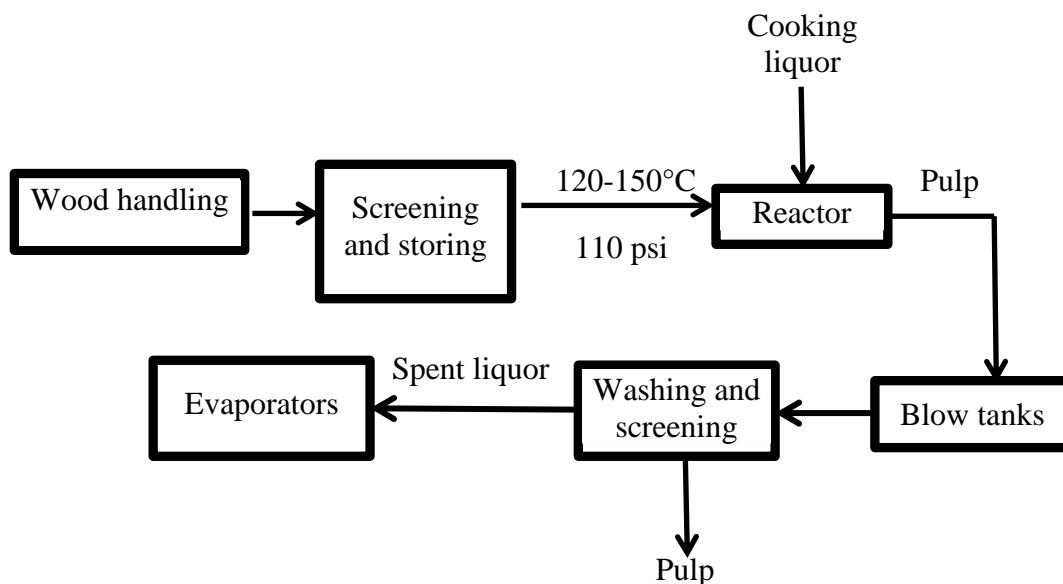


Figure 8: Main unit processes of manufacturing of spent liquor (Benjamin, Douglass, Hansen, Major, Navarre and Yerger, 1969).

2.5.3. Organosolv Lignin Process

The term “Organosolv” refers to a group of pulping processes and it used since 1970s (Zhao, Cheng and Liu, 2009). Organosolv lignin is acquired by the treatment of wood with either different organic solvents or their aqueous solution, the most used solvents for Organosolv lignin process include alcohol with low boiling point such as ethanol, methanol

and organic acids such as formic and acetic acid. High boiling point organic solvents also have been used (Azadi, Inderwildi, Farnood and King, 2013). The catalyst can be used in this process using acid and base. For example; sulfuric acid, although pure Organosolv (without catalyst) can be performed at high temperature (Sun and Ren, 2010). Figure 9 shows the flow chart for Organosolv process.

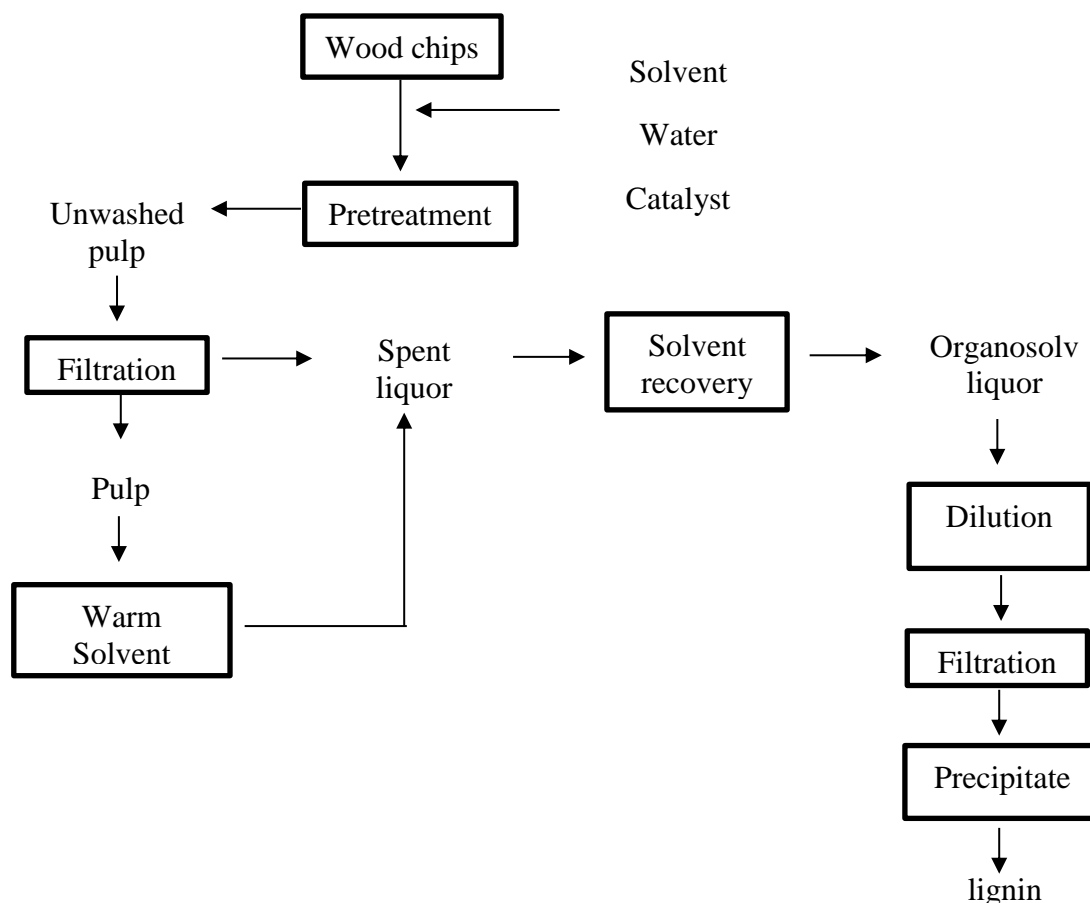


Figure 9: Flow Chart for Organosolv Process (Sannigrahi and Ragauskas, 2013).

This process is characteristic by utilizing sulfur-free chemicals. It has low molecular weight between (3960–4340 g/mol), hydrophobic nature so the solubility in water is slightly, more environmentally and higher purity compared to lignosulfonate and Kraft process. Moreover Organosolv solvents are easily recovered by distillation (Sun and Ren, 2010, Zhao, Cheng and Liu, 2009).

Despite of these advantages, organic solvents still more expensive and recovering it considered energy consuming. In addition, extremely tight and efficient control must be

performed on Organosolv process because of volatility of organic solvents. Care must be taken of any leakage that happened because of inherent fire and explosion hazard (Zhao, Cheng and Liu, 2009) and although of many pilot-scale Organosolv pulping plants have been established in the past it doesn't find commercial application to the current time (Sun and Ren, 2010).

There are two steps for isolating lignin by Organosolv process, beginning with pulping process which followed by separating Organosolv lignin. These two steps are discussed below.

Organosolv Pulping Process

A wide range of organic solvents has been proposed for Organosolv pulping process. These solvents are divided into two methods which are alcohol (methanol and ethanol) and acid (formic acid and acetic acid) Organosolv pulping. Table 3 contains the main characteristics of these solvent but doesn't provide a comprehensive list of known lignin solvents. Organosolv pulping process is shown below in Figure 10.

Table 3: Organosolv Pulping Methods Proposed (Johansson, Aaltonen and Ylinen, 1987).

Solvent	% in H ₂ O	Catalysts	Wood Liquor	Raw Material	Temperature
Methanol	50-100	None, HCl, H ₂ SO ₄	1:10	Spruce, Pine, Beech, Aspen	130-220°C
Ethanol	40-60	None HCl, H ₂ SO ₄ , organic acid	1:6-15	Spruce, Pine, Birch, Bagasse, Rice straw	120-240°C
Formic acid	80	Not specified	Not specified	Hardwood, Softwood	100°C (atmospheric pressure)
Acetic acid	50-95	None, HCl	1:4-8	Spruce, Beech, Aspen	110-220°C

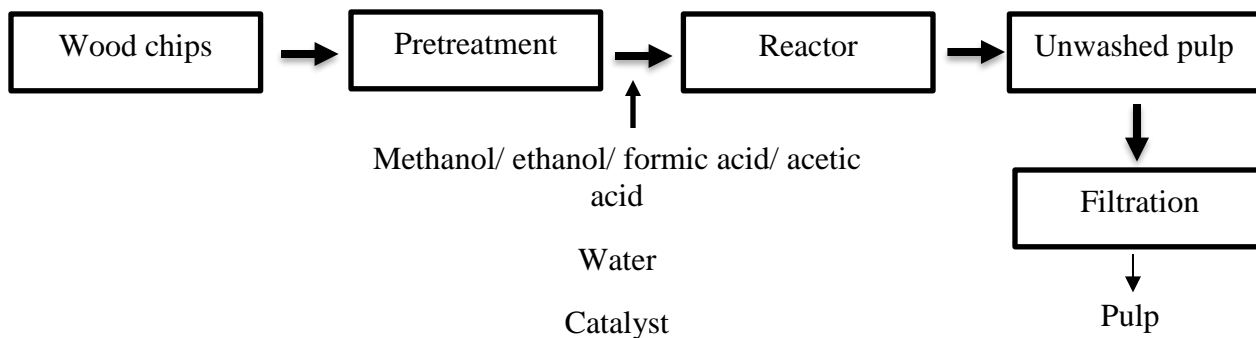


Figure 10: Flow Chart for Organosolv Pulping (Zhao, Cheng and Liu, 2009).

1) Alcohol Organosolv

Alcohol contains ethanol and methanol has been used to study lignin in terms of dividing lignocelluloses into its components (cellulose, hemicellulose and lignin).

The highest ratio of lignin to carbohydrate degradation is achieved with ethanol and with higher water content the wood is almost totally liquefied. There are many traditional methods of alcohol developed (Aittamaa and Sundquist, 2000).

- **Ethanol**

Ethanol pulping in more Organosolv pulping process is more suitable for hardwood than for softwood. Delignification (isolation of lignin) by ethanol solvent is more supported than other solvent in regard to the cost, easy recovery and less toxic. The process of aqueous ethanol penetrates easily into the structure of wood resulting in uniform delignification (Aittamaa and Sundquist, 2000). The Alcell process is the most advanced process of the ethanol pulping. During 1987-1997 Alcell process was developed by Repap Enterprises Inc, a demonstration Organosolv pulp mill operated in Miramichi, New Brunswick, Canada from 1989 to 1996 using the Alcell process (Pye, 2006). In this process, lignin extracted from mixed hardwoods by an Organosolv process using aqueous ethanol as the pulping liquor (Gosselink, Abächerli, Semke, Malherbe, Käuper, Nadif and van Dam, 2004, Liu, Carriero, Pye and Argyropoulos, 2000).

Wood chips were impregnated with 40-60 vol. % ethanol/water as pulping liquor in reactor. The mixture was heated up at 180 °C for 60-100 min with or without 1 wt. % sulfuric acid catalyst with liquor pH (2-4). Pulps were separated from the pulping liquor by vacuum filtration and washed with aqueous 70 wt. % (w/w) and finally washed with 1L hot deionized water, air dried and saved for further process (Nitsos, Stoklosa, Karnaouri, Vörös, Lange, Hodge, Crestini, Rova and Christakopoulos, 2016, Pan, Arato, Gilkes, Gregg, Mabee, Pye, Xiao, Zhang and Saddler, 2005).

- **Methanol**

Methanol is most widely used as an additional chemical in pulping. Methanol increases the delignification rate by enhancing the penetration the pulping liquor. It also prevents lignin from condensing during the process. Moreover, it can be relatively easily recovered by distillation because it has a low boiling alcohol. However, methanol is a toxic chemical and at a relatively low temperature it forms flammable vapors. Therefore, a pulping process using it has to be carefully designed and operated (Aittamaa and Sundquist, 2000). Organocell process is most common method for methanol pulping. In Germany in 1992 a different form of Organosolv pulping technology was commercialized under the name of Organocell (Pye, 2006). Few number of alkali-Organosolv process appeared including Organocell process because a variety of different auto-catalyzed and acid-catalyzed Organosolv processes often fail to produce softwood pulp at reasonable yields and acceptable strengths. It performed in two sequential stages. In the first one Extraction of wood chips with methanol solution at elevated temperature (170-190) °C. The second stage cook with aqueous alkali in the presence of residual alcohol from the first stage (Azadi, Inderwildi, Farnood and King, 2013, Lindner and Wegener, 1988).

2) Acid Organosolv

Isolated lignin from wood in organic solvent is facilitated at higher hydrogen content. Formic and acetic acids are good solvent for lignin. This process requires peroxides or catalyst such as HCl at low temperature at (80-100) °C pulping is carried out at atmospheric

or low operating pressures. Delignification in presence of formic acid was found to proceed faster than in presence of acetic acid (Azadi, Inderwildi, Farnood and King, 2013).

- **Formic Acid**

Formic acid is a good solvent for the lignin and extractives present in wood. It causes hydrolytic breakdown of wood polymers into smaller and more soluble molecules as well. Moreover, formic acid used for pulping is also used as solvent to study wood components. Peroxyformic acid oxidized lignin and making it more hydrophilic this makes lignin more soluble. In addition, peroxyformic acid does not react with cellulose and other wood polysaccharides. Subsequently, it is highly selective chemical. Russian researchers introduced the idea to use peroxyformic acid as a pulping solvent. Then Finnish development this idea by used Milox process (Aittamaa and Sundquist, 2000).

Formic acid process is a three-stage pulping process which use 80% concentration of formic acid and employs hydrogen peroxide in the first and third stages to form peroxyformic acid, which is a good delignifying agent because it has a strong oxidizing power. Since lignin is susceptible to oxidation, this operational procedure can be used either directly for delignification or for bleaching purposes. The reaction temperature in the first and third stages 80 °C while it increased to 100 °C in second stage (Azadi, Inderwildi, Farnood and King, 2013).

- **Acetic Acid**

Acetic acid can be used to produce pulp like most of the organic chemicals, was originally used to isolate lignin from wood. In addition to pulping and bleaching, acetic acid can be used to inhibit color reversion in pulp (Aittamaa and Sundquist, 2000).

➤ Acetosolv

The Acetosolv cooked wood chips at 100-112 °C with aqueous acetic acid include in the starting of the cook 0.05-0.2% HCL and at the end 0.5-2 wt. % hydrogen peroxide.

Acetosolv pulps is washed with hot 93 wt. % acetic acid. Then, pulps can be bleached in two stages. The first bleaching stage is a treatment with hydrogen peroxide followed by washing with hot acetic acid. The second one is an ozone treatment. 93% aqueous acetic acid containing 0.1-0.2 % hydrochloric acid as solvent can be used in Acetosolv process. The pulping is achieved at 110 °C for 4-6 hours and it is prebleached with ozone, final bleaching is done with peroxyacetic acid. A rotating extractor is used as digester. Because of higher solubility of ozone, reduced formation of OH radicals and higher solubility of lignin degradation products, ozone in acetic acid is a more selective bleaching agent than ozone in aqueous systems (Aittamaa and Sundquist, 2000).

Although a wide range of solvent and acid systems have been investigated a small number of leading processes have been developed. Table 4 contains these Organosolv lignin processes.

Table 4: Orgnosolv Lignin Process Developed (Calvo-Flores and Dobado, 2010).

Lignin Type	Solvent
Alcell	Ethanol/water
Acetosolv	Acetic acid/ HCl pulping
Formcancell	Acetic acid/formic acid/water
Milox	Formic acid/hydrogen peroxide
Organocell	Methanol pulping, followed by methanol, NaOH, and anthraquinone pulping

2.6. Separation Lignin from Liquor

2.6.1. Separation Kraft Lignin from Black Liquor

There are different methods for isolating and purifying Kraft lignin from black liquor such as:-

- **Acidification**

Acidification has been considered as an effective and economical process for isolating Kraft lignin from black liquor. In acidification process, the pH of black liquor is reduced to 9, on this value of pH the lignin can be formed as granules and thus can be separated from liquor by filtration. The lignin precipitation at pH 9 has molecular weight between 3000 and 13000 g/mol.

Procedure of lignin isolation by acidification consists mainly of three steps, acidification, filtration, and washing (Lin and Dence, 2012). Table 5 below shows the properties of precipitated lignin after washing and purification. (Fatehi and Chen, 2016).

Table 5 Properties of lignin precipitated at pH 9 *via* acidification of a black liquor (Fatehi and Chen, 2016).

Carbohydrate wt. %	Klason lignin, wt. %	Acid soluble Lignin, wt. %	Precipitated materials, wt. %	Ash, wt. %	S, wt. %	C, wt. %	H, wt. %
2.7	93.7	2	3.3	0.1	1.69	64.88	6.3

- **Membrane**

Other method of extracting lignin from the black liquor is use of membrane filtration which included ultrafiltration and nanofiltration. Previous results showed that the use of Ultrafiltration leads to the retention of a small amount of lignin but a high retention of high molecular weight hemicellulose because its size is greater than lignin. Also, the retention of lignin on the nanofilter was few. There are different types of membranes that are used for isolating lignin, for example polymeric or ceramic (Fatehi and Chen, 2016). Also tend

to isolate other components having a similar size of lignin therefore it generated lignin with fewer purities (Fatehi and Chen, 2016). The use of membrane technology to separate Kraft lignin from black liquor is very limited due to its high suspension solids concentration, high temperature and high pH value (da Silva, Zabkova, Araújo, Cateto, Barreiro, Belgacem and Rodrigues, 2009).

- **Solvent**

Solvents are one of the most famous methods of separating Kraft lignin from black liquor. Water-insoluble organic solvents such as chloroform or dichloromethane were added to black liquor to precipitate lignin. After the solvent is added, hydrogen bonds are formed between it and lignin this leads to improved filtration of black liquor. Also, alcohol such as ethanol, methanol and isopropanol are considered effective in separation of Kraft lignin from black liquor (Fatehi and Chen, 2016).

2.6.2. Separating Lignosulfonates from Spent Liquor

The spent liquor of the sulfite pulping process is generally acidic and diluted containing lignosulfonate, sugars and residual pulping chemicals. Typical compositions for hardwood and softwood spent sulfite liquors are given in Table 6.

Table 6: Compositions of Spent Sulfite Liquors (Li, 2011)

Component	Percentage of total solids	
	Softwood	Hardwood
Lignosulfonate	55	42
Sugars	20	25
Other chemicals	14	21
Ash	10	10

Lignosulfonate needs to be separated from spent liquor of the sulfite pulping process. As lignosulfonates are water soluble products, they may not be precipitated by acidifying the spent liquor (Aro and Fatehi, 2017). Therefore, there are various methods for isolating and

purifying lignosulfonates. The oldest and the most widely used industrial processes is Howard process which is one of the earliest and most efficient ways for isolating lignin product from spent liquors that involves the precipitation of calcium lignosulfonate by the addition of lime as shown in Figure 11. Lignosulfonate is precipitated from spent liquor by addition of excess lime (calcium oxide) (Li, 2011). Other laboratory scale methods are ultrafiltration (UF) and amine extraction. ultrafiltration (UF) as shown in Figure 12 is extremely promising tool for recovery of the dissolved materials in the liquors (lignosulfonates, sugars, etc.) that required due to their economic value (Bottino, Capannelli, Kuiper and Salvemini, 1983). Amine extraction is among the most commonly used separation methods. As a first step spent sulfite Liquor is treated with a long-chain alkyl amine to form a water insoluble lignosulfonic acid-amine complex which is then extracted with solvents such as Amyl alcohol, Butanol, pentanol, methyl, isobutyl ketone, 1,2 dichloroethane and cyclohexane to separate it from sugars, Oligosaccharides, inorganic salts, and other nonlignin contaminants (Lin and Dence, 2012, Fatehi and Chen, 2016). Lignosulfonate is obtained following the procedure as shown in Figure 13.

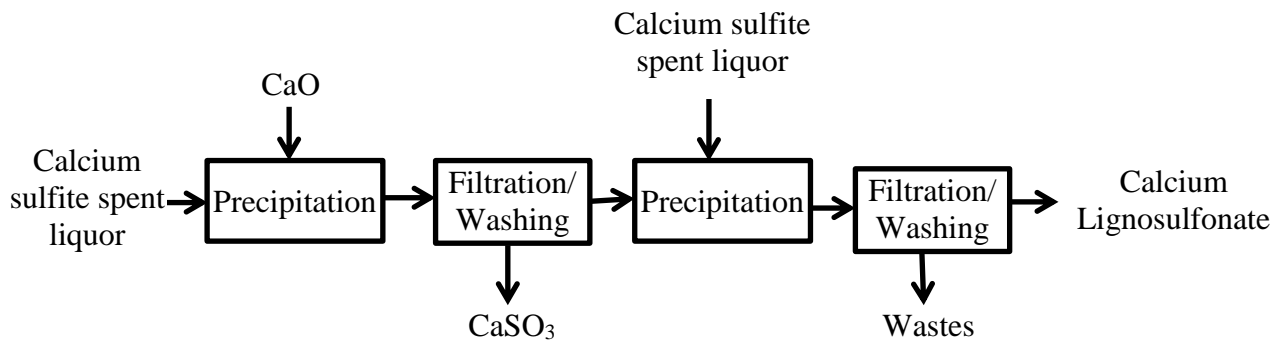


Figure 11: Howard method to separate calcium lignosulfonates from calcium sulfite spent liquor (Benjamin, Douglass, Hansen, Major, Navarre and Yerger, 1969).

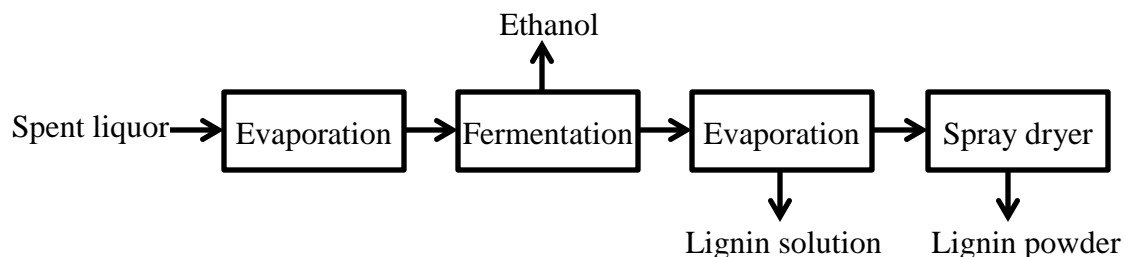


Figure 12: A process for producing lignosulfonate from spent liquor (Fatehi and Chen, 2016).

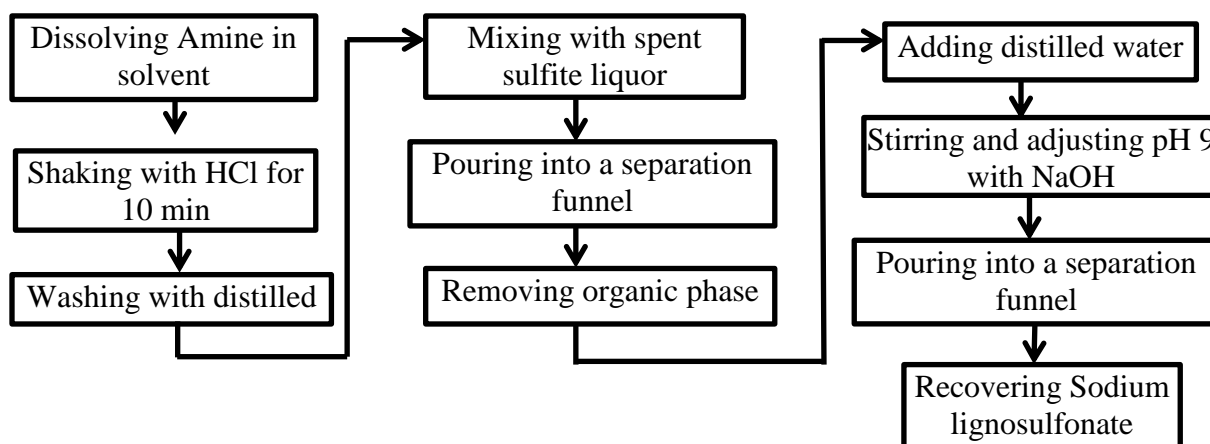


Figure 13: Steps of amine extraction for producing sodium lignosulfonate (Lin and Dence, 2012).

2.6.3. Separation Organosolv Lignin from Liquor

The pulp obtained from Organosolv process, it separated by filtration and washes three times with 200 ml solvent to recover all dissolved lignin. Solvent was removed with a rotary vacuum evaporator at 40 °C. Lignin recovery from Organosolv liquor. Firstly, the Organosolv liquor was diluted (3:1 w/w dilution ratio cold deionized H₂O: Organosolv liquor), added to chilled water. Then, the Organosolv lignin was precipitated from Organosolv liquor by acidification with addition 2 M HCl to pH= 1.5. Finally, the Organosolv liquor was centrifuged at 4000 rpm for 15 min and the precipitated Organosolv lignin was filtered and dried under vacuum at 40 °C overnight. Organosolv lignin was obtained in form of solid brown powder (Koumba-Yoya and Stevanovic, 2017, Nitsos, Stoklosa, Karnaouri, Vörös, Lange, Hodge, Crestini, Rova and Christakopoulos, 2016). Figure 14 demonstrate separation of Organosolv lignin.

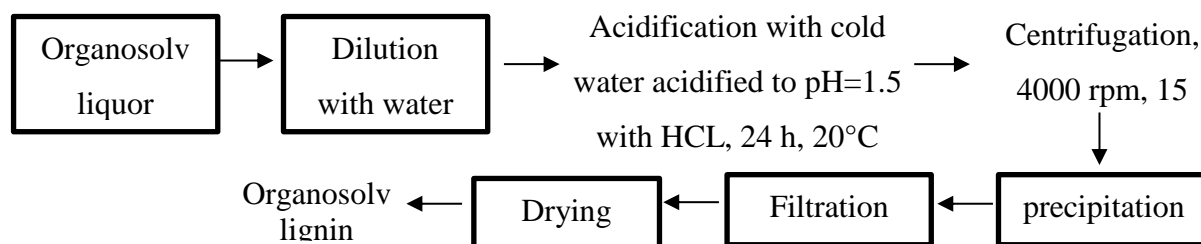


Figure 14: Separation lignin from Organosolv liquor (Amendola, De Faveri, Egües, Serrano, Labidi and Spigno, 2012).

2.7. Lignin Applications

Lignin has influential and economical adhesive properties, which can be improved through phenol and aldehyde or through modification of lignin itself (Agrawal, Kaushik and Biswas, 2014). Most good new applications are related to sulfur-free lignins, because it can be treated thermally without the appearance of irritating odor that commonly observed with commercial Kraft lignins (Lora and Glasser, 2002).

In the past, only 1% of the lignin was used to produce paper in the world and the remaining 99% were burned for power generation or disposal in waste streams (Thielemans, Can, Morye and Wool, 2002). But there are currently many useful and effective applications of lignin such as:

- **Substitute for Phenolic Powder Resins:** The lignin is added to phenolic resin at a specific ratio. The previous experiment showed that 20% for lignin: 80% phenolic resin resulted in competitive advantages (included the stability of the friction coefficient to temperature variations) relative to 100% phenolic resin, are used as the binder in the manufacture of friction products (Lora and Glasser, 2002).
- **Use of Lignin in polyurethane Foam** (Lora and Glasser, 2002).
- **Use of lignin as an anti-bacterial agent in paper industry** (Gosselink, De Jong, Guran and Abächerli, 2004).
- **Lignin as Binder:** for glass wool building insulation. Binder yields low cost composite materials (Agrawal, Kaushik and Biswas, 2014).
- **Lignin as Dispersant:** Chemically modified lignin has been used as a dispersing agents, thickener or helpful agents for coatings and paints (Agrawal, Kaushik and Biswas, 2014).
- **Lignin in Battery:** Lignin promotes performance of energy storage devices. It forms a fine layer on the graphite powder surface and so on preventing the graphite powder from decreasing overvoltage without affecting its condition (Agrawal, Kaushik and Biswas, 2014).

- **Lignin as Dust Suppressant:** Lignosulfonate acts as a dust suppressant through its affinity for binding with other polar and non-polar compounds. It is also used for dust control in ceramic manufacture, synthetic fertilizer production and application, and concrete mixing (Agrawal, Kaushik and Biswas, 2014).
- **Lignin as Food Additives:** Additions of alkali lignins to food and feed as a fiber source (Agrawal, Kaushik and Biswas, 2014).
- **Lignin in Cement:** Decrease damage of external wall due to moisture and acid rain. Some selective lignin can develop the compressive strength of cement pastes (Agrawal, Kaushik and Biswas, 2014).
- **Lignin Blends:** Thermoplastic materials containing lignin and protein blended with natural rubber show improved impact resistance compared to lignin-free formulations (Agrawal, Kaushik and Biswas, 2014).

2.8. Analytical Process

Lignin isolation methods from wood (Kraft, lignosulfonate and Organosolv) have been analyzed regarding their application as a chemical resource and polymeric material. The analytical methods can be divided into two categories: chemical methods (Klason, Kappa, acetyl bromide method and Chlorine Consumption Method (CCM)) and physical methods (ultraviolet (UV) spectroscopy, Nuclear Magnetic Resonance (NMR) and Fourier Transformed Infra-Red spectroscopy (FTIR)) which are discussed below (El Khaldi-Hansen, Schulze and Kamm, 2016).

Chemical Methods

1. Klason method which is the most widely method used for determining the concentration of lignin, most reliable and probably the simplest. This method is obtained by treating wood with sulfuric acid to hydrolyze and solubilize the polysaccharides; the insoluble residue is dried and weighed as lignin (Jingjing, 2011, Kirk and Obst, 1988).

2. Kappa number method used to estimate the amount of lignin by measuring the oxidant demand of the pulp. Kappa numbers were performed on air-dried pulp samples, which were dried on a heated balance to acquire their oven-dried weight. This is an indirect method in which the pulp sample is oxidized with an excess of potassium permanganate under specified conditions. The Kappa number is calculated from the consumption of permanganate. Other oxidizable structures in wood do contribute to the Kappa number but a linear relationship has been found to exist between the Klason lignin content and the kappa number, at least for softwood Kraft pulps (Sjöström and Alén, 2013).
3. Acetyl bromide method which is a convenient method of quantifying lignin in small samples (mg scale). A wood or pulp sample is treated with acetyl bromide in acetic acid in the presence of perchloric acid, resulting in the complete dissolution of the sample. The lignin is determined by UV measurement at 280 nm (Sjöström and Alén, 2013).
4. Chlorine Consumption Method, because the lignin reacts easily with chlorine, sample of consumption chlorine can form the basis to determination of lignin. The pulp is reacted with acidified sodium or calcium hypochlorite and the chlorine consumption is measured then the amount of lignin is calculated (Sjöström and Alén, 2013).

Physical Methods

1. Fourier Transform Infrared (FTIR) which is a versatile and rapid technique for identification and determining lignin content. FTIR spectra can be obtained directly on solid samples such as wood, pulp. The FTIR is a method of obtaining infrared spectra by first collecting an interferogram of a sample signal using an interferometer. The interferometer consists of a beam splitter, a fixed mirror, and a mirror that translates back and forth; radiation from the source strikes the beam splitter and separates into two beams. One beam is transmitted through the beam splitter to the fixed mirror and the second is reflected off the beam splitter to the moving mirror. The fixed and moving mirrors reflect the radiation back to the beam splitter. Again, half of this reflected radiation is transmitted,

and half is reflected at the beam splitter. Also, to elucidate the structure of lignin and also to investigate the differences in the structure of the lignin FTIR is used (Li, 2011).

2. Ultraviolet UV spectrophotometric method which is the most suitable method for determining the concentration and purity of lignin in wood and most user friendly and adaptable analytical techniques. The UV method is based on the difference spectra obtained when subtracting the UV spectrum of the sample in neutral solution from the spectrum obtained in alkaline solution. The measurement is carried out in the range 250-350 nm, the quantitative determination of lignin concentration typically relies on the macromolecule's adsorption (280 nm) (Jingjing, 2011, Lee, Bédard, Berberi, Beauchet and Lavoie, 2013).

3. Nuclear Magnetic Resonance (NMR) which is another powerful technique utilized in measuring total lignin content. ^{13}C -NMR became popular in lignin characterization, which is the most reliable, comprehensive techniques and a powerful way capable of revealing a large amount of lignin structural information including the presence of aryl ethers, as well as condensed and uncondensed aromatic and aliphatic carbons. The characterization of lignin by ^{13}C -NMR spectroscopy, in particular, furnishes rather comprehensive data about the nature of all carbons in lignin in terms of chemical structure. By contrast, the other physical and chemical analytical methods only provide incomplete information on the chemical structure of lignin. The major disadvantage of ^{13}C -NMR spectroscopy is the inherent low sensitivity because of the extremely low abundance of the natural ^{13}C isotope so it requires a high sample concentration and a long acquisition time, especially for quantitative ^{13}C -NMR, and thus limiting its application (Sciencedirect, 1988, Wen, Sun, Xue and Sun, 2013).

CHAPTER 3

3. METHODOLOGY

Two isolation methods of Organosolv lignin (ethanol-water (50 vol. %) and formic acid-acetic acid (85 vol. %)) from wood were carried out. Also, lignin was analyzed by Klason method as acid insoluble residue (AIR) and by UV spectrophotometer as acid soluble lignin (ASL). The sawdust was collected from a local carpentry workshop in Nablus.

3.1. Organosolv Lignin Isolation using Ethanol-Water (50 vol. %) Mixture

After the sawdust was pretreated by sieving and washing with deionized water, the following procedure was followed for the isolation of Organosolv lignin (Figure 15) (Koumba-Yoya and Stevanovic, 2017, Nitsos, Stoklosa, Karnaouri, Vörös, Lange, Hodge, Crestini, Rova and Christakopoulos, 2016, Pan, Arato, Gilkes, Gregg, Mabee, Pye, Xiao, Zhang and Saddler, 2005, Amendola, De Faveri, Egües, Serrano, Labidi and Spigno, 2012).

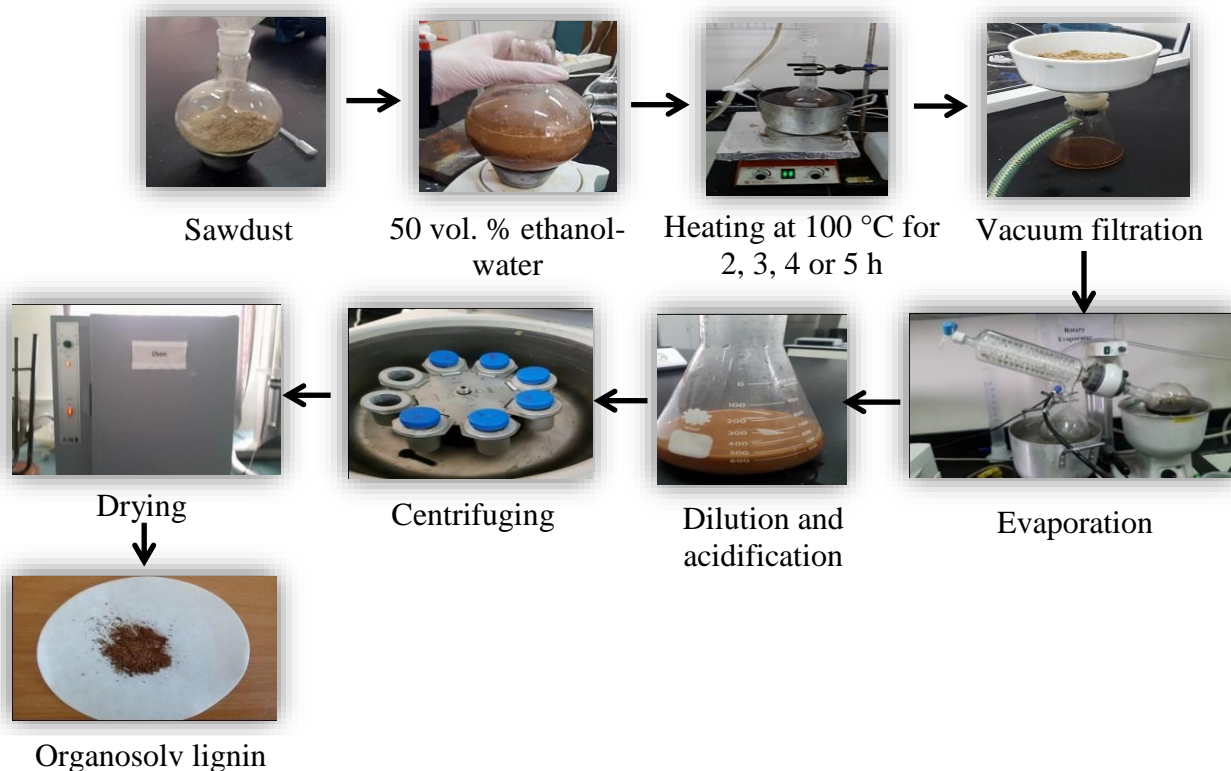


Figure 15: Procedure for lignin isolation using ethanol-water (50 vol. %) mixture

1. In a 500 ml round-bottom flask with condenser, 22 g of sawdust wood was mixed with 110 ml ethanol-water (50 vol. %) mixture and 1 wt. % H₂SO₄ as a catalyst. Then pH of the solution is measured (range 3-4) using litmus paper.
2. To study the effect of reaction time of lignin isolation process, the mixture was heated up with various periods of time; 2 h, 3 h, 4 h and 5 h at 100 °C using hot plate with continuous stirring using magnetic stirrer as shown in the Figure 16.
3. The solution then slowly was cooled down to below 40 °C.
4. The Residue (Pulps; solid part) was separated from the pulping liquor (liquor; liquid part) by vacuum filtration.
5. Ethanol was removed from pulping liquor by using rotary evaporator.
6. The Organosolv liquor was diluted in beaker (3:1 w/w dilution ratio, cold deionized H₂O: Organosolv liquor).
7. Organosolv lignin was precipitated from Organosolv liquor by acidification (addition of 2 M HCl) until pH = 1.5.
8. The sample was then placed into centrifuge tubes of volume 50 ml.
9. Organosolv liquor was centrifuged at 4000 rpm for 15 min.
10. The precipitated Organosolv lignin was dried by oven at 25 °C overnight.
11. The centrifuge tubes were put directly into a desiccator and cooled for 30 min.
12. Organosolv lignin was obtained in the form of solid brown powder.

Lignin yield was calculated at various periods of time; 2 h, 3 h, 4 h and 5 h according to equation 1 and the effect of reaction time on the delignification was explored

$$\text{lignin yield (\%)} = \frac{\text{weight of lignin sample (g)}}{\text{weight of total lignin in wood(g)}} * 100 \dots\dots\dots (1)$$

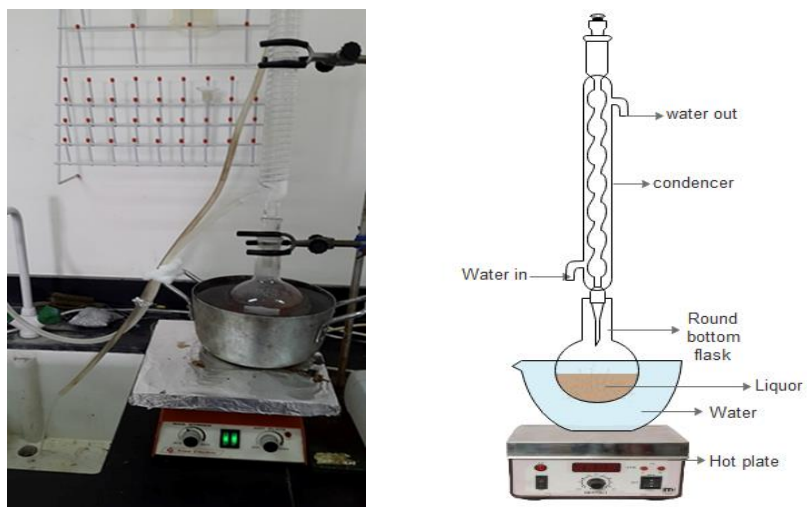


Figure 16: Flask reactor with reflux condenser

3.2. Organosolv Lignin Isolation using Formic acid-Acetic acid Mixture

Lignin was isolated by following the procedure shown in Figure 17. (Watkins, Nuruddin, Hosur, Tcherbi-Narteh and Jeelani, 2015).

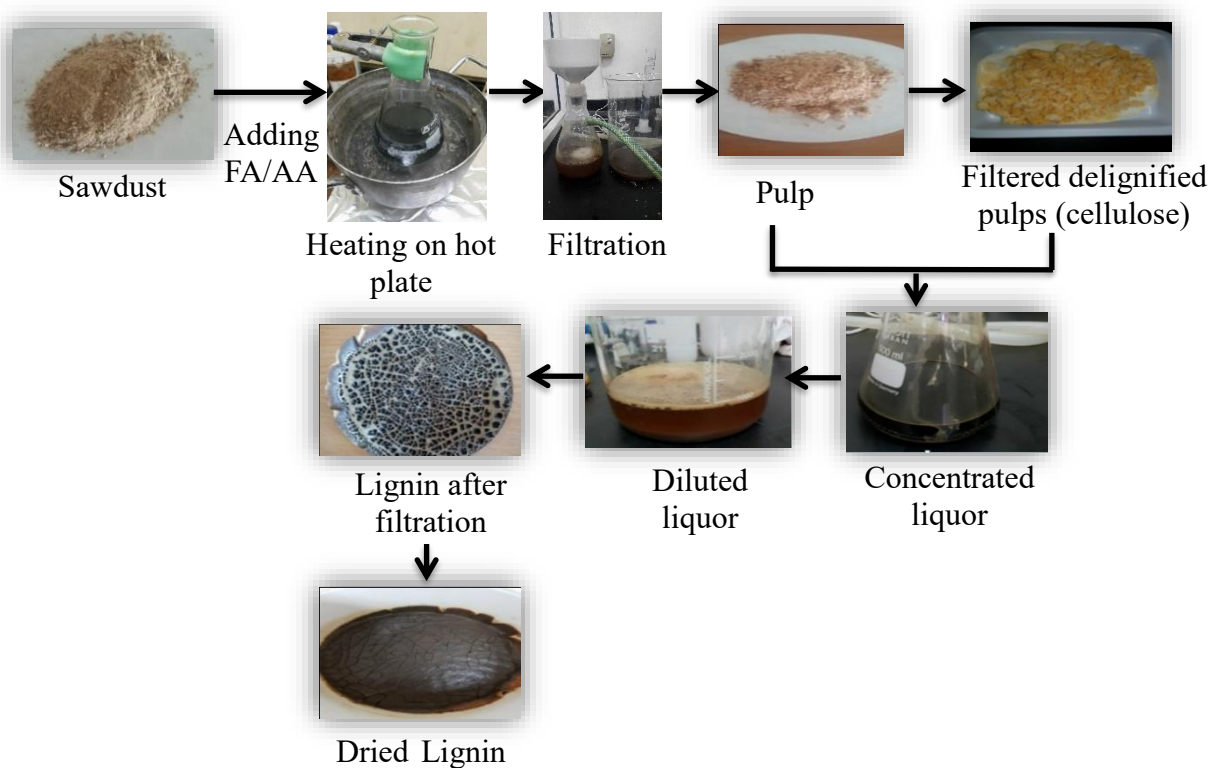


Figure 17: Procedure for lignin isolation using formic acid-acetic acid (85 vol. %) mixture

❖ Formic acid-acetic acid (FA/AA) treatment:

1. 22 g of sawdust was weighed and placed in a round-bottom flask.
2. A mixture of 85 vol. % organic acid (ratio of formic acid-acetic acid mixture was 70:30 by volume) was prepared.
13. The mixture was added to the sawdust in the flask (1:8 w/v ratio, sawdust: Organosolv liquor)
3. The mixture was allowed to boil on a hot plate for 2.0 h with continuous stirring.
4. The mixture was allowed to cool to ambient temperature.
5. The mixture was filtered using vacuum filtration and washed with 80 wt. % formic acid followed by hot deionized water producing pulp (pulp; solid part) and liquor (liquor; liquid part).

❖ Peroxyformic acid/peroxyacetic acid (PFA/PAA) treatment:

1. PFA/PAA mixture solution was prepared by adding 35 wt. % H₂O₂ with 85 wt. % formic acid/acetic acid mixture (ratio of formic acid-acetic acid to H₂O₂ mixture was 70:30 by volume).
2. FA/AA treated pulps were further delignified by treating them with a mixture of PFA/PAA solution in hot water bath at 80°C for 2.0h.
3. The delignified pulps were filtered to separate cooking liquor from cellulose and washed with hot water.

❖ Isolation of lignin:

1. The spent liquor was heated at 105 °C after pulping and delignification process.
2. The lignin dissolved in formic acid was precipitated by adding deionized water (5 times more than volume of concentrated liquor).
3. The precipitate was filtered using vacuum filtration.
4. The precipitated lignin was washed with deionized water and finally dried at ambient temperature.
5. Lignin yield was calculated according to Equation 2.

$$\text{lignin yield (g)} = \text{weight of filter paper final (g)} - \text{weight of filter paper initial (g)} \dots\dots\dots (2)$$

3.3. Lignin Content measurement in Sawdust and Pulp:

Klason method was used for the determination of acid-insoluble residue (AIR) while acid soluble lignin (ASL) was determined by using UV spectrophotometer, in wood and pulp samples using TAPPI T222. The main procedure for lignin measurement in wood and pulp was illustrated in Figure 18 (Holzforschung, 2016, Jingjing, 2011, Tappi, 2011).



Figure 18: Procedure for lignin content measurements in sawdust and pulp.

- 1) 1.0 g of sawdust and 2.0 g of pulp were placed in a porcelain crucible. The crucible was weighed and dried at 105 °C for one hour. After that, the oven dried samples were placed in a desiccator then weighed.
- 2) Dried samples (1.0 g wood or 2.0 g pulp) were placed into glass beakers.
- 3) To each glass beaker, exactly 15 ml of cold 72 wt. % sulfuric acid was added for sawdust sample while 40 ml for the pulp sample.

Sulfuric acid Preparation

To prepare 72 wt. % sulfuric acid; 30 ml of deionized water was added to 100 ml volumetric flask, then 67 ml of concentrated sulfuric acid (specific gravity= 1.84) was added slowly while cooling and dilute to the mark and mix until the concentration reaches 72 wt. % (specific gravity 1.63 from 10-15 °C). The specific gravity and sulfuric acid concentration were recorded using the densitometer.

- 4) The contents of the beaker were stirred with a glass rod until the solution was dissolved.
- 5) Samples were placed in a (30 ± 2) °C water bath for 1.0 h with slightly shaking. Then, 280 ml of deionized water was added for sawdust while 560 ml for the pulp sample.
- 6) The beakers were covered with aluminum foil and placed in autoclave at (120 °C for 1.0 h. Subsequently, it was cooled to about 80°C.

Determination of acid-insoluble residue (AIR)

- 1) The solution after autoclaved was filtered while still hot through a pre-weighed glass fiber filters by using vacuum filtration.
 - 2) The filtrate was placed in a separate beaker (this filtrate is used for the determination of acid-soluble lignin).
 - 3) The retained residue in filter was washed with hot water until acid free (neutral pH, the pH was checked by indicator paper).
 - 4) The filter with residue in the filter container was dried overnight at 25°C in oven. After that, it was placed in a desiccator and the weight was determined and recorded.
- Figure 19 shows acid-insoluble residue.



Figure 19: Acid-insoluble residue (AIR).

Acid-insoluble residue was calculated according to equations 3 and 4:

Weight the residue after drying(m) = weight glass filter after drying – weight of empty glass filter..... (3)

$$AIR = \left(\frac{m}{M}\right) * 1000 \left(\frac{mg}{g}\right)..... (4)$$

where:

m: the weight residue after drying (g).

M: the oven dry weight of sample (1.0 g) before sulfuric acid hydrolysis/suspension, in g.

Determination of acid-soluble lignin (ASL)

The content of acid-soluble lignin (ASL) in the first filtrate was determined by UV spectrophotometry at wavelength 205 nm. The absorption should be in the range 0.2–0.7 otherwise dilution is needed.

Acid-soluble lignin was calculated according to equation 5:

$$ASL = \left(\frac{A.D.V}{a.b.M}\right). 1000 \left(\frac{mg}{g}\right)..... (5)$$

where:

A: the absorption at 205 nm.

D: the dilution factor.

V: the volume of filtrate in L.

a: the extinction coefficient of lignin, in g/L.cm (110 g/L.cm). Extinction coefficient: it is a measurement of how strongly a molecular species absorbs light at a given wavelength.

b: the cuvette path length, in cm (1.0 cm).

M: the oven dry weight of sample (1.0 g).

Determination of total lignin

The total lignin and lignin yield was calculated according to equation 6, 7 respectively:

$$\text{Total lignin content (TL)} = \text{AIR} + \text{ASL} \left(\frac{\text{mg}}{\text{g}} \right) \dots\dots\dots (6)$$

$$\text{lignin yield} = \frac{\left(\frac{\text{total lignin content (TL)}}{M} \right)}{1000} * 100\% \dots\dots\dots (7)$$

Determination of total Sugar Content

The concentration of total sugar in liquor (liquor; the filtrate (180 ml) after filtration for ethanol-water (50 vol. %) mixture isolation method for the 4.0 h sample) was determined by phenol sulfuric acid method in the following procedure (Cuesta, Suarez, Bessio, Ferreira and Massaldi, 2003):

- The liquor was diluted with deionized water to 100 times.
- 5% w/v phenol was prepared by dissolving 5.0 g phenol in 100 ml deionized water.
- 1.2 ml from liquor and 0.6 from 5% phenol were placed in tubes and mixed. Blank containing only deionized water was also prepared.
- 3 ml of concentrated H₂SO₄ (98 wt. %) was added directly to each tube.
- After about 10 minutes, the tubes were shaken vigorously (Figure 20).
- The absorbance was recorded by UV at 490 nm after at least 30 minutes.



Figure 20: Samples for Sugar Content.

CHAPTER 4

4. RESULTS AND DISCUSSION

Lignin yield was calculated using different isolation methods, the results received from the experiments were illustrated in tables and figures. Analytical method was performed in triplicate while the others in duplicate.

4.1. Lignin Isolation using Ethanol-Water (50 vol. %) Mixture

❖ Effect of reaction time on the delignification process

Lignin yield was calculated by comparing the amount of lignin precipitated; with 1 wt. % H_2SO_4 as a catalyst at pH 3-4; from the liquor after changing reaction time. Table 7 presents yields of Organosolv lignin isolated with ethanol-water (50 vol. %) mixture at different reaction times.

Sawdust was pretreated by sieving to obtain the chips in almost the same size and washing with only deionized water. The pretreated solids were preferred to be washed with organic solvent previous water washing in order to avoid the reprecipitation of dissolved lignin, which leads to cumbersome washing arrangements, but organic solvents are always expensive, so it should be recovered as much as possible, but this causes increase of energy consumption. In addition, Organosolv pretreatment must be performed under extremely tight and efficient control due to the volatility of organic solvents.

Table 7: Lignin yield using ethanol-water (50 vol. %) mixture at different reaction times.

Reaction Time	Wt. of isolated lignin (g)	Lignin content (wt. %) of 22 g sawdust ($\frac{\text{Wt. of lignin (g)}}{22 \text{ g}} * 100$)
2 h	0.30 ± 0.015	1.37 ± 0.07
3 h	0.52 ± 0.040	2.38 ± 0.18
4 h	0.68 ± 0.1281	3.11 ± 0.58
5 h	1.27 ± 0.1796	5.05 ± 0.23

Duplicate samples were taken.

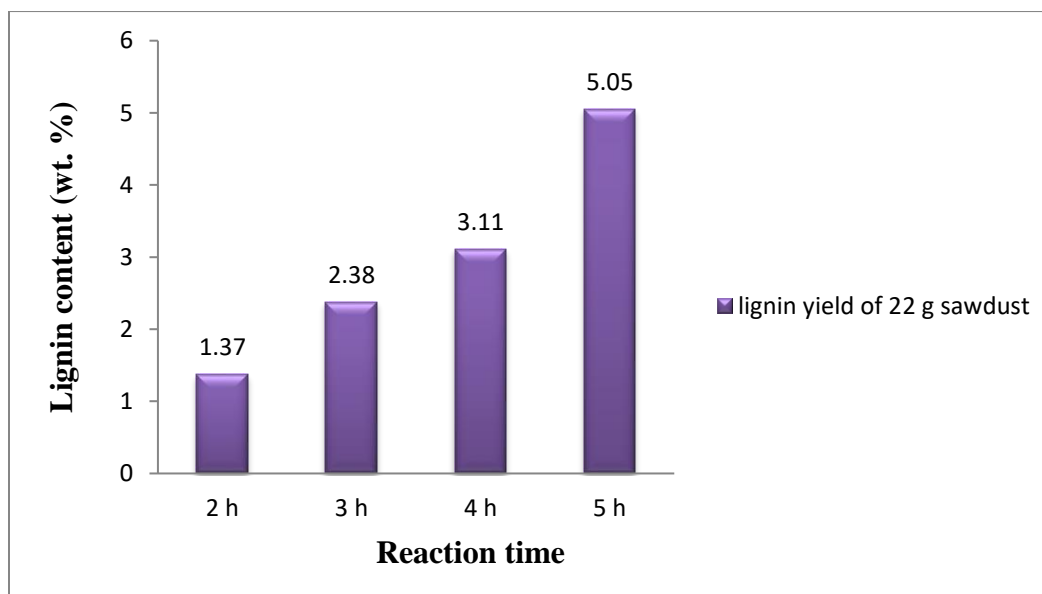


Figure 21: The effect of reaction time on the delignification using ethanol-water (50 vol. %) mixture.

The lignin contents as measured with the procedure at different reaction time are compared in Figure 21. The differences between the results as well as 85 wt. % confidence limits were calculated, and the results are given in Table 7. As it was observed that the yield of delignification increased from 1.37 wt. % to 5.05 wt. % as the reaction time was increased

from 2 h to 5 h at 100 °C. This is probably due to exposing sample to the temperature for a longer period. Treatment temperature is an important parameter affecting lignin separation (Sun, Lawther and Banks, 1997). As a result, heating the mixture for 5 h exhibited a relatively the highest lignin content (5.05 %).

Isolation of lignin from sawdust used in this study was carried out using a mixture of ethanol/water (50% vol. %) for pulping. Organosolv pulping is the process that used to extract lignin from lignocellulosic feedstocks with organic solvents or their aqueous solutions. Ethanol was selected in this study as a solvent for its cost effective, easy recovery and less toxicity comparing to other types of organic solvents. Also, the process of aqueous ethanol penetrates easily into the structure of wood resulting in uniform delignification.

In this study the catalyzed Organosolv cooking was explored. Where a process involving a water and ethanol mixture with addition of sulfuric acid (H_2SO_4) due to its effect on the pH of the Organosolv liquor and its expected role in increasing the delignification (Aittamaa and Sundquist, 2000). The mixture was heated up at 100 °C with various periods of time 2 h, 3 h, 4 h and 5 h to study the effect of time on the delignification process.

Organic solvent, alkaline solution and volume ratio are other variables that were expected to affect the extent of delignification. Sun, Lawther and Banks, 1997 reported that there was no significant difference in the Organosolv lignin yields among the various organic solvents used (methanol, ethanol, 1-propanol, n-butanol, dioxane, and acetone) in a relatively high concentration (60/40 vol. %) and low temperature (75°C) for 2 h which produced (24-28%) of Organosolv lignins. Increase of ethanol-water volume ratio from 40/60 to 70/30 resulted in raising lignin yields from 25 to 31%, while the reverse yield from 31 to 24% appeared in the increase of volume ratio from 70/30 to 90/10 (Sun, Lawther and Banks, 1997)

4.2. Organosolv Lignin Isolation using Formic acid-Acetic acid Mixture

Isolation of lignin from 22 g sawdust used in this study was carried out using a mixture of formic acid-acetic acid (85 vol. %) for pulping with the main objective of degrading the

lignin molecules by dissolving them in the solution and consequently retrieving by washing them. Organosolv (acetic acid-formic acid) under acidic condition cleaves ether bonds between lignin and hemicellulose, thereby accelerating the delignification process (Watkins, Nuruddin, Hosur, Tcherbi-Narteh and Jeelani, 2015). The lignin content was calculated according to the total lignin amount (29.93 %). This method was carried out to be compared with ethanol-water (50 vol. %) mixture method according to the amounts of lignin isolated.

As a result, the average weight of isolated lignin was 2.164 g depending on equation 6 in methodology; which is (9.13 wt. %) of 22 g sawdust. It was observed that the lignin isolated by formic acid-Acetic acid (85 vol. %) was higher than that isolated by ethanol-water (50 vol. %).

The presence of H_2O_2 in organic acid solution enhanced the delignification process due to the combined effect of formic acid-acetic acid as solvent and peroxy acid as an oxidizing agent to dissolve the lignin in formic acid/acetic acid/hydrogen peroxide media (Watkins, Nuruddin, Hosur, Tcherbi-Narteh and Jeelani, 2015). It also acts as a primary delignification to increase the brightness of the fiber as shown in the Figure 22.



Figure 22: Delignified fibers after filtration

Watkins, Nuruddin, Hosur, Tcherbi-Narteh and Jeelani, 2015 reported that the formic acid treatment at atmospheric pressure dissolved 90% of the lignin. But the obtained pulp after formic acid treatment still contains some lignin, hemicellulose and ash (Watkins, Nuruddin, Hosur, Tcherbi-Narteh and Jeelani, 2015).

4.3. Analytical method

Two analytical methods were used, such as Klason (a chemical method which involves the use of 72 wt. % H₂SO₄ to dissolve all the carbohydrates in the extracted free-wood sample leaving lignin as the insoluble residue) and UV spectrophotometer (a physical method which is the most suitable method for determining the concentration and purity of lignin in wood) (Jingjing, 2011, Kirk and Obst, 1988), in order to determine the total lignin, sugar and others contents in wood also to show the yield of lignin using the two previous isolation methods according to the total amount.

❖ Determination of total lignin in wood

Acid insoluble residue (AIR) and acid soluble lignin (ASL) were determined to figure out the total lignin content in wood (Table 8):

Table 8: Total Lignin content in wood

Lignin	Average (mg/g)
Acid-insoluble residue (AIR)	298.37 ± 0.15
Acid-soluble lignin (ASL)	0.897 ± 0.12
Total lignin (TL)	299.26 ± 0.10

The samples were done in triplicate.

Table 8 shows that, the total lignin content depending on the amounts of acid insoluble residue (AIR) and acid soluble lignin (ASL). As a result, Figure 23 shows the percentage of total lignin in the wood sample which was about 30 wt. % according to equation 6. (Tappi, 2011) was reported that the percentage of total lignin in wood was 34 wt. %, which

is a little higher. This is probably due to the non-identification of wood type (hardwood or softwood) in addition to the lack of standard lignin for measuring (ASL) using UV spectrophotometer.

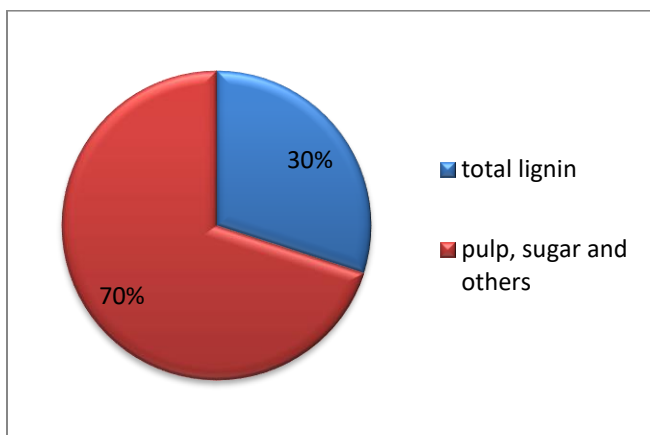


Figure 23: Percentage of wood components

72 wt. % sulfuric acid was used to accelerate the dissolving of wood components especially lignin. Also, it was observed that when cooled 72 wt. % sulfuric acid (10-15 °C) was used the solution was dissolved completely and better results were obtained.

The yield of lignin using the two previous isolation methods according to the total amount was measured (Table 9)

Table 9: Lignin yield of two Organosolv methods according to total content

Method of isolation		Lignin yield (wt. %) of (29.93 wt. %) of total lignin in wood
Ethanol-water (50 vol. %) mixture at different reaction times	2.0 h	4.59 ± 0.22
	3.0 h	7.96 ± 0.61
	4.0 h	10.41 ± 1.95
	5.0 h	16.88 ± 0.81
Formic acid-acetic acid (85 vol. %) mixture (FA-AA)		30.49 ± 1.00

The samples were done in duplicate

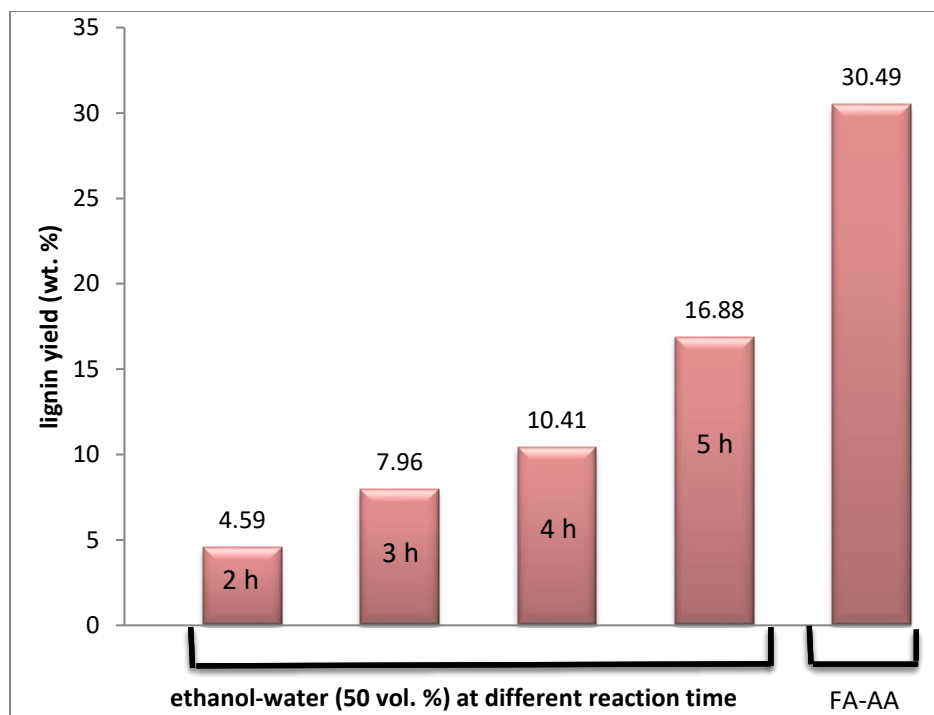


Figure 24: Lignin yield using two different isolation methods of total lignin

The lignin contents as measured with the procedure at different isolation methods in Figure 24. The differences between the results as well as 85 wt. % confidence limits were calculated, and the results are given in Table 9. As it was observed that the highest yield of delignification was obtained by using formic acid-acetic acid (85 vol. %) method which increased from 4.59 wt. % using ethanol-water (50 vol. %) at 2 h reaction time to 30.49 wt. % of total lignin presented in wood.

Determination of lignin content in pulp

Lignin content in pulp (pulp; solid residue) after filtration was determined by using Klason method, as shown in Table 10.

Table 10: Lignin content in pulp sample.

Lignin	Average (mg/g)
Acid-insoluble residue (AIR)	100.98 ± 18.42
Acid-soluble lignin (ASL)	1.39 ± 0.18
Total lignin (TL)	102.36 ± 18.24

The samples were done in triplicate.

Table 10 above shows the lignin content that remained in pulp depending on the amounts of acid insoluble residue (AIR) and acid soluble lignin (ASL). As a result, 5.12 wt. % of lignin content was found in the pulp sample according to equation 6, which leads to one of the reasons that led to the inability to isolate the entire lignin amount presented in wood (30 wt. %) by using the two different isolation methods.

Determination of total sugar

In the case of sugar content, the compounds were reacted to form complex compound and produce a green-blue color. it was considered that the total sugar was converted completely to glucose that used to create the calibration curve and the absorption was measured at 490 nm by using UV spectrophotometer. To obtain better result dilute the solution using deionized water was used (dilution factor; 100). From calibration curve (Figure 35) in appendices was found that the total sugar was 1.1 ± 0.09 g/L which equals to 0.9 wt. % of 22 g sawdust.

Mass balance for 22 g sawdust:

Wood is composed of carbohydrates (cellulose, hemicellulose), lignin and extractives (Figure 25). Mass balance was applied on ethanol-water (50 vol. %) method of sample (4.0 h) and the details were illustrated in appendices.

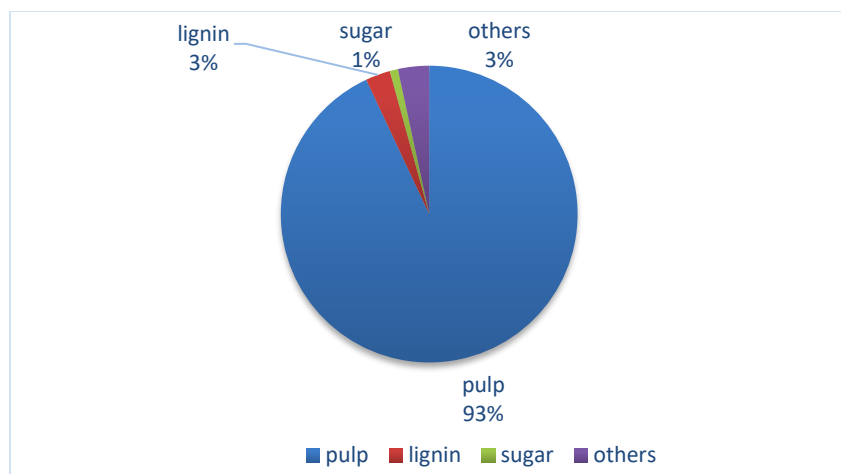


Figure 25: The composition of sawdust.

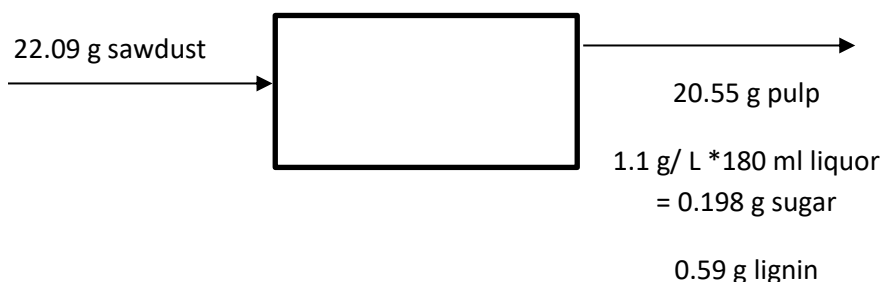


Figure 26: Mass balance for wood component.

As shown in Figure 26 (22.09 g) of sawdust was used as input. Output (21.34 g) were not matched to input so the other (0.75 g) were considered as extractives. This is probably due to many reasons such as; the extinction coefficient in acid-insoluble lignin determination wasn't measure using lignin standard, unavailability of HPLC for measuring the total sugar (less efficient method which consider all sugar as glucose only, regardless of other sugar types was used) and removing all lignin from pulp was very hard. Moreover, personal errors were also considered.

4.4.Comparisons of two Organosolv Lignin Methods

The two methods of Organosolv lignin isolation are compared according to many aspects as shown in Table 11 and Figure 27 below

Table 11: comparison between Organosolv isolation methods

	Ethanol-water (50 vol. %) mixture	Formic acid-acetic acid mixture	Ref.
Lignin yield	16.87 wt. %	30.45 wt. %	This study
Process time needed	7 h	6 h	This study
Productivity (lignin yield/time needed)	0.16 g/h	0.34 g/h	This study
Safety	<ol style="list-style-type: none"> 1. Ethanol is highly flammable liquid. 2. Less Hazardous in case of skin contact (irritant). 3. Slightly hazardous in case of inhalation. 4. Less toxic 	<ol style="list-style-type: none"> 1. Combustible and corrosive liquid. 2. Very hazardous in case of skin contact (irritant). 3. Extremely hazardous in case of inhalation. 4. More toxic. 	(Regulations, 2009, Science Lab, 2013)
Environment	The Combustion of ethanol produces carbon oxides (CO, CO ₂).	Decomposes more rapidly under fire conditions, forming carbon monoxide	(Regulations, 2009, Science Lab, 2013)
Cost of material / L	Ethanol (10 NIS)	Formic acid (7 NIS) Acetic acid (7NIS)	(Brothers for Chemicals Raw Materials Co., 2018)



Lignin using formic acid-acetic
acid mixture

Lignin using ethanol-water
mixture

Figure 27: Organosolv lignin of the two isolation methods (ethanol-water, formic acid-acetic acid mixtures)

As can be seen from the Figure 27, there were some differences in a number of aspects, such as shape and color. The color of lignin using formic acid-acetic acid mixture is darker brown comparing with lignin isolated using ethanol-water mixture. In addition, there were obvious differences in the shape and size.

By contrast between the two Organosolv lignin isolation methods, the formic acid-acetic acid mixture could be selected for its higher content of lignin, but in terms of safety ethanol-water mixture is preferred as shown in Table 11.

CHAPTER 5

5. Conclusion

In this work isolated lignin from sawdust by Organosolv process was the primary aim, it was done by two different mixtures ethanol-water and formic acid-acetic acid. The extracted lignin was analyzed by the Klason method.

The Organosolv pretreatment process covers a wide range of organic solvents such as ethanol, acetic acid, and so on. The process involves mixing an organic liquid and water together in different ratios and adding them to the sawdust. Then this mixture is heated to dissolve the lignin. Also, a catalyst (1 wt. % H_2SO_4) is sometimes added to reduce the operating temperature. Ethanol can be easily recovered during reaction by distillation; it can be used again in the process.

Results showed that, the total amount of lignin in sawdust was about 30 wt. % which was analyzed by Klason method (the most widely method used for determining the concentration of lignin).

As the results of the ethanol method showed, the percentage of extracted lignin increased with increase time of reaction with the stability of the other factors, the maximum percentage was about 17 wt. % of total lignin at time of heating equal 5 hours. While this percentage was much greater when formic acid-acetic acid mixture was used, this was about 30.5 wt. % of the total lignin.

The future development of Organosolv pretreatment should be concentrated on the integrated and interrelated use of raw materials components (e.g sawdust) and decrease of the pretreatment costs. This can be achieved by reducing of energy and chemicals consumption, reducing heat of the reaction by using catalyst, reducing the amount of organic liquid used in pretreatment.

Recommendations and Future Works

➤ Analytical method:

1. Moisture content should be measured and sample with high moisture content should be dried prior to use.
2. If the pulp doesn't dissolve in the 72 wt. % sulfuric acid, put the solution in a vacuum desiccator for a few minutes to facilitate wetting and dispersion.
3. The extinction coefficient in acid-insoluble lignin determination must be measured using lignin standard (according to TAPPI UM 205).
4. High-performance liquid chromatography (HPLC) could be used for sugar content measurements.
5. Other analysis methods such as Fourier-transform infrared spectroscopy (FTIR), Gas chromatography (GC) should be used for analyzing lignin.

➤ Ethanol/ water method:

1. Oil bath could be used for higher temperature operation process.
 2. Optimization of the ethanol/water ratio for delignification process.
 3. Alkaline solution could be used instead of Organosolv.
- The experiment containing formic acid/acetic acid mixture used large quantities of water, it is recommended to recycle so it is economically feasible to try to find a way to recycle this water and use it for other purposes.
6. Lignin could be isolated from known sources of wood (hardwood and/or softwood).
- The feasibility study and economic issue must be considered.
- Safety Recommendations
1. Do not expose ethanol directly to the fire.
 2. The experiments containing formic acid/acetic acid mixture must be done in the fume hood.
 3. Personal protective and safety equipment should be used (suitable respiratory equipment, goggles and gloves).

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Appendices

➤ Apparatus

Apparatus followed where used in the experiment part :

1. Water bath (labTech)



Figure 28: Water bath.

2. Autoclave (mrc)



Figure 29: Autoclave.

3. Ultraviolet-visible (UV-Vis) Spectroscopy (genesys 10S)



Figure 30: UV spectroscopy.

4. Vacuum filtration apparatus.



Figure 31: Vacuum Filtration Apparatus.

5. Rotary Evaporator.



Figure 32: Rotary Evaporator.

6. Centrifuge (universal 320 R)



Figure 33: Centrifuge.

7. Densitometer (METTLER TOLEDO)



Figure 34: Densitometer.

8. Oven



Figure 35: Oven.

9. Desiccator



Figure 36: Desiccator

The total lignin in sawdust and its calculation are represented in Table 12.

Table 12: Total lignin for wood sample.

Sample	Wt. filter (empty)(g)	Wt. filter after drying (g)	residue after drying(g)	AIR(mg/g)	ASL(mg/g)	TL(mg/g)
1	38.11	38.41	0.2985	298.5	0.88	299.38
2	42.67	42.97	0.2984	298.4	0.79	299.19
3	39.32	39.62	0.2982	298.2	1.02	299.22

The total lignin in pulp sample and its calculation were illustrated in Table 13.

Table 13: Total lignin for pulp sample.

sample	Wt. filter (empty)(g)	Wt. filter after drying (g)	Residue after drying (g)	AIR (mg/g)	ASL (mg/g)	TL (mg/g)
1	40.95	41.13	0.18	87.95	1.51	89.46
2	38.12	38.35	0.23	114	1.26	115.26

Figure 37 represents calibration curve (a relationship between absorbance and concentration (g/L)) to determine the total sugar (Hassoun and Maraabe, 2018).

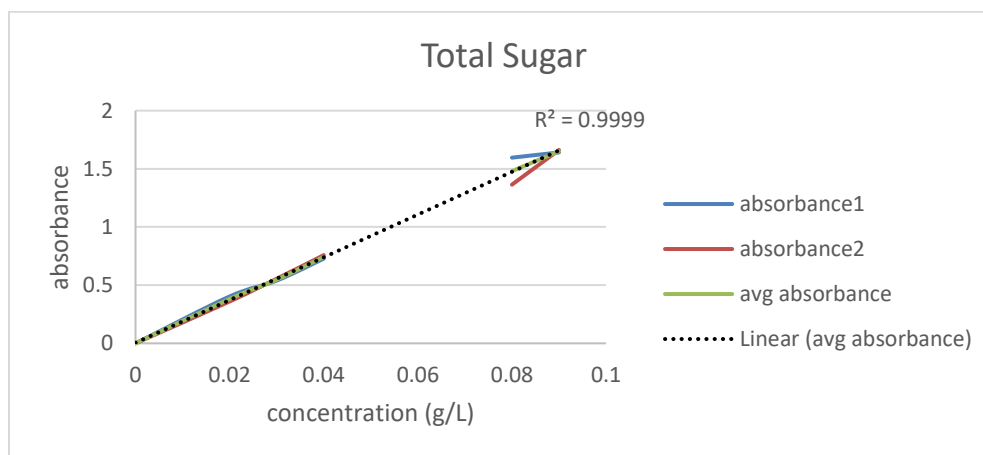


Figure 37: Calibration curve for determine the total sugar.

Mass balance was applied on sample 4.0 h Ethanol-Water (50 vol. %) mixture from (22.0g) sawdust. Table 14 shows amount of total input and total output for wood components. Mass balance applied as shown in Figure 38.

Table 14: Amount of total input and total output for wood components.

Wood Component	Amount
Wt. of sawdust	22.09 g
Wt. of pulp after drying	20.55 g
Wt. of lignin	0.59 g
Volume of liquor before rotary	180 ml
Total sugar	1.1 g/L
Total output	21.34 g

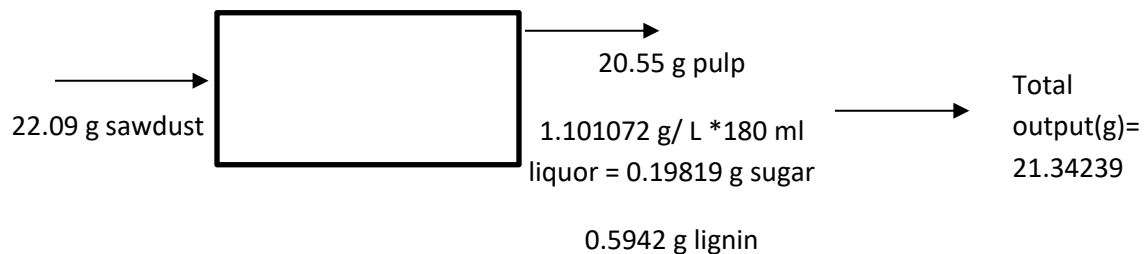


Figure 38: Mass balance for wood components