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LIGNIN EXTRACTION FROM HAZELNUT SHELLS

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Yenilenebilir ve sürdürülebilir çevre insanoğlunun temel hedeflerinden biridir. Sanayi Devrimi'nden bu yana, kağıt endüstrileri katlanarak büyümekte ve çoğunlukla selülozun eldesine odaklanmakta, bunun yanında oluşan yan ürünleri henüz değerlendirilmesini sağlayamamaktadır. Bunlardan en önemlilerinden biri, esas olarak p-kumaril, koniferil ve sinapil alkollerden oluşan organik bir polimer olan lignindir. Ligninden çoğunlukla yanma sırasında yakıt olarak kullanılmaktadır ve bu uygulama alanı ligninin gerçek potansiyelini karşılamamaktadır. Ligninin ekstraksiyonu ve geri kazanımı için farklı tipte ön arıtma yöntemleri literatürde çalışılmış ve bunlardan alkali ön işlemi yaygın olarak kullanılmakta ve dikkat çekmektedir. Alkali ön işlemi, özellikle sodyum hidroksit (NaOH) ve kalsiyum hidroksit ($Ca(OH)_2$) gibi çözücüler açısından nispeten ucuz bir yöntemdir.

Bu çalışmada, fındık üretiminin en fazla olduğu ülke olan Türkiye göz önüne alınarak, fındık kabukları biyokütle olarak seçilmiş ve alkali ön işlem yöntemleri ile 3 farklı ajan; sodium hidroksit, kalsiyum hidroksit ve potasyum hidroksit kullanılarak farklı artan sıcaklık (20°C, 40°C, 60°C), artan önişlem süresi (1s, 2s, 4s) ve artan biyokütle; çözücü oranı (0.1 g/g, 0.2 g/g, 0.3 g/g, 0.4 g/g) denemeleri ile geri kazanılmıştır ve sonuçlar, NaOH'ın su ve gliserol ile kombine edilmesinin en iyi; %14.34 lignin geri kazanımını gösterdiğini göstermektedir.

Anahtar Kelimeler: lignoselülozik biyokütle, fındık kabukları, alkali ön-işlem, kalsiyum hidroksit, sodyum hidroksit, potasyum hidroksit

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ABSTRACT

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Renewable and sustainable environment is the one of the key goals of the continually growing communities. Since the Industrial Revolution, paper industries have been exponentially growing and focusing mostly on extraction of cellulose. The remaining parts of lignocellulosic biomass (LB) such as lignin and hemicellulose proportions do not receive as much emphasis as cellulose. Lignin is an organic polymer mainly composed of p-coumaryl, coniferyl and sinapyl alcohols. Lignin composed biomass utilized as fuel for combustion and this application area doesn't fully exploit true potential of lignin. To evaluate the potential of lignin, extraction and some preliminary steps must be performed. In literature, different types of pre-treatment methods are described, and alkali pre-treatment is commonly used due to high delignification and improved enzymatic accessibility. Sodium hydroxide and (NaOH) and calcium hydroxide ($Ca(OH)_2$) are the most common reagents owing to economically feasible.

In this thesis, considering most abundant hazelnut production country as a Turkey, hazelnut shells were subjected to alkali pretreatment with three different reagents; NaOH and $Ca(OH)_2$ and KOH at different temperatures (20°C, 40°C 60°C), operation times (1 h, 2 h, 4 h) and hazelnut: alkali solvent ratios (0.1 g/g , 0.2 g/g, 0.3 g/g, 0.4 g/g). Effect of deionized water and glycerol on alkali solvents discussed in terms of lignin recovery. Results showed that NaOH combined glycerol pretreatment achieved the highest lignin recovery which was 14.34 %.

Keywords: lignocellulosic biomass, hazelnut shells, alkali pre-treatment, calcium hydroxide, sodium hydroxide, potassium hydroxide

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ÖNSÖZ VE TEŞEKKÜR

Bu tez, Literatür araştırmaları, Giriş, kullanılan Gereçler ve Yöntem, Bulgular ve Tartışma, Sonuç ve Öneriler ve Başvurular olmak üzere 6 ana bölümden oluşmaktadır. Bu çalışma kapsamında, üç farklı bazik önişlem yönteminin çeşitli çözücü konsantrasyonlarında, sıcaklıklarında, operasyon sürelerinde optimize edilmesi hedeflenmiştir. Ayrıca, gliserolün çözeltiye eklenmesinin fındık kabukları üzerinde lignin geri-kazanımı üzerindeki etkisinin belirlenmesi için farklı bir yaklaşım sağlayabilmektedir.

Yüksek lisans yoğun emek gerektirir ve bu bilinç, kişi tarafından bilinmesi, farkına varılması ve öneminin benimsenmesi gereklidir. Yüksek lisans tez çalışmaları belirlenirken, deneysel yöntemler hayata geçirilirken ve tezin dökümantasyon kısımları hazırlanırken, en temel ihtiyaçlarımızdan biri moral ve motivasyondur. Moral ve motivasyon kaybının minimuma indirilip, yüksek lisans bilincinin sağlanması ve sürdürülmesinde, sadece bir rehber değil, aynı zamanda bir akıl hocası, yol gösterici, örnek alınacak bir rol model olan Doç. Dr. Irem DENIZ CAN hocama gönülden teşekkürlerimi sunarım.

Maddi ve manevi desteklerini daima yanımda hissettiren, her sarsıcı zorlukta bana güç vererek yeniden ayağa kalkmamı sağlayan ve her koşulda bana güvenen pek değerli annem Sevda GEZEN'e sonsuz teşekkürlerimi sunarım.

Manevi destekleriyle her zaman yanımda olan, gerektiğinde ellerinden gelen her şeyi hiçbir karşılık beklemeden bana sunan DENIZ Lab'ın çalışkan öğrencileri, pek sevdiğim arkadaşlarım Çağlar ÖZGENER, Aleyna FİLİZFİDANOĞLU'na da teşekkürü borç bilirim.

Son olarak şunları da eklemek isterim ki, "En mühim ve feyizli vafizelerimiz, milli eğitim işleridir. Milli eğitim işlerinde kesinlikle başarılı olmak gerekir. Bir milletin gerçek kurtuluşu ancak bu şekilde" sözüyle, her açıdan nesillere örnek olan yegane Başöğretmen Ulu Önderimiz Gazi Mustafa Kemal Atatürk'e saygı, sevgi ve minnetlerimi sunarım.

Özcan GEZEN
Manisa, 2024

LIST of ABBREVIATIONS

AFEX	Ammonia Fibre Expansion
BCE	Before the Common Era
$CaCl_2$	Calcium Chloride
$Ca(OH)_2$	Calcium Hydroxide
DP	Degree of Polymerization
FTIR	Fourier Transform Infrared Spectroscopy
GHG	Greenhouse Gas
HPLC	High Pressure Liquid Chromatography
HS	Hazelnut Shells
ILS	Ionic Liquids
KOH	Potassium Hydroxide
LB	Lignocellulosic Biomass
LCC	Lignin Carbohydrate Complex
MCR	Macroalgae Cellulosic Residue
NaOH	Sodium Hydroxide
NMR	Nuclear Magnetic Resonance
OVAT	One Variable at a Time
PEG	Polyethylene Glycol
PHA	Polyhydroxyalkanoates
RPM	Round per Minute
SEM	Scanning Electron Microscope
S/G Ratio	Syringyl (S), Guaiacyl (G) Unit Ratio
WS	Wheat Straw

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SECTION ONE

INTRODUCTION

1.1. Literature Research

Fossil fuels are the main concern for toxic air pollutants and carbon dioxide (CO_2). Based on this evidence, it is imperative to decrease the consumption of fossil fuels and transition to more environmentally sustainable energy sources (Perera, 2017). In this scope, renewable energy attracts too much attention and day by day dependency on renewable energy increases. This leads to minimization of the harmful impacts of fossil fuels on the ecosystem (Xu et al.,2023). Bioconversion of lignocellulosic biomass (LB) into bioenergy, is one of the promising candidates for taking the throne of fossil fuels (Jiang et al.,2017). LB is mainly composed of three different units known as cellulose, hemicellulose, and lignin (Zhao et al., 2012; Kumar and Sharma, 2017). Hazelnut shells (HS), one of the suitable biomass for investigation of lignin recovery. The following properties make HS more attractive; abundance in Turkey it approximately 70% of the world, (Islam, 2018), easy to reach, cheap and has a suitable composition mainly consist of 40-51% lignin, 13-32% hemicellulose and 17-27% cellulose (Demirbaş, 2005; Demirbaş, 2006; Surek and Buyukkileci, 2017; Rivas et al.,2020; Hoşgün and Bozan, 2019).

Paper and most LB dependent industries, cellulose has received great research interest as the most abundant source of polysaccharide and environmentally friendly properties. (Sharma et al.,2019; Sofiah et al.,2023). While evaluating the true potential of cellulose, lignin is often disregarded due to its complex structure and significantly greater resistance to enzymatic attacks compared to both hemicellulose and cellulose. They are generally employed for generating heat and electricity and these are equal to more than 50 million tons of lignin (Collins et al.,2019).

Most of the cellulosic processes involve an early pretreatment step. This is important for overcoming the enzymatic recalcitrance of lignin and hemicellulose, as cellulose is surrounded by lignin and hemicellulose. Pre-treatment step is mainly employed for solubilizing lignin, hemicellulose and while doing that, most of the cellulose remains in solid fraction. In literature, different types of pretreatment methods described; alkaline pretreatment, acid pretreatment, organosolvent, steam

explosion, cold plasma etc., and depending on the cost-effectiveness, popularity, alkaline pretreatment attracts too much attention toward itself (Razali et al., 2022). The most common alkaline reagents are sodium hydroxide (NaOH), calcium hydroxide Ca $(OH)_2$ and potassium hydroxide (KOH). These reagents are commonly employed for the breakdown of ester bonds between lignin and hemicelluloses. After alkali NaOH, Ca $(OH)_2$ and KOH, pretreatment, lignin solubilized in aqueous media and by adding flocculation agents (such as calcium chloride ($CaCl_2$) lignin can be recovered (Wang et al.,2017). The effectiveness of the alkali pretreatment is mostly influenced by solvent to material ratio, temperature, operation time and (Sayakulu & Soloi, 2022).

In this thesis, optimum solvent to raw material ratio, temperature, operation time of alkali pretreatments of NaOH, $Ca(OH)_2$ and KOH investigated hazelnut shells in terms of lignin recovery.

1.1.1. Historical background of using lignocellulosic biomass

Lignocellulosic biomass has been widely used for both as a material and fuel purposes since Neanderthal (Aranguren et al., 2018; Berna et al., 2012). However, the early usage of LB may have had some restrictions due to the invention of fire approximately a million years ago (Aranguren et al., 2018; Berna et al., 2012). Along with the years (to 15000 BCE, the development of mastery of fire allowed the development composite materials, such as plywood and veneer from plant biomass and LB were also used in early textiles and linens (Wu et al., 2012; Harrar et al., 1947; Poot et al., 1964; Alves et al., 2016).

Throughout the early years of the industrial revolution and the development of modern chemistry, new chemical processing methods and novel usage of LB emerged. For instance, development of industrial scale pulping technologies, mass production of paper from LB became financially feasible and viable (Dard et al.,1967). Alongside the advances in paper industries, fermentation processes occurred in alcohol distilleries enabled formation of one of the most required fuels; “large scale ethanol”. Ethanol gave a very wide carrousel for humans for industrial fuels; “camphene” also known as “burning oil” which mainly produced from

blending ethanol with turpentine (derived from pine trees) was led to first patented internal combustion engine by Samuel Morey (Songstad et al., 2011; Carolan et al., 2009; Daum et al., 1959). Today, developing technologies of effective LB conversions into the bioproducts are still in the table with high intensity and most of them falling into one of two groups: thermochemical or biochemical; and even combination of both such as hydrothermal-syngas fermentation (Salan, 2017; Verma et al., 2012, Kundu et al., 2018; Song et al., 2015; Pang, 2018; Brethauer & Studer 2015, Saxena et al., 2009). Within the scope of thermochemical processes, the aim is to liquefy or gasify LB directly into biogas, bio-oils, or biochar and then in near future, it can be replaced with petroleum-based fossil fuels. On the other hand, in biochemical scope, the aim is break down of LB into fermentable sugars thus enable them upgradable to alcohols and by-products, derived from the non-sugar part of LB. However, both strategies are highly suffered to heterogeneity, complexity of LB. LB is mainly composed of lignin, hemicellulose, and cellulose. Lignin acts as a barrier to cellulose, hemicellulose which hemicellulose connects lignin with cellulose and innermost cellulose exists (Figure 1). Complexity of assembly of polymers, LB naturally recalcitrant to enzymatic conversion.

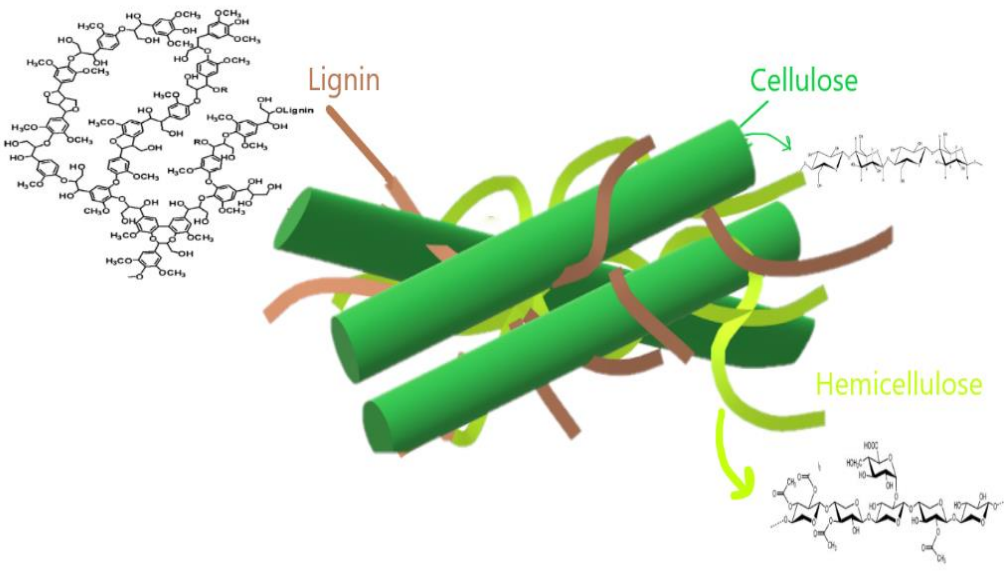


Figure 1. Structure of Lignocellulosic Biomass (Claus et al.,2017; Prieur et al., 2017; Zhang & Naebe 2017; Lisong et al.,2020).

1.1.2. Recalcitrance of lignocellulosic biomass to enzymatic hydrolysis

There are different important parameters described in literature for recalcitrance of LB against enzymatic hydrolysis and they can be divided into two main groups: structural factors and chemical factors. The structural factors are specific surface area of cellulose, crystallinity of cellulose, pore size, distribution, volume while chemical factors can be listed as; percentage of lignin (%) and composition of lignin; p-coumaryl, coniferyl and sinapyl alcohol distribution, hemicellulose and acetyl groups (Zoghlami & Paës ,2019), following Figure 2. illustrates the chemical structure of lignin.

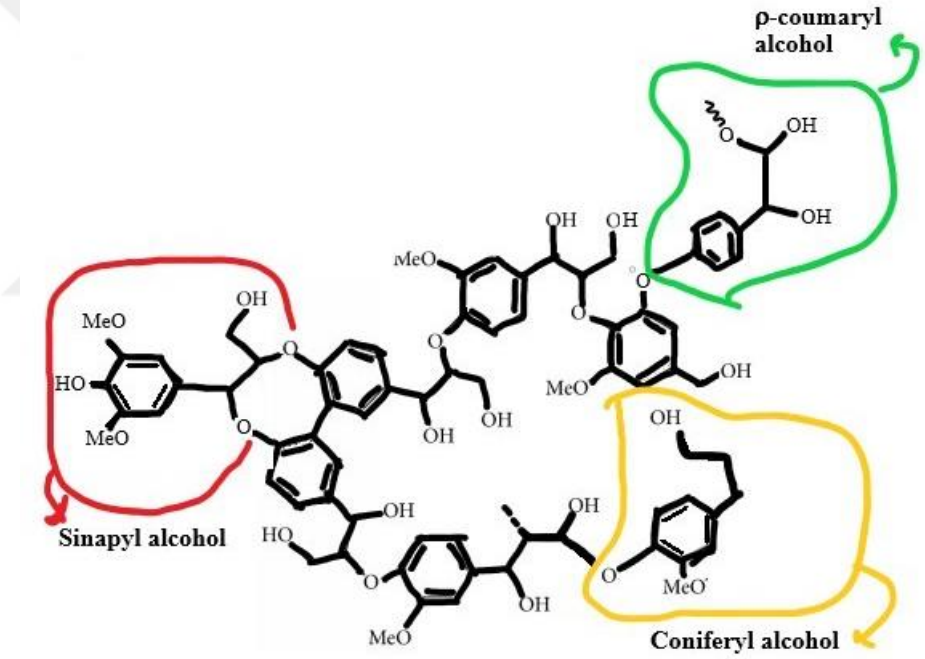


Figure 2. Organic structure of lignin (Lee et al., 2014).

1.1.3. Recalcitrance of lignin

Lignin is the most abundant polymer in LB after cellulose. Generally, takes a piece of total 15-40% percentage of dry weight (Ragauskas et al., 2014). Lignin binds the hemicellulose to cellulose units in the cell wall and lignin is responsible for

structural rigidity and hydrophobicity. the following Figure 3. shows the structural units of lignin. Lignin acts as a physical barrier that blocks the access of enzymes to cellulose and limits polysaccharide accessibility.

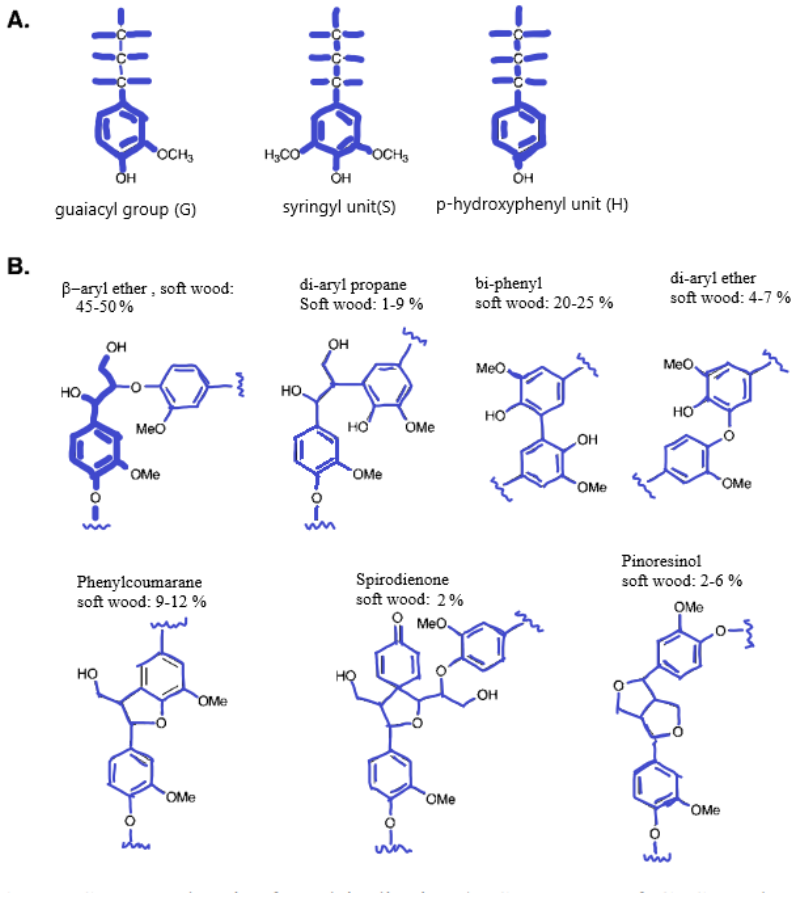


Figure 3. Structural units in lignin. (A) abbreviations of monomeric units of lignin and (B) illustrate the chemical linkages between lignin subunits.

In depth, hydrophobic structural features of lignin such as methoxy groups, polyaromatic structures and hydrogen bonding, enables that irreversible adsorb cellulase and other enzymes when enzymatic attack (Santos et al., 2012; Kumar & Wyman, 2009; Zeng et al., 2014). In literature, different studies had reported for effect of lignin portion on enzymatic digestibility, results are showed lignin content was negatively correlated with enzymatic digestibility in poplar (Meng et al., 2017; Yoo et al.,2017a), wheat straw (Herbaut et al.,2018). Lignin removal and recovery usually focuses on disrupting lignin carbohydrate complex (LCC) thus increasing the porosity and enables enzymes reach to cellulose and hemicellulose.

(Pihlajaniemi et al., 2016; Kruyeniski et al., 2019). Phenolic groups of lignin can be reversibly inhibited of cellulases (Yu et al., 2014; Yang and Pan, 2016; Yao et al., 2018) and blocking free phenolic hydroxyl group significantly reduces (65-91%) negative impact of lignin on enzymatic hydrolysis (Yang and Pan, 2016). Furthermore, Yoo et al. showed that lignin syringyl (S), guaiacyl (G) ratio (S/G) are directly related with recalcitrance to enzymatic attacks. S and G units interact together to form the backbone of the lignin polymer via non-uniform arylglycerol-β-aryl ether (β-O-4) bond, which is most common linkages a found at generally C_2 of the C_3 chain of lignin (Ahmad et al., 2011; Santos et al.,2012; Yoo et al.,2016) and carbon bonds such as β-β, 5-5 and β-5 linkages also formed and have effect on biomass degradation (Kishimoto et al.,2009). Studer et al. reported that S/G ratio of lignin likely important and varies from 1.0-3.0 in undomesticated *Populus trichocarpa* known as black cotton (Studer et al.,2011). However, the general considerations between S/G and lignin recalcitrance are still uncertain. For instance, Herbaut et al. (2018) and Yu et al. (2014) showed a positive correlation between S/G ratio and recalcitrance in miscanthus and woody chips. They highly relate these results to higher binding capacity of G over S to cellulase (Guo et al.,2014; Yoo et al., 2017.). The formation of each of these linkages is mostly dependent on polymerization or radical dimerization of cinnamyl alcohol precursors (Freudenberg, 1965; Higuchi, 1971). In summary, lignin is strongly recalcitrant to enzymatic hydrolysis and this recalcitrance is influenced by its chemical composition and structure.

1.1.4. Recalcitrance of cellulose

Cellulose is the most abundant LB biopolymer, which mainly 40-60% in weight (Sharma et al., 2019). Consists of β-D-glucopyranose units linked between β-1-4- glycosidic bonds with cellobiose repeating units. Cellulose fibrils are directly embedded in an LB matrix that makes it recalcitrant to enzymatic hydrolysis. Yoo et al. (2017) reported that glucose release was positively correlated with the cellulose content (Yoo et al., 2017). One of the different perspectives, the degree of polymerization (DP) which numbers of glucose units in the polymer playing a crucial role on recalcitrance. However, effect of the DP is still insufficient and not clear.

Indeed, the mains factors that affects DP accompanied such as crystallinity and porosity. For instance, a study carried out Sinitsyn et al. (1991) and results showed that γ-irradiation had a minor reduction effect on saccharification rate and Ioelovich & Morag. (2011) also achieved a similar conclusion. In contrary, Lu et al. (2019) found that the cellulose DP was negatively correlated to the cellulose hydrolysis.

1.1.5. Hemicellulose

Hemicellulose is a heterogeneous polymer that consists of xylan, glucan, xyloglucan, mannans, glucomannans and others. Hemicellulose is amorphous polymer that have little physical strength and have much lower DP than cellulose. Hemicellulose represents 20-35% of total LB (Chandel et al.,2018; McKendry, 2002). It can be easily hydrolyzed by alkali, dilute acids as well as hemicellulase enzymes (Isikgor and Becer, 2015). In literature, different studies have been reported for removal of hemicellulose by different types of pre-treatment methods such as steam explosion pretreatment, alkali pre-treatment, dilute acid treatments. Dilute acid pretreatment improves enzymatic accessibility to cellulose (Auexenfans et al.,2017; Herbaut et al.,2018; Santos et al.,2018). Several studies performed for hemicelluloses removal were more efficient than the lignin removal in terms of improving enzymatic hydrolysis rate (Yoshida et al., 2008; Leu and Zhu, 2013; Lv et al., 2013).

1.1.6. Interaction between these polymers

Cellulose and hemicelluloses are associated together through hydrogen bonds. On the other hand, there are ester and ether linkages between holocellulose parts; carbohydrate fraction of LB basically consists of hemicellulose and cellulose between lignin (Lee et al., 2014). The most critical aspect to be addressed for lignin recovery. lignin is covalently linked to hemicellulose and forms lignin-carbohydrate complex (LCC) (Tarasov et al., 2018; Giummarella and Lawoko, 2019).

1.1.7. History of lignin carbohydrate complex (LCC) and importance

Back in the 19th Century, Erdmann. (1866) suspected a phenomenon that bonds must be presence in lignin and carbohydrate to explain the inability in separating these two components completely and named as “glycolignose”. Later that, the low yield of lignin obtained via ethanol extraction on to wood and referred to “Brauns native lignin” rest was bound to carbohydrates (Brauns & Seiler, 1952). In 1957, Merewether was published a comprehensive study on LCC and suggested that “impossible to extract just a fraction unchanged lignin from unhydrolyzed wood and without pretreating wood, it is totally impossible to complete fermentation of the carbohydrate. The development of important characterization devices such as scanning electron microscope (SEM), Fourier transform infrared spectroscopy (FTIR), X-ray diffraction analysis (XRD), nuclear magnetic resonance (NMR) enabled that the widened knowledge about native structure of lignin and interaction between other polymers in LB complex. However, even in the 21st Century, debates about the complete structure of native lignin continues (Giumarrella & Lawoko, 2019). It is widely accepted that a protocol developed by Björkman (Björkman, 1956), using dioxane/water co-mixtures treatment at ambient conditions on milled wood, commonly termed milled wood lignin (MWL), that nearest lignin structure to ideal native lignin structure. After that LCC obtained, residuals after MWL isolation were referred as “Bjorkman LCC” and after Bjorkman’s works, different studies emerged, included detailed studies on the residues remained after extraction and different characterization methods are described. Following studies have suggested that, virtually all residual lignin in the sulphide pulps as well as lignin in both bleached and unbleached oxygen, delignified softwood kraft pulps (Lawoko et al., 2006) is covalently bound to polysaccharides. Furthermore, these results are consistent with the final phase of kraft pulping; during final phase, delignification selectivity is considerably low.

Similarly, recalcitrance to delignification during pulping process was proposed to directly be related with presence of alkaline stable LCC bond while enzymatic hydrolysis occurred and studied by Size exclusion chromatography (SEC) (Yamasaki et al., 1981; Hortling et al., 1990; Tenkanen et al., 1999)

In literature, typically five different types of LCC bonds suggested; benzyl ethers (BE), γ-esters to esters (GE), phenyl- glycosides (PG), hemiacetal / acetal

linkages and ferulate / coumarate esters (FE/CE) that are linked to lignin at a 4-OH and 4-O positions (Giummarella and Lawoko, 2019) and Figure 4 shows the suggested structure of these linkages.

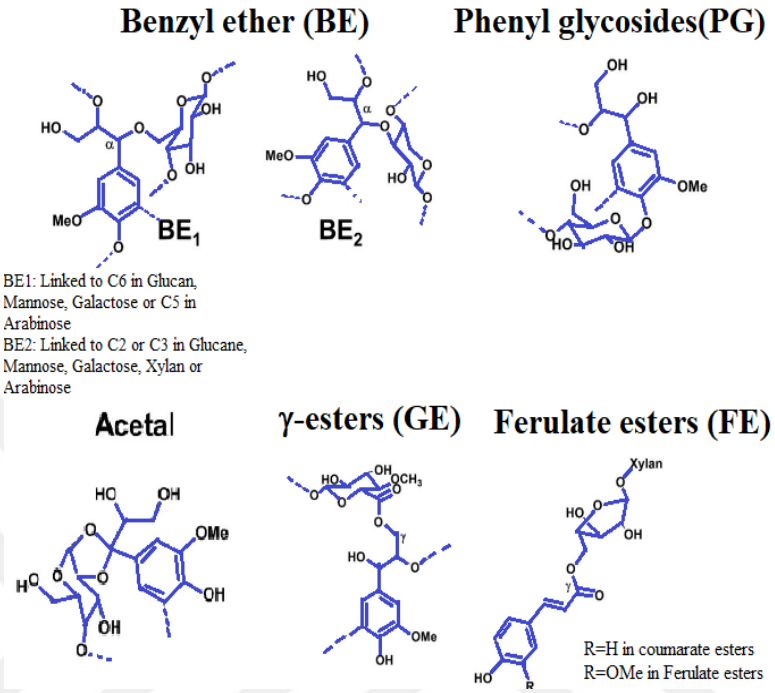


Figure 4. Suggested structures of LCC bonds in wood and grass (Giumarella and Lawoko 2019).

Despite the uncertainty on LCC, this phenomenon does not mean that valorization of lignin through various pretreatment methods is impossible. Alkali pretreatment, one of the most common and appliable method for lignin recovery and potentially lignin valorization. The following section is directly related with the pretreatment methods and difference of alkali pretreatment from different methods.

1.1.8. Pretreatment methods

LB is mainly composed of lignin, hemicellulose, and cellulose. These complex polymers have assembling recalcitrance to enzymatic hydrolysis, that is why pretreatment is mandatory to make cellulose more accessible. However, this can

be appliable to another frame, lignin recovery. Pretreatment methods typically enable the delignification of lignin and solubilization of hemicellulose. While doing that, cellulose accessibility considerably improved, and the following figure (Figure 5.) illustrates basic pretreatment effect on LB.

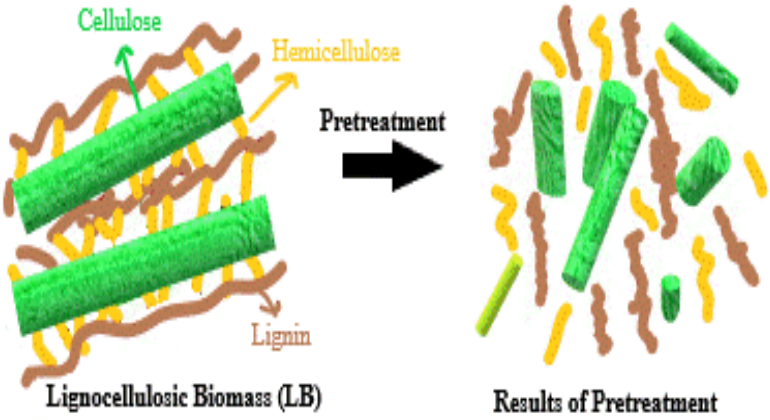


Figure 5. Basic illustration of pretreatment effect on biomass. (Rahmati et al., 2021)

In literature, different types of pretreatment methods are described as a three main group: physical, chemical, and biological. Some of the hybrid systems which contain two or more of them and the following Figure 6. summarize the different types of pretreatment methods.

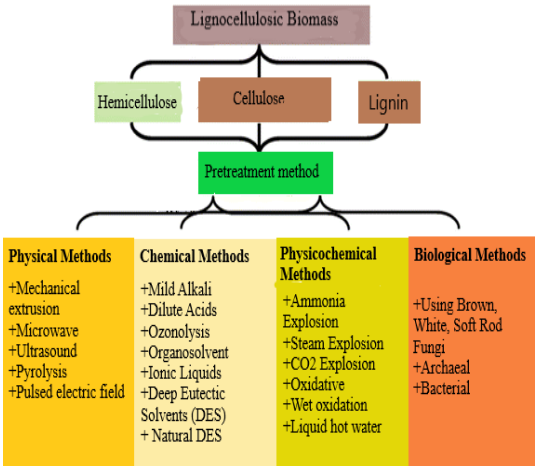


Figure 6. Summary of different types of pretreatment methods (Lee et al., 2014).

However, all pretreatment methods have their own advantages and major drawbacks, these are summarized in the following Table 1.

Table 1. Advantages and disadvantages of different types of pretreatment methods.

Methods	Advantages	Disadvantages	References
Dilute Acid	Low operation time	Furfural formation	Chen (2012)
	Higher yield of pentose	Risky acid recovery	
Oxidation Agents	Depolymerize lignin	Expensive	Sun and Cheng., (2002)
	No inhibitory	High amount of agent	
Organosolv	Depolymerize lignin	Inhibitory	Monavari et al., (2009)
	Higher yield of pentose	Expensive	
Ionic Liquids	High accessible surface area	Toxicity of some ILs	Rezania et al., (2017)
	Lignin rec. and reuse	Expensive	
Biological Pretreatment	Low use of energy	lower hydrolysis	Bak et al., (2010)
	Environmental friend	rate of reaction low	Singh and Chen (2008)
Alkali Pretreatment	Lignin recovery and reuse	Washing required	Alexandropoulou et al., (2017)
		Salt formation	

1.1.8.1. Acidic pretreatment methods

Acidic pre-treatments, the most employed method for the breakdown of rigid structure of LB typically breakdown of hydronium ions and intermolecular, intramolecular bonds between cellulose, hemicellulose, and lignin in LB hierarchy structure (Lee et al., 2014). These methods usually operated using concentrated acids such as HCl, H_3PO_4, HNO_3 and mostly H_2SO_4 to hydrolyze the LB (Sun et al., 2011; Zhang et al., 2012). These agents are effective agents for biomass degradation to maximize the accessibility of enzymes to reach monomeric sugars, thus improving the yield of monomeric and oligomeric sugars for different types of processes such as bioethanol production (Lee et al., 2014). However, in larger scales, requirements of higher amounts of concentrated acids usually leads the considerably high economical investment and operational costs; also, these agents are toxic, hazardous to health and corrosive, thus highly corrosion resistant equipment must be required. Extreme reaction conditions will cause uncontrollable degradation of biomass to sugar and undesired byproducts can be formed such as alcohols and acids (Pedersen & Meyer, 2010; Harmsen, 2010). For these reasons, further optimizations and research were conducted, then dilute acid hydrolysis method emerged. In this method, the usage of acids decreased considerably, approximately < 4 wt.% and has become a more cost-effective method when compared to concentrated acids. In addition, dilute acid hydrolysis method enhances LB separation. For instance, by using H_2SO_4 < 4 wt.% can dissolve and recover most of the hemicellulose part of LB as the dissolved sugars up to completely conversion under low temperature and low acid concentration (Lie et al., 2010; Moe et al., 2012).

There is different major optimization conditions studied in literature for acidic pretreatments, and these are: acid concentrations, solids loadings, temperature, and residence time. As a response of these parameters, the solid recovery, hemicellulose conversion, glucose, xylose, and furfural concentrations of wide variety of raw materials have been evaluated (Yoon et al., 2015; Triana et al., 2011; (Juan Carlos López-Linares et al., 2013; Martínez-Patiño et al., 2017; Cara et al., 2008). For these purposes, wide scopes of investigations have been performed and day by day well developed. Operation parameters such as economical, technical, and environmental aspects have been investigated. These parameters can be counted for

the change of the operating conditions: acid concentrations, pressure, temperature, pH, environment that treatment will occur for example reactor and configuration of this reactor: for instance, plug-flow reactor and effective catalyst (use of Lewis acid or solid catalyst). According to the solid loadings, the acid pretreatment methods can be classified as wet or dry acid pretreatment. The wet acid pretreatment is mainly characterized using solid loading below 15% w/w (Jung & Kim, 2015). Wet acidic treatment usually contains both mineral acids (e.g., HCl, H_2SO_4 and H_3PO_4 and organic acids (e.g., CH_2O_2, $C_2H_4O_2$, $C_4H_4O_4$) as a catalyst. This method is the most used way to hemicellulose degradation of secondary generation raw materials (Solarte-Toro et al., 2019). In literature two different types of wet acidic treatment are described: concentrated acid and diluted acid process.

Concentrated acid pretreatment method is reliant to use of a large amounts of acids such as phosphoric acid (H_3PO_4), sulphuric acid (H_2SO_4) in high concentrations, moderate temperature (< 100°C) (typically higher than 30% v/v) to obtain higher sugar yields to avoid enzymes and lignin (Jung & Kim, 2015). This reaction consists of three steps; (1) hemicellulose removal, (2) cellulose disruption to cello-oligosaccharides, crystalline structure of cellulose converted into amorphous form and finally (3) cellulose to monomeric sugars such as xylose, arabinose, mannose, glucose, galactose. However, in larger scales this method has serious issues; high inhibitory compound generation, the salt formation after reaction and neutralization step mandatory; increased salinity leads corrosion and then leads to extra operation cost and healthy safety issues.

On the other hand, dilute acid pretreatment is the second method of wet acidic pretreatments, and this method is closest one for commercialization due to the simplicity, low reagent costs, relatively low operation costs and effectiveness (Jung & Kim, 2015). In the same way with concentrated acid pretreatment, this process is typically carried out at 1:10 solid to liquid ratios. However, in this method only hemicellulose fractions are removed therefore enzymatic saccharification process is required for completely cellulose hydrolysis (Liu et al., 2016; Ask et al., 2012). Once hemicellulose and lignin fractions are removed, sugar yields can be increased up to 90% through enzymatic hydrolysis. Compared with concentrated acid method, conditions are slightly high when compared with other methods; operation time 10-120 min, temperature 120-215 ºC, pressures 2-10 atm and low

acidic concentrations which 1% w/w to 5% w/w) (Solarte-Toro et al., 2019). Dilute acid pretreatment has more advantages from an environmental and economical perspective than concentrated acid methods in terms of low reagent cost, amount of acidic waste formation, less corrosivity (Jung & Kim, 2015). Nevertheless, the perpetual quest for improvement persists, and in recent years, solid catalysts such as zeolites, carbon-based catalysts, and ion exchange resins have been extensively researched. For instance, Tan and Lee (2015) investigated the Dowex (TM) Dr-G8 catalyst to pretreat *Eucheuma cottonii* red macroalgae cellulosic residue (MCR), obtained 4% w/w catalyst loading is optimal and most important part there is no inhibitory compound formation observed. Moreover, results of the study showed catalyst could be used six times without any performance fluctuations, reaching glucose yields higher than 80%. The study concluded that due to the easy catalyst recovery in the first case the environmental impact of the use of solid catalyst is lower than the use homogeneous catalyst. Finally, at higher levels of catalyst usage, pretreatment process negatively affected due to the presence of sufficient acid sides, thus improved undesired side reactions. While evaluating wet acid pretreatments, be useful to remind that water is another important phenomenon that should not be overlooked easily.

In order to reduce the amount of wastewater formed using the traditional diluted acid methods, dry dilute acid pretreatment method (DDAP) emerged. DDAP operates high solid loadings approximately two times greater than liquid fraction (He et al., 2014). Theoretically, in this method different acid solutions (e.g., H_2SO_4, H_3PO_4 and HNO_3) can be used. Mass transfer between the LB matrix and the acid catalyst can be improved by adding presoaking or impregnation step with the DDAP but this time process adaptation to industrial scale can be disrupted because presoaking step is usually taking 12 hours at room temperature. Moreover, additional costs related to mixing equipment, storage tanks and system transportation should be economically taken into account. The operation conditions of DDAP method involves 175 to 190 ºC, 2-5% w/w acid concentrations and 3 to 5 minutes of residence time. This pretreatment method is usually investigated on corn stover using sulphuric acid as a catalyst (He et al., 2014; Zhou et al., 2017). Therefore, this method must be evaluated using another LB feedstocks for the extent of knowledge about DDAP then feasibility of applying this process on the industrial scale. He et al.

(2013) reported that DDAP pretreatment has a cellulose conversion 72.1% to 87.1% while furfural formation acceptable about 0.18% to 0.90% of pretreated solid and finally following figure (Figure 7.) review of main process variables, reactor configurations, eco-friendly parameters, and industrially potential products.

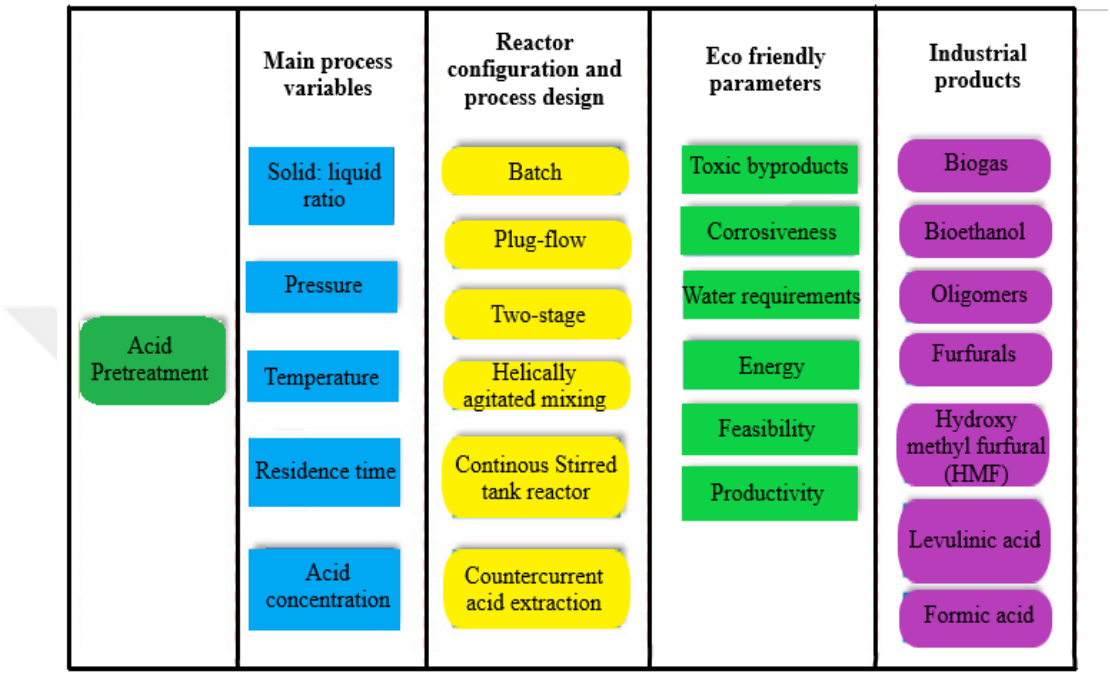


Figure 7. Review of acid pretreatment of main process variables. Main process variables, reactor configuration and effect on process design, economic considerations, and industrial potential applications (Solarte-Toro et al., 2019).

1.1.9. Oxidation agents

Oxidation agent method is another method to catalyze delignification process by attacking specific ring formation of lignin and cleaves it. This method typically uses oxidation agents such as hydrogen peroxide (H_2O_2), ozone (O_3), glycolic acid ($C_2H_4O_3$). For instance, Lawrence et al. (1980) concluded that peracetic acid converts lignin to soluble manner by two different main pathways. First pathway, directly cleaving of β–aryl ether bonds and C-C, C-O bonds linked to the aromatic rings of lignin, thus this cleavage results as the reducing in the molecular weight of

lignin polymer and by that way lignin can be eliminated from LB. In the second method, peracetic acid increases the solubility of lignin in water, thus dealkylation of O-methyl groups, introduced of hydroxyl groups to aromatic rings to lignin and cleavage of aromatic ring to muconic acids (Lawrence et al.,1980). Further, these reactions lead to increasing the polarity of lignin, increases the solubility of lignin in water, then lignin fragments washed out from the LB. However, when working with peracetic acid, operators must be careful due to the explosive nature of peracetic acid in concentrated forms and be useful to note that peracetic acid is not economically feasible.

1.1.10. Organosolvent pretreatment

The presence of organic solvents in aqueous solutions which contain LB, simultaneously lignin, hemicellulose degradation, solvation and solubilization will occur. This method was first developed to eliminate shortcomings; -air and water pollution- of conventional pulping process, kraft and sulfite processes. Organosolv pretreatment can be miscible with organosolv pulping, but the main different between these two methods, in organosolvent treatment, the degree of delignification of the treatment is not demanded to be as high as pulping. This method has some advantages as follows: (1) can isolate lignin and carbohydrate complex in two different phases, thus they can be valorized as a chemicals or renewable materials (Lora and Aziz, 1985; Johansson et al., 1987; Aziz and Sarkanen, 1989). The distillation (2) applied for organic solvents recovery and can be reusable. When compared with different treatment methods, organosolvent method seems more feasible for biorefinery of LB, which considers all the biomass components.

However, this method has tremendous major drawbacks; cumbersome washing arrangements are required; the pretreated solid must be washed with water and immediately after washed with organic solvent to avoid reprecipitation of lignin in the presence of water. Due to the organic solvent usage and solvent must be recovered for chemical usage regulation and saving but this time recovering of organic solvent needs too much energy, the organosolv method can be listed as one of the most expensive pretreatments in total (Zhao et al.,2009). In addition, volatility

of organic solvents must be extremely important because most of the organic solvents can cause explosions, fire hazardous (Aziz and Sarkanen, 1989).

1.1.11. Ionic liquids

Ionic liquids (ILs) are another important and trendy pretreatment method. Unlike the other methods, ILs makes the catalytic sites highly accessible to the β–glycosidic bonds, thus facilitates the hydrolysis of cellulose (Xiong et al., 2014; Morales-delaRosa et al.,2012). ILs can dissolve lignin, hemicellulose, and cellulose under considerably mild conditions without degrading the chain structure. They are generally tunable for target components, organic cation and inorganic anion and they are reusable salts such as 1-alykl-2,3-dimethylimidazolium $[C_nmmim]^+$ with "n" indicates that number of carbons in alkyl chain (Tadesse and Luque, 2011; Zavrel et al.,2009). Ideal ILs for LB degradation usually possess three important requirements: (1) varying organic cation high dissolution capacity; (2) low viscosity, low melting point, low or non-toxicity (3) high stability. ILs pretreatment typically reduces crystallinity of cellulose to amorphous structure.

1.1.12. Biological pretreatment

Biological pretreatment mostly depends on the metabolic reactions of microorganisms that enable degradation of lignin and hemicellulose in LB. In biological pretreatment, brown, white and soft rot fungi were used and using white rot, seems energy saving and considerably more environmentally friendly (Chen et al.,2010). Currently, different types of research and developmental studies are ongoing to detect alterations in chemical structure of LB and accessibility of enzymatic hydrolysis. The byproducts of biological pretreatment typically do not inhibit enzymatic hydrolysis, as the conditions are usually carried out under mild conditions.

In biological pretreatment, depending on the lignolytic enzymes that are provided by basidiomycete, such as manganese peroxidase, *laccase* and lignin peroxidase, lignin

degradation efficiency varies considerably. Nowadays, to improve the lignin degradation, different types of consortiums are trying to discover, for this purpose, synergistic effect of specific fungi and bacteria strains are taking too much attention towards them. There are different types of advantages these consortiums such as increased adaptability, improving productivity and enzymatic saccharification efficiency, enables the controlled pH and increased substrate utilization during sugar utilization (Kalyani et al.,2013). When compared to ammonia fibre expansion (AFEX) and organosolvent, biological pretreatment, biological pretreatments in lab to pilot scale productions, it can be considerable to more inexpensive process. However, in larger scales due to the sterile conditions and high operational costs; maintaining temperature, oxygen presence/absence modulating devices, aeration (if it necessary), also time consuming and relying on these properties, industrial applicability to this method still in investigation (Chaturvedi and Verma, 2013).

Laboratory and pilot scale, different types of microorganisms investigated lignin removal. For instance, Suhara et al. (2012) investigate *Ceriporiopsis subvermispora* FP90031 and *Phanerochaete sordida* YK624 specific strains. Results showed that preferential lignin removal reached up to 50% percent (Suhara et al.,2012). Promising effect of white rot fungus; *Irpex lacteus*, reported by Du et al. 2011, on corn stalks, the hydrolysis yield was achieved 82% at 28 days (Du et al.,2011). However, in industrial applications, these amounts of operation times cannot be feasible and appliable. Taha et al. 2015, reported that co-cultivation of lignocellulose degrading organisms resulted in a seven folds increase in saccharification rate (Taha et al.,2015).

1.1.13. Alkali pretreatment

Alkaline pretreatment is a common and effective method for solubilizing lignin while neutralizing the acidic products released from the lignin-carbohydrate complex (Hendricks and Zeeman, 2009; Chen et al., 2012). Compared with other types of pretreatment methods, alkali hydrolysis occurs under relatively mild conditions (below 140°C). Alkaline pretreatment typically involves two steps: solvation and saponification. These reactions make cellulose and hemicellulose more accessible to

enzymes and bacteria, while lignin is dissolved in the supernatant. It is useful to note that this method preserves a higher amount of pentose sugars than other methods; therefore, sometimes this results in higher sugar production and more of the desired product (Sasmal & Mohanty, 2018).

There are different types of alkali reagents that can be applied in this method: sodium hydroxide (NaOH), calcium hydroxide (Ca $(OH)_2$), and potassium hydroxide (KOH). In aqueous potassium hydroxide, xylan can be selectively removed at low temperatures (room temperature or below) to prevent the cleavage of end groups (Hon and Shiraishi, 2001). The cleavage of end groups is typically associated with higher monomeric hemicellulose fractions, thereby reducing the overall recovery of hemicellulose (Lase et al., 2002). However, if the main consideration is achieving higher lignin recovery, the peeling process may not need to be avoided, potentially increasing the lignin recovery rate (%). On the other hand, NaOH pretreatment specifically cleaves ether and ester bonds in lignin-related structures in the lignin-carbohydrate complex (LCC) and lignin biopolymer (LB), while Ca $(OH)_2$ pretreatment particularly cleaves acetyl groups. The depolymerization of lignin depends on the ester and aryl ether bonds, categorized as aryl ether bonds; $OC_{aromatic}$, $C_{aromatic}$ and $C_{aliphatic}$ (from least stable to most stable). In alkaline media, oxygen is reduced through reactions with superoxide radicals (-O2) and phenolic hydroxyl groups of lignin. Alkaline oxidative pretreatment methods primarily involve single radical reactions, leading to the formation of various acids during the degradation of hemicellulose and cellulose fragmentation, which introduces hydrophilic groups into the lignin structure (Klinke et al., 2002). Nucleophilic attacks also occur to some extent, causing ring openings that promote degradation and solubilization. Figure 8. illustrates the involvement of OH- anions in the delignification process. This mechanism essentially describes the entire process: acidic protons of phenolics in lignin become phenolate ions in highly basic environments. These phenolate ions then break down, removing ether from the benzylic position to form a transition state. This transition can result in two different pathways: (1) the formation of highly conjugated arylvinyl-phenol after cleavage of the CH2O group, and (2) the restoration of aromaticity in lignin fractions. Furthermore, in the presence of oxygen, lignin oxidizes to 1,4-dicarboxylic acids, which are soluble in bases.

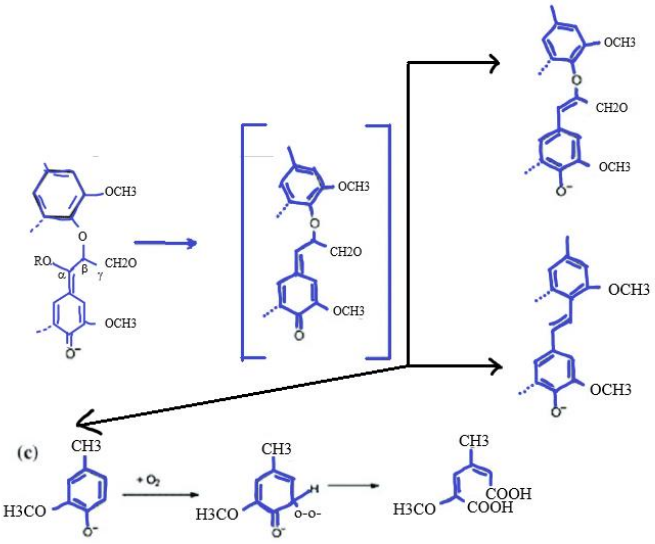


Figure 8. Alkaline conditions lignin degradation typically α and β ether linkages (Sasmal & Mohanty, 2018).

Therefore, Ca $(OH)_2$ facilitates the reaction (delignification) steadily presence of oxygen without any damage to the hemicellulose, cellulosic fractions. Chang et al., (1998) investigated the effect of Ca $(OH)_2$ on enzymatic digestibility of two crop residues: bagasse and wheat straw. Results of this study indicate that optimum load for Ca $(OH)_2$ was 0.1 g Ca $(OH)_2$ g^{-1} dry biomass and water loading have little effect on the digestibility, 14% of lignin became solubilized.

On the other hand, Potassium hydroxide is another appliable alkali for pretreatment of LB. KOH has higher expenditure compared with other alkalis such as NaOH and Ca $(OH)_2$ (Kim et al., 2016; Rodrigues et al., 2016). The KOH pretreatment has an advantage against NaOH pretreatment; in NaOH pretreatment, sodium use, and discharge have an enormous potential to negatively affect environment such as water pollution and soil salinization (Zheng et al., 2014). KOH has potential soil amendment derived from black liquor (Liu et al., 2015).

1.1.14. Glycerol pretreatment

Glycerol pretreatment partially removes lignin and hemicellulose, thereby enhancing the accessible surface area of cellulose fibrils. Due to these properties,

glycerol is often combined with various types of pretreatment methods. For example, Trinh et al. found that under pretreatment conditions of 190°C for 10 hours, glycerol pretreatment of rice straw improved sugar digestibility up to 4-5 times compared to untreated samples (Trinh et al., 2017). However, these high temperature conditions necessitate increased energy inputs and are time-consuming on a larger scale. Another study by Sun et al. demonstrated improved cellulase enzyme susceptibility towards sugarcane bagasse, achieving 90% hydrolysis in 72 hours (Sun et al., 2016).

1.1.15. Raw material consideration

Lignocellulosic biomass is generally categorized into four different groups based on its source: (1) agricultural residues such as rice, wheat, wheat straw, barley, sugarcane bagasse, corn stover, grape pomace, grape stalks, walnut shells, and hazelnut shells; (2) energy crops including switchgrass, short rotation hardwood, and miscanthus; (3) woody biomass like pine wood and oak wood; and (4) municipal solid waste and pulping wastes (Kim et al., 2016). These types of lignocellulosic biomass have varying fractions of lignin, hemicellulose, and cellulose due to factors such as growth environment, plant species, soil composition, and pesticide use.

Hazelnuts, the largest cultivated in Turkey (Corylus avellana L.), account for approximately 75% of the world's production (Erdogan, 2018). This abundance makes hazelnut shells relatively easy to obtain for different lignocellulosic biomass applications. The lignin content of hazelnut shells ranges between 20% and 40% of the total shell weight (Avci et al., 2013; Alessandro Di Michele et al., 2021), with values significantly influenced by growth conditions, nut size, shape, shell thickness, and vigor (Alessandro Di Michele et al., 2021)

1.1.16. Potential valorization areas of lignin

It has been estimated that approximately $3x10^{11}$ metric tons of lignin are present on planet Earth, with a yearly biosynthesis of lignin estimated at around 2 x 10^{10} metric tons (Matsushita, 2015). On the industrial scale, lignin is often considered a low-value residue, with more than 50 million tons used annually as a

material for electrical generation through combustion. Lignin possesses complex aromatic compounds and a heterogeneous chemical structure, which may be among the main reasons why industries hesitate to use it (Zhang et al., 2020).

However, there is growing interest and research in various applications of lignin across different sectors such as surfactants, electrodes for energy storage devices, hydrogels, and bio composites. In this section, detailed discussions on different applications and future trends of lignin are presented. Additionally, various parameters and considerations are illustrated in Figure 9. Lignin can be utilized for synthesizing promising hydrogel networks using different polymerization techniques including semi-interpenetrating networks, penetrating networks, atom transfer radical polymerization, catechol reduction chemistry, crosslinking copolymerization, etc. (Sethupathy et al., 2022).

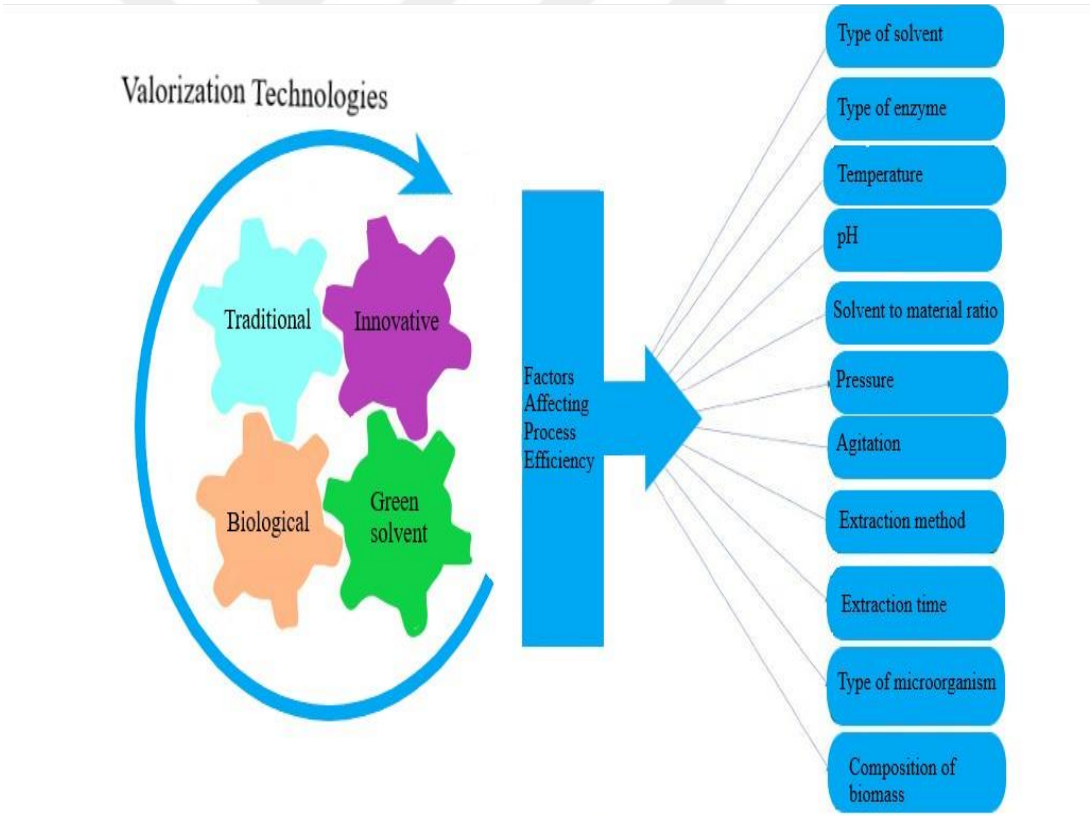


Figure 9. Different considerations for valorization of molecule of interest.

Owing to its biocompatible and biodegradable nature, these types of hydrogel networks can be usable in wound healing/dressing, superabsorbent of heavy metals, food packaging, oxygen scavenging and too much investigation already been performed by researchers, following Table (Table 2.) represents lignin-based studies for hydrogels.

Table 2. Representation of different lignin-based hydrogel studies (Sethupathy et al.,2022).

Lignin based hydrogels	Applications	References
Keratin-lignin hydrogels	3D and 4D printing	Grigsby et al., 2020
Alkali Lignin-cellulose nanofibers carbon dots-based hydrogels	Adsorption of Cr (VI)	Yuan et al.,2021
Hybrid double-crosslinked lignin hydrogels	Foldable capacitor	Liu et al., 2020

1.1.17. Lignin based hydrogels

The global demand for agriculture has drastically increased due to the rising global population, resulting in restricted food accessibility and a sharp decrease in arable lands. This increase has led to higher use of chemical fertilizers in agriculture, affecting the quality of farmlands. In response, biopolymer-based hydrogels have emerged as promising solutions due to their eco-friendly nature and excellent water holding, retention, and release properties. Among biopolymer-based hydrogels, lignin-based hydrogels exhibit superior mechanical properties such as compression resistance, water retention capacity, and tensile strength. These properties make them directly applicable to soils, improving water availability and retention, thereby

enabling crop cultivation in drought conditions. For instance, alkali lignin and polyethylene glycol (PEG)-based diglycidyl ether hydrogels have been shown to promote crop growth in severe drought conditions, resulting in higher yields due to improved soil water availability and retention (Mazloom et al., 2020). Similarly, lignin/konjac flour/alginate-based hydrogels enhance water holding and retention abilities of soils under drought conditions, leading to increased growth of tobacco plants (Song et al., 2020). Additionally, lignin-polyvinyl alcohol hydrogels exhibit better swelling and water retention capacities compared to conventional hydrogels, while black liquor lignin-hydroxyethyl cellulose hydrogels are effective for dye removal from wastewater (Morales et al., 2020; Huang et al., 2019).

Recently, there has been significant interest in lignin-based hydrogels for biomedical applications. One critical criterion for biomedical applications is cytotoxicity; materials must not be toxic to be considered safe. Lignin exhibits very low cytotoxicity and has been extensively investigated in this regard. For instance, lignin-dependent chitosan alkali hydrogels (Ravishankar et al., 2019) and lignin-PEG methyl ether methacrylate hydrogels (Afewerki et al., 2020) have been reported for their non-toxic nature and biocompatibility. Additionally, silver (Ag)-lignin nanoparticles (NPs) incorporated into peroxyacetic acid (PAA)-pectin hydrogels (Ag-lignin NPs-PAA-Pectin) exhibit excellent mechanical, wound healing, and antibacterial properties. When combined with epidermal growth factor on the surface, these hydrogels have shown increased wound healing activity (Gan et al., 2019).

1.1.18. Lignin based surfactants

Lignin based surfactants are another hot topic investigation area nowadays. Surfactants/surface active agents are compounds that accumulate at the interface between two distinctive phases via alteration on the surface tension of liquid. Surfactants are classified as ionic surfactants, non-ionic surfactants, polymeric surfactants, natural and unnatural (synthetic) surfactants. They are widely used in different industries from petroleum, pharmaceuticals, personal care, laundry and agricultural. Lignosulfonates basically consist of sulfonated lignin particles and offer

a great opportunity to apply them to biosurfactants. Biosurfactants are comprised of hydrophilic sulfonate groups and hydrophobic aromatic rings (Alwadani & Fatehi, 2018). However, applications of lignosulfonates as a biosurfactants still have drawbacks; heterogeneous and complex structure of lignin limits applications but be useful to note different types of improved extraction methods were shown to reduce the heterogeneity and polydisperse effect lignin (Rumpf et al., 2020). On the other hand, the physical property of lignin is very important for applicability as biosurfactants. For instance, Sekeri et al. (2020) were operated a study in soda lignin from oil palm empty fruit bunch and prepared nanosized lignin particles then compared with commercially available emulsifying agent, and results are showed that nanosized lignin superior to commercially available agent. Similarly, Perkins et al. (2017) combined kraft lignin with cyclohexane, methane sulfonic anhydride, triethylamine, and polyethylene glycol (PEG). Results show that PEGylated lignin possessed good emulsifying ability, and they propose that, can be appliable to water-in-oil emulsions.

1.1.19. Valuable products by degradation of lignin via microorganisms

Lignin can be degraded by microorganisms. In recent years, by using different types of microorganisms such as fungi, bacteria. Lignin and lignin-derived aromatic compounds can be transformed into vanillin, pyrogallol, lipids, monolignols, furfural, pyruvate, lactate, succinate, polyhydroxyalkanoates (PHA), pyridine-2,5-dicarboxylic acid (Wang et al., 2022). However, adaptation of microbial dependent procedures to industrial scale is mostly stubborn and some of the essential parameters can be listed as biofilm formation, iron oxidation, corrosion, cell development, cost of growth medium, fermentation strategy, optimum strain for degradation of desired valuable product, genetic manipulation scenarios and others.

SECTION TWO

MATERIALS AND METHODS

In this section, the required agents, raw materials, devices, and chemicals are explained in detail. The general experimental steps and analyses are reported under this section. Before the experiments, a general flow diagram can be useful to understand the process more efficiently, and Figure 10. summarizes the general procedure.

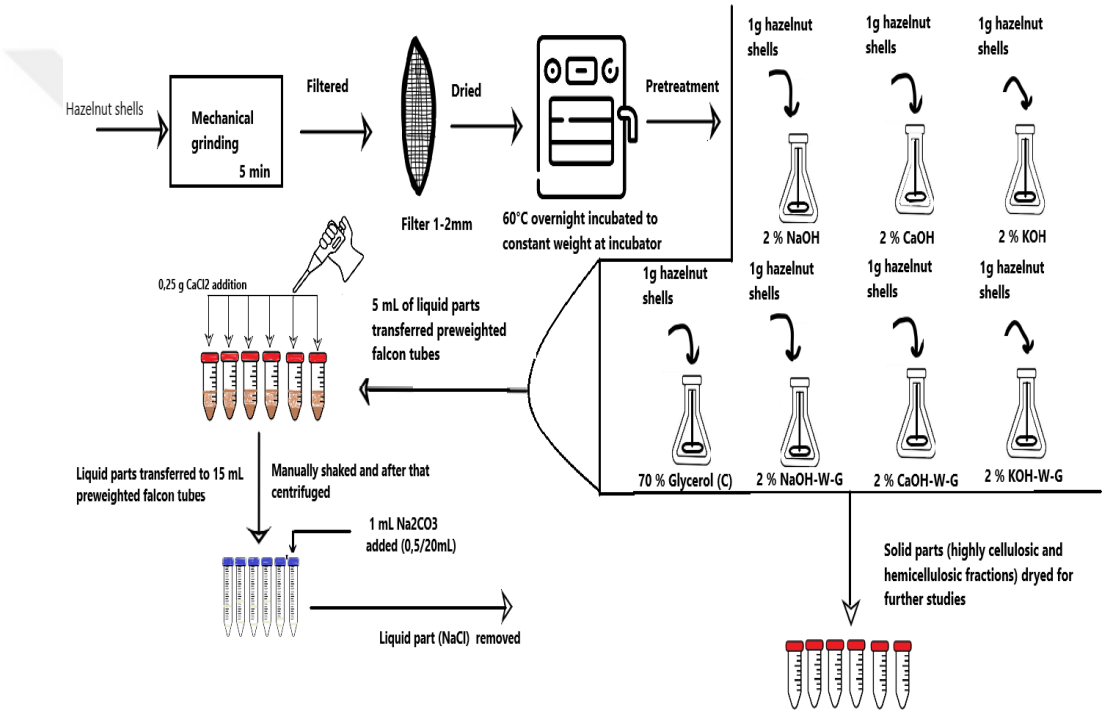


Figure 10. Flow diagram of performed procedure.

HS residues were provided by a local market and were ground with a cereal grinder. Chemicals NaOH, Ca $(OH)_2$, KOH, $CaCl_2$, Na_2CO_3 were purchased from Sigma-Aldrich (St. Louis, MO, USA). Fourier transform infrared spectroscopy (FTIR) analysis was performed via Nicolet Nexus 870, Thermo Fisher Scientific, Waltham, MA, USA. For effective incubation, Mikrotest MSC55 shaking and cooling incubator was used.

Table 3. Equipment used in this thesis.

Equipment	Model
Cereal grinder (for smash of HS)	Lavion HC100
Fourier transform infrared spectroscopy (FTIR)	Nicolet Nexus 870, Thermo Fisher Scientific
Heating magnetic stirrer	Daihan WiseStir MSH-20-A
High precision balance	ACU HJ-150
Microanalytical balance	SHIMADZU AP-225WD
Shaking and cooling incubator	Mikrotest MSC55-A
Orbital shaking incubator	HEIDOLPH Unimax 1010
Vacuum horizontal flow oven	DAIHAN WiseVen WOF-105
Tabletop centrifuge	Nüve NF800 and NF800R

2.1. Raw material and alkali solvents preparation

Hazelnut shells were smashed and sieved to a particle diameter of 1–2 mm and then were oven-dried at 60 °C to a reach constant mass. For effective alkali pretreatment and determination of effect of glycerol and water on the pretreatment lignin recovery (%) , NaOH, $Ca(OH)_2$, KOH are dissolved in water and water-glycerol (30:70 water: glycerol) solutions at total 2% of concentration; 2% NaOH-Water, 2% NaOH-glycerol solution, 2% $Ca(OH)_2$-Water, $Ca(OH)_2$-glycerol, KOH-Water, and KOH-W-G solutions are prepared and as a control group, 70% Glycerol solution was used.

2.2. Alkali pretreatment and optimization parameters

Pretreatment studies are carried out by the modified method of Chang et al. (2017). solvent ratio (w/w), temperature and operation time optimization parameters are operated at one variable at a time (OVAT). Dried 1 g of HS transferred to pre weighted 50 mL falcon tubes and increasing concentrations are described to 5:1, 10:1, 15:1 and 20:1 and these values equal to 0.1, 0.2, 0.3, 0.4 g NaOH g / g, $Ca(OH)_2$ g / g, KOH g /g dry mass respectively, while temperature constant at 60ºC and operation time 2 hours and Figure 11. shows the operation.

Shortly after the optimum solvent ratio determined for each different groups, (solvent with water and glycerol) ratio and operation time was set to 2 hours. After that optimum temperature optimization performed at increasing temperatures 20ºC, 40ºC, 60ºC. In the end, operation time was determined at 20ºC and 0.3 g solvent / g dry biomass.



Figure 11. Operation picture of alkali pretreatment in shaking incubator. (samples are labeled and noted for different varying parameters: temperature, operation time and solvent ratio)

2.3. Lignin recovery

Optimization was carried out, and optimum conditions were determined based on lignin recovery (%). Pretreated samples were centrifuged at 4100 rpm for 10 minutes, Figure 12. illustrates the samples after centrifugation. Immediately after centrifugation, the supernatant was transferred to new 50 mL falcon tubes, and 5 mL of each sample was transferred to pre-weighed 50 mL redcap falcon tubes. The pretreatment liquor was then weighed. Furthermore, 0.25 g of calcium chloride ($CaCl_2$) was added to these samples and thoroughly mixed manually to ensure it was homogeneously dissolved. Then, the samples were centrifuged once more at 4100 rpm for 10 minutes, as shown in Figure 13. The solid residue was washed with tap water and centrifuged 3 times at 4100 rpm for 10 minutes each time, then dried at 60°C overnight to reach a constant weight.

Supernatants were transferred pre-weighted 15 mL redcap bluecap falcon tubes, 1 mL of Na_2CO_3 (0.5 g / 20 mL prepared) added to determine unreacted $CaCl_2$ in the supernatants and they were measured by the sedimentation of $CaCO_3$ and reaction that occurred between $CaCl_2$ and Na_2CO_3:

$$CaCl_2 + Na_2CO3 \rightarrow CaCO_3 + 2NaCl \qquad (1)$$

This essentially indicates that 1 mole of $CaCO_3$ was formed while 1 mole of $CaCl_2$ was consumed and the excess sodium and chloride ions formed $NaCl_2$ at liquid phase and then subsequently, removed as a NaCl after 4100 rpm 5 minutes. Figure 12. shows the $CaCO_3$ formation in the bottom of falcon tube:

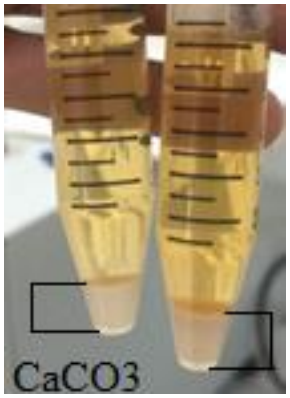


Figure 12. Visible $CaCO_3$ formation after adding Na_2CO_3 to glycerol (C) group.

Solid residue settled in incubator at 60ºC to reach constant weight and sedimented $CaCO_3$ weighted. After incubation, samples are weighted and lignin recovery (%) was determined by the following equation Eq. (2) (Chang et al.,2017).

$$Lignin\ Recovery\ (\%) = \frac{Lignin\ precipitate\ (g) - (0.25 - unreacted\ CaCl2) * 0.36}{Pretreatment\ Liquor\ (g)} x100 \qquad (2)$$

Where 0.36 is the molecular weight ratio of calcium ions (Ca^{2+}) to $CaCl_2$.

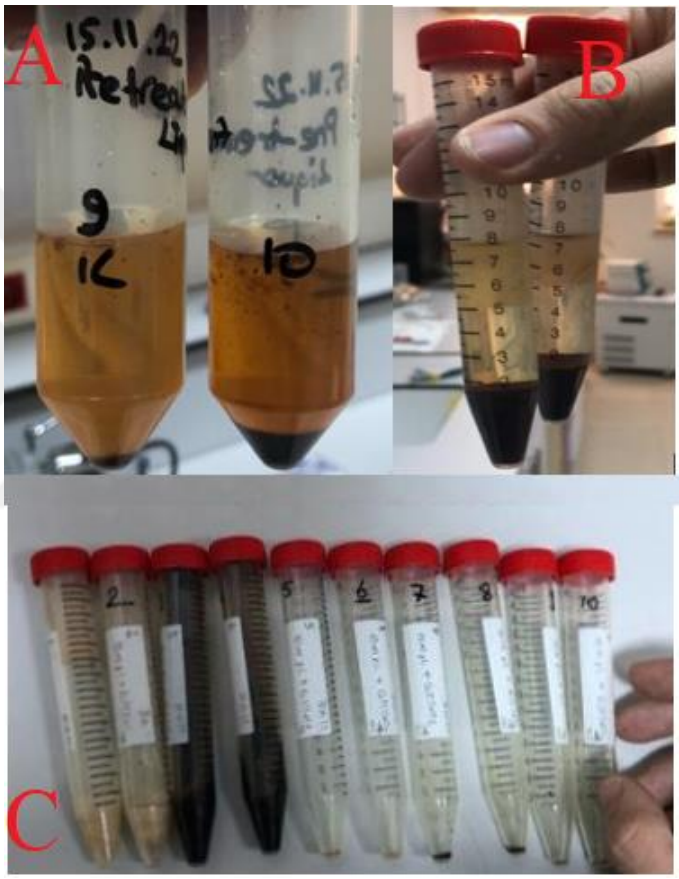


Figure 13. **(A)** Samples after pretreatment and centrifuge. (9) label is abbreviating the control groups (just glycerol) and (10) label is NaOH-W. **(B)** After $CaCl_2$ addition, the sedimentation of NaOH-W-G solution. Both falcon tubes contain NaOH-W-G, the recovery is considerably higher than other groups so stored and takes place in two falcon tubes. **(C)** Picture of samples dried overnight for lignin precipitate.

2.4. Fourier transform infrared spectroscopy

Fourier transform infrared spectroscopy was utilized to validate the lignin fractions. Recovered fractions of 200 mg lignin powder were pressed and placed on the probe. The analysis was performed in the range of 400 – 4000 cm^{-1} using 32 scans, with a resolution of 4 cm^{-1} for each of three samples (Figure 14.).

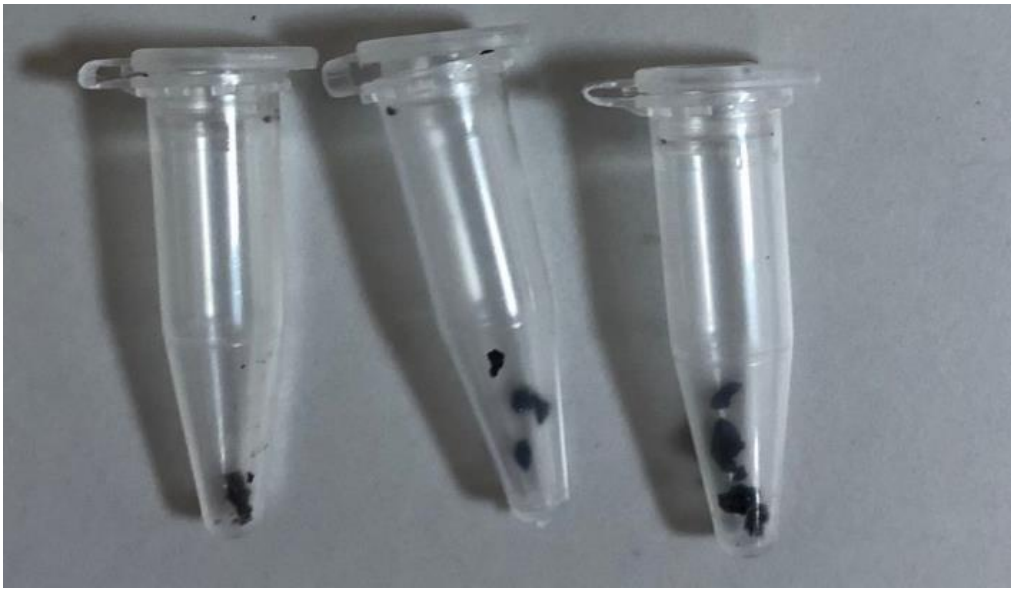


Figure 14. Recovered and overnight dried lignin fractions; one triplet.

2.5. Statistical Analysis

The experiments were carried out in duplicate, and statistical analysis was performed using one-way analysis of variance (ANOVA). The results are presented as means ± standard deviation, and statistically significant differences were defined at a significance level of $p < 0.05$.

SECTION THREE

RESULTS AND DISCUSSION

3.1. Effect of NaOH-W pretreatment conditions on lignin recovery (%) from hazelnut shells

This section comprehensively examines the effect of temperature, solvent ratio and operation time on three different alkali solvents. The subsequently, subsections begins with an investigation of NaOH, followed by $Ca(OH)_2$ and KOH pretreatments and effect of both glycerol and optimization conditions on lignin recovery.

3.1.1. Effect of increasing solvent ratio on lignin recovery of NaOH-W

The results indicated that, increasing the solvent ratio improves lignin recovery.0.1 g NaOH/ g dry biomass, lignin recovery was considerably low at 1.80% compared to 0.2 g NaOH / g dry biomass, 0.3 g NaOH / g dry biomass and 0.4 g NaOH / g dry biomass which 5.3%, 7.006%, 10.36% respectively (Figure 15.).

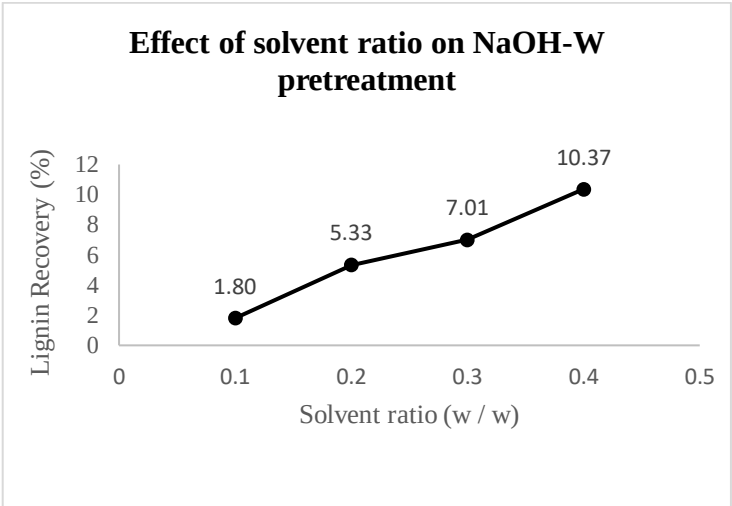


Figure 15. Effect of NaOH-W solvent ratio on lignin recovery.

These findings suggest that increasing concentration positively influences lignin recovery. Furthermore, it is likely that increase in solvent concentration could enhance lignin recovery, up to certain point. However, the use of solvent is known to

be a critical parameter for the feasibility and scalability of industrial scale production; thus, solvent usage must be carefully considered. Additionally, the environmental impact of chemical usage, particularly the formation of potential byproducts such as furfurals, is another significant concern (Wang et al.,2022).

Comparable studies conducted by Wang et al. (2023) applied 0.3 g NaOH/g dry mass, at 70°C for a 1.5-hour pretreatment on rice straw, resulting in the removal of approximately 10% lignin. Similarly, Harun & Geok. (2016) pretreated rice straw with varying 2%, 6%, 12% (w/v) NaOH at concentrations of 0.4, 1.2, 2.4 g NaOH / g dry biomass at 55ºC, 1-3 hours. Harun & Geok reported that the maximum delignification efficiency of 79.6 %, was achieved with 2.4 g NaOH / g dry biomass after a 1 h pretreatment. Furthermore, Gomes et al. (2021) applied NaOH pretreatment on imperial and colombina at varying concentrations of 0.4, 0.6, 0.8 g NaOH / dry biomass at increasing operation times of 30, 60, 90 min and they found that, at higher NaOH concentrations, lignin recovery decreased and regenerated up to 85-95%. Umikalson et al. (2018) treated oil palm empty fruit bunch fibers at different NaOH concentrations of 0.05 g / g and 0.2 g NaOH / g dry biomass and they reported that 0.2 g NaOH/ g dry biomass reached 63% lignin removal.

On the other hand, at lower alkali concentrations and operation temperatures (lower than 100°C), lignin is more likely to be affected, means that higher lignin recovery can be achievable. (Modenbach & Nokes, 2014). At higher concentrations, (higher than 6% of alkali solvent) morphological alterations began on the structure of lignin. (Eronen et al.,2009; Mittal et al.,2011).

3.1.2. Effect of temperature on lignin recovery (%) NaOH-W

The investigation into the impact of temperature on lignin recovery during NaOH-W pretreatment involved the assessment of three distinct temperature levels: 20°C, 40°C, and 60°C. Findings showed that exponential increase in lignin recovery from 2.25% to 10.37% as the temperature elevated from 20°C to 60°C (Figure 16.).

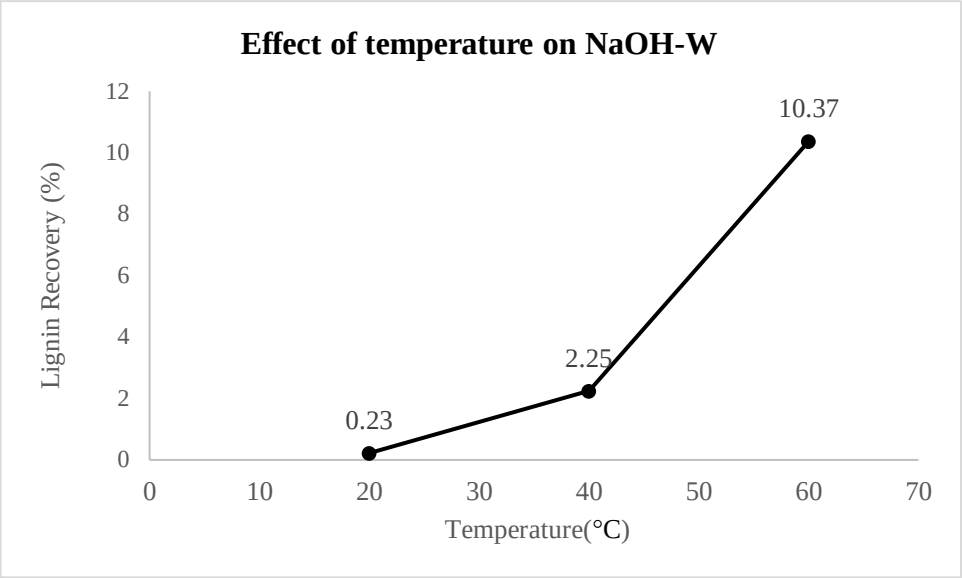


Figure 16. Effect of increasing temperature on lignin recovery.

This phenomenon can be attributed to the enhanced molecular interactions between alkyl ether bonds or aryl ether bonds and the NaOH-W solution at increasing temperatures, leading to the accelerated breakdown of the lignin carbohydrate complex (LCC). Menezes et al. (2014) demonstrated a maximum lignin recovery of 76.73% from coffee pulp under conditions of 30 minutes, 120°C, and a 6.37 w/v NaOH at 3.185 g NaOH / g dry biomass. Similarly, Umikalsom et al. (1998) conducted an alkali pretreatment process on oil palm empty fruit bunch fibers at a concentration of 0.5 NaOH (w/v); 0.05 g NaOH / g dry biomass and revealing that the absence of an autoclave process at 30°C for 4 hours produced no lignin. In contrast, the application of an autoclave (121°C, 15 psi for 5 minutes) under the same solvent and NaOH concentrations resulted in a 17% removal of lignin. This inference can be supported by Fuertez Córdoba et al. (2021) where a conducted series of investigations on alkaline delignification of LB at 40ºC and 70ºC were performed. Maximum lignin removal of 36.70% was achieved at 40ºC for African oil palm fiber, 34.95% for coffee pulp waste at 40ºC, 32.75% for sugarcane bagasse at 40ºC. However, although these values considerably higher than pretreatment of hazelnut shells, these studies primarily focused removal rather than recovery of lignin. Moreover, the different parameters also must be considered such as particle sizes. Fuertez Córdoba et al., (2021) conducted experiments with 0.25 mm particle size,

while in this thesis the particle size considerably higher 1-2 mm, thus directly affects pretreatment efficiency, alkali concentrations; different 0.1 g $Ca(OH)_2$ / g dry biomass and raw materials nature is different and certain dependence to these differences leads to lignin removal. For instance, Menezes et al. (2014) reported that alkali pretreatment on coffee pulp can vary between 20.74% minimum to 76.73% maximum. For maximum lignin removal, pretreatment operated at 2 mm fiber length, 0.05 g NaOH/g dry mass at 30ºC 4 hours. While adding the autoclave before treatment (121ºC, 15 psi for 5 min), the lignin removal decreased to 17 %. In addition, at lower operation temperatures, lignin is more affected that can be achievable higher lignin recovery (Modenbach & Nokes, 2014).

Finally, to investigate the effect of the following operation time on pretreatment, temperature was kept constant at 20°C. In normally, at OVAT method, every variable tested while the other parameters kept constant, and results of that experiments determine optimum conditions. While performing OVAT, parameters are usually optimized step by step and optimum desired value is considered. However, if there wasn't too much difference between lignin recovery, the possible outcomes; in this case lignin recovery increase by increasing temperature can be simulated by using lower temperatures; and this can be economically feasible in industrial scale.

3.1.3. Effect of increasing operation time on lignin recovery (%) NaOH-W

Experimental setups were conducted at constant temperature of 20°C, 0.3 g NaOH / g dry biomass and 150 rpm. The results indicate that an increase in operation time led to a lignin recovery up to 6,9-fold from 0.22% to 1.52% (Figure 17.). This increase is closely associated with the accessibility of NaOH to the hazelnut shells and the breakdown of ester, C-C C=C, bonds, alkyl ester, ether bonds, as well as some nucleophilic attacks to backbone of hazelnut shells. However, once the reaction reaches saturation, further increase of the operation time will result in only a small increase in lignin recovery percentage or even negligibly low. Similarly, Chang et al. (1998), conducted a series of experiments and optimized four different operation times: 1, 3, 6, 24 hours. The results indicated that increasing operation times led to enhanced lignin recovery with 14% of lignin removed at the 24 hours of pretreatment time.

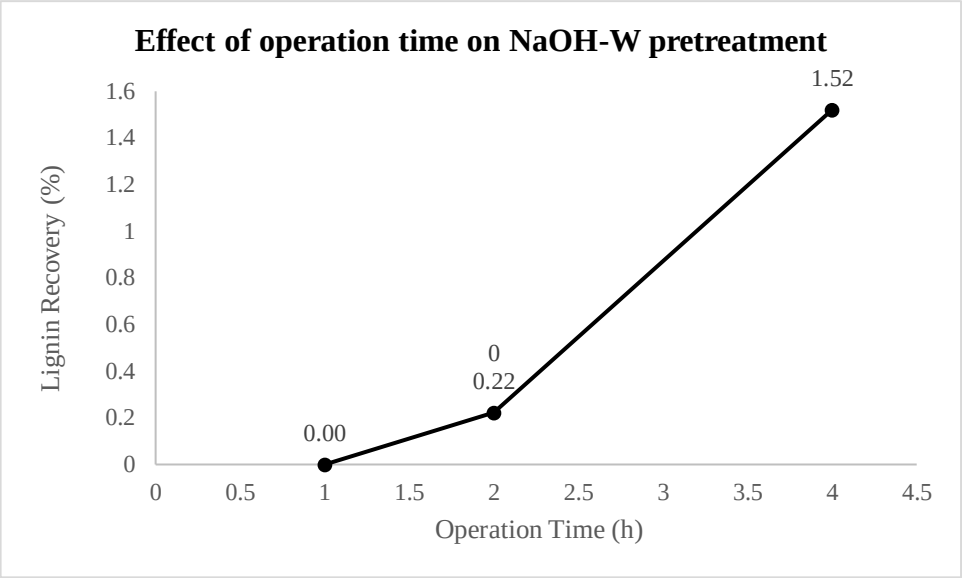


Figure 17. Effect of increasing operation time on lignin recovery (%) via NaOH-W pretreatment.

Guo et al. (2019) used NaOH pretreatment on tobacco stalks and varying alkaline pretreatment time 15, 30, 60, 90 minutes and found that maximum lignin removal at 60 minutes. However, in this case, there was no considerable lignin recovery observed due to the not observed flocculation, thus this is highly relatable with low temperature conditions (20ºC).

3.2. Effect of sodium hydroxide-glycerol (NaOH-W-G) pretreatment conditions on lignin recovery (%) from hazelnut shells

Effect of increasing solvent ratio, temperature and operation time on glycerol mediated NaOH pretreatment investigated in this section. The results showed that lignin recovery (%) increased in the presence of glycerol for NaOH pretreatment. This can be highly related to glycerol improved accessibility of NaOH to ester bonds of LCC thus improved lignin removal. In literature, Chen. (2010) showed that in the presence of high temperature (210°C) glycerol pretreatment for 1 hour could be appliable for lignin removal and glycerol and NaOH combined pretreatment could improve lignin recovery. On the other hand, NaOH completely dissolved in glycerol

70% (rest of water) this is highly endowing the stronger of NaOH solution then solubility of lignin improved (Rodrigues et al., 2016).

Hassan & Badri (2014) investigate the lignin recovery from alkali hydrolysis and glycerolysis of oil palm fiber with 10% NaOH solution, 20 g NaOH / g dry biomass for 48 h. Immediately after, 70% glycerol solution catalyzed using 5% (w/v) 1 M NaOH for 3 h at 600 rpm constant stirring. The results showed that, up to 16% total of klason lignin and lignin in filtrate was recovered.

3.2.1. Effect of solvent ratio on lignin recovery (%) of NaOH-W-G treatment

Results indicate that increasing concentrations of solvent over dry weight increased lignin recovery (%) on HS (Figure 18.). Maximum lignin recovery 14.34% at 0.3 g NaOH / g dry biomass, and after this point, increase in ratio of solvent resulted as slightly decreased to 13.96 (%) lignin recovery at higher concentration 0.4 g NaOH/ g dry biomass. This can be highly related to lignin removal by reaching their limit with using NaOH-W-G pretreatment. Glycerol possessed a positive effect on lignin recovery when compared with NaOH-W. Maximum lignin recovery (%) is 10.37 (%) at 60ºC 2 h 0.3 g NaOH / g dry biomass. This is highly related to glycerol addition improved viscosity, density of solvent and this enabled the retention time of NaOH with LCC complex thus increased interaction time NaOH with LCC then more lignin removed. Figure 19. and Figure 20. express the obtained results for different solvent ratios at constant temperature and operation time. The lowest lignin recovery was obtained at 0.1 g / g ratio with 8.72% and even compared with NaOH-W, the effect of glycerol was considered 1.81% (NaOH-W) to 8.72% (NaOH-W-G). However, in NaOH-W, the increase is sharper than NaOH-W-G; at NaOH-W about 5.37-fold increase observed at 0.1 g / g to 0.4 g / g while NaOH-W-G only 1.6-fold increase observed 0.1 g / g to 0.4 g / g. Also, water combined NaOH pretreatment seems to be able to obtain higher lignin recovery rates when increased solvent ratio but this time economical feasibilities must be considered because increase in solvent directly leads to increased operation cost. On the other hand, glycerol combined NaOH pretreatment seems to reach saturation of maximum lignin recovery and increasing solvent ratio probably does not increase lignin recovery % drastically.

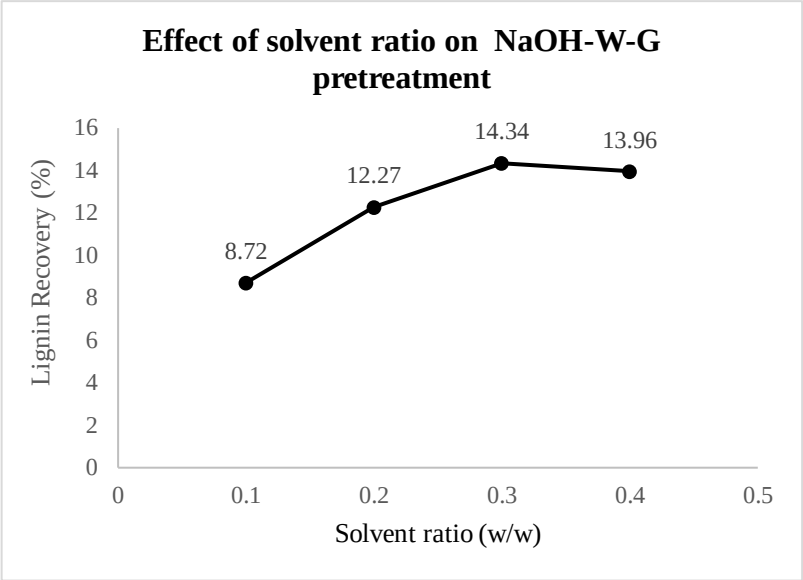


Figure 18. Effect of increasing solvent ratio on lignin recovery (%) via NaOH-W-G pretreatment.

In literature, crucial studies have been conducted to determine the effect of increasing concentration of NaOH. Gomes et al. (2021) investigated the effect of increasing concentrations of NaOH pretreatment however, their increase method was slightly varied. They were investigating 2-8 %(w/v) NaOH-water solutions prepared at constant solvent ratio 0.2 g NaOH / g dry biomass on two different brewer's grain. They found out that at higher concentrations of NaOH; 0.8 g NaOH / g dry matter, lignin removal increased approximately 95.5% for imperial brewer's grain.

Chatkaew et al. (2021) is an alternative group that investigated the effect of increasing solvent concentration on NaOH pretreatment. Their finding similarly showed that, up to a certain point, lignin removal was enhanced. Experiments revealed that the maximum lignin recovery was achieved at a solvent concentration of 2.17 M using RSM. The results also suggest that increasing solvent concentration enhances lignin recovery. Moreover, Chen et al. (2013) conducted experiments on corn stover for improve enzymatic saccharification via NaOH alkali pretreatment. The investigation consists of different concentrations of NaOH addition from 0.06 to 0.10 g NaOH / g corn stover and highest lignin removal obtained at 0.1 g NaOH / g corn stover loading approximately 80% of lignin removal. They also added that, the main reactions that play a crucial role in lignin removal was cleavage of α and β-

ether bonds in phenolic groups and β-ether linkages in non-phenolic units. In these reactions, NaOH particularly attacks C1 and/ or C2 hydroxyl groups of monosaccharide rings, hydroxyl groups and free phenolic hydroxyl groups at α, β and γ positions in lignin monomers (Chen et al., 2013).

3.2.2. Effect of temperature on lignin recovery of NaOH-W-G pretreatment

In the scope of this thesis, it was found that the effect of temperature on the glycerol mediated NaOH pretreatment positively influenced lignin recovery. Maximum lignin recovery was achieved at 60ºC with 14.34% at 0.3 g/g solvent ratio at 2 h operation time (Figure 19.). The combination of glycerol and NaOH was improved lignin removal and results also support this inference. Compared with water dissolved NaOH treatment, the lignin recovery increased 10.37% to 14.34% this can be related with high viscosity of glycerol enables that higher retention time with NaOH and LCC complex and glycerol should be changing the composition of HS fibers then while enhancing the cleavage of hemicellulose especially xylans related lignin fractions.

Chen et al. (2013) highlighted that temperature was the second most important parameter affects hydrolysis conversion and lignin removal. They set a set of experiments that investigate temperature, solvent concentration ratio, operation time of alkali pretreatment on corn stover, and the temperature variables are between 90-120ºC, delignification improved by increasing temperature. However, it seems severe pretreatment conditions can lead to condensation reaction of lignin thereby formation of carbon-carbon bonds between lignin subunits which limits the removal and reduce glucan/xylan conversion (Pan et al., 2004; Chen et al.,2013).

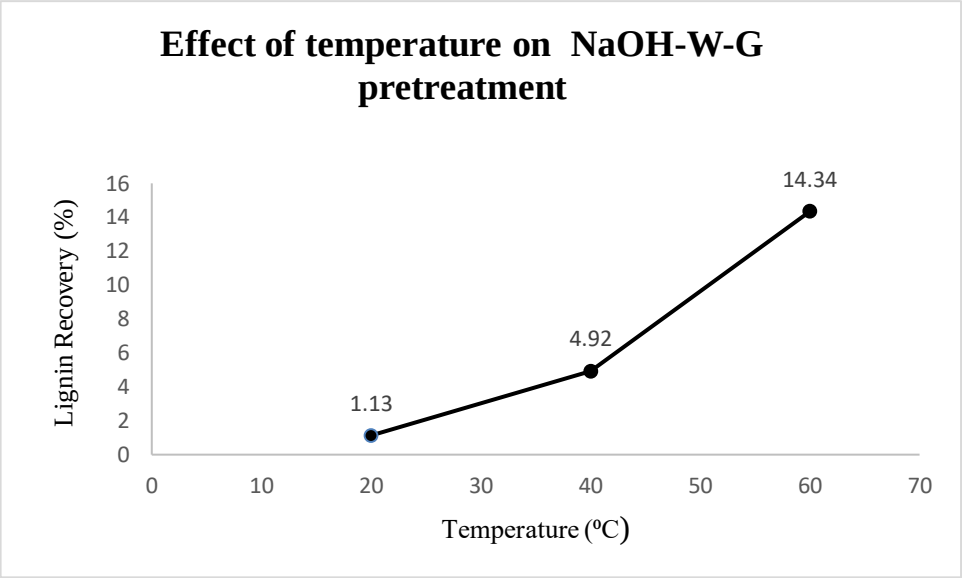


Figure 19. Effect of increasing temperature on lignin recovery (%) via NaOH-W-G pretreatment.

3.2.3. Effect of operation time on lignin recovery (%) of NaOH-W-G pretreatment

Maximum lignin recovery obtained at 4 hours with 9.99%. At an hour of operation time, there was no significant lignin precipitation observed by adding $CaCl_2$ (Figure 20.). This can be related with the NaOH pretreatment conditions didn't satisfy, NaOH retention time was not enough for interaction and breakage of α and β ether bonds thus there was no significant recovery observed. On the other hand, maximum recovery obtained at 4 hours and about ~8.55-fold increased lignin recovery of 1.13 to 9.99%. This is closely associated with the improved accessibility to NaOH-W-glycerol, which facilitates increased delignification of lignin. Simultaneously, the accessibility of $CaCl_2$ for flocculation is also facilitated. Similarly, Guo et al. (2019) studied tobacco stalks with alkali NaOH pretreatment, investigate reaction time, alkali concentration and temperatures on saccharification. Results indicate that increased operation time (15 to 90 minutes) at 0.1 g NaOH / g dry biomass concentration resulted in increased lignin removal.

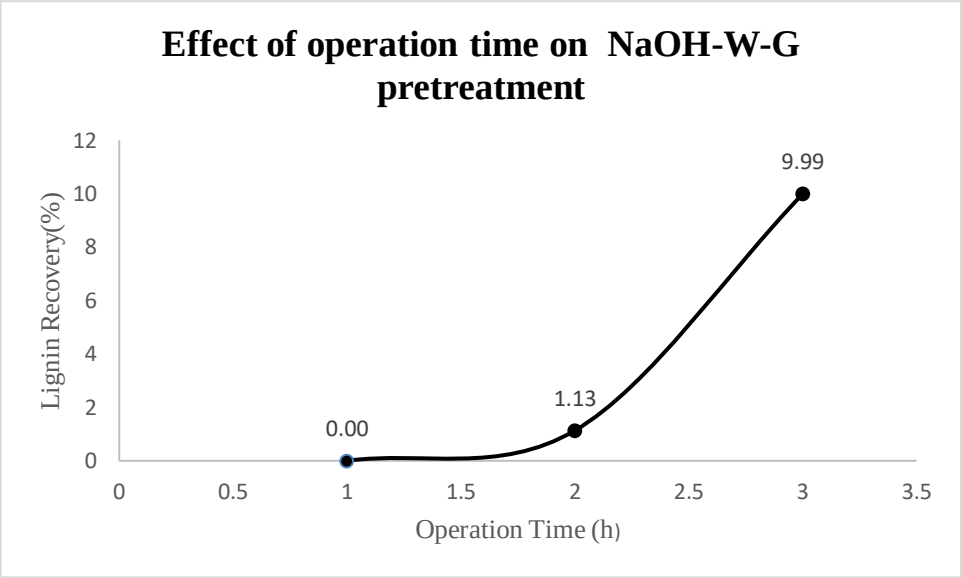


Figure 20. Effect of increasing temperature on lignin recovery (%) via NaOH-W-G pretreatment.

3.3. Ca $(OH)_2$ pretreatment effect on lignin recovery (%) from hazelnut shells

$Ca(OH)_2$ pretreatment also known as lime pretreatment first studied by Chang et al. (1997) and investigated conditions are varied between 60-130 °C, 2 hours of operation time and 0.1 g $Ca(OH)_2$ / g dry biomass. In this section, similar to NaOH solvents, 0.1 g/g, 0.2 g/g, 0.3 g/g and 0.4 g $Ca(OH)_2$/ g dry biomass and operation time 1, 2, 4 hours, 20-60 ºC investigated for $Ca(OH)_2$ and effect of glycerol on calcium hydroxide pretreatment investigated. The results are showed that the lignin recovery by using $Ca(OH)_2$ pretreatment considerably lower when compared with NaOH pretreatment and effect of glycerol on $Ca(OH)_2$ is also directly opposite with NaOH. This can be concluded that increased viscosity depending on the glycerol, limited mass transfer of $Ca(OH)_2$ pretreatment and negatively affect the accessibility of $Ca(OH)_2$ bond breaking of acyl groups. $Ca(OH)_2$ dissolved in glycerol highly turbid which can impede mass transfer and hinder reaction between $Ca(OH)_2$ and lignin, lignin related complexes (Ming Xian Chang et al., 2017).

3.3.1. Effect of solvent ratio on lignin recovery of Ca (OH)2-W pretreatment

Maximum lignin recovery was achieved 0.4 g $Ca(OH)_2$ / g dry biomass (Figure 21.). Similar to NaOH-W and NaOH-W-G pretreatment, increasing concentrations of $Ca(OH)_2$ concentrations, leads to increased levels of lignin recovery. However, in this case the increase of lignin recovery (%) sharply increased 0.1 g/g to 0.3 g/g from 2.84 to 8.33% about ~ 2.95-fold and increases between 0.3 g/g to 0.4 g/g considerably low; just 8.33% to 8.88% (Figure 21.). This can be noted that in larger scales, by considering multiple times of feasibility, 0.3 g $Ca(OH)_2$ / g dry biomass seems more feasible but be useful to note that feasibility reports must be reconsidered multiple times. This can be highly related to during pretreatment, uncontrollable and simultaneous reactions should be occurred between lignin complexes and calcium ions and similar results are obtained by Xu et al. (2014). In addition, when compared with NaOH-W pretreatment, $Ca(OH)_2$ pretreatment have unique advantage that can be change the cards: by injection CO_2 to solution, $Ca(OH)_2$ can be recovered from system as a form of calcium carbonate ($CaCO_3$) (Park et al., 2010; Rodrigues et al., 2016). Furthermore, Chang et al. (1998) performed $Ca(OH)_2$ pretreatment on crop residue bagasse and wheat straw, showed that mainly 18.93% of lignin pretreated and washed away 4.89% percent of lignin, totally 0.22 g / g raw material became solubilized. Additionally, they demonstrated that at higher concentrations of Ca $(OH)_2$ loadings, specifically higher than, 0.1 g $Ca(OH)_2$ per gram of dry biomass, the increase in lignin removal is negligibly low. This observation is consistent with our findings. In final, 86% of the added calcium was removed from the bagasse by ten washing and could be recovered by carbonating the water with CO_2 at 9.5 pH.

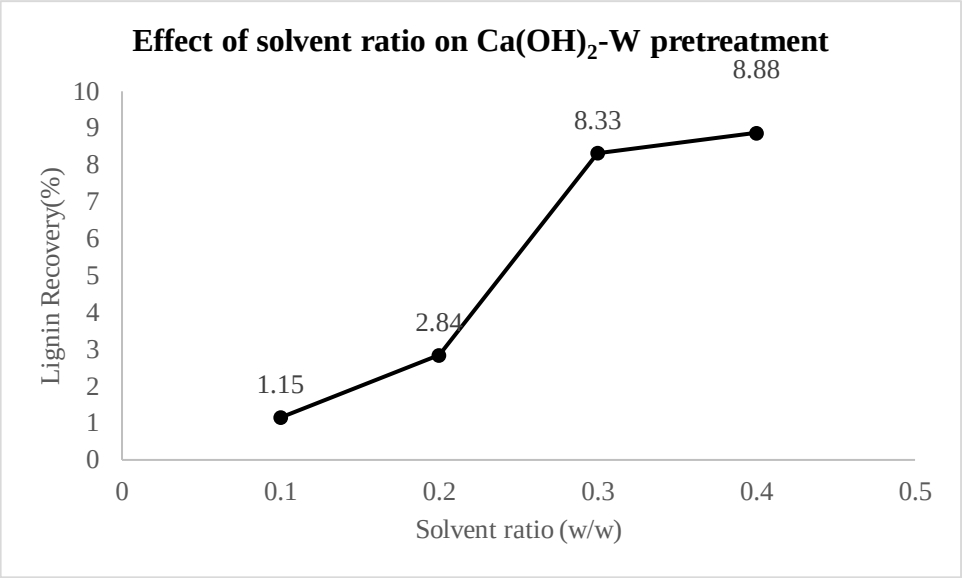


Figure 21. Effect of solvent ratio on calcium hydroxide water pretreatment.

3.3.2. Effect of temperature on lignin recovery of Ca $(OH)_2$-W pretreatment

The maximum recovery rate was obtained at 60ºC, which is 8.33% about 3.95-fold of 40ºC value: 2.11% (Figure 22.). Minimum lignin recovery was obtained at 20ºC, drastically lower than other temperatures; approximately 0.02% and this indicates that temperature is a very important parameter for lignin recovery of $Ca(OH)_2$-W pretreatment. This should be highly related to increased temperature leads to the atomic structure more energetic and availability of $Ca(OH)_2$ to reach lignin related carbohydrate complexes, then lignin removal improved and by the addition of $CaCl_2$ flocculation of lignin particles occurred, lignin recovery increased. On the other hand, according to Fuertez Córdoba et al. (2021), which investigate the effect of NaOH and $Ca(OH)_2$ pretreatments on different types of LB's and found that increased temperature augments the lignin removal. While doing that, their results showed that increasing temperature with increasing operation time can negatively impact the saccharification efficiency due to the inhibitors formation from phenolic groups. Thus, this can affect lignin removal; structure of lignin can be degraded, and then phenolic groups formed. In the final, they obtained maximum lignin recovery at 40°C, approximately 36.79%.

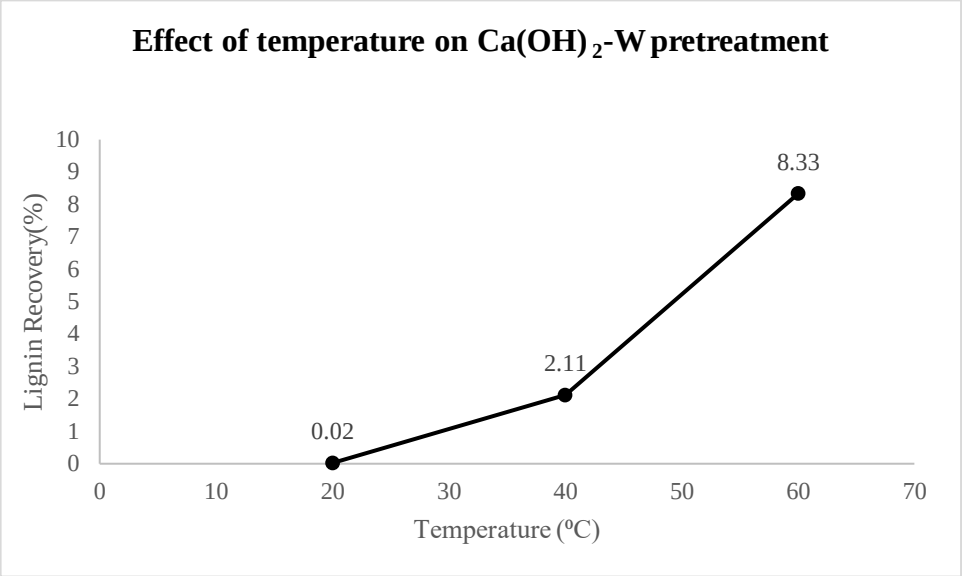


Figure 22. Effect of temperature on calcium hydroxide-water pretreatment.

3.3.3. Effect of operation time on lignin recovery of Ca $(OH)_2$-W pretreatment

In this thesis it was found that the maximum lignin recovery was achieved after 4 hours of operation time, with a value of 5.75% (Figure 23.). In $Ca(OH)_2$-W pretreatment, there is no flocculation of lignin particles was observed in falcon tubes at an hour operation. Depending on the pretreatment conditions, insufficient lignin solubility leads to lignin recovery to zero, similar results also achieved other pretreatment conditions at 20 ºC 1 h. On the other hand, increasing the operation time to 2 hours resulted in a slight increase in lignin recovery 0.02% which is also insufficient. However, after couple of hours, at 4 h, the lignin recovery drastically (compared to 0.02%) increased to 5.75% and considerably higher than at other operation times. In addition, compared with NaOH-W pretreatment, the increasing operation time have more sharper increases from 0.02% to 5.75% this accelerated increase is drastically higher than NaOH-W and NaOH-W-G and since it was, it can be concluded that operation time have greater influence on calcium hydroxide pretreatment.

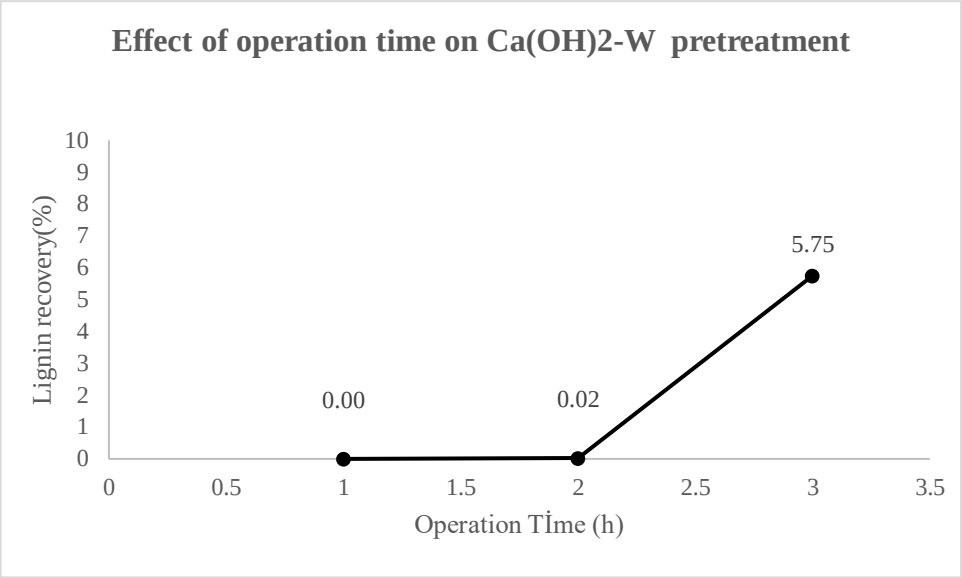


Figure 23. Effect of operation time on calcium hydroxide-water pretreatment.

3.4. $Ca(OH)_2$-W-G Pretreatment and effect on lignin recovery (%)

The effect of glycerol on lime pretreatment was investigated. The results indicate that increasing the concentration of $Ca(OH)_2$ leads to a significant increase in lignin recovery up to a certain point. However, beyond this point, while lignin recovery continues to increase, the rate of increase slows down. Increasing temperature leads to more lignin recovered. Similarly, increasing operation time leads to increased lignin recovery.

3.4.1. Effect of solvent on lignin recovery of $Ca(OH)_2$-W-G pretreatment

Increasing solvent ratio leads to increased lignin recovery (Figure 24.). Lignin recovery (%) ratios were obtained at 0.1, 0.2, 0.3, 0.4 g $Ca(OH)_2$ / g dry biomass to 0.97%, 2.72%, 3.10%, 3.36% respectively. The maximum lignin recovery achieved was 3.36%. If glycerol-assisted lime pretreatment is to be implemented in larger-scale productions, 0.3 g $Ca(OH)_2$ / g dry biomass and even 0.2 g $Ca(OH)_2$ / g dry biomass may be more economically feasible. On the other hand, when compared with $Ca(OH)_2$-W pretreatment, the effect of glycerol obtained negative impact,

highly dependent to solubility of $Ca(OH)_2$. $Ca(OH)_2$ forms a highly turbid liquid in glycerol, thus should be hinder/limiting mass transfer of calcium hydroxide to environment and lead to negative effect of lignin recovery (%). Controversially, Wang et al. (2017) performed lignin recovery on sugarcane bagasse and investigate NaOH and $Ca(OH)_2$ combined glycerol pretreatment, and they found that glycerol has negative impact on NaOH and positive impact on $Ca(OH)_2$. However, eventually they also support that effect of glycerol hinder reaction between solvent and lignin. In Figure 24, the increased lignin recovery as a result of increased solvent ratio was illustrated. Moreover, it can be concluded that the pretreatment with Ca $(OH)_2$-W had shown more lignin recovery than the one with Ca $(OH)_2$-W-G.

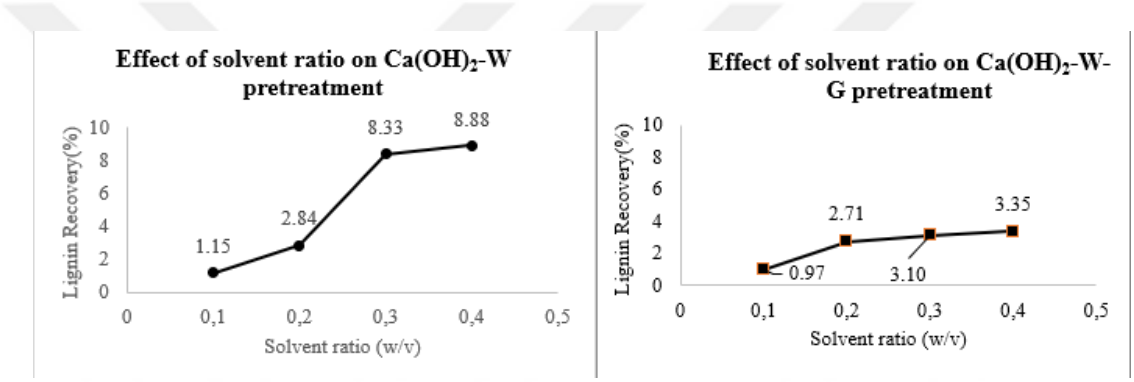


Figure 24. Effect of glycerol addition to $Ca(OH)_2$ pretreatment.

Interestingly, both recovery values are slightly increased between 0.3 g/g and 0.4 g/g 8.33 to 8.88% for $Ca(OH)_2$-W and 3.10 to 3.36 $Ca(OH)_2$-W-G, respectively. This can be concluded that, in larger scales the use of higher concentrations of solvent wouldn't be feasible thus by decreasing use of less chemicals can be more beneficial for economical perspective.

3.4.2. Effect of temperature on lignin recovery of Ca $(OH)_2$-W-G pretreatment

The results showed that, similar to Ca $(OH)_2$-W pretreatment, increasing temperature leads to increased lignin recovery from 0.58% to 3.10% (Figure 25.).

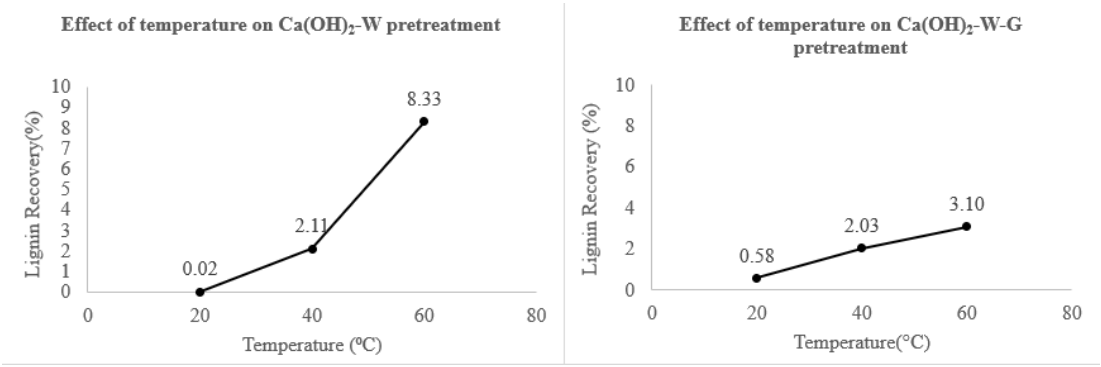


Figure 25. Comparison of the effect of glycerol on temperature of $Ca(OH)_2$ pretreatment.

Compared to $Ca(OH)_2$-W pretreatment, the increase of temperature leads to a more stable increase, also at 20ºC, the lignin recovery a way higher than 20ºC of $Ca(OH)_2$-W pretreatment. It seems that the glycerol addition at lower temperatures can improve lignin recovery for calcium hydroxide pretreatment. However, the maximum total lignin recovery of $Ca(OH)_2$-W-G was considerably lower than $Ca(OH)_2$ pretreatment 3.10 and 8.33 (%) respectively. This can be concluded that the higher viscosity of glycerol and very turbid liquid of $Ca(OH)_2$-W-G solution, negatively affect the mass transfer thus lignin recovery of $Ca(OH)_2$-W.

3.4.3. Effect of operation time on $Ca(OH)_2$-W-G

Operation time is another important parameter for improving lignin removal and lignin recovery. Results showed that, at 20°C 1 hour of operation time, these conditions were not satisfied for lignin recovery from hazelnut shells. In both pretreatments; $Ca(OH)_2$-W and $Ca(OH)_2$-W-G, the lignin recovery increased by approximately 0.02% for $Ca(OH)_2$-W and 0.58% for $Ca(OH)_2$-W-G pretreatment between 1 and 2 hours of operation time (Figure 26.). Lignin recovery after 2 h was higher than $Ca(OH)_2$-W and increased more exponentially, however after 2 hours, the increase in $Ca(OH)_2$-W drastically increased when compared with $Ca(OH)_2$-W-G from 0.02% to 5.75% and this can be related with higher viscosity of glycerol negatively affect the attack of $Ca(OH)_2$ to acyl bonds and then lignin removal and recovery affected. In depth, the operation time and temperature have controversial relationship; for instance, Menezes et al. (2014) operated at $Ca(OH)_2$-W pretreatment of coffee pulp at autoclave conditions; 121ºC, 5 psi and varying operation times and found that at 30 minutes of operation time, lignin removal was 23.27% and at 25

minutes, was 39.92% at 6% $Ca(OH)_2$ and 6% NaOH concentrations. However, while at 6.37% $Ca(OH)_2$ and no NaOH combination at 30 minutes, lignin removal ramped to 79.26%. This can be concluded that, at higher concentrations and higher operation times, the effect of $Ca(OH)_2$ pretreatment ramped up in terms of lignin recovery in coffee pulps. However, the initial lignin content is very important for effect of pretreatment and sometimes even with increase in temperature and operation time, the lignin removal wouldn't increase due to the structure of lignin potential differential on lignin structure.

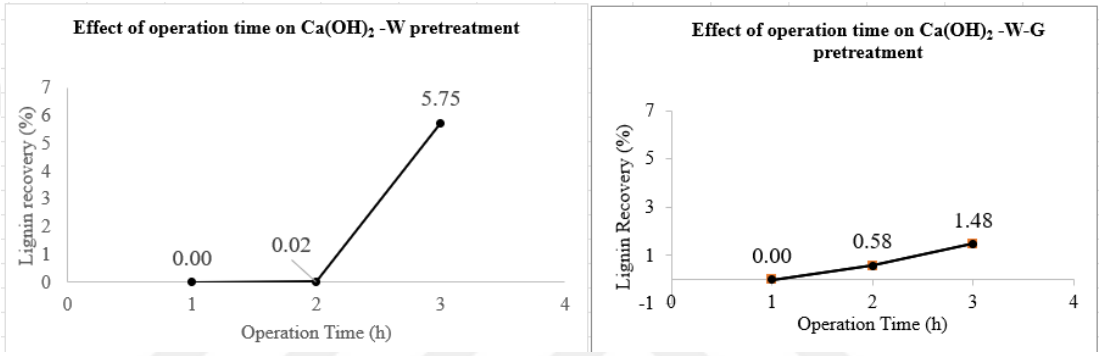


Figure 26. Comparison of the effect of glycerol on operation time of $Ca(OH)_2$ pretreatment.

3.5. Effect of potassium hydroxide – water (KOH-W) pretreatment on lignin recovery from hazelnut shells

The lignin recovery from HS by using KOH pretreatment and effect of temperature, operation time and solvent investigated, also effect of glycerol investigated.

3.5.1. Effect of solvent ratio on KOH-W pretreatment

Same concentrations applied for KOH pretreatment; 0.1, 0.2, 0.3, 0.4 g KOH / g dry biomass. Results are shown in Figure 27. and minimum lignin recovery

obtained at 0.1 g/g ratio as an 8.02%. Increasing solvent ratio up to 0.3 g/g, lignin recovery systematically increased to maximum value 9.97% and after increasing concentrations, the lignin recovery slightly decreased to 8.70% at 0.4 g/g ratio. This can be concluded that higher concentrations of KOH should negatively impact lignin removal and recovery, thus having negative influence on economic feasibility. Liu et al. (2019) also found similar results; increasing KOH loads positively leads to the higher lignin reduction from wheat straw and maximum lignin reduction was observed at 54.7% in 50% w/w dry basis of KOH loading.

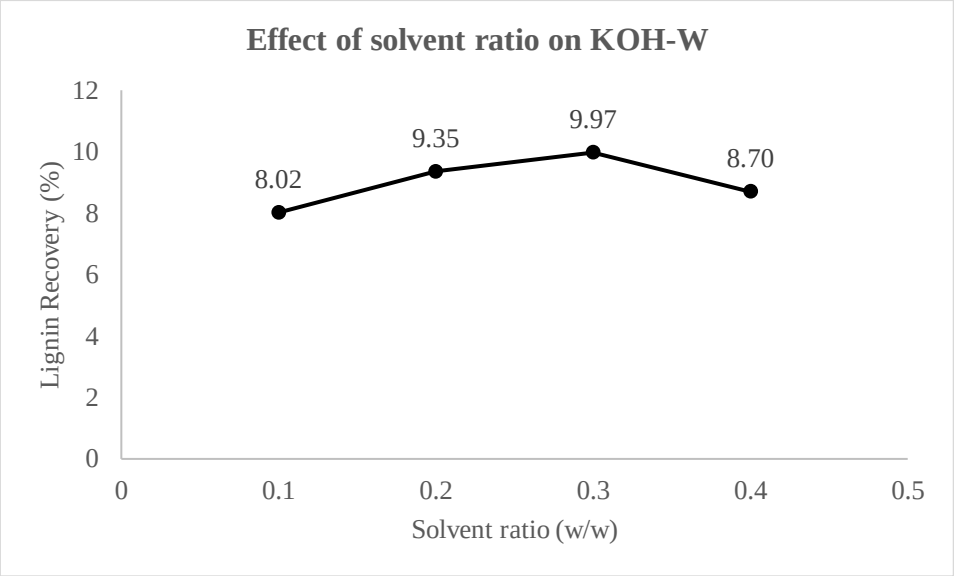


Figure 27. Effect of increasing solvent ratio on potassium hydroxide pretreatment of hazelnut shells.

3.5.2. Effect of temperature on KOH-W pretreatment

Results showed increasing temperature led to the increased lignin recovery (%). Minimum lignin recovery (%) was observed at 20ºC, as a value of 0.70%. The temperature increase from 20ºC to 40ºC, leads to lignin recovery 0.70% to 1.06%. At 60 ºC, maximum lignin recovery obtained 9.97% (Figure 28.).

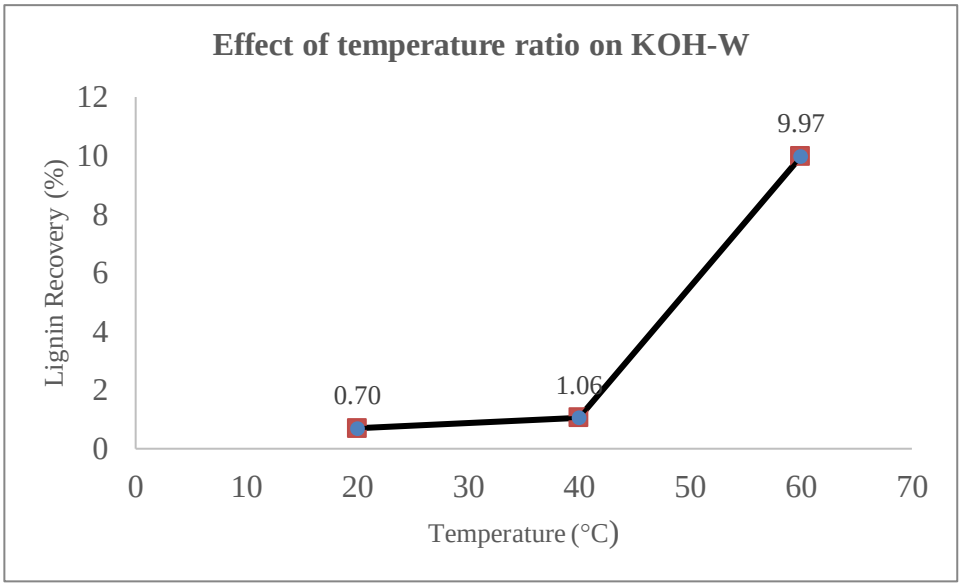


Figure 28. Effect of increasing temperature on potassium hydroxide pretreatment of hazelnut shells.

The increase between 40ºC to 60ºC is considerably higher than 20ºC to 40ºC. Increased temperature exponentially improves lignin recovery between 40 to 60ºC and. The following studies support the findings of the effect of temperature on KOH pretreatment. For instance, Bensah et al. (2015) was investigated the effects of KOH pretreatment on different types of West African biomasses and especially for bamboo. They found that the elevated temperature in alkali conditions directly leads to increase of lignin dissolution, on the other hand, hemicelluloses, ashes, and accumulation of glucan also augmented.

3.5.3 Effect of operation time on KOH-W pretreatment

The positive effect of operation time was observed in KOH-W pretreatment. Results indicated that at 1 hour of operation time, lead to undetectably lower amounts of lignin fractions (Figure 29.). At 2 hours of KOH-W pretreatment, the lignin recovery increased to 0.70% and at 4 hours, maximum lignin recovery obtained 1.84%. Similar results obtained Oruganti et al. (2023), conducted kraft lignin recovery experiments via KOH-W pretreatment, while optimizing optimal concentrations, temperature and operation time using response surface methodology.

Investigated effect of 6.36, 20, 40, 60 ,73.63 mins of pretreatment and maximum lignin recovery obtained between 40 60 mins (51.89 mins) approximately 31.3%. However, compared with our findings, the pretreatment times considerably higher and lignin recoveries considerably lower. This is highly related to other parameters: concentration of KOH and high temperature conditions highly affects lignin recovery efficiency, Oruganti et al. (2023) conducted experiments between 80ºC and 160ºC at 0.8 g KOH / g dry biomass.

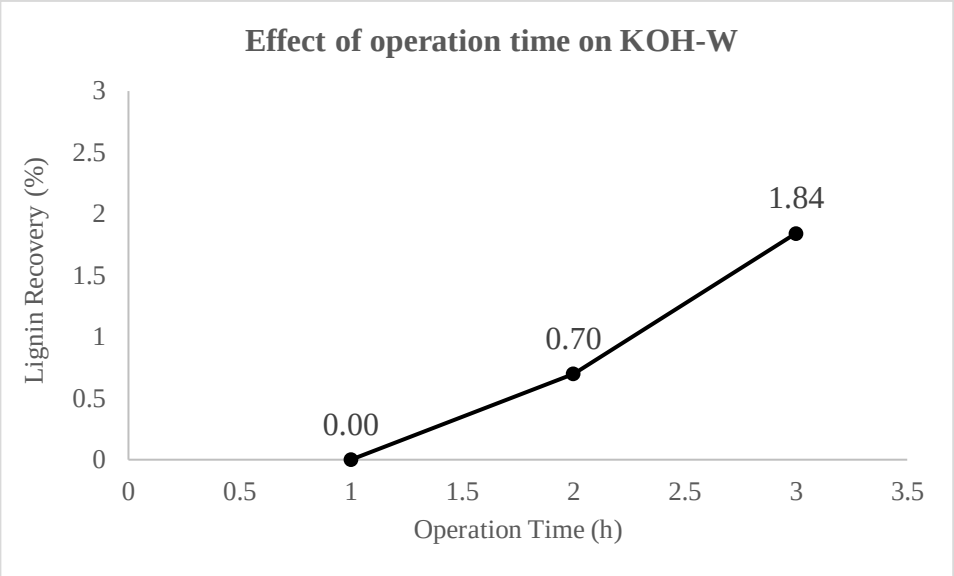


Figure 29. Effect of increasing operation time on KOH-W pretreatment.

3.6. Effect of potassium hydroxide – glycerol (KOH-W-G) pretreatment on lignin recovery from hazelnut shells

In this section, the effect of glycerol on potassium hydroxide pretreatment was investigated. Similar to other pretreatment conditions, three different parameters were considered: solvent ratio, temperature and operation time. The results revealed that, effect of glycerol addition has negative impact on the lignin recovery.

3.6.1 Effect of solvent ratio on KOH-W-G pretreatment

Four different solvent ratios were considered: 0.1 g / g, 0.2 g/g, 0.3 g/g, 0.4 g KOH / g dry biomass. The minimum concentration of KOH-W-G, 0.1 g/g, resulted in a lignin recovery of approximately 1.09%, which was lower compared to other KOH-W-G concentrations (Figure 30.). At 0.2 g/g, lignin recovery increased by approximately ~2.2-fold relative to 0.1 g/g, reaching 2.32%. Further increasing the concentration to 0.3 g / g led to a slight rise in lignin recovery from 2.32% to 2.76%, with the maximum recovery observed at this concentration (Figure 30.). However, after the 0.3 g/g, the concentration ramped to 0.4 g/g, and this time the lignin recovery suffered and decreased to 2.15%. Similar results are obtained Chi et al. (2019), which conducted a study for investigate effect of potassium hydroxide dosage on corncob residue for enhance enzymatic hydrolysis and the increase of dosage of KOH up to 1.6 g/g leads to 89.4 ± 0.5% delignification at 70ºC and at 90ºC, 96.1 ± 0.4 respectively and after the ramped increase in KOH loads, the lignin recovery decreased.

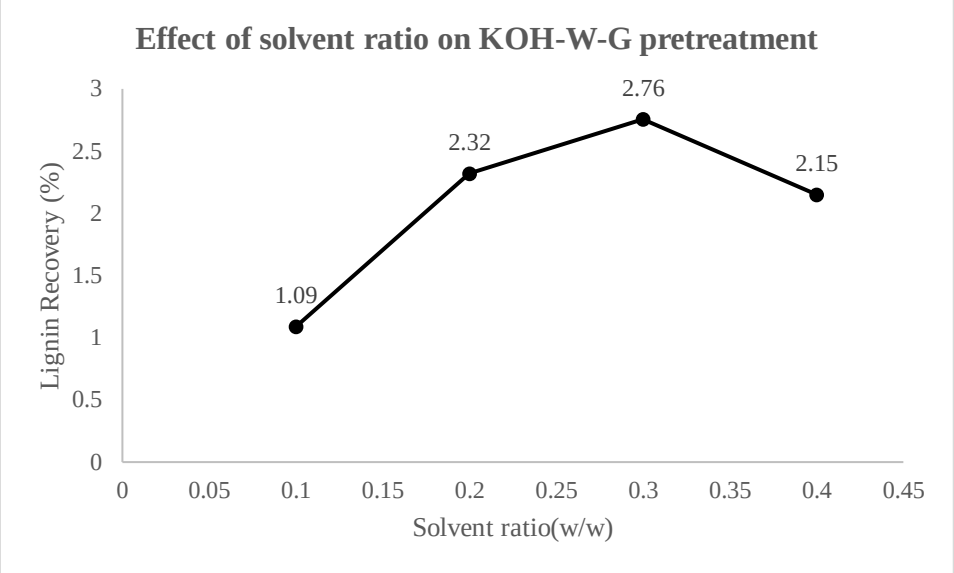


Figure 30. Effect of increasing solvent ratio on glycerol mediated potassium hydroxide pretreatment.

3.6.2. Effect of temperature on KOH-W-G

Three different temperature values (20ºC, 40ºC and 60ºC) were investigated and the results were shown in Figure 31. Maximum lignin recovery received at 60ºC with 2.75%. At 20ºC and 40ºC conditions, lignin recovery obtained 1.76% and 2.55%. Lignin recovery rates between 40°C and 60°C did not show significant difference: 2.55% and 2.75% respectively. Compared with KOH-W pretreatment, increase between 20°C and 40°C comparably higher; 0.70% to 1.06% in KOH-W and 1.76% to 2.55%. However, at 60°C, lignin recovery of KOH-W considerably higher than KOH-W-G, which 9.97% and 2.75% respectively. Higher viscosity of solution should be affecting lignin recovery. In higher viscosities of pretreatment liquor, can hinder effective mixing, limiting the accessibility of solvent to lignin related complexes (Asghar et al., 2017; Bimestre et al., 2022; Hernández-Beltrán et al., 2019).

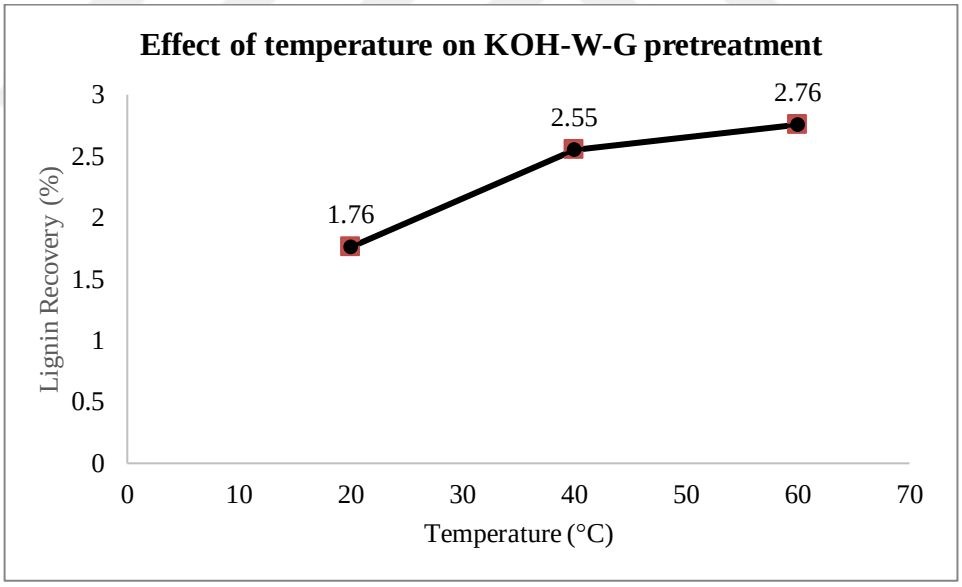


Figure 31. Effect of temperature on glycerol mediated potassium hydroxide pretreatment.

3.6.3 Effect of operation time on KOH-W-G

In this thesis, increasing operation time leaded to improved lignin recovery (Figure 32.). Maximum lignin recovery was achieved at 4 hours of pretreatment with a value of 2.23% (Figure 32.). At 1 hour of pretreatment, due to the insufficient pretreatment time, there was no significant flocculation of lignin fractions observed. 2 hours of pretreatment had resulted in a lignin recovery of 1.76% and further increase in pretreatment time: 4 h, ramped lignin recovery to 2.23%. While compared with KOH-W pretreatment, the increase between 1 h and 2 h of operation times was more rapid: approximately 0.70% in KOH-W and 1.76% for KOH-W-G. In comparison to KOH-W, the addition of glycerol appears to result in an improved lignin recovery rate.

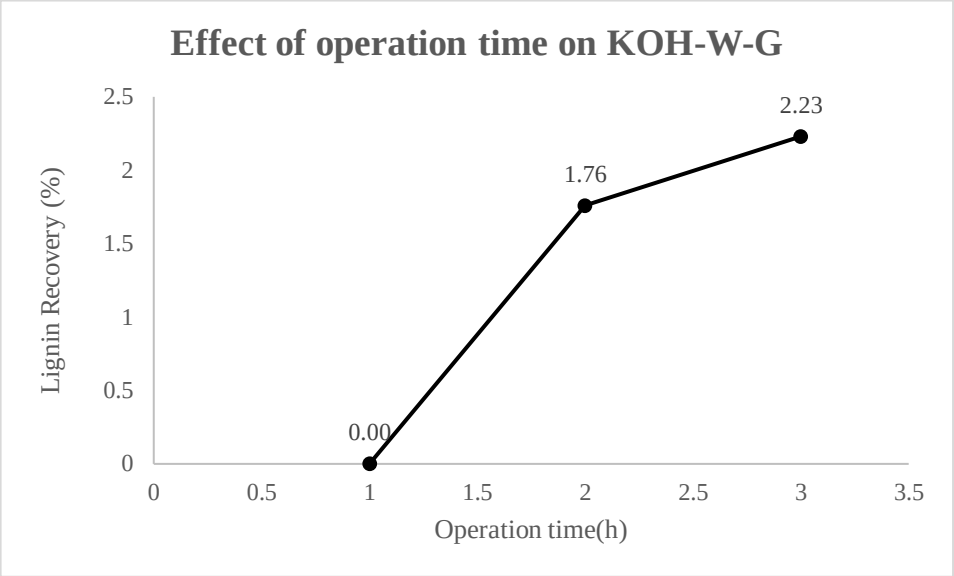


Figure 32. Effect of increasing operation time on KOH-W pretreatment.

3.7. Comparison of lignin recovery of three water combined solvents

In this part, the water dissolved NaOH, KOH and $Ca(OH)_2$ solutions was compared.

3.7.1. Comparison of solvent ratio of three water dissolved alkali treatment

In contrast, maximum lignin recovery was achieved with NaOH, reaching up to 10.37% (Figure 33.). However, at 0.4 g/g concentrations of KOH, the lignin recovery tends to decrease slightly from 9.97% to 8.70%. Minimum lignin recovery achieved at 0.1 g/g $Ca(OH)_2$; 1.15 % (Figure 33.). In terms of low-dosage usage, KOH seems to be more favorable due to its considerably higher lignin recovery. While NaOH and $Ca(OH)_2$ recovered 1.15% and 1.81% of lignin fractions, respectively, KOH recovered 8.07% of the lignin fraction. Compared with KOH and NaOH pretreatment, most rapid increase of lignin recovery observed at $Ca(OH)_2$ at concentrations between 0.2 g/g to 0.3 g/g approximately ~2.93 fold. In KOH and NaOH, increase between same concentrations only resulted ~1.05 fold and ~1.31 fold respectively. Similar results were achieved by Wang et al. (2017), which were investigated the comparison of NaOH pretreatment (glycerol mediated) and $Ca(OH)_2$ pretreatment on sugarcane bagasse. Results indicate that NaOH-W mediated pretreatment superior to $Ca(OH)_2$-W. They suggest that due to the ionic replacement between Ca^{+2} and lignin-Ca complex, the $CaCl_2$ could not play the same role as for the NaOH-W pretreatment.

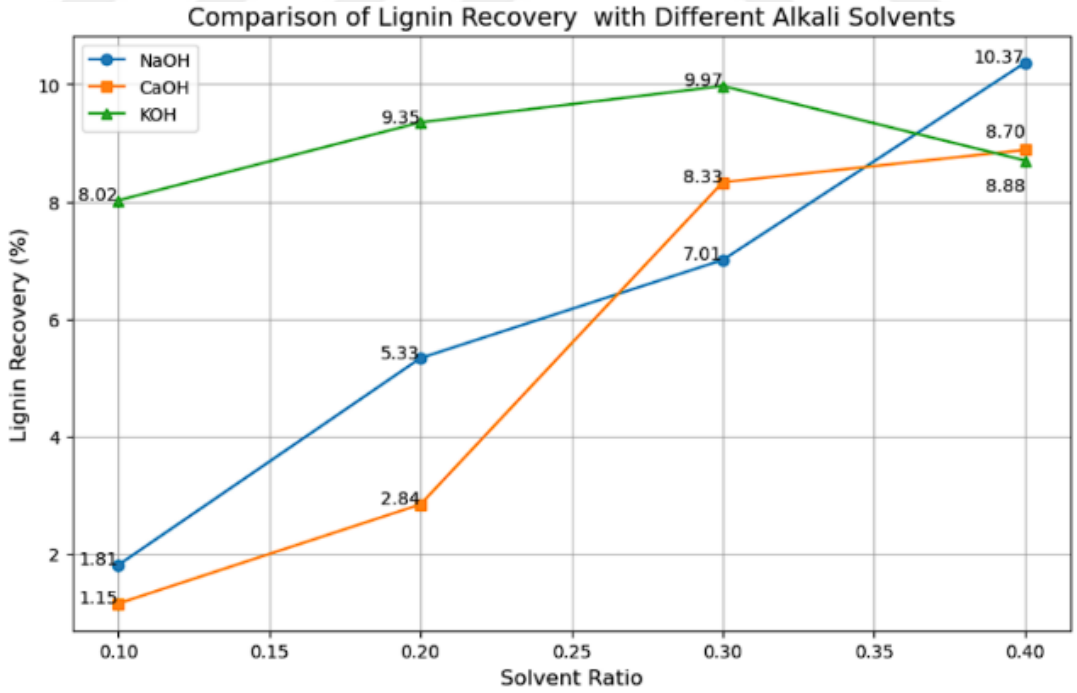


Figure 33. Comparison of three different alkali solvents in terms of their solvent ratios.

3.7.2. Comparison of temperature of water dissolved alkali treatment

The maximum lignin recovery was observed in NaOH pretreatment at 60°C with a value of 10.37% while KOH and $Ca(OH)_2$ pretreatment achieved 9.97% and 8.33% respectively (Figure 34.).

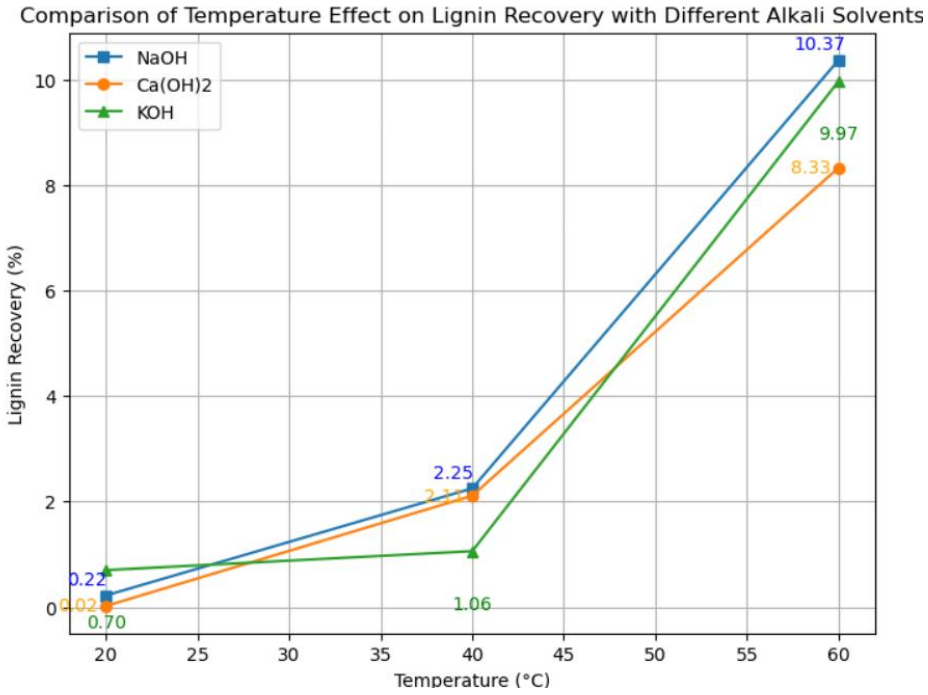


Figure 34. Comparison of effect of temperature on lignin recovery of three alkali solvents.

A minimum lignin recovery rate was obtained $Ca(OH)_2$ pretreatment at 20°C with a value of 0.02%, while NaOH and KOH pretreatment resulted slightly higher values; 0.22% and 0.70% respectively. Between 40°C and 60°C temperatures, the most rapid increase was observed in KOH treatment about ~ 9.40 fold; 1.06% to 9.97% while NaOH and $Ca(OH)_2$ ~4.60 fold; 2.11% to 8.33% and ~3.94 fold; 1.06% to 9.97% respectively. Similar results were obtained by Chang et al. (2017),

conducted experiments on sugarcane bagasse. $_2$pretreatment methods and it was suggested that at same temperature (80°C), the highest lignin recovery was observed with NaOH pretreatment.

3.7.3. Comparison of operation time of three water dissolved alkali treatments

Maximum lignin recovery was observed at 4 hours of $Ca(OH)_2$-pretreatment time as 5.75% Increase in operation time facilitated lignin recovery. Similar outcomes were obtained in all of three solvents; at 1 hour of pretreatment, lignin flocculation was not observed. At the same time, as the operation time increased to 2 hours, lignin recovery showed slight increase for NaOH, $Ca(OH)_2$ and KOH with values of approximately 0.22%, 0.02%, and 0.70 %, respectively. After the 4 hours of pretreatment operation, the lignin recovery was rapidly increased: for NaOH, from 0.22% to 1.52%, for $Ca(OH)_2$ from 0.02% to 5.75% and for KOH, from 0.70% to 1.84% (Figure 35.).

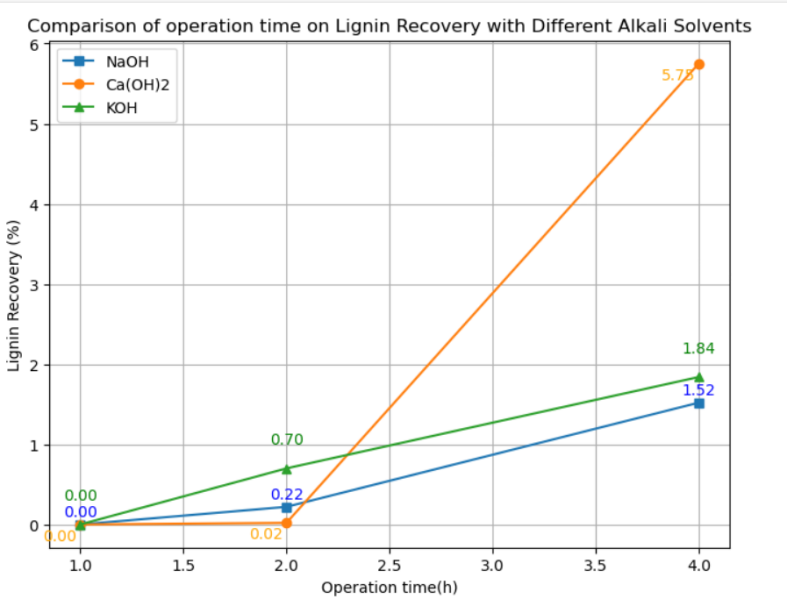


Figure 35. Comparison of operation time of three different alkali solvents.

Maximum lignin recovery was achieved through $Ca(OH)_2$ pretreatment and it seems that $Ca(OH)_2$ has greater potential for treating higher amounts of lignin fractions

compared to NaOH and KOH. However, at 2 hours of pretreatment, lignin recovery remained significantly lower than 4 hours. Therefore, further improvements and optimizations are necessary to improve lignin recovery for more developed industrial applications.

3.8. Comparison of glycerol combined pretreatment

Three distinctive glycerol mediated alkali sets were compared.

3.8.1. Comparison of solvent concentration of three different glycerol mediated alkali solvent

Maximum lignin recovery was achieved through NaOH-W-G pretreatment with a value of 14.34 % at a concentration of 0.3 NaOH g / g dry biomass. $Ca(OH)_2$-W-G and KOH-W-G pretreatments recovered approximately 3.10% and 2.75%, at the same concentration of 0.3 g / g. Importantly, the increase in solvent concentration for $Ca(OH)_2$-W-G had a positive effect on lignin recovery, and enhancing recovery from 3.10% to 3.55%.In contrast, NaOH-W-G and KOH-W-G pretreatments were negatively affected by increasing solvent concentration, NaOH-W-G treatment decreasing to 13.96%, and KOH-W-G treatment recovering only 2.15% of lignin fractions (Figure 36.).

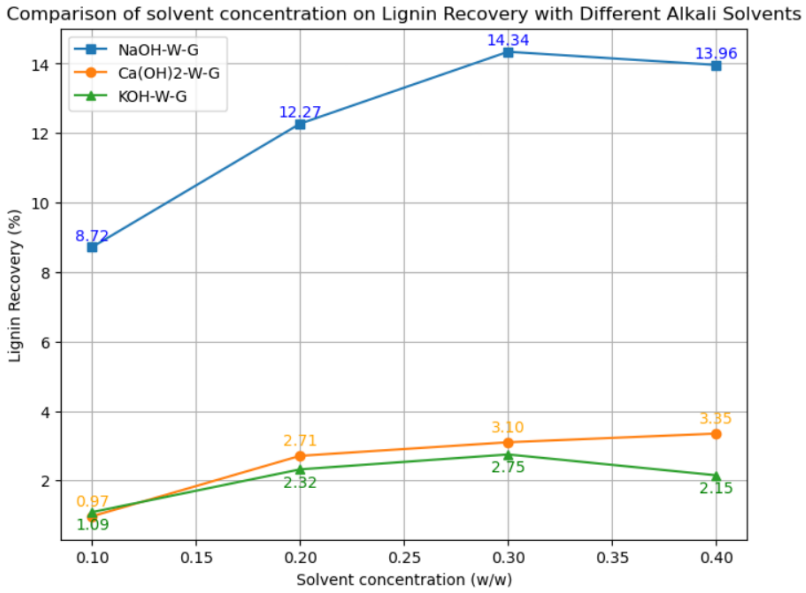


Figure 36. Comparison of the effect of glycerol on three different alkali solvents.

In addition, maximum lignin recovery at minimum solvent concentration was also achieved through NaOH-W-G pretreatment. It can be concluded that the glycerol mediated NaOH pretreatment is more favorable than other alkali solvents for hazelnut shells.

3.8.2. Comparison of effect of temperature on glycerol mediated alkali solvents

Maximum lignin recovery was obtained at 60°C with significant difference observed; 14.34% for NaOH-W-G, 3.10% for $Ca(OH)_2$-W-G, and 2.75% for KOH-W-G respectively. The lowest lignin recovery was observed at temperature 20°C, with a recovery rate of .0.59% for $Ca(OH)_2$-W-G. At the same temperature, NaOH-W-G pretreatment achieved a recovery of 1.13 % and $Ca(OH)_2$-W-G 0.58%. KOH-W-G pretreatment seems to be more favorable at lower temperatures. However, for industrial applications, further economic analysis is necessary. Figure 37. summarize the results of effect of temperature on glycerol mediated alkali pretreatments.

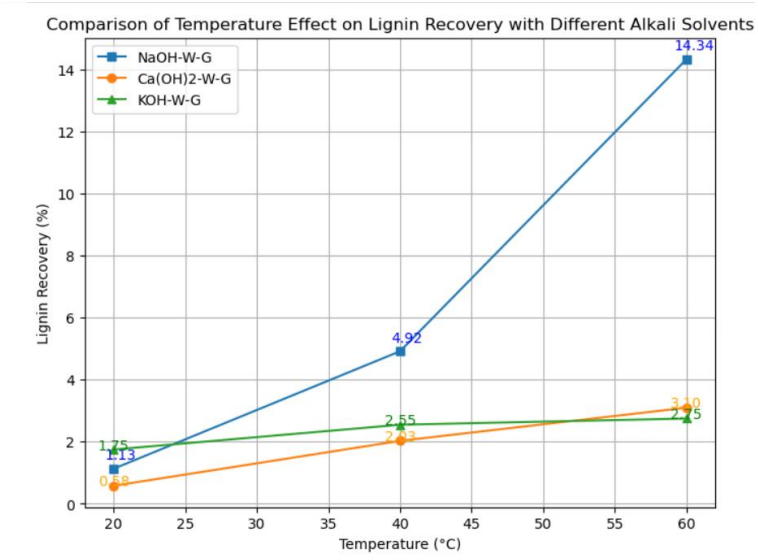


Figure 37. Comparison of the effect of temperature on glycerol mediated alkali solvents.

3.8.3. Comparison of effect of operation time on glycerol mediated alkali solvents

Maximum lignin recovery was obtained at 4 hours of pretreatment. NaOH-W-G achieved 9.99 %, $Ca(OH)_2$-W-G and KOH respectively achieved 1.46 % and 2.23 % (Figure 38.). At the shortest operation time, lignin flocculation was not observed due to the insufficient contact time with a temperature of 20°C. The most rapid increase was observed in NaOH-W-G pretreatment: from 1.13% to 9.99% and this can be concluded as, the most sensitive glycerol mediated alkali solvent is NaOH-W-G.

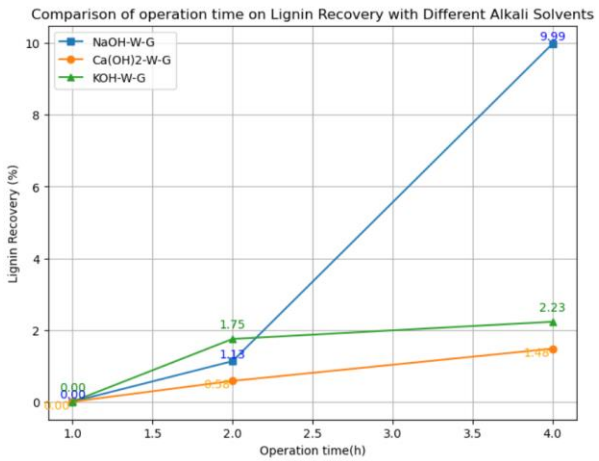


Figure 38. Comparison of operation time on glycerol mediated alkali solvents.

3.9. FTIR analysis

FTIR analysis was conducted on the recovered lignin fractions, revealing several characteristic lignin peeks (Figure 39.). Between 850 and 922 cm^{-1} peaks indicate the presence of hemicellulose and carbohydrate residues (Nascimento et al., 2014). Similarly, Nduru et al. (2019) support this finding, and reporting that at 869.89 cm^{-1} suggests the presence of β–1-4 glycosidic bonds. Additionally, Xu et al. (2006), associates 855 cm^{-1} with Aromatic C-H out-of-plane deformation. In

addition, at the peak of 898 cm^{-1} C-H deformation can be resulted in cellulose (Pandey & Pitman., 2003).

The peak at 1034 cm^{-1}, generally associated with C-H in-plane deformation in guaiacyl unit and C-O deformation in primary alcohol (Derkacheva & Sukhov., 2008; Chen et al., 2010). According to Nduru et al. (2019), the peaks around 1060 cm^{-1} are strongly correlated with C-O symmetric stretching of primary alcohol.

The peak 1108 cm^{-1} corresponds to C-O-C stretching, and symmetric vibration of the ester linkages, with CH stretching in the aromatic ring (syringylic) is also being detectable. (Lupoi et al.,2015).

The peak 1210 cm^{-1} associated with C-O and glucopyranosic cycle syringilic symmetric vibrations (Faix, 1991; Carballo et al., 2004). On the other hand, Nduru et al. (2019) detected C-O stretching of ether linkages in 1251.80 cm^{-1}. The peaks between 1317 cm^{-1} to 1550 cm^{-1} are strongly related with skeletal vibrations in guaiacyl and syringly groups (Manara et al, 2014; Sun et al., 2021). Moreover, between 1317-1321 cm^{-1}, condensation reactions of guaiacyl and syringyl units, syringyl unit and CH_2 bending stretching was also reported by Chen et al. (2010). Also, Xu et al. (2006) was reported that 1325 cm^{-1} is highly associated with syringyl ring breathing with C-O stretching.

At 1650 cm^{-1}, According to Pandey & Pitman. (2003), Absorbed O-H and conjugated C-O should be present. Between 1734-1738 cm^{-1}, unconjugated C=O in xylans (hemicellulose) represented (Lupoi et al., 2015; Pandey & Pitman., 2013; Faix. 1991) and 2935 cm^{-1} band was associated with CH stretching. CH_2 asymetric vibration of guaiacyl-syringyl (Nduru et al., 2017; Carballo et al., 2004; Derkacheva & Sukhov, 2008). In final, 3312 cm^{-1} represented the O-H stretching: between 3400-3430 cm^{-1} (Lupoi et al., 2015; Pandey & Pitman., 2013; Faix, 1991).

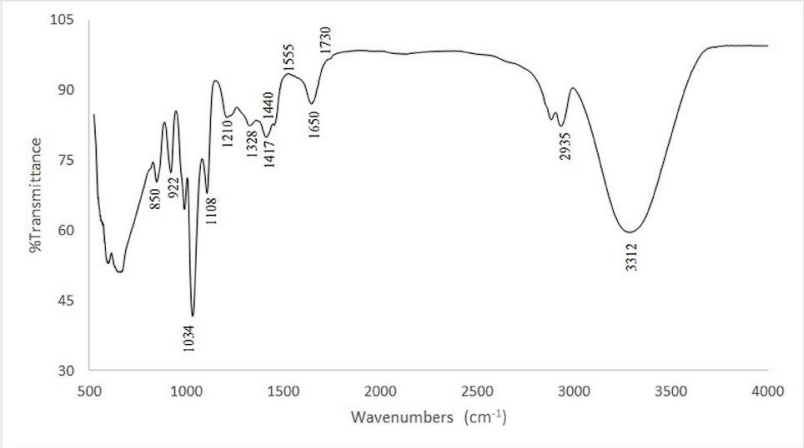


Figure 39. FTIR spectra of the lignin fractions.

CONCLUSION & RECOMMENDATIONS

Hazelnuts, one of the most abundantly produced lignocellulosic biomass source in Turkey and globally. Worldwide, tons of lignocellulosic biomass, including hazelnut shells, are used as heating material. However, these shells can be valorized into higher value-added products. As the global population continues to grow, the energy demand of human beings increases. To minimize the harmful effects of fossil fuels, the use of petroleum-based fuels must decrease. Biomass-dependent facilities currently utilize untreated waste from lignocellulosic biomasses. Soon, by applying these types of pretreatment methods, we may increase the efficiency of energy production, reduce the reliance on petroleum-based fuels, and even advance cutting-edge biorefinery concepts.

Within the scope of this thesis, the highest lignin recovery from hazelnut shells was achieved through KOH-W pretreatment, reaching 9.97 % at at 20°C and 2 hours of pretreatment at constant stirring speed of 150 rpm.Glycerol addition positively affect NaOH pretreatment, while $Ca(OH)_2$ and KOH pretreatment suffered .In literature, limited studies have explored the combination of alkali solvents and glycerol and comparison of three different alkali solvents, and within the scope of this thesis, this combination could serve as a notable resource for further studies in this field.However, in higher temperatures, further studies must be performed.The recovered lignin particles were characterized using FTIR spectroscopy, and the presence of lignin particles was confirmed. In literature, limited studies have explored the combination of alkali solvents and glycerol and comparison of three different alkali solvents, and within the scope of this thesis, this combination could serve as a notable resource for further studies in this field.

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