

CHAPTER 2

Theory

This chapter is about the theory of lignocellulose, lignocellulose material preparation, method of pretreatment and the toxicity. The details are as follows;

2.1 Lignocellulose

Lignocellulose is the main component of plant cells, which consists of cellulose, hemicellulose and lignin.

Cellulose is polymer which connects structures as line of cellulose of glucose of 8,000 to 12,000 units articulating with the bonds β -1, 4-Glucosidie linkage. Because cellulose has β -configuration which consists of carbon position number 1(C1), the chains could connect together with hydrogen bonds, the characteristic is similar to crystalline filament cells which is low solution and very hard except these cellulose are besieged by hemicellulose and is encompassed by lignin.

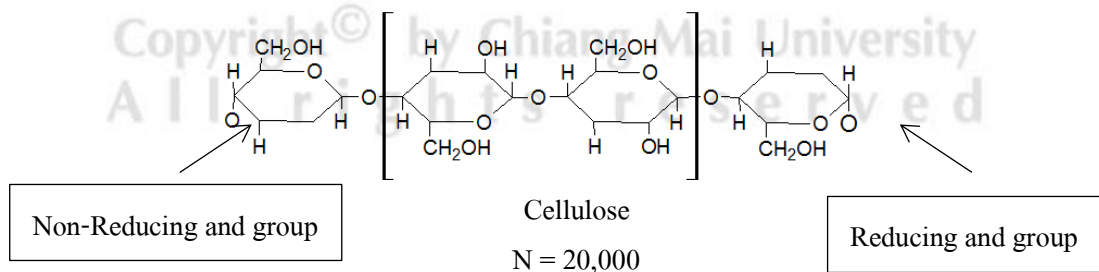


Figure 2.1 Structure of cellulose

Cellulose is insoluble in water or any organic substances and in diluted base or acid but it could be soluble well in both strong base and strong acid solutions so we could divide cellulose into the following solubility in 3 characteristic types;

1. Alpha cellulose (α -Cellulose) is cellulose which is not soluble in NaOH solution intensity as 17.5% at room temperature.

2. Beta cellulose (β -Cellulose) is cellulose which is soluble in NaOH solution intensity as 17.5% at room temperature but could precipitate easily in acid condition.

3. Gamma cellulose (γ -Cellulose) is cellulose which is soluble in NaOH solution intensity as 17.5% at room temperature and could be soluble in acid solution but could precipitate with alcohol.

Hemicellulose is polymer saccharide which is underweight molecule which composes mainly of sugar such as D-xylose, D-inanmose, D-galactose and L-arabinose so hemicellulose division could be separated upon type of sugar such as xylan, mannan, arubinan. Many lines of hemicellulose would be xylose which link to the bonds of β -1,4, and branches are units of arabinose, mannose, glucose, glucuronic acid. Pentose sugar which links to the bonds β -1,4 with the exception of galactose which has specific characteristics to connect at position of bonds β -1,3.

The difference between hemicellulose and cellulose is that hemicellulose can be digested by diluted acid solutions and could be well dissolved in strong NaOH solution as 17.5%. Beside this polymer line of hemicellulose is featured as many more branches but the length of chain is shorter at only about 40 glucose units.

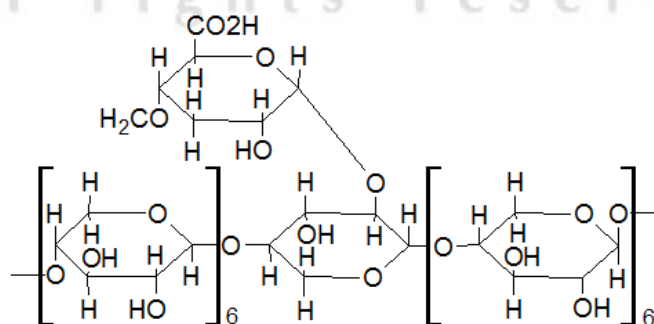


Figure 2.2 Hemicellulose structure.

Lignin is polymer of p-hydroxyphenylpropane unit connects with C-C and C-O-C linkage, could be referred that lignin is the polymer in three dimensional phenolic compound.

Lignin has strong structure which is not be able to dissolve in water but could dissolve in some organic solvents such as ethanol or hot methanol and NaOH solution.

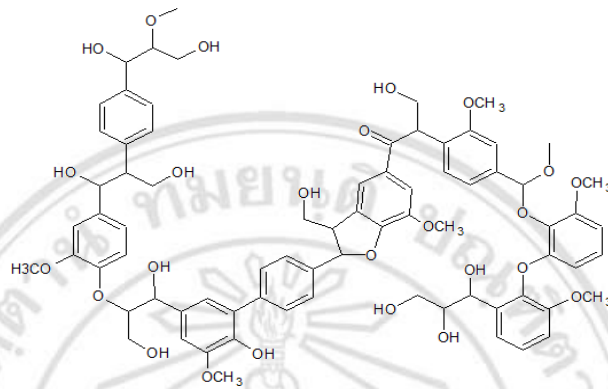


Figure 2.3 Lignin structure.

Lignocellulose materials pretreatment is destroying the hard structures of lignocellulose in order to open the easy way for enzyme, acid, and microorganisms to gain access, decay and activate.

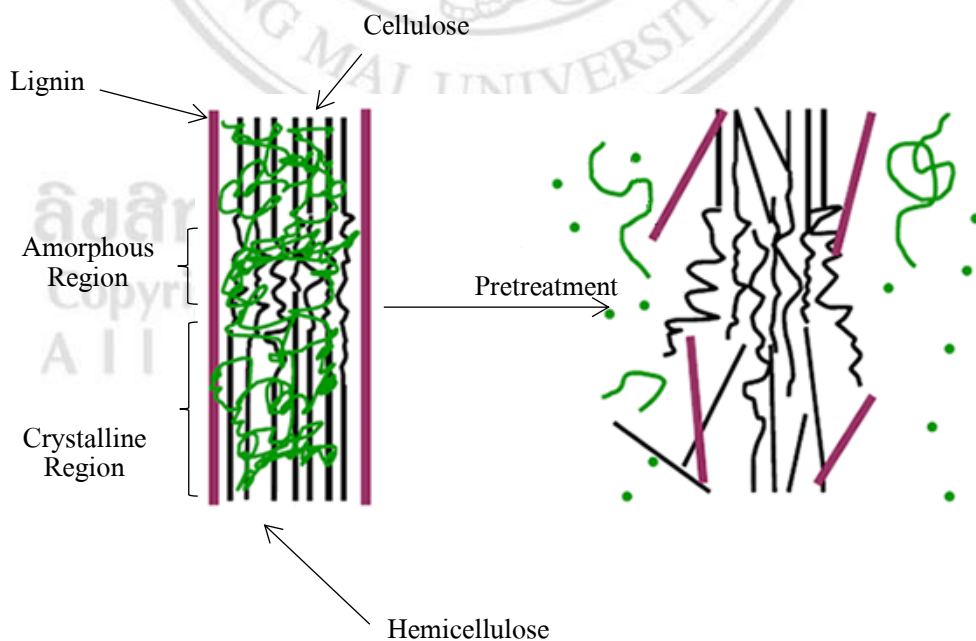


Figure 2.4 Lignocellulose materials pretreatment.

Material pretreatment process shall stimulate the changing attribute conditions in physical result. Sun matrix of lignocellulose material is destroyed which can affect catalysis activated enzyme effect with hydrolysis reaction easily. Studying performed pretreatment process is the most significant criteria of technological achievement in cellulose pretreatment because it is explicated in commercial worthiness related to cellulose and hemicellulose change.

2.2 Purpose of lignocellulose material preparation

2.2.1 Size of particle or surface area

Enzyme could be digested when it exposes to initial substance. If it proceeds to the initial substance by being smaller or increases more surface of weight, these could make enzyme to absorb or expose to initial substance more. Hydrolysis proceeds faster and increases initial preparation using woods, it is found that hydrolysis ranking increase with downsizing particles and the preparation seems to change the characteristics of initial substance which were going to be for good hydrolysis process.

2.2.2 Crystallization of cellulose

Cellulose in crystal part could be hard and resistant to hydrolysis with enzyme, by that hydrolysis; non-crystal part shall be used for hydrolysis process first, follow by with crystal part, which would lower hydrolysis.

2.2.3 Polymerization

Level of Polymer shall be either high or low is up to glucosyl number in cellulose so low polymer level would have much tip of cellulose which polymer level is up to initial substance and result of preparation.

2.2.4 Amount of lignin

The amount of lignin in lignocellulose materials causes capability for increased hydrolysis. Clearly, lignin would protect enzyme exposure to sugar polymer. To separate lignin from cellulose during preparation, it is important that hydrolysis efficiency should increase but lignin would not be separated mostly and completely prepared condition that

is average serve would remain the high lignin. It would create exposure between enzyme-cellulose which is less if compare to aggressive prepared condition which is more exposure but hemicellulose would remain just a little.

2.2.5 Degree of o-acetylation

Level of o-aectyl of o-acetylation affects hydrolysis result so much which acetyl would obstruct hydrolysis of enzyme.

2.3 Lignocellulose pretreatment

Lignocellulose pretreatment would make physical, chemical and biological processes

2.3.1 Physical pretreatment

2.3.1.1 Mechanical commination

It is the reduction of material by cutting, grinding to reduce crystallization of cellulose. The size from cutting is approximately 10-30 mm.; the grinded size is approximately 0.2-2 mm. Grinding can destroy crystallization of cellulose and increase digestion.

2.3.1.2 Pyrolysis

Pyrolysis process is the processes for digesting materials at approximated temperature 200 °C to 600 °C under pressure of temperature which is the chemical process that is irreversible reaction. Product produced from pyrolysis process is upon natural material, humidity, period, temperature, particle size, by general products are divided to be battery 30%-50%, gas 20%, liquid of organic substance 18%-20% such as acetic acid. Methanol which is soluble in pyrolysis will be used to proceed in lignocellulose pretreatment at temperature over 300 °C, causing cellulose to decay rapidly and when bringing battery that produced form hydrolysis with acid 1 N H₂SO₄, 97 °C at 2.5 hrs. would make lignocellulose changed to be reduced sugar at 80%-85% which is composed of glucose over than 50%.

2.3.1.3 Physio-chemical pretreatment

1) Steam explosion (Autohydrolysis)

Steam explosion is the most regular used method for lignocellulose preparation. In this method, material would be prepared at high steam pressure then reduce pressure immediately for explosion and decomposition.

An advantage of steam explosion is when compares with grinding. Physical process shall use energy more than steam explosion at 70%. Steam explosion would have high efficiency with hardwood and agriculture crops but has low efficiency with softwood, resulting in breaking of xylene, lignin and carbohydrate incompletely, produced microorganism inhibitor.

2) Ammonia fiber explosion (AFEX)

AFEX is process that puts material into Ammonia solution at high temperature and pressure rapidly which is similar to steam explosion. The amount of Ammonia is 1-2 kg. of ammonia per material weight 1 kg. at temperature 90 °C for 30 mins. long. AFEX preparation process would increase the transformed sugar and to be able to prepare lignocellulose from various groups such as legumes, rice straw and corn cobs, waste, paper, grass and etc.

3) CO₂ explosion

Proceeding in the same way as steam explosion and ammonia explosion. Using CO₂ would be similar to Carbonic acid, this method is used for legumes preparation (4 kg. CO₂/kg. fiber at pressure 5.62 MPa) and Non-enzyme hydrolysis produces glucose sugar at 75% within 24 hrs.

2.3.2 Chemical pretreatment

2.3.2.1 Ozonolysis

Ozone could decompose lignin as well as hemicellulose in various kinds of material such as rice straw, bagasse, grass, peanut, cotton and saw dust. Decomposing is not specific

only to lignin and hemicellulose but cellulose would be harder for this method. Advantage of preparation with ozone is to decompose lignin, not produce toxin or inhibitor at normal temperature and pressure but to use ozone in a large quantity would increase the cost because of high price.

Molecule of Ozone is O_3 and molecule weight is equal to 48 in Gas state at normal temperature and pressure, boiling point is $-111.9\text{ }^\circ\text{C}$ at the pressure and instability.

Ozone is oxidation/oxidizing agent that is aggressive, has much efficiency in obstructing smell, color and taste of water and could dissolve in water on average 20 times of oxygen dissolving in water but is unstable in water. Ozone has more stability in air than in water especially in cool and dry climate (Weber, 1972; Cheremisinoff, 1993, referenced by Isree Rodtassana, 2007). Dissolving ability of ozone rely on temperature and pressure of ozone in gas condition. Ozone is unstable gas, can be decomposed to become Oxygen by splitting for any radical such hydroxyl radical (OH^\bullet), OH_3 , HO_4 and Super oxide (O^{2-}). Decomposition of ozone would increase and alkalinity also increase, the result is that ozone can react with water and hydroxide ion (OH^-) gets product is hydroxyl radical (OH^\bullet) which this process would have hydroxide ion (OH^-) is the promoter of enzyme decomposing reaction so half-life of ozone would be quite short in Alkalinity condition as pH 10. Ozone half-life in pure water is about 30 mins long. Organic compound from natural inhibitor and promoter of enzyme decomposing reaction (Langlais et al,1991; Gottschalk et al.,2000, referenced by Isree Rodtassana, 2007) .Oxidation react mechanism between organic substance and Ozone (Sumeth, 1998, Gottchalk et al., 2000 (reference by Isree Rodtassana 2007) . It is divided into 2 algorithms as follows;

1) Direct attack

Organic substance is reacted with molecule of ozone directly which is reacted by electrophilic or dipolar cyclo addition, ozone would react with organic substance at double bond ($C=C$, $C=C-O-R$, $C=C-X$) or negative ion atom (N, P, O, S and nucleophilic C) aromatics substances which have OH, CH_3 or OCH_3 at ortho position would be high reactivity with Ozone, if it has NO_2 , $COOH$ or CHO group, the reactivity is lower.

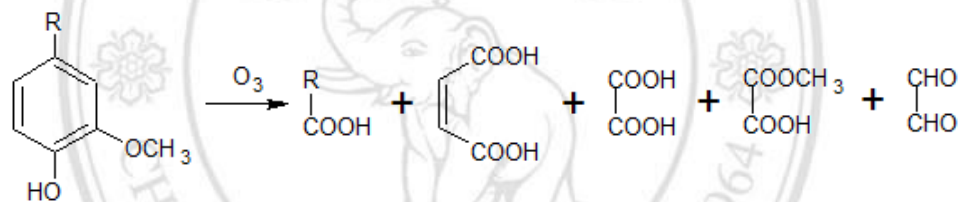
2) Indirect attack

Organic substance would react with free radicals that are from reaction process 1 such as OH° OH_2° which these charges would respond in oxidation again and could oxidize the organic substance type acids aldehydes ketones and less highly activated aromatic efficiently.

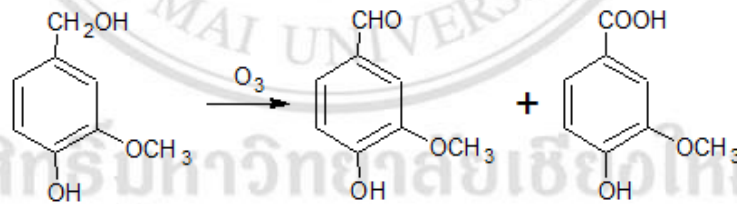
Ozone using in decomposing organic substance bond that is complex structure would produce product which can be decayed easily.

Ozone reacts with molecule of lignin in the group of olefin, aromatic and carbon-hydrogen (Thomas E.Lyse) as the following;

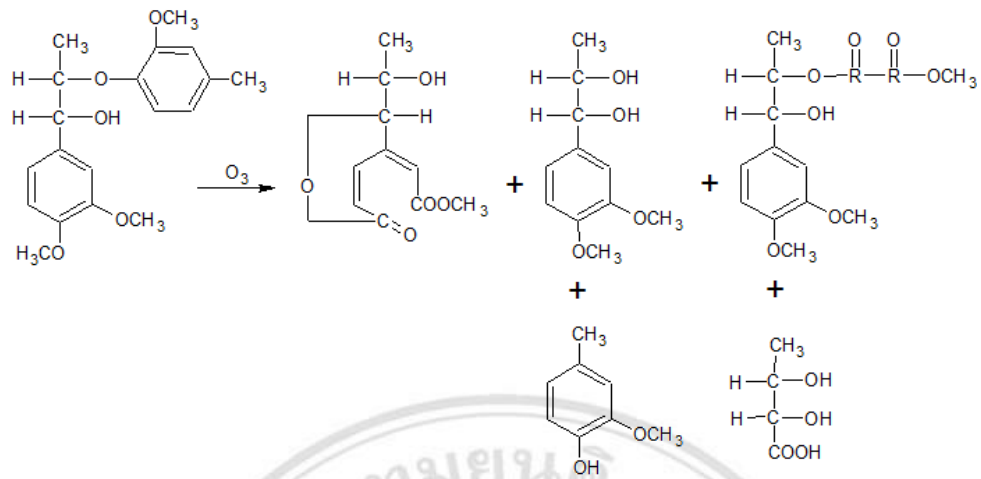
(1) Ozone-oxidation of lignin model to form acidic reaction products



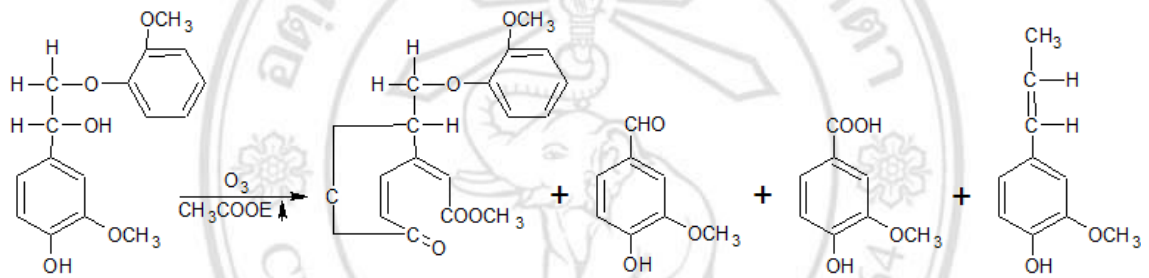
(2) Ozonation of side-chain in lignin model compound



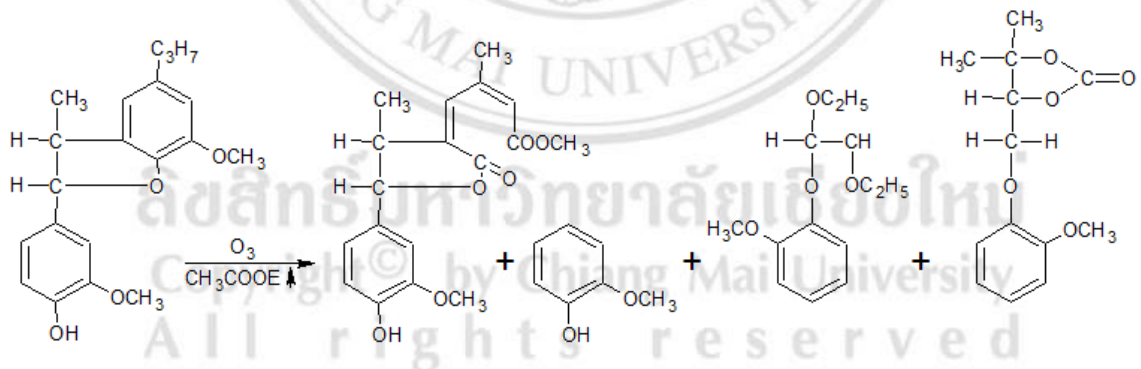
(3) Ozonation of β -aryl ether lignin model according to Balousek.



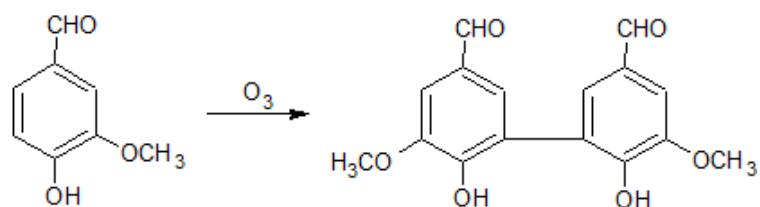
(4) Ozonation of β -aryl ether model according to Kojima et al.



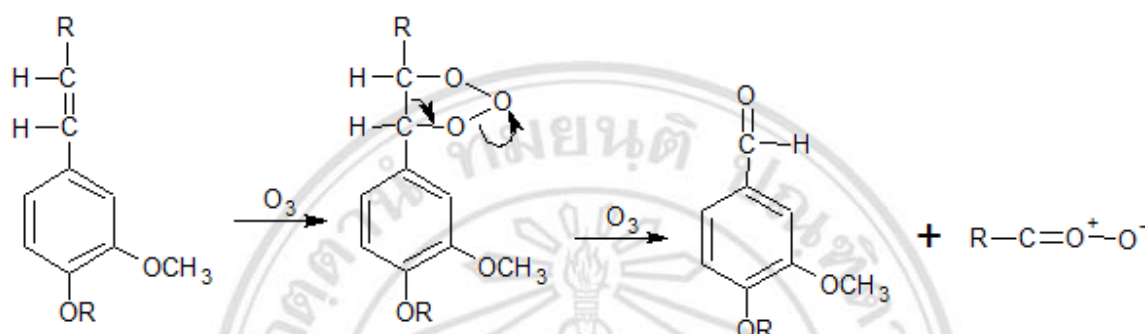
(5) Ozonation of phenyl coumaran lignin model according to Kojima, et al.



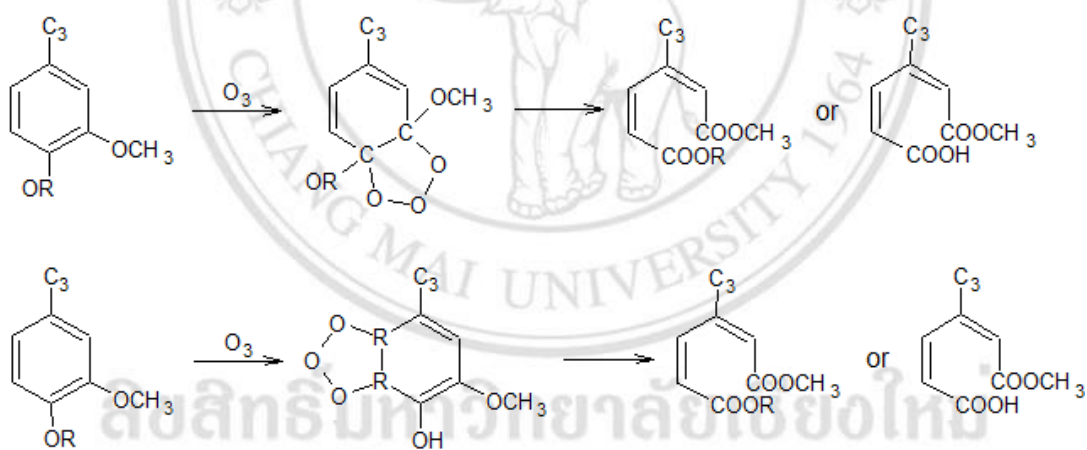
(6) Formation of polycondensed reaction product through ozonization of lignin Model Compound



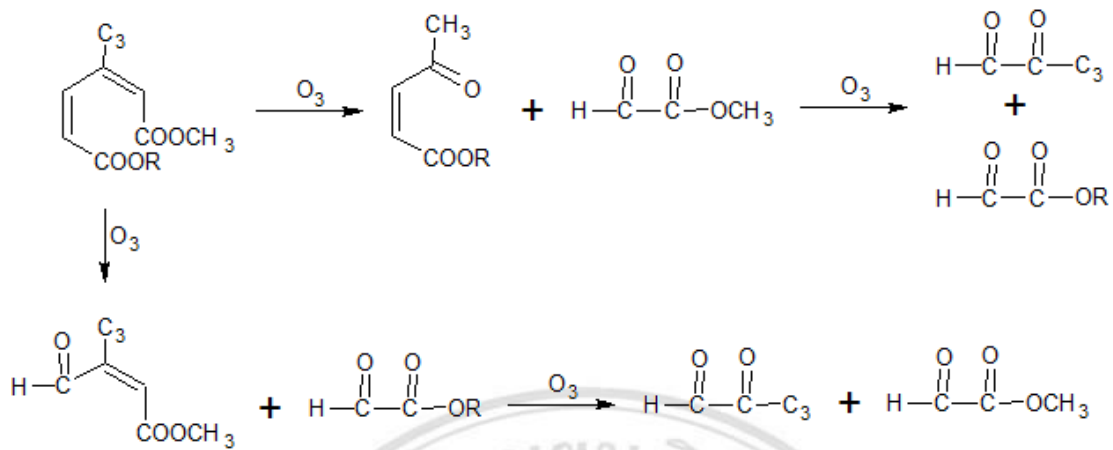
(7) Reaction of olefinic bond in lignin with ozone as proposed by Soteland



(8) Reaction of aromatic bonds in lignin via 1,3 dipolar addition of ozone



(9) Ozone degradation of muconic acid derivatives in lignin as proposed by soteland



(10) Reaction of lignin with ozone via aromatic electrophilic substitution mechanism proposed by Singh

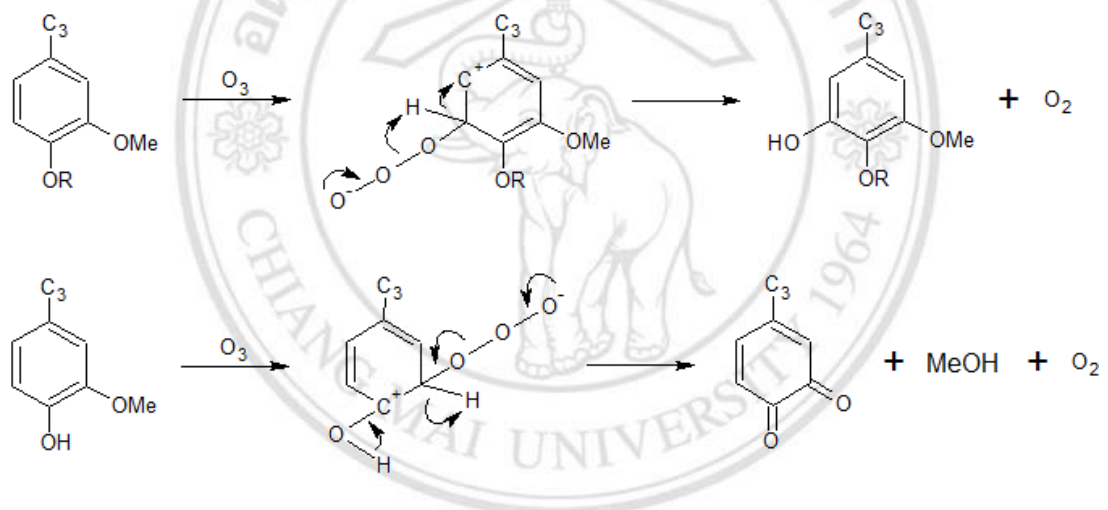


Figure 2.5 Forming between ozone and lignin molecule

2.3.2.2 Acid pretreatment

There is the use of acid as well such as sulfuric acid (H₂SO₄) and hydrochloric acid (HCl) in lignocellulose pretreatment although acid is chemical that affects cellulose in hydrolysis process greatly but concentration is harmful, corroded and dangerous. Thus, there is the need to use reactor which endures corrosion. At present, there is a development in diluting acid for lignocellulose pretreatment until it could increase the hydrolysis of the cellulose but acid use would make high cost and pH would effect in fermentation as well.

2.3.2.3 Alkaline pretreatment

Some alkaline could be used in material preparing for lignocellulose type and factors of pretreatment depend on lignin quantity. Lignocellulose preparation with diluted NH_4OH would be diluted. This results in the increased surface, decompose to be polymer, reduce crystallization and destroy lignin structure. NH_4OH solution would reduce lignin number in hardwood from 24%-55% and leave only 20% but for diluted acid would leave lignin over 26%. Some alkaline solutions are able to destroy bonds between lignin and other lignin. They can also destroy bonds between lignin and polysaccharide. To prepare corn stalk, cassava bark and peanut husk by radiation together with NaOH solution at 2%. The effect of corn stalk produces yield of glucose increasingly. Glucose at 20% increases to 43%. Cassava bark and peanut husk produce yield average 3.5% and 2.5% respectively.

To use NH_4OH concentration at level of 2.5%-20% in pretreatment the corn cob/stover and switch- grass at temperature 170 °C. The period of time is 1 hr. It shows that ammonia concentration could decompose lignin in corn cob/stover at 60%-80% and lignin in switchgrass at 65%-85%

2.3.3 Oxidative delignification

To decompose lignin with hydrogen peroxide that is able to occur with peroxidase enzyme catalysis. To pretreatment bagasse with hydrogen peroxide could increase hydrolysis lignin average 50 percentiles and large number of hemicellulose could be able to decompose by hydrogen peroxide using 2% at temperature 30 °C within 8 hrs. and when Na-lignocellulose that prepared with this method to hydrolysis with enzyme cellulose would results that cellulose changes to be glucose sugar at 95% at temperature 45 °C for 24 hrs.

2.3.4 Organosolv process

Organosolv process (organic substance or organic substance mixed with inorganic acid such as HCl or H_2SO_4 use for decomposing lignin and hemicellulose, the organic solvent is such as methanol, ethanol, acetone, ethylene glycol. Organic acid that is used for being catalysts such as oxalic acid, acetylsalicylic acid and salicylic acid, and high temperature (185 °C) is not necessary to put more catalysts, solvent is necessary to discharge from

reactor, evaporate in order to be intensive and could reuse to reduce cost. To remove solvent out from system is important because solvent is the inhibitor in microorganisms growing of hydrolysis and fermentation.

2.3.5 Biodegradation pretreatment

In lignocellulose pretreatment by biodegradation process has used microorganisms such as brown-rot and soft-rot fungi that decompose lignin and hemicellulose of brown-rot fungi materials, specified using when white-rot fungi and soft-rot fungi could be used for both cellulose and lignin. White-rot fungi are groups of much efficient fungi for lignocellulose pretreatment.

White-rot fungi which is able to decay lignin and release cellulose is produced to be brown-rot fungi could decompose cellulose to be sugar for fermentation, but for yeast could be fermented then changed to be sugar produced ethanol.

Table 2.1 Comparison strengths/weaknesses of any pretreatments process.

	Main advantages	Main disadvantages
Milling	Reduces cellulose crystallinity; increase surface area	Need of combination with other treatments; high energy consumptions
Torrefaction	Easier biomass storage; no formation of inhibitors; moderate energy consumption; easier grindability	Need of combination with other treatments; still incomplete investigation
Steam explosion	Increase of accessible surface area; higher substrate digestibility; depolymerization of lignin; solubilization of hemicellulose	Need of combination with other treatments; formation of inhibitors
LHW	Enhanced substrate digestibility; low formation of inhibitors; low-cost plant	High energetic requirements; high water input
AFEX	Low formation of inhibitors; increase of accessible surface area	High cost of plant and ammonia
CO ₂ Explosion	No toxicity; easy recovery; increase of accessible surface area; efficient hydrolysis of hemicellulose	High cost of plant; high pressure involved lignin remains
Alkaline	Hemicellulose and lignin hydrolysis; mild conditions; increased substrate digestibility	Long reaction times; salts formation and incorporation; base consumption
Acid	Increased substrate digestibility; hemicellulose solubilization	Formation of degradation products; formation of inhibitors; corrosion; need of acid recovery
Ozonolysis	No toxicity; no formation of inhibitors; lignin solubilization	High cost of ozone
Organosolv	Hemicellulose and lignin solubilization	High cost for plant and solvents
ILs	Low toxicity and flammability; high selective solubilization of biomass components	High cost for plant and ILs; high viscosity

Table 2.2 Results of pretreatment for structure in Lignocellulose materials.

	Milling	Torrefact.	S.E.	LHW	AFEX	CO ₂ Expl.	Alkaline	Acid	O ₃	Organo solv.	ILs
Increase of accessible surface area	H	H	H	H	H	H	H	H	H	H	H/-
Cellulose decrystallization	H	n.d.	-	n.d.	H	L	H	-	n.d.	n.d.	H/-
Hemicellulose solubilization		L	H	H	L	L	L	H	H	H	H/L
Lignin solubilization		-	L	L	L	L/-	H	L	H	H	H/-
Generation of inhibitors		-	H	L	L	-	L	H	-	-	-
Alteration lignin Structure		L	H	L	H	L/-	H	H	H	H	H/-



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2.4 Inhibitor

Lignocellulose pretreatment might be produced from products that are inhibitors with ethanol fermentation process. These inhibitors are poisonous for microorganisms which are used in ethanol fermentation process in order to produce ethanol. Level of poisoning is upon factors that ferment an Ethanol along with cell conditions of microorganisms, concentration of oxygen that dissolve in water and pH. Beside that, microorganism might adapt to endure the inhibitors. However the best method is to protect inhibitors from condition of pretreatment process or other processes.

Results from inhibitors effect capability of microorganism that resist toxin, could not decide expect concentration because of results of many inhibitors' factor such as microorganisms, cultured condition of fermented food, fermentation types and toxin types of solution and etc. Total poisoning of any elements such products are from sugar decomposition, products are from lignin decomposition included derivatives from lignocellulose materials group and ion of metals, cause bad effects for growing, producing cell mass and number of reduced production.

Inhibitor substances that effect to fermentation such as Furfural, 5-hydroxymethylfurfural (HMF), Acetic acid, Phenols, Levulinic acid and Formic acid and etc.

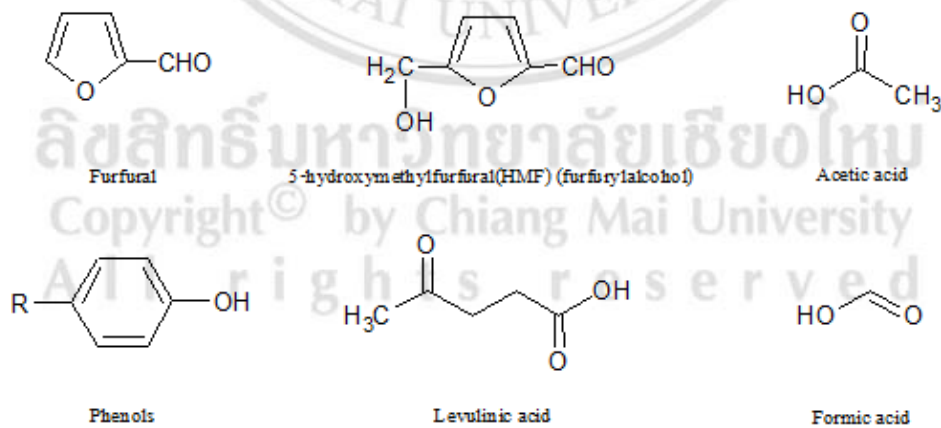


Figure 2.6 Inhibitor types and chemical structures (Mussatto and Roberto, 2004)

2.4.1 Sugar degradation products

To decay hemicellulose molecule, monomer pentose sugar might be changed to be inhibited furfural as same as glucose Sugar is degradation to be hydroxymethyl-furfural (HMF) Furfural and HMF affects to growth, cells and respiration for HMF, this has less toxin than furfural.

2.4.2 Lignin degradation products

Lignin elements degradation such as aromatic, poly-aromatic, phenolic, aldehydic might be released or reacted. Phenolic substance types are inhibitors and toxin, even though it has lower concentration than furfural and HMF. Phenolic compound might cause damage with cell membrane of fermented microorganisms and reduce sugar absorption.

2.4.3 Acetic acid

Acetic acid consisted from acetylene elements which are in hemicellulose on acid fermented condition, Acetic is in the form of undissociated is liposoluble that spread to Microorganisms cells, effect to inhibited working of cells. Toxicities would be different follow fermented states; however pH pretreatment in neutral process would help to reduce affectations from Acetic acid.

2.4.4 Inhibitory extractives

Deposits in lignocellulose materials such as acidic resins, tanninic anterpene acids are inhibited substances but toxicities are less than inhibited substances which produced from degradation of lignin or acetic acid.

Table 2.3 Origin of inhibited substances and the highest concentration that was accepted in Ethanol fermented conditions (Klinke et al.2004)

Component	Inhibitor	Maximum Concentration [g/l]	Remarks
Hemicellulose >> Xylose >> Mannose,galactose,glucose	Acetic acid Furfural HMF	3 < 0.25 0.25	Inhibition of acetic acid Depends on pH during fermentation
Cellulose >> Glucose	HMF	0.25	HMF may further degrade to formic acid and levulinic acid
Lignin	Aromatics Poly-aromatics Aldehydes Phenolic compounds	< 0.1	Vanillic acid, caproic acid, caprylic acid, pelargonic acid, palmitic acid

Table 2.4 compared any process of Lignocellulose materials pretreatment that effect to inhibited substances.

Pretreatment	Mode of action (in addition to increasing the surface area)	Potential Sugar yield	Inhibitor formation	Residue formation	Need for recycling chemicals	Low Investment costs	Low Operational Costs	Applicable to various biomass	Proven at pilot scale	Additional remarks
Mechanical		-	++	++	++	+	-	+	+	
Liquid hot water	Removal of hemicellulose	++	-	++	++	+			++	
Weak acid	Removal of hemicellulose (major) Alteration lignin structure (minor)	++	-	-	-	+-	+	+	++	Specially suitable for biomass with low lignin content
Strong acid	Hydrolysis of cellulose and hemicellulose	++	-	-	-	-	+-	++	++	Strong acid is hazardous, toxic and corrosive
Alkaline	Removal of lignin (major) and hemicellulose (minor)	++	++	-	-	++		+-	+-	
Organosolv	Removal of lignin (major) Removal of hemicellulose (minor) depending on solvent used	++	++	+	-	-	-	+	++	High quality lignin. Solvent used may be inhibitor for cell growth
Wet oxidation	Removal of lignin (major) Dissolve hemicellulose Decrystallization cellulose	++	++	+	++	+			-	
Steam explosion	Removal hemicellulose (major) Alteration lignin structure (minor)	+	-	+	++	+	+	+-	++	Low environmental impact
AFEX	Removal of lignin (major) and hemicellulose (minor) Decrystallization cellulose	++	++		-					No need for small particle size for efficacy
CO ₂ -explosion	Removal of hemicellulose Decrystallization cellulose	+	+	++	++	-			-	More cost effective than AFEX
Combined mechanical/alkaline	Removal of lignin (major) and hemicellulose (minor)	++	++	-	-	+-	+-	+	+	

2.5 Lignin elimination from lignocellulose materials

The most important of lignin property is dissolving in solvent because lignin is not soluble in water and solvents regularly thus could not extract lignin with organic solvents that have high polar while some groups of alkaline lignin could dissolve in dioxane and pyridine solvents and diluted alkaline solvents beside these when add methylation and acetylation groups replace any benzene ring positions of lignin, affected lignin could absorb ultraviolet was produced at wave length 280 nms. All these to add NaOH is hydroxyl group addition for lignin structure, effects for lignin could absorb light as well.

Regarding Lignin combines with cellulose of woods causes plants structure to be strong naturally included effects to microorganisms and enzyme could not damage plants structure easily by a structure as lignin coexists with cellulose would have covalent bonds connect with lignin and hemicellulose, thus to be more useful from lignocellulose materials must pretreat these materials, this also protecting the damage caused from lignin included cellulose and hemicellulose in proper condition for being usable.

Lignin number in lignocellulose is causing for reduced hydrolysis efficiency by lignin would protect touching of enzymes and polymer of sugar. Separating lignin out from cellulose during pretreatment is the important thing to increased efficiency for hydrolysis but most of lignin would not be separated completely. The high and medium states of pretreatment would be left high number of lignin, would effect to touching between enzyme and hemicellulose are tiny when compares with great pretreatment states which effect touching more but a number of hemicellulose are left in small amount.

Lignocellulose materials pretreatment effect lignin detoxification highly such pretreating by alkaline, ozone, organic solvent with AFEX methods.

Table 2.5, 2.6 and 2.7 shows Researched report of lignocellulose material pretreatment in cellulose that affects to lignin reduction

Table 2.5 Lignocellulose materials pretreatment by alkaline method effects to lignin number reduction

Reference	Biomass	Handling	Chemicals	Conc.	L/S	Temperature (°C)	Pressure	Time	Cellulose removal (%)	Hemicellulose removal (%)	Lignin removal (%)	EH cellulose conversion (%)
(Zhao et al.,2008)	Spruce chips	Refining	NaOH NaOH-urea	1-12 1.7-12	20	-	4bar	2-24 hrs	91	40	19	70
(Chen et al.,2007)	Barley and wheat straw	Milling (<1mm)	NaOH	0.5-2.0	9	15,23,60	15 Psi	1 hr	70-90	7-4	20-85	
(Sun et al.,2004;2005a;2005b)	Wheat straw	Cutting Steam explosion (2-3 cm)	NaOH	2	20	50		2.5 hrs	31-40	8-13	14-15	
(Kim et al., 2003)	Corn stover	Grinding	Aqueous NH ₃ /ARP)	15	15	170	-	20 min	>95%	40-60%	70-85	-
(Vrije de et al., 2002)	Miscant Hus	Milling (<1 mm)	NaOH	12	6	70		various	>95%	44%	77	69

Table 2.6 Lignocellulose materials pretreatment by ozone method effects lignin number reducing

Reference	Biomass	Experimental	Cellulose	Hemicellulose	Lignin Removal
Susimoto et al	Sugi sawdust	Never-dried sample (25 g o.d.) was treated with 0.5lmin of O ₂ flow containing 3.5% of O ₃ in a rotating flask. O ₃ consumption was determined from remaining O ₃ trapped in 10% KI solution.			60%
R. Travaini et al	Sugarcane bagasse	The bagasse was oxidized passing and ozone-air flow through a bagasse fixed bed.	Increase 6 to 50%	Increase 2 to 32%	Decreases the total lignin concentration
Ujjal Kaur et al	Cotton stalks	milled cotton stalks were subjected to alkali pretreatment with NaOH at 1-4% (w/v) concentrations at 121 °C for time ranging from 30 to 90 min. Ozone pretreatment was performed by passing 45 mg.L of ozone gas over 2 mm cotton stalks for 150 min at a flow rate of 0.37 L.min.			46.6%

Table 2.7 Shows lignocellulose materials pretreatment by organic solvent method effects lignin number reduction

Reference	Biomass	Remarks	Organic solvent	Catalyst	L/S (%w/w)	Temperature (°C)	Pressure (bar)	Cooking time (hr)	Pulp Yield (%)	Lignin Removal (%)	Cellulose recovery (%)	Hemicellulose removal (%)
(Pasquini et al, 2005)	Sugarcane (Ptaeda) Pine Bagasse /		Ethanol, dioxane, methanol, acetic acid,	sc CO ₂		142 - 198	147 - 232	0.5 - 2.5	33 - 44	88 - 93		
(Papatheofanous et al, 1995)	Wheat straw	Pre-treated Hydrolysis with acid	Ethanol	H ₂ SO ₄		81		1.5	63	>70	>98	50
(Sun et al, 2007)	Wheat straw		Glycerol		15	240	Atm	4		>70	95	>90
(Arato et al, 2005)	Woody biomass		Ethanol		9 - 20	180 - 195	30	0.5 - 1.5		>90		
(Zhang et al, 2007)	Corn stover	Knife milled And screened.	H ₃ PO ₄ / acetone			50	Atm	0.5 - 1		50	95	79
(O'Connor et al, 2007)	Corn stover	Chopped, Pre-soaked.	Ethanol	H ₂ SO ₄	6	170		0.5	40	85	92	91
(Carioca et al, 1985)	Elephant grass		Ethanol		3 - 14	180		1 - 3		70	95	90
(Ibrahim et al, 1999)	Oak (red)	After steam Pre-treatment	Acetic acid		11	60		1		~60		
(Hasegawa et al, 2004)	Oil palm/ Apricot tree Shell wastes		Acetone			200				High		High