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Acetosolv delignification of Dichrostachys cinerea



By:

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Acetosolv delignification of *Dichrostachys cinerea* biomass for ethanol production

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Preface

Submitting this master thesis is the final stage before getting the degree of Master of Science in Sustainable Technology and Resource Recovery. The work has been performed for University of Matanzas (Cuba) and experimental works were performed at University of Matanzas (Cuba) and University of Borås (Sweden). The extent of the project is 30 credits and it was carried out between January and June 2009. I would like to thank the following persons for their contributions to this work.

First of all I would like to thank especially to Dr. Mohammad Taherzadeh (University of Borås, Sweden), and Dr. Carlos Matin Medina (University of Matanzas, Cuba) for the opportunity to do my master thesis in the field of bio-fuels. It has created a genuine interest to me for further research in biofuel production. Secondly, I want to express my sincere gratitude to my supervisor Professor Dani Rodríguez Alvarez (University of Matanzas, Cuba) for guiding me through this project. I appreciate his enthusiasm, great helping nature, and never ending source of ideas. I wish him and his family the best in becoming future life.

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Abstract

The interest in production of fuel ethanol from lignocellulosic materials is continuously increasing due to the urgency of finding non-food substrates for production of bio-fuels. Marabou (*Dichrostachys cinerea*) is one of the abundant lignocellulosic bio-resources in Cuba, and it could be useful to produce bio-ethanol. Pre-treatment is an important step to produce ethanol from lignocellulosic materials since it allows the separation of cellulose, hemicellulose and lignin, and activates cellulose towards enzymatic hydrolysis. During the past few years, organosolv methods have been reported for effective separation of the main components of lignocellulosic materials and improvement of the enzymatic hydrolysis of cellulose. By using acetosolv method lignin is separated under mild conditions and many of the lignin properties are well preserved.

The present work was aimed to perform a chemical characterisation of marabou biomass and to evaluate acetosolv delignification of the material. In this work the content of moisture, ash, extractives, easy-to-hydrolyze polysaccharides, difficult-to-hydrolyze polysaccharides, and Klason lignin of marabou biomass were analyzed. Klason lignin of the marabou biomass was 23.4% of the mass. Acetosolv delignification was performed at normal boiling temperature (NBT) and 121°C, using 50-50, 70-30 and 90-10 acetic acid – water mixtures with 10% of solids load during 1h. Hydrochloric acid (0.2g / 100g of mixture) was used as catalyst. The delignification of marabou biomass was also evaluated for the combination of dilute acid pre-hydrolysis (DAPH) and acetosolv with the same reaction conditions. This investigation proved that acetosolv pretreatment was effective for solubilizing lignin contained in marabou biomass. The degree of lignin solubilisation increased with increasing acetic acid concentration in the reaction mixture. Lignin removals above 80% were achieved consistently both at NBT and 121°C with 90% acetic acid, while only around 44.6 and 6.8% of the initial lignin was removed using, respectively, 70 and 50% acetic acid at 121°C. The effect of temperature on delignification was only marginal when acetosolv was conducted with 90% acetic acid, but it was remarkable for lower acetic acid concentrations. A two-fold decrease of lignin removal was observed for the NBT acetosolv compared with the process performed at 121°C using both 70 and 50% acetic acid. The insertion of a DAPH step prior to acetosolv considerably improved lignin removal using 70 and 50% acetic acid at both temperatures, but its effect on the processes using 90% acetic acid was minimal. High lignin yields were achieved upon its precipitation from acetosolv liquors.

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1. Introduction

1.1 Biofuels and the environment

It is confirmed that the world is facing a climate change. During the period 1906-2005 over 100 years the global surface temperature increased by approximately 0.7 degrees⁵. Effects of the increasing in atmosphere temperature are raising sea levels, melting glaciers, unusual monsoons and shifting climate zones. Increasing green house gases in the atmosphere are the one reason for increase in atmosphere temperature. Carbon dioxide (CO₂), methane (CH₄), and nitrous oxide (N₂O) are the three major green house gases; in these CO₂ is the most important. These gases affect the absorption, scattering and emission of solar radiation with in the atmosphere and the earth's surface, which causes the atmospheric temperature to rise. Between 1970 and 2004 emissions of the green house gases have increased to 70 percent.⁵

Most of the CO₂ that is released in the atmosphere is due to the combustion of fossil fuels. Fossil fuels are mostly using in transportation sector, and small amounts are using in industrial and commercial sectors. Today, most of the transportation is depending on gasoline and diesel as main fuels. The world consumption of oil is 30 billion barrels per year (2005)⁵ and the demand will continue to increase because of the development that is taking place around the world. It is believed that fossil fuels are limited and these can't be renewable in a short period of time. These fuels can't be replenished at the rate that is consumed⁶. There are indications that in a near future, the oil reserves that are easiest to extract will start to deplete. And there is more reserves exist but they are more expensive to exploit and the extraction will have a large environmental impacts and release more carbon dioxide. The decrease in oil supply is indeed a problem for transportation sector⁵. A great challenge is therefore to develop the non fossil-fuel production which would also result in reduced CO₂ emissions⁵, for energy, environmental and economical security of all the nations.

Using biofuels is one of the solutions to increase energy independence and security and a sustainable way to reduce greenhouse gas emissions. Biofuels are combustible fuels that are derived directly or indirectly from biomass like plants or organic wastes. Biofuels are renewable which can replace or reduce the use of fossil fuels. There are a lot of renewable fuels on the market already, such as bio-diesel from rape seed oil (RME), biogas produced from anaerobic digestion of waste and ethanol from sugar and starch⁵. These are called first generation Biofuels and are based upon farm products¹. When bio-fuels are combusted it release CO₂ as like fossil fuels; but the CO₂ that release by the combustion of bio-fuels will not accumulate in the atmosphere it will absorb and transform from CO₂ to O₂ in the process of photosynthesis by the plant biomass to re growth. Figure1 shows the carbon cycle in the usage of bio-fuels. Liquid bio-fuels (ethanol and diesel) can readily replace conventional transportation fuels without major modifications in current transportation technologies. The usage of bio-fuels has been increasing drastically during the past few years. Figure 2 and 3 showing the projected global bio-ethanol

and diesel production. It is estimated that by 2010, global biodiesel production will be three times that of 2005, and the global biofuel ethanol production in 2010 is expected to be close to double to the production in 2005⁸.

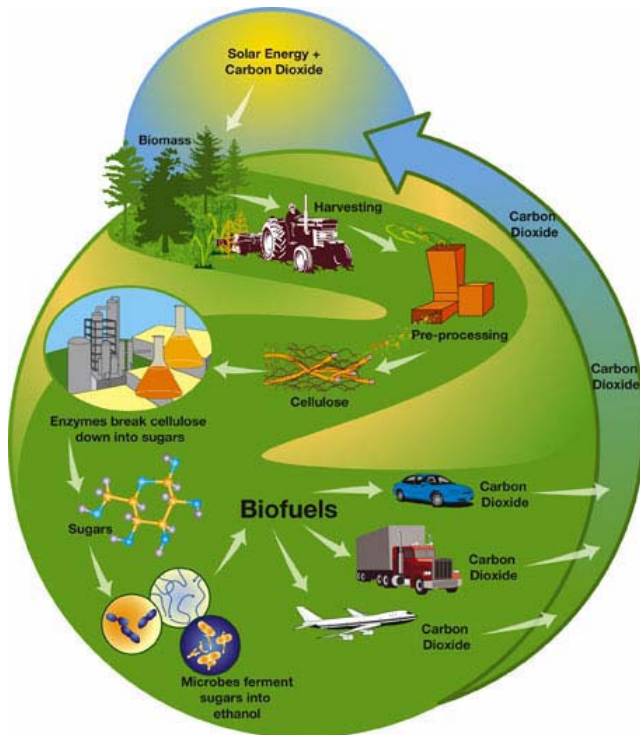


Figure 1: Carbon cycle of Biofuels. Adapted from [7]

Production of fuels with farm products by using farm lands has becoming a controversial issue; whether farmlands should be used for food or fuel production, even if other factors are more important for the increase in food prices. During the past few years prices of corn and wheat has been increased since they are using in ethanol production. During the last year, the price of wheat has doubled in Sweden and today it is not economically feasible to produce ethanol from wheat^{5,6}.

Lignocellulosic materials from forestry and agriculture are an alternative feed stock to produce bio-fuels. Production of fuels from lignocellulosic materials does not compete with food production. This group is called the second generation of Biofuels that are include ethanol from lignocellulose and fuels that are synthesized from gas

produced by gasification of biomass. This is an area that needs further development^{5,6}.

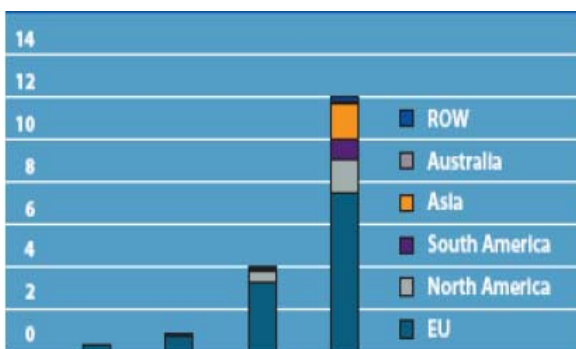


Figure 2: Global biodiesel production, 1995 – 2010
In million tones. Adapted from [8]

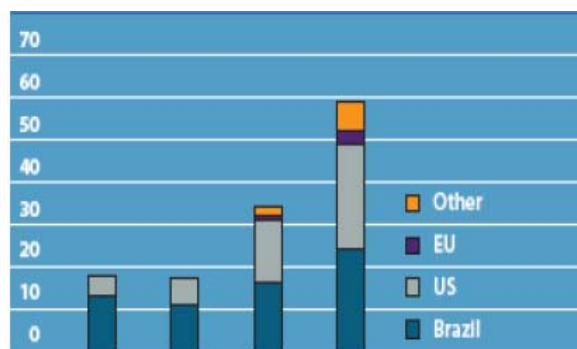


Figure 3: Global fuel ethanol production, 1995 -2010
in billion liters. Adapted from [8]

1.2 Bio-ethanol

Bio-ethanol can play an important role as a renewable alternative for fossil fuels. Ethanol is one of the modern forms of energy derived from biomass, it has the potential to replace gasoline as a transportation fuel as well as fuel oxygenate. Bio-ethanol is regarded as a promising biofuel substitute for gasoline in the transportation sector because of its environmental benefits. The main feed stocks for ethanol production can be conveniently classified in to three types; they are sucrose-containing feed stocks (e.g. sugar beet, sweet sorghum and sugar cane), starchy materials (e.g. wheat, corn, and barley) and lignocellulosic biomass (e.g. wood, straw, and grasses)¹⁰. In 2006 world ethanol production has reached about 51,000 million liters, in this 73% corresponds to fuel ethanol, 17% to beverage ethanol and 10% to industrial ethanol. USA and Brazil are the leading ethanol producing countries in the world they are contributing about 62% of world ethanol production. The main feed stock for ethanol in Brazil is sugar cane while corn grains are in USA. China and India are the major ethanol producers in Asia; while Spain, France, and Sweden are in Europe.^{6, 10}

Availability of feedstock is one of the major problems to bio ethanol production it can vary from season to season and depends on geographic locations. Ethanol can be produce from any sugar and starch based crops. Now a day's most of the ethanol has been producing from sugar and starch based materials around the world. Figure 4 illustrates the development of biodiesel and bio-ethanol demand and the incorporation rate until 2020 in the EU-27. It is well known that the bio-ethanol demand would increase each and every year. And it is not possible that sugar and starch based materials will not cover the demand and this will increase the price of foods which will leads to other problems. It is necessary to produce bio-ethanol with lower cost to be competitive with gasoline and the raw material cost has great impact in the bio-ethanol production.⁶

However there are some conflicts between the human food use and industrial use of crops, and agricultural lands used to energy crops dedicated to the bio fuels. To solve this problem it is necessary to produce bio-ethanol from other alternative feed stocks. Lignocellulosic biomass is the most promising biomass feed stock because of its great availability and low cost. And these materials are considered to be the main source of feedstock for low cost bio-ethanol production. Utilization of lignocellulosic biomass which includes materials such as agricultural residues (e.g. corn Stover, rice straw, sugar cane bagases, etc...), herbaceous crops (e.g., alfalfa, Switch grass), forestry wastes, wastepaper, etc... is still in under development and it is believed that the research work with in this area will decrease the cost of bio-ethanol production from lignocellulosic materials.

The available lignocellulosic biomass could produce up to 442 GL of bio-ethanol per year; totally around 491 GL year-1 of ethanol can be potentially produce from crop residues and wasted crops^{9, 10}. This lignocellulosic ethanol is about 16 times higher than the current world ethanol production. This amount of bioethanol can replace about 32% of global gasoline usage and from the lignin which is a byproduct of ethanol production from crop residue can potentially generate 458 TW h of electricity which is equal to about 3.6% of world electricity production and 2:6 EJ of steam. To produce ethanol from crop residues Asia has the largest potential; it could produce 291 GL year-1 of bio ethanol⁹. Rice straw, wheat straw, and corn stover are the

most favorable lignocellulosic feed stocks for ethanol production in Asia while wheat straw is in Europe and corn stover in North America. In Europe around 69.2% GL year-1 of bioethanol can produce from wheat straw while 38.4 GLyear-1 of bioethanol can potentially produce from corn stover in North America.^{9,10}

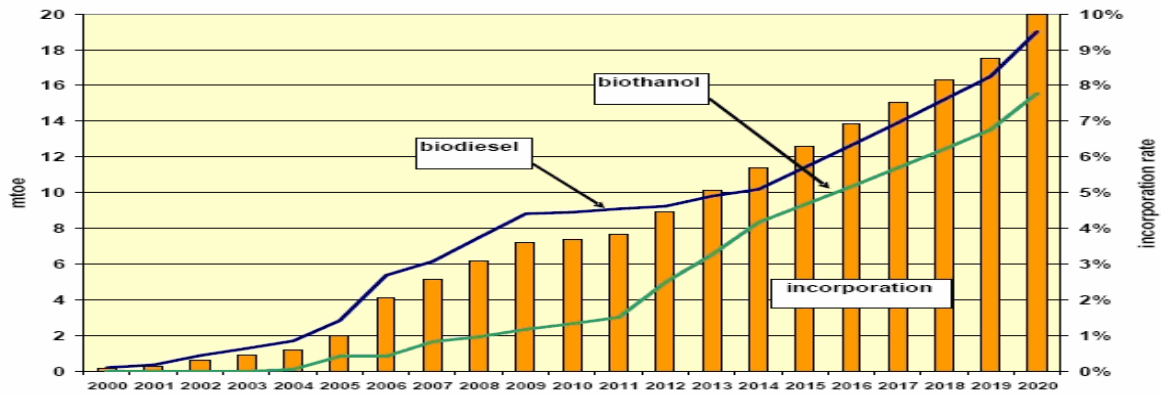


Figure 4: Illustrative development of biodiesel and bio-ethanol demand and the incorporation rate until 2020 in the EU-27. Adapted from [9]

Due to the environmental, social, and energy security issues the interest in fuel ethanol on a global scale has been growing in the past few years. The market for ethanol is predicted to increase greatly in the future due to the rise of energy demand in transportation; the rise of oil due to its slower production; and global concerns about environmental and energy security issues. The predicted ethanol demand in 2010 is double compare to 2006 where the share of ethanol increases from 5% to 54% by 2050 respectively. However ethanol from lignocellulosic materials is still in underdevelopment and even though ethanol has not been largely produced from lignocellulosic materials, it is predicted that the use of lignocellulosic feedstock will increase dramatically in the near future and it will become the main resource for ethanol production.¹¹

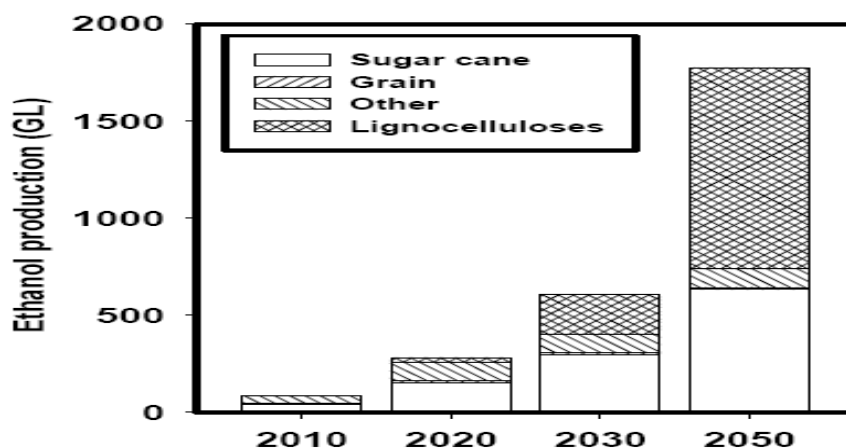


Figure 5: Ethanol potential production from different feed stocks. Adapted from [11]

1.3 Lignocellulosic Materials & Composition

Lignocellulosic biomass which has source range from trees to agricultural residues is one of the most abundant materials in the world, it represents the major fraction of most plant matter.^{12, 13} From the past these materials has been using as fire wood, building materials and animal food. Nowadays, applications of lignocellulosic materials have expanded into the fiber level as in pulp and paper products. In some cases lignocellulosic materials are also using to produce chemical components. For example cellulose can be used for fibers in the textile industry and lignin is used as adhesive component in the composite industry.¹²

The main composition of the lignocellulosic materials contains four chemical components they are cellulose, hemicelluloses, lignin and extractives¹². Genetic variation of species, growth conditions and age of the plant are influences the compositions of the woods cell wall. In general, cellulose, hemicelluloses and lignin have the high molecular weights and contribute much mass of the lignocellulosic materials. And the extractives are of small molecular size and these available in little quantity. Lignocellulosic materials are made up of high complex crystalline structure with the matrix of cellulose and lignin bound by hemicellulose chains. The basic polymers of the lignocellulosic biomass structure consists: cellulose $(C_6H_{10}O_5)_x$, hemicelluloses such as xylan $(C_5H_8O_4)_m$, and lignin $[C_9H_{10}O_3 - (OCH_3)_{0.9-1.7}]_n$ ¹⁰. Generally cellulose and hemicelluloses are higher in hard woods (e.g. alder, aspen and birch) compared to soft woods (e.g. pine and spruce), while soft woods have higher lignin content compare to hard woods. The major fraction of lignocellulosic materials contains 35-50% cellulose, 20-35% hemicellulose and 15-25% lignin and small amounts of other compounds such as plant oils, proteins, ash¹³.

1.3.1 Cellulose

Cellulose is the primary structural component of green plants. Cellulose content of wood varies between the species¹²; and the cellulose fibers comprise 40-50 wt% of dry wood that strength's the wood¹⁰. The cellulose in a plant consists of parts with a crystalline (well organized) structure and parts with an amorphous (not well organized) structure. Generally cellulose strains are 'bundled' together and forms cellulose fibrils or cellulose bundles.¹⁴

Cellulose is a homo-polysaccharide that composed of β -D-glucopyranose units linked together by (1-4)-glycosidic bonds. The cellulose molecules are linear; the β -D-glucopyranose chain units are in a chair conformation and the substituent's HO₂, HO₃ and CH₂OH are oriented equatorially. Glucose anhydride forms by the removal of water from each glucose, the anhydride glucose polymerized into long cellulose chains which contain 5000-10,000 glucose units¹⁰. The basic repeating unit of the cellulose polymer consists of two glucose anhydride units, which called as cellobiose units. The β - linkages form linear chains that are resistant to chemical attack because of the forming intra-molecular and intermolecular hydrogen bonds between OH groups within the same cellulose chain and the surrounding cellulose chains, the chains tend to arrange in parallel and form a crystalline super-molecular structure^{6, 12}. Figure 6 shows the schematic illustration of cellulose structure.

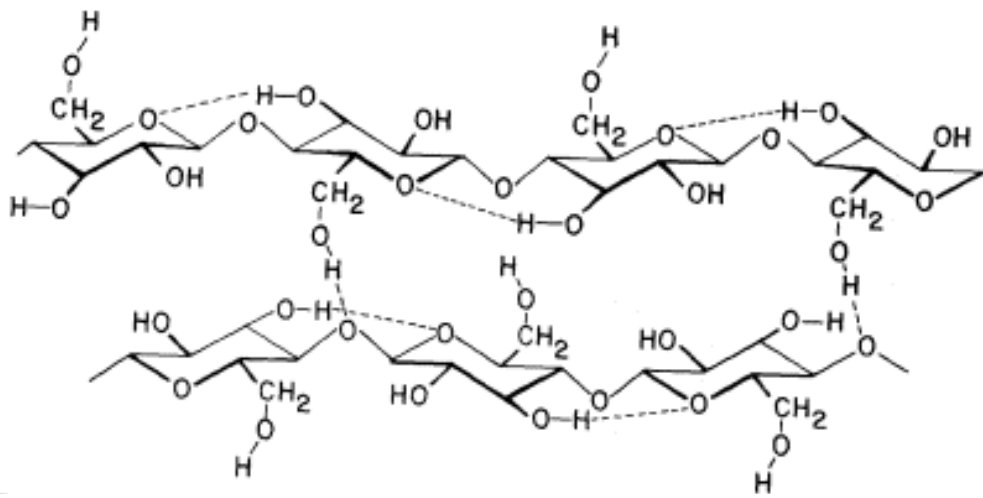


Figure 6: β -1,4 linked glucan chains of cellulose portions of two associated chains are illustrated by conformational line drawings. Adapted from [12]

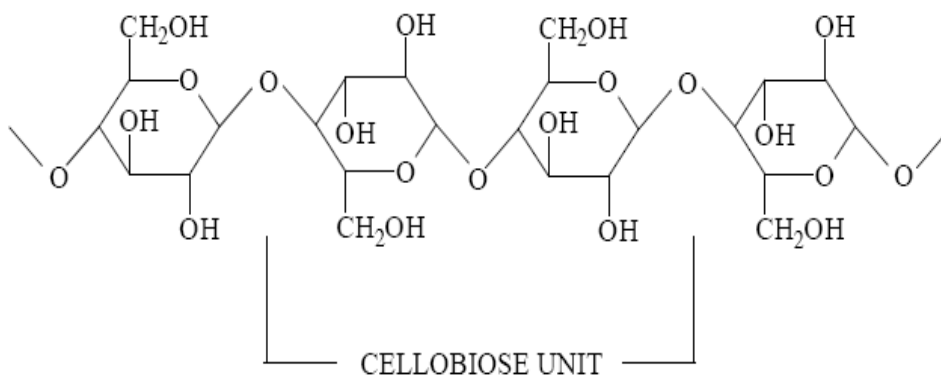


Figure 7: Schematic illustration of cellulose chain. Adapted from [12]

1.3.2 Hemicelluloses

The second major chemical constituents of wood are the hemicelluloses, which are also known as polyoses¹⁰. They have a complex carbohydrate structure and their molecular weight is lower than that of cellulose⁴. Unlike cellulose, hemicelluloses consist of different monosaccharide units. In addition, they contain shorter polymer chains than cellulose and are amorphous. Due to their amorphous morphology hemicelluloses are partially soluble or swellable in water.¹² Hemicelluloses serve as a connection between the cellulose and lignin fibers and give the more rigidity network of whole cellulose-hemicellulose-lignin. Hemicellulose is the most thermal-chemical sensitive component in cellulose, hemicellulose and lignin¹⁴.

Hemicelluloses are branched polymers of various monosaccharides like pentoses (D-xylose and L-arabinose), hexoses (D-mannose, D-glucose and D-galactose)^{6,10} and sugar acids like 4-O-methyl glucuronic acid and galacturonic acid. The main component of the chains of hemicelluloses can be a homopolymer (single sugar repeat unit) or a heteropolymer (mixture of different sugars). Hemicelluloses usually share about 25-35% of the dry wood mass, 28% in softwoods and 35% in hardwoods¹⁰. Xylose is the most important sugar component of the hemicelluloses and it is the predominant pentose sugar derived from the hemicellulose of most hardwood feed stocks and arabinose and other hexoses can constitute significant amounts. The dominant component of hemicellulose from hardwood is xylan, while this is glucomannan for softwood. The solubility of different hemicellulose compounds is in decreasing order of mannose, xylose, glucose, arabinose, and galactose. The solubility's of these compounds increase with increasing temperature.¹⁴

The backbone chain in hardwood xylan consists of xylose units which are linked by β -(1, 4)-glycosidic bonds and branched by α -(1, 2)-glycosidic bonds with 4-O-methylglucuronic acid groups. Some times O-acetyl groups replace the OH-groups in position C2 and C3. Acetyl groups are fewer in the backbone chain for softwood xylan and softwood xylan has additional branches with arabinofuranose units linked by α -(1, 3)-glycosidic bonds to the backbone. Starch is the only structure that has linear and branched chains among the carbohydrates components of hemicellulose. The linear chain is known as amylose and the branched chains are known as amylopectin. The anhydroglucose units of linear chain (amylose) are linked by α -(1, 4)-glycosidic bonds. And backbone of the amylopectin is like amylose but it also has α -(1, 6)-glycosidic bonds at branch position¹². Figure 8 shows the different sugar units of hemicelluloses.

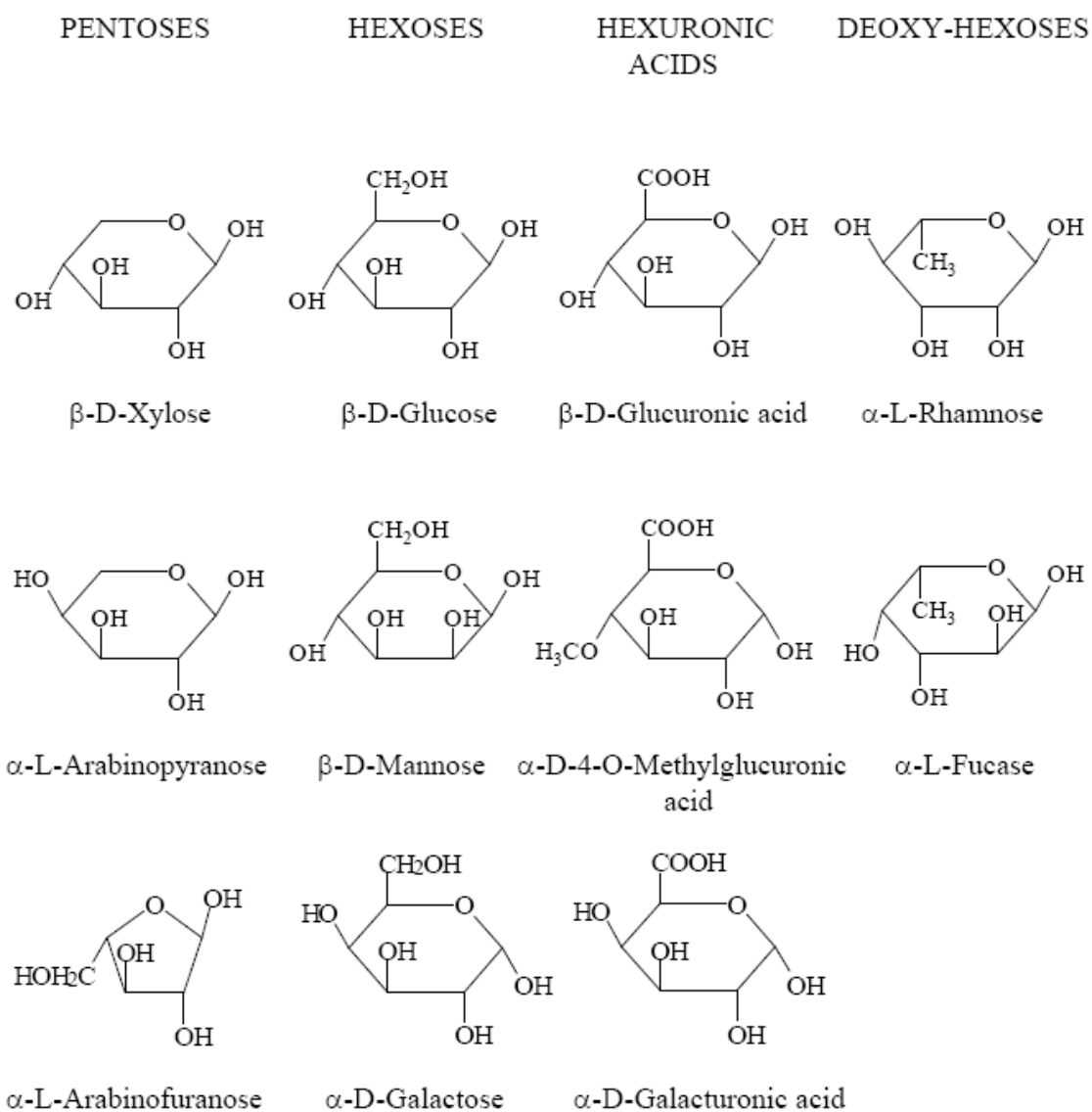
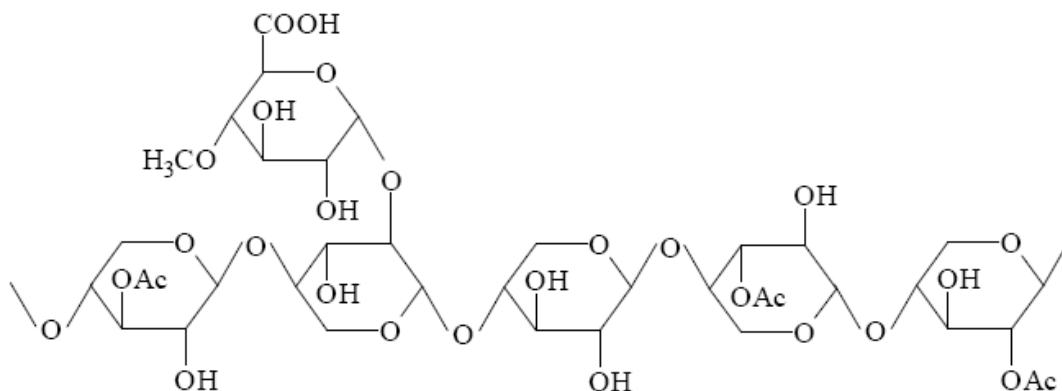
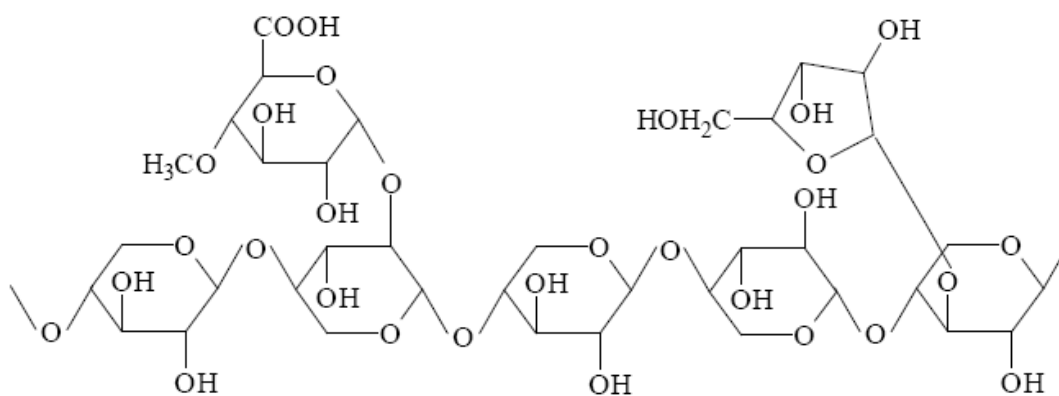


Figure 8: Schematic illustration of sugar units of hemicelluloses. Adapted from [12]



A



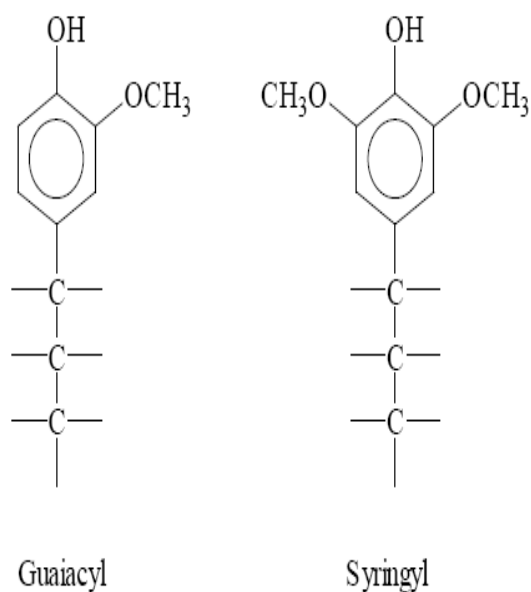
B

Figure 9: A – Partial xylan structure from hardwood chain.

B – Partial xylan structure from softwood chain.

1.3.3 Lignin

After cellulose and hemicelluloses lignin is one of the most abundant biopolymers in the nature¹⁴. It is a highly branched, substituted, mononuclear aromatic complex polymer and one of the slowly decomposing components. Lignin is made up of three different phenyl-propane units (p-coumaryl, coniferyl and sinapyl alcohol)¹⁴, these units are cross-linked to each other with different chemical bonds that form a large molecular structure. Lignin works as a reinforcing agent for gluing the fibers together¹²; it fills the space between cellulose and hemicellulose of cell walls to form a lignocellulosic complex, mostly by hydrogen bonds but also by covalent bonds. Because of these bonds it is especially resistant to chemical attack or enzymatic degradation. The amorphous heteropolymer is also non-water soluble and optically inactive; all this makes the degradation of lignin very tough¹⁴. There are some fungi and bacteria that are able to biodegrade lignin but it can persist for long time.^{6, 10}



Lignin gives mechanical strength to wood. The main function of lignin is to give the plant structural support, impermeability, and resistance against microbial attack and oxidative stress¹⁴. The lignin contents in both softwoods and hardwoods in the range from 20% to 30% by dry weight basis¹⁰. In hardwoods lignin syringyl units are dominant while guaiacyl units are dominant in the softwoods¹². The monomeric building units of lignin are shown in Figure 9.

Figure 10: Schematic illustration of building units of lignin. Adapted from [12]

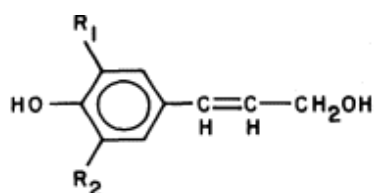


Figure 11: Lignin. Adapted from [6]

1.3.4 Extractives

Extractives are organic substances which have low molecular weight¹²; and these are a group of chemicals that exists in their monomers, dimers or polymers forms in cell wall. The chemicals mainly consists of fats, fatty acids, fatty alcohols, phenols, terpenes, steroids, resin acids, rosin, waxes, and many other minor organic compounds^{6,12}. Extractives represent only between 4-10% of the total weight of dry wood¹², the content of extractives is higher in softwoods than hardwoods and it varies among the species, geographical site and season. These extractives can be extracted from the woods by using solvents. The extractives can be classified by the solvents used to extract them, for example, water soluble or toluene-ethanol soluble or ether soluble extractives⁶. Some extractives contains toxic property this is an advantage for the wood to resist the attack by fungi and termites¹².

1.4 Bio-ethanol production from lignocellulosic materials

Bioconversion of lignocellulosic materials following a bio-refinery philosophy allows producing fuel bioethanol plus added value co-products. For example, sugars may be subjected to bacterial fermentation under aerobic and anaerobic conditions to produce a variety of products, including lactic acid that can be used to produce other products and the non-carbohydrate components of lignin also have potential for use in value added applications.¹⁰

However, production of ethanol from lignocellulosic materials is a complex process because of the high crystalline matrix structure of the materials. The production process mainly consists in four different steps, which are Pretreatment, Hydrolysis, Fermentation, Product separation/distillation, and Post treatment of the liquid fraction^{14,10}. Pretreatment is a necessary step to improve the production rate and the total yield of monomeric sugars in the hydrolysis step. In hydrolysis process, cellulose and hemicelluloses (unconverted fraction from the pretreatment) are converted in to monomeric sugars. It can be done chemically by acids or enzymatically by addition of cellulases (enzymes that are responsible for hydrolysis of cellulose). The produced monomeric sugars include both pentoses and hexoses, which can be fermented to ethanol. Hexoses can be fermented quite easily, but the fermentation of pentoses is only done by a few organisms. There are some problems that can occur in fermentation which can puts a limit to the concentration of fermentable sugars and can inhibit, or even stop the fermentation. Ethanol itself is an inhibitor for the yeasts/bacteria that perform the fermentation and the compounds like furans, phenolic, carboxylic acids and other soluble lignin compounds can form in the process which can inhibits the fermentation. After the fermentation ethanol recovers from the fermentation broth by distillation process.¹⁴ The residuals' (cellulose, hemicellulose, lignin and other solid materials) that are left from the process can be burned as boiler fuel to provide energy and power, can convert into octane boosters, or can be use as feed stocks for production of chemicals¹³.

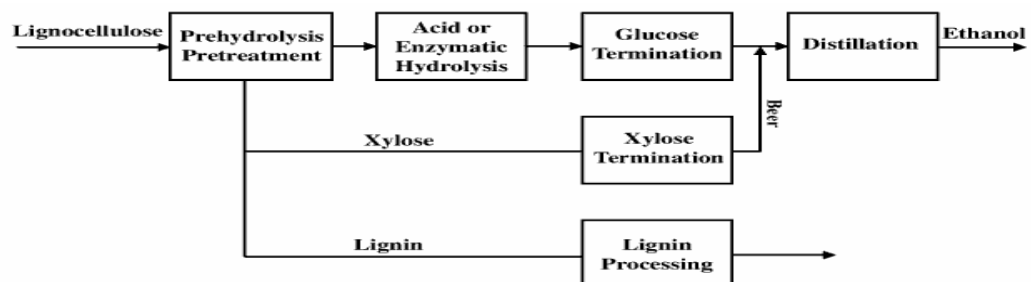


Figure 12: Schematic diagram of main units in ethanol production from lignocellulosic materials

1.5 *Dichrostachys cinerea* (Marabou):-

Dichrostachys cinerea is also known as a Bell mimosa, Chinese lantern tree, Kalahari Christmas tree and sicklebush, etc... The generic name 'Dichrostachys' obtained from the Greek 'konis' it refers to '2-coloured spike' and 'cinerea' obtained from the Latin 'cineres' it refers to the grayish hairs of the typical subspecies, which is confined to India. It is native mostly to the Africa and parts of Southeast Asia, during the 19th century the tree was introduced to parts of the Caribbean and is known to have been introduced mainly to parts of Cuba, Hispaniola, Guadeloupe, Marie-Galante, and Martinique.

Dichrostachys cinerea is a semi-deciduous to deciduous tree. These trees can typically grow up to 7 meters in height and contains strong alternate thorns; these thorns generally have up to 8 cm long. The *Dichrostachys cinerea* tree generally grows at (6-8 cm per year) medium to slow rate. Flowers of the *Dichrostachys cinerea* are characteristically in bi-colored cylindrical spikes that resemble Chinese lanterns and are 6-8 cm long and fragrant. The pods are usually mustard brown and are generally twisted or spiraled and may be up to 100 x 15 mm ^[21]. The species has subcategorized in to *D.cinerea* ssp, *africana* and *D. cinerea* ssp, and *nyassana* with two slight variations that have been recognized, which is typically larger and less hairy in its foliage. ^[21, 23]

Natural Habitat: - *Dichrostachys cinerea* can easily penetrates clear cut areas far into the rainforest zone. It occurs in areas with strong seasonal climate, clayey soils, in brushwood, thickets, hedges, teak forest and grassland, dense hammocks on lateritic soils. In India it occurs in dry deciduous forest. *Dichrostachys cinerea* is fire resistant and it does not tolerate water logging. And it can be an indicator of overgrazing in low rainfall areas. ^[21, 22, 23]

Biophysical limits: -

The species grows in rainforest zones and in altitudes up to 2,000 meters. It often occurs in strong seasonal climate areas with a wide ranging mean annual temperature -2 to 50 deg. C, and with a mean annual rainfall ranging from 200-400 mm. And it occurs in brushwood, thickets, hedges, teak forest and grassland and generally takes to poorer quality clay soils or deep and sandy soils with a wide pH scale range. ^[21, 23]

Propagation:

Seeds, root cuttings, root suckers has been majorly playing an important role in *Dichrostachys cinerea* propagation. In general about 1 million seeds are expected from a plant in a year and these trees produce seeds almost all year. Because of advancing horizontal root system and by means of the seeds this tree spreads in all directions. The distribution of the tree is probably effected by the driven or transportation of seeds over the island and also animals like cattle greatly relish the seeds, and it is possible that they are capable of germination after passing through the animal. Marabou is a well developed tap root that it penetrates to a considerable depth in the soil and lateral horizontal root extends unusual distance in all directions, these roots that makes eradication difficult. The marabou tree develops even if the parent tree is cut down and their removal is attempted, any small section of the root left in the soil. ^[21, 23, 25]

Uses:-

Fruits and seeds of *Dichrostachys cinerea* are edible, these fruits, seeds and immature twigs, leaves of the tree that are rich in protein (11-15%) and minerals are feed to the animals like cattle, camels, game such as giraffe, buffalo, kudu, hartebeest, nyala, red duiker and damara dikdik, etc... The flowers of the *Dichrostachys cinerea* can be a valuable source of honey. And the wood of *Dichrostachys cinerea* is of a dense nature and in burning slowly with no toxicity; it is often used as fuel wood. The species yields has a medium to heavy, durable hard wood with a density of 600-1190 kg/cubic m at 15% mc, and are often used in smaller domestic items as walking sticks, handles, spears and tool handles, etc... In medicine uses, the bark is used to alleviate headache, toothache, dysentery, elephantiasis and the root infusions are useful to treat leprosy, syphilis coughs, as an anthelmintic, purgative and strong diuretic. The leaves of the tree are particularly useful and can be beaten to treat epilepsy and can also be taken as a diuretic and laxative, and its powder can be used to massage in the treatment of fractures. The roots of the tree have been used to treat bites or stings. As these roots are rich in nutrients they are often used as manure in the Sahel region of Africa along the riverbanks. Particularly in India the plant is widely used for soil conservation, for shallow soils, and in arid western and sub humid alluvial plains. Although *Dichrostachys cinerea* has its uses, it is considered as a threat to agricultural production and is listed on the global invasive species database. ^[21, 23]

Management:-

It is necessary to control the propagation of *Dichrostachys cinerea* because it causes losses in agricultural production. And frequent management is necessary even if it is heavy and expensive work. In certain situations physical or chemical methods are only ways to control the propagation of this species. Physical management involves cutting, burning of the plants but it is not an efficient control method, since the seeds survive in the soil, and growth is very fast. Usage of dangerous herbicides is often necessary in chemical management. ^[22,23]

In Cuba *Dichrostachys cinerea* forms dense, impenetrable thickets. And it invades fields, wastelands, road sides, and other disturbed areas. Another correspondent wrote that whole farms in central Cuba have been rendered useless by this foreign nuisance without any effort being made to check the curse and that good farm lands are being abandoned in disgust. In Cuba whenever the farm lands for cane fails because of one cause or another, to produce the required tonnage, it is frequently allowed to run wild or revert to pasture. On such lands, on such areas, the Marabu (*Dichrostachys cinerea*) finds optimum conditions for growth. In Cuba small patches of the Marabu tree make their appearance here and there over the areas. *Dichrostachys cinerea* which is natively called as Marabu in Cuba is a problematic species in Cuba. And risk assessment results showing that it has high risk which has 16 score. ^[22, 23, 25]

1.6 Pretreatment

Pretreatment is an important step in bioconversion of lignocellulosic materials to bioethanol. In the pretreatment process, the cellulose matrix should be broken in order to reduce its crystallinity, to increase the fraction of amorphous cellulose and to increase the porosity of the material¹⁵. The main goal of pretreatment step is to open the crystalline structure of the substrate, to make cellulose more accessible to the enzyme.¹⁰ It should hydrolyze most of the hemicellulose fraction, and lignin should be released or even degraded¹⁶.

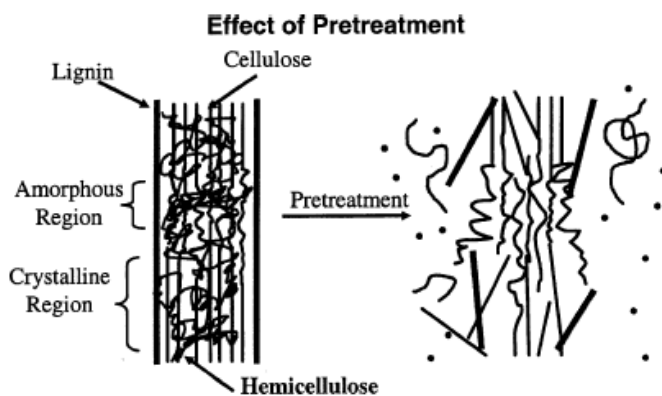


Figure 13: Goals of pretreatment on lignocellulosic materials. Adapted from [17]

Pretreatment promotes the physical disruption of the lignocellulosic matrix in order to facilitate acid or enzyme catalyzed hydrolysis. Pretreatment can be carried out in different ways, there are several methods have been proposed and developed to pre-treat the lignocellulosic materials. Pretreatment methods have been categorized mainly in four groups they are Physical, Physical-Chemical, Chemical and Biological pretreatment methods^{15, 16}. Different pretreatment methods have their specific advantages and

disadvantages. The main pretreatment methods have shown in table 1.

Table 1: Pretreatment methods for lignocellulosic materials.

Physical methods	Physical-chemical Methods	Chemical Methods	Biological methods
<ul style="list-style-type: none"> ➤ Mechanical Pre-treatment. ➤ Pyrolysis 	<ul style="list-style-type: none"> ➤ Steam explosion. ➤ Liquid hot water (LHW). ➤ Ammonia fiber explosion (AFEX). ➤ CO₂ explosion. 	<ul style="list-style-type: none"> ➤ Ozonolysis. ➤ Dilute-acid hydrolysis. ➤ Concentrated-acid hydrolysis. ➤ Alkaline hydrolysis. ➤ Oxidative delignification. ➤ Wet oxidation. ➤ Organosolv process. 	<ul style="list-style-type: none"> ➤ Fungal pretreatment.

A successful pre-treatment must meet the following requirements:

- (i) Improve formation of sugars or the ability to subsequently form sugars by hydrolysis
- (ii) Avoid degradation or loss of carbohydrate
- (iii) Avoid formation of byproducts inhibitory to subsequent hydrolysis and fermentation processes

(iv) Should be cost effective¹⁰

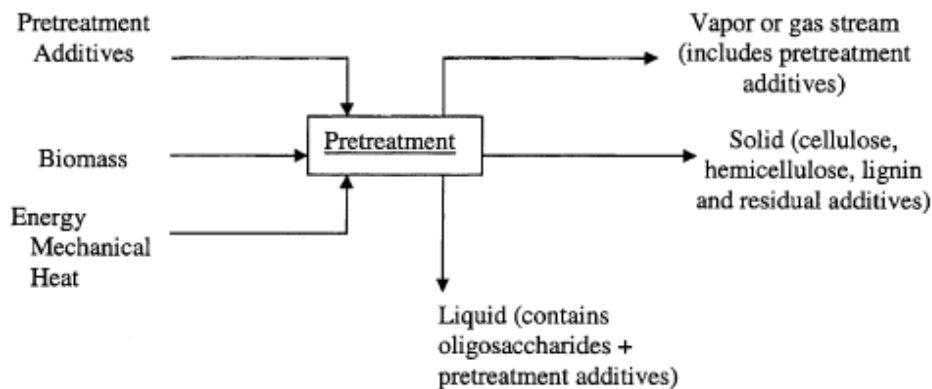


Figure 14: Schematic of pretreatment process. Adapted from [17]

The choice of pretreatment technology highly influences the cost and performance in subsequent hydrolysis and fermentation. Techno economical analyses have shown that pretreatment is the most significant determinant thing in the success of cellulosic bioethanol technology because it defines the extent to and cost at which the carbohydrates of cellulose and hemicellulose can be converted to bioethanol. Cost effective pretreatment of cellulosic biomass is a major challenge in the cellulosic bioethanol production. Through extensive research and development there is a huge scope to lowering the cost of pretreatment process.

1.6.1 Physical Pretreatment methods

Mechanical operation such as chipping, grinding and milling can be reduce the crystallinity of cellulosic materials. This facilitates the access of enzymes to the biomass surface increasing the cellulose conversion. Mechanical pretreatment methods increase cellulose reactivity towards enzymatic hydrolysis¹⁶. Lignocellulosic materials can be pre-treat with the combination of different mechanical methods and the energy requirement for these operations depends on the final particle size and biomass characteristics^{16, 18}. The size of the materials is generally 10-30 mm after chipping and 0.2-2 mm after milling or grinding¹⁸. Energy requirement with respect to size for the mechanical pretreatment of hardwood materials has shown in table 2.

Table 2: Energy requirement of mechanical comminution of agricultural lignocellulosic materials with different size reduction (Source 2)

Lignocellulosic materials	Final size (mm)	Energy consumption ($\frac{\text{kWh}}{\text{ton}}$)	
		Knife mill	Hammer mill
Hardwood	1.60	130	130
	2.54	80	120
	3.2	50	115
	6.35	25	95

The main objectives of these processes are: size reduction of lignocellulosic materials to increase available surface area, reduce the degree of polymerization and reduction of crystallinity that lead to improve cellulose hydrolysis. However mechanical pretreatment methods are unattractive due to their high energy consumption and capital costs. And these do not remove lignin or hemicelluloses finally and consequently produce inhibitors for further processes^{19, 20}.

Cellulose rapidly decomposes when it is treated at high temperature. Since it decomposes at higher temperatures pyrolysis has also been tested as a physical method to pretreatment the lignocellulosic biomass¹⁶. When the lignocellulosic materials are treated at temperatures greater than 300°C, cellulose rapidly decomposes to produce gaseous products and residual char¹⁸. At low temperatures the decomposition of cellulose is much slower and less volatile products are formed. The decomposition of pure cellulose can occur at low temperatures when zinc chloride or sodium carbonate is added as a catalyst. However under mild acid hydrolysis (1N H₂SO₄, 97°C, 2.5 h) of the residues from pyrolysis pretreatment has given 80–85% conversion of cellulose to reducing sugars with more than 50% glucose. And with the presence of oxyzen the process can be enhanced.¹⁸

1.6.2 Physical-Chemical pretreatment methods:

Some pretreatment methods are incorporates both physical and chemical effects these methods are called physical-chemical pretreatment methods. Physical-Chemical pretreatment methods are considerably more effective than physical methods¹⁶. As shown in table 1 the main physical-chemical methods are Steam explosion, Liquid hot water (LHW) method, Ammonia fiber explosion (AFEX), and CO₂ explosion.

Steam Explosion:

In physical-chemical pretreatment methods steam explosion is the most studied one¹⁶. Steam explosion is one of the biomass fractionation processes and it involves extrusion of the biomass at high temperature and pressure¹⁰. Steam explosion process uses saturated steam at high pressure that causes auto-hydrolysis reactions in which part of the hemicellulose and lignin are converted into soluble oligomers. In this process high pressure and high temperature steam introduces in to a sealed chamber which contains lignocellulosic material, after 1-5 min the pressure is releases through a small open that causes the steam to expand with the lignocellulosic matrix, this separates the individual fibers with minimum fibers loss of the material. To hydrolyze hemicellulose, the biomass/steam mixture is held's for a period of time. During this process hemicelluloses though to be hydrolyzed by the acetic and other acids released during steam explosion pretreatment. Residence time, temperature, chip size and moisture content are the affective factors in steam explosion.

This method is recognized as one of the most cost-effective for hardwoods and agricultural residues, but it is less efficient for soft woods¹⁶. Pretreatment that involves lignocellulosic biomass is rapidly heated by high pressure steam without addition of any chemicals is refers to un-catalyzed steam explosion. With the addition of an acid catalyst such as H₂SO₄ or SO₂ is a prerequisite to reach high sugar yields with softwood. Using acid as a catalyst in steam explosion increases the recovery of hemicellulose sugars and it improves the enzymatic hydrolysis of the lignocellulosic material solid fraction. The acid catalyst in this steam explosion pretreatment functions similar to acid pulp cooking. H₂SO₄ is a strong catalyst it highly improves the hemicellulose removal but it also generates inhibitory substances. SO₂ is a mild catalyst it gives less inhibitors but also a less extended hemicellulose hydrolysis.¹⁰ “Shahbazi et al. (2005) proposed a fractionation procedure for softwood based on steam explosion and alkaline delignification in order to produce ethanol and related co-products. So“derstro“m et al. (2003) propose a two-step steam pretreatment of softwood by dilute-acid impregnation that includes a partial hydrolysis of cellulose during the second step. According to these authors, this variant of pretreatment is a promising method for increasing the overall yield during ethanol production”¹⁶. The common temperature of steam in this process is in the range of 160 to 260° C and pressure is 0.69 to 4.83 Mega Pascal at starting, and with a residence time of several seconds to a few minutes. The main advantages of steam explosion includes lower energy consumption in the comparison with mechanical methods that are need more than 70% of energy, effective for hard

woods and agriculture residuals and it hydrolysis hemicelluloses perfectly. The main disadvantages includes less effective for soft wood, destruction of xylan, incomplete disruption of lignin, production of inhibitors so that removes 20 to 25% of dry matters by washing out by water, increase crystallinity of amorphous parts and the cost of reactor due to high pressure and high temperature.

Liquid hot water (LHW):

Liquid Hot Water (LHW) pretreatment is one of the most promising methods for lignocellulosic materials. Water pretreatments use pressure to maintain the water in the liquid state at elevated temperatures¹⁷. Flow of the water through the processes maintained in the liquid state at elevated temperatures through cellulosic portion. This type of pretreatment has been called as hydrothermolysis, aqueous or steam/aqueous fractionation, un-catalyzed solvolysis, and aquasolv¹⁷. In this process hot water contacts with biomass for up to 15min at temperatures of 200-300°C.

“Laser et al. (2002) mention that under optimal conditions, this method is comparable to dilute acid pretreatment but without addition of acids or production of neutralization wastes”¹⁶. LHW pretreatment presents elevated recovery rates of pentose’s and it does not generates inhibitors. In LHW pretreatment process between 40% and 60% of the total biomass is dissolved, this is with 4-22% of the cellulose, 35%-60% of the lignin and all of the hemicellulose being removed and when acid was used to hydrolyze the resulting liquid, over 90% of the hemicellulose is recovered as monomeric sugars¹⁷. The pretreatment results from LHW were found to be virtually independent of temperature and time. The results are varies depend on the biomass type with high lignin solubilization impeding recovery of hemicellulose sugars.

In general there are three types of liquid hot water pretreatment systems they are Co-current, Counter-current, and flow through the biomass. These three systems have shown in below diagrams. In co-current system, slurry of biomass and water is heated to the desired temperature and held at the pretreatment conditions for a controlled residence time. In counter-current system water and biomass moves in opposite directions through the pretreatment reactor. In a flow-through system, hot water passes over a stationary bed of lignocellulosic material and dissolves the components and carries them out of the system. LHW pretreatments are helps and hinders by the cleavage of O-acetyl and uronic acid substitutions from hemicellulose to generate acetic and other organic acids and release of these acids helps to catalyze formation and removal of oligosaccharides.¹⁷

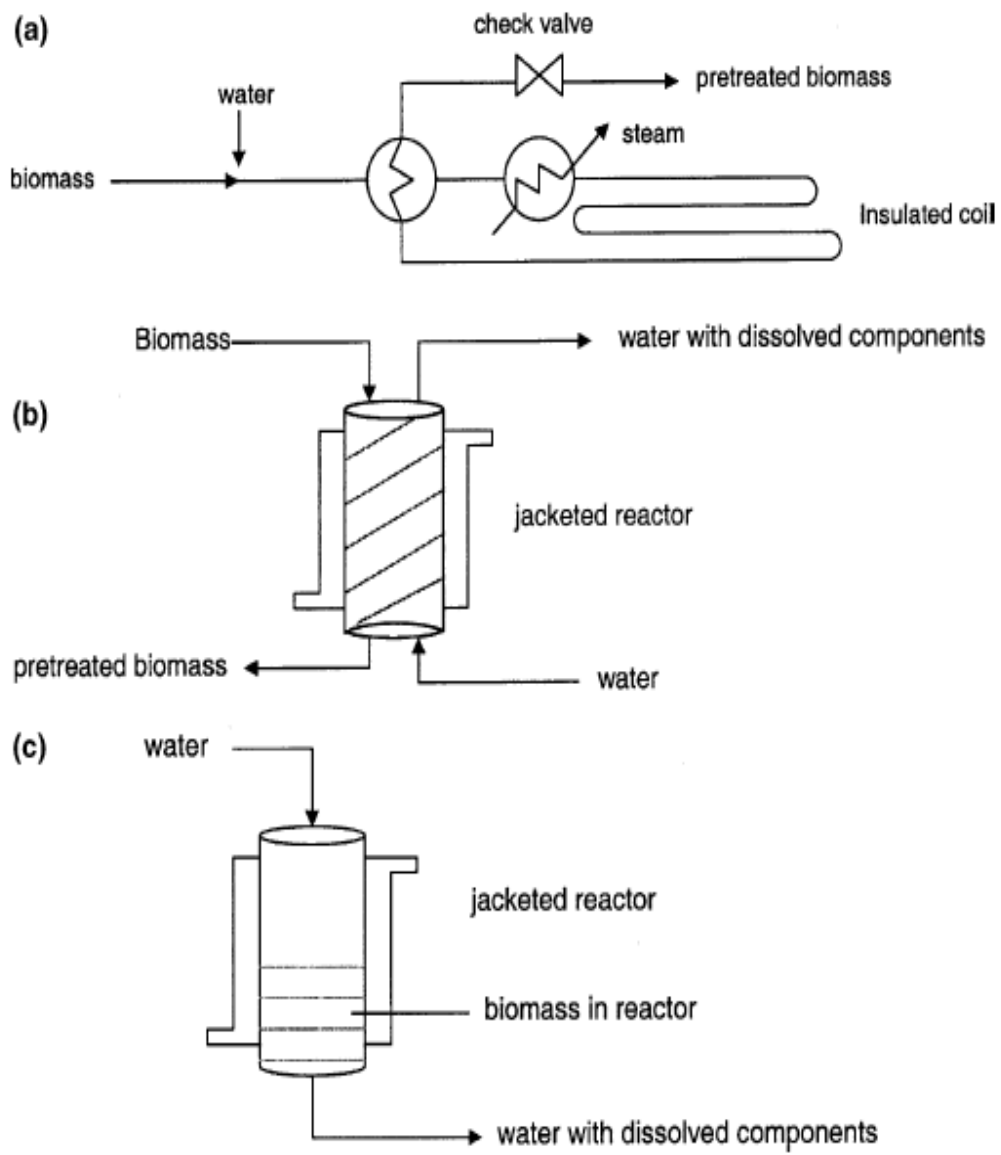


Figure 15: Schematic illustration of a) co-current b) counter-current c) flow through LHW pretreatment methods. Adapted from [17]

Ammonia fiber explosion (AFEX):

Ammonia fiber explosion (AFEX) is another type of physical-chemical pretreatment method, and its principle is similar to steam explosion. In this process lignocellulosic material is exposed to liquid ammonia at high temperature and pressure for a certain period of time, and then the pressure is swiftly reduced. In a typical AFEX process, 1-2 kg of liquid ammonia uses per kg of dry biomass at 90°C (up to 120 °C) temperature and 30 min residence time^{14, 18, 10}. In AFEX process aqueous ammonia reacts primarily with lignin and causes de-polymerization of lignin and cleavage of lignin-carbohydrate linkages. While de-crystallizing cellulose through this process, it removes lignin content and some hemicelluloses.¹ This pretreatment yields optimal hydrolysis rates for pretreated lignocellulosic materials with close to theoretical yields at low enzyme loadings (<5 FPU per gram of biomass or 20FPU/g cellulose)^{10, 17}.

AFEX process can significantly improve the saccharification rates of various herbaceous crops and grasses. AFEX process is well studied for herbaceous and agricultural residues. This process can be use for the pretreatment of different lignocellulosic materials including alfalfa, wheat straw, wheat chaff, barley straw, corn stover, rice straw, municipal solid waste, softwood news paper, etc.. However AFEX process works only moderately well on hardwoods and it is not attractive for softwoods¹⁷. Compared to acid pretreatment and acid catalyzed steam explosion AFEX process does not significantly solubilize hemicelluloses. In the comparison of steam explosion and AFEX process for enzymatic hydrolysis of aspen wood, wheat straw, wheat chaff, and alfalfa stems, it was found that steam explosion solubilized the hemicelluloses while AFEX did not¹⁸. In a study over 90% of hydrolysis of cellulose and hemicellulose has been obtained after AFEX pretreatment of Bermuda grass and bagases. However AFEX pretreatment process is not effective for the high lignin contained biomass such as newspaper (18–30% lignin) and aspen chips (25% lignin). It is necessary to recycle the ammonia after the pretreatment to make the process economically and protect environment. Since ammonia is recycling in this process it is also known as ammonia recycled percolation (ARP) process¹⁷. The best advantage of AFEX process is, it does not produce any inhibitors which is useful for next downstream biological processes for ethanol production. This process does not require small particle size of biomass and the water wash for the pretreated material is not necessary.³

CO₂ explosion:

CO₂ explosion is similar to steam and ammonia explosion pretreatment methods. In the pretreatment of lignocellulosic materials CO₂ would form carbonic acid and increase the hydrolysis rate³. “Explosive steam pretreatment with high pressure carbon dioxide causes the liquid to be acidic and this acid hydrolyses especially the hemicellulose”¹⁴. CO₂ explosion uses the same principle as like steam explosion and AFEX process but the yields are relatively low¹⁶. The glucose yields are relatively low (from the pretreatment experiment of alfalfa) compared to steam or ammonia explosion pretreatment methods, but the yields are high compared to the enzymatic hydrolysis without pretreatment¹⁸. From the pretreatment of recycled paper mix, sugarcane bagases, and re-pulping waste of recycled paper have shown that CO₂ explosion is more cost-effective than ammonia explosion and did not cause the formation of inhibitor compounds that could occur in steam explosion¹⁸.

1.6.3 Chemical pretreatment methods:

Ozonolysis:

Ozone is a powerful oxidant, it is readily available by soluble in water. Over the last two decades ozone applications have increased substantially both in number and diversity. And it has been used in the treatment of ground and industrial waste waters. Ozone has been widely used in pulp bleaching in the paper industry and it has evidenced high delignification efficiency³⁰. Lignin and hemicellulose in many lignocellulosic materials such as wheat straw, bagases, green hay, peanut, pine, cotton straw, and poplar sawdust can be degrade by ozone¹⁰. In recent decades, ozonolysis has shown its efficiency in lignin polymer degradation and also slightly solubilising hemicellulose content of lignocellulosic biomass³⁰. In ozonolysis lignin and hemicelluloses can be slightly attacked but cellulose can be hardly affected¹⁰. Ozone is highly reactive with the compounds which incorporating conjugated double bonds and functional groups with high electron densities. Ozone attacks the lignin releasing soluble compounds of less molecular weight those are mainly organic acids such as formic and acetic acid³⁰. The rate of enzymatic hydrolysis of ozone pretreated wheat straw has increased by a factor of 5 following 60% of the lignin removal. As a percentage of lignin decreased from 29% to 8% enzymatic hydrolysis increased from 0% to 57%, after ozonolysis pre-treatment of poplar sawdust¹⁰. The main advantages from ozonolysis are, it removes lignin effectively, it does not produce any toxic residues for the downstream processes, and lack of degradation products which might interfere with subsequent hydrolysis or fermentation and the reactions can be carried out at room temperature and pressure. However in this process a large amount of ozone is required that makes the process expensive and it can produce the carboxylic acids from the wide range of lignin degradation^{10, 30}.

Acid hydrolysis:

In acid pre-hydrolysis of lignocellulosic materials, concentrated or dilute mineral acids like sulphuric acid can be used. The main function of acid hydrolysis is breaking down hemicelluloses into monomeric sugars and simultaneously removing a part of the lignin. This technology was first developed in Germany in 1898. Since this process required a small amount of energy it needs small amount of water to get an optimum temperature. This process contains some advantages and disadvantages, the main advantages of this methods includes, high yield of hemicellulose sugars, removing the heavy metals in the raw materials and removal of lignin and hemicelluloses in this method increases exposing the cellulose to enzymes prior to hydrolysis. Some of disadvantages of this process are neutralization of acids is necessary, Degradation of hemicelluloses sugar produce some inhibitors like acetic acid and furfural, High cost reactor due to high pressure and temperature.^{31, 32}

Wet Oxidation:

Wet oxidation has been using commercially from last 60 years. “The process in which an organic material is oxidized with gaseous oxygen in water is called wet oxidation”³⁴. Wet oxidation is a form of hydrothermal treatment which is the oxidation of dissolved or suspended components in water using oxygen as the oxidizer. The process is called as wet air oxidation when air is used in this process. The process can be carried out between the temperatures range from 100°C to 374°C. Wet oxidation has been used commercially for around 60 years and it is used predominately for treating wastewater. The main commercial wet oxidation systems are used to treat industrial wastewaters, bio solids³⁵. When lignocellulosic material treats with wet oxidation process, cellulose and lignin disrupts and oxidizes in to CO₂, H₂O, and carboxylic acids³⁴.

Organosolv process:

By using organic or aqueous organic solvents the network of the lignin and also possibly a part of the hemicelluloses in lignocellulosic materials can be remove or decompose. Organosolv pre-treatment can be used to provide suitable treated cellulose for enzymatic hydrolysis. In organosolv pretreatment methods lignocellulosic material mixed with organic liquid and water and heated to dissolve the lignin and a part of the hemicelluloses and the reactive cellulose leaves in solid phase. To reduce either the operating temperature or to enhance the delignification process a catalyst may be added to this process. In this process a large number of organic or aqueous organic solvents such as alcohols, esters, ketones, glycols, organic acids, phenols, and ethers can be used with or without addition of catalysts at temperatures of 150-200°C. One of the main advantage of the use of solvents over other chemical pre-treatment's is that relatively pure, low molecular weight lignin is recovered as a valuable by product. This pretreatment process can be used together with acid hydrolysis to separate hemicellulose and lignin in a two stage fractionation. To make the process economic, the applied solvents should be separate and recycled.³⁶

Alkaline hydrolysis:

Using NaOH, Ca(OH)₂ (lime) or ammonia to remove lignin and a part of the hemicellulose from the lignocellulosic materials to improve the accessibility of enzymes to the cellulose is refers as alkaline pretreatment. These pretreatment can be performed at low temperatures but with a relatively long time and high base concentration. Alkali treatment is the most effective method compared to acid or oxidative reagents to break the ester bonds between lignin, hemicellulose and cellulose and avoid fragmentation of the hemicellulose polymers. However, alkaline pretreatment method was shown more effective on agricultural residues than wood materials. Alkaline peroxide is one of the effective pretreatment methods that can improve the enzymatic hydrolysis by delignification of lignocellulosic materials. “In this method, the lignocelluloses are soaked in pH-adjusted water (e.g. to pH 11-12 using NaOH) containing H₂O₂ at room temperatures for a period of time (e.g. 6-24 h)”³⁶.

1.6.4 Biological pretreatment methods:

In biological pretreatment methods lignin content of the lignocellulosic biomass solubilize by fungi. Generally microorganisms such as brown-, white-, and soft-rot fungi are used in this pretreatment method to degrade lignin and hemicellulose of the lignocellulosic materials¹⁸. “Brown rots mainly attack cellulose, while white and soft rots attack both cellulose and lignin. White-rot fungi are the most effective basidiomycetes or biological pretreatment of lignocellulosic materials”¹⁸. In 1984 it is mentioned that the use of biological pretreatment methods in future, although during that time it was expensive and inadequate, required a long process time and the microorganisms were poisoned by lignin derivatives¹⁰. There is some little experience is exists with these approaches, however these technologies could greatly simplify pretreatment, but the rates are slow and the yields are low¹⁰. The main advantages of biological pretreatment are low energy requirement and mild environmental conditions, but the rate of hydrolysis in most biological pretreatment processes is very low.¹⁸

1.7 Acetosolv Pretreatment:-

Organosolv methods have attracted growing interest in recent decades to produce cellulose pulp from lignocellulosic materials. Organosolv gets its name from its use of organic solvents in the processing with a view to taking full advantage of the primary material. Kleinert and Taenthal (1931) were started the pioneering work to the utilization of organic solvents as fractionation agents and they proposed the utilization of ethanol – water media for delignification. Since then, a variety of organic solvents have proposed as pulping agents. The principle used in these methods is that any organic solvent is capable of dissolving lignin or lignin oligomers. The important aspect of all organosolv processes is to use raw material more efficiently by improved separation of its major fractions lignin, cellulose and hemicellulose or investigation of quantitative fractionation of the material. The feasibility of organosolv pulping technology depends on the efficiency of the solvent and byproduct recovery.

Every year large amounts of lignin is producing from the pulping processes as a by-product of wood and non-wood delignification. The commercial pulping processes like kraft and sulphite gives high quality pulps, but around 50-55% of the dry weight of wood and straw is wasted or employed in low added value applications such as power generation ^[28, 29]. Organosolv methods have been recognized as viable sulphur-free alternatives to traditional pulping methods. Since organosolv processes leads to enrich cellulose in solid phase and hemicellulose-degradation products, lignin-degradation products in liquors. In these Oxoacid-based methods are most successful to efficient delignification of both woody and non-woody materials, including straws. Large number of organosolv systems has been tested experimentally in past few years. However only a small amount of those processes has shown high selectivity and efficiency. Organosolv processes using acetic acid and formic acid have achieved particularly good delignification results. Utilization of the acetic acid in pulping has been assayed in HCl-catalyzed media (acetosolv process), formic acid-catalyzed media (Formacell process) and un-catalyzed media (Acetocell process). All three processes have proved to be promising processes to achieve complete utilization of lignocellulosic materials with minimal environmental impacts. Under mild conditions these processes have ability to cause extensive removal of lignin and hemicelluloses with no significant cellulose degradation ^[27, 29]. The organosolv methods based on acetic acid have been applied successfully to hardwood, softwood and non-woody plant material ^[1, 26]. In acetosolv process wood and non-wood can be simply fractionated into pulp, lignin and hemicellulosic-derived monosaccharides or degradation products, it makes easy to utilize these fractionated compounds for more valuable products ^[29].

The cooking liquor becomes acidified due to the acetic acid released from the wood in non-catalyzed organosolv pulping (Autocatalyzed). In catalyzed pulping depending on the nature of the additives employed the liquor can be acidic, neutral or alkaline. The acetosolv process shows comparative advantages with other delignification technologies. Under mild operational conditions, extensive delignification and hemicellulose removal can be achieved in a single

reaction step and the pulp shows good characteristics. Delignification proceeds towards cellulose degradation, and enabling an almost complete cellulose recovery in pulp. When hemicelluloses are hydrolyzed acetyl groups are simultaneously splits off leading to the formation of acetic acid thus facilitating the recovery of solvent. This is an important factor in the economical analysis of organosolv-based processes. The hemicellulosic fraction is converted into marketable products (sugars and/or furfural, in relative proportions dependent on severity of the operational conditions). Under harsh conditions, furfural (a chemical intermediate employed for manufacture of polymers, furfuryl alcohol and tetra-hydro-furan) is the main reaction byproduct derived from hemicelluloses. The pulp can be used to produce cellulose derivatives, such as carboxymethyl-cellulose, cellophane, viscose, cellulose acetate, fuels, paper. The dissolved lignin fragments can be easily precipitated from the pulping liquors by water addition, enabling an effective separation of the compounds derived from hemicelluloses. And the lignin can be converted to valuable products such as carbon fiber, activated carbon, and adhesives. On the other hand acetosolv process has some drawbacks, such as the corrosion caused by the HCl-containing reaction media and the esterification of cellulose with acetic acid leading to pulps with comparatively high contents of saponifiable groups. ^[28, 29]

2. Materials and Methods

2.1 Raw material

Marabou biomass was donated by Limonar Delegation of the Ministry of Agriculture (Limonar, Matanzas, Cuba). The material was air-dried for a week, and then it dried at 50°C for 48 h. After that, two different portions of the biomass were milled to two different particle sizes. A portion of 100-g was milled to pass a 1-mm screen and used for compositional analysis; another portion of 1-kg was milled to pass from 2-mm screen and used for acetosolv experiments.

2.2 Characterisation

The contents of total solids, moisture, ash, extractives, Klason lignin, easy-to-hydrolyze polysaccharides and difficult-to-hydrolyze polysaccharides of marabou biomass were analyzed. First the material was air-dried for a week, and then it dried for 48 h at 50°C. After that a portion of 100-g was milled to pass a 1-mm screen and used for compositional analysis. The content of total solids, moisture, ash, extractives, Klason lignin in marabou biomass was analyzed according to NREL standards^[37 to 40]. Easy-to-hydrolyze polysaccharides and difficult-to-hydrolyze polysaccharides were determined according to GOST protocols.^[45]

2.2.1 Determination of total solids and moisture in *Dichrostachys cinerea*:-

Biomass samples are hygroscopic materials and these can contain large and varying amounts of moisture. To be meaningful, the results of chemical analyses of *Dichrostachys cinerea* are typically reported on a dry weight basis. Convection Oven Procedure was used to determine the total solids in the raw material. This standard method is developed by National Renewable Energy Laboratory (NREL).^[37]

Convection Oven Procedure has been used to analyze the solid content in the *Dichrostachys cinerea*. In this procedure all the volatile matter has been removed by heating the sample at 105°C to constant weight. The total solids content of the *Dichrostachys cinerea* sample is the amount of solids remaining after all volatile mater has been removed by heating the sample at 105°C. The moisture content is a measure of the amount of water and other components volatilized at 105°C, which are present in the sample. Triplicate samples were run in this experiment. The apparatus used in this procedure are Analytical balance (sensitive to 0.1 mg), Convection (drying) oven with temperature control of 105±3°C, Desiccator, Aluminum foil weighing dishes. The results of the analysis of *Dichrostachys cinerea* samples are typically reported on a 105°C dry weight basis.

The percent total solids on a 105°C dry weight basis

$$= \left(\frac{\text{weight dried sample plus dish} - \text{weight dish}}{\text{weight sample as received}} \right) \times 100$$

The percent of moisture present in sample

$$= \left(1 - \frac{(\text{Weight dried sample plus dish} - \text{Weight dish})}{\text{Weight sample as received}} \right) \times 100$$

2.2.2 Ash Determination in *Dichrostachys cinerea*:-

Ash content in biomass is an approximate measure of the mineral content and other inorganic mater; it is used in the conjunction with other assays to determine the total composition of biomass samples. Determination of ash in *Dichrostachys cinerea* has expressed as the percentage of residue remaining after dry oxidation of material at 550⁰C to 600⁰C. The method used to determine the ash content is developed by National Renewable Energy Laboratory (NREL).^[38]

The apparatus used in this procedure are porcelain crucibles, muffle furnace, analytical balance (sensitive to 0.1 mg), desiccator, drying oven. In this experiment first the crucibles have marked with unique identification numbers using porcelain marker. Then these crucibles have brought to constant weight by igniting at 590⁰C in a muffle furnace for 4 hours. After 4 hours these crucible removed from the furnace and cooled to room temperature in a desiccator. Then the weights of the dried crucibles have recorded to the nearest 0.1 mg as the tare weights and crucibles have kept in desiccator until used. Air dried samples of *Dichrostachys cinerea* samples of 1 gm nearest to 0.1 mg have took in to the crucibles and recorded the weights of the samples as W2. The weight of the sample corrected for its moisture content prior to calculating the ash. Then the crucibles along with sample material have placed in to the muffle furnace and ignited at 590⁰C for 4 hours until the carbon eliminated, the furnace has heated slowly to avoid flaming. After complete ignition the crucibles with its contents removed from the furnace and placed in a desiccator. These samples were cooled to room temperature in desiccator and recorded the weights as W1 (ash weight) to nearest 0.1mg. All the experimental results have calculated and recorded.

Determination of ash in *Dichrostachys cinerea* has expressed as the percentage of residue remaining after dry oxidation of material at 590⁰C. Air dried *Dichrostachys cinerea* samples which were used in this experiment and following calculations have used to report the results on a 105⁰C dried weight basis.

$$\text{Ash\% in } *Dichrostachys cinerea* \text{ samples} = (W1 / (W2 \times T / 100)) \times 100$$

Where:

W1 = weight of ash,

W2 = initial weight of sample,

T = Percent total solids of sample, on a 105⁰C oven dried weight basis. ('T' is the average value of the results has been calculated in the experiment of determination total solids in the *Dichrostachys cinerea*).

2.2.3 Determination of Extractives in *Dichrostachys cinerea*:-

Biomass contains non-structural materials which potentially could interfere in the biomass analysis. These should be removed from biomass to prevent interference with later analytical steps prior to compositional analysis. Removal of these non-structural compounds from biomass uses a two step extraction process to remove water soluble and ethanol soluble materials. Inorganic materials, non-structural sugars, and nitrogenous materials includes in water soluble materials. Inorganic materials in water soluble material may come from both biomass and any soluble material that it is associated with the biomass, such as soil or fertilizer. Chlorophyll, waxes, or other minor components includes in ethanol soluble materials. It is necessary to use both extraction steps for some biomass, while some biomass may only require ethanol extraction process.

Samples of *Dichrostachys cinerea* are the representatives of hard wood materials. Extractives of these samples have been extracted by using one step extraction process which includes ethanol as an extractive solvent. The method used to determine the extractives in *Dichrostachys cinerea* is provided by National Renewable Energy Laboratory (NREL) Apparatus have been used in this experiment are Soxhlet extraction apparatus (A glass Soxhlet extraction apparatus of suitable size (100 ml) for containing the sample and a 250 ml collection flask), alundum extraction thimbles, analytical balance, rotary evaporator with vacuum and water bath, drying oven, Buchner funnel, Desiccator, Ethyl alcohol (95%).^[39]

In this experiment three samples of *Dichrostachys cinerea* have been tested for the extractive materials. In this experiment first the thimbles have been weighed in order to use them in further steps. Approximately 5 gm of sample material has added to the each extraction thimble. The samples of the material have been added to the thimbles by taking care to maintain proper gap between top of the thimble and sample to prevent the sample loss during the extraction. Weights of the thimbles along with samples have recorded to the nearest 0.1 mg. 250 ml balloon flasks have used as extractive containers; first weights of the flasks have been measured and recorded as tare weights of the flask. After that 160 ml of 95% ethanol added to each the flask. Then conventional soxhlet extraction apparatus has assembled by inserting the thimbles. The soxhlet extraction apparatus has heated for 6 hours to give around 130 solvent exchanges in the soxhlet thimble. After the extraction time thimbles and extractive containers have been removed

carefully from soxhlet apparatus. Thimbles along with samples were allowed to air dry. All the solvent from the extractive flask has been removed by using rotary evaporator under vacuum and flask has heated to $42 \pm 3^{\circ}\text{C}$ in the water bath during the evaporation. After all of the visible solvent is removed by the rotary evaporator the flasks and thimbles from the soxhlet apparatus were placed in to oven for 24 ± 1 hour at 105°C . After this time flasks and thimbles have removed from the oven and allowed to cool to room temperature and weights of the flasks along with the non-volatile extractives and thimbles with extractive free material. % Non-volatile extractives, % Volatile extractives and % Total extractives were analyzed in this experiment.

The amounts of extractives in the *Dichrostachys cinerea* samples have been calculated on a percent dry weight basis.

Oven dry weight of the sample (ODW) =

$$\frac{(\text{weight of the thimble plus sample} - \text{weight of the thimble})}{100} \times \% \text{ Total solids}$$

Total solids = (From the experiment Determination of total solids in *Dichrostachys cinerea*).

$$\% \text{ Extractives} = \frac{\text{weight of the extractives}}{\text{ODW of the sample}} \times 100$$

2.2.4 Determination of Klason Lignin in Extractive free *Dichrostachys cinerea* Biomass:-

Three samples of *Dichrostachys cinerea* have been tested in this experiment. The residue collected from this method is defined as the acid insoluble lignin content of the sample. This could contain small fraction of condensed proteins from the original sample and separation of this fraction is outside scope of this procedure. Klason lignin of *Dichrostachys cinerea* was analyzed for the samples with extractives and without extractives. The *Dichrostachys cinerea* samples used in this experiment are extractive free samples collected from the previous experiment which determined the extractive fraction of the *Dichrostachys cinerea* and the raw *Dichrostachys cinerea* biomass.

All the analyses have been performed according to the guidelines established in the Ethanol Project Quality Assurance Plan (QAP). Apparatus, reagents and materials used in this procedure are Analytical balance readable to 0.1 mg, Convection oven with temperature control of $105 \pm 3^{\circ}\text{C}$, Autoclave capable of maintaining $121 \pm 3^{\circ}\text{C}$, Water bath set at $30 \pm 1^{\circ}\text{C}$, Filtration crucibles with medium porosity, Filtration setup including vacuum source and vacuum adapters for crucibles, Desiccator, 72% sulfuric acid, Deionized water, glass test tubes, 125 ml glass serum bottles, Filtration flasks.

In this method 0.5 gm of three *Dichrostachys cinerea* extractive free material and three raw biomass samples have been taken in different test tubes and the weight of the samples have

recorded to its nearest 0.1 mg. Then 5 ml of 72% H₂SO₄ solution added to each sample and the solution has stirred with a glass rod for couple of minutes to the sample material thoroughly wetted in test tube. The samples have hydrolyzed for 1 hour in water bath at 30°C temperature and the samples have stirred in every 15 minutes to assure complete mixing and wetting. After 1 hour, some distilled water was added to the reaction mixture to stop the reaction and all the hydrolyzate (residual solids along with the hydrolysis liquor) of the samples has carefully transferred in to 250 ml different glass bottles and diluted the acid concentration to 4% by adding 148.67 gm of deionized water. Stopper each of the bottles and crimp aluminum seals in to place to prevent the loss of sample from bottle during the experiment. These bottles were put in to the autoclave for 1 hour at 121 ± 3°C. After completion of autoclave cycle the bottles have left to cool for room temperature to remove stoppers and seals of the bottles. Then vacuum filter the hydrolysis solution in bottles through the filter crucibles which were marked and weighed before. The filtrate samples were collected and stored in refrigerator at 4°C for further analysis to determine the carbohydrates and acid-soluble lignin. Deionized water was used to transfer all the particles to the filter crucibles that are remained in the glass bottles and to wash the filter residue for free of acid using vacuum filtration. The crucibles along with filter residue were dried for 24 hours at 105 ± 3°C in convection oven to get constant weight. Then the crucibles were took out from the oven and placed in a desiccator to cool and recorded the weights of crucibles along with residues. All the experimental values were recorded and calculated.

The results of acid-insoluble lignin analysis can be effected by incomplete hydrolysis of biomass sample and the timing of the acid digestion steps due to this the samples were mixed thoroughly at the beginning and periodically throughout the concentrated acid hydrolysis and the timing with in this procedure of acid digestion steps was followed closely to dissolve insoluble lignin into solution in an irreproducible fashion. All results are reported relative to the 105± 3°C oven dried weight of the sample and an extractive free basis. Weight of the extractive free matter of the samples and the raw biomass samples were corrected to the total weight of the samples to obtain the actual acid-insoluble fraction of the total *Dichrostachys cinerea* biomass samples.

$$\% \text{ lignin} = \frac{\text{weight of the lignin}}{\text{total weight of the sample}} \times 100$$

2.2.5 Analysis of total reducing sugars:-

Total reducing sugars were analyzed for all the liquid streams and for analytical acid hydrolysates of the solid streams of each experiment and of the raw material. Fifty µl of the sample were placed in a test tube, and 450 µl of distilled water was added to get 10X dilution. A reference tube was filled with 500 µl of distilled water to prepare the blank. Five hundred µl of 3,5-DNSA was added to the test tubes, and the mixtures were placed for 10 min in a boiling water bath. The tubes were brought out from the bath and cooled to room temperature. After that,

1200 µl of distilled water was added to each tube and the absorbance was read at 545 nm using a spectrometer. Triplicates determinations were made for each sample.

$$\text{Concentration of total reducing sugars} = \left(\frac{\text{Absorbance} + 0.0009}{1.2571} \right) \times 10$$

The concentrations of the sugars in the samples were calculated using the above formula.

2.2.6 Determination of polysaccharides:-

2.2.6.1 Easy to hydrolyse polysaccharides:

Determination of easy-to-hydrolyse polysaccharides is based on gravimetric analysis of the solid residue remaining after hydrolysis of hemicelluloses (Obolenskaia, 1986). Triplicate 2-g samples of extractive free marabou biomass were mixed with 20 mL 5% hydrochloric acid in round bottom flasks. After that the reaction mixtures were refluxed at boiling temperature for 3 h in a water bath. After completion of reflux time water bath was stopped and the equipment was left to reach room temperature. Then the reaction mixtures in each flask were vacuum filtered. The filtration residues were dried at 105°C for 24 h in digital oven. After that, the residues were cooled in a desiccator and weighed in an analytical digital balance Easy to hydrolyse polysaccharides (EHP) from the three samples were calculated by using the formula below.

$$EHP = \frac{(M1 - M0)}{M0} \times 100$$

Where M0 = Initial mass of the sample in grams. M1 = Final mass of the sample (mass of the residues) in grams.

Easy-to-hydrolyse polysaccharides were also determined by analysis of total reducing sugars in the hydrolyzate.

2.2.6.2 Difficult to hydrolyse polysaccharides:

In this analysis triplicate samples were run to analyze difficult to hydrolyze polysaccharides. First the residues that are collected from the determination of easy to hydrolyze polysaccharides were weighed and took in to three different 100ml beakers that were marked before. After that 15ml of 72% sulfuric acid was added to each beaker and the reaction mixtures in three beakers were stirred properly with a glass rod for every 20min during 2hr of reaction time at room temperatura. After that the reaction mixtures were transferred in to three different 500ml bubble flasks that were marked before. And 135ml of distilled water was added to each flask and the reaction mixtures in the flasks were refluxed for 2 hr in the water bath. After completion of reflux time the water bath was stopped and the equipment was left to reach room temperature. Then the reaction mixtures in each flask were filtered by vacuum filtration with Buchner filters (that have marked before) and filter papers (that are weighed before). The residues from filtration

were dried at 105°C for 24hr in digital oven. After that the residues were collected in a desiccator and left to reach room temperature. After that the residues were weighed in an analytical digital balance to its nearest 0.1 mg and the values were recorded. Difficult to hydrolyse polysaccharides (DHP) from the three samples were calculated by using the formula below.

$$DHP = \frac{(M2 - M3)}{M0} \times 100$$

Where M0 = Initial mass of the sample in grams, M2 = Initial mass of the residues from the easy to hydrolyse polysaccharides in grams, M3 = Final mass (mass of the residues from this process) in grams. Difficult-to-hydrolyse polysaccharides were also determined by analysis of total reducing sugars in the hydrolyzate.

2.2.6.3 HPLC analysis

For carbohydrates analysis, 2 mL aliquots of the hydrolysate obtained after analytical acid hydrolysis were eluted through an extraction cartridge (Sep Pak C₁₈, Waters) for removal of the aromatic compounds, followed by samples' analysis by HPLC (Shimadzu, Kyoto, Japan). Glucose, cellobiose, xylose, arabinose and acetic acid were separated with an Aminex HPX-87H column (Bio-Rad, Hercules, CA), on using 5 mM H₂SO₄ as a mobile phase, at a 0.6 mL min⁻¹ flow at 45°C, and detected on an RI detector (Shimadzu RID-6A). The obtained concentrations were used for calculating the content of cellulose, polioses and acetyl groups, by conversion factors, on also considering water addition during hydrolysis.

2.3 Acetosolv delignification of *Dichrostachys cinerea*

Acetosolv delignification was performed at normal boiling temperature (NBT) and 121°C, using 50-50, 70-30 and 90-10 acetic acid-water mixtures at a 10-% concentration of solids load during 1 h. Hydrochloric acid (0.2 g/100 g of mixture) was used as catalyst. Combination of dilute-acid pre-hydrolysis (DAPH) with acetosolv was also investigated with respect to the same pretreatment conditions that those were used for Acetosolv delignification. The experimental approach of the acetosolv delignification of marabou biomass is shown in Figure 16. In this work first the marabou biomass with 1mm size has been used for characterisation to analyze the moisture content, lignin, carbohydrates (easy to hydrolyze polysaccharides, difficult to hydrolyze polysaccharides, total reducing sugars), and extractives that are present in the marabou biomass.

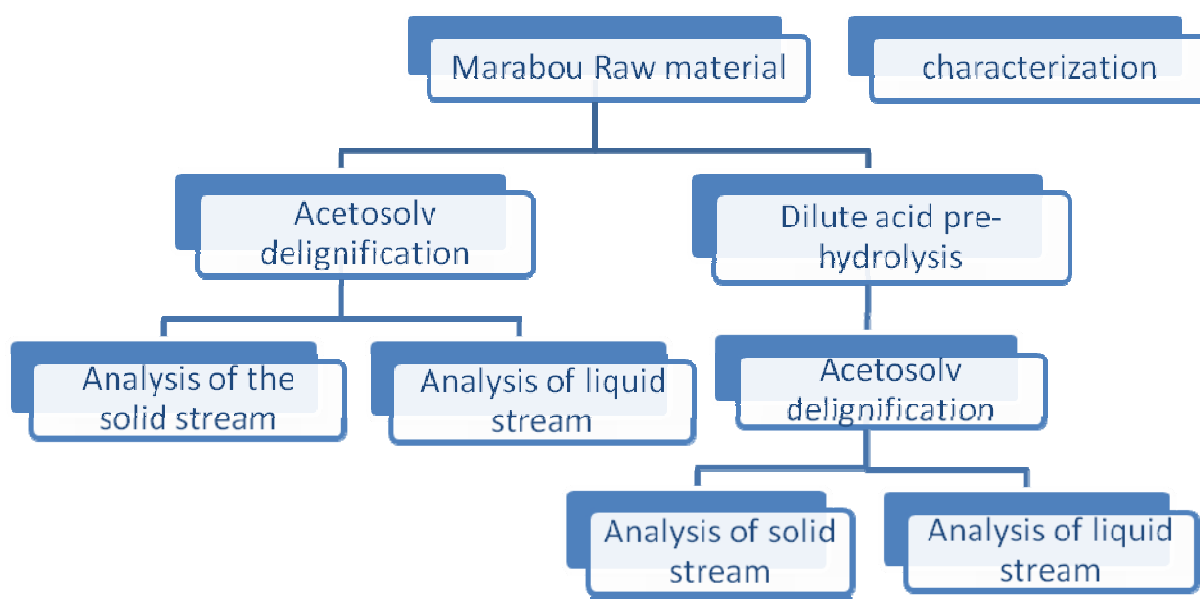


Figure 16: Experimental approach for acetosolv delignification of the marabou biomass.

After that Acetosolv experiments was performed for the delignification of marabou biomass with and without of DAPH (Dilute acid pre-hydrolysis). All the solid and liquid streams were analyzed from the each and every set of reactions.

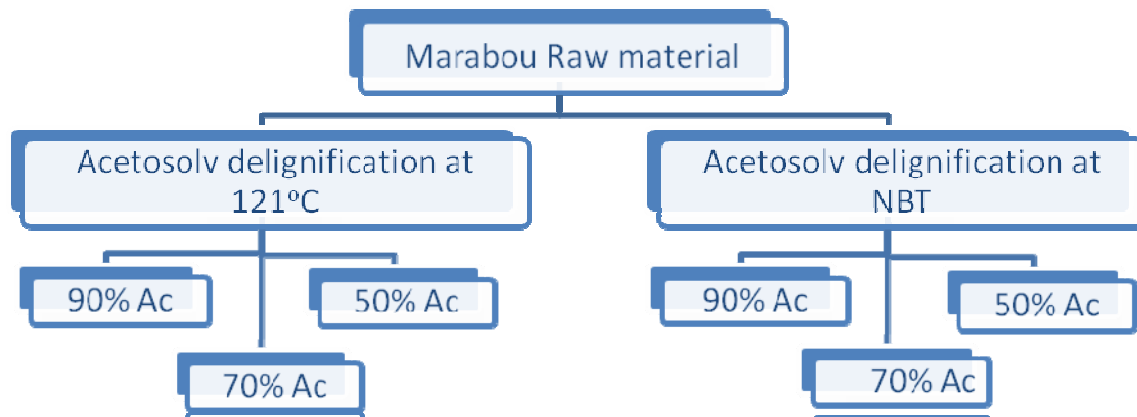


Figure 17: Experimental conditions for acetosolv delignification of marabou biomass.

From the solid streams pulp yield, lignin removal, lignin non removal, total reducing sugars, extractives, easy to hydrolyze polysaccharides and from liquid streams volume of the liquor, pH, total reducing sugars were analyzed. Lignin has been recovered from the acetosolv liquors and yields were analyzed. As shown in figure 17, the marabou biomass has been treated with acetosolv system at different experimental conditions to identify the optimal conditions for efficient delignification. All the experiments have been done with 1hr reaction time 0.2% HCl as a catalyst. And the experiments were carried out at different reaction temperatures and acetic acid concentrations. All the summarized experimental conditions that were used in this work has shown in the below table 3.

Summary of the experimental conditions used for acetosolv delignification of marabou biomass.

Table 3

Experiment No	Acetosolv system	Catalyst	Temperature (°C)	Solid to liquor ratio (g/g)	Reaction time (hr)
1	Acetic acid–H ₂ O (50/50, v/v)	0.2% HCl	NBT	10:1	1
2	Acetic acid–H ₂ O (70/30, v/v)	0.2% HCl	NBT	10:1	1
3	Acetic acid–H ₂ O (90/10, v/v)	0.2% HCl	NBT	10:1	1
4	Acetic acid–H ₂ O (50/50, v/v)	0.2% HCl	121	10:1	1
5	Acetic acid–H ₂ O (70/30, v/v)	0.2% HCl	121	10:1	1
6	Acetic acid–H ₂ O (90/10, v/v)	0.2% HCl	121	10:1	1
7	(DAPH) + Acetic acid–H ₂ O (50/50, v/v)	0.2% HCl	NBT	10:1	1
8	(DAPH) + Acetic acid–H ₂ O (70/30, v/v)	0.2% HCl	NBT	10:1	1
9	(DAPH) + Acetic acid–H ₂ O (90/10, v/v)	0.2% HCl	NBT	10:1	1
10	(DAPH) + Acetic acid–H ₂ O (50/50, v/v)	0.2% HCl	121	10:1	1
11	(DAPH) + Acetic acid–H ₂ O (70/10, v/v)	0.2% HCl	121	10:1	1
12	(DAPH) + Acetic acid–H ₂ O (90/10, v/v)	0.2% HCl	121	10:1	1

2.3.1 Acetosolv Delignification of *Dichrostachys cinerea* at 121°C:-

Acetosolv delignification of *Dichrostachys cinerea* biomass was performed using 50%, 70% and 90% of acetic acid concentrations either at 121°C or at normal boiling temperature using 0.2% HCl as a catalyst and at a 1:10 solid-liquid ratio. All the experiments were performed in duplicates. Approximately 5 g of raw material were placed in 250-ml blue-cap flasks, and 45 g of previously prepared acetosolv liquor was added to reach the desired acetic acid concentrations. The acetosolv liquor contained acetic acid, water and 0.2% of hydrochloric acid added to the all

samples in different glass bottles. After that, the flasks were placed in an autoclave at 121°C for 1 hour. After completion of the reaction time the autoclave was stopped. When the temperature was below 70°C the flasks were brought out from the autoclave and left to cool to room temperature. After that, the slurries were separated with vacuum filtration. The volume and pH of the obtained filtrate were measured and recorded. The filtration residues were washed with two volumes of fresh liquor. The washing liquors were mixed with the respective filtrates and stored in labeled 1-L bottles. Then, the residues were washed with three volumes of distilled water. The washed solids were dried at room temperature for 4–5 h and then at 105°C for 24 h. The dry solids residues were cooled to room temperature in a desiccator, and weighed. The pulp yield was calculated as:

$$\% \text{ Residues} = \frac{\text{Weight of the residues}}{\text{Actual weight of the sample}} \times 100$$

Lignin and total polysaccharides in the acetosolv pulps were determined by analytical acid hydrolysis combined with total reducing sugars analysis. Extractives and easy-to-hydrolyze polysaccharides were also analyzed. In the acetosolv liquors total reducing sugars were determined.

2.3.1.1 Lignin recovery from the liquors of acetosolv delignified *Dichrostachys cinerea* biomass samples at 121°C:-

Lignin recovery from the acetosolv liquors of *Dichrostachys cinerea* biomass was carried out in two stages. In the first step, the liquors were concentrated to half of their volumes by vacuum rotor-evaporation. After that, 10 volumes of distilled water were added to the each concentrated liquor. In the second stage, the liquors were heated in water bath at 80°C for 2 hours and left at room temperature for 24 hours to precipitate the lignin. The precipitated lignin was separated by vacuum filtration, dried overnight at 105°C, cooled to room temperature in a desiccator and weighed in an analytical balance. Lignin recovery was calculated using the following expression:

$$\text{Lignin yield from the total lignin in the sample} = \frac{\text{Weight of the recovered lignin}}{\text{Total amount of lignin in sample}} \times 100$$

2.3.2 Acetosolv Delignification of *Dichrostachys cinerea* at Normal Boiling Temperature (NBT):-

In this experiment delignification of *Dichrostachys cinerea* biomass samples have been studied with acetosolv process which is used acetic acid media and Hydrochloric acid as a catalyst. 50%, 70% and 90% of acetic acid concentrations at Normal Boiling Temperature (NBT) have studied with 0.2% HCl as a catalyst and 1:10 solid-liquid ratios.

Duplicate samples have run for each concentrations of acetic acid at NBT. Approximately 5 gm of *Dichrostachys cinerea* biomass samples have been taken in 250 ml balloon flasks.

First 20gm previously prepared different concentration acetosolv liquors were added to the flasks then 0.2% (0.270 gm of 37% HCl) Hydrochloric acid added to the all samples in different glass bottles. After that all the flasks were filled with respective concentrations of the acetic acid liquors until the reaction mixture weight reaches to 50gm. Then these flasks were placed in conventional heater. The reaction mixtures in the flasks were made to reflux for 1 hour at Normal Boiling Temperature. After completion of reaction time the conventional heaters were stopped and the flasks were left to cool at room temperature. After that, the slurries were separated with vacuum filtration. The volume and pH of the obtained filtrate were measured and recorded. The filtration residues were washed with two volumes of fresh liquor. The washing liquors were mixed with the respective filtrates and stored in labeled 1-L bottles. Then, the residues were washed with three volumes of distilled water. The washed solids were dried at room temperature for 4–5 h and then at 105°C for 24 h. The dry solids residues were cooled to room temperature in a desiccator, and weighed. The pulp yield was calculated as:

$$\% \text{ Residues} = \frac{\text{Weight of the residues}}{\text{Actual weight of the sample}} \times 100$$

Lignin and total polysaccharides in the acetosolv pulps were determined by analytical acid hydrolysis combined with total reducing sugars analysis. Extractives and easy-to-hydrolyze polysaccharides were also analyzed. In the acetosolv liquors total reducing sugars were determined. The lignin removed from the acetosolv treated *Dichrostachys cinerea* biomass samples at Normal Boiling Temperature were recovered from the liquors as like in the section 2.3.1.1 and the lignin yields were calculated and recorded.

2.4 DAPH & Acetosolv delignification

Dichrostachys cinerea bio-mass samples were pre-treated with dilute sulphuric acid (H₂SO₄) to make prior the solid residues for acetosolv pre treatment. 1:8 solid – liquid ratio and 1.25% w/v of sulphuric acid was used in this experiment. Duplicates of the each sample have run in this experiment. 125 gm dry weight *Dichrostachys cinerea* biomass sample were taken in to two different glass bottles. Then 200 gm of distilled water added to each of the bottle. Previously prepared sulphuric acid solutions (14.4 gm of sulphuric acid (98% H₂SO₄) and 300 gm of distilled water) were added to each flask. After that distilled water was added to the each bottle to make the total weight of reaction mixture for 1125 gm which contains 1:8 solid – liquid ratios and 1.25% sulphuric acid concentration.

Then these two bottles were covered tightly with caps and put in the autoclave for 17 min at 121°C. After completion of reaction time the bottles with reaction mixtures were put out from the

autoclave and cooled to room temperature. Then the samples were vacuum filtered to separate the solid and liquid fractions.

The volume and the pH of the liquid fraction of the each sample were measured and duplicates of the filtrate samples were collected for further analysis. The residues were collected and dried at 105°C for 24 hr. After drying process the residues were collected in a desiccator and cooled to room temperature. Then the residues were weighed to its nearest of 0.1 mg and recorded the values.

$$\% \text{ of the residues} = \frac{\text{weight of the residues}}{\text{actual weight of the sample}} \times 100$$

The solid residues obtained from the DAPH were further analyzed to evaluate the total solids and moisture. Total reducing sugars from both the solid and liquid streams of the dilute acid prehydrolysis (DAPH) and the liquid streams of the analytical acid hydrolysis (raw material was DAPH residues) were analyzed.

The pulp obtained from the Dilute Acid Pre-hydrolysis (DAPH) was further tested with acetosolv system for delignification. First the pulps from the DAPH were mixed carefully and a portion of sample was taken to the acetosolv pretreatments. Acetosolv pre-treatments for the DAPH pulps were carried out at 121°C and Normal Boiling Temperature (NBT). Acetosolv pretreatment of DAPH pulp at 121°C were carried out according to the procedure specified in 2.3.1 and the acetosolv pretreatment at NBT were carried out according to the procedure specified in 2.3.2. Lignin and total polysaccharides in the acetosolv pulps were determined by analytical acid hydrolysis combined with total reducing sugars analysis. Extractives and easy-to-hydrolyze polysaccharides were also analyzed. In the acetosolv liquors total reducing sugars were determined. The lignin was recovered from the liquors as like in the section 2.3.1.1.

3. Results & Discussion

Table 4 shows the general composition of dry marabou biomass. The content of total polysaccharides, which was calculated after total reducing sugar determination in the hydrolysate obtained in the analytical acid hydrolysis, was 67.4%. This result matched very well with the sum of the easy-to-hydrolyze polysaccharides (30.1%) and difficult-to-hydrolyze polysaccharides (37.6%) fractions determined in separate analyses (Figure 18). The validity of these results was confirmed by a parallel HPLC analysis of the analytical acid hydrolysates, which gave cellulose content (39.5%) very close to the percentage of difficult-to-hydrolyze polysaccharides shown Table 4(b). The HPLC analysis also revealed details about the composition of marabou hemicelluloses, including 19.4% of pentosans and 2.3% of acetyl groups.

Table 4: Composition of marabou raw material

Component	% (d. b) Raw material
EHPS	30.1
DHPS	37.6
Lignin	23.4
Extractives	4.1
Ash	2.7
unknowns	2.1

Klason lignin content resulted 23.4% when it was determined as the solid residue remaining after determination of difficult-to-hydrolyze polysaccharides (Figure 18). It was also determined as the residue after analytical acid hydrolysis, resulting 23.0% (Table 5), which indicates a good correlation between the two analytical methods used. Other components of marabou raw material were extractives (4.1%) and ash (2.7%).

Figure 18: Main components of the Marabou biomass

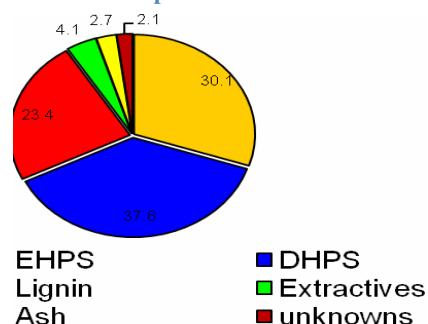


Table 4(b): Polysaccharide composition of un-debarked marabou

Component	%Content
Cellulose	39.5
Pentosans	19.39
Acetyl groups	2.26
Hemicelluloses	20.84

From the acetosolv experiments all the solid and liquid streams were analyzed for each and every set of experiments. Duplicate samples were run for all the experiments. From the solid streams pulp yield, lignin removal, lignin non-removal, total reducing sugars, extractives, EHPS were analyzed and from the liquid streams volume of the liquor, pH and the total reducing

sugars were analyzed. Finally lignin yields from all the acetosolv experiments were calculated. Lignin yields were calculated with respect to weight of the raw material and weight of the lignin in the raw material. All the experimental and calculated values for all acetosolv experiments are given in the table 5 below.

In the first set of delignification marabou raw material was de-lignified with acetic acid – water mixture with respect to 50-50, 70-30, 90-10 percentages at 120°C. For this set of experiments, average values of experimental results from the duplicate samples have shown in table 6. The pulp yields were decreased with respect to the increasing acetic acid concentrations. 69.05%, 60.35%, 48.1% pulp yields were obtained from the respective 50%, 70% and 90% acetic acid concentrations at 121°C. And the lignin removal from the marabou biomass was increased with respect to the increasing acetic acid concentrations, while the lignin non removal was decreased. 84.75% lignin was removed from the marabou biomass with 90% acetic acid concentration at 121°C, while 6.8% and 44.6% was removed with 50%, 70% respective acetic acid concentrations. Lignin non removal percentages were 31.55, 21.45, and 7.4 with respective 50%, 70% and 90% acetic acid concentrations.

Table 6: Results from acetosolv delignification of marabou biomass at 121°C

Pretreatment conditions	Solid fraction						Liquid fraction				
	Pulp yield %	Lignin%	Lignin removal %	TRS-AAH g/L C	% Extractives	% EHPS	Volume mL	pH	TRS g/L C	Yield (Lig/MP)	Yield (Lig/Lig MP)
AS-121-50	69.05	31.55	6.8	2.05	2	15.9	28	1	2.8	2.85	12.35
AS-121-70	60.35	21.45	44.6	2.6	1.7	14.9	29	0.475	2.1	8.4	35.85
AS-121-90	48.1	7.4	84.75	3.55	1.2	13.5	28.5		2.15	16.8	71.85

The total reducing sugars in solid fraction was 2.05, 2.6, 3.55 g/l, in liquid fraction was 2,8, 2.1, 2.15 g/l while the easy to hydrolyze polysaccharides in the solid fraction was 15.9%, 14.9%, 13.5% with respect to 50%, 70% and 90% acetic acid concentrations. Extractives that are present in the de-lignified pulps from acetosolv pretreated with 50%, 70% and 90% acetic acid concentrations were 2%, 1.7%, 1.2% respectively. That shows the extractives were removed effectively along with the acetosolv liquors while the acetic acid concentrations were increased.

Pretreatment conditions	Solid fraction						Liquid fraction				
	Pulp yield %	Lignin%	Lignin removal %	TRS-AAH g/L C	% Extractives	% EHPS	Volume mL	pH	TRS g/L C	Yield (Lig/MP)	Yield (Lig/Lig MP)
AS-121-50	68.6	32.1	5.8	2.0	2.0	15.9	26	1	3.0	2.7	11.7
AS-121-50	69.5	31.0	7.8	2.1			30	1	2.6	3.0	13.0
AS-121-70	61.2	21.0	44.9	2.4	1.7	14.9	29	0.5	2.0	8.6	36.6
AS-121-70	59.5	21.9	44.3	2.8			29	0.45	2.2	8.2	35.1
AS-121-90	47.8	7.6	84.4	3.5	1.2	13.5	27		2.1	16.6	70.9
AS-121-90	48.4	7.2	85.1	3.6			30		2.2	17.0	72.8
AS-NBT-50	86.4	25.8	4.5	1.6	1.4	29.0	29	1.2	0.45	1.3	5.4
AS-NBT-50	87.3	25.8	3.8	1.9			28	1	0.42	1.3	5.4
AS-NBT-70	82.3	21.9	23.0	2.0	2.0	26.4	32	0.7	0.62	3.6	15.6
AS-NBT-70	83.1	22.3	20.7	2.2			30	0.65	0.59	3.9	16.5
AS-NBT-90	58.4	7.5	81.4	3.2	2.8	19.9	31		0.67	14.6	62.3
AS-NBT-90	57.9	7.6	81.1	3.0			31		0.66	14.8	63.2
AS/DAPH-121-50	74.8	23.94	49.3	2.2	1.4	15.8	32	1.3	1.3	5.5	15.7
AS/DAPH-121-50	73.8	23.97	49.9	2.2			30	1.4	1.2	5.9	16.6
AS/DAPH-121-70	64.6	17.64	67.7	2.6	1.4	13.7	30	0.95	1.0	12.2	34.6
AS/DAPH-121-70	64.7	17.65	67.7	2.8			30	0.95	1.1	12.3	34.8
AS/DAPH-121-90	52.3	5.96	91.2	3.0	0.8	13.1	35	0.1	1.1	22.0	62.5
AS/DAPH-121-90	52.5	5.64	91.6	3.4			35	0.2	1.2	22.2	62.9
AS/DAPH-NBT-50	90.8	26.4	32.0	1.8	1.0	20.6	30	1	0.95	3.3	9.3
AS/DAPH-NBT-50	90.4	26.6	31.9	1.7			29	0.95	0.93	3.4	9.6
AS/DAPH-NBT-70	82.6	23.7	44.4	2.0	1.0	19.8	30	0.5	0.99	6.0	17.1
AS/DAPH-NBT-70	83.7	23.8	43.5	2.2			31	0.52	0.98	6.0	17.1
AS/DAPH-NBT-90	60.1	10.2	82.7	3.0	1.1	13.2	34	0	1.1	18.4	52.0
AS/DAPH-NBT-90	60.5	10.1	82.7	3.2			34	0.2	1.1	18.9	53.4
DAPH-121	83.5	34.9		2.5	2.0	24.5	656	1.3	5.6		
DAPH-121	84.4	35.6		2.3	2.0	24.9	654	1.4	6.7		

Table 5: Experimental results from the Acetosolv delignification of marabou biomass.

Volumes of the acetosolv liquors were almost same. Lignin yields from the 50%, 70%, 90% acetosolv liquors were 2.85%, 8.4%, 16.8% with respect to the mass of marabou biomass sample and 12.35%, 35.85%, 71.85% with respect to the total lignin in the sample. Majorly 84.75% lignin was removed and 71.85% lignin was recovered with 90% acetic acid concentration at 121°C.

Table 7 shows the experimental results from acetosolv delignification of marabou biomass at normal boiling temperature (NBT). In this set of experiments the results were also quite similar in manner like acetosolv delignification of marabou biomass at 121°C. Pulp yields were decreased with respect to increase in acetic acid concentrations and the lignin removal was increased. Obtained pulp yields, lignin removal, lignin non-removal, and total reducing sugars, extractives and polysaccharides in the pulps were shown in the table 6. And the recovered lignin yields from the acetosolv liquors are also shown in the table. Pulp yields and lignin non-removal were almost same from the acetosolv de-lignified marabou raw material at NBT with 50%, 70% acetic acid concentrations. And the lignin yields were also very small. But with 90% acetic acid concentration at normal boiling temperature has shown high results to remove lignin from marabou raw material. With 90% acetic acid concentration at NBT, 81.25% lignin was removed and 14.7%, 62.75% yields were obtained with weight of the mass and lignin in the mass respectively. Acetosolv delignification at 121°C has shown good and better results compare to the acetosolv delignification at NBT. Especially at 70% acetic acid concentration, acetosolv delignification of marabou biomass has shown better results at 121°C compare to at NBT. Volumes of the acetosolv liquors were almost same from the all experiments at NBT.

Table 7: Results from acetosolv delignification of marabou biomass at NBT

Pretreatment conditions	Solid fraction						Liquid fraction				
	Pulp yield %	Lignin%	Lignin removal %	TRS-AAH g/L C	% Extractives	% EHPS	Volume mL	pH	TRS g/L C	Yield (Lig/MP)	Yield (Lig/Lig MP)
AS-NBT-50	86.85	25.8	4.15	1.75	1.4	29	28.5	1.1	0.435	1.3	5.4
AS-NBT-70	82.7	22.1	21.85	2.1	2	26.4	31	0.675	0.605	3.75	16.05
AS-NBT-90	58.15	7.55	81.25	3.1	2.8	19.9	31		0.665	14.7	62.75

A comparison of lignin removal, and recovered lignin yields of acetosolv delignification of marabou biomass at 121°C and NBT has shown in figure 19. At 50% acetic acid concentrations lignin removal and recovery was very less in both of the experiments at 121°C and NBT. Acetosolv delignification with 70% acetic acid at 121°C has shown good results compare to at NBT. Lignin removal of 70% acetic acid at 121°C was 44.6% and yield was 35.85% lignin/lignin MP, while lignin removal at NBT was only 21.9%.

Acetosolv delignification at 121°C & NBT

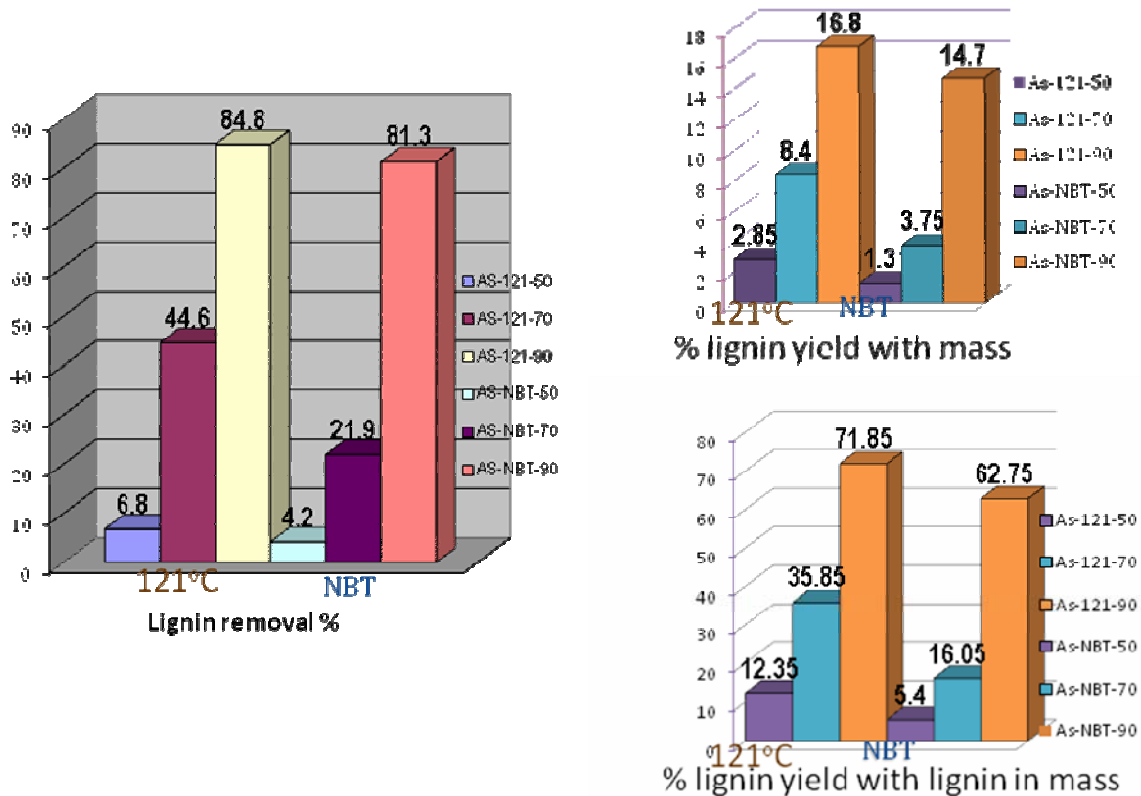


Figure 19: Lignin removal, lignin yield per mass of the sample and lignin yield per lignin in the mass of the sample from acetosolv delignification of marabou biomass at 121°C & NBT

But highest lignin removal was achieved in both acetosolv experiments at 121°C and NBT with 90% acetic acid concentrations. 84.8% lignin was removed from the marabou biomass with 90% acetic acid concentration at 121°C and the lignin yield was 71.85% lignin/lignin MP while lignin removal at NBT was 81.3% and the lignin yield was 62.75% lignin/lignin MP. However marabou biomass was delignified effectively with 90% acetic acid concentration at 121°C and NBT.

Acetosolv delignification of marabou biomass has given better results with combination of Dilute Acid Prehydrolysis (DAPH) compare to without DAPH. Duplicate samples were run to obtain the marabou pulp from DAPH, prior to acetosolv delignification. All the experimental results from the DAPH of marabou biomass have given in table 8. The average pulp yield from DAPH was 83.95% on dry basis. And the pulp contains 35.25% of lignin, 2.4 g/l total reducing sugars, 2% extractives and 24.9% of easy to hydrolyze polysaccharides. And the liquid fraction

from DAPH contains 6.15 g/l total reducing sugars and the pH of the liquid fraction was 1.35 in average.

Table 8: Results from Dilute Acid Pre-hydrolysis of marabou biomass

Pretreatment conditions	Solid fraction					Liquid fraction		
	Pulp yield %	Lignin%	TRS-AAH g/L C	% Extractives	% EHPS	Volume mL	pH	TRS g/L C
DAPH-121-1	83.5	34.9	2.5	2	24.5	656	1.3	5.6
DAPH-121-2	84.4	35.6	2.3	2	24.9	654	1.4	6.7

The pulp obtained from the DAPH was tested with acetosolv systems at 121°C and NBT with 50-50, 70-30, and 90-10 acetic acid – water system as same as the acetosolv pretreatment steps that made before. All the average results of duplicate samples from the combination of DAPH and acetosolv delignification of marabou biomass have given in table 9. From the combination of DAPH and acetosolv the pulp yields and lignin non removal has decrease with respect to the acetic acid concentration, while the lignin removal has increased, in both at NBT and 121°C temperature. 91.4% lignin was removed and 22.1% Lig/MP, 62.7 Lig/Lig MP was recovered with DAPH – acetosolv system at 121°C and 90% acetic acid concentration while the lignin removal was 49.6% and 67.7% with 50%, 70% acetic acid concentrations respectively.

With DAPH – acetosolv delignification of marabou biomass at NBT has given 31.95%, 43.95%, 82.7% lignin removal with respect to 50%, 70% and 90% acetic acid concentrations. Easy to hydrolyze polysaccharides was less in the solid fraction of DAPH – acetosolv treated pulp in all respective concentrations of acetic acid at 121°C compare to at NBT. DAPH with acetosolv system has better results at 121°C compare to at NBT especially with 70% acetic acid system (lignin removal was very high at 121°C compare to at NBT). However combined DAPH and acetosolv delignification of marabou biomass has shown good results compare to acetosolv delignification without DAPH and high amount of lignin was removed and recovered with 90% acetic acid concentration in both the experiments at 121°C and NBT in the acetosolv delignification of marabou biomass with the combination of DAPH.

Table 9 Results from DAPH & acetosolv delignification of marabou biomass at 121oC and NBT.

Pretreatment conditions	Solid fraction						Liquid fraction				
	Pulp yield %	Lignin%	Lignin removal %	TRS-AAH g/L C	% Extractives	% EHPS	Volume mL	pH	TRS g/L C	Yield (Lig/MP)	Yield (Lig/Lig MP)
DAPH/AS-NBT-50	90.6	26.5	31.95	1.75	1	20.6	29.5	0.975	0.94	3.35	9.45
DAPH/AS-NBT-70	83.15	23.75	43.95	2.1	1	19.8	30.5	0.51	0.985	6	17.1
DAPH/AS-NBT-90	60.3	10.15	82.7	3.1	1.1	13.2	34	0.1	1.1	18.65	52.7
DAPH/AS-121-50	74.3	23.955	49.6	2.2	1.4	15.8	31	1.35	1.25	5.7	16.15
DAPH/AS-121-70	64.65	17.645	67.7	2.7	1.4	13.7	30	0.95	1.05	12.25	34.7
DAPH/AS-121-90	52.4	5.8	91.4	3.2	0.8	13.1	35	0.15	1.15	22.1	62.7

DAPH & Acetosolv delignification at 121°C & NBT

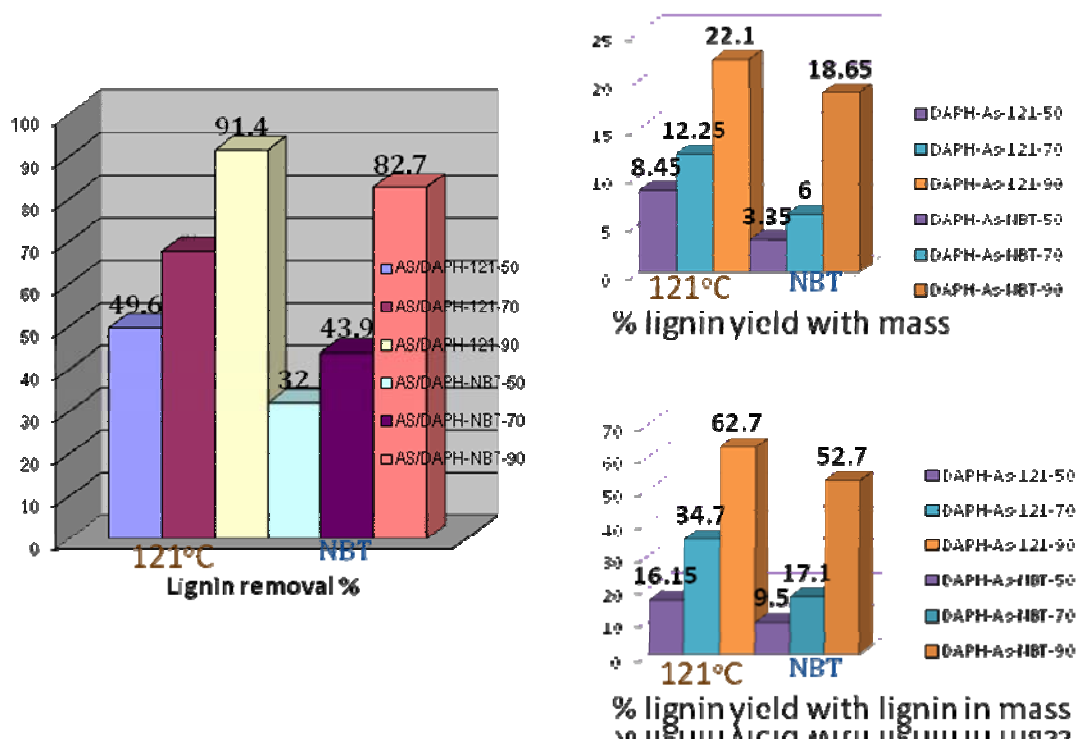


Figure 20 Lignin removal, lignin yield per mass of the sample and lignin yield per lignin in the mass of the sample from the combination of DAPH and acetosolv delignification of marabou biomass at 121°C & NBT

Lignin removal and the lignin yields of marabou biomass with the combination of DAPH and acetosolv at both 121°C and NBT have given in below figure 20. As like in the acetosolv experiments without DAPH, in these experiments high amount of lignin was removed and recovered with 90% acetic acid concentrations compare to 50% and 70% in both of the experiments at 121°C and NBT. Lignin removal with the combination of DAPH and acetosolv delignification of marabou biomass has shown good results at 121°C compare to at NBT. The lignin removal at 121°C was 49.6%, 67.7%, 91.4% with respect to 50%, 70% and 90% acetic acid concentrations, while lignin removal was 31.95%, 43.95% and 82.95% at NBT. However maximum 91.4% of lignin was removed from marabou biomass with the combination of DAPH and acetosolv delignification at 121°C and 90% acetic acid concentration and the recovered lignin yield was 22.1% lignin/mass of the sample.

One experimental design (statistical analysis) program has run with all the experimental results from the acetosolv delignification of marabou biomass with and without DAPH. Temperature, concentration of acetic acid and type of pretreatment conditions (without DAPH and with DAPH) were taken as variables pulp yield, lignin non removal, lignin removal TRS in solids, TRS in liquids lignin yield with respect to mass of the sample and mass of the lignin in the sample were taken as response factors. All the graphics that has got from this design has shown below in figure 21.

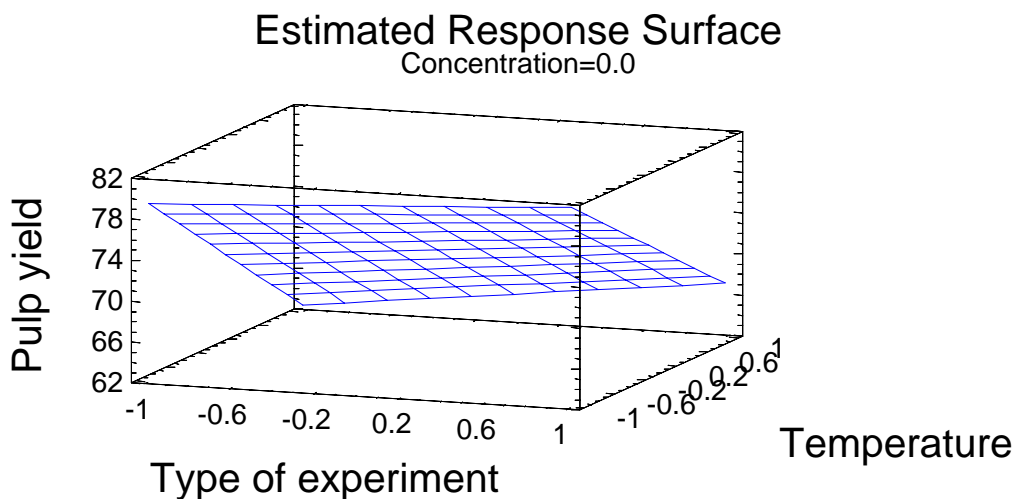


Figure 21 (a) Pulp yields from the acetosolv delignification of marabou biomass.

In all this analysis type of experiments is -1 and 1 where -1 is shows the experiments without DAPH and 1 shows the experiments with DAPH. Temperatures are -1 and 1 where -1 is at NBT

and 1 at 121°C. And the concentrations of acetic acid are -1, 0, 1 where -1 is 50%, 0 is 70% and 1 is 90% acetic acid concentrations.

Figure 21 (a) shows the pulp yield from all the acetosolv delignification experiments of marabou biomass in this less pulp yield is optimum.

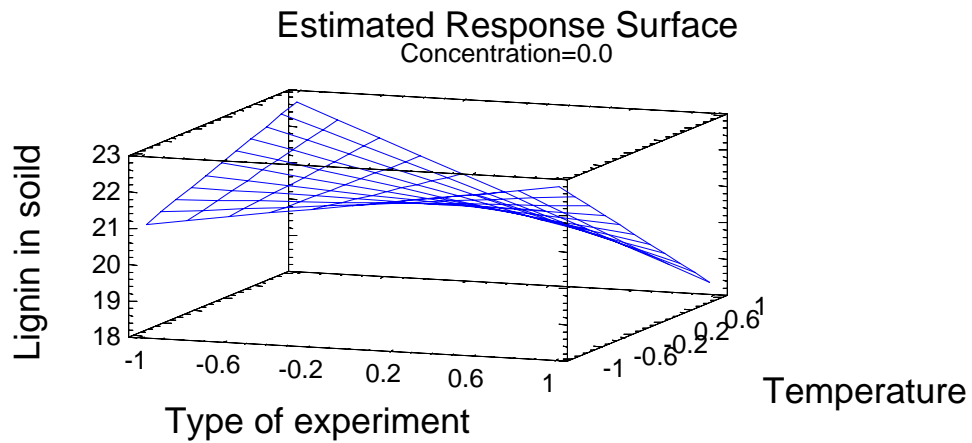


Figure 21 (b) Lignin non-removals from the marabou biomass with acetosolv pretreatment.

Figure 21 (b) shows the non removal of lignin from the marabou biomass with acetosolv pretreatment in this the optimum condition is the less lignin non removal and it has obtained with the combination of DAPH – acetosolv delignification of marabou biomass and that is only 5.8% of lignin couldn't removed with this system.

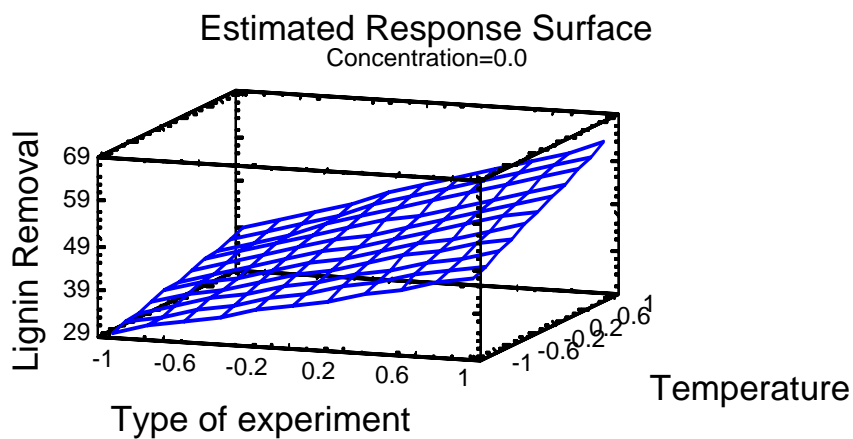


Figure 21 (c) Lignin removals from the marabou biomass with acetosolv pretreatment.

Figure 21 (c) shows the lignin removal from the marabou biomass with acetosolv pretreatments. Maximum lignin removal is the optimum in these experiments, which has obtained with the combination of DAPH – acetosolv pretreatment at 121°C with 90% acetic acid concentration, and the lignin removal was 91.4%.

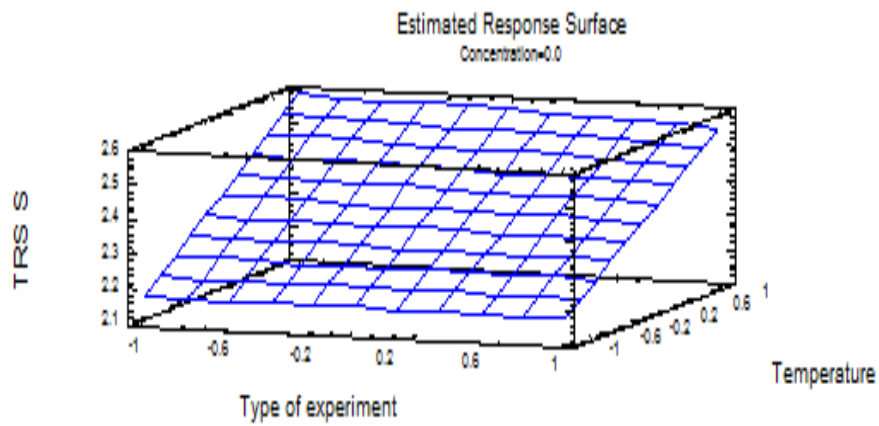


Figure 21 (d) 1 Total reducing sugars present in the solid fraction of acetosolv pretreated marabou biomass

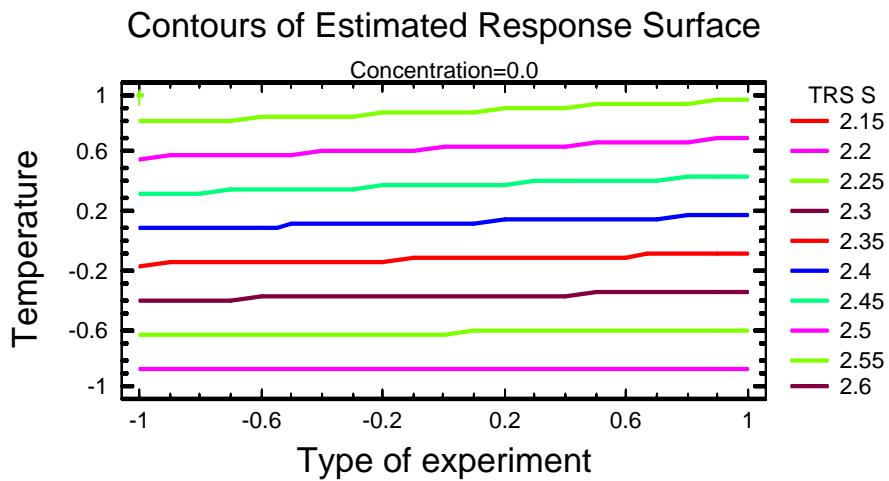


Figure 21 (d) 2 Total reducing sugars present in the solid fraction of acetosolv pretreated marabou biomass

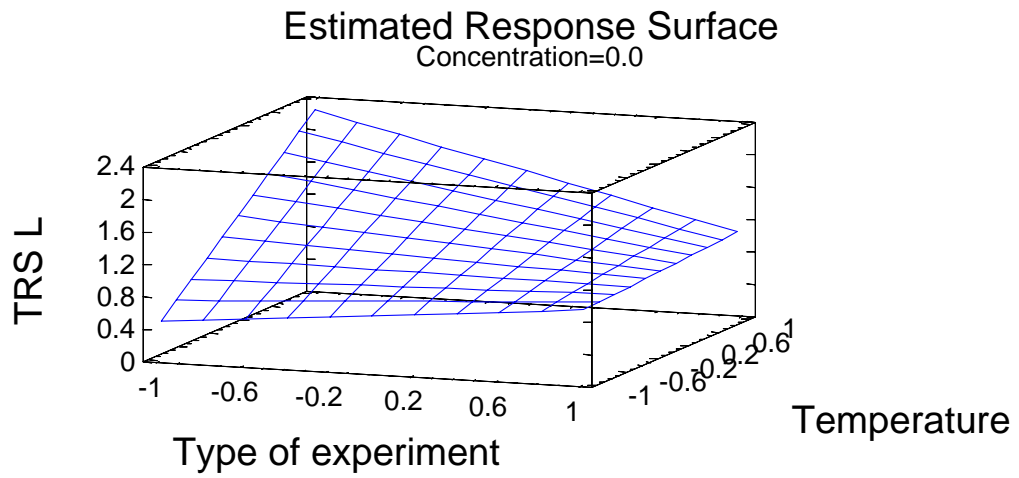


Figure 21 (e) 1 Total reducing sugars present in the liquid fraction of acetosolv pretreated marabou biomass

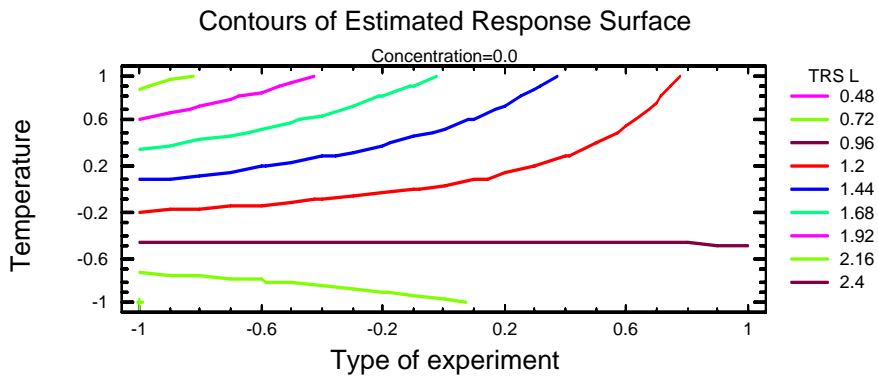


Figure 21 (e) 2 Total reducing sugars present in the liquid fraction of acetosolv pretreated marabou biomass

Figure 21 (d) and 21 (e) showing the total reducing sugars that are present in the solid fraction and the liquid fraction of acetosolv pretreated marabou biomass with and without DAPH at different operational conditions that have specified above in all experiments.

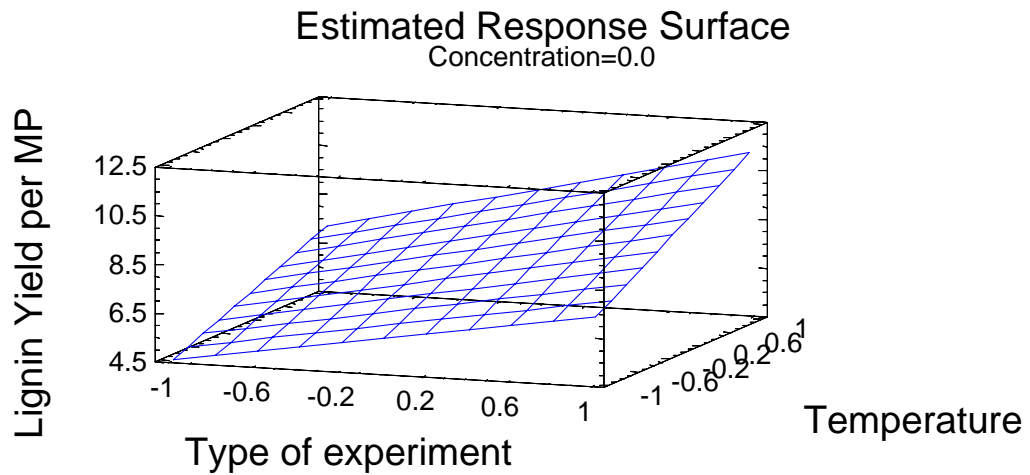


Figure 21 (f) 1 Lignin yields per mass of the sample from the acetosolv delignification of marabou biomass.

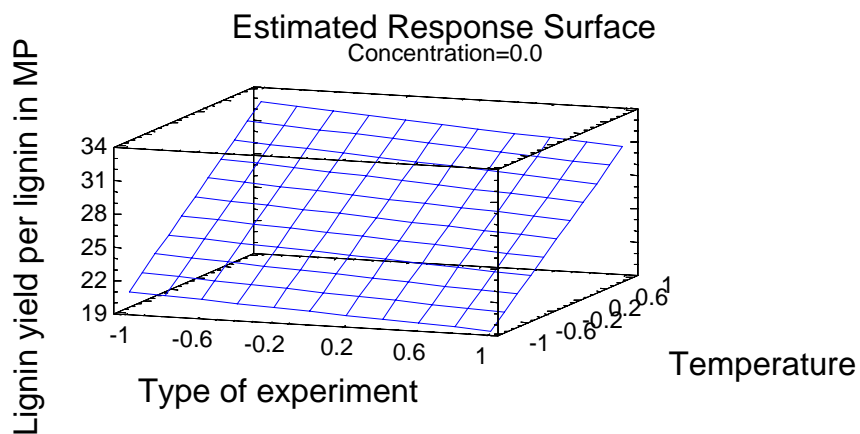


Figure 21 (f) 2 Lignin yields per lignin in the sample from the acetosolv pretreatment of marabou biomass.

Figure 21 (f) shows the lignin yields (with respect to mass of the sample and mass of the lignin in the sample) from the acetosolv delignification of marabou biomass with and without DAPH and in different experimental conditions that has specified in this work. The optimum in this work is maximum lignin yield. 22.1% lignin/MP has obtained from the set of experiments with the combination of DAPH – acetosolv delignification of marabou biomass with 90% acetic acid concentration and at 121°C.

4. Conclusion

Delignification of *Dichrostachys cinerea* (marabou) biomass with 50%, 70% and 90% of acetic acid concentrations with and without dilute acid prehydrolysis (DAPH) at 121°C and NBT have studied with 0.2% HCl as a catalyst and 1:10 solid-liquid ratios. Acetosolv delignification of marabou has shown good results in many set of experiments with deferent reaction conditions. Figure 22 shows the lignin removal from marabou biomass from different acetosolv experiments. As shown in figure maximum lignin was removed from the marabou biomass with 90% acetic acid concentration in all acetosolv experiments that have been studied. Very less amount of lignin was removed with 50% acetic acid concentrations compare to 70% and 90% in all acetosolv experiments. The combination of DAPH and acetosolv has removed more lignin from marabou biomass compare to acetosolv without DAPH with 50%, 70% and 90% acetic acid concentrations at 121°C and NBT.

Lignin Removal From Marabou

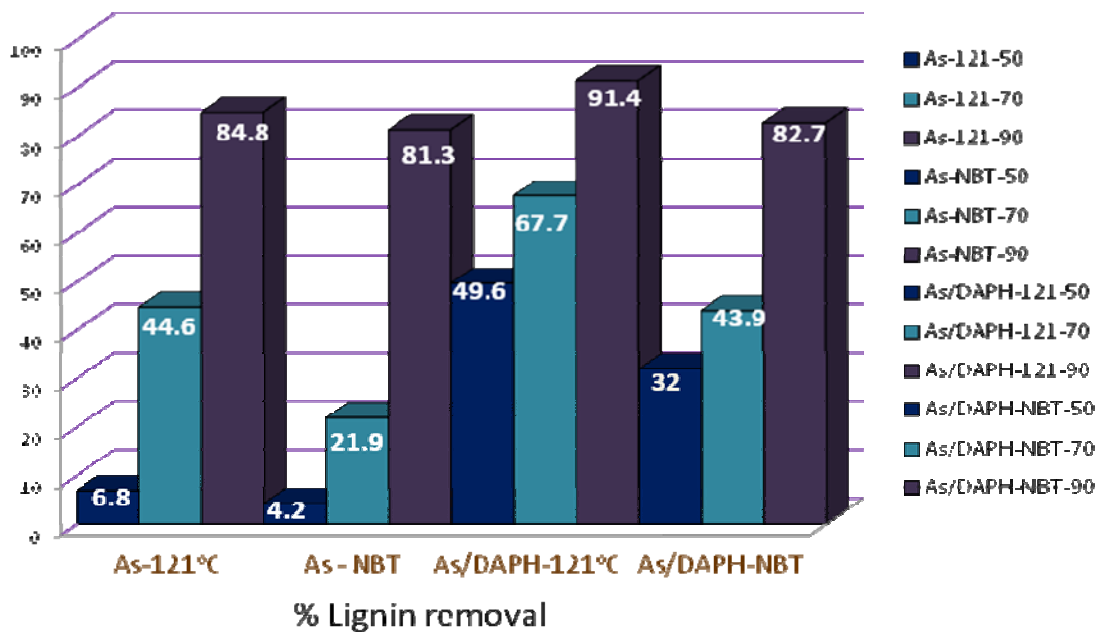


Figure 22: Delignification of marabou biomass with different acetosolv experimental conditions

Acetosolv delignification of marabou biomass at 121°C, NBT and acetosolv delignification with the combination of DAPH at 121°C, NBT with 90% acetic acid concentration has removed

respectively 84.8%, 81.3%, 91.4%, and 82.7% lignin. Acetosolv with the combination of dilute acid prehydrolysis have removed lignin effectively from marabou biomass at 121°C with 50%, 70% and 90% acetic acid concentrations compare to all other acetosolv processes that have been tested with the respective acetic acid concentrations. Increase in acetic acid concentration and temperature has shown significant effect in these experiments. However majorly 91.4% of lignin was removed from marabou biomass with the combination of DAPH and acetosolv at 121°C and 90% acetic acid concentration, and the yield was 22.1% of the mass.

5. Future works

This work has shown that lignin can be removed effectively from marabou biomass with the increasing acetic acid concentration in the reaction mixture. It would be interesting to test with 95% acetic acid concentration in the acetosolv system with and without DAPH at 121°C and NBT. And this work would be interesting to continue as like the points given below.

- Evaluation of the enzymatic convertibility of acetosolv cellulose
- Obtention of acetosolv lignin derivatives
- Further characterisation of acetosolv lignin
- Alcoholic fermentation of cellulose hydrolysates
- Alcoholic fermentation of hydrolysates from all the polysaccharides
- Lactic acid fermentation of hemicellulose hydrolysates

6. Nomenclatures:

AS	Acetosolv Pretreatment
DAPH	Dilute acid pre-hydrolysis
AAH	Analytical acid hydrolysis
EHPS	Easy to hydrolyse polysaccharides
DHPS	Difficult to hydrolyse polysaccharides
TRS	Total reducing sugars
MP	Mass of the sample
NBT	Normal boiling temperature

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