

**OPTIMIZATION OF ACID PRETREATMENT
PROCEDURES FOR OIL PALM TRUNK FIBERS
USED IN PRODUCTION OF SUGAR**

PRESENTED BY

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Optimization of acid pretreatment procedures for oil palm trunk fibers used in production of sugar:
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CERTIFICATION

I hereby certify that this work titled "**OPTIMIZATION OF ACID PRETREATMENT PROCEDURES FOR OIL PALM TRUNK FIBERS USED IN PRODUCTION OF SUGAR**" is original and written by me.

It is an accurate record of my research work and has not been presented before for the award of any other degree.

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Signature

(Student)

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(Asst. Project Supervisor)

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DECLARATION

We declare that this his entitled "**OPTIMIZATION OF ACID PRETREATMENT PROCEDURES FOR OIL PALM TRUNK FIBERS USED IN PRODUCTION OF SUGAR**" by **Onwuka Obasi Kalu (20074585478)** carried out under our supervisor has been examined and found to have met the regulations of the Federal University of Technology, Owerri (FUTO) for the award of M.Sc. in Chemistry (Analytical Chemistry option).

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DEDICATION

This work is dedicated to my beloved and wonderful Parents for
everything...

ACKNOWLEDGEMENT

I wish to express my profound gratitude to God Almighty for seeing me through in this academic study.

Special acknowledgment is due to my supervisor, Prof. E. N. Ejike, whose interest, patience, constructive criticism, intellectual guidance and thorough supervision the secret behind the completion of this work.

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Finally, I am most grateful to the following: Mr. Ayika, Mr. Emmanuel, Nkechi, and Uzo who aided in the success of this work, by giving me materials and all needed assistance. My course mates, lecturers and my friends who in one way or the other contributed to the success of this work, I also say thank you.

OPTIMIZATION OF ACID PRETREATMENT PROCEDURES FOR OIL PALM TRUNK FIBERS USED IN PRODUCTION OF SUGAR

ABSTRACT

The Oil Palm Trunk (OPT) fiber was pretreated, using Tetraoxosulphate (vi) acid H_2SO_4 , Hydrochloric acid HCl , and Trioxonitrate (v) acid HNO_3 , at temperature of $80^\circ C$, and residence time varying from 15 to 45 minutes, and acid concentration between 1-2.5%. It was found that the optimum acid concentration and residence time for highest sugar yield, was 1% w/w and 30minutes for the three acids investigated. Hydrolysis with HCl gave the highest sugar yield. The effect of additives such as 0.5M $ZnCl_2$, and $(NH_4)_2SO_4$ showed little or no improvement. $ZnCl_2$ had a marginal effect on the increase in sugar concentration.

Keywords: *Acid hydrolysis, Oil Palm Trunk (OPT), Optimization, Additives*

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LIST OF ABBREVIATIONS

OPT	Oil Palm Trunk
OPF	Oil Palm Fronds
EFB	Empty Fruit Bunch
POME	Palm Oil Mill Effluent
H_2SO_4	Tetraoxosulphate (vi) acid
HCl	Hydrochloric
HNO_3	Trioxonitrate (v) acid
ZnCl_2	Zinc Chloride
$(\text{NH}_4)_2\text{SO}_4$	Ammonium Sulphate
AFEX	Ammonia Fiber Explosion

CHAPTER 1

INTRODUCTION

1.1 Oil Palm Industry in Nigeria

The Oil Palm (*Elaeis guineensis*) is an important economic crop in the tropics (Anyawu *et al.*, 1982). Oil Palm locally called “Nkwu” (Igbo) and “Ope” (Yoruba) in Nigeria is a native of West Africa humid tropics, the Congo basin and Central Africa, growing wild in secondary forest (Ugochukwu *et al.*, 1999, Akinyoseye, 1976). Presently Oil Palm exists in a wild, semi-wild and cultivated state in the three land areas of the equatorial tropics, Africa, East Asia and America (Hartley, 1988).

Oil Palm is also indigenous of the Nigerian plain, having migrated inland as a stable crop. In the case of Nigeria, oil palm cultivation is part of the way of life-indeed it is the culture-of millions of people (World Rainforest Movement Bulletin, 2001). Over the decades, oil palm production in Nigeria has declined compared to it previously. While in the early 1960’s, Nigeria’s oil palm production accounted for 43% of the world production, nowadays it only accounts for 7% of

total global output (World Rainforest Movement Bulletin, 2001). Even in Nigeria today, the greatest bulk of oil palm and products is not derived from the cultivated oil palm but the groves of palms growing wild, often in a state of semi cultivation (International Potash Institute, 1957).

It was in an attempt to restore the oil palm to its prime position as well as agriculture as the mainstay of the economy, that the Federal and State governments started revamping and establishing oil palm agencies (www.otal.com). In doing this, the countries oil palm production has increased, and so is the waste.

1.2 Oil palm Waste

In an oil mill, fruits bunches are treated mainly in four stages of a semi-continuous process involving bunch cooking, bunch stripping, oil extraction, and oil refining (Hartley, 1988). Various by-products such as bunch, sludge, and fiber, are produced through the process and almost all of them are discarded at a site in the factory without further use. In these wastes, bunch alone is used as a potassium-rich

fertilizer after ignition, but sludge and fiber have not been reused up to now.

The waste includes Oil Palm Fronds (OPF), Oil Palm Trunks (OPT), Empty Fruit Bunch (EFB), Palm kernel, Palm press fiber, cake, palm kernel shell, and Palm Oil Mill Effluent (POME). More than 70% (by weight) of the processed fruit bunch in the palm oil mill are left over as the oil palm waste (Prasertsan and Prasertsan, 2004).

These wastes are Lignocellulose materials, and so contain cellulose, hemicelluloses, lignin, and other substances from were sugar can be obtained from by hydrolysis and subsequent fermentation/distillation for ethanol production.

Figure 1.1 shows the flowchart of the production of oil palm waste from a standard oil palm industry.

Schematic flow diagram of a palm oil mill

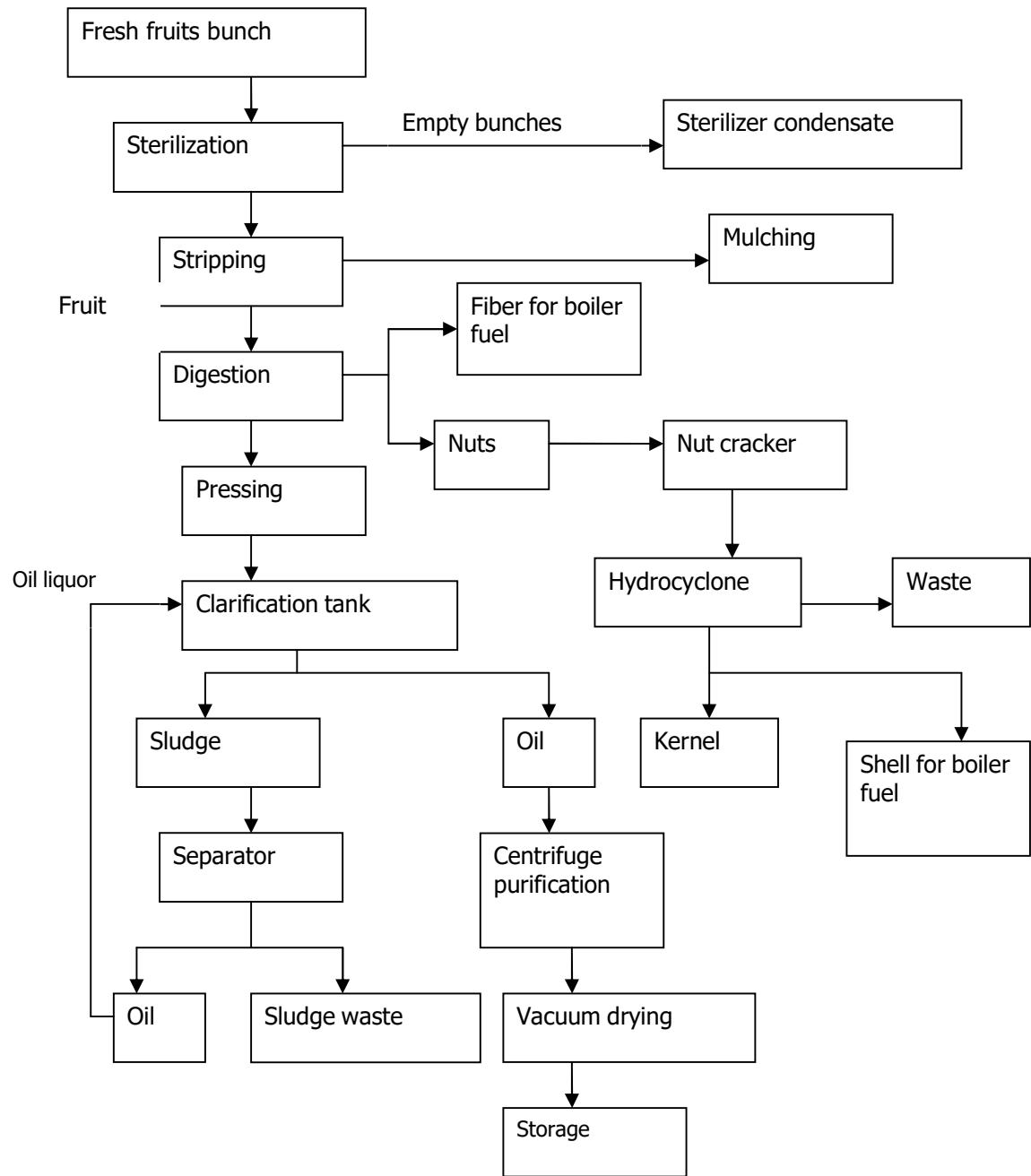


Figure 1.1 The production of oil palm waste from oil palm industries (Prasertsan and Prasertsan, 2004).

1.3 Renewable Energy

The development of a technological society is closely linked to humankind's growing energy needs. Fossil fuels, namely oil, natural gas and coal, have been the fundamental sources of energy during the 20th and early 21st centuries. At the same time, they have been increasingly used as raw materials for chemical industries.

With the cost of Petroleum continuously rising and the over dependence upon fossil fuel resources, attention is now focused on alternative energy sources which are renewable sources of energy. These renewable energy sources include; Photovoltaic (PV), wind, water, geothermal, and bio-fuels. On top of the list of renewable energy sources are the bio-fuels with particular attention on ethanol; in other that it might replace gasoline in the near future.

Ethanol is a promising alternative fuel which can be produced biologically from a variety of waste materials such as paper products, corn fiber, sawmill waste, straw, and rice. Ethanol has been made from grapes, barley and potatoes for thousands of years. The

production of ethanol from non-starch, lignocellulosic materials is, however, a fairly recent development. The ethanol can be blended with gasoline or used neat in combustion engines. As a fuel, ethanol burns cleaner than gasoline, is completely renewable, and relatively less toxic to the environment (Dowe *et al*, 2001; Wyman, 2007). The emission and toxicity of ethanol is lower than those of petroleum ().

Before now the conventional production of bio-ethanol utilizes sugar resources supplied by sugar cane or corn grain (Wyman, 2007). These sources of bio-ethanol are not widely accepted, because of it competing with the need for human food, but these days it is produced from lignocellulosic biomass. Ethanol production from lignocellulosic biomass is far better than that from other sources. This is because the chemical composition of lignocellulosic biomass differs greatly from products with high sugar or starch content (Galbe and Zacchi, 1993; Galbe *et al.*, 1997; McMillan, 1997). The input/output relation between the energy released during ethanol combustion and the energy needed to produce it from lignocellulosic residues is six, a little lower than the process using sugars and honeys, but quite

superior than the process using grains (Van Zessen *et al.*, 2003). And also its extensive distribution, renewable character and availability in great quantities at low cost, makes it a better option.

Oil Palm Trunk (OPT) which is one of the major wastes from oil palm industry is one of the cheapest sources of lignocellulosic biomass. And this work is based on it being used as a feedstock for the production of ethanol.

1.4 Problem statement

With the increase in Palm oil production in the country, and the subsequent waste – OPT fiber in particular – generated as a result of it, there is need to devise a waste disposal mechanism that is cost effective. This is where the use of OPT fiber as a feedstock in ethanol production comes in, because it is both cost effective and attractive, being a source of renewable energy – a solution to our energy crisis, and a waste disposal mechanism at the same time.

There are two methods of conversion of lignocellulose to ethanol. These are acidic or enzymatic hydrolysis. But first it has to be

pretreated in other to obtain a better sugar yield. There are various pretreatment procedures as shown in Table 2. For this work acid pretreatment is used. In acid pretreatment of lignocellulose, and subsequent acid hydrolysis to get ethanol, the following factors: residence temperature, acid concentration, and residence time; influence the ethanol yield. So this work is an investigation to optimize the acid pretreatment procedure in order to get the highest ethanol yield from the lignocellulosic material.

1.5 Fermentation Products and inhibition of process

OPT fiber hydrolysate like other lignocellulosic materials have been shown to contain other products, not only fermentable sugars, but also a wide spectrum of by-products including furfural, acetic acid and phenolic compounds (Rahman *et al.*, 2007; Herrera *et al.*, 2003; Aguilar *et al.*, 2002). The inhibitory effect of these by-products has become one of the major impediments to the commercialization of ethanol from biomass. According to Delgenes *et al.*, (1996) since the toxic components effects are cumulative, efficient methods must be developed to overcome either the single or recombined effect

resulting from even low residual concentration of individual components. The concentration of these by-products after hydrolysis is dependent on the material's internal structure, which in turn can be affected by the degree of pretreatment, so it becomes very imperative that a good pretreatment procedure be carried out on the material.

The major problem in this work was to develop an optimized acid pretreatment method that will help eliminate or decrease the concentration of these by-products thereby increasing the ethanol yield.

Figure 1.2 shows the overall process of ethanol production using sugars form lignocellulosic material.

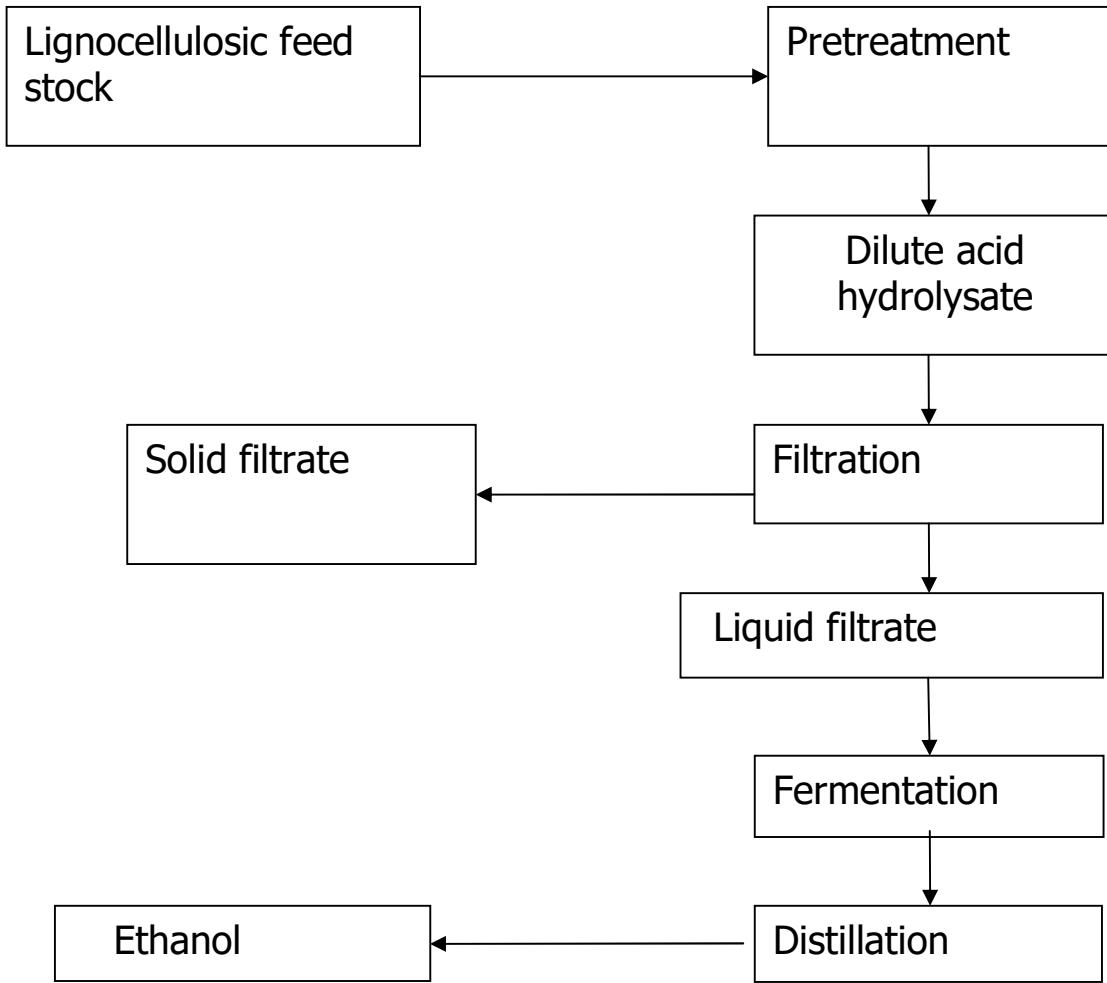


Figure 1.2 Ethanol production using sugars from lignocellulosic material.

CHAPTER 2

LITERATURE REVIEW

2.1 Lignocellulosic Biomass

Lignocellulosic biomass refers to plant biomass that is composed of cellulose, hemicelluloses, and lignin. The carbohydrate polymers (cellulose and hemicelluloses) are tightly bound to the lignin, by hydrogen and covalent bonds (Yan *et al.*, 1996).

2.2 Lignocellulosic structure

Lignocellulose is the primary building block of plant cell walls. Plant biomass is mainly composed of cellulose, hemicelluloses, and lignin, along with smaller amounts of pectin, protein, extractives (soluble nonstructural materials such as nonstructural sugars, nitrogenous material, chlorophyll, and waxes), and ash (Jorgensen *et al.*, 2007). The composition of these constituents can vary from one plant species to another. For example, hardwood has greater amounts of cellulose, whereas wheat straw and leaves have more hemicelluloses (Table 1). In addition, the ratios between various constituents within

a single plant vary with age, stage of growth, and other conditions (Perez *et al.*, 2002).

Table 1: Cellulose, Hemicelluloses, and Lignin Contents in Common Agricultural Residues and Wastes (Jorgensen et al, 2007)

Lignocellulosic material	Cellulose (%)	Hemicelluloses (%)	Lignin (%)
Hardwood stems	40-55	24-40	18-25
softwood stems	45-50	25-35	25-35
Nut shells	25-30	25-30	30-40
Corn cobs	45	35	15
Grasses	25-40	35-50	10-30
Paper	85-99	0	0-15
Wheat straw	30	5	15
Sorted refuse	60	20	20
Leaves	25-20	80-85	0
Cotton seed hairs	80-95	5-20	18-30
Newspaper	40-55	25-40	18-30
Waste papers from chemical pulp	60-70	10-20	5-10
Primary waste water solids	8-15	-	-
Solid cattle manure	1.6-4.7	1.4-3.3	2.7-5.7

Coastal grass	Bermuda	25	35.7	6.4
Switch grass		45	31.4	12

2.2.1 Cell Wall Structure

Plant cell walls are the source of lignocellulosic materials, which are referred to here as biomass, whose structure is chiefly represented by the physio-chemical interaction of Cellulose (a linear glucose polymer), with Hemicelluloses (a highly branched hetero-polymer), and Lignin (a very high molecular weight and cross-linked aromatic Macromolecule), (Himmel *et al.*, 2007; Howard *et al.*, 2003; Joseleau *et al.*, 1992, Meshitsuka and Isogai, 1996; Sakakibara, 1991). Oil palm fiber just like every other known lignocellulose materials is also made up of these three major components. In general, lignocellulosic material is structured as can be seen in Figure 2.1.

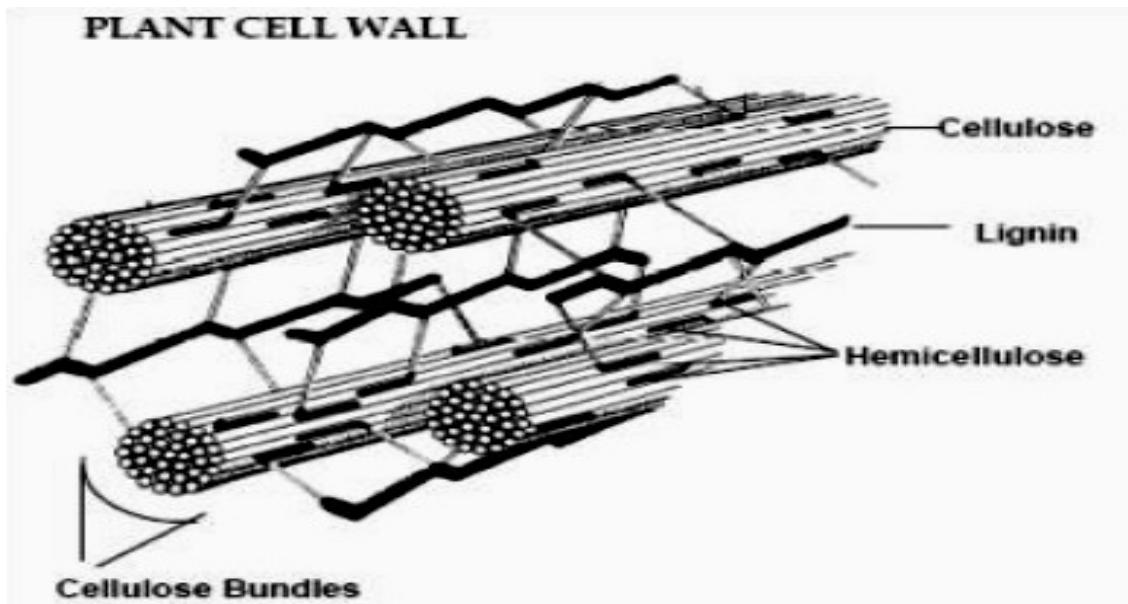


Figure 2.1 Structure of a plant cell wall

2.2.2 Chemical Structure

Biomass and especially oil palm trunk, is essentially composed of compounds containing only carbon, hydrogen, and oxygen. Such woody substances, are composed predominantly of carbohydrates, (cellulose, hemicelluloses), aromatic component-lignin, and extractives. The carbohydrates are all polymers or polysaccharides and are the most highly oxygenated constituents of wood.

2.2.2.1 Cellulose

Cellulose is the most abundant organic material on earth, comprising approximately 50% of all biomass for an annual production of about 100 billion tones. It is a polysaccharide with a high molecular weight and also the structural component of the primary cell wall of green plants, many forms of algae and the oomycetes. Some species of bacteria secrete it to form bio-films. About 33 percent of all plant matter is cellulose (the cellulose content of cotton is 90 percent and that of wood is 50 percent); (Encyclopedia Britannica Online, 2008). Many hundreds or thousands monomeric units are bound covalently. The monosaccharide is a glucose unit as well as in starch oligomers. In the cellulose polymer the glucose monomers are bound together with β -1,4 orientation. The basic compound is a cellobiose, a disaccharide of two β -1,4 bonded glucoses.

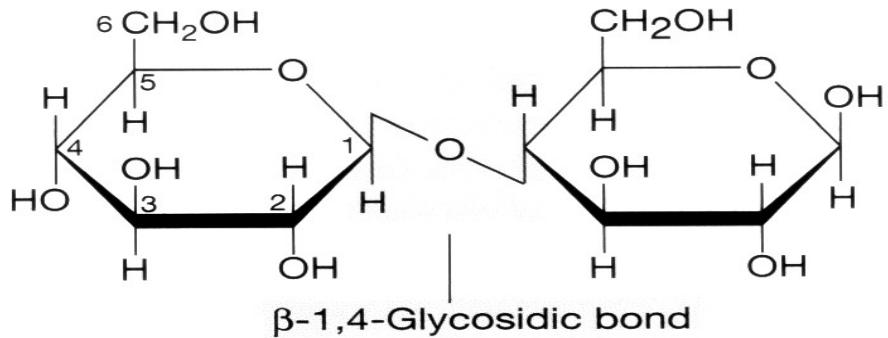


Figure 2.2 Cellubiose (Medigan *et al*, 2000)

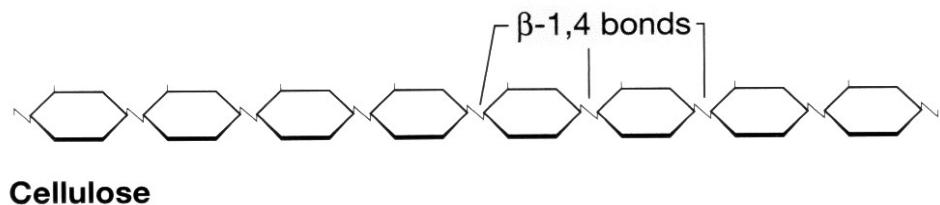


Figure 2.3 Cellulose Cellubiose (Medigan *et al*, 2000)

Despite the presence of three hydroxyl groups on each anhydroglucose residue in the cellulose chain, cellulose is completely insoluble in water. Strong acids or alkalis, concentrated salt solutions, and various complexing reagents can swell and disperse, or even dissolve the cellulose, breaking up the highly ordered crystallites. On regeneration from dispersion or solution the cellulose assumes a different crystalline structure. The degree of crystallinity of celluloses varies with their origin (Goldstein, 1981).

For industrial use, cellulose is mainly obtained from wood pulp and cotton. It is mainly used to produce cardboard and paper; to a smaller extent it is converted into a wide variety of derivative products such as cellophane and rayon. Converting cellulose from energy crops into bio-fuels such as cellulosic ethanol is a source of much socio-economic and technical controversy.

Some animals, particularly ruminants and termites, can digest cellulose with the help of symbiotic micro-organisms that live in their guts. Humans cannot digest cellulose; however it is often referred to as 'dietary fiber' or 'roughage' (e.g. outer shell of Maize) and acts as a hydrophilic bulking agent for feces (<http://en.wikipedia.org>).

2.2.2.2 Hemicelluloses

Closely associated with the skeletal polysaccharide cellulose in the wall are other structural polysaccharides collectively called Hemicelluloses (Schurech, 1963). Hemicelluloses are branched polymers of xylose, arabinose, galactose, mannose, and glucose, of which xylose is the largest amount. Hemicelluloses bind bundles of

cellulose fibrils to form micro-fibrils, which enhance the stability of the cell wall. Hemicelluloses consist of shorter chains - around 200 sugar units, and also branched, whereas cellulose is un-branched. They also cross-link with lignin, creating a complex web of bonds which provides structural strength, but also challenge microbial degradation of the plant material. They differ from the cellulose in that although water insoluble, they can be dissolved in strong alkali. This property may be used to separate the hemicelluloses, leaving essentially pure or α -cellulose behind. Hemicelluloses are also more readily hydrolyzed by acid than cellulose (Goldstein, 1981).

There are only a few basic hemicelluloses structures found in all plants, which show small modifications in different plants, and often within the same plants in different tissues. In softwoods which contain about 25% hemicelluloses, the principal constituent sugars in descending abundance are mannose, galactose, xylose, glucose, and arabinose. In hardwoods which contain about 30% hemicelluloses, the principal constituent sugars in decreasing abundance are xylose, galactose, mannose, with minor amounts of rhamnose and

arabinose. Both types contain 4-O-methylglucuronic acid. In general, annual plants and hardwoods are rich in pentosans while in softwoods, the hexosans predominate (Goldstein, 1981).

Some monomers of hemicellulose are shown Figure 2.4

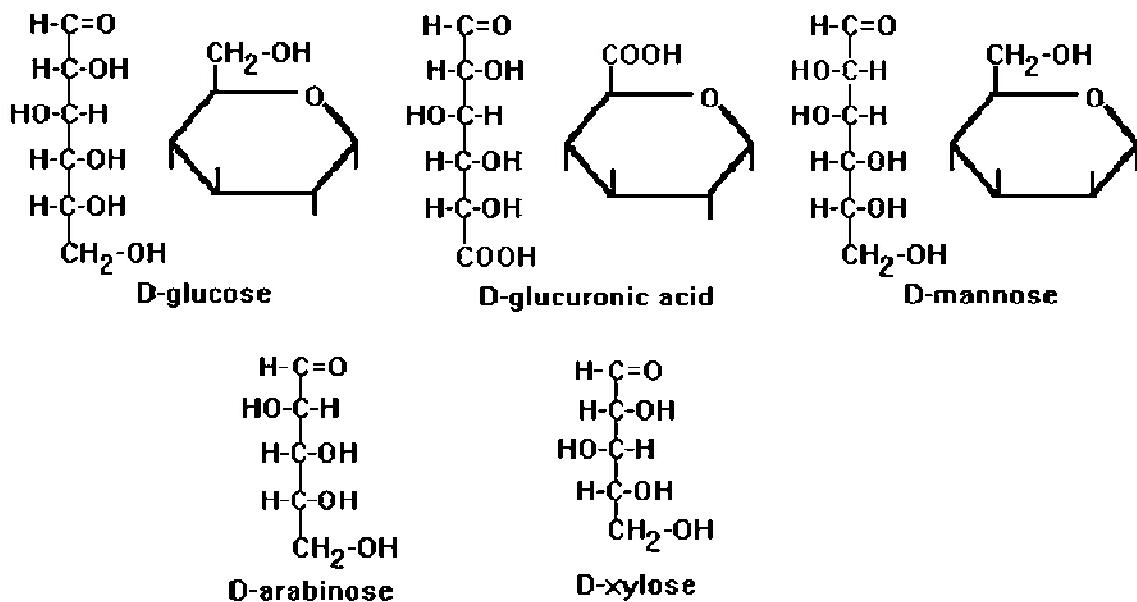


Figure 2.4 Some monomers of Hemicelluloses

2.2.2.3 Lignin

The third major cell wall component in plants is Lignin, comprising approximately the remaining 25% of the cell wall material. It serves as the cement between the wood fibres, as a stiffening agent within the fibres, and as a barrier to enzymatic degradation of the cell wall.

Lignin is a three dimensional network polymers formed from

phenylpropane units that have randomly grown into a complicated large molecule with many different kinds of linkages between the monomers. It is formed by the condensation of cinnamyl alcohol monomers in Figure 2.5 (Goldstein, 1981).

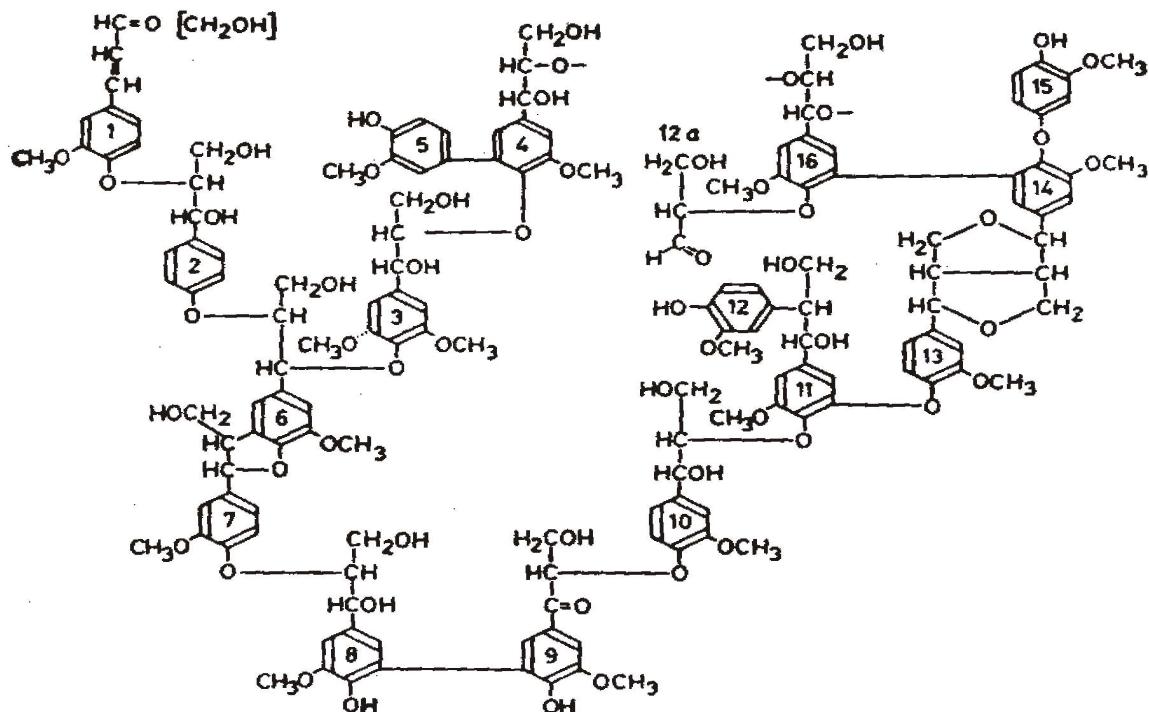


Figure 2.5 The structure of Lignin (Goldstein, 1981).

Lignin condenses in acid, especially in strong mineral acids, whereas the carbohydrate fraction of wood is hydrolyzed into soluble sugars. The acid-insoluble lignin residue is more condensed than the original lignin; i.e., it has an increase in carbon-carbon bonding among the monomeric units, which is highly undesirable for most

uses. Lignin is soluble in hot alkali and bisulfite-sulfite solutions, which is the basis for delignification in chemical pulping. Lignin is not as oxygenated as the carbohydrate portion of wood, giving it a higher fuel value. Lignin, however, is the readily oxidized component of wood, and this property is utilized in holocellulase preparations and in pulp bleaching (*Chum et al., 1985*).

Despite their importance and tremendous natural abundance, second only to cell wall carbohydrates, lignin resisted structural characterization until quite recently, and still poses a major problem for their utilization (*Sarkanen et al., 1971*).

2.2.2.4 Extractives

Extractives are the term applied to the extraneous plant components that may be separated from the insoluble cell wall material by their solubility in water or organic solvents (Buchanan, 1963; Hillis, 1962). They include many different kinds of chemicals and a large number of individual compounds. Traditional uses of chemical from biomass have involved such extractive components as turpentine, rosin, tall oil

fatty acids, tanning materials, camphor, volatile oils, gums, and rubber.

Classification of extractives is made difficult by their great variety. Although related species often contain similar compounds, distinct differences occur even between closely related species so the total number of compounds is very large. Even within the same plant, different tissues contain different extractives (Goldstein, 1981). Major categories of extractives include volatile oils, terpenes (turpentines, tropolones, cymene, resin acids, and steroids), fatty acids, unsaponifiables, polyhydric alcohols nitrogen compounds, and aromatic compounds (acids, aldehydes, alcohols, phenylpropane dimmers, stilbenes, flavonoids, tannins, and quinones).

2.3 Pretreatment of Lignocellulosic Biomass

Although lignocellulose is the most abundant plant material resource, its susceptibility has been curtailed by its rigid structure. As a result, an effective pretreatment is needed to liberate the cellulose from the

lignin seal and its crystalline structure so as to render it accessible for a subsequent hydrolysis step (Mosier *et al.*, 2005).

Pretreatment refers to the solubilization and separation of one or more of the four major components of biomass—hemicelluloses, cellulose, lignin, and extractives. It makes the remaining solid biomass more accessible to further chemical or biological treatment. Its consists of the collection, transportation, manipulation, storage, grinding or chipping to reduce the particle size and opening the fibrous material in order to transform it into a suspension that may be pumped and enable the further penetration of the chemical hydrolysis agents (Muzzy *et al.*, 1983). It also includes a thermal-chemical treatment, in order to achieve soften lignin and hemicelluloses to enable the further action of enzymes or microorganisms (Millett *et al.*, 1975).

The goal of the pretreatment process is to break down the lignin structure and disrupt the crystalline structure of cellulose, so that the acids or enzymes can easily access and hydrolyze the cellulose

(Mosier *et al*, 2005). Besides effective cellulose liberation, an ideal pretreatment has to minimize the formation of degradation products because of their inhibitory effects on subsequent hydrolysis and fermentation processes (Olsson *et al.*, 1996). The presence of inhibitors will not only further complicate the ethanol production but also increase the cost of production due to entailed detoxification steps.

Pretreatment can be the most expensive process in biomass-to-fuels conversion but it has great potential for improvements in efficiency and lowering of costs through further research and development (Mosier *et al.*, 2005; Lynd *et al.*, 1996; and Lee, 1997). Pretreatment is an important tool for biomass-to-ethanol conversion processes and is the main subject of this work. The goal of pretreatment in biomass-to-biofuels conversion is depicted in Figure 2.6.

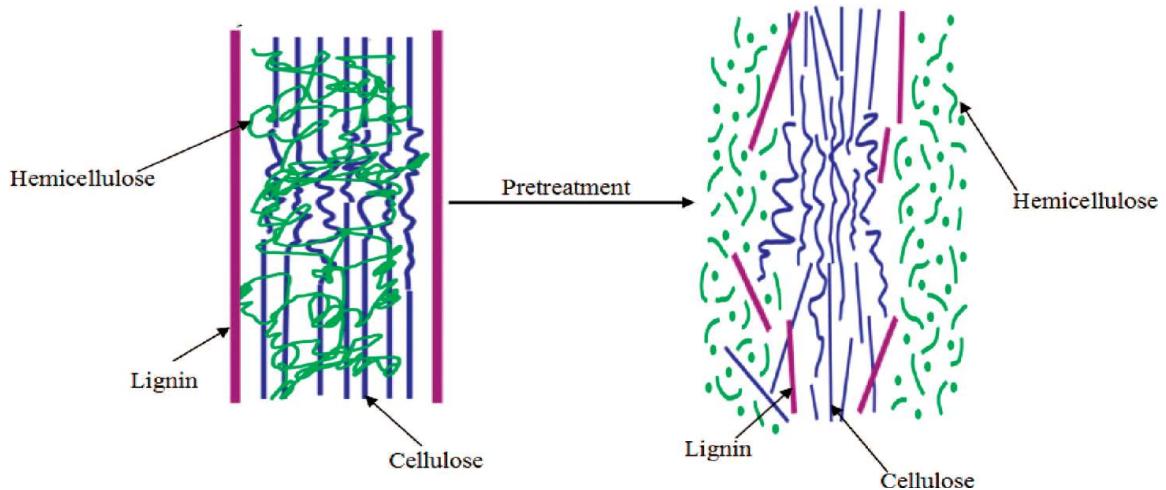


Figure 2.6 Schematic of the role of pretreatment in the conversion of biomass to fuel. (Hsu *et al*, 1980).

2.4 Methods of pretreatment

The beneficial effects of pretreatment of lignocellulosic materials have been recognized for a long time (Himmel *et al*, 1994). Its goal is to remove lignin and hemicelluloses, reduce the crystallinity of cellulose, and increase the porosity of the lignocellulosic materials.

Pretreatment must meet the following requirements:

- Improve the formation of sugars or the ability to subsequently form sugars by hydrolysis
- Avoid the degradation or loss of carbohydrate

- Avoid the formation of byproducts that are inhibitory to the subsequent hydrolysis and fermentation processes, and
- Be cost-effective.

Various pretreatment techniques change the physical and chemical structure of the lignocellulosic biomass and improve hydrolysis rates. During the past few years a large number of pretreatment methods have been developed, and these methods of pretreatment can be roughly divided into the different categories:

- Physical - milling and grinding, pyrolysis;
- Physicochemical - steam pretreatment/autohydrolysis, hydrothermolysis, and wet oxidation, AFEX (Ammonia fiber explosion), carbon dioxide explosion;
- Chemical - alkali, dilute acid, oxidizing agents, and organic solvents;
- Biological- fungi, bacteria, enzymes;

The following pretreatment technologies have promise for cost-effective pretreatment of lignocellulosic biomass for biological conversion to fuels and chemicals.

In this work, chemical pretreatment (acid hydrolysis pretreatment in particular), is considered.

Table 2: Summary of Various Processes Used for the Pretreatment of Lignocellulosic Biomass (Parveen, *et al*, 2009)

Pretreatment process	Advantages	Limitations and disadvantages
Mechanical comminution	Reduce cellulose crystallinity	Power consumption usually higher than inherent biomass energy
Steam explosion	Causes hemicelluloses degradation and lignin transformation; cost effective	Destruction of a portion of the xylan fraction; incomplete disruption of the lignin-carbohydrate matrix generation of compounds inhibitory to microorganisms
AFEX (Ammonia fiber explosion)	increases accessible surface area, removes lignin and hemicellulose to an extent; does not produce inhibitors for downstream processes	not efficient for biomass with high lignin content
CO ₂ explosion	Increases accessible surface area; cost effective	Does not modify lignin or hemicelluloses
Ozonolysis	Reduces lignin content;	Large amount of ozone

	does produce toxic residues	required; expensive
Acid hydrolysis	Hydrolyzes hemicellulose to xylose and other sugars; alters lignin structure	High cost; equipment corrosion; formation of toxic substances
Alkaline hydrolysis	Removes hemicelluloses and lignin; increases accessible surface area	Long residence times required; irrevocable salts formed and incorporated into biomass
Organosolv	Hydrolyzes lignin and hemicelluloses	Solvents need to be drained from the reactor, evaporated, condensed, and recycled; high cost
Pyrolysis	Produces gas and liquid products	High temperature; ash production
Pulsed electrical field	Ambient conditions; disrupts plant cells; simple equipment	Process needs more research
Biological	Degradates lignin and hemicelluloses; low energy requirements	Rate of hydrolysis is very low

2.4.1 Acid Hydrolysis Pretreatment

Acid pretreatment has received considerable research attention over the years, with reviews given by Tsao et al. (1982), Bienkowski *et al.*,

(1984), McMillan (1994), Hsu (1996), Jacobsen and Wyman (1999), and Lee *et al.*, (1999). Dilute sulfuric acid has been added to cellulosic materials for some years to commercially manufacture furfural (Root *et al.*, 1959; Zeitsch, 2000). Dilute sulfuric acid is mixed with biomass to hydrolyze hemicellulose to xylose and other sugars and then continue to break xylose down to form furfural. The furfural is recovered by distillation. The volatile fraction contains the furfural which is purified and sold. The acid is mixed or contacted with the biomass, and the mixture is held at temperatures of 160–220°C for periods ranging from minutes to seconds.

Addition of sulfuric acid has been initially applied to remove hemicellulose either in combination with breakdown of cellulose to glucose or prior to acid hydrolysis of cellulose (Ruttan, 1909; Faith and Hall, 1944; Sherrard and Kressman, 1945; Harris *et al.*, 1945; Faith, 1945; Harris and Begliner, 1946). Hemicellulose is removed when sulfuric acid is added and this enhances digestibility of cellulose in the residual solids (Knappert *et al.*, 1981; Brownell and Saddler, 1984; Converse and Grethlein, 1985; Grous *et al.*, 1985). The most

widely used and tested approaches are based on dilute sulfuric acid (Grohmann *et al.*, 1985; Torget *et al.*, 1992; Nguyen et al., 2000; Kim *et al.*, 2000). However, nitric acid (Brink, 1993, 1994), hydrochloric acid (Israilides *et al.*, 1978; Goldstein et al., 1983; Goldstein and Easter, 1992), and phosphoric acid (Israilides *et al.*, 1978) have also been tested.

Pretreatment of lignocellulosic materials with acid at a high temperature can efficiently improve the enzymatic/chemical hydrolysis. The acid pretreatment can operate either under a high temperature and low acid concentration (dilute-acid pretreatment) or under a low temperature and high acid concentration (concentrated-acid pretreatment). The lower operating temperature in concentrated-acid pretreatment (e.g. 40 °C) is a clear advantage compared to dilute-acid processes. However, high acid concentration (e.g. 30-70%) in the concentrated-acid process makes it extremely corrosive and dangerous. Therefore, this process requires either specialized non-metallic constructions or expensive alloys. The acid recovery, which is necessary in the concentrated-acid process for

economical reasons, is an energy demanding process. On the other hand, the neutralization process produces large amounts of gypsum. The high investment and maintenance costs also reduce the commercial interest in this process as a commercial option (Wyman, 1996; Sun et al., 2004; Jones, *et al.*, 1984).

Dilute-acid hydrolysis is probably the most commonly applied method among the chemical pretreatment methods. It can be used either as a pretreatment of lignocellulose for enzymatic hydrolysis, or as the actual method of hydrolyzing to fermentable sugars. Different types of reactors such as batch, percolation, plug flow, countercurrent, and shrinking-bed reactors, for either pretreatment or hydrolysis of lignocellulosic materials by the dilute-acid processes, have been applied.

These processes and different aspects of dilute-acid hydrolysis and pretreatment have recently been reviewed (Taherzadeh, *et al.*, 2007). At an elevated temperature (e.g. 140-190 °C) and low concentration of acid (e.g. 0.1-1% sulfuric acid), the dilute-acid

treatment can achieve high reaction rates and significantly improve cellulose hydrolysis. Almost 100% hemicellulose removal is possible by dilute-acid pretreatment. The pretreatment is not effective in dissolving lignin, but it can disrupt lignin and increases the cellulose's susceptibility to enzymatic hydrolysis (Wyman, 1996; Yang, *et al.*, 2004).

Dilute-acid pretreatment can be performed either in short retention time (e.g. 5 min) at high temperature (e.g. 180 °C) or in a relatively long retention time (e.g. 30-90 min) at lower temperatures (e.g. 120 °C). Sun and Cheng (2005) pretreated rye straw and Bermuda grass for ethanol production by enzymatic hydrolysis at 121°C with different sulfuric acid concentrations (0.6, 0.9, 1.2 and 1.5%, w/w) and residence times (30, 60, and 90 min). Emmel *et al.*, (2003) pretreated Eucalyptus grandis impregnated with 0.087 and 0.175% (w/w) H₂SO₄ at 200–210°C for 2–5 min. The best conditions for hemicellulose recovery were obtained at 210°C for 2 min, while a lower pretreatment temperature of 200°C was enough to obtain the highest yield of cellulose conversion (90%) by enzymatic hydrolysis.

The optimum conditions for the highest hemicellulosic sugars recovery do not necessarily mean the most effective conditions for enzymatic hydrolysis. Cara *et al.*, (2007) reported the maximum hemicellulose recovery (83%) of olive tree biomass to be obtained at 170 °C and 1% sulfuric acid concentration, but the enzyme accessibility of the corresponding pretreated solid was not very high. The maximum enzymatic hydrolysis yield (76.5%) was obtained when pretreated at 210 °C with 1.4% acid concentration. The maximum total sugars, 75% of all sugars present in olive tree biomass, were obtained when the feedstock was pretreated by dilute acid at 180 °C with 1% sulfuric acid concentration. This indicates that the highest overall sugars, higher hemicellulose recovery and higher enzymatic hydrolysis yield can be achieved under respectively different conditions.

Pretreatment with acids such as acetic and nitric were also used to remove lignin from waste newsprints (Xiao *et al.*, 1997) and activated sludge (Chen *et al.*, 2007) for biogas production. Cellulose-lignin association is considered to be the major limiting factor on long-term

anaerobic digestion of newsprint. Pretreatment of bagasse and coconut fibers with HCl improved the formation of biogas from these materials by 31% and 74%, respectively (Kivaisi *et al.*, 1994). Acetic acid cannot dissolve lignin even at a very high concentrations, e.g. as high as 80%, at elevated temperature (in a boiling water bath) for 30 minutes. In order to effectively dissolve significant amounts of lignin, nitric acid should be added. A treatment of newsprints with 30% acetic acid and 2% nitric acid resulted in removing 80% of the lignin and increasing the cellulose/lignin ratio from 1.6 to 9.9. This treatment gave improved digestion of the newsprints. The production of biogas increased by three times within 60 days incubation, from 97 ml CH₄/g VS for the untreated newsprints to 364 ml CH₄/g VS for the treated ones. A portion of the nitric acid might be replaced by another strong acid like hydrochloric acid; however, a longer reaction time may be required for pretreatments with lower concentrations of nitric acid (Xiao *et al.*, 1997).

Dilute-acid hydrolysis can be combined with other chemical treatments. Azzam (1987) studied pretreated bagasse in a solution of

ZnCl_2 and 0.5% hydrochloric acid, heated at 145°C for 10 min, cooled and precipitated with acetone. The pretreated biomass was highly hydrolysable (yield of 93%) by cellulase. The major drawback of some pretreatment methods, particularly at low pH is the formation of different types of inhibitors such as carboxylic acids, furans and phenolic compounds (Taherzadeh *et al.*, 2007; Taherzadeh 1999). These chemicals may not affect the enzymatic hydrolyses, but they usually inhibit the microbial growth and fermentation, which results in less yield and productivity of ethanol or biogas (Taherzadeh *et al.*, 2007). Therefore, the pretreatments at low pH should be selected properly in order to avoid or at least reduce the formation of these inhibitors.

2.5 Hydrolysis of Lignocellulosic Biomass

Generally, hydrolysis of lignocellulosic material was performed by acid, enzymatic and microbial hydrolysis (Humphrey, 1979). This is intended to transform the pretreated mass obtained in the previous stage into a solution of oligomeric sugars. These oligomeric sugars

are then converted into monomeric sugars, hexoses (glucose) and pentoses (xylose). For this work acid hydrolysis is considered.

2.5.1 Acid (catalyzed) Hydrolysis

Acid hydrolysis breaks down the hydrogen bonds in the hemicellulose and cellulose fractions into their sugar components: pentoses and hexoses. These sugars can then be fermented into ethanol. It is the most widely used in industrial processes and have the advantage of separating monomeric sugars from hemicelluloses, and expose cellulosic fibers to further hydrolytic action (Farone and Cuzens, 1996a). Nevertheless, as a disadvantage, it generates some fermentation inhibitor compounds; therefore a detoxification step is needed (Farone and Cuzens, 1996b). Inhibitor substances are formed as a result of hydrolysis of the different components, of esterified organic hemicellulose acids, and the solubilized phenolic lignin derivatives. Inhibitors are also formed from degradation products of soluble sugars and lignin (Lynd, 1996; Palmqvist and Hahn-Hagerdal, 2000a,b). Therefore, depending on the type of pre-treatment and

hydrolysis used, it is essential to carry out a detoxification step prior to fermentation.

There are two acid hydrolysis processes commonly used are dilute acid and concentrated acid. The dilute acid process is conducted under high temperature and pressure, and has a reaction time in the range of seconds or minutes, which facilitates continuous processing.

The concentrated acid process uses relatively mild temperatures, and the only pressures involved are those created by pumping materials from vessel to vessel. Reaction times are typically much longer than for dilute acid.

2.5.1.1 Dilute Acid Hydrolysis

The dilute acid process involves two reactions. First, the cellulosic materials are converted to sugar. However, if the reaction continues, these sugars will convert into other chemicals. The conditions that cause the first reaction are also the right conditions for the second to occur. Once the cellulosic molecules are broken down, the reaction proceeds rapidly to convert the sugars into other products – typically

furfural. The sugar degradation not only reduces the sugar yield, but the furfural and other by-products can inhibit the fermentation process.

Because hemicellulose (5-carbon) sugars degrade more rapidly than cellulose (6-carbon) sugars, one way to decrease sugar degradation is to implement a two-stage process. The first stage is conducted under mild process conditions to recover the 5-carbon sugars while the second stage is conducted under harsher conditions to recover the 6-carbon sugars. Both of these hydrolyzed solutions are then fermented to alcohol. The first reaction converts the cellulosic materials to sugar and the second reaction converts the sugars to other chemicals. Unfortunately, the conditions that cause the first reaction to occur also are the right conditions for the second to occur (Demirbas, 2005). Lime is used to neutralize residual acids before the fermentation stage.

Diluted hydrolysis of α -cellulose with high temperature has been investigated by Xiang and his coworkers (Xiang, *et al.* 2003a), it was

indicated that at above 215°C, the chemical reaction was more sensitive to temperature than the physical factors of cellulose. The physical factors appeared to be eliminated while the true chemical reaction will be a major influential factor in the overall process. It has proved that most of the cellulose was dissolved in 65 % of H₂SO₄ or higher (Xiang, *et al.* 2003b). It was indicated that beyond this concentration, the hydrolysis rate was gradually increased with respect to acid concentration. Most of the hydrolysis of lignocellulose may take place with the presence of hydrogen ions. The suitable range of acid concentration should be considered in order to reduce the formation of decomposed products (Yan, *et al.* 1996)

The biggest advantage of dilute acid processes is their fast rate of reaction, which facilitates continuous processing. The combination of acid and high temperature and pressure dictate special reactor materials, which can make the reactor expensive.

2.5.1.2 Concentrated Acid Hydrolysis

Hydrolysis of cellulosic materials by concentrated sulfuric or hydrochloric acids is a relatively old process. The concentrated acid process uses relatively mild temperatures, and the only pressures involved are those created by pumping materials from vessel to vessel. Reaction times are typically much longer than for dilute acid. This method generally uses concentrated sulfuric acid followed by a dilution with water to dissolve and hydrolyze or convert the substrate into sugar (Demirbas, 2005). This process provides a complete and rapid conversion of cellulose to glucose and hemicelluloses to 5-carbon sugars with little degradation. The critical factors needed to make this process economically viable are to optimize sugar recovery and cost effectively recovers the acid for recycling.

The solid residue from the first stage is dewatered and soaked in a 30 to 40% concentration of sulfuric acid for 1 to 4 hours as a pre-cellulose hydrolysis step. The solution is again dewatered and dried, increasing the acid concentration to about 70%. After reacting in another vessel for 1 to 4 hours at low temperatures, the contents are

separated to recover the sugar and acid. The sugar/acid solution from the second stage is recycled to the first stage to provide the acid for the first stage hydrolysis (Demirbas, 2005).

The primary advantage of the concentrated acid process is the potential for high sugar recovery efficiency. The acid and sugar are separated via ion exchange and then acid is re-concentrated via multiple effect evaporators. The low temperatures and pressures employed allow the use of relatively low cost materials such as fiberglass tanks and piping. The low temperatures and pressures also minimize the degradation of sugars.

Unfortunately, it is a relatively slow process and cost effective acid recovery systems have been difficult to develop. Without acid recovery, large quantities of lime must be used to neutralize the acid in the sugar solution. This neutralization forms large quantities of calcium sulfate, which requires disposal and creates additional expense.

2.6 Significance of Study

Although many studies have been made of pretreatment processes, there is need to subject Palm Oil fiber (OPT) to rigorous pretreatment/hydrolysis regime so that optimal conditions can be obtained. There is lack of data which shows a surface contour of the factors disposition in a response surface map of sugar yield from OPT.

2.7 Research Objectives

The objectives for this are as follows:

1. Pretreatment of OPT fibers with H_2SO_4 , HCl , HNO_3 ,
2. Pretreatment of OPT fibers with additives $[ZnCl_2, (NH_4)SO_4]$
3. Total Hydrolysis of Pretreated fibers to sugars
4. Isolation of factor interaction factors by ANOVA
5. Development of a Model as a result of factor interaction.

2.8 Scope of Study

The scope of this study for the different objectives above is:

For objective 1)

- Acids at 4 concentrations each gives 12 experiments, at 3 observations = 36 experimental runs;
- For objective 2)
- 12 experiments with 2 additives gives 24 runs, at 3 observations= $24 \times 3 = 72$ experimental runs
- For objective 3)
- Evaluation of objective 1) and 2); require total hydrolysis = 108 experimental runs
- For objective 4)
- ANOVA from objectives 1-3
- For objective 5)
- Development of a relationship describing objectives i.e. a Polynomial.

CHAPTER 3

MATERIALS AND METHODS

3.1 Materials

Many chemicals, reagents, apparatus and equipments were used at different stages of this work. They are all listed below.

3.1.1 Chemicals and Reagents

The Chemicals and Reagents used in the work include the following:

1. Tetraoxosulphate (vi) acid (H_2SO_4);
2. Hydrochloric acid (HCl);
3. Trioxonitrate (iv) acid (HNO_3);
4. Zinc Chloride (ZnCl_2) solution;
5. Ammonium Sulphate ($(\text{NH}_4)_2\text{SO}_4$) solution;
6. Distilled Water;
7. Sodium Hydroxide solution;

3.1.2 Apparatus and Equipments

The Apparatus and Equipments used in the work include the following:

1. Weighing balance
2. 200ml Beakers
3. Thermometer
4. Spatula
5. Heating Apparatus
6. Measuring Cylinders
7. Grinding machine
8. Sieve
9. Filter paper
10. Refractometer (Abbe, 60)
11. Water bath
12. Stirrer
13. Sample bottles

3.2 Overall Experimental Flowchart

Below is the overall experimental flowchart, in which all the stages in the experimental, from OPT fiber preparation to down to the estimation of the percentage Sugar concentration is represented.

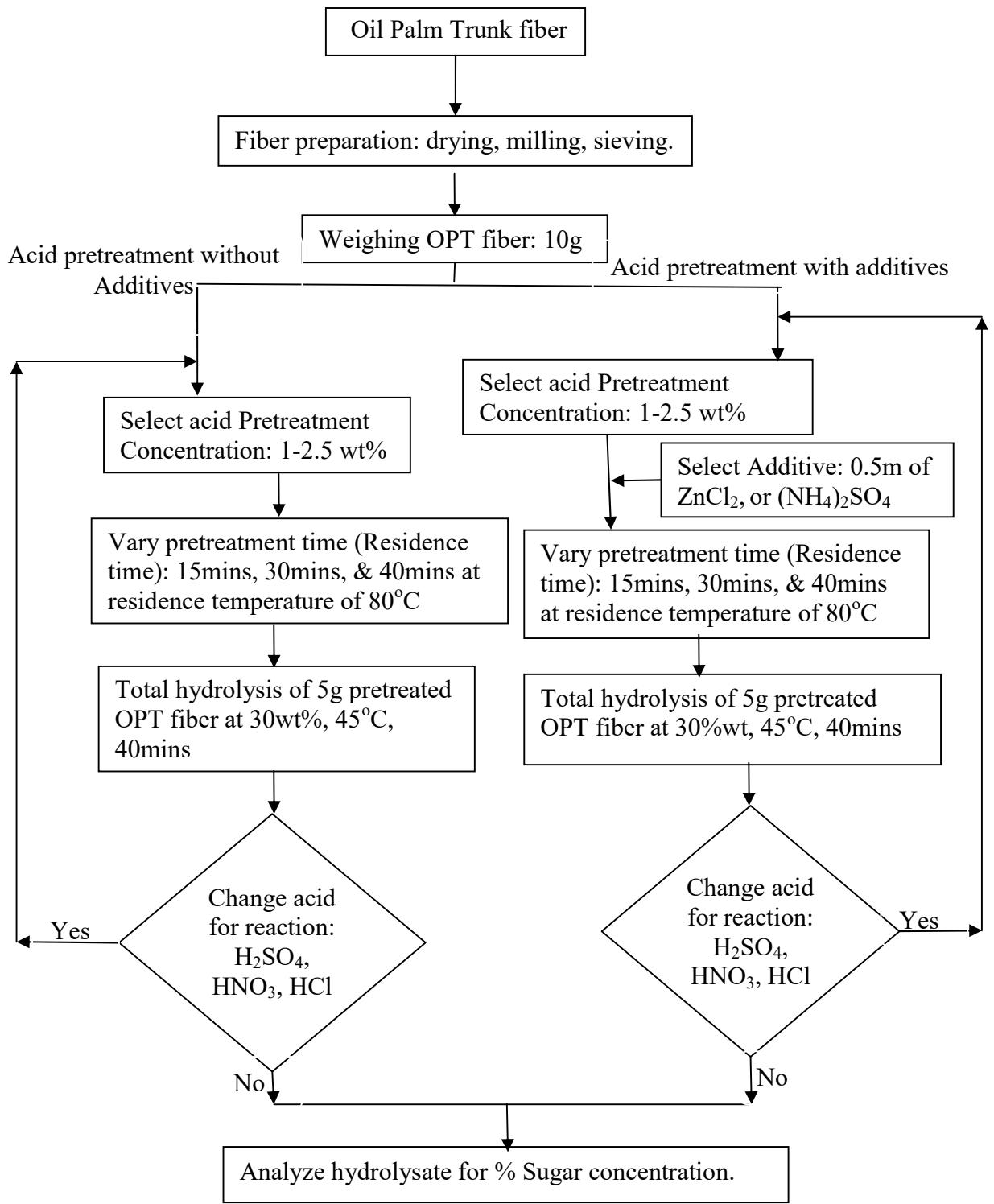


Figure 3.1 Flowcharts for the Experimental Design

3.3 Experimental Procedures

3.3.1 Oil Palm Trunk (OPT) Fiber Collection and Preparations

The OPT fiber was collected from a farm in Aba, Abia State Nigeria. A serrated disc grinder was used to reduce the OPT fiber into very small sizes of particle, with the aim of achieving a large surface area of cellulose for pretreatment and hydrolysis reaction. The OPT fiber was then, dried and sieved to obtain average particle sizes of 500 μ m in diameter.

3.3.2 Pretreatment procedures for the Oil Palm Trunk (OPT) Fiber

Pretreatment as described here, entails acid steeping-soaking of fiber in acid solution at a specific temperature and time, filtering, and drying the fiber.

3.3.2.1 Pretreatment with Acids

The acid pretreatment of OPT fiber was carried out by weighing 10g of the fiber into a 200ml Beaker, and firstly 100ml of 1%, H₂SO₄ was added to it. After which it is then immersed in a water bath with

residence temperature maintained at 80°C, and residence time at 15 minutes. The setup was continuously agitated in order to maintain uniform temperature throughout the pretreatment process. The content was cooled, neutralized with NaOH and filtered to recover the solids after which it is filtered and dried. The whole process above was repeated at the same residence temperature of 80°C for acid concentrations of 1.5%, 2%, 2.5%, and residence time of 30 minutes and 40 minutes.

The whole pretreatment process above was also repeated with HCl, and HNO₃, at the same acid concentrations, residence temperatures and residence times.

3.3.2.2 Pretreatment with Acids and additives

10g of OPT fiber was weighed into a 200ml beaker and 50ml of 1% H₂SO₄ added to it. 50ml of 0.5m ZnCl₂ was also added to it. The whole setup was pretreated at residence temperature maintained at 80°C, and residence time of 15 minutes. The reaction was also continuously agitated in order to maintain uniform temperature

throughout the pretreatment process. The content was cooled, neutralized with NaOH and filtered to recover the solids after 15 minutes after which it was dried as in the case of pretreatment with acids alone. Later also pretreatment with acids and additives was also repeated at the same temperature of 80°C for acid concentrations of 1.5%, 2%, 2.5%, 0.5m of ZnCl₂ and residence time of 30 minutes and 40 minutes with H₂SO₄.

Two other acids HCl, and HNO₃, was also used in the pretreatment with acids at the same acid concentrations, residence temperatures and residence times.

The effect of 0.5m (NH₄)₂SO₄ as an additive was also investigated, at the same acids concentrations, residence time and residence temperature as in using ZnCl₂ as an additive.

3.3.3 Total Acid Hydrolysis

The total acid hydrolysis of 5g of the pretreated OPT fiber was first carried out with H₂SO₄ at acid concentration of 30% with residence temperature of 45°C and residence time of 40 minutes (Ghasem *et al.*

2007). The reaction was quenched with cool water in order to stop the reaction. It was filtered and the sugar content of the filtrate analyzed with a Refractometer in order to get the refractive index, which was later compared with that of a standard so as to get the percentage sugar content of the hydrolyzate.

3.3.4 Method for Sugar content estimation

Sugar content estimation of the hydrolyzate was carried out with the aid of a Refractometer (Pavia *et, al.* 1985). The refractive indices were taken using Abbe 60 Refractometer, Bellingham and Stanley Limited, England, after which it was compared with that of standard sugar solutions (1%-10% w/w).

In the analysis for theb refractive indices, two drops of the hydrolysate (which was taken as soon as the residence time for the hydrolysis was reached) was put on the fixed prism and covered by hinged prism. The field eye piece was observed. The borderline was adjusted until it came to the point of intersection of the cross hairs. The reading on the scale moved simultaneously to the corresponding

refractive index. This was read through the scale eyepiece of the Refractometer. The temperature of the hydrolysate during the analysis was 28⁰C.

3.3.5 Experimental Design and Optimization

To investigate the effect of pretreatment conditions on glucose yield, an experimental matrix was designed using full factorial design. The variables were varied and studied. The pretreatment conditions used as continuous variables were acid concentrations (alone and with additives) and residence time (for acid treatment). The temperature was kept constant. The significance was evaluated at significance level of 0.05 multiple regression models were developed to predict the percent fermentable sugar yield and validated using ANOVA test.

Origin (version 8) software were used for the regression and graphical analyses of the data obtained. The model used for the regression is shown in equation 1 below;

$$Y_i = \beta_0 + \beta_1 x_1 + \beta_{11} x_1^2 + \beta_2 x_2 + \beta_{22} x_2^2 + \beta_{12} x_1 x_2 \quad \text{equation 1.}$$

Where; \mathbf{Y}_i =Response variable (sugar concentration) β_0 =constatant coefficient (intercept); β_1 and β_2 =linear coefficients; β_{11} and β_{22} =quadratic coefficients; β_{12} =interaction coefficient; \mathbf{x}_1 =acid concentration; \mathbf{x}_2 =Residence Time

CHAPTER 4

RESULTS AND DISCUSSIONS

4.1 Optimum Pretreatment Conditions

The results of the acid hydrolysis experiments which were conducted using the three acids (H_2SO_4 , HCl , HNO_3) as the reaction catalyst at acid concentration of 30%, residence time of 40 minutes, and residence temperature of $45^\circ C$, and the results is shown in Table 4.1-4.10 and figure 4.1-4.10 below.

In the pretreatment of OPT fiber using the three acids (H_2SO_4 , HCl , HNO_3) at acid concentrations of 1.0%, 1.5%, 2.0%, and 2.5%, different residence time of 15 minutes, 30 minutes, 40 minutes; and at the same residence temperature of $80^\circ C$, it was found that the sugar concentration after hydrolysis was highest for acid pretreatment concentration of 1.0% and lowest for 2.5% i.e after 1.0% the sugar started to decreased. Also in the case of the different residence time, the highest sugar concentration was obtained for the

residence time of 30 minutes, having been increasing from 0 to 30minutes, after which it started to decrease.

Among the three acids (H_2SO_4 , HCl , HNO_3), the acids with the highest sugar yield was HCl , whose sugar concentration at residence time of 30 minutes at acid pretreatment concentration of 1.0% was 5.74g/l while H_2SO_4 and HNO_3 at the same residence time of 30minutes and acid pretreatment concentration of 1.0% was 5.45g/l and 5.44g/l respectively, making it a percentage conversion of 57.4%, 54.5% and 54.4% for HCl , H_2SO_4 , and HNO_3 acids respectively.

Also 0.5M ZnCl_2 , and $(\text{NH}_4)_2\text{SO}_4$ where also added differently as additives to the acids to investigate whether it has any effect on the percentage of sugar released, at the same acid pretreatment concentration of 1.0%, 1.5%, 2.0%, and 2.5%, residence times of 15, 30, and 40 minutes respectively, and at residence temperature of 80°C and later hydrolyzed as in the case of acids only.

It was found that these additives at this concentration has little or no effect on the sugar concentration of the OPT fiber after hydrolysis. And even the effect of the additives were not was not even constant, in that the sugar concentration was not uniform as that of acids only.

Residence time (minutes)	Sugar concentrations (g/l) at different H₂SO₄ concentrations			
	1.0%	1.5%	2.0%	2.5%
0	0.00	0.00	0.00	0.00
15	4.63	3.92	3.72	3.38
30	5.45	4.93	4.83	4.44
40	4.35	4.00	3.73	3.25

Table 4.1; *Sugar concentration of OPT fiber pretreated at different H₂SO₄ concentrations of 1-2.5%, and residence time of 15-40minutes (hydrolysis was at temperature of 45°C, residence time of 40 minutes and acid concentration of 30%).*

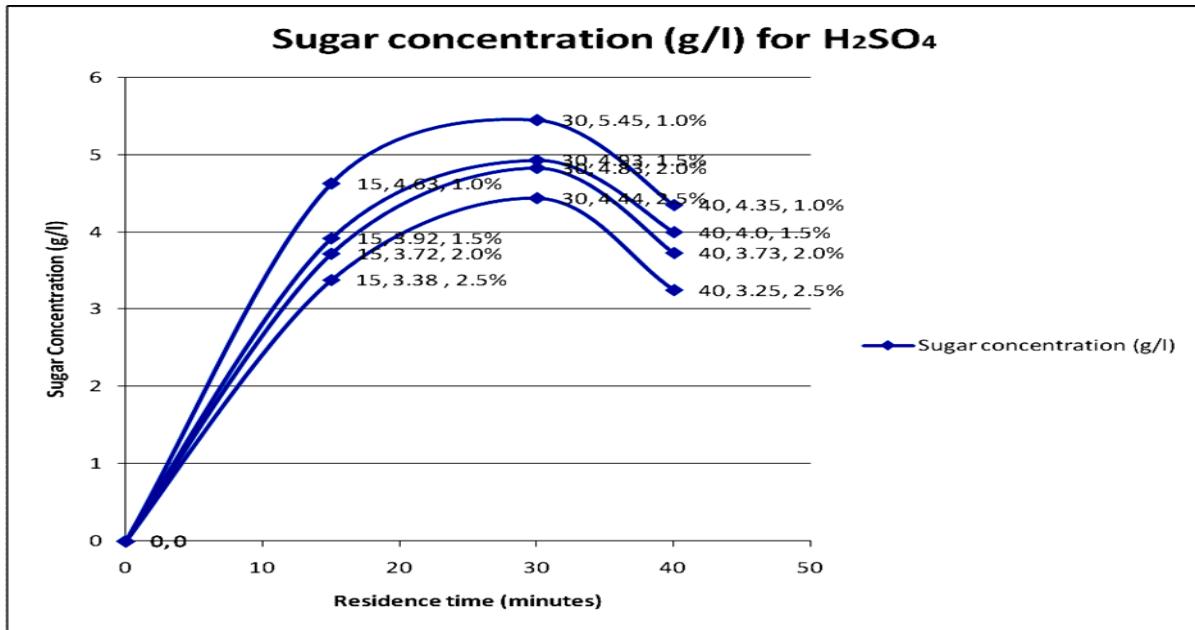


Figure 4.1; Sugar concentration of OPT fiber pretreated at different H_2SO_4 concentrations of 1-2.5%, and residence time of 15-40minutes (hydrolysis was at temperature of $45^{\circ}C$, residence time of 40 minutes and acid concentration of 30%).

Residence time (minutes)	Sugar concentrations (g/l) at different H_2SO_4 concentrations +0.5M $ZnCl_2$			
	1.0%	1.5%	2.0%	2.5%
0	0.00	0.00	0.00	0.00
15	3.81	3.66	3.45	3.11
30	5.53	5.34	4.78	4.41
40	5.42	5.12	4.51	3.33

Table 4.2; Sugar concentration of OPT fiber pretreated at different H_2SO_4 concentrations of 1-2.5%, 0.5M $ZnCl_2$ and residence time of 15-40minutes (hydrolysis was at temperature of $45^{\circ}C$, residence time of 40 minutes and acid concentration of 30%).

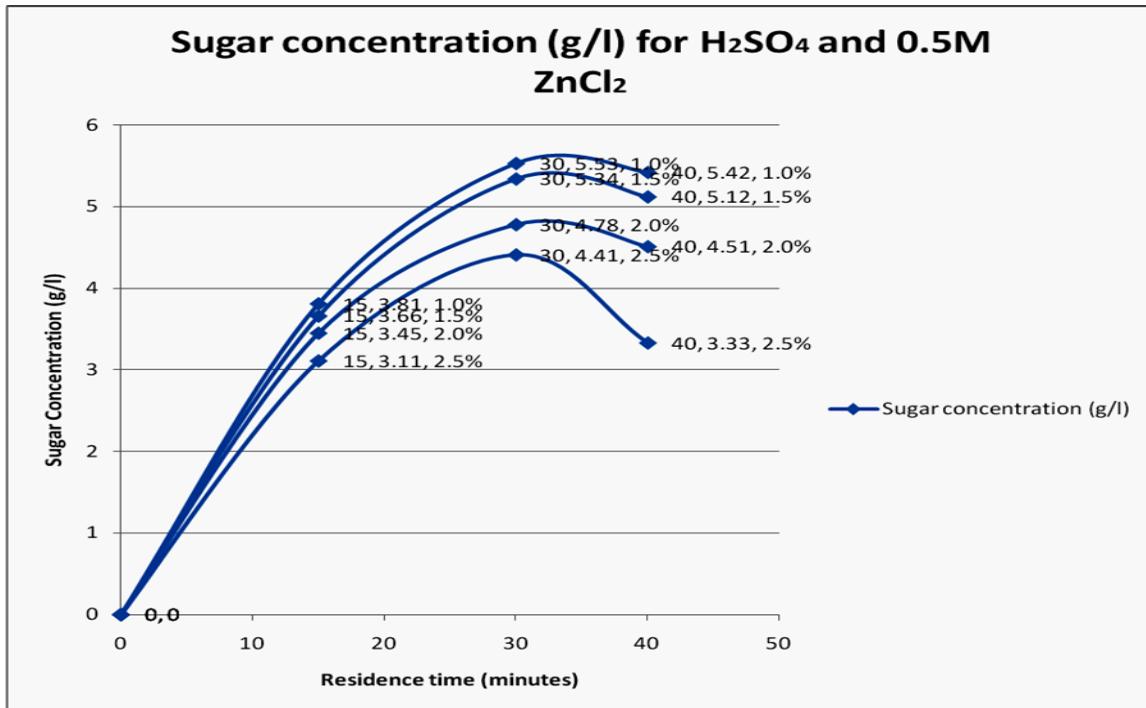


Figure 4.2; Sugar concentration of OPT fiber pretreated at different H_2SO_4 concentrations of 1-2.5%, 0.5M $ZnCl_2$ and residence time of 15-40 minutes (hydrolysis was at temperature of $45^{\circ}C$, residence time of 40 minutes and acid concentration of 30%).

Residence time (minutes)	Sugar concentrations (g/l) at different H_2SO_4 concentrations +0.5M $(NH_4)_2SO_4$			
	1.0%	1.5%	2.0%	2.5%
0	0.00	0.00	0.00	0.00
15	5.21	4.25	3.49	3.16
30	4.82	4.72	4.37	3.73
40	4.98	4.61	3.89	3.74

Table 4.3; Sugar concentration of OPT fiber pretreated at different H_2SO_4 concentrations of 1-2.5%, 0.5M $(NH_4)_2SO_4$ and residence time of 15-40 minutes (hydrolysis was at temperature of $45^{\circ}C$, residence time of 40 minutes and acid concentration of 30%).

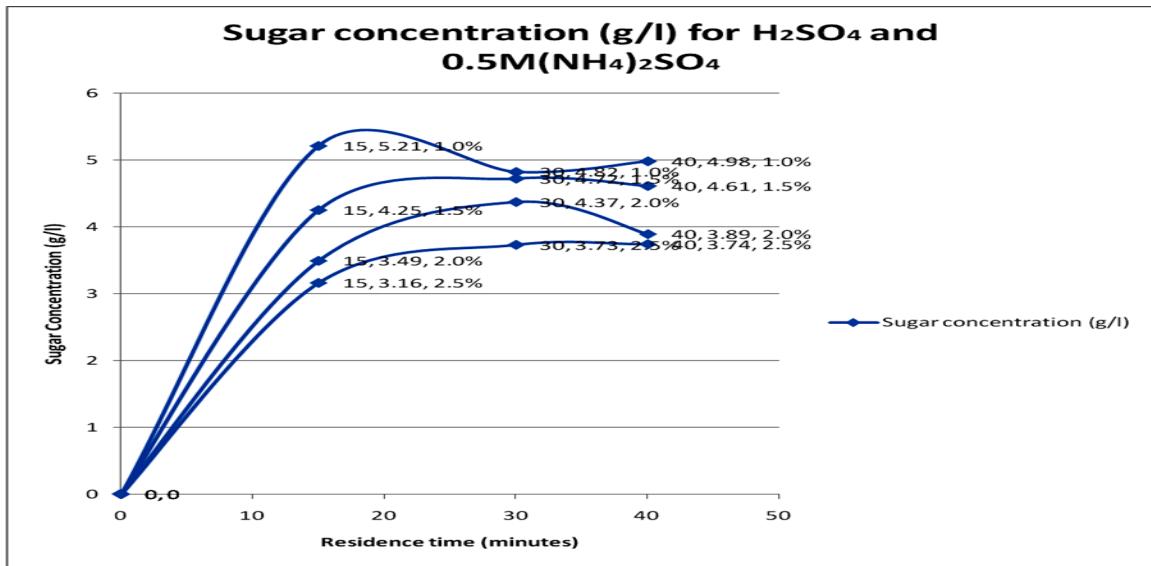


Figure 4.3; Sugar concentration of OPT fiber pretreated at different H_2SO_4 concentrations of 1-2.5%, 0.5M $(NH_4)_2SO_4$ and residence time of 15-40minutes (hydrolysis was at temperature of $45^{\circ}C$, residence time of 40 minutes and acid concentration of 30%).

Residence time (minutes)	Sugar concentrations (g/l) at different HCl concentrations			
	1.0%	1.5%	2.0%	2.5%
0	0.00	0.00	0.00	0.00
15	5.29	4.93	4.58	4.35
30	5.74	5.41	5.32	5.00
40	4.86	4.50	4.35	4.00

Table 4.4; Sugar concentration of OPT fiber pretreated at different HCl concentrations of 1-2.5%, and residence time of 15-40minutes (hydrolysis was at temperature of $45^{\circ}C$, residence time of 40 minutes and acid concentration of 30%).

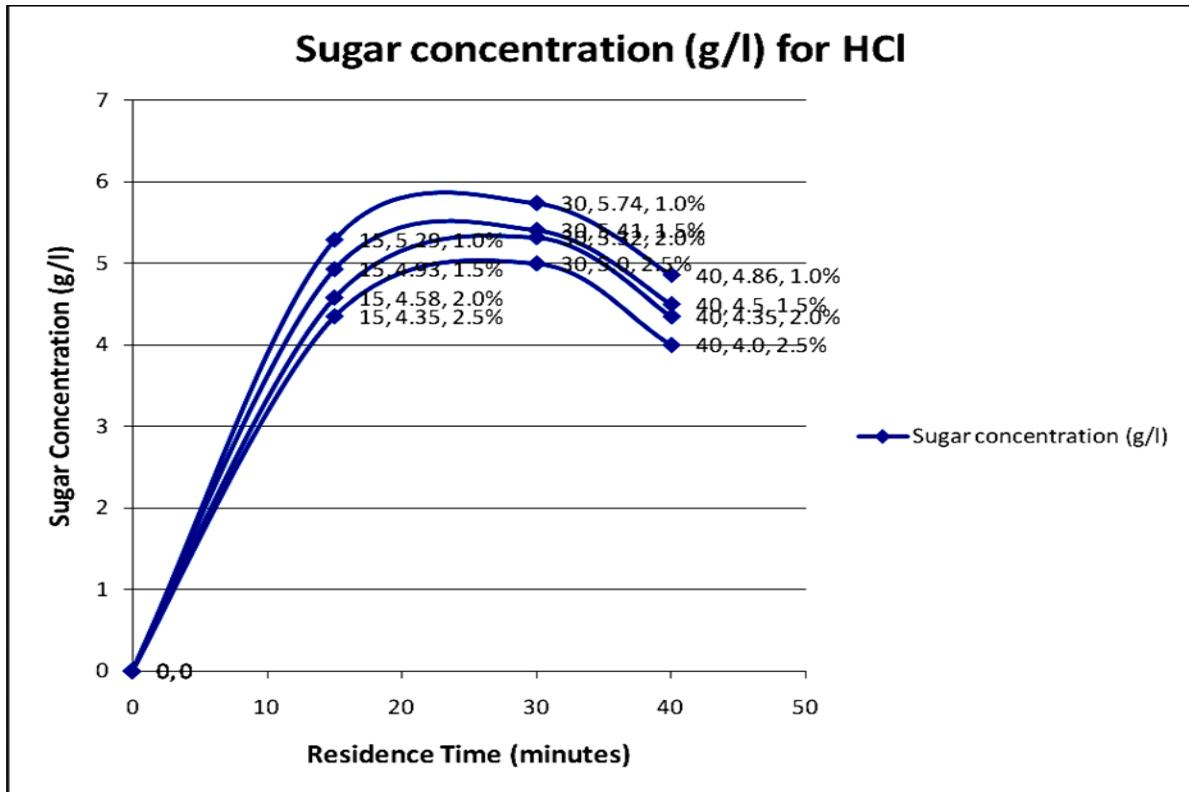


Figure 4.4; Sugar concentration of OPT fiber pretreated at different HCl concentrations of 1-2.5%, and residence time of 15-40minutes (hydrolysis was at temperature of 45°C , residence time of 40 minutes and acid concentration of 30%).

Residence time (minutes)	Sugar concentrations (g/l) at different HCl concentrations +0.5M ZnCl_2			
	1.0%	1.5%	2.0%	2.5%
0	0	0	0	0
15	4.69	4.32	3.92	3.53
30	5.65	4.85	4.55	4.13
40	5.03	4.41	4.34	3.71

Table 4.5; Sugar concentration of OPT fiber pretreated at different HCl concentrations of 1-2.5%, 0.5M ZnCl_2 and residence time of 15-40minutes (hydrolysis was at temperature of 45°C , residence time of 40 minutes and acid concentration of 30%).

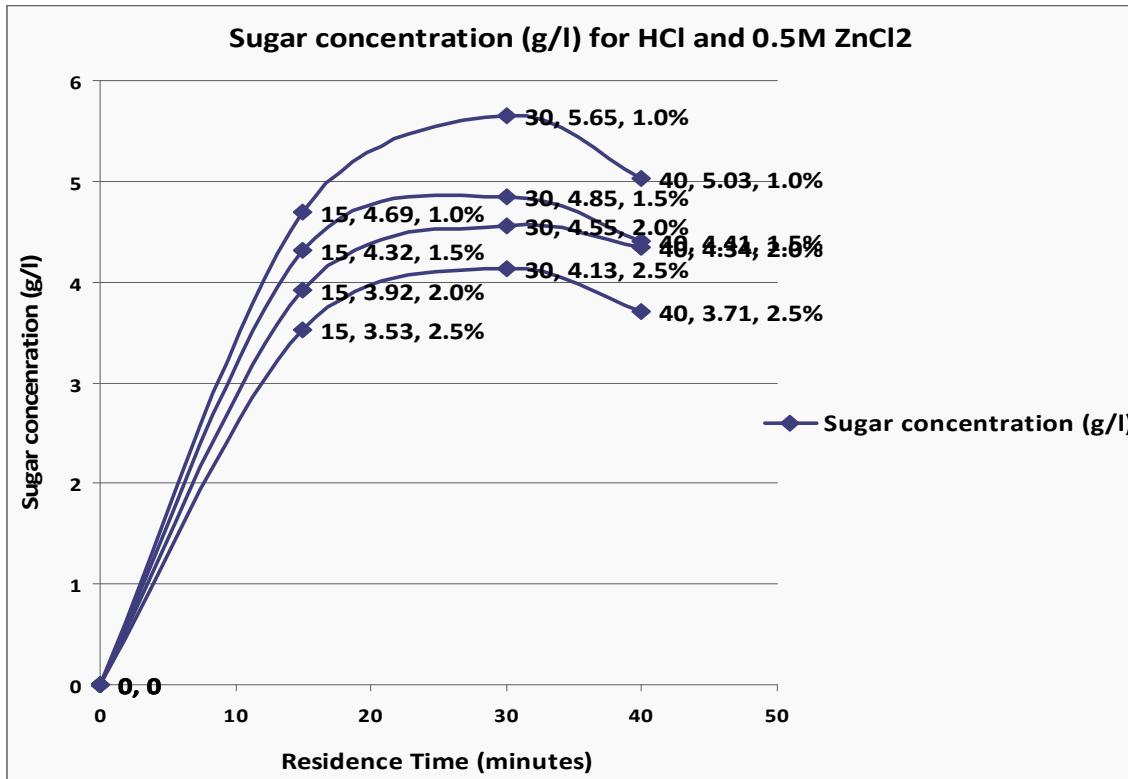


Figure 4.5; Sugar concentration of OPT fiber pretreated at different HCl concentrations of 1-2.5%, 0.5M ZnCl₂ and residence time of 15-40 minutes (hydrolysis was at temperature of 45°C, residence time of 40 minutes and acid concentration of 30%).

Residence time (minutes)	Sugar concentrations (g/l) at different HCl concentrations +0.5M (NH ₄) ₂ SO ₄			
	1.0%	1.5%	2.0%	2.5%
0	0	0	0	0
15	5.46	4.56	4.31	3.30
30	5.57	5.25	4.76	4.51
40	4.00	4.96	4.53	4.36

Table 4.6; Sugar concentration of OPT fiber pretreated at different HCl concentrations of 1-2.5%, 0.5M (NH₄)₂SO₄ and residence time of 15-40 minutes (hydrolysis was at temperature of 45°C, residence time of 40 minutes and acid concentration of 30%).

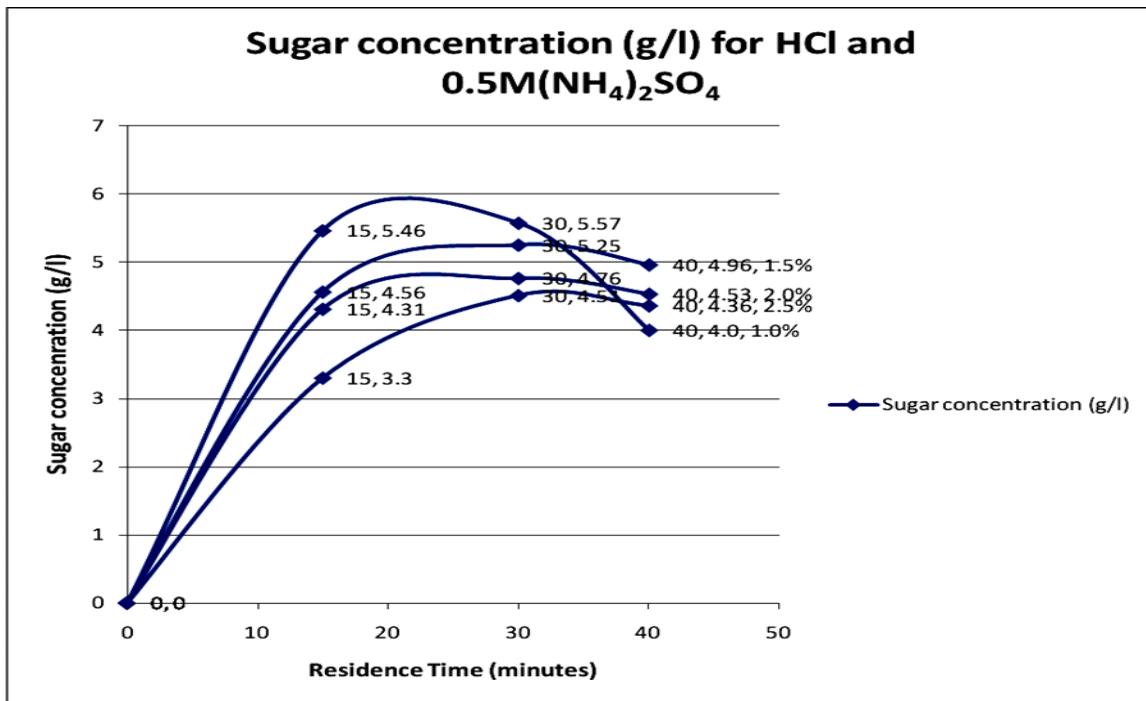


Figure 4.6; Sugar concentration of OPT fiber pretreated at different HCl concentrations of 1-2.5%, 0.5M (NH₄)₂SO₄ and residence time of 15-40 minutes (hydrolysis was at temperature of 45° C, residence time of 40 minutes and acid concentration of 30%).

Residence time (minutes)	Sugar concentrations (g/l) at different HNO ₃ concentrations			
	1.0%	1.5%	2.0%	2.5%
0	0	0	0	0
15	4.93	4.62	4.25	3.72
30	5.44	5	4.63	4.44
40	4.50	4.15	3.76	3.63

Table 4.7; Sugar concentration of OPT fiber pretreated at different HNO₃ concentrations of 1-2.5%, and residence time of 15-40 minutes (hydrolysis was at temperature of 45° C, residence time of 40 minutes and acid concentration of 30%).

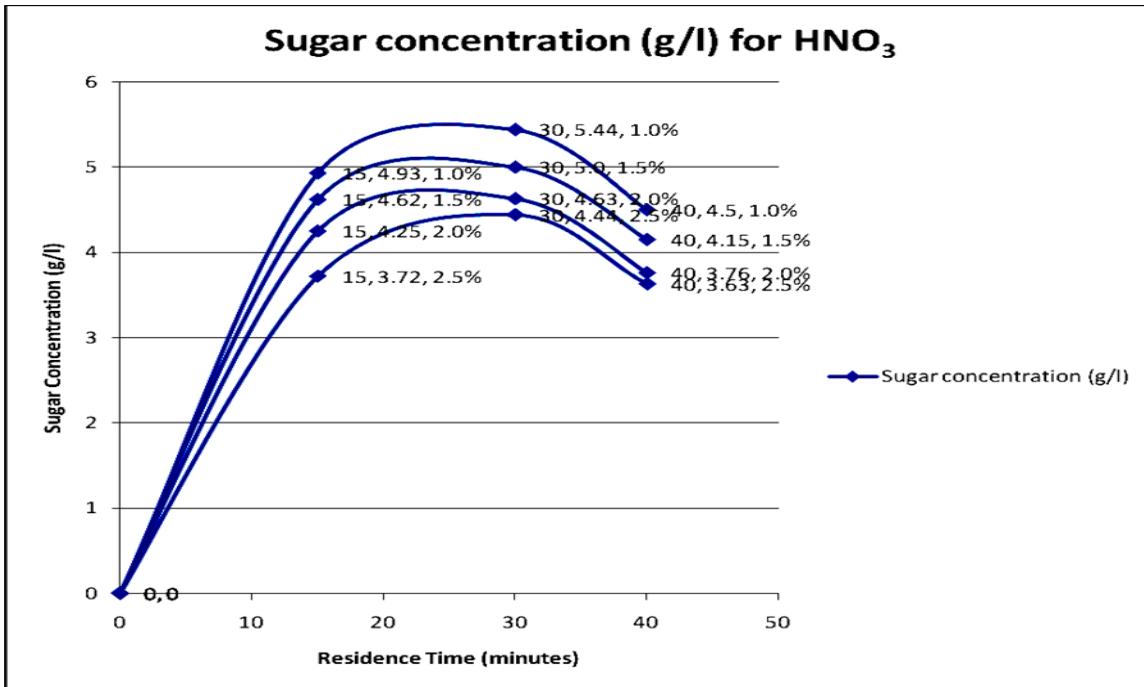


Figure 4.7; Sugar concentration of OPT fiber pretreated at different HNO_3 concentrations of 1-2.5%, and residence time of 15-40 minutes (hydrolysis was at temperature of 45°C , residence time of 40 minutes and acid concentration of 30%).

Residence time (minutes)	Sugar concentrations (g/l) at different HNO_3 concentrations +0.5M ZnCl_2			
	1.0%	1.5%	2.0%	2.5%
0	0	0	0	0
15	4.42	4.12	3.89	2.99
30	5.10	4.43	4.09	3.49
40	4.91	4.36	4.00	3.68

Table 4.8; Sugar concentration of OPT fiber pretreated at different HNO_3 concentrations of 1-2.5%, 0.5M ZnCl_2 and residence time of 15-40 minutes (hydrolysis was at temperature of 45°C , residence time of 40 minutes and acid concentration of 30%).

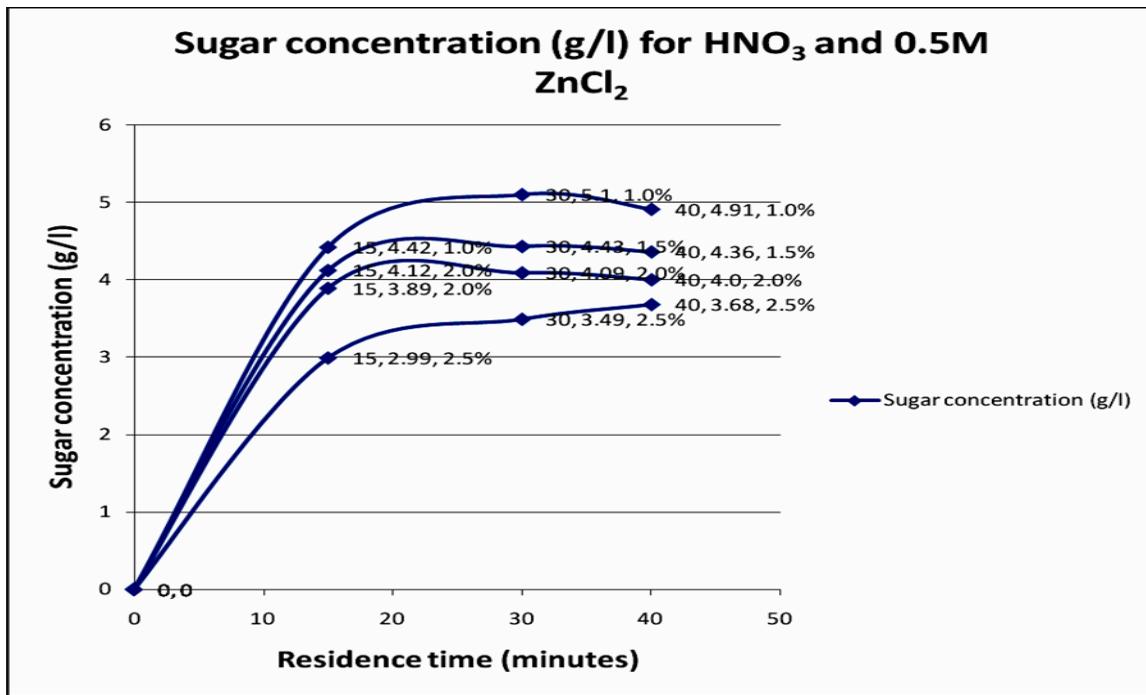


Figure 4.8; Sugar concentration of OPT fiber pretreated at different HNO_3 concentrations of 1-2.5%, 0.5M ZnCl_2 and residence time of 15-40 minutes (hydrolysis was at temperature of 45°C , residence time of 40 minutes and acid concentration of 30%).

Residence time (minutes)	Sugar concentrations (g/l) at different $\text{HNO}_3 + 0.5\text{M} (\text{NH}_4)_2\text{SO}_4$			
	1.0%	1.5%	2.0%	2.5%
0	0	0	0	0
15	4.75	4.32	3.94	3.43
30	4.84	4.43	4.25	3.29
40	4.66	3.91	3.12	2.91

Table 4.9; Sugar concentration of OPT fiber pretreated at different HNO_3 concentrations of 1-2.5%, 0.5M $(\text{NH}_4)_2\text{SO}_4$ and residence time of 15-40 minutes (hydrolysis was at temperature of 45°C , residence time of 40 minutes and acid concentration of 30%).

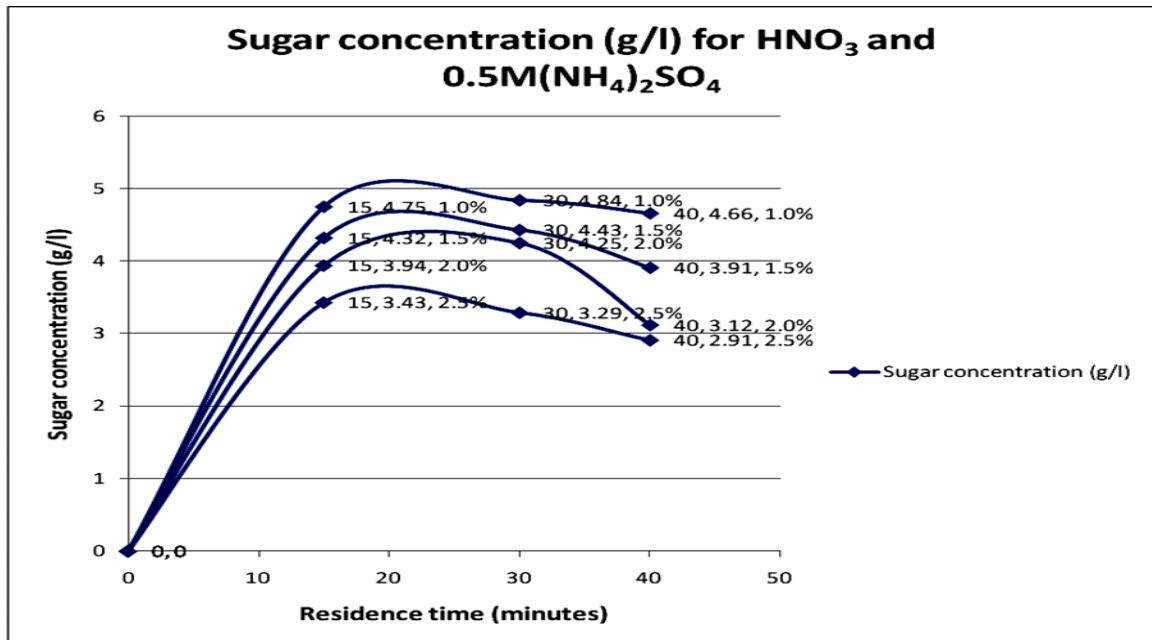


Figure 4.9; Sugar concentration of OPT fiber pretreated at different HNO_3 concentrations of 1-2.5%, 0.5M $(\text{NH}_4)_2\text{SO}_4$ and residence time of 15-40 minutes (hydrolysis was at temperature of 45°C , residence time of 40 minutes and acid concentration of 30%).

Residence time (minutes)	Sugar concentrations (g/l) at different H_2SO_4 , HCl and HNO_3 1.0% concentration		
	H_2SO_4	HCl	HNO_3
0	0	0	0
15	4.63	5.29	4.93
30	5.45	5.74	5.44
40	4.35	4.86	4.5

Table 4.10; Sugar concentration of OPT fiber pretreated at 1% w/w H_2SO_4 , HCl , and HNO_3 concentrations of 1-2.5%, 0.5M $(\text{NH}_4)_2\text{SO}_4$ and residence time of 15-40 minutes (hydrolysis was at temperature of 45°C , residence time of 40 minutes and acid concentration of 30%).

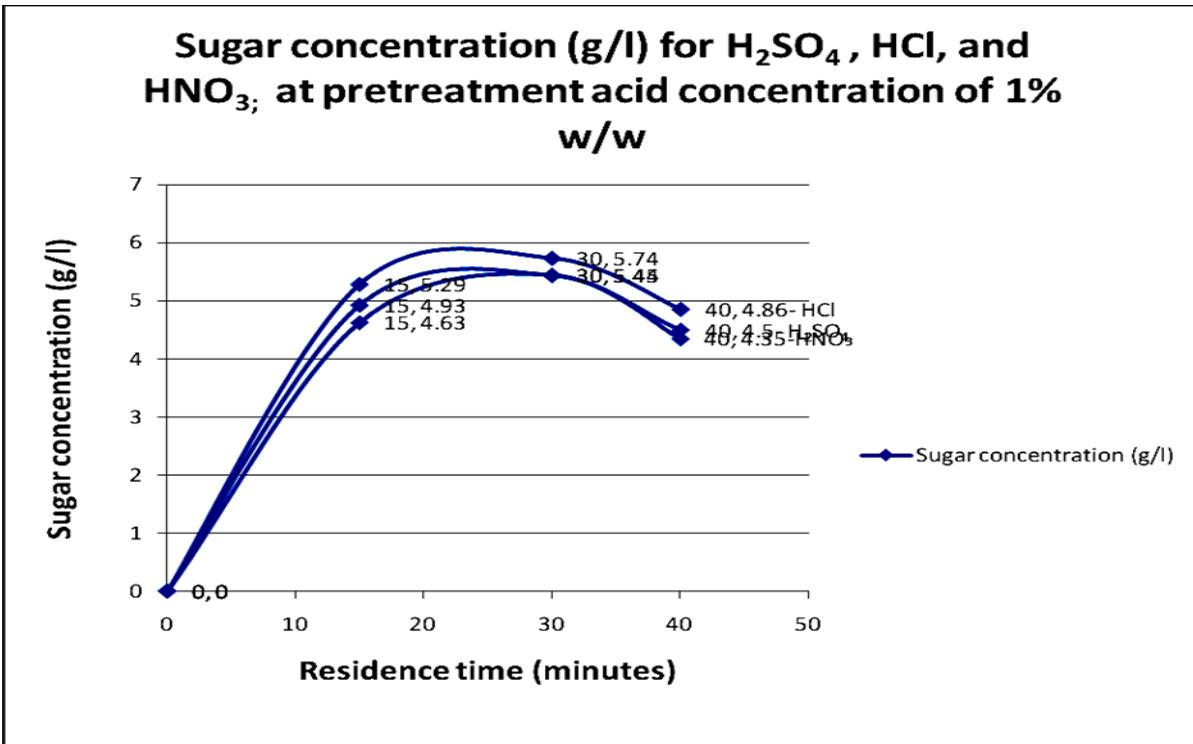


Figure 4.10; Sugar concentration of OPT fiber pretreated at different HNO_3 concentrations of 1-2.5%, 0.5M $(NH_4)_2SO_4$ and residence time of 15-40 minutes (hydrolysis was at temperature of 45^0C , residence time of 40 minutes and acid concentration of 30%).

As can be seen from the results above, in the pretreatment using three acids (H_2SO_4 , HCl, HNO_3), the sugar concentration after hydrolysis was highest for acid pretreatment concentration of 1.0% and lowest for 2.5%, i.e. after 1.0% the sugar started to decreased. Also in the case of the different residence time, the highest sugar concentration was obtained for the residence time of 30 minutes,

having been increasing from 0 to 30minutes, after which it started to decrease.

4.2 Statistical Analysis and Model for Optimization of Sugar Concentration

In the analysis of variance (ANOVA) of the three acids on the interaction of the residence time, acid concentration and sugar concentration, (results of the Two-way Anova are shown in the Appendices) it was also found that at 0.05 confidence level, the interaction between acid concentration and time is significant.

Also when 0.5M ZnCl₂, and (NH₄)₂SO₄ where added differently as additives to the acids to investigate whether it has any effect on the percentage of sugar released, at the same experimental conditions as that of the acids, it was found that the acid concentration and time was also significant at 0.05 confidence level and there was little effect of the additives on the sugar concentration released.

To evaluate the results, the data in Table 4.1-4.10 were subjected to regression analysis, and the following models for all the

pretreatments (both for acids only and with additives) were obtained as shown below;

Polynomials models for the sugar concentrations (\mathbf{Y}) obtained after hydrolysis at different pretreatment conditions of the OPT fiber as calculated by Origin (version 8) software, which was used for the analyses of the sugar concentration data obtained.

$$\mathbf{Y}_1 = 1.827314 + 0.050828x_1 - 0.11849x_1^2 + 12.61073x_2 - 1.3709x_2^2 + 0.54006x_1x_2$$

$$\mathbf{Y}_2 = 1.85892 + 0.68763x_1 - 0.14013x_1^2 + 15.42729x_2 - 1.96015x_2^2 + 0.31486x_1x_2$$

$$\mathbf{Y}_3 = 1.85907 + 0.75825x_1 - 0.16924x_1^2 + 16.45451x_2 - 2.24686x_2^2 + 0.513966x_1x_2$$

$$\mathbf{Y}_4 = 1.6795 + 0.42708x_1 - 0.09405x_1^2 + 5.92067x_2 - 0.12794x_2^2 + 1.38516x_1x_2$$

$$\mathbf{Y}_5 = 1.47245 + 0.87425x_1 - 0.20155x_1^2 + 11.44483x_2 - 1.08071x_2^2 + 1.43491x_1x_2$$

$$\mathbf{Y}_6 = 1.69968 + 0.77643x_1 - 0.17317x_1^2 + 8.71514x_2 - 0.51074x_2^2 + 0.78111x_1x_2$$

$$\mathbf{Y}_7 = 1.49718 + 0.61768x_1 - 0.13085x_1^2 + 12.42309x_2 - 1.32311x_2^2 + 2.24751x_1x_2$$

$$\mathbf{Y}_8 = 1.69828 + 0.94401x_1 - 0.22514x_1^2 + 8.63324x_2 - 0.41521x_2^2 + 0.9356x_1x_2$$

$$\mathbf{Y}_9 = 2.02371 + 0.87828x_1 - 0.21456x_1^2 + 18.50873x_2 - 2.81139x_2^2 + 1.25791x_1x_2$$

Where; $Y_1 = H_2SO_4$; $Y_2 = HCl$; $Y_3 = HNO_3$; $Y_4 = H_2SO_4 + 0.5mZnCl_2$; $Y_5 = H_2SO_4 + 0.5m(NH_4)_2SO_4$; $Y_6 = HCl + 0.5mZnCl_2$; $Y_7 = HCl + 0.5m(NH_4)_2SO_4$; $Y_8 = HNO_3 + 0.5mZnCl_2$; $Y_9 = HNO_3 + 0.5m(NH_4)_2SO_4$;

Experimental ranges and levels of independent process variables; acid concentrations (X_1 ; 1-2.5% w/v), and residence time (X_2 ; 15-40minutes) as well as dependent process variables- Sugar yields (Y_i ; 2.91-5.47g/l).

The ANOVA quadratic regression demonstrated that the models was significant, as evidence from a low probability ($P<0.1$) - all results shown in the appendices.

CHAPTER 5

CONCLUSIONS

From the results and discussions, it can be seen that in the pretreatment of OPT fiber, using H_2SO_4 , HCl, and HNO_3 , at temperature of 80^0C , residence time of 15-45 minutes, and acid concentration of 1-2.5%, the optimum acid concentration and residence time for highest sugar yield, is 1% w/w and 30minutes, after the pretreated OPT fiber has undergone acid hydrolysis at residence temperature of 45^0C , residence time of 40^0C and acid concentration of 30%. Above this acid pretreatment concentration of 1%, and pretreatment residence time of 30 minutes, the sugar concentration starts to decrease tremendously.

When comparing the pretreatment and subsequent hydrolysis of the three acids, H_2SO_4 , HCl, and HNO_3 , at the same conditions, HCl seem to be the best acid for pretreatment and subsequent hydrolysis in that it gives the highest sugar yield than the other two acids, H_2SO_4 , and HNO_3 .

When the effect of ZnCl_2 , and $(\text{NH}_4)_2\text{SO}_4$ were investigated as additives, at the same parameters as that of the acids H_2SO_4 , HCl , and HNO_3 , in relation to their acid pretreatment concentrations, residence time and residence temperature, it was found that the concentration of these additives-0.5M, has little or no effect to the amount of sugar released. It was only ZnCl_2 that showed slight increase in the sugar concentration released. This suggests that if the concentration is increased, there might be more sugar released.

REFERENCES

- Aguilar, R., Ramírez, J. A., Garrote G. and Vázquez, M., (2002). *Kinetic study of the acid hydrolysis of sugar cane bagasse*. Journal of Food Engineering, Vol. 55, 309-318.
- Akinyosoye, V. A., (1976). *Senior Tropical Agriculture for West Africa*. First Edition, London and Basingstoke: Macmillan Education Limited.
- Anyawu, A. C., Anyawu, B. O. and Anyawu, V. A., (1982). *A Textbook of Agriculture for School Certificate*, 4th Edition. Africana Educational Publishers Ltd. Nsukka, Nigeria.
- Azzam, A. M., (1987). *Saccharification of bagasse cellulose pretreated with ZnCl₂ and HCl*. Biomass Bioengineering. 12, 71-77.
- Background Information on Oil Palm. <http://www.otal.com/palm油>
- Bienkowski, P., Ladisch, M. R., Voloch, M., Tsao, G. T., (1984). *Acid hydrolysis of pretreated lignocellulose from corn residue*. Biotechnology and Bioengineering Symposium Series 14, 512–524.
- Brink, D. L., (1993). *Method of treating biomass material*. US Patent 5,221,357.
- Brink, D. L., (1994). *Method of treating biomass material*. US Patent 5,366,558.
- Brownell, H. H., Saddler, J. N., (1984). *Steam explosion pretreatment for enzymatic hydrolysis*. Biotechnology and Bioengineering Symposium 14, 55–68.
- Buchanan, M. A., (1963). *Extraneous components of wood, in the Chemistry of Wood*. Browning, B. L., Ed., Interscience, New York. Chapter 7.
- Cara, C., Ruiz, E., Oliva, J. M., Saez, F., Castro, E., (2007). *Conversion of olive tree biomass into fermentable sugars by dilute acid*

pretreatment and enzymatic saccharification. Bioresource Technology. 99, 1869-1876.

Cellulose, (2008). In *Encyclopedia Britannica*. Retrieved January 11, 2008, from Encyclopedia Britannica Online.

Cellulose. <http://en.wikipedia.org/wiki/Cellulose>

Chen, Y. G., Jiang, S., Yuan, H. Y., Zhou, Q., Gu, G. W., (2007). *Hydrolysis and acidification of waste activated sludge at different pHs.* Water Resources. 41, 683-689.

Converse, A. O., Grethlein, H. E., (1985). *Process for hydrolysis of biomass.* US Patent 4,556,430.

Delgenes, J., Moletta, R., Navarro, J., 1996. Effects of lignocellulose degradation products on ethanol fermentation of glucose and xylose by *Saccharomyces cerevisiae*, *Zymomonas mobilis*, *Pichia stipitis*, and *Candida shehatae*. *Enzyme and Microbial Technology* 19: 220-225.

Demirbas, A., (2005) *Bioethanol from Cellulosic Materials: A Renewable Motor Fuel from Biomass.* Department of Chemical Engineering Selcuk University, Konya, Turkey; Taylor & Francis Inc.

Dowe, N. and McMillan, J., (2001) *SSF Experimental Protocols — Lignocellulosic Biomass Hydrolysis and Fermentation, Laboratory Analytical Procedure (LAP).* National Renewable Energy Laboratory.

Emmel, A., Mathias, A. L., Wypych, F., Ramos, L. P., (2003). *Fractionation of Eucalyptus grandis chips by dilute acid- catalysed steam explosion.* Bioresources Technology. 86, 105-115.

Faith, W. L., Hall, J. A., (1944). *Ethyl alcohol from waste wood by a modified Scholler Process.* Chemical Engineering News 22, 525–526.

Faith, W. L., (1945). *Development of the Scholler process in the United States.* Industrial and Engineering Chemistry 37 (1), 9–11.

Farone, W. A., Cuzens, J. E., (1996a). *Method of Separating Acids and sugars resulting from Strong Acid Hydrolysis*. U.S. Patent No. 5,580,389.

Farone, W. A., and Cuzens, J.E., (1996b). *Method of Producing Sugars Using Strong Acid Hydrolysis of Cellulosic and Hemicellulosic Materials*. U.S. Patent No. 5,562,777.

Fengel, D. and Wegener, G., (1984). *Wood Chemistry, Ultrastructure, Reactions*. Walter de Gruyter, Berlin, Germany.

Galbe, M., Larsson, M., Stemberg, K., Tenborg, C. and Zacchi, G., (1997). *Ethanol from wood: Design and operation of a process development unit for techno-economic process evaluation*. In: ACS Symposium Series 666. American Chemical Society, Washington DC, USA.

Galbe, M., and Zacchi, G., (1993). *Simulation processes for conversion of lignocelluloses*. In: J. N. Saddler (Ed.). Bioconversion of Forest and Agricultural Plant Residues. CAB International. Wallingford, UK. 291-319.

Ghasem, N., Asmida, I., Sadegh, S., and Mohammad, N. (2007) *Acid Hydrolysis of Pretreated Palm Oil Lignocellulosic Waste*. IJE Transactions B: Applications, 148 - Vol. 20, No. 2.

Goldstein, I. S., Easter, J. M., (1992). *An improved process for converting cellulose to ethanol*. TAPPI Journal 75 (8), 135–140.

Goldstein, I. S., Pereira, H., Pittman, J. L., Strouse, B. A., Scaringelli, F. P., (1983). *The hydrolysis of cellulose with superconcentrated hydrochloric-acid*. Biotechnology and Bioengineering. 13, 17–25.

Goldstein, I. S., (1981). *Organic Chemicals from Biomass*. CRC Press, Inc., Boca Raton Florida, USA. 10-15.

Grohmann, K., Torget, R., Himmel, M., (1985). *Dilute acid pretreatment of biomass at high solids concentrations*. Biotechnology and Bioengineering Symposium 15, 59–80.

Grous, W. R., Converse, A. O., Grethlein, H. E., (1985). *Effect of steam explosion pretreatment on pore size and enzymatic hydrolysis of poplar*. Enzyme and Microbial Technology 8, 274–280.

Harris, E. E., Beglin, E., (1946). *Madison wood sugar process*. Industrial Engineering Chemistry 38, 890–895.

Harris, E. E., Beglin, E., Hajny, G. J., Sherrard, E. C., (1945). *Hydrolysis of wood: Treatment with sulfuric acid in a stationary digester*. Industrial Engineering Chemistry 37 (1), 12–23.

Hartley, C. W. S., (1988). *The Oil Palm*. Third Edition. Longman. Harlow, England.

Herrera, A., Téllez-Luis, S. J., Ramírez, J. A. and Vázquez, M. (2003). *Production of xylose from sorghum straw using hydrochloric acid*. Journal of Cereal Science, Vol. 37, 267-274.

Hillis, E. W., (1962). *Wood Extractives and Their significance to the Pulp and Paper Industry*. Academic Press, New York.

Himmel, M. E., Ding, S. Y., Johnson, D. K., Adney, W. S., Nimlos, M. R., Brady, J. W., Foust, T. D., (2007). *Biomass recalcitrance: engineering plants and enzymes for biofuels production*. Science, 315: 804-807.

Howard, R. L., Abotsi, E., Jansen van Rensburg, E. L., Howard, S., (2003). *Lignocellulose biotechnology: issues of bioconversion and enzyme production*. African Journal of Biotechnology, 2: 602-619.

Hsu, T. A., (1996). *Pretreatment of Biomass*. In: Wyman, C.E. (Ed.), *Handbook on Bioethanol, Production and Utilization*. Taylor & Francis, Washington, DC, USA.

Hsu, T. A., Ladisch, M. R., Tsao, G. T., (1980). *Alcohol from cellulose*. Chem. Technology. 10 (5), 315–319.

Humprey, A. E., (1979). *The hydrolysis of cellulosic materials to useful product*. In: Brown, R. D. and Jurasek, L. Ed., Hydrolysis of cellulose: mechanism of enzymatic and acid catalysts advances in chemistry series 181, Washington, D. C. USA

http://www.mpopb.gov.my/oilpalm_env/oilnew10.htm

International Potash Institute, (1957). *The Oil Palm, Its Culture, Manuring and Utilization*. Berne, Switzerland: IPI.

Israilides, C. J., Grant, G. A., Han, Y. W., (1978). *Sugar level, fermentability, and acceptability of straw treated with different acids*. Applied Environmental Microbiology 36 (1), 43–46.

Jacobsen, S. E., Wyman, C. E., (1999). *Hemicellulose and cellulose hydrolysis models for application to current and novel pretreatment processes*. Applied Biochemistry and Biotechnology 84–86, 81–96.

Jones, J., Semrau, K., (1984). *Wood hydrolysis for ethanol production previous experience and the economics of selected processes*. Biomass. 5, 109-135.

Jorgensen, H., Kristensen, J. B., and Felby, C., (2007). *Enzymatic conversion of lignocellulose into fermentable sugars: Challenges and opportunities*. Biofuels, Bioprod. Bioref.1, 119–134.

Joseleau, J. P., Comtat, J., Ruel, K., (1992). *Chemical structure of xylans and their interaction in the plant cell walls*. In Xylan and xylanases, Eds. J. Visser, G. Beldman, M. A. Kusters- van Someren and A. G. J. Voragen, Elsevier Science Publishers, Amsterdam. 1-15.

Kim, J. S., Lee, Y. Y., Park, S. C., (2000). *Pretreatment of wastepaper and pulp mill sludge by aqueous ammonia and hydrogen peroxide*. Applied Biochemistry and Biotechnology 84/86, 129–139.

- Kivaisi, A. K., Eliapenda, S., (1994). *Pretreatment of bagasse and coconut fibres for enhanced anaerobic degradation by rumen microorganisms*. Renewable Energy. 5, 791-795.
- Knappert, H., Grethlein, H., Converse, A., (1981). *Partial acid hydrolysis of poplar wood as a pretreatment for enzymatic hydrolysis*. Biotechnology and Bioengineering Symposium 11, 67-77.
- Lee, J., (1997). *Biological conversion of lignocellulosic biomass to ethanol*. Journal of Biotechnology. 56, 1-24.
- Lee, Y. Y., Iyer, P., Torget, R. W., (1999). *Dilute-acid hydrolysis of lignocellulosic biomass*. Advances in Biochemical Engineering and Biotechnology. 65, 93.
- Lin, Y., and Tanaka, S., (2006). *Ethanol fermentation from biomass resources: current state and prospects*. Applied Microbiology and Biotechnology 69.6; 627-642
- Lynd, L. R., (1996). *Overview and evaluation of fuel ethanol from cellulosic biomass: technology, economics, the environment and policy*. Annual Rev. Energy Environ. 21:403-465.
- Lynd, L. R., Elander, R. T., Wyman, C. E., (1996). *Likely features and costs of mature biomass ethanol technology*. Applied Biochemistry, Biotechnology. 57 (58), 741-761.
- McMillan, J.D., (1994). *Pretreatment of lignocellulosic biomass*. In: Himmel, M.E., Baker, J.O., Overend, R.P. (Eds.), Enzymatic Conversion of Biomass for Fuels Production, ACS Symposium Series, vol. 566. ACS, Washington, DC, USA. 292-324.
- McMillan, J. D., (1997). *Bioethanol production: Status and prospects*. Renewable Energy 10:295-302.
- Medigan, M. T., Martinko, J. M., Parker, J., (2000). *Brock Biology of Microorganism, 9th Edition*. New Jersey, USA: Prentice Hall Inc.

Meshitsuka, G., Isogai, A., (1996). *Chemical structure of cellulose, hemicellulose, and lignin*. In: Chemical modification of lignocellulosic materials. Ed. D. N-S. Hon, Marcel Dekker, Inc., New York, Basel, Hong Kong.11-34.

Millett, M. A., A. J. Baker, and L. D. Satter., (1975). *Pre-treatments to enhance chemical, enzymatic and microbiological attack of cellulosic materials*. Biotechnol. Bioeng. Symp. 5:193-219.

Mosier N, Wyman C, Dale BE, Elander R, Lee YY, Holtzapple M, Ladisch M., (2005). *Features of promising technologies for pretreatment of lignocellulosic biomass*. Bioresour Technol 96:673-686.

Mosier, N. S., Wyman, C., Dale, B., Elander, R., Lee, Y. Y., Holtzapple, M., Ladisch, M. R., (2005). *Features of promising technologies for pretreatment of lignocellulosic biomass*. Bioresources Technology. 96, 673-686.

Muzzy, J. D., Roberts, R. S., Fieber, C. A., Faass, G. S. and Mann, T. M., (1983). *Pretreatment of hardwood by continuous hydrolysis*. 351-368. In: J. Soltes (ed.). Wood and Agricultural Residues. Academic Press. NY, USA.

Nguyen, Q. A., Tucker, M. P., Keller, F. A., Eddy, F. P., (2000). *Two stage dilute-acid pretreatment of softwoods*. Applied Biochemistry and Biotechnology 84–86, 561–576.

Olsson L, Hahn-Hägerdal B. (1996). *Fermentation of lignocellulosic hydrolysates for ethanol fermentation*. Enzyme Microb Technol 18:312–331.

Palmqvist, E., and B. Hahn-Hagerdal. (2000a). *Fermentation of lignocellulosic hydrolysates. I: inhibition and detoxification*. Bioresources Technology. 74: 17-24.

Palmqvist, E., and B. Hahn-Hagerdal., (2000b). *Fermentation of lignocellulosic hydrolysates. II: inhibitors and mechanisms of inhibition*. Bioresources Technology. 74: 25-33.

Parveen, K., Diane, M. B., Michael, J. D., and Pieter, S., (2009). *Methods for Pretreatment of Lignocellulosic Biomass for Efficient Hydrolysis and Biofuel Production*; <http://pubs.acs.org> on March 26, 2009.

Perez, J., Dorado, J. M., Rubia, T. D., Martinez, J. (2002). *Biodegradation and biological treatment of cellulose, hemicellulose and lignin: An overview*. Int. Microbiology. 5, 53–63.

Prasertsan, P., and Prasertsan, S., (2004). *Palm oil industry residues*. In: Pandey, A. (Ed) Concise encyclopedia of bioresource Technology. Haworth Press, New York. 460-465.

Rahman, S. H. A., Choudhury, J. P., Ahmad, A. L, Kamaruddin, A. H., (2007). *Optimization studies on acid hydrolysis of oil palm empty fruit bunch fiber for production of xylose*. Bioresource Technol. 98(3): 554-559.

Rodríguez-Chong, A., Ramírez, J. A., Garrote, G. and Vázquez, M., (2003). *Hydrolysis of sugar cane bagasse using nitric acid: A kinetic assessment*. Journal of Food Engineering. 1-10.

Root, D. F., Saeman, J. F., Harris, J. F., (1959). *Kinetics of the acid catalyzed conversion of xylose to furfural*. Forest Products Journal, 158–165.

Ruttan, R. F., (1909). *Ethyl alcohol from sawdust and other wood waste*. Society of Chemical Industries 28, 1290–1294.

Sakakibara, A., (1991). *Chemistry of lignin*. In: Wood and cellulosic chemistry. Eds. D. N-S. Hon and Shiraishi. N., Marcel Dekker, Inc., New York, Basel.113-175.

Sarkanen, K. V. and Ludwig, C. H., (1971) *Lignins: Occurrence, Formation, Structure and Reaction*. Wiley-Interscience, New York.

Scherrard, E. C., Kressman, F. W., (1945). *Review of processes in the United States prior to World War II*. Industrial Engineering Chemistry 37 (1), 5–8.

Schurech, C., (1963). *The hemicelluloses, in the Chemistry of Wood*, Browning, B. L., Ed., Interscience, New York. chapter 5.

Sun, X.F., Xu, F., Sun, R. C., Wang, Y. X., Fowler, P., Baird, M. S., (2004). *Characteristics of degraded lignins obtained from steam exploded wheat straw*. Polym. Degrad. Stabil. 86, 245-256

Taherzadeh, M. J. (1999). *Ethanol from lignocellulose: physiological effects of inhibitors and fermentation strategies*. Ph.D. Thesis in Biotechnology, Chemical Reaction Engineering, Chalmers University of Technology.

Taherzadeh, M. J., Karimi, K. (2007). *Acid-based hydrolysis processes for ethanol from lignocellulosic materials: A review*. BioResources. 2, 472-738.

Torget, R., Himmel, M., Grohmann, K., (1992). *Dilute-acid pretreatment of two short-rotation herbaceous crops*. Applied Biochemistry and Biotechnology 34/35, 115–123.

Tsao, G. T., Ladisch, M. R., Voloch, M., Bienkowski, P., (1982). *Production of ethanol and chemicals from cellulosic materials*. Process Biochemistry 17 (5), 34–38.

Ugochukwu, O. G., Otegbade, J. O., Ifeonu, P., Okeke, E. U. and Idris, S. A., (1999). *STAN Agricultural Science for Senior Secondary Schools*. First Edition; Ikeja, Nigeria: Longman Nigeria Plc.

Van Zessen, E., Weismann, M., Bakker, R. R. C., Elbersen, H. W., Reith, J.H., Den Uil, H., (2003). *Ligno-cellulosic-ethanol, a second opinion*. Energy Research Center of the Netherland, ECN. Utrecht, The Netherlands.

World Rainforest Movement Bulletin, (2001). *Nigeria: Palm oil deficit in a traditional palm oil producing country*. Maldonado 1858 - 11200 Montevideo – Uruguay. June, 2001.

- Wyman, C. E., (1996). *Handbook on bioethanol: production and utilization*. Taylor & Francis: Washington DC, USA.
- Wyman, C. E. (1999) Biomass ethanol: Technical progress, opportunities, and commercial challenges. *Annu. Rev. Energy Environ.*, 24, 189–226.
- Xiang, Q., Kim, J. S. and Lee, Y. Y., (2003a). "A comprehensive kinetic model for dilute-acid hydrolysis of cellulose", *Applied Biochemical Biotechnology*, Vol. 105, No. 108, 337-52.
- Xiang, Q., Lee, Y. Y., Pettersson, P. O. and Torget, R. W., (2003b). "Heterogeneous aspect of acid hydrolysis of α -cellulose", *Applied Biochemistry and Biotechnology*, Vol. 105, No. 108, 505-513.
- Xiao, W. P., Clarkson, W. W., (1997). Acid solubilization of lignin and bioconversion of treated newsprint to methane. *Biodegradation*. 8, 61-66.
- Yan, Y., Li, T., Ren, Z. and Li, G., (1996). "A study on catalytic hydrolysis of peat", *Bioresource Technology*, Vol. 57, 269-273.
- Yang, B., Wyman, C. E., (2004). Effect of xylan and lignin removal by batch and flow-through pretreatment on the enzymatic digestibility of corn stover cellulose. *Biotechnol. Bioengineering*. 86, 88-95.
- Zeitsch, K. J., (2000). *The Chemistry and Technology of Furfural and Its Many By-Products*. Sugar Series. Elsevier, New York. Vol. 13.

APPENDICES

LIST OF PUBLICATIONS & SEMINAR

Optimization of acid pretreatment procedures for oil palm trunk fibers used in production of bio-ethanol.



Optimization of acid pretreatment procedures for oil palm trunk fibers used in production of sugar:
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