OPTIMIZATION OF XYLOSE PRODUCTION FROM SUGARCANE BAGASSE USING RESPONSE SURFACE METHODOLOGY (RSM)

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ABSTRACT

Xylose is a monosaccharide containing five carbon atoms, including an aldehyde functional group. It is a pentose sugar which has chemical formula $C_5H_{10}O_5$. Hemicellulose is present in plant cell walls and is associated with the cellulose. Its chemical formula is $(C_5H_8O_4)n$ and in some cases is $(C_6H_{10}O_5)n$. It is possible to hydrolyze hemicellulose by several processes (enzymatic, physical and chemical) for producing monomer sugars with great purity and high yield. The aim of this study is to optimize the xylose production from sugarcane bagasse by manipulating the temperature, agitation rate and enzyme concentration using Response Surface Methodology (RSM) based on central composite design (CCD). In this study, producing xylose from sugarcane bagasse contributes to reduce the environmental impact and bioprocess cost. Alkaline and acid hydrolysis method was used for the pretreatment of sugarcane bagasse. After the pretreatment, the screening process was constructed to determine the best range of parameters to be used in optimization process. Seventeen experiments have been arranged by RSM for optimization. The optimized conditions of parameters were 50°C of temperature, 180 rpm of agitation rate and 2 mg/ml of enzyme concentration with the predicted xylose production was 0.367 mg/ml. The actual xylose production was 0.373 mg/ml. Before the optimization, the xylose production was 0.228 mg/ml. As a conclusion, the optimization of xylose production from sugarcane bagasse by using RSM was successfully done with 63.6% of increment.

ABSTRAK

Xilosa adalah monosakarida yang mengandungi lima atom karbon, termasuk kumpulan berfungsi aldehid. Ia adalah gula pentosa yang mengandungi formula kimia $C_5H_{10}O_5$. Hemiselulosa hadir dalam dinding sel tumbuhan dan bercampur dengan selulosa. Formula kimianya adalah $(C_5H_8O_4)n$ dan dalam beberapa kes adalah $(C_6H_{10}O_5)n$. Adalah mungkin untuk menghidrolisis hemiselulosa oleh beberapa proses (enzimatik, fizikal dan kimia) untuk menghasilkan gula monomer dengan ketulenan dan hasil yang tinggi. Tujuan kajian ini adalah untuk mengoptimumkan penghasilan xilosa daripada hampas tebu dengan memanipulasikan suhu, kadar agitasi dan kepekatan enzim menggunakan Kaedah Tindak Balas Permukaan (RSM) berdasarkan Rekabentuk Komposit Pusat (CCD). Dalam kajian ini, xilosa dihasilkan daripada hampas tebu dapat mengurangkan kesan pada persekitaran dan kos bioproses. Kaedah alkali dan asid hidrolisis digunakan untuk pra-rawatan hampas tebu. Setelah dirawat, proses saringan telah dilakukan untuk menentukan julat terbaik pembolehubah - pembolahubah yang akan digunakan dalam proses pengoptimuman. Sebanyak 17 eksperimen telah ditetapkan oleh RSM untuk pengoptimuman. Kondusi pembolehubah – pembolehubah yang telah dioptimumkan adalah pada suhu 50 °C, kadar agitasi 180 rpm dan kepekatan enzim sebanyak 2 mg/ml dengan penghasilan xilosa yang diramalkan adalah 0.367 mg/ml. Penghasilan xilosa yang sebenar adalah 0.373 mg/ml. Sebelum pengoptimuman, penghasilan xilosa adalah 0.228 mg/ml. Kesimpulannya, pengoptimuman penghasilan xilosa daripada hampas tebu dengan menggunakan RSM telah berjaya dilakukan dengan peningkatan sebanyak 63.6%.

TABLE OF CONTENTS

CHAPTER	TITLE	PAGE
	DECLARATION	ii
	DEDICATION	iii
	ACKNOWLEDGEMENTS	iv
	ABSTRACT	v
	ABSTRAK	vi
	TABLE OF CONTENTS	vii
	LIST OF TABLES	x
	LIST OF FIGURES	xi
	LIST OF APPENDICES	xiii
	LIST OF SYMBOLS/ ABBREVIATIONS	xiv
1	**INTRODUCTION**	1
	1.1 Overview	1
	1.2 Problem Statement	4
	1.3 Objective	5
	1.4 Scopes of Research	5
	1.5 Rationale and Significance	6
2	**LITERATURE REVIEW**	**7**
	2.1 Sugarcane	7
	2.2 Hemicellulose	10
	2.3 Lignin	12
	2.4 Cellulose	13

	2.5	Acid Hidrolysis	14
	2.6	Alkaline Hydrolysis	17
	2.7	Enzymatic Hydrolysis	19
	2.8	Production and Properties of Xylose	21
	2.9	Factor Affecting the Production of Reducing Sugar	23
	2.9.1	Effect of Temperature on Production of Reducing Sugar	23
	2.9.2	Effect of Agitation Rate on Production of Reducing Sugar	24
	2.9.3	Effect of Enzyme Concentration on Production of Reducing Sugar	25
	2.10	Response Surface Methodology	27
3	**METHODOLOGY**		29
	3.1	Procedure on Optimization of Xylose Production From Sugarcane Bagasse Using Response Surface Methodology	29
	3.2	Chemicals, Apparatus and Equipments	30
	3.3	Pretreatment Process	31
	3.4	Standard Curve of Xylose	35
	3.5	Screening Process for Temperature, Agitation Rate and Enzyme Concentration on Xylose Production Using Conventioanal Method	35
	3.5.1	Effect of Temperature on Xylose Production	36
	3.5.2	Effect of Agitation Rate on Xylose Production	36
	3.5.1	Effect of Enzyme Concentration on Xylose Production	36
	3.6	Reducing Sugar Analysis	37
	3.7	Optimization of Xylose Production	38

4	**RESULT AND DISCUSSION**		39
	4.1	Standard Curve of Xylose	39
	4.2	Screening Process of Temperature, Agitation Rate and Enzyme Concentration on Xylose Production Using Conventional Method	40
	4.2.1	The Effect of Temperature on Xylose Production	41
	4.2.2	The Effect of Agitation Rate on Xylose Production	43
	4.2.3	The Effect of Enzyme Concentration on Xylose Production	45
	4.3	Analysis of Temperature, Agitation Rate and Enzyme Concentration on Xylose Production Using Response Surface Methodology (RSM)	47
	4.4	Optimization of Temperature, Agitation Rate and Enzyme Concentration on Xylose Production using Response Surface Methodology (RSM)	55
5	**CONCLUSION AND RECOMMENDATIONS**		57
	5.1	Conclusion	57
	5.2	Recommendations	58
	REFERENCES		59
	APPENDIX A		68
	APPENDIX B		69

LIST OF TABLES

TABLE NO.	TITLE	PAGE
3.1	Experimental ranges of the independent variables with different levels	38
4.1	The low and high values from screening process	46
4.2	Combination data arranged by Response Surface Methodology (RSM)	48
4.3	The residual between actual and predicted value	49
4.4	Analysis of Variance (ANOVA) for response surface quadratic model of xylose production	50
4.5	Summary of optimization of xylose concentration using Response Surface Methodology	55

LIST OF FIGURES

FIGURES NO.	TITLE	PAGE
2.1	Secondary Cell-Wall (CW) Structure of cellulose, hemicellulose and lignin in lignocellulosic materials	8
2.2	Composition of pectin, cellulose and hemicelluloses in plant cell wall	11
2.3	Three different types of C9 units are present in lignin	12
2.4	Scanning electron micrograph of wood cellulose	14
2.5	Effects of reaction temperature on hydrolysis yield	24
2.6	Production by *Bacillus Licheniformis NK-27* at different agitation speed	25
2.7	Effects of enzyme dosage on the enzymatic hydrolysis	26
3.1	Process flow for xylose production from sugarcane bagasse	30
3.2	The sugarcane bagasse was dried to the sunlight.	32
3.3	The grinded bagasse after dried for a day	32

3.4	Sugarcane bagasse during the alkaline hydrolysis before incubates in water bath	33
3.5	Sugarcane bagasse during the alkaline hydrolysis after incubated in water bath.	33
3.6	Sugarcane bagasse during acid hydrolysis with 1% sulphuric acid.	34
3.8	Comparison between of sample before and after DNS reaction.	37
4.1	The standard curve of xylose.	40
4.2	The effect of temperature on xylose production	41
4.3	The effect of agitation rate on xylose production	43
4.4	The concentration of xylose at different enzyme concentration.	45
4.5	Response surface plot of the combined effect of temperature and agitation rate on the xylose production	52
4.6	Response surface plot of the combined effect of agitation rate and enzyme concentration on the xylose production	53
4.7	Response surface plot of the combined effect of temperature and enzyme concentration on the xylose production	54

LIST OF APPENDICES

APPENDIX	TITLE	PAGE
A1	Preparation of Enzyme Concentration in Sodium Citrate Buffer	68
B1	Experiment Data for Standard Curve of Xylose	69
B2	Screening Process the Effect of Parameters on Xylose Production (Conventional Method)	70
B3	Enzymatic hydrolysis using RSM suggested parameters	72
B4	RSM Analysis	73

LIST OF SYMBOLS/ABBREVIATIONS

ANOVA	-	Analysis of Variance
CCD	-	Central Composite Design
CCRD	-	Central Composite Rotable Design
DNS	-	Dinitrosalicylic
g	-	gram
g/L	-	Gram per Litre
L	-	Litre
mg	-	miligram
mL	-	mililitre
OD	-	Optical density
rpm	-	revolution per minute
RSM	-	Response Surface Methodology
°C	-	Degree Celcius
µL	-	microlitre
%	-	Percentage

CHAPTER 1

INTRODUCTION

1.1 Overview

Among several potential sources of biomass, the sugarcane bagasse has been one of the most promising industrial residues obtained from the sugar and alcohol industries (Saska & Ozer, 1994). In recent years, an increasing effort has been made towards a more efficient utilization of renewable agro-industrial residues, including sugarcane bagasse. Lignocellulosics are an abundant and inexpensive source of carbohydrates that can be used to produce high-value chemicals (Carvalho *et al*., 2005). Biotechnological production of xylitol could be economically attractive using hemicellulosic hydrolysates as potential substrates, instead of pure xylose, to reduce the cost of production (Pessoa *et al.*, 1996). Sugarcane bagasse is composed approximately of 40% cellulose, 24% hemicellulose and 25% lignin.

Hemicellulose is a plant cell wall polysaccharide and in some plants, comprises up to 40% of the total dry material. In Brazil, the bagasse is a particularly convenient source for carbohydrate conversion because it is produced in large amounts (5.8 x 10^7 tonne in a year). Each ton of milled sugarcane gives 180-280 kg of bagasse residues (Pessoa *et al*., 1997). It is possible to hydrolyse hemicellulosic materials by several processes (enzymatic, physical and chemical) for producing monomer sugars with great purity and high yield. The hemicellulosic fraction can be easily hydrolysed by acid treatment. If cellulose and hemicellulose were utilized in an efficient hydrolysis process, the hemicellulose would be completly hydrolyzed to D-xylose (50-70% w w-1) and L-arabinose (5-15% w w-1) as well as the cellulose would be converted to glucose (Puls and Schuseil, 1993). Hemicellulose can be converted to carbohydrates, particularly simple sugar. The dilute acid hydrolysis of bagasse hemicellulose to produce xylose, arabinose, glucose, acid-soluble lignin (ASL) and furfural were conducted using a temperature-controlled digester (Lavarack *et al*., 2002). However, the bagasse needs to undergo pretreatment process to increase the yield of production.

Pretreatment is one of the most important steps in the process in the production of ethanol from lignocellulosic materials. The most common chemical pretreatment methods used for cellulosic feedstock are dilute acid, alkaline, organic solvent, ammonia, sulfur dioxide of other chemicals to make the biomass more digestible by enzymes. The main goal of pretreatment is to alter or remove structural and compositional impediments to hydrolysis and subsequent degradation processes in order to enhance digestibility, improve the rate of enzyme hydrolysis and increase yields of intended products (Hendriks and Zeeman, 2009). These methods cause mechanical, physical, chemical or biological changes in the plant biomass in order to achieve the desired products.

The complete hydrolysis of hemicellulose into monosaccharides requires the concerted action of several enzymes. These include xylanases, galactanases, mannanases, as well as glycosidases xylosidase, galactosidase and mannosidase. The used of xylanase enzyme improving the yield of xylose production from hemicelluloses from 70-74% (Ohgren *et al*., 2007).

Xylose is a hemicellulosic sugar mainly used for its bioconversion to xylitol. It is a major product of the hydrolysis of hemicellulose from many plant materials. Rahman *et al.*, (2007) had done a research entitle optimization studies on acid hydrolysis of oil palm empty fruit bunch fiber for production of xylose. The objective of the study was to determine the effect of H_2SO_4 concentration, reaction temperature and reaction time for production of xylose. Batch reactions were carried out under various reaction temperature, reaction time and acid concentrations. Response Surface Methodology (RSM) was followed to optimize the hydrolysis process in order to obtain high xylose yield. The optimum reaction temperature, reaction time and acid concentration found were 119 C, 60 min and 2%, respectively. Under these conditions xylose yield and selectivity were found to be 91.27% and 17.97 g/g, respectively.

1.2 Problem Statement

Xylose is highly demand in industry and expensive sugar. It is relatively expensive by about $7/kg (Leathers, 2003) comparatively with other natural sweeteners. It is found in the fibers of many fruits and vegetables, including various berries, corn husks, oats, and mushrooms. It also can be found in chewing gum, toothpaste and corn sweeteners.

Xylose is used to produce xylitol, which is a sugar alcohol sweetener used as a naturally occurring sugar substitute. Xylitol has been used in foods since the 1960's. In the U.S., xylitol is approved as a food additive in unlimited quantity for foods with special dietary purposes. It is suitable for diabetes, recommended for oral health and paranteral nutrition (Makinen, 2000). On an industrial scale, xylitol is currently produced through chemical reduction of xylose derived from birchwood chips and sugarcane baggase hemicelluloses hydrolysate. The chemical process is expensive because of the high temperature and pressure required for hydrogenation of xylose. Furthermore, extensive steps for separation and purification had increased the cost of production. Instead of using pure xylose, the biotechnological production of xylose could be economic and attractive method, which could reduce the cost of production.

Xylose also can be fermented to ethanol by many bacteria, yeasts and fungi. Production of ethanol from sugars or starch from sugarcane and cereals, respectively, impacts negatively on the economics of the process, thus making ethanol more expensive compared with fossil fuels. Hence, the technology development focus for the production of ethanol has shifted towards the utilization of residual lignocellulosic materials to lower production costs (Howard *et al*., 2003).

Besides, open burning of bagasse is one of the topics in environmental issues. Instead of burning for disposal purpose, sugarcane baggase can be reuse for xylose and others production. This could improve the waste management process of the country.

1.3 Objective

The aim of this study is to optimize the production of xylose from sugarcane bagasse using Respond Surface Methodology (RSM).

1.4 Scope of Research

In order to achieve the stated objective, the following scopes of research have been identified:

- To study the effect of temperature on xylose production from sugarcane bagasse.
- To study the effect of agitation rate on xylose production from sugarcane bagasse.
- To study the effect of enzyme concentration on xylose production from sugarcane bagasse.
- To optimize the production of xylose from sugarcane bagasse by using Response Surface Methodology (RSM).

1.5 Rationale and Significance

The rationale of using bagasse in xylose production is based on commercially unproven and new technology and hence with considerable potential risks as well as direct and indirect benefits, arising from adaptive technological research. It is directly linked and interrelated with agriculture as well as the sugar industry. Besides, this method use innovative characteristic by doing experimentation with new ideas possible.

The rational and significance of this study is to reduce the air pollution from sugarcane bagasse open burning. Usually, the agriculture waste is dispose by burning but nowadays, the waste had been reuse to become other beneficial product such as diesel, sugar and ethanol. In this study, sugarcane bagasse will be use as a source of xylose production which has high demand in industry. Besides, sugarcane bagasse is very economical source since it is a waste product.

One of natural fibres with high availability is sugarcane bagasse, a residue of the sugarcane milling process. In Malaysia, the annual production of sugarcane reaches a million tonnes, which is less than 0.1% of the world annual production (Wirawan *et al*., 2010) Nearly 30% of that number will turn into bagasse when it is crushed in a sugar factory. This procedure produces a large volume of bagasse wastes that may have an extremely harmful effect upon the environment if not suitably treated (Reis, 2006). Moreover, the stock is abundant, and the price of sugarcane bagasse is less expensive than that of other natural fibres (Vazquez *et al.,* 1999)

CHAPTER 2

LITERATURE REVIEW

2.1 Sugarcane

Sugarcane (*saccharum officinarum*) is a grass that is harvested for its sucrose content. Sugarcane is any of six to thirty-seven species (depending on taxonomic system) of tall perennial grasses of the *genus saccharum* (family *poaceae*, tribe *andropogoneae*). Native to warm temperate to tropical regions of Asia, they have stout, jointed and fibrous stalks that are rich in sugar and measure two to six meters (six to nineteen feet) tall. All sugar cane species interbreed and the major commercial cultivars are complex hybrids. Brazil produces about one-third of the world's sugarcane. After extraction of sugar from the sugarcane, the plant material that remains is termed bagasse. Currently, the bagasse production in the United States is about 8.6 million tons per year. Sugarcane bagasse found at sugar mills contain both relatively easy and hard to degrade materials. The easily degraded materials appear to be from the leaf matter and the hard to degrade from the rind (Fox, 1987). Bagasse is cheap, readily available and has high carbon content (Martín *et al*, 2002).

Sugarcane bagasse is composed approximately of 40% cellulose, 24% hemicelluloses and 25% lignin. In plant cells, including sugarcane plants, a secondary wall, consisting of three layers (S1, S2 and S3) is surrounded by a thin primary wall. The secondary wall is surrounded by lignin. The S1 and S3 layers contain mainly amorphous cellulose and hemicellulose. The S2 layer contains crystalline cellulose. However, amorphous regions also exist in the cellulose. Amorphous cellulose, hemicelluloses and lignin are present between the layers (S1, S2 and S3) (Fox, 1987). An illustration of the structure of the cell wall with its component organization is shown in Figure 2.1. At the sugarcane bagasse, the basic composition is 40% cellulose, 24% hemicelluloses and 25% lignin. S1 and S3 layers have more amorphous cellulose and hemicellulose contents. S2 layer has more crystalline cellulose regions (Bidlack *et al*, 1992).

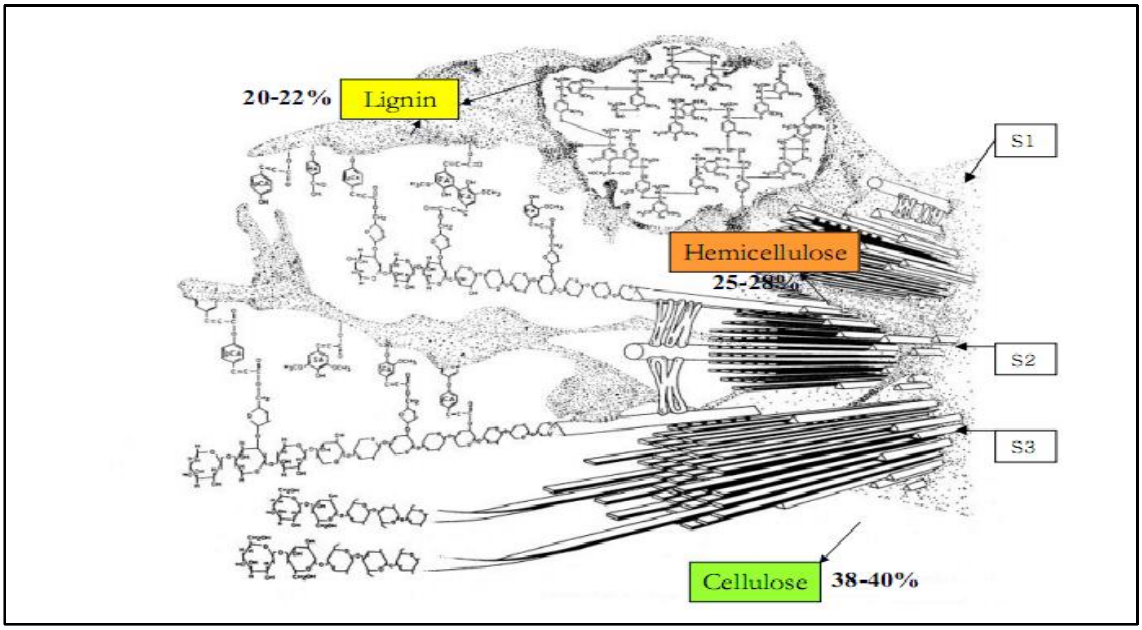

Figure 2.1: Secondary Cell-Wall (CW) Structure of cellulose, hemicellulose and lignin in lignocellulosic materials.

According to Brienzo *et al*., (2009), sugar mills generate approximately 135 kg of bagasse (dry weight) per metric ton of sugarcane. Bagasse is a rich source of not only cellulose, but also hemicellulose, represented by L-arabino-(4-O-methyl-D-glucurono)-D-xylan. The two polysaccharides represent about 70% of bagasse. Nowadays about 50% of generated sugarcane bagasse is used to generate heat and power to run the sugar mills and ethanol plants. The remaining portion is usually stockpiled. However, because the heating value of carbohydrates is approximately half of that of lignin, it would be beneficial to develop a more economical use of carbohydrates.

In the Brazilian economic context an effective utilization of sugarcane bagasse for bioproduction is very important. More than 60,000,000 tons of bagasse containing 50% moisture can be produced annually during the ethanol production season. This waste has been used as a raw material to produce hydroxymethyl furfural, paper pulp, acoustic boards, pressed woods and agricultural mulch (Dominguez *et al.*, 1996). About 70% of the dry mass in lignocellulosic biomass consists of cellulose and hemicellulose. If these two carbohydrates were utilized in an efficient hydrolysis process, the hemicellulose would be completely hydrolyzed to D-xylose (50-70% w/w) and L-arabinose (5-15% w/w), while the cellulose would be converted to glucose (Cao *et al.*, 1995).

Lavarack *et al*., (2002) studied that the acid hydrolysis of sugarcane bagasse hemicelluloses could produce xylose, arabinose, glucose and other products. The experimental were conducted using a temperature-controlled digester. The reaction conditions varied at temperature (80–200^{o}C), mass ratio of solid to liquid (1:5–1:20), type of bagasse material (bagasse or bagacillo), concentration of acid (0.25–8 wt% of liquid), type of acid (hydrochloric or sulphuric) and reaction time (10–2000 min). Kinetic modelling of the global rates of formation of products was performed. The most accurate kinetic model of the global reaction for the decomposition of xylan was a simple series hydrolysis of xylan to xylose followed by xylose decomposition. Similar schemes were used to model the production of arabinose, glucose and furfural from the hemicellulose. The yield of xylose is about 80% of the theoretical xylose available from the bagasse.

2.2 Hemicellulose

The term hemicellulose refers to a group of homo- and heteropolymers consisting largely of anhydro-β-(1, 4)-D-xylopyranose, mannopyranose, glucopyranose, and galactopyranose main chains with a number of substituent. Hemicellulose is present in plant cell walls and is associated with the cellulose. It is a complex polysaccharide that is soluble in both alkali and acid solutions. Its chemical formula is $(C_5H_8O_4)n$ and in some cases is $(C_6H_{10}O_5)n$. Hemicellulose is a heterogeneous polymer, unlike cellulose and is usually composed of 50 to 200 monomeric units of a few simple sugars. Xylose, a C5 sugar is the most abundant component in hemicellulose. Xylan contains of D-xylose units and is linked from the number one to the number four carbon of each residue. Arabinose is normally the next most plentiful component in hemicellulose. Minor components, including mannose, galactose and uronic acids may also be present (Jeffries *et al*., 1994).

Hemicelluloses are generally found in association with cellulose in the secondary walls of plants, but they are also present in the primary walls. The principal component of hardwood hemicellulose is glucuronoxylan and glucomannan in the softwoods. Some hemicelluloses contain both glucomannans and galactoglucomannans. The glucomannans contain D-glucose and D-mannose units in a ratio of 30:70. The galactoglucomannans contain D-galactose, D-glucose and D-mannose units in a ratio of 2:10:30 (Paster, 2003). Hemicellulose utilization is considered difficult because the branched structure of hemicellulose slows enzymatic hydrolysis. Among biomass components, hemicelluloses which are mainly composed of xylans, provide an important source of interesting molecules such as xylose and xylo-oligosaccharides which have potential applications in different areas, notably in chemical, food and pharmaceutical industries (Fooks *et al*., 1999).

Herrera *et al*., (2003) studied that xylose can be convert from sorghum straw by using hydrochloric acid. The main composition of the sorghum straw was cellulose, 35%; xylan, 19%; hemicelluloses, 24% and lignin, 25%. The objective of the research was to study the xylose production by hydrolysis of sorghum straw with hydrochloric acid at 122^{o}C. Several concentrations of HCl (2 - 6%) and reaction time (0 - 300 min) were evaluated. Kinetic parameters of mathematical models for predicting the concentration of xylose, glucose, acetic acid and furfural in the hydrolysates were found. Optimal conditions for hydrolysis were 6% HCl at 122^{o}C for 70 min, which yielded a solution with 162 g xylose/L, 38 g glucose/L, 20 g furfural/L and 19 g acetic acid/L.

Figure 2.2 shows the composition of pectin, cellulose and hemicelluloses in primary cell wall of a plant. Cellulose and hemicellulose are arranged into at least three layers in a matrix of pectin polymers.

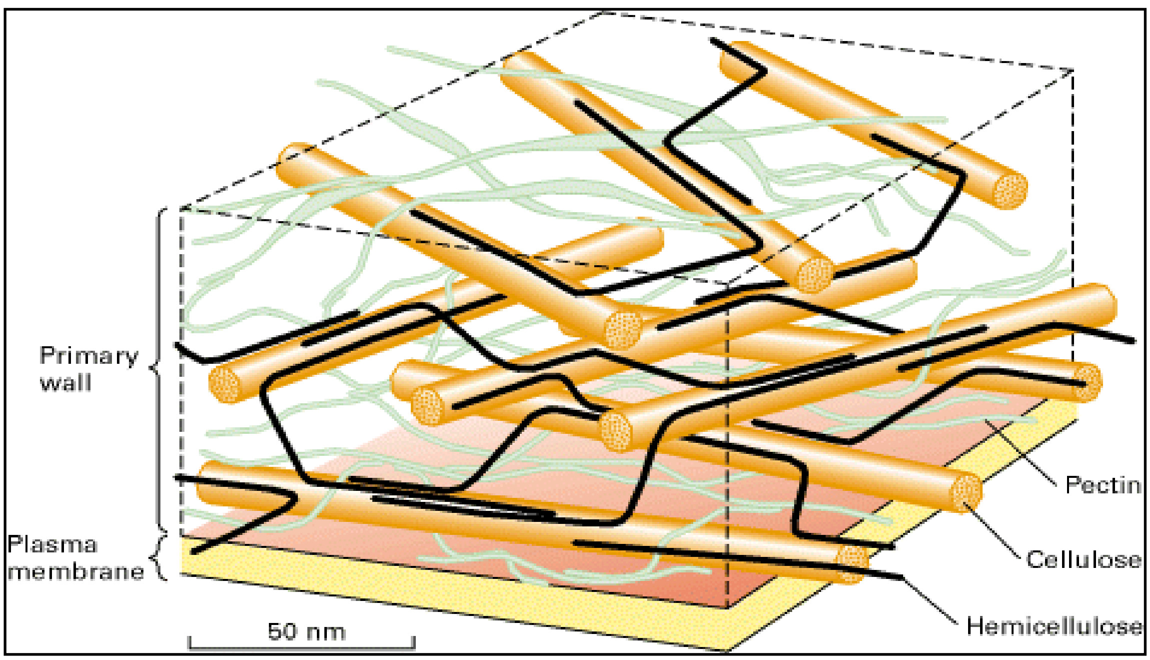

Figure 2.2: Composition of pectin, cellulose and hemicelluloses in plant cell wall.

2.3 Lignin

Lignin are a highly branched polymers formed in plant cell walls (Pareek *et al.*, 2000). Lignin resists the growth of microorganisms and stores more solar energy than either cellulose or hemicelluloses (Hu, 2002). The structure of lignin is complex, disordered, and random and consists mainly of ether linked aromatic ring structures, which adds elasticity to the cellulose and hemicellulose matrices (Paster, 2003). In pulp industries, the emphasis is removal not utilization of lignin. However, lignin is in the spotlight as a useful source for phenolic compounds for plastics and other materials (Wallis, 1971).

Lignin is mainly composed of phenylpropane or C9 units. Three different types of C9 units are present in lignin (Figure 2.3). These are p-hydroxyphenylpropane, guaiacylpropane and syringylpropane units (Gratzl, 2000). In hard wood, lignin consists mainly of guaiacylpropane and syringylpropane units with a small amount of p-hydroxyphenylpropane units. Lignin is composed principally of guaiacylpropane units with traces of p-hydroxyphenylpropane units in soft wood (Baucher, 1998). In grasses, lignin is composed of both guaiacylpropane and syringylpropane units. P-hydroxyphenylpropane units exist as a minor component of lignin in grasses (Grabber *et al.*, 2004).

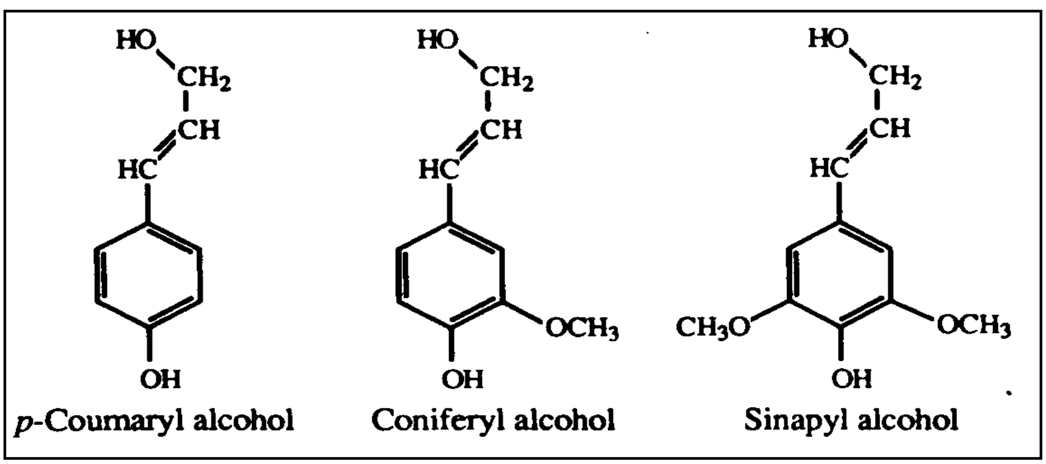

Figure 2.3: Three different types of C9 units are present in lignin.

2.4 Cellulose

Cellulose is the most abundantly available carbohydrate polymer in nature (Imai *et al*., 2003) and has therefore long been pursued as a source of providing plentiful food and energy resources. Cellulose is a complex carbohydrate polymer comprising of d-glucose units linked together by β-1,4 bonds. It comprises approximately 45% of dry wood weight. Cellulose can be hydrolyzed enzymatically or with acid. Cellulose, the major fraction of lignocellulosic biomass, can be hydrolyzed to glucose by cellulase enzymes (Jeya *et al.*, 2009). Cellulose in the primary walls of dividing and elongating cells fulfills severa1 functions, the most obvious being to provide strength. In most primary walls, cellulose exists as elementary fibrils that form a complex with xyloglucan (Hayashi, 1989).

Cellulose is the substance that makes up most of a plant's cell walls. Aside from being the primary building material for plants, cellulose has many others uses. According to how it is treated, cellulose can be used to make paper, film, explosives, and plastics, in addition to having many other industrial uses. A plant uses glucose to make cellulose when it links many simple units of glucose together to form long chains. These long chains are called polysaccharides and form very long molecules that plants use to build the walls (http://www.scienceclarified.com).

Figure 2.4 illustrated the Scanning Electron Micrograph (SEM) of cellulose in wood cell wall.

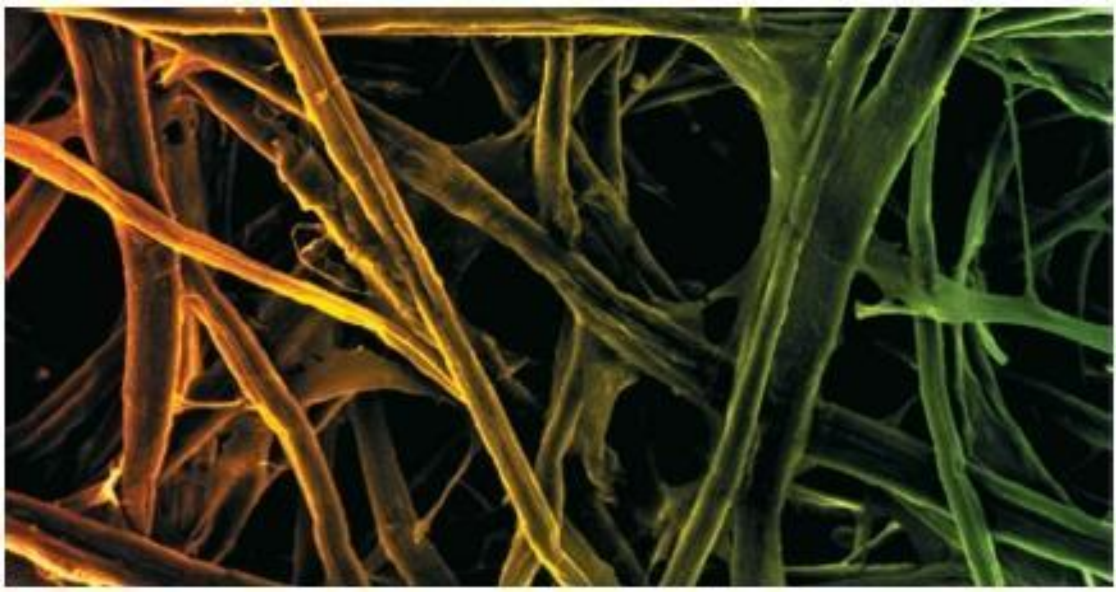

Figure 2.4: Scanning electron micrograph of wood cellulose.

2.5 Acid Hydrolysis

In general, acid treatment is effective in solubilizing the hemicellulosic component of biomass. Proper combinations of pH, temperature, and reaction time can result in high yields of sugars, primarily xylose from hemicellulose (Elander and Hsu, 1995). Acid hydrolysis has been investigated as a possible process for treating lignocellulosic materials such as wood chips, rice straw, sugar beet pulp and wheat straw. According to Parisi (1989), the mineral acids act simply and rapidly as reaction catalyzers of polysacharide fractions. Sugarcane bagasse can be hydrolyzed using dilute acid to obtain a mixture of sugars with xylose as the major component. However, in the hydrolyzate some by-products generated in the hydrolysis, such as acetic acid, furfural, phenolic compounds, or lignin degradation products, can be present. These are potential inhibitors of a microbiological utilization of this hydrolyzate (Dominguez *et al.,* 1996).