

**KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY**

**COLLEGE OF SCIENCE**

**DEPARTMENT OF CHEMISTRY**



**ASSESSMENT OF AVAILABILITY AND CHARACTERIZATION OF  
CORN STOVER AS FEEDSTOCK FOR CELLULOSIC BIOETHANOL  
PRODUCTION IN GHANA**

**A Thesis submitted to the Department of Chemistry, Kwame Nkrumah  
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requirements for the degree of**

**MASTER OF PHILOSOPHY (M.Phil.)  
PHYSICAL CHEMISTRY**

**By**

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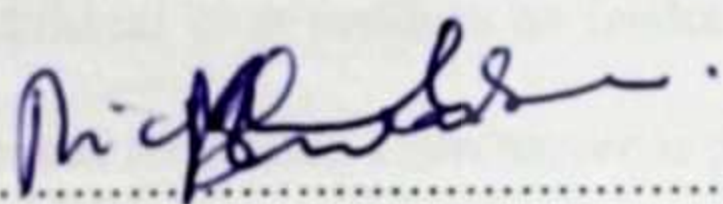
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**June, 2013**

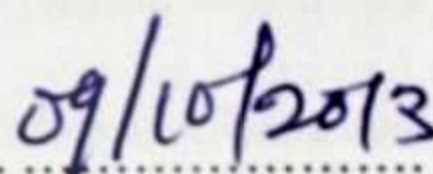


## CERTIFICATION

I hereby declare that this submission is my own work towards the M.Phil. and that, to the best of my knowledge, it contains no material previously published by another person nor material which has been accepted for the award of any other degree of the University, except where due acknowledgment has been made in the text.



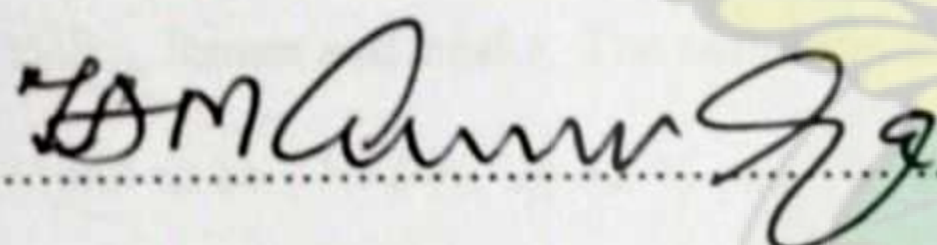
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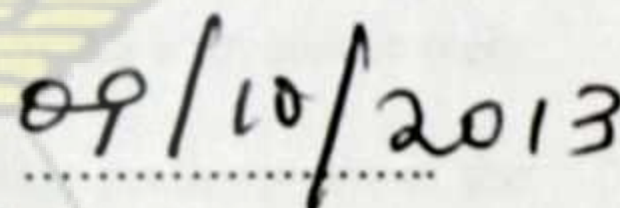


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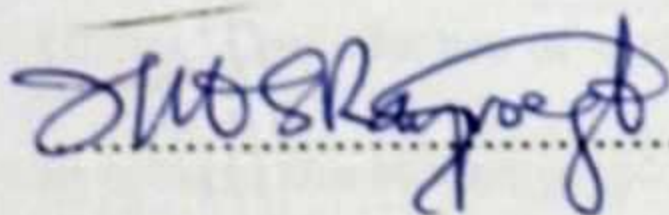


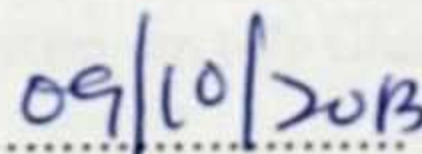


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## ABSTRACT

Bioethanol is a renewable fuel used as blend or an alternative to gasoline. It burns clean in the internal combustion engine compared to gasoline. Currently, bioethanol is produced from food sources such as sugar and starchy crops. Use of these sources of foods as feedstock for fuel production raises concerns of food – fuel competition. Recently, focus has been on use of agricultural crop residues as feedstock. Corn stover has received great attention worldwide as potential feedstock. Corn stover is perceived to be available in large quantities in Ghana and its use as feedstock for bioethanol production will be more beneficial than food sources. In this study, an investigation was carried out to evaluate the quantities and the chemical characteristics of the corn stover available in Ghana. The corn stover yield per hectare was determined to be 3.35 MT. The average quantity of corn stover production per year was estimated to be  $1,512.22 \times 10^3$  MT. The dry weight distribution of the maize plant was 46.3 % corn, 9.4 % cobs and 44.3 % stalks, leaves and husks. The cellulose, hemicellulose and lignin content of the corn stover were found to be 37.72 %, 20.62 % and 30.50 % respectively. Sodium hydroxide pretreatment of the corn stover yielded a biomass with high cellulose content of 81.27 % and low lignin content of 4.83 %. Enzymatic hydrolysis of the pretreated corn stover with cellulase enzyme (*Cellic CTec2*) resulted in total reducing sugar concentration of 157.96 mg/mL. Based on the carbohydrate content of the raw corn stover, the theoretical ethanol yield per 1000 Kg of the dry stover was calculated to be 198.95 L. Theoretically, it was found that 40 % of annual production of corn stover could yield bioethanol to blend or replace 16.67 % of the annual gasoline consumption in Ghana. Thus, the use of corn stover alone as feedstock for bioethanol production can help Ghana to achieve the bioenergy policy target of substituting 10 % of petroleum fuels consumption with biofuels by 2020 and 20 % by 2030.



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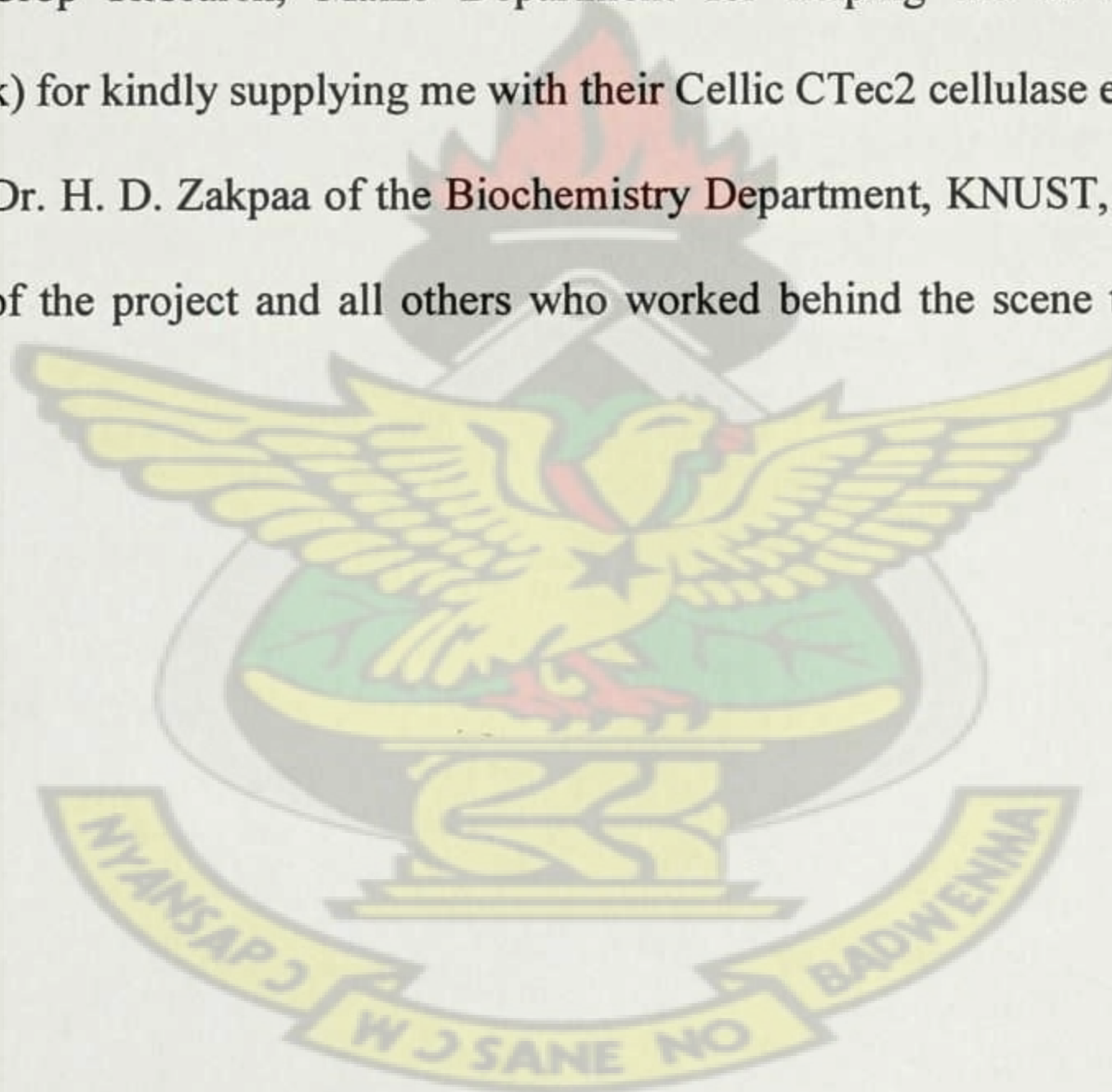
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## DEDICATION

This project is dedicated to Mr. Sulemana Issah, CEO of Tropical Agricultural Marketing and Consultancy, (TRAGRIMACS), Tema, for his immense interest and effort in commercialization of biofuels in Ghana. It is also dedicated to Mr. Raphael Osei Bobie, my uncle and Nana Adubofour, my grandfather, who gave me guidance and support during my second degree education. I also dedicate this work to my beloved parents, Mr. Seth Mensah and Mrs. Sarah Mensah and my siblings. Finally, I dedicate this work to anyone who in any way has contributed towards my education.





## 1.0 INTRODUCTION

### 1.1 BACKGROUND

#### 1.1.1 Bioethanol

Bioethanol is simply ethanol or ethyl alcohol ( $\text{CH}_3\text{CH}_2\text{OH}$ ) derived from biomass by biochemical or thermo-chemical conversion routes. Ethanol in its anhydrous form (i.e. 200 proof or 100 %) is fuel and it is currently receiving much attention globally as alternative transportation fuel to gasoline due to the fact that petroleum is non-renewable and world fuel prices are on the rise. However, the use of ethanol as fuel has its history in the 1800s when Henry Ford and others built engines that run on ethanol. The interest in ethanol fuel declined after World War I because of cheap source of petroleum fuels. Consequently, owing to the oil crises in the 1970s as a result of OAPEC (Organization of Arab Petroleum Exporting Countries) embargo on oil production and exportation, interest in fuel ethanol was rekindled. 'Gasohol' was introduced in 1971 as the brand name for fuel made up of a mixture of ethanol and gasoline (Solomon et al, 2007).

Bioethanol has similar fuel characteristics with gasoline and can serve as a renewable alternative fuel (refer Appendix 1). It is also used to blend gasoline to improve the fuel properties. Because of its higher oxygen content, when blended with gasoline, it allows better oxidation of the gasoline hydrocarbons with consequent reduction in the emission of carbon monoxide (CO) and aromatic compounds (Sanchez and Cardona, 2008). An example of bioethanol blend is E15 which means the fuel contains 15 % bioethanol and 85 % gasoline. Bioethanol is also employed as gasoline enhancer since the discovery in the 1990s that proved that methylene tertiary butyl ether (MTBE) used as octane booster pollutes underground water. The principal use of



bioethanol is in the spark ignition engine as transportation fuel because it has high octane rating, implying very good anti-knock characteristics. However, the energy content of bioethanol is about two-thirds of that of gasoline. Apart from environmental effect of using bioethanol, other factors such as energy security, socio-economy and agriculture development motivate many countries into bioethanol research and production.

### 1.1.2 Bioethanol Production

Bioethanol is presently produced from food sources such as sugar crops (sugarcane, sugar beets, and sweet sorghum) and starchy crops (corn, cassava, and wheat). These sugar and starchy crops (feedstock) chemically consist of polymers of fermentable sugars (especially glucose) which can be easily hydrolyzed and fermented to ethanol using fermenting yeast. Currently, the great interest in bioethanol is to use it as blend in gasoline. Many countries have set blending targets. Brazil has bioethanol blending mandate of E22 – E25 by 2013, India has E5 by 2008 and E20 by 2018, South Africa has proposed E8 – E10, UK has E2.5 by 2008 and E5 by 2020 and US has E10 in Iowa, Hawaii, Missouri, and Montana; E20 in Minnesota, and E2 in Louisiana and Washington State (Timilsina and Shrestha, 2011).

High energy demand in the world, because of increasing human population, high level of commercialization and advancement in technology are the main drivers for bioethanol production. Bioethanol is required in substantial amounts to be able to sustainably blend with gasoline or replace gasoline as transportation fuel. Global production of bioethanol grew from 30.8 billion liters in 2004 to 76 billion liters in 2009 (Timilsina and Shrestha, 2011). People argue that diverting food crops into producing bioethanol will give rise to food – fuel competition. Balat, (2011), reported that using corn for bioethanol production increased the price



of beef, chicken, pork, eggs, breads, cereals, and milk from 10 % to 30 % in the US. Thus, production of bioethanol from food sources only is not sustainable, but a more sustainable way will be utilizing agricultural waste and fast growing grass.

### 1.1.3 Cellulosic Bioethanol and Pathways of Production

Due to the food – fuel competition, much focus on bioethanol production has recently been on utilizing waste from agriculture (corn stover, rice straw and other crop residues), forest (dead wood, broken wood branches), wood processing industries (sawdust, wood chips), paper industries (waste process liquor, waste papers), and fast growing grass such as switch grass, and water hyacinth. These wastes contain cellulose (a polymer of glucose), hemicelluloses (a polymer mainly of xylose) and lignin (a polymer of phenylpropane) and are termed lignocellulosic biomass. Cellulose and hemicelluloses are carbohydrates which can be hydrolyzed to release fermentable sugars (mainly glucose and xylose) for fermentation with yeast to ethanol. The bioethanol produced from these feedstocks is termed Cellulosic Bioethanol.

However, these carbohydrates form a strong molecular network with lignin and are in a form which makes them difficult to hydrolyze and in most cases pretreatment of the biomass is necessary to obtain high yield of bioethanol. Obtaining high yield of bioethanol also depends on the efficiency of the process used for the production. The flow chart of cellulosic bioethanol production using various processing pathways is shown in Figure 1.1 below.



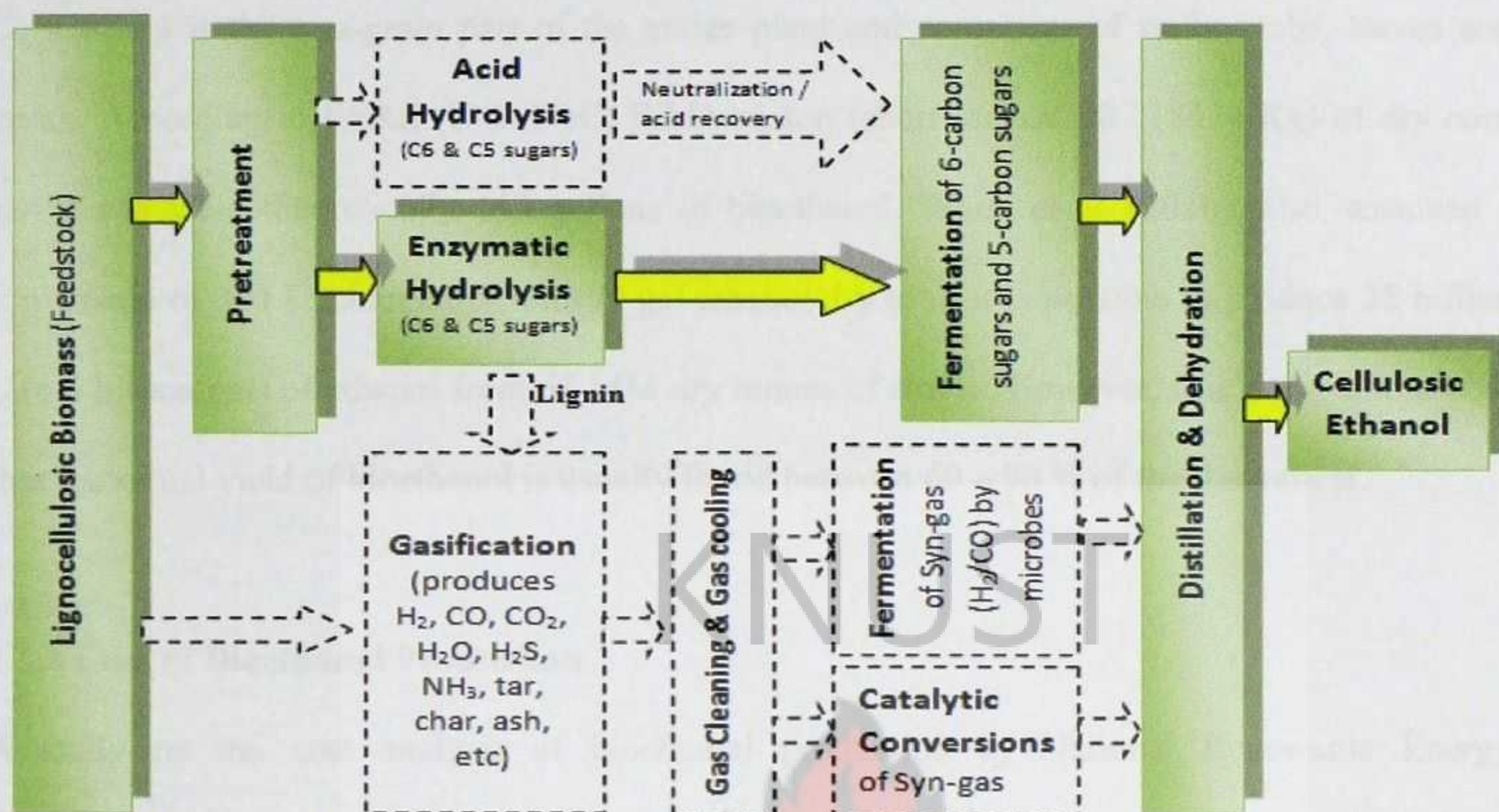


Figure 1.1: Emerging Commercial Cellulosic Ethanol Technologies

(Sources: Biorefinery Workshop, Washington D.C, 2007 and Dwivedi et al, 2009)

#### 1.1.4 Corn Stover as Potential Feedstock

Corn stover is an example of lignocellulosic biomass which has received much attention as bioethanol production feedstock in recent times. This is because corn stover is strongly associated with corn or maize production and that it is substantially available which makes it promising. Kim and Dale, (2004), estimated corn stover to corn (or maize) ratio to be 1.00, i.e. with the quantity of corn produced, the same quantity of corn stover is generated. In Ghana alone, maize production for the past eleven years (2000 – 2010) has been recorded to be in the thousand metric tonnes ( $10^3$  MT) and therefore suggests that corn stover is also available in the same amount (SRID-MoFA, 2011).



Corn stover is the non-grain part of the maize plant and comprises of stalks, cobs, leaves and husks. According to EERE (U.S. DoE) R&Ds, a ton (short ton i.e. 907.18474 Kg) of dry corn stover can yield theoretically 113 gallons of bioethanol. Weiss et al, (2010), also, assumed a conversion of 374 L ethanol/dry ton (99 gal ethanol/dry ton), it is possible to produce 25 billion L (6.6 billion gal) of ethanol from 68 MM dry tonnes of stover. However, it is important to note that the actual yield of bioethanol is usually found between 60 – 90 % of the theoretical.

### 1.1.5 Cost of Bioethanol Production

A study on the cost analysis of bioethanol production by National Renewable Energy Laboratory, U.S. reviewed that producing bioethanol from corn starch cost \$ 0.88 (¢ 1.66, i.e. considering a conversion of GH¢1.89) per liter and producing bioethanol from corn stover cost \$ 1.50 (¢ 2.84) per liter (McAloon et al, 2000). Balat, (2011), also estimated cellulosic bioethanol to cost US\$ 0.80 – 1.10 (GH¢ 1.51 – 2.08) per liter. However, recent advancement in enzyme technology worldwide (e.g. Novozymes) makes cellulosic bioethanol from lignocellulosic biomass more attractive because enzymes able to hydrolyze hexosans (six-carbon sugar polymers) and pentosans (five-carbon sugar polymers) and withstand several processing conditions are being developed to meet the needs of the world's future cellulosic bioethanol industries. Hence, there are considerable efforts to drastically reduce the cost of production of bioethanol from corn stover, making it competitive with producing from corn.



## **1.2 PROBLEM STATEMENT**

### **1.2.1 Ghana Bioenergy Policy**

The Bioenergy Policy for Ghana has been drafted. The national target on biofuels (e.g. bioethanol) as described in the policy is to substitute the national petroleum fuels (e.g. gasoline) consumption with biofuel by 10 % by 2020 and 20 % by 2030 (Energy Commission, 2010). Government policies and programmes to develop renewable energy to contribute to the energy mix in Ghana over the years have concentrated on biogas, solar energy, biodiesel, hydropower and wind. Bioethanol as an alternative to gasoline (petrol), the second most consumed transportation fuel in Ghana is unexploited compared to the other sources of renewable energy. Kemausuor et al, (2011), stated that, there is a growing demand for energy in Ghana but there is less attention given to exploitation of renewable sources of energy.

In bioethanol production, the chemical characteristics (cellulose, hemicelluloses and lignin content) of the feedstocks are necessary in predicting the theoretical ethanol yield expected in the process. Also feedstocks from different places have different physical and chemical characteristics which pose several technological challenges to the process. Setting a national biofuels target requires that, there should be research into the characteristics of some major cellulosic bioethanol feedstocks such as corn stover in order to ascertain how much percentage of the annual national gasoline consumption those feedstocks can replace. However, that information about corn stover and other biomasses are not known.

### **1.2.2 Research and Development (R&D) Gaps**

Duku et al, (2011), identified some research and development (R&D) gaps which are required to be addressed in order to enable Ghanaian scientists and other stakeholders in the biofuel industry



to better understand current and future availability of biomass resources, and also biofuels production and utilization potential in Ghana. The gaps identified are

- i. Biomass feedstock assessment,
- ii. The characterization of potential biomass feedstock for second-generation biofuels production,
- iii. Data collection on agricultural and forestry biomass,
- iv. The development of second-generation biofuels technologies particularly, bio-chemical and thermo-chemical routes, including fast pyrolysis,
- v. Biomass process and kinetic modeling,
- vi. The establishment of pilot and demonstration plants,
- vii. The development of supply chain concepts,
- viii. Analysis of production costs, and
- ix. The use of bio-technology to develop new feedstock varieties for biofuels production.

This project seeks to assess and characterize and provide data on corn stover biomass in Ghana useful for producing cellulosic bioethanol, thereby, creating the platform for closing R&D gaps i, ii, and iii stated above.



1.3 JUSTIFICATION

1.3.1 Ghana’s Dependence on Petroleum

Ghana depends heavily on petroleum for transportation fuels and energy/power generation and, therefore, there is the need to explore renewable sources of fuels such as bioethanol. In 2010, crude oil importation and production (from Jubilee field) accounted for about 90.89 % and 9.11 % respectively. Gasoline accounts for about 29 – 37 % of the total petroleum product consumption (Energy Commission, 2010). Figure 1.2 shows the share of petroleum products consumption in Ghana. The major consumption of gasoline is in the transportation sector. This sector accounts for about 78 % of the consumption of the petroleum products (Duku, et al, 2011). Some percentage of the petroleum products are also used in electricity generation. All the thermal plants in the country use petroleum fuels for power generation.

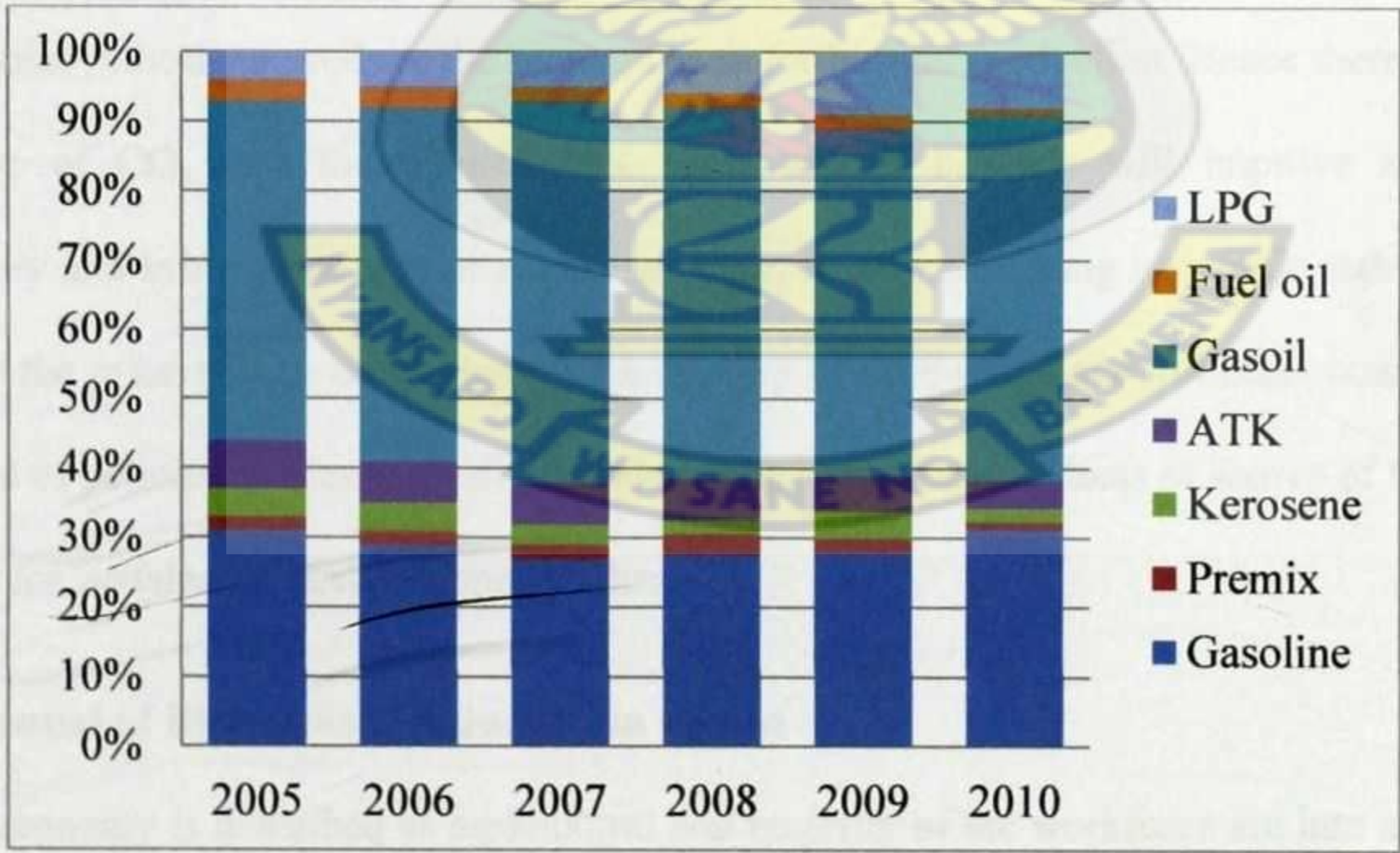


Figure1.2: Petroleum Products Consumption in Ghana  
(Source: Energy Commission, 2010)



Overdependence on petroleum has serious implications on the energy security, socio-economy and environment of the country. There have been fluctuations in crude oil prices worldwide and this destabilizes the economy. Prices of petroleum products in the country have been on the rise rendering high cost of goods and services, high cost of production, high cost of energy generation and high cost of living. Petroleum is non-renewable and its availability has been predicted to be on the decrease worldwide and as a result many countries have biofuels targets. Notwithstanding, petroleum reserves have recently been discovered in Ghana but that should not prevent the country from venturing into exploration of renewable sources of energy (biofuels) since they have several advantages over non-renewable sources of energy.

Use of petroleum products implies high emissions of Green House Gases (GHGs) into the environment and this leads to global warming and climate change. Use of biofuels introduces what is known as carbon neutrality which means the CO<sub>2</sub> emissions released as the fuel is combusted is almost similar to the amount of CO<sub>2</sub> taken from the environment by the plants during photosynthesis to produce the biomass used for biofuel production. Hence there is little or no release of CO<sub>2</sub> into the environment. Also use of biofuels will improve agricultural productivity and bring some sort of energy security. It may also bring economic stability in the sense that the price will be controlled by the country of production and not other countries as is in the case of petroleum. Hence, total dependence on petroleum products as source of fuels is not favorable for sustainable development in Ghana.

### **1.3.2 Potential of Bioethanol Production in Ghana**

Ghana's economy is described as agricultural and majority of the workforce are into agriculture. Therefore, agricultural crop residues are perceived to be substantially available. Due to the problem of food – fuel competition in the arena of using food sources as feedstocks, production



of bioethanol from agricultural crop residues will be beneficial especially to developing countries like Ghana. Most of the population will be encouraged into agriculture and consequently increase agricultural productivity.

## **1.4 OBJECTIVES**

### **1.4.1 Main Objective**

The main objective of this work is to assess the quantities, chemical composition, sodium hydroxide pretreatment and enzymatic hydrolysis of corn stover useful for production of cellulosic bioethanol in Ghana.

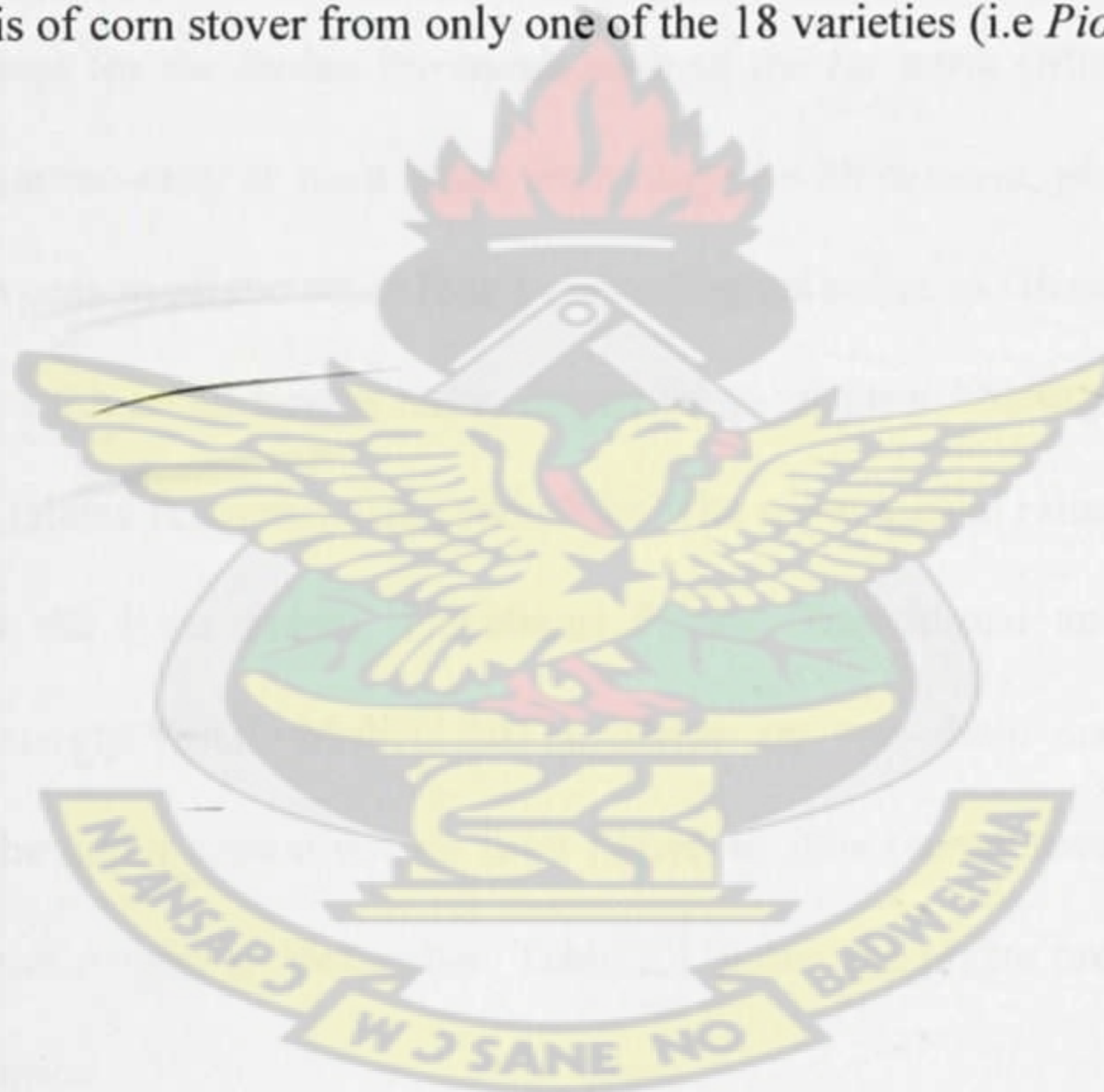
### **1.4.2 Specific Objectives**

1. To estimate the average quantities of corn stover produced per year in Ghana
2. To investigate the cellulose, hemicelluloses, lignin, ash and extractive composition of the corn stover from a local maize variety
3. To determine optimal NaOH pretreatment process for corn stover
4. To compare the FT-IR (Fourier Transform Infrared Spectroscopy) spectra of the raw and pretreated corn stover
5. To determine the effect of enzymatic hydrolysis of the pretreated corn stover by measuring the concentration of the total reducing sugars released into solution
6. To determine some kinetic constants of the enzymatic hydrolysis of the corn stover (maximum reaction velocity,  $V_{\max}$ , and Michaelis-Menten constant,  $K_m$ )
7. To estimate the theoretical ethanol yield based on the characteristics of the corn stover



## 1.5 SCOPE OF PROJECT

Although there are many local maize varieties available in Ghana, this project is limited to (i) assessing the quantities of corn stover using 18 local maize varieties cultivated at CSIR – Crop Research, Maize Department, Fumesua, Kumasi and (ii) assessing the chemical characteristics and enzymatic hydrolysis of corn stover from only one of the 18 varieties (i.e *Pioneer 30W40*).





## 2.0 LITERATURE REVIEW

### 2.1 MAIZE PRODUCTION IN GHANA

Maize is one of the most important cereals in Ghana. It is the major staple food in almost all the ten regions of Ghana. The maize crop is grown by the vast majority of rural households in all parts of the country except for the Sudan Savannah zone of the far north (Morris et al, 1999). Maize is cultivated as a mono-crop or most often intercropped with cassava, plantain, cocoyam, yam, groundnut and cowpeas in all the other four agro-ecological zones in Ghana. Agriculture in Ghana is predominantly on a small holder basis. About 90 % of farm holdings are less than 2 hectares. The climate in Ghana favours maize production. The mean annual rains are 2200, 1500, 1300 and 800 mm for the Rain Forest, Deciduous Forest, Transitional and Coastal agro-ecological zones respectively (SRID-MoFA, 2011). There are two main seasons for maize production in Ghana. The major season occurs from March to June (sometimes ending in July) and the minor occurs from August to November. Table 2.1 shows the maize production (2000 – 2010) and area of cultivation.

Annual mean production of maize in Ghana is about  $1,304 \times 10^3$  MT. The total maize production for the year 2010 was  $1,872 \times 10^3$  MT and it represented about 58.5 % of all the major cereals (rice, sorghum and millet) cultivated. The distribution of maize productions across the country in 2010 were Western 74.2, Central 195.4, Eastern 380.5, Greater Accra 3.6, Volta 93.9, Ashanti 253.4, Brong Ahafo 510.2, Northern 202.3, Upper West 96.0, and Upper East 62.3  $\times 10^3$  MT (SRID – MoFA, 2011). As these data show, maize cultivation in 2010 was more concentrated in Brong Ahafo followed by Eastern and then Ashanti. (Maps showing geographically maize land suitability and maize production in Ghana are provided in Appendix 8). The southern parts of



Ghana are the most suitable lands for maize production and high maize production figures are recorded there.

Table 2.1: Maize: Area Planted and Output (2000 – 2010)

Year	Area Planted (10 <sup>3</sup> ha.)	Production (10 <sup>3</sup> MT)
2000	695	1,013
2001	713	938
2002	940	1,400
2003	792	1,289
2004	733	1,158
2005	740	1,171
2006	793	1,189
2007	790	1,220
2008	846	1,470
2009	954	1,620
2010	992	1,872

(Source: SRID – MoFA, 2011)

Maize production in Ghana is expected to increase with increasing efforts by both local and international determinations to introduce improved technologies to farmers in attempt to increase food productivity. Stakeholders in maize production in Ghana are Ministry of Food and Agriculture (MoFA), Council for Science and Industrial Research – Crop Research Institute (CSIR-CRI), Savannah Agricultural Research Institute (SARI) and international project initiatives such as Ghana – Canadian International Development Agency (CIDA) Grains Development Project. These agencies over the years have contributed immensely to maize production in Ghana. Example of their efforts is the development of local maize varieties such as shown in Table 2.2 below with high productivity and improved seeds.



Table 2.2: Some Maize Varieties Developed in Ghana

Name	Maturity (days of flowering)	Yield (T/ha)
<i>Aburotia</i>	105	4.6
<i>Dobidi</i>	120	5.5
<i>Kawanzie</i>	95	3.6
<i>Golden Crystal</i>	110	4.6
<i>Safita-2</i>	95	3.8
<i>Okomasa</i>	120	5.5
<i>Abeleehi</i>	105	4.6
<i>Dorke SR</i>	95	3.8
<i>Obatanpa</i>	105	4.6
<i>Mamaba</i>	110	6
<i>Dadaba</i>	110	6
<i>Cidaba</i>	110	6

Source: Morris et al, 1999

There are more than about twenty high yielding local maize varieties. Examples (in the local dialect) are *Mamaba*, *Obatampa*, *Etubi*, *Enibi*, *Aburohema* and *Okomasa*. *Okomasa*, is a normal, full-season open pollinated maize variety (OPV) and has its origin from CIMMYT Population 43-SR. It was released in 1988 and has a yield potential of 6.5 T/ha. It has acceptable agronomic characteristics including resistance to streak and lodging, good ear position and good stand ability (Tengan et al, 2011).

## 2.2 AVAILABILITY OF CORN STOVER IN GHANA

### 2.2.1 Ethanol Potential of Corn Stover

Corn stover consists of the stalk, leaves, husks and cobs of the maize plant left on the farms after harvest. Duku et al, (2011), assessed the residue to product ratio of maize crop to be 1.5. This



means that with the  $1,872 \times 10^3$  MT of maize produced (in 2010),  $2,808 \times 10^3$  MT of corn stover (i.e.  $1.5 \times 1,872 \times 10^3$  MT) were made available or produced in the process. These quantities of corn stover can theoretically yield about 350.3 million gallons of ethanol. The total consumption of gasoline in Ghana for 2010 was 737.8 kilotonnes or  $737.8 \times 10^3$  MT (Energy Commission, 2010). The gasoline consumption represents 26.27 % of the  $2,808 \times 10^3$  MT of corn stover. From this analysis, production of ethanol is shown to have the possibility of replacing reasonable percentage of the annual gasoline consumption. Table 2.3 shows the production of corn stover in the ten regions of Ghana and their ethanol production potential.

Table 2.3: Regional Distribution of Corn Stover Production, (2010)

REGION	Maize Production ( $10^3$ MT)	Corn Stover ( $10^3$ MT)	Ethanol Potential (Million gallons)
Western	74.2	111.3	13.9
Central	195.4	293.1	36.5
Eastern	380.5	570.8	71.1
Greater Accra	3.6	5.4	0.7
Volta	93.9	140.8	17.5
Ashanti	253.4	380.1	47.3
Brong Ahafo	510.2	765.3	95.3
Northern	202.3	303.5	37.8
Upper West	96.1	144.1	18.0
Upper East	62.3	93.4	11.6
Total	1871.8	2807.6	349.7

Source: Data generated from production figures provided by SRID – MoFA, 2011, and residue to product ratio 1.5 (Duku et al, 2011)

### 2.2.2 Estimation of Availability of Corn Stover

The estimates based on residue to product ratio of 1.5 for corn stover availability are given in Table 2.4. These estimates assume a whole stover removal i.e. 100 % removal of the maize plant



after harvest of the corn. However, estimates vary widely depending on assumptions about what fraction of agricultural crop residues can be sustainably collected. Some residue must be left in the field or farm to protect the soil against water and wind erosion. The amount of stover that should be left on the ground is dependent on many factors including: tillage practice, topography (especially the slope and extent of sloped land), soil type, and crop rotation (Kadam and McMillan, 2003).

A 3 – year study on the effect of stover removal on three different soils revealed that the magnitude of the stover removal has impacts on both the macro- and micro- scale properties of soil. The impact varied due to site-specific differences in soil (e.g. texture, drainage) and topographic characteristics. Retaining stover on soil surface is essential to maintain the structural and hydrological properties of aggregates, which comprise the elemental building units of the whole soil. Aggregate properties such as stability, strength and subcritical water repellency were impacted with stover removal at rates as low as 25 %, while other aggregate properties such as density and water sorptivity and retention were impacted only when stover was removed completely. Thus, the most detrimental effect on the soil properties was when stover was completely removed regardless of the soil and topographic conditions. The study supports only about 25 % of stover removal (Blanco-Canqui and Lal, 2008).

Several studies on the subject as reported by Kadam and McMillan, (2003), suggest stover removal of 20 – 60 %, while some 76 – 82 %. For commercial balers in the U.S., 70 % is their limit. However, the percentage of stover removal as reported by other studies show that it depends on conditions and practices in a given farm, residue coverage of 20 – 65 % is advised for soil erosion control. US Department of Agriculture guidelines for residue management require at least 30 % residue. Hence, approximately 40 % removal of stover on a sustainable



basis is reasonable. Table 2.4 shows corn stover removal at 40 % from farms and the theoretical ethanol potential.

Table 2.4: Estimation of Corn Stover Based on Percentage Removal from Farms

Maize and Corn Stover Production, (10 <sup>3</sup> MT)					
Year	Maize	Corn Stover (100 % Removal)	Ethanol Potential (Million gallons)	Corn Stover (40 % Removal)	Ethanol Potential (Million gallons)
2000	1,013	1520	189	608	76
2001	938	1407	175	563	70
2002	1,400	2100	262	840	105
2003	1,289	1934	241	773	96
2004	1,158	1737	216	695	87
2005	1,171	1757	219	703	88
2006	1,189	1784	222	713	89
2007	1,220	1830	228	732	91
2008	1,470	2205	275	882	110
2009	1,620	2430	303	972	121
2010	1,872	2808	350	1123	140
Total	14,340	21510	2679	8604	1072

Source: Data generated from production figures provided by SRID – MoFA, 2011, and residue to product ratio 1.5 (Duku et al, 2011)

### 2.2.3 Other Potential Uses of Corn Stover

Corn stover has no significant use in Ghana, apart from its use as fuel to supplement or augment ignition when the main fuel is forest wood or charcoal. The usual practice of getting rid of corn stover after massive production is to gather them and burn on the farms. Much value can be added to corn stover. The following are some other potential uses of corn stover apart from its use as bioethanol feedstock.



**Compost:** Corn stover can be made into compost as a source of manure for improving the physical and chemical composition of soil.

**Erosion prevention:** Sometimes corn stover is kept on farms especially farm lands that are sloppy as a source of soil erosion prevention.

**Animal feed:** Corn stover is a potential feed for dairy cattle. However, it is not a high quality feed; its biggest drawback is its physical character. Ensiling stover while it is still green or mixing dry material with higher moisture hay-crop forage may make it more acceptable to cattle. Stover may provide 20 – 30 % of the forage dry matter for dairy cattle. Use as bedding for farm animals is another low-value application (Kadam and McMillan, 2003).

**Composite products:** Particleboard, which is used mainly as a furniture core stock, is produced commercially from bagasse and wheat straw as well as other types of fiber. Building panels have been made from several crop residues including wheat straw, rice straw, flax shives and bagasse, and can also be made from corn stover. Particleboard from corn stover can be applied in non-structural applications like furniture, cabinets, store displays, door panels, moldings, countertops, and case good items. A drawback to manufacturing particleboard using agricultural fiber like corn stover is the far expensive resin binders, particularly methyl diphenyl isocyanate; however, soybean-based adhesives may be used (Kadam and McMillan, 2003).

**Pulp and paper:** Drawing an analogy to other agricultural residues, corn stover-based pulp and paper production is a viable option. Because agricultural residues-based pulp (agric-pulp) can be bleached without using chlorine, dioxins are not produced, a major environmental benefit. Also, the lower lignin content requires less bleach than that needed for wood pulp.



**Chemicals:** Corn cobs are currently used as a feedstock for producing furfural. Another possibility is to make dissolving pulp (essentially pure cellulose pulp containing very little lignin or hemicelluloses) from corn stover. Dissolving pulp is used to produce high-value cellulose derivatives, such as rayon, cellulose acetates and cellulose nitrates (Kadam and McMillan, 2003).

**Fuels:** Corn stover can be used as a fuel after milling directly in a boiler furnace.

## 2.3 CHEMICAL COMPOSITION OF CORN STOVER

Corn stover consists of cobs, stalks, leaves and husks. The cobs contain 31.7 % cellulose, 34.7 % hemicelluloses, 20.3 % lignin and 3.4 % acetyl groups (Torre et al, 2008). Chen et al (2010) also reported corn cob composition as 40.67 % cellulose, 31.10 % hemicelluloses and 11.70 % lignin. Chen et al (2009) reported composition of corn stover as 19.3 % lignin, 21.7 % hemicelluloses and 38.7 % cellulose. Buranov and Mazza (2008) also reported composition of corn stover as 17 % lignin, 21.4 % hemicelluloses, 36.1 % cellulose, 7.1 % ash and 3.2 % acetyl. Dongen et al, (2011), reported corn stover characteristics as 57 % total sugar and 30.9 % lignin. Li et al, (2012), reported corn stover characteristics as 39.4 % cellulose (corn stalk rind), and 20.1 % lignin. Depending on the origin, maturity and variety, the corn stover characteristics differ from one location to another.

### 2.3.1 Cellulose

Cellulose is the most abundant biopolymer in nature followed by hemicelluloses and lignin. It is considered as the major source of feedstock for cellulosic bioethanol production. Cellulose forms the main part of plant cell walls. Together with hemicelluloses, they act as supporting and



scaffold substances. Cellulose is a polydisperse linear homopolymer consisting of regio- and enatio-selective  $\beta$ -1,4-glycosidic linked D-glucose units (anhydroglucose units). It contains three reactive hydroxyl groups at the C-2, C-3, and C-6 atoms, which are, in general, accessible to the typical conversions of primary and secondary alcoholic OH groups. Based on its molecular structure, i.e. the 'tacticity' and the uniform distribution of the hydroxyl groups, ordered hydrogen bond systems form various types of supra-molecular semicrystalline structures. In addition to the crystallinity, the hydrogen bonding patterns have a strong influence on the whole chemical behavior of cellulose. A further consequence of the supra-molecular structure is that it is insoluble in water and common organic liquids (Heinze and Liebert, 2001). The hydrogen bonding in cellulose makes it recalcitrant to *rapid* hydrolysis compared to starch or sugar materials and this poses technical problems such as pretreatment of the material before hydrolysis making the process expensive (Alvira et al, 2010). Figure 2.1 shows a section of the chemical structure of cellulose.

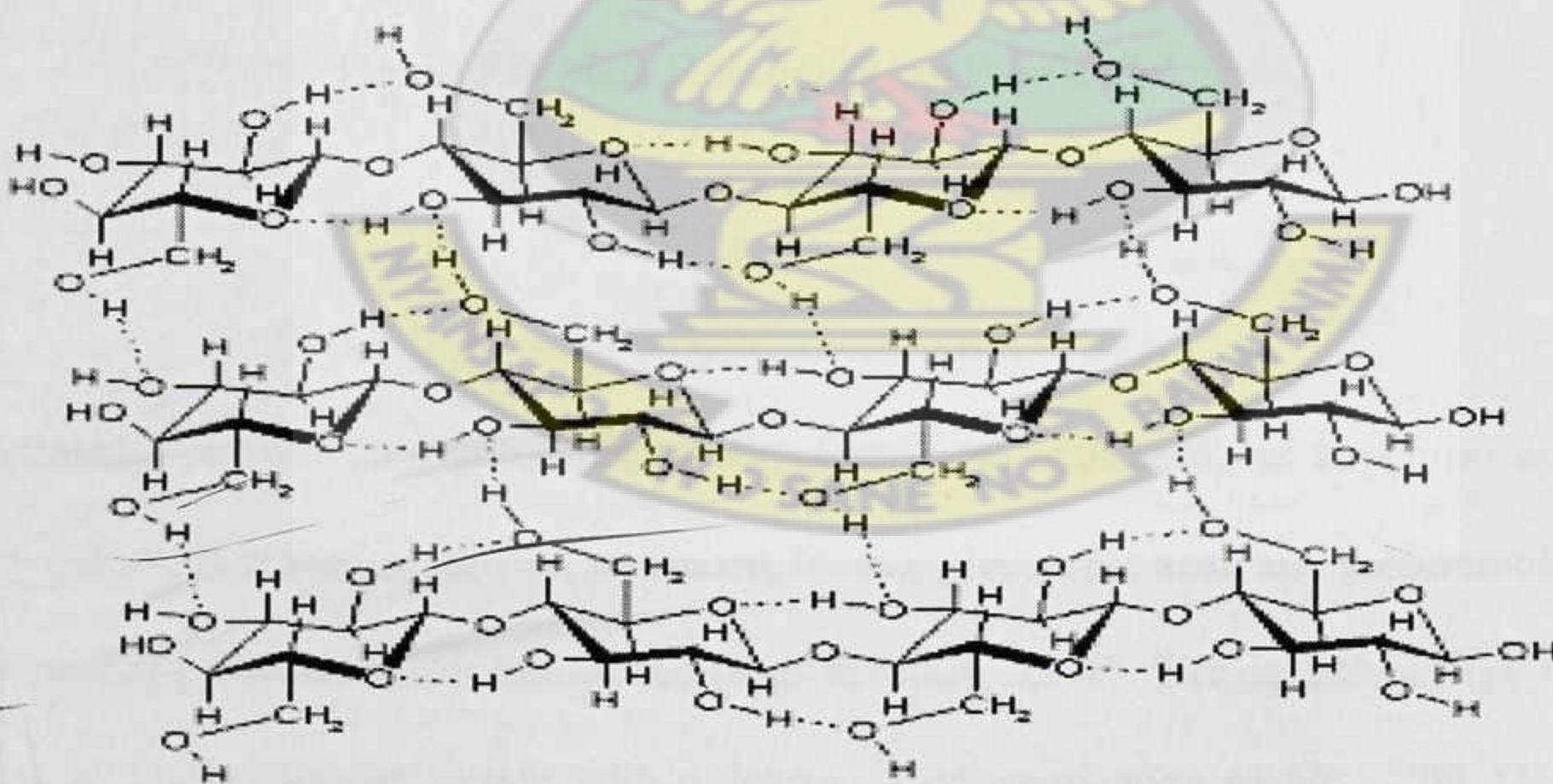


Figure 2.1: Section of Chemical Structure of Cellulose

(source: [www.doitpoms.ac.uk/tlplib/wood/structure\\_wood\\_ptl.php](http://www.doitpoms.ac.uk/tlplib/wood/structure_wood_ptl.php))



### 2.3.2 Hemicellulose

Hemicellulose is one of the most abundant natural polysaccharides and comprise roughly one-fourth to one-third of most plant materials. Together with cellulose and pectic polysaccharides, hemicelluloses belong to the building components of the cell walls of higher plants, where they are associated with varying levels of proteins and phenolics. They differ from cellulose in the main cell wall constituent, which is a highly uniform 1-4- $\beta$ -linked polyglucan. Figure 2.2 shows the main constituents of hemicelluloses.

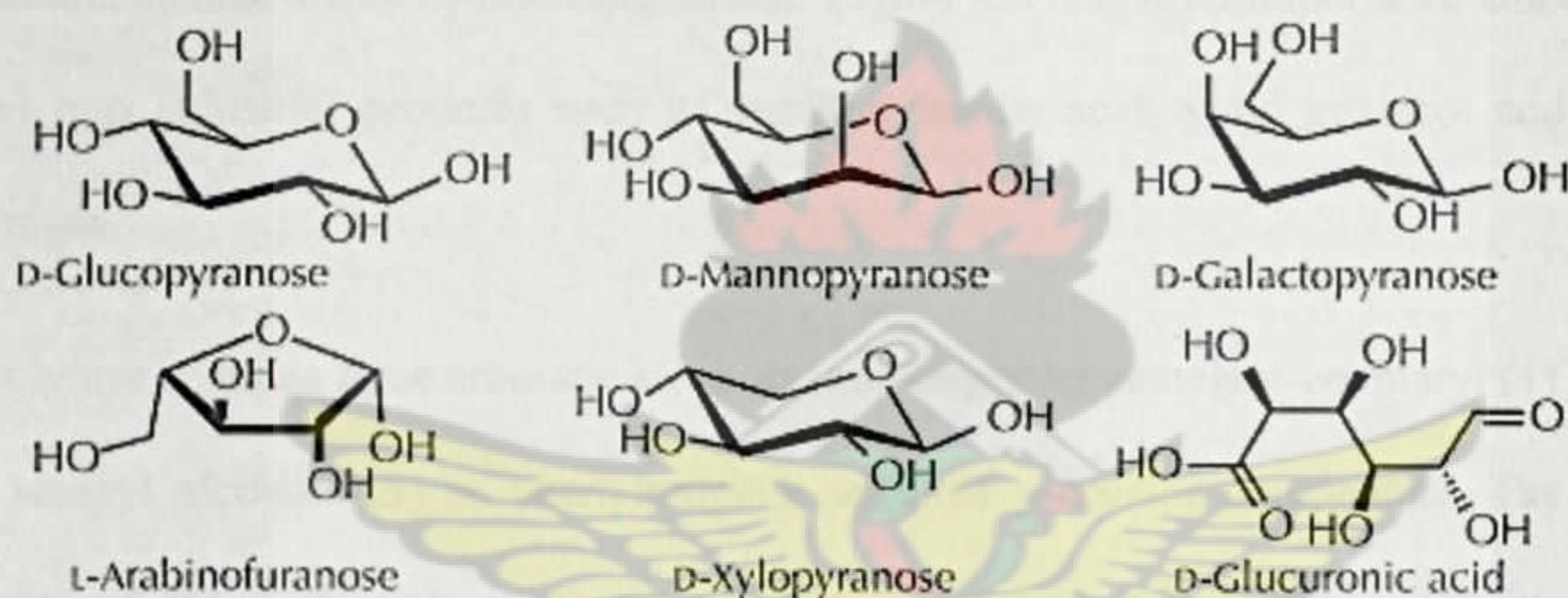


Figure 2.2: Main Constituents of Hemicelluloses

(Source: Ren and Sun, 2010)

Hemicelluloses represent a type of hetero-polysaccharides with complex structures containing glucose, xylose, mannose, galactose, arabinose, fucose, glucuronic acid, and galacturonic acid in various amounts depending on the source (Ren and Sun, 2010). Hemicelluloses are branched polymers of low molecular weight with a degree of polymerization of 80 – 200. Girio et al, (2010), however, reported degree of polymerization of 40 – 600. Their general molecular formulas are  $(C_5H_8O_4)_n$  and  $(C_6H_{10}O_5)_n$ , which are called pentosans and hexosans, respectively.

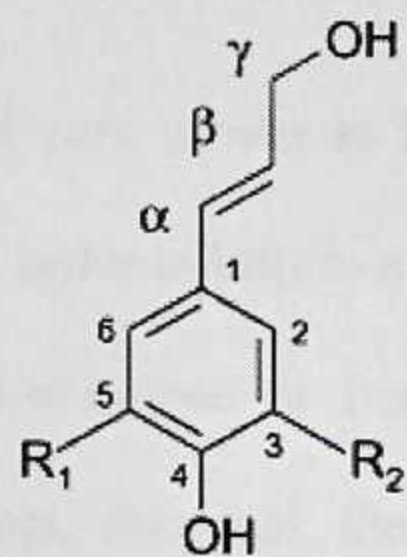


### 2.3.3 Lignin

Lignin is the third most abundant natural polymer present in nature after cellulose and hemicelluloses. Lignin is not a carbohydrate and cannot be hydrolyzed and fermented to ethanol compared to cellulose and hemicelluloses. Lignin is an amorphous, thermoplastic, three-dimensional polymer network based on phenylpropane subunits (aromatic monomers), which is incorporated in the plant cell wall causing so-called lignification of the cells (Kamm et al, 2006). As a major cell wall component, lignin provides rigidity, internal transport of water and nutrients and protection against attack by microorganisms. Lignin has industrial importance since it can be converted into industrial products such as vanillin, ferulic acid, vinyl guaiacol and optically active lignans.

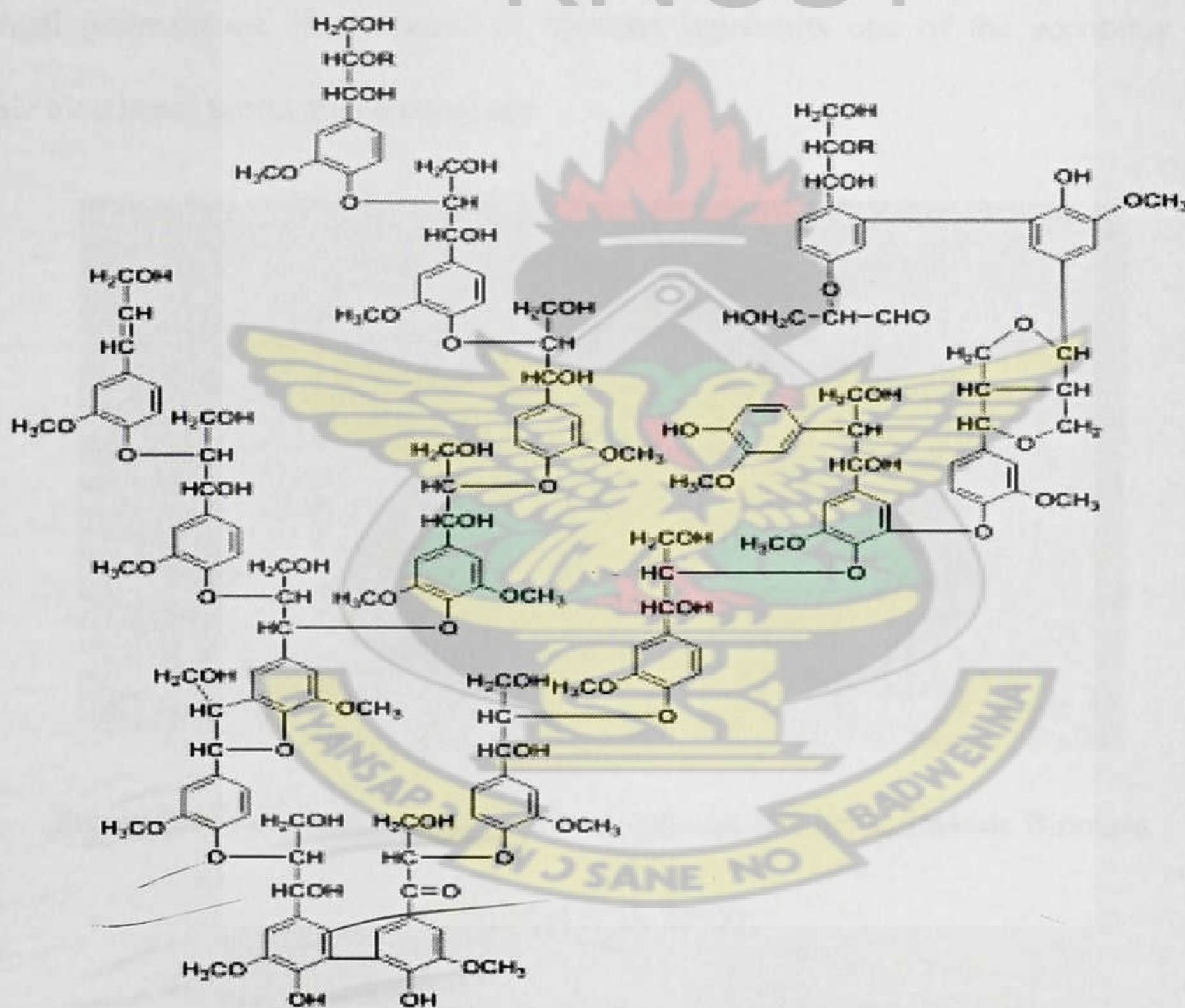
Lignin polymer contains three aromatic alcohols (monolignols) namely p-coumaryl (1), coniferyl (2) and sinapyl alcohols (3) as phenylpropane subunits precursor compounds. The aromatic constituents of these alcohols in lignin polymer are called p-hydroxyphenyl, guaiacyl and syringyl moieties respectively. During the lignification process, these monolignols produce a complex three-dimensional amorphous lignin polymer via  $\beta$ -O-4,  $\alpha$ -O-4,  $\beta$ -1, 5-5, 4-O-5 and  $\beta$ - $\beta$  linkages which lack the regular and ordered repeating units found in other polymers such as cellulose. This biosynthesis process consists of mainly radical coupling, and creates a unique lignin polymer in each plant species. Corn stover lignin contains all the three units in significant amounts. Figure 2.3 shows the chemical structure of lignin.





1.  $R_1=R_2=H$
2.  $R_1=OCH_3$ ;  $R_2=H$
3.  $R_1=R_2=OCH_3$

a. **Lignin monolignols** (p-coumaryl (1), coniferyl (2), and sinapyl alcohols (3)),  
(Source: Buranov and Mazza, 2008)



b. **Section of Chemical Structure of Lignin**  
(Source: Arumugam et al, 2008)

Figure 2.3: a and b: Structure of Lignin



## 2.4 PRETREATMENT OF CORN STOVER

The goal of pretreatment of corn stover is to alter or remove structural and compositional impediments to hydrolysis in order to improve the rate of enzyme hydrolysis and increase yields of fermentable sugars from cellulose or hemicelluloses (Figure 2.4) (Mosier et al, 2005). Pretreatment can be a physical, chemical, thermochemical or biological process. Examples of pretreatment processes are alkali, acid, organosolv, ionic liquids, steam explosion, liquid hot water, ammonia fiber explosion (AFEX), wet oxidation, microwave, ultrasound, CO<sub>2</sub> explosion and fungal pretreatment. Pretreatment of biomass represents one of the economic costs in cellulosic bioethanol production technology.

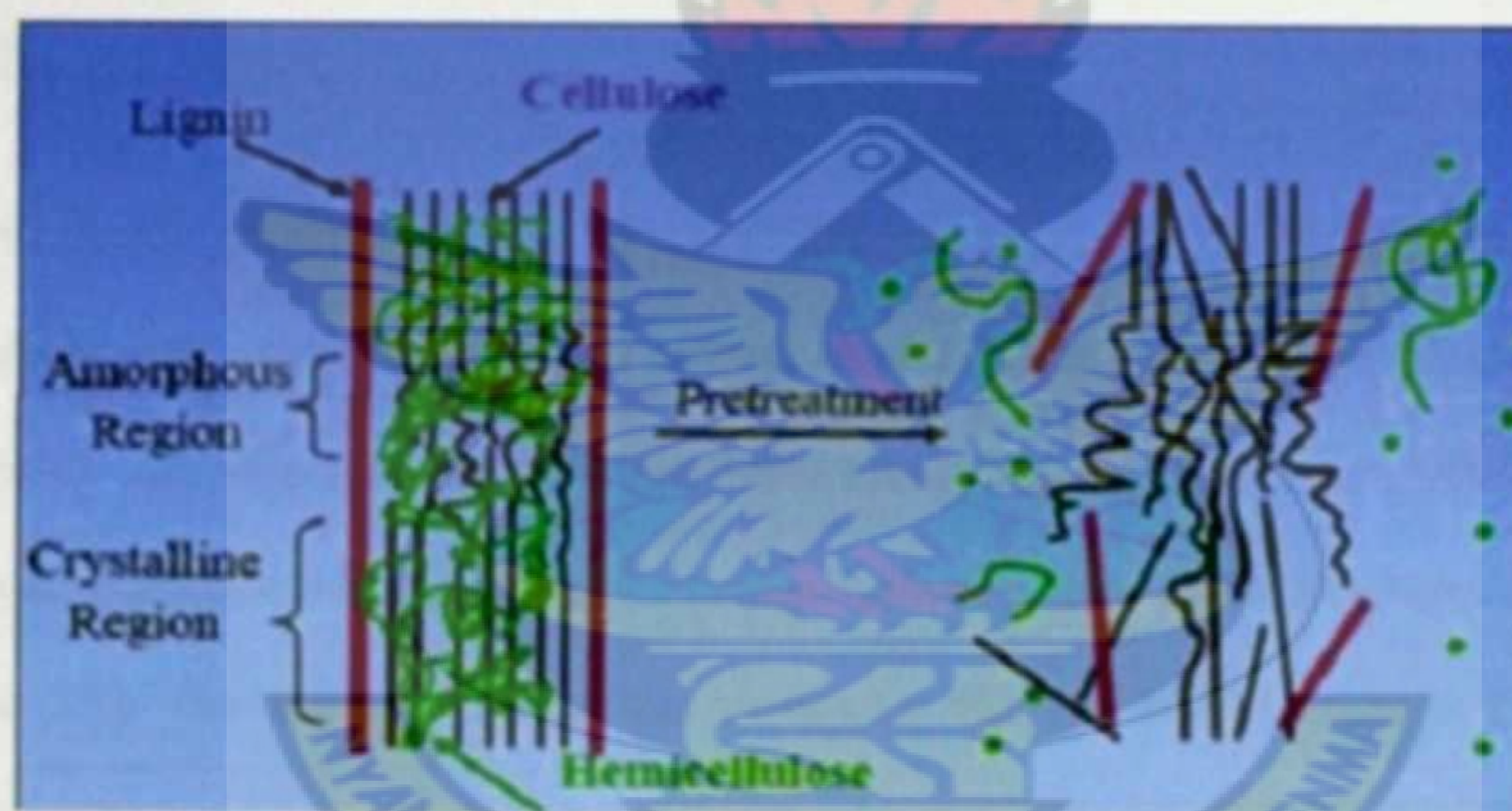


Figure 2.4: Schematic Diagram of Pretreatment of Lignocellulosic Biomass

(Mosier et al, 2005)

An effective pretreatment process has the potential of high carbohydrate (cellulose and hemicellulose) content of the pretreated sample that leads to improving the efficiency of the enzymatic hydrolysis process, thereby, lowering of the overall cost of the technology. Unless a



very large excess of enzyme is used, the enzymatic digestibility of the cellulose in an untreated biomass is low (< 20 %) because of its structural characteristics, whereas the yield of pretreated biomass often exceeds 90 % of theoretical (Mosier et al, 2005, Sanchez and Cardona, 2008).

#### 2.4.1 Sodium Hydroxide Pretreatment of Corn Stover

Sodium hydroxide pretreatment causes swelling, increasing the internal surface of cellulose and decreasing the degree of polymerization and crystallinity, and this provokes lignin structure disruption (Alvira et al, 2010). Sodium hydroxide pretreatment is found to be effective on agricultural residues such as corn stover.

Corn stover according to Buranov and Mazza (2008) contains more of alkali-labile bonds in its lignin – carbohydrate complexes and less amount of acid-labile bonds. NaOH cleaves the alkali labile bonds i.e. ester linkages that links the hemicellulose to the lignin and in the process the lignin content of the biomass is reduced drastically, however, some amount of hemicelluloses are lost (Figure 2.5). The disadvantage of this process is that the NaOH leads to formation of irrecoverable salts.

Dilute acid and sodium hydroxide pretreatment of corn stover were compared by Zhao and Xia, (2010). The main composition of the raw corn stover was found to contain cellulose 38.7 %, hemicelluloses 21.7 %, lignin 19.3 % and others 20.3 %. For the acid pretreatment, the corn stover was pretreated at 108 °C with 1.5 % sulphuric acid for 6 hours and solid loading of 1:10 (w/v). For sodium hydroxide pretreatment, the corn stover was pretreated at 80 °C with 2 % NaOH for 75 mins with and solid loading of 1:8 (w/v).



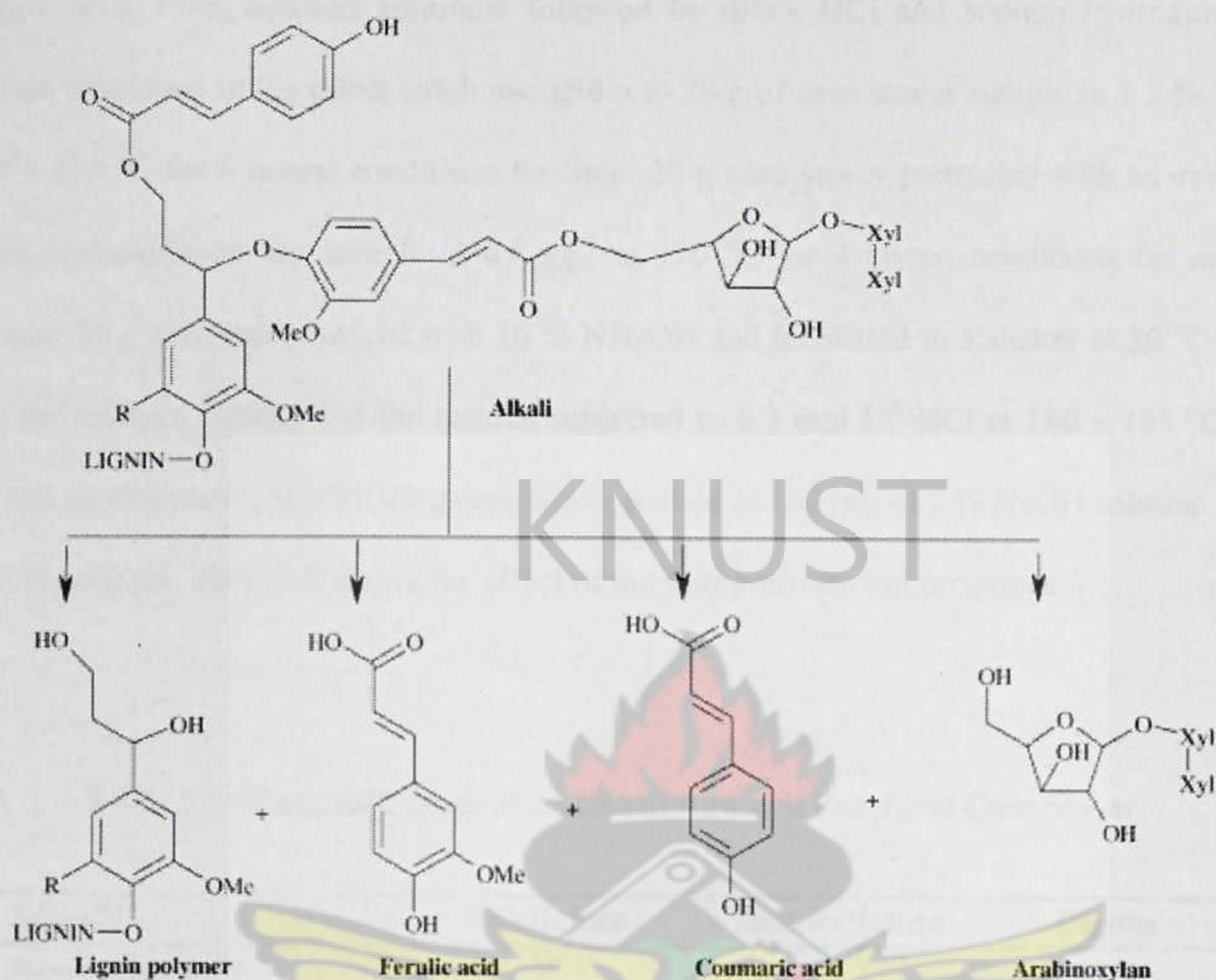


Figure 2.5: Lignin-Carbohydrate Complex from Corn Cell Walls and its Cleavage with Alkali (Buranov and Mazza, 2008)

The composition of the acid-pretreated corn stover residue was found to be cellulose 62.4 %, hemicelluloses 12 %, lignin 24.9 % and others 0.7 %. The composition of the alkaline – pretreated corn stover residue was found to be cellulose 64.1 %, hemicelluloses 24.6 %, lignin 8.6 % and others 2.7 %. The recovery for cellulose and hemicelluloses was 92.3 % and 32.2 % for acid pretreatment, 97.2 % and 66.5 % for alkaline pretreatment respectively.

Chen et al (2009), made a comparison of four different chemical pretreatments of corn stover for enhancing enzymatic digestibility. The four pretreatment processes compared were dilute



sulphuric acid, lime, aqueous ammonia followed by dilute HCl and sodium hydroxide. The condition employed in the dilute sulphuric acid was 20 g of corn stover sample in 1.5 % H<sub>2</sub>SO<sub>4</sub> at 106 – 108 °C for 6 hours; conditions for lime: 20 g corn stover pretreated with an excess of calcium hydroxide on dry sample at 0.4 gg<sup>-1</sup> at 120 °C for 4 hours; conditions for aqueous ammonia: 20 g corn stover mixed with 10 % NH<sub>4</sub>OH and incubated in a shaker at 26 °C for 24 hours, the mixture filtered and the residue subjected to 0.3 mol L<sup>-1</sup> HCl at 100 – 108 °C for 1 hour; and conditions for NaOH: 20 g corn stover soaked in 160 mL of 2 % NaOH solution at 120 °C for 30 minutes. Table 2.5 shows the effect of the four pretreatment processes.

Table 2.5: Chemical Composition (%) of Raw and Pretreated Corn Stover

Sample	Cellulose	Hemicellulose	Lignin
Raw corn stover	38.7	21.7	19.3
Dilute H <sub>2</sub> SO <sub>4</sub> –pretreated	61.9	8.5	28.4
Lime-pretreated	53.3	27.3	18.6
NH <sub>3</sub> /HCl-pretreated	65.7	12.9	21.0
NaOH-pretreated	64.1	24.6	8.6

(Source: Chen et al, 2009)

According to Table 2.5, the dilute H<sub>2</sub>SO<sub>4</sub> pretreatment was able to remove most of the hemicelluloses whereas the alkaline pretreatment retained most of the hemicelluloses but removed most of the lignin. Comparing all the four pretreatment processes according to Chen et al (2009), NaOH pretreatment was the most effective pretreatment process for pretreating corn stover because it removed most of the lignin and leaved most of the carbohydrate intact.



Corn stover was pretreated using different NaOH loadings (1%, 2.5%, 5.0% and 7.5 % w/w) and the lignin degradation during the pretreatment was found to increase from 9.10 % to 46.2 % when the NaOH concentration increased from 1.0 % to 7.5 % (Zhu et al, 2010).

#### 2.4.2 FT-IR Analysis of Pretreated Corn Stover

Fourier Transform Infrared (FT-IR) Spectroscopy is frequently used for the investigation of the chemical changes in pretreated corn stover. The main constituents in corn stover are cellulose, hemicellulose and lignin. The characteristic or prominent peaks for pure cellulose, hemicellulose and lignin were shown by Adapa et al, (2009), in attempt to design a methodology of FT-IR for quantitative determination of biomass components. The cellulose spectrum they found had three distinct peaks at wavenumbers of 1634, 1427 and 899  $\text{cm}^{-1}$ ; hemicellulose (xylan) spectrum showed four prominent peaks at wavenumbers of 1646, 1563, 1044 and 899  $\text{cm}^{-1}$ ; and lignin spectrum showed characteristic peaks at wavenumber of 1697, 1603, 1514, and 837 $\text{cm}^{-1}$ .

Cellulose, hemicellulose and lignin components of biomass are most likely made up of alkene, esters, aromatics, ketone and alcohol, with different oxygen-containing functional groups, e.g., OH (3600 – 3000  $\text{cm}^{-1}$ ), C=O (1510 – 1560  $\text{cm}^{-1}$ ), C-O-C (1232  $\text{cm}^{-1}$ ) and C-O-(H) (~1050  $\text{cm}^{-1}$ ) (Table 2.6). However, they showed different IR structures according to Yang et al, (2007). The highest IR absorbance of OH and C-O was found with cellulose while hemicellulose contained higher C=O compounds. Comparatively, lignin was found to show a big difference in the finger print region (1830 – 730  $\text{cm}^{-1}$ ) IR spectra. Yang et al, (2007), also found a group of complex IR absorbance of lignin, indicating that lignin might be rich in -O-CH<sub>3</sub>, C-O-C stretching and C=C



stretching (aromatic ring) containing compounds. Figure 2.6 shows the IR spectra for pure cellulose, hemicellulose, and lignin determined by Yang et al, (2007).

Table 2.6: The Main Functional Groups of the Three Main Components of Biomass

Wave number (cm <sup>-1</sup> ) <sup>a</sup>	Functional groups	Compounds
3600–3000 (s)	OH stretching	Acid, methanol
2860–2970 (m)	C–H <sub>n</sub> stretching	Alkyl, aliphatic,
1700–1730 (m),	C=O stretching	aromatic
1510–1560 (m)		Ketone and carbonyl
1632 (m)	C=C	Benzene stretching ring
1613 (w), 1450 (w)	C=C stretching	Aromatic skeletal mode
1470–1430 (s)	O–CH <sub>3</sub>	Methoxyl–O–CH <sub>3</sub>
1440–1400 (s)	OH bending	Acid
1402 (m)	CH bending	
1232 (s)	C–O–C stretching	Aryl-alkyl ether linkage
1215 (s)	C–O stretching	Phenol
1170 (s), 1082 (s)	C–O–C stretching vibration	Pyranose ring skeletal
1108 (m)	OH association	C–OH
1060 (w)	C–O stretching and C–O deformation	C–OH (ethanol)
700–900 (m)	C–H	Aromatic hydrogen
700–400 (w)	C–C stretching	

(Source: Yang et al, 2007)

Kim et al, (2003), also determined the FT-IR spectra for untreated corn stover and ammonia recycle percolation pretreated corn stover. They observed the lignin characteristic peaks at 1218, 1268 (C–O of guaiacyl ring), 1315 (C–O of syringyl ring), and 1502 – 1600 cm<sup>-1</sup> (aromatic skeletal vibration). Kim et al, (2003), found the band intensities at all lignin peaks (1502 – 1600 cm<sup>-1</sup>) of untreated corn stover sample to be higher than those which are Ammonia Recycled



Percolation pretreated. Sun et al, (2011), also determined FT-IR spectra of corn stover, and found bands at 1630, 1140 and 835  $\text{cm}^{-1}$  peaks which according to them were characteristic of grass lignin. Thus, it is expected that the FT-IR spectra of a pretreated corn stover should have a major reduction in the intensities of peaks at 1630 – 835 nm.

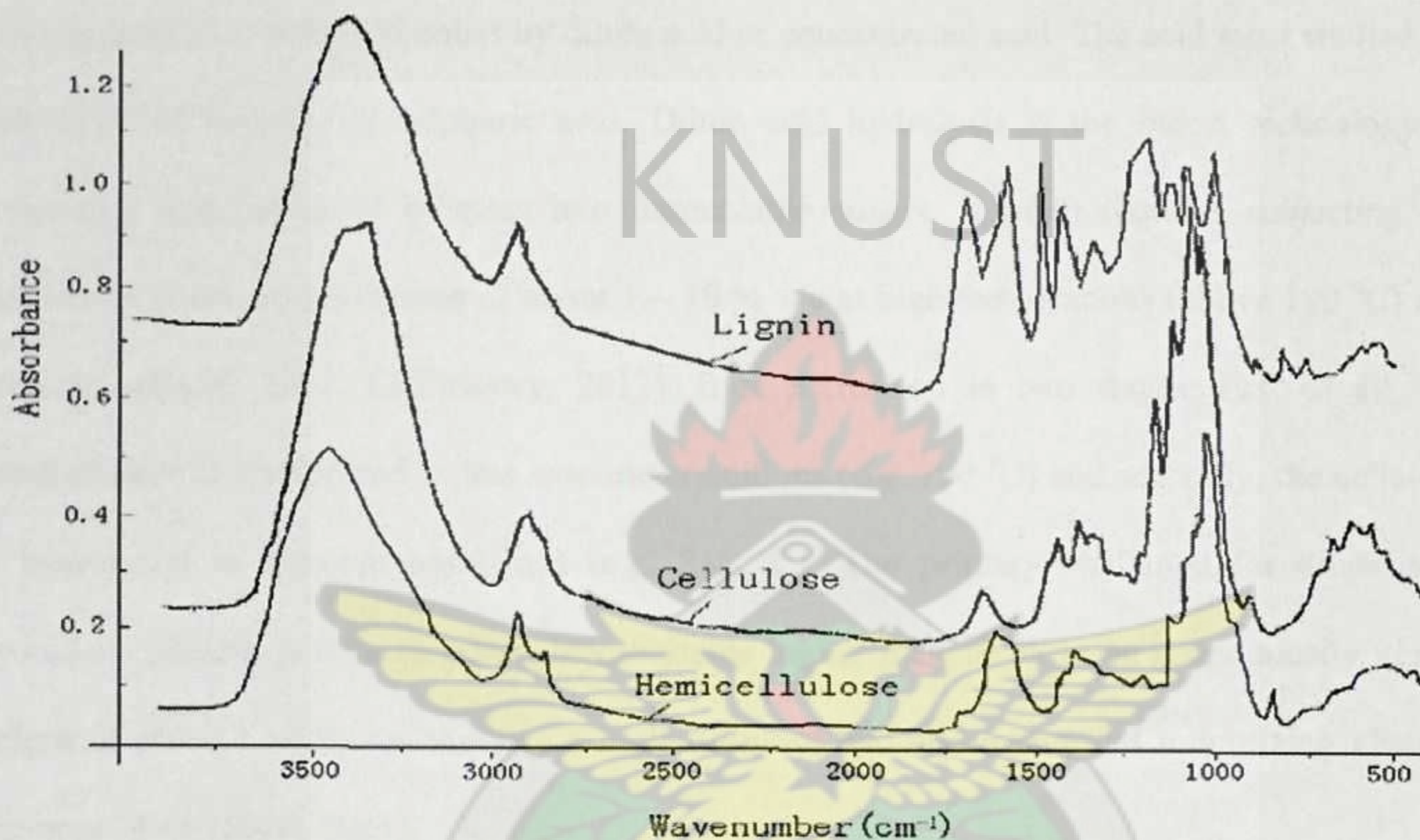


Figure 2.6: FT-IR Spectra for Pure Cellulose, Hemicellulose and Lignin  
(Yang et al, 2007)

## 2.5 ENZYMATIC HYDROLYSIS OF CORN STOVER

### 2.5.1 Hydrolysis

The next step after the pretreatment process is the hydrolysis of the cellulose and hemicelluloses content of the lignocellulosic biomass into fermentable sugars. Cellulose is hydrolyzed into



glucose whilst hemicelluloses are hydrolyzed into xylose, mannose, galactose and glucose (depending on the nature of the hemicelluloses in the biomass). This is achieved either by an acid or an enzyme.

### 2.5.2 Acid Hydrolysis

Acid hydrolysis is achieved either by dilute acid or concentrated acid. The acid most studied for hydrolysis of biomass is sulphuric acid. Dilute acid hydrolysis is the oldest technology of converting lignocellulosic biomass into fermentable sugars. It often requires subjecting the biomass in dilute acid solutions of about 1 – 10 % v/v at high temperatures (above 180 °C) and pressures (Balat, 2011, El-Zawawy, 2011). It is performed in two stages; first of all, the hemicellulose is hydrolyzed at less extreme conditions (e.g. 190 °C) and secondly, the cellulose is hydrolyzed at extreme conditions (e.g. 215 °C). The primary challenge for dilute acid hydrolysis process is how to raise glucose yields higher than 70 % in an economically viable industrial process while maintaining a high cellulose hydrolysis rate and minimizing glucose decomposition (Balat, 2011).

Comparatively, concentrated acid hydrolysis employs concentrated acids (about 70 – 90 % acid) to hydrolyze the biomass into fermentable sugars. The concentrated acid disrupts the hydrogen bonding between cellulose chains, converting it to a completely amorphous state. At this decrystallized state of cellulose it is easily converted to fermentable sugars (Binod et al, 2011). This process is usually done at low temperatures (~30 °C) and pressures. It gives sugar yields of nearly about 100 % with little sugar degradation. However, environment and corrosion problems and high cost of acid consumption and recovery present major barriers to its economic success (Balat, 2011).



### 2.5.3 Enzymatic Hydrolysis

The challenges met in acid hydrolysis are somewhat overcome when enzymes favouring 100 % selectivity of conversion of cellulose and hemicellulose to monomeric sugars are used. Enzymatic hydrolysis are performed at mild conditions; temperatures (30 – 50 °C), and pH (4.8) with conversions of about 75 – 85 %. It is very slow (days) compared to acid hydrolysis (minutes). However, it has low utility cost and it is environmentally friendly. Considering the economic and commercial viability of converting corn stover (as well as lignocellulosic biomass materials) into fermentable sugars, enzymatic hydrolysis is promising and is currently operated in many demonstration commercial scale plants (e.g. Iogen, Ottawa, Canada) (Solomon et al, 2007). Enzymatic hydrolysis of 2 % NaOH pretreated corn straw (at 80 °C, 1 hour) by cellulase of *Trichoderma reesei* ZU-02 and cellobiase of *Aspergillus niger* ZU-07 resulted yields of sugar of 86 g/L (glucose 56.7 g/L, xylose 23.6 g/L and arabinose 5.7 g/L) (Sarkar et al, 2012). Also enzymatic hydrolysis of a lactic acid pretreated corn stover using *Cellubrix* (Novozymes) yielded glucose of 29.6 g per 100 g of dry corn stover representing 80 % of the theoretical (Xu et al, 2009).

### 2.5.4 Mechanism of Enzymatic Hydrolysis

#### *Cellulase hydrolysis*

Cellulose is enzymatically hydrolyzed into glucose by the synergistic action of three different cellulases known as endoglucanases (EGs), exoglucanases (EC) or cellobiohydrolases (CBHs) and  $\beta$ -glucosidases (BG) or  $\beta$ -glucoside glucohydrolases. Endoglucanases hydrolyze internal  $\beta$ -1, 4-glucosidic linkages randomly in the cellulose chain away from the chain ends. Exoglucanases or cellobiohydrolases cleaves of cellobiose units from cellulose chain ends (Cellobiohydrolase I



(Cel7A) acts from the reducing ends and Cellobiohydrolase II (Cel6A) acts from the non-reducing ends of the cellulose chains).  $\beta$ -glucosidases hydrolyze the cellobiose to glucose and also cleave off glucose units from cello-oligosaccharides (Binod et al, 2011; Bansal et al, 2009).

Figure 2.7 below shows the mechanism of cellulases action on cellulose substrate.

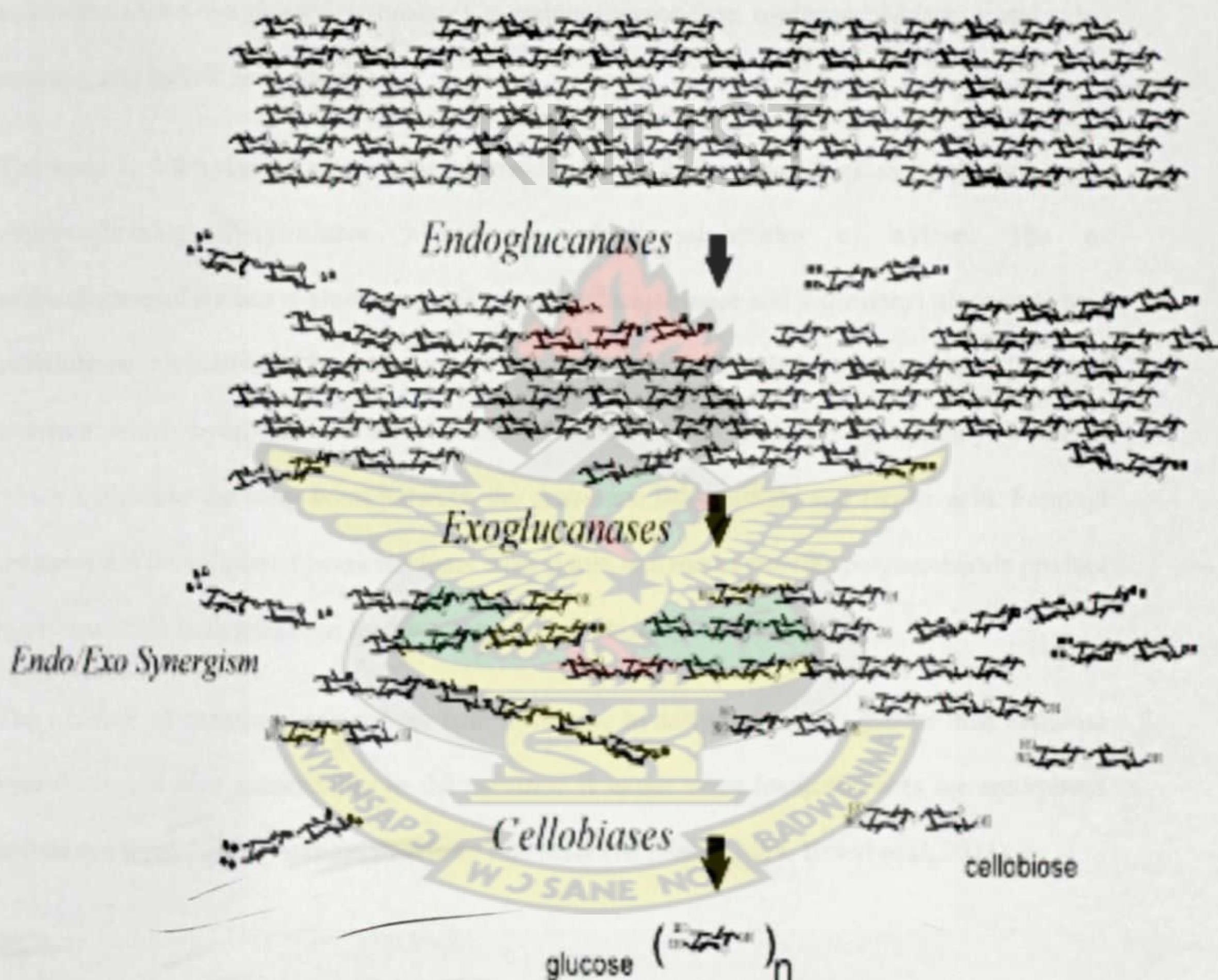


Figure 2.7: Synergism among Cellulases during Hydrolysis of Cellulosic Materials

(Spencer and Ragout de Spencer, 2004)



### ***Hemicellulase hydrolysis***

Hemicelluloses are enzymatically hydrolyzed into xyloses, glucose, galactose (depending on the nature of the hemicellulose in the biomass) by hemicellulase. Hemicellulase is a multi-enzyme system, including endo-1,4- $\beta$ -xylanase (1,4- $\beta$ -d-xylan xylanohydrolases), exoxylanase,  $\beta$ -xylosidase (1,4- $\beta$ -d-xylan xylohydrolase),  $\alpha$ -arabinofuranosidase,  $\alpha$ -glucuronisidase, acetyl xylan esterase, and ferulic acid esterase.

The endo-1, 4- $\beta$ -xylanase cleaves the glycosidic bonds in the xylan backbone releasing xylo-oligosaccharides.  $\beta$ -xylosidase hydrolyzes xylooligosaccharides to xylose. The  $\alpha$ -arabinofuranosidase and  $\alpha$ -glucuronisidase remove the arabinose and 4-O-methyl glucuronic acid substituents, respectively, from the xylan backbone. Hemicellulolytic esterases include acetyl esterases which hydrolyze the acetyl substitutions on xylose moieties, and feruloyl esterases which hydrolyze the ester bond between the arabinose substitutions and ferulic acid. Feruloyl esterases aid the release of hemicelluloses from lignin and render the free polysaccharide product more amenable to degradation by the other hemicellulases (Binod et al, 2011).

The number of enzymes required for hemicelluloses hydrolysis is much greater than cellulose hydrolysis and also accessibility to the substrate is easier since hemicelluloses are amorphous and do not form tight crystalline structures like cellulose (Balat, 2011, Binod et al, 2011).

### **2.5.5 Kinetics of Enzyme Hydrolysis of Biomass**

The rate of cellulose hydrolysis is observed to increase significantly at initial conversions and reduces drastically at high conversions. This has been attributed to the increase of the



crystallinity of the cellulose during conversions. Attempts to model this observation have been complicated since several factors come into play and this is as a result of the heterogeneous nature of the reaction. The cellulases action on the cellulose is categorized into primary hydrolysis and secondary hydrolysis. The primary hydrolysis involves endoglucanases and exoglucanases and occurs on the surface of solid substrate releasing soluble sugars with a degree of polymerization up to 6 into the liquid phase. This depolymerization step is the rate-limiting step for the whole cellulose hydrolysis process. The secondary hydrolysis occurs in the liquid phase involving primarily the hydrolysis of cellobiose to glucose by  $\beta$ -glucosidases (Bansal et al, 2009).

Classical homogeneous enzyme catalysis is modeled by Michaelis-Menten kinetics and heterogeneous catalysis on a catalyst support, by Langmuir-Hinshelwood kinetics. Cellulase kinetics on insoluble lignocellulosic substrates is a combination of both Michaelis-Menten and Langmuir-Hinshelwood kinetics and also involvement of other factors such as product inhibition, enzyme deactivation, substrate crystallinity, substrate accessibility changes, substrate reactivity changes, fractal nature of the reaction, changes in enzyme synergism and lignin inhibition (Bansal et al, 2009).

## **2.6 SIMULTANEOUS SACCHARIFICATION AND FERMENTATION OF CORN STOVER**

SSF is a process where both hydrolysis and fermentation processes are carried out in a single reactor. In this process, fermentable sugars (glucose and xylose) released by the hydrolyzing enzymes is consumed immediately by the fermenting microorganism present in the culture, and a



low concentration of sugars is maintained in the media, thus reducing the problem of end product inhibition of cellulase. The optimal temperature for SSF is maintained around 38 °C, which is a compromise between the optimum temperature for hydrolysis (45 – 50 °C) and fermentation (30 °C). The following are the advantages of SSF.

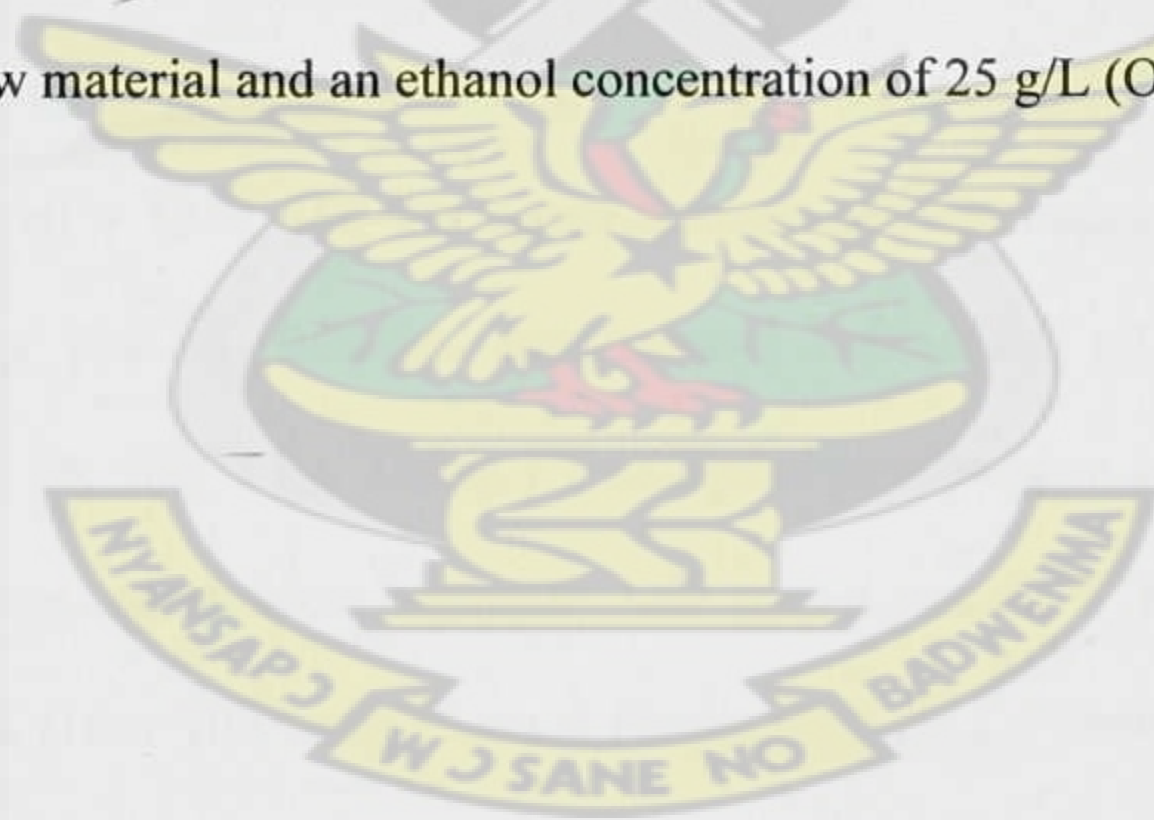
1. Increase of hydrolysis rate by reducing end product inhibition of cellulase
2. Lower enzyme requirement
3. Higher ethanol yield
4. Lower requirement for sterile conditions
5. Shorter process time
6. Cost reductions by eliminating expensive reaction and separation equipment

The main disadvantage of SSF is the inhibition of cellulase enzyme by ethanol produced after fermentation, and ethanol inhibition may be a limiting factor in obtaining high ethanol yield. Ethanol concentration of 30 g/l ethanol reduces the enzyme activity by 25 % (Binod et al, 2011). Another major drawback is that the incomplete hydrolysis of the substrates at the end of the reaction which causes the close association of the yeast and adsorbed cellulases with the recalcitrant residue. This restricts the reuse of the high concentrations of yeasts that are necessary to ensure good ethanol production in the subsequent batch. As a result, much of the sugars released by cellulose hydrolysis are used to grow the yeast rather than fermenting the sugars to ethanol. Despite these disadvantages, SSF is the preferred method in many pilot-scale studies for ethanol production.

Simultaneous saccharification and fermentation (SSF) of corn stover pretreated with acetic acid at 195 °C for 15 minutes gave a high ethanol yield of 88.7 % of the theoretical based on glucose



in the raw material. The raw corn stover without any pretreatment yielded 24.6 % ethanol (6.1 g/l). The estimated total ethanol production reported is 241.1 kg/tonne corn stover by assuming fermentation of both C-6 and C-5, and 0.51 g ethanol/g sugar (Xu et al, 2009). SSF of 1 wt% H<sub>2</sub>SO<sub>4</sub> dilute acid pretreatment of corn stover at 190 °C, using an enzyme loading of 15 filter paper units (FPU)/g cellulose, gave ethanol yields in excess of 85 % (Tucker et al, 2003). SSF of steam pretreated corn stover at solid load of 10 % with 2 g/L hexose fermenting *Saccharomyces cerevisiae* (ordinary compressed baker's yeast) resulted in an ethanol yield of 74 % based on glucose content in the raw material and an ethanol concentration of 25 g/L (Ohgren et al, 2006).





### 3.0 MATERIALS AND METHODS

#### 3.1 MATERIALS

##### 3.1.1 Equipment

Convection Drying Oven, Analytical Balance, Desiccator (containing silica gel), Porcelain Ashing Crucibles, Muffle Furnace, Tongs, Soxhlet apparatus (tubes, 500 mL round bottom flask, heating mantle – 500 mL size, condenser, source of water or tap for condensation, cotton-nylon thimbles), Rotary Evaporator, Buchner Funnels, 250 mL Volumetric Flasks, Water bath, Spectrophotometer (UVmini – 1240, UV-Vis Spectrophotometer, Shimadzu), Goosch Filtering Crucibles, Vacuum Pump, 250 mL Beakers, Autoclavable bottles 250 mL, 10 L Autoclave, Measuring Cylinders (10, 50, 100, 1 L), Timer, FT-IR Spectrometer, Mortar and Pestle, Sieves (16, 32 mesh size), Clamp, Mechanical Hammer Mill (sieve size of 1 mm), Corn Milling Machine, Glass test tubes (16 x 100 mm), Conical Flasks (250 mL), Funnels, Whatman Filter Papers, Stirring Rod, Quartz Cuvettes, and Moisture Analyzer Machine.

##### 3.1.2 Chemicals

Distilled Water, Ethanol (99.97 %),  $\text{H}_2\text{SO}_4$  (95 % w/w, analytical reagent), Nitric acid (68 %, AnalaR), Ethanol (general purpose grade), Acetic acid (analytical reagent), Sodium Hydroxide (general purpose grade), Potassium bromide (analytical reagent) and Methanol (general purpose grade), Sodium acetate, Potassium dichromate (analytical reagent)



### 3.1.3 Enzymes

Commercial cellulase mixtures (Enzyme test kit), Cellic CTec2, was kindly donated by Novozymes, Denmark for this work. The enzyme combination used was Cellulase, Xylanase and  $\beta$ -Glucosidase. Bakers' yeast (*Saccharomyces Cerevesie*) was used for the fermentation

## 3.2 METHODS

### 3.2.1 Sampling for Estimation of Quantities of Corn stover

#### Choice of maize variety

Different maize varieties are cultivated in almost all the ten regions of Ghana. Examples of the maize varieties include, *Aburohema*, *Okomasa*, *Omankwa*, *Aburotia*, *Dobidi*, *Golden Crystal*, *Obatanpa*, *Mamaba*, *Dadaba*, *Cidaba*, etc. These maize varieties are high yielding. The CSIR – Crop Research, Maize Department has the mandate to improve on the local maize varieties and to develop hybrids. The biomass yield (corn or grain, cobs and stover) of the crop depends on factors such as the variety, agricultural practices, nature of the soil and climate. Also, a particular variety will have different physical and chemical characteristics in its biomass. Because these factors differ from region to region, the present work was limited to sampling from experimental farms of the CSIR – Crop Research, Maize Department, Fumesua, Kumasi. Thus, the choice of the maize varieties used in the work was influenced by the maize variety grown or experimented by the Maize Department as of September, 2011.

There were several trials of different maize varieties on the Maize Department farm. Of all the trials, the “Station Variety Trial 2” was said to contain high yielding maize local varieties. So the



sampling was done on “Station Variety Trial 2” which was ready to harvest of land size 25 by 27 m (675 m<sup>2</sup>). The period of cultivation was 3 months 21 days (111 days). The plantation was made up of eighteen (18) high yielding local varieties of maize. They include *Dorke SR*, *Fu 20-90 DWDP*, *Fu 20-90 DYFP*, *GH 90 DWD Pop*, *GH 90 DYF Pop*, *TZE-Y-Pop DT STR C4*, *TZE-W-Pop DT STR C3*, *Omankwa*, *Aburohemaa*, *Pioneer 30K73*, *Pioneer 30B80*, *Pioneer 30Y87*, *Pioneer 30B74*, *Pioneer 30F32*, *Pioneer 30F96*, *Pioneer 30W40*, *Pioneer 30D55*, *Kwadaso local*. These are intermediate materials cultivated in the minor season. The major season starts from March to June and the minor season, August to November. However, the climate pattern observed in 2011 was a bit different from the normal rainfall pattern in Ghana. There was rainfall almost throughout the year. And as a result, the cultivation of the varieties was done on 27<sup>th</sup> May to 14<sup>th</sup> September, 2011.

### **Sampling field**

The total sampling field was 675 m<sup>2</sup> (27 x 25 m<sup>2</sup>) (Figure 3.1). The field was divided into two blocks. Each block was made up of two reps (or representatives). One rep was further divided into 18 plots (0.75 x 5 m<sup>2</sup>) with spacing between plots by 0.75 x 5 m<sup>2</sup>. Two rows of maize plants separated by 0.75 m from each other constituted a plot.

### **Harvesting of corn stover**

#### ***Cobs with Corn (or head of grain)***

The harvesting was done according to plot numbers. The ears of maize (husks + cobs + corn) for a plot were harvested, dehusked and weighed on the field (i.e. field weight) and some of the corn or grains from the cobs removed for moisture analysis. A plot was to contain about 48 – 49 maize plants but some plots recorded less than 20. In total 2,732 plants were harvested with



2,460 ears of maize obtained. After the harvesting of all the 72 plots, the heads (cob with corn or grain) of maize for each plot were bagged in sacks and sent to the yard (CSIR-CR, Maize Department) for removal of the grains from the cobs.

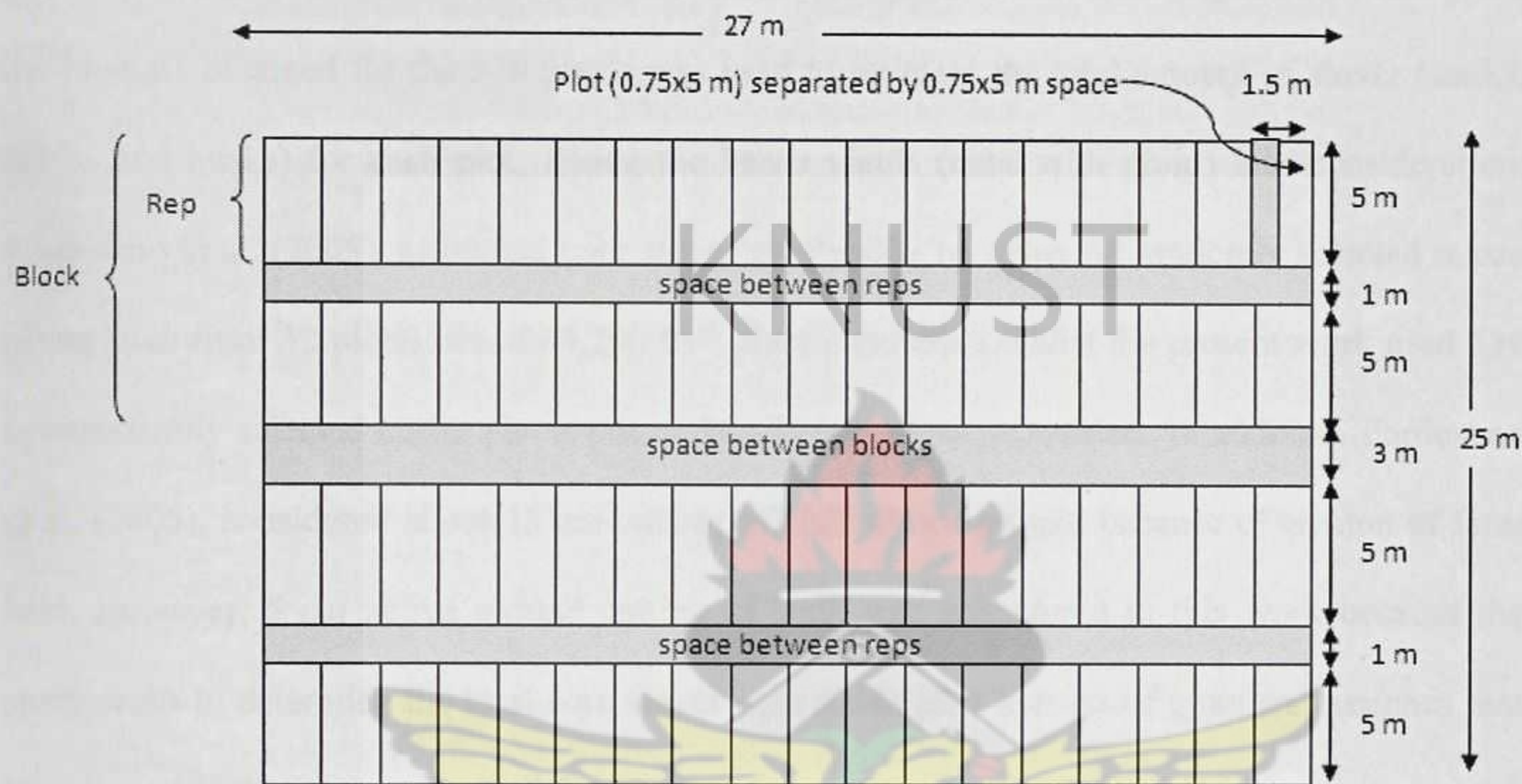


Figure 3.1: Sketch of sampling field ('rep' means representative)

The maize heads were dried in an open for 3 days and the corn or grain removed from the cobs using a Grain/Corn Removal Machine. The weight of the cobs for each plot was determined. Since most of the grains were lost during the removal from the cobs, the weight of the cobs was taken and deducted from the field weight (cobs with grain) to obtain the weight of the grain.

**Stover (stalks, leaves, and husks)**

The corn stalks, leaves and husks remaining on the field were also analyzed by cutting all the plants about 5 cm above ground, arranged, tied and weighed (field weight) for thirteen systematically selected plots (530 standing plants). The criterion for the systematic selection of



the plots was that most of the plants within the plot should be standing or if most of the plants were broken, the biomass should be available within the plot area. This criterion was assumed because most of the plants were found broken as a result of the weather condition during the period (raining season) and some trampled underfoot by the workers during the harvesting. So the biomass obtained for the 530 plants was used to estimate the total amount of stover (stalks, leaves and husks) for each plot, taking the heads yields (cobs with grain) into consideration. Pordesimo et al, (2005), estimated corn stover availability by using 64 randomly selected maize plants (two from 32 plots) out of 64,200 ha<sup>-1</sup> plant population whilst the present work used 530 systematically selected maize plants out of 40,474 ha<sup>-1</sup> plant population. In addition, Pordesimo et al, (2005), considered about 15 cm cutting of stalk above ground because of erosion of farm land. However, 5 cm above ground cutting of stalk was considered in this work because the study seeks to determine the total corn stover collectable after harvest of grain and assumes that represents 100 % corn stover available.

#### **Moisture content determination**

The moisture content of corn from each plot was determined using a Moisture Analyzer Machine. The cobs were dried in open air for one month six days and the moisture content determined by deducting the weight after the drying period from the field weight. The corn stalks, leaves and husks were also dried in open air for one month six days and the moisture content determined by deducting the weight after the drying period from the field weight.



### 3.2.2 Characteristics of Corn Stover

Total solids, ash content, extractives, cellulose, hemicellulose and lignin are the major characteristics of lignocellulosic biomass for bioethanol production. The corn stover from plot one in representative one which contained *Pioneer 30 W 40* maize variety was collected and characterized. Prior to the analysis, the sizes of the corn stalks, leaves and husks were reduced using a mechanical hammer mill (sieve size of 1 mm) and the corn cobs were also ground using a commercial corn milling machine. The milled samples were passed through a 16 mesh screen or sieve. The milled samples were then stored in polyethylene bags and sacks at room temperature for the compositional analyses.

#### (a) Total solids

Biomass mostly contains moisture. The moisture is a measure of the amount of water (and other components volatilized at 105 °C) present in the sample. Total solids content is defined as the amount of solids remaining after heating the sample at 105 °C to constant weight. It is reported on a 105 °C dry weight basis. The total solids of the corn stover was determined following the procedure provided by National Renewable Energy Laboratory (NREL, U.S. DoE, 2008).

#### *Procedure*

1. Pre-drying of the porcelain crucibles for four hours at 105 °C and cooling in a dessicator was done. The weight of each porcelain crucible was recorded.
2. The biomass sample was thoroughly mixed and about 1.0 g weighed into the porcelain crucible. The weight of the sample plus porcelain crucible was recorded.



3. The sample was placed into a convection oven at  $105 \pm 3$  °C for five hours. The sample was removed and cooled to room temperature in a desiccator. The porcelain crucible containing the oven-dried sample was weighed and the weight recorded.
4. The sample was placed back into the convection oven at  $105 \pm 3$  °C for one hour to dry to constant weight (Constant weight is defined as  $\pm 0.1$  % change in the weight percent solids upon one hour of re-heating the sample).
5. The analysis was replicated and the average and standard deviation determined.

#### Calculation

$$\% \text{ Total Solids} = \frac{\text{Weight}_{(\text{dry crucible plus sample})} - \text{Weight}_{(\text{dry crucible})}}{\text{Weight of sample before drying}} \times 100 \dots\dots (3.1)$$

$$\% \text{ Moisture} = 100 - \% \text{ Total Solids} \dots\dots\dots (3.2)$$

#### (b) Ash content

The ash content is a measure of the mineral content and other inorganic matter in biomass. The inorganic material may be structural or extractable. Structural ash is inorganic material that is bound in the physical structure of the biomass, while extractable ash is inorganic material that can be removed by washing or extracting the material. The ash is the inorganic residue left after dry oxidation at 575 °C. The ash content in the corn cobs and husks were determined following the procedure provided by National Renewable Energy Laboratory (NREL, U.S. DoE, 2008).



**Procedure**

1. The crucibles were placed into the muffle furnace set at  $575 \pm 25\text{ }^{\circ}\text{C}$  for four hours. They were removed and cooled for one hour in a desiccator and weighed. The cooling period was also recorded. The crucibles after weighing were returned into the furnace set at  $575 \pm 25\text{ }^{\circ}\text{C}$  for one hour, then cooled in a desiccator and the weight recorded. This was done to ensure constant weight of less than 0.3 mg from previous measurement.
2. About 2.0 g of the air-dried biomass sample was weighed into each crucible and the weight recorded (crucible plus sample).
3. The crucibles were placed in the muffle furnace at  $575 \pm 25\text{ }^{\circ}\text{C}$  for  $24 \pm 6$  hours.
4. The crucibles were removed directly into a desiccator and cooled for one hour and the weight recorded. The samples were placed back into the furnace at  $575 \pm 25\text{ }^{\circ}\text{C}$  and ashed to constant weight (constant weight is defined as less than  $\pm 0.3\text{ mg}$  change in the weight upon one hour of re-heating the crucible).
5. The analysis was replicated and the average and standard deviation determined.

**Calculations**

The % Ash is reported relative to the 105°C oven dry weight (ODW) of the sample:

$$ODW = \frac{Weight_{(air\ dry\ sample)} \times \% \text{ Total Solids}}{100} \dots\dots\dots (3.3)$$

$$\% \text{ Ash} = \frac{Weight_{(crucible\ plus\ ash)} - Weight_{(crucible)}}{ODW_{(Sample)}} \times 100 \dots\dots\dots (3.4)$$



### (c) Extractives

Extractives are non-structural materials in the biomass sample. Non-structural components include sucrose, nitrate/nitrites, protein, ash, chlorophyll, and waxes. The National Renewable Energy Laboratory describes an effective extraction process with 95 % ethanol which removes ethanol soluble material (NREL U.S. DoE, 2008).

#### *Procedure*

1. The total solids content of the biomass sample was determined in order to calculate the oven dry weight (ODW) of the sample to be used for the extraction.
2. The boiling flasks (500 mL round bottom flask) were dried in a drying oven at  $105 \pm 5$  °C for 12 hours, cooled in a desiccator and then weighed (Weight of flask).
3. The thimble was weighed and about 10 g of the sample was placed into it and the total weight recorded (Weight of thimble plus sample).
4. The Soxhlet apparatus was assembled and the thimble inserted into the tube. About 200 mL of ethanol was added to the receiving flask and the heating mantle adjusted to provide 4 siphon cycles per hour.
5. Refluxing was done for overnight and the heating mantle was turned off and the glassware allowed to cool to room temperature.
6. The thimble was removed and the extracted solids transferred quantitatively onto a cellulose filter paper in a Buchner funnel. The solids were washed with ethanol and then air dried. The solvent in the Soxhlet tube was combined with the extractives in the receiving flask.



7. The ethanol in the ethanol extractives were removed using the Soxhlet apparatus. The flask containing the extractives was placed in a drying oven at  $40 \pm 2$  °C for 24 hours and then cooled to room temperature in a desiccator and the weight recorded.
8. The analysis was replicated and the average and standard deviation determined.

### Calculations

The % Extractive is reported relative to the 105 °C oven dry weight (ODW) of the sample:

$$ODW = \frac{Weight_{(thimble\ plus\ sample)} - Weight_{(thimble)}}{100} \times \% Total\ Solids \dots\dots\dots (3.5)$$

$$\%Extractives = \frac{Weight_{(flask\ plus\ extractives)} - Weight_{(flask)}}{ODW_{(sample)}} \times 100 \dots\dots\dots (3.6)$$

### (d) Lignin

Lignin is a complex phenolic polymer mostly insoluble in mineral acids. The total lignin of a biomass is the sum of the acid-insoluble lignin and acid-soluble lignin. The acid-insoluble lignin is the residue remaining after extensive acid hydrolysis of the biomass corrected for its ash content. Acid-soluble lignin refers to a small portion of the lignin that solubilized during the hydrolysis process. Total lignin of the corn stover was determined by applying a method described by the National Renewable Energy Laboratory (NREL U.S. DoE, 2008).

### Acid-insoluble lignin

#### Procedure

1. 72% w/w H<sub>2</sub>SO<sub>4</sub> was required for the hydrolysis, so it was prepared by diluting 76 ml of the 95 % w/w H<sub>2</sub>SO<sub>4</sub> with 24 ml distilled water.



2. The total solids as the sample was received and after the sample was prepared for the acid-insoluble lignin analysis were determined as  $T_{as\ received}$  and  $T_{prep}$  respectively.
3. The crucibles were ignited at  $575 \pm 25\ ^\circ\text{C}$  in a furnace and then cooled in a desiccator to room temperature and the weight measured.
4. About  $0.3 \pm 0.01\ \text{g}$  of the extracted sample ( $W_i$ , initial weight) was weighed into a test tube. The total solids of the extracted sample was determined as  $T_{final}$  also at the time weighing this amount.
5.  $3.00 \pm 0.01\ \text{mL}$  of 72% w/w  $\text{H}_2\text{SO}_4$  was added to the test tube and thoroughly mixed to make sure the sample is wetted.
6. The test tube was placed in a water bath ( $30 \pm 1\ ^\circ\text{C}$ ) and hydrolyzed for 2 hours. The sample was stirred every 15 minutes to ensure complete mixing and wetting.
7. The content was transferred into an autoclavable bottle and diluted to 4 % acid concentration by adding  $84.00 \pm 0.04\ \text{mL}$  water.
8. The bottle containing the sample was closed and autoclaved for 1 hour at  $121 \pm 3\ ^\circ\text{C}$  and afterwards the sample allowed to cool for 20 minutes.
9. The hydrolysis solution was filtered through a previously oven dried glass goosch filtering crucible using a vacuum pump.
10. About 30 mL of the filtrate was taken for acid-soluble lignin.
11. The bottle was washed with hot distilled water of any particles clinging to it into the goosch filtering crucible.
12. The goosch filtering crucible and lignin residues were dried at  $105 \pm 3\ ^\circ\text{C}$  for 3 hours to constant weight.



13. The goosch filtering crucible and lignin residues were cooled in a desiccator and the weight recorded as  $W_2$  (weight of crucible, acid-insoluble lignin and acid-soluble ash).
14. The goosch filtering crucible contents (lignin residues) were transferred into a previously weighed porcelain crucible (which is pre-ignited in the furnace) and placed in a furnace and ignited at  $575 \pm 25^\circ\text{C}$  for 3 hours. The weight of the transferred lignin residues in the goosch filtering crucible was determined by subtracting the weight of the porcelain crucible from the total weight of the porcelain crucible plus lignin residues.
15. The porcelain crucible and its ashed content were then cooled in a desiccator to room temperature and weight recorded. The weight of the ash corresponding to the actual weight of the lignin residues (i.e. the difference between the weight of the empty dried goosch filtering crucible and empty goosch crucible plus lignin residues) was recorded as  $W_3$  (weight of crucible and acid-insoluble ash).
16. The analysis was replicated and the average and standard deviation were determined.

### Calculation

The % Acid-Insoluble Lignin is reported relative to the  $105^\circ\text{C}$  oven dry weight (ODW) of the sample.

$$\% \text{acid} - \text{insoluble lignin} = \frac{W_2 - W_3}{W_1 \times \frac{T_{(as\ received)}}{T_{(prep)}}} \times 100\% = \frac{W_2 - W_3}{W_1 \times \frac{T_{(final)}}{100}} \times 100\% \dots (3.7)$$

$$\% \text{acid} - \text{insoluble residue}_{(extractives\ free)} = \frac{W_2 - W_3}{W_1 \times \frac{\%T_{(final)}}{100\%}} \times 100 \dots \dots \dots (3.8)$$

$$\% \text{acid} - \text{insoluble residue}_{(whole\ sample)}$$

$$= \%AIR_{(extractive\ free)} \times \frac{(100\% - \% \text{Extractives})}{100\%} \dots \dots \dots (3.9)$$



Acid-soluble lignin

Procedure

- 1. The Spectrophotometer was set up and calibrated.
- 2. A 4 % sulfuric acid solution was used as a reference blank. The absorbance was measured at 205 nm.
- 3. The absorbance of the hydrolyzate (filtrate) reserved at step 9, procedure for acid-insoluble lignin analysis, was measured at 205 nm.
- 4. The analysis was repeated three times and the average and standard deviation reported.

Calculation

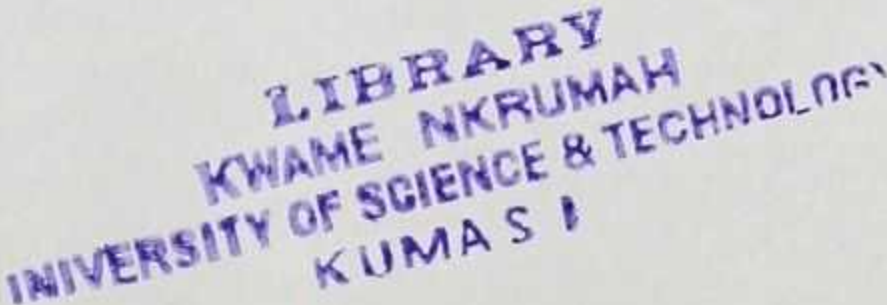
The % Acid-Soluble Lignin (%ASL) is reported relative to the 105 °C oven dry weight (ODW) or extractives free basis of the sample.

$$\%ASL = \frac{\frac{A}{b \times a} \times df \times V \times \frac{L}{1000 \text{ mL}}}{\frac{W \times T_{final}}{100}} \times 100 \dots \dots \dots (3.10)$$

Where:  $A$ = absorbance at 205 nm,  $df$ = dilution factor,  $b$  = cell path length, 1 cm,  $a$  = absorptivity, equal to 110 L/g-cm unless experimentally determined for a given biomass material,  $V$  = filtrate volume, equal to 87 mL,  $W$  = initial biomass sample weight in grams,  $\%T$  = % total solids content of the biomass sample.

(e) Carbohydrate

The carbohydrate (holocellulose) consists of the cellulose and hemicelluloses portions of the biomass. The cellulose was determined gravimetrically using the nitric acid-acetic acid-ethanol method described by Technical Association of Pulp and Paper (TAPPI). Wright and Wallis,





(1998), analyzed cellulose extracted with this method with High Performance Anion-Exchange Chromatography with pulsed amperometric detector (HPAE-PAD) and found the non-cellulosic impurity to be 5.5 %, hence, this method is considered approximate. The same method was applied by Zhao et al, (2008) and Chen et al, (2009), to determine cellulose content of croften weeds and corn stover respectively.

The hemicellulose content was determined as the difference of summing the cellulose content, lignin content, extractive content and ash content and deducting from 100 %. This approach assumed the cellulose, lignin, ash and extractives as the major component of the corn stover. However, chemical compositions such as protein, starch, lipids etc. are in relatively smaller amounts which are ignored in these determinations.

### ***Determination of Cellulose***

#### ***Procedure***

1. The nitric acid – acetic acid mixture was prepared by mixing 90 ml of nitric acid and 732 mL acetic acid in a 1 L volumetric flask and making up to the mark with distilled water (the mixture was allowed to sit for more than 8 hours to ensure homogeneity).
2. About 0.1 g of the corn stover was weighed into the 250 mL beaker and about 25 mL of the nitric acid – acetic acid mixture was added. (The total solid of the corn stover was determined alongside).
3. The content in the beaker was refluxed for about 30 minutes on a hot plate and then cooled to room temperature.



4. After cooling, the content in the beaker was filtered in a pre-weighed goosch filtering (or sintering) crucible and the residues (i.e. cellulose) dried at 105 °C for 3 hours and then weighed.
5. The analysis was repeated three times and the average and standard deviation reported.

### Calculation

$$\% \text{ Cellulose content} = \frac{\text{Weight}_{(\text{crucible plus cellulose})} - \text{Weight}_{(\text{crucible})}}{\text{ODW}_{(\text{sample})}} \times 100 \dots \dots \dots (3.11)$$

### 3.2.3 Sodium Hydroxide Pretreatment of Corn Stover

The main factors affecting the sodium hydroxide pretreatment process are temperature, residence time and concentration of alkaline solution (alkalinity). All the sodium hydroxide pretreatments were done using an autoclave at temperature 121 °C. The catalyst load or alkalinity of 0.5 %, 1 %, 2 %, 3 %, and 4 % of NaOH were varied at residence times of 30, 60, 90, 120 minutes autoclaving. The extent of pretreatment was assessed by measuring the (1) solid yield (i.e. the solid remaining after pretreatment), (2) the cellulose content of the pretreated sample, and (3) extent of delignification – i.e. the residues remaining after acid hydrolysis of the pretreated biomass (acid insoluble lignin).

### Procedure

1. The total solids content of the corn stover was determined by drying in oven at 105 °C.



2. Sodium hydroxide solutions of concentrations 0.5 %, 1.0 %, 2.0 %, 3.0 % and 4.0 % were prepared by dissolving the respective grams of NaOH into 500 mL volumetric flasks and making up the volume with distilled water.
3. About 2.5 g of corn stover was weighed into the autoclavable bottle and 47.5 ml of the respective NaOH solution added.
4. The autoclavable bottles with their contents were then packed into a preconditioned autoclave for autoclaving at 121 °C for the said residence time.
5. After the autoclaving, the bottles were removed and cooled in water bath to room temperature.
6. The solids remaining were washed with water to neutrality (NB: Enough water was added to the contents in the bottles and allowed to settle and the top layer decanted. This was done repeatedly to ensure that most of the lignin has been washed away since it will hinder filtering through the filtering goosch crucible).
7. The solids remaining were dried in an oven at 105 °C for 3 hours to constant weight and the weight recorded as solid yield.
8. Analysis was done in triplicates and the average and standard deviation reported.
9. The pretreated samples were assessed by determining the cellulose contents of the pretreated samples since cellulose content has very good correlation with the solid yield. Also the lignin (acid-insoluble lignin) contents of the pretreated samples were determined to enable calculation of the extent of delignification.



### 3.2.4 FT-IR Analysis of Raw and Pretreated Corn Stover

The chemical changes in the raw and pretreated corn stover were analyzed using the FT-IR spectrometer.

#### *Procedure*

1. The corn stover samples were sieved with the 32 mesh sieve
2. The pellet was made by weighing about 0.001g (1.0 mg) of the corn stover sample and about 0.149 g (149 mg) of KBr which represented a ratio of 1:149 and total weight of pellet of about 150 mg. The weighed masses were ground and pressed to form pellet in the sample holder
3. The FT-IR spectrum for pure KBr was taken for the background correction before the readings were taken for the samples.
4. After each determination, the sample holder was washed with water and methanol and then dried in an oven.
5. The FT-IR spectra for the raw and pretreated corn cobs were determined.

### 3.2.5 Enzymatic Hydrolysis and ~~SSF~~ of Corn Stover

The enzymatic hydrolysis of the pretreated corn stover was done by employing enzyme test kit kindly donated by Novozymes, Denmark. The cellulase, xylanase and  $\beta$ -glucosidase enzyme loading in the hydrolysis system were kept at 5, 0.25 and 0.6 % respectively of the total mass of the system. Common bakers' yeast (*Saccharomyces cerevisie*) was employed at 1.0 % of the total weight of the SSF system. The total solids of the pretreated corn stover used were 19.09 % for corn cobs, 21.84 % for corn stalk and 22.70 % for corn leaves. The extents of hydrolysis and



SSF were determined by measuring the reducing sugars and ethanol respectively. The following procedure was followed:

### Procedure

1. Portions of the individual pretreated corn stover fractions were weighed into the respective hydrolysis or SSF bottles.
2. The required amount of sterilized distilled water was added and the pH was adjusted with dilute solutions of  $\text{H}_2\text{SO}_4$  and  $\text{KOH}$  to 5.0.
3. The whole hydrolysis or SSF system was autoclaved at  $121^\circ\text{C}$  to ensure a sterile condition and the systems were allowed to cool to room temperature.
4. The respective amounts of enzymes were added to the hydrolysis systems, whereas, the respective amount of enzymes and yeast were added to the SSF systems. The systems were stirred properly to ensure a uniform mixture of the enzymes and yeast and initial samples (about 3 ml) were taken as at time zero (The reactions in the samples were stopped by denaturing the enzymes and yeast in boiling water).
5. The systems afterwards were placed in water bath adjusted to  $38^\circ\text{C}$ . Samples from the systems were taken at 0, 24, 48, 72, 96, 120, 144, and 168 hours. For kinetic studies in the hydrolysis systems, samples were taken at 0, 1, 2, 3, 4, 6, 8, 12, 18, 24, 36, and 48 hours from hydrolysis systems containing different substrate concentrations.
6. The samples after denaturing of the enzymes and yeast were centrifuged at 4000 rpm for 10 minutes to separate the supernatant.
7. The samples (supernatant) were then analyzed for reducing sugars using 3, 5-dinitrosalicylic acid (DNS) colorimetric method.



- a. The DNS reagent was prepared by making a 1 L solution of 10 g of 3, 5-dinitrosalicylic acid, 10 g of NaOH, 2 g of phenol and 0.5 g sodium sulphite. The reagent was kept at 4 °C in a freezer.
  - b. A calibration curve was developed from a 10 mg/ml glucose solution (stock).
  - c. Aliquots of 0.1, 0.3, 0.5, 0.7, and 0.9 ml of the stock were taken and made up to 1ml with distilled water in centrifuge tubes. 2 ml of the DNS reagent was added to each aliquot and then heated in boiling water for 10 minutes to develop the red dark colour. 1 ml of the resulting solutions were taken and diluted to 50 ml in a volumetric flask and the absorbance read at 510 nm with the spectrophotometer.
  - d. 0.5 ml of the hydrolysis and SSF samples were taken and diluted to 50 ml with distilled water. 0.5 ml of the diluted sample was taken and 2 ml of DNS reagent added and heated in boiling water to develop the dark red colour. The absorbances were read at 510 nm and the reducing sugar concentrations calculated using the calibration curve.
8. The ethanol content in the SSF samples was also analyzed using potassium dichromate colorimetric method. Reagents or solutions for the analysis were 40 mg/mL potassium dichromate solution, 4.3 (pH) acetate buffer and sulphuric acid solution.
- a. 40 mg/mL potassium dichromate reagent was prepared by weighing 4 g of the potassium dichromate into 100 ml volumetric flask and making the total volume to the mark with distilled water.
  - b. The 4.3 (pH) acetate buffer was prepared by preparing 2.0 M acetic acid solution and adjusting the pH to 4.3 with sodium acetate.



- c. 1.0 M sulphuric acid solution was prepared by measuring 54 ml of concentrated sulphuric acid into 1.0 L and making the volume up to the mark with distilled water.
  - d. Aliquots of 0.2, 0.4, 0.6, 0.8, and 1.0 ml of the stock (1.574 mg/ml ethanol solution) were taken and made up to 1ml with distilled water in volumetric flasks. 2.5 ml of the dichromate reagent, 2.5 ml of buffer and 12.5 ml of sulphuric acid solution were added to each aliquot and then heated in boiling water for 10 minutes to develop the dark green colour. A blank containing only distilled water and reagents was also prepared. The absorbance was read at 578 nm with the spectrophotometer and the calibration curve determined.
  - e. 0.3 ml of each SSF samples was taken and the reagents added and heated in boiling water to develop the dark green colour. The absorbances were read at 510 nm and the reducing sugar concentrations calculated using the calibration curve.
9. Analyses were done in triplicates.



## 4.0 RESULTS AND DISCUSSIONS

### 4.1 ESTIMATION OF QUANTITIES OF CORN STOVER

#### 4.1.1 Field Dried Matter Yield

Field dried matter represents the weight of biomass dried on the field or weight of biomass at the time of harvesting. Table 4.1 shows the field dried matter obtained from the 675 m<sup>2</sup> field at the CSIR – Crop Research, Maize Department, Fumesua, Kumasi. Whilst this study considered 18 different maize varieties, other studies considered only about two to four maize varieties in the corn stover estimation (Pordesimo et al, 2005, Shinnars and Binversie, 2007). The 18 maize varieties were randomly distributed in each of the four land representatives (reps). Since the maize varieties were cultivated together on the same piece of land, there was the possibility of cross pollination which was conceivable to affect the yield and physical and chemical characteristics of the individual maize variety. This effect would have been reduced if the varieties were grown individually on different fields. Thus, the yield of dried matter is assumed to be an average yield obtained for the 18 different maize varieties.

Table 4.1: Field Dried Matter Yield per Land Representative (rep)

Land Rep.	Land Size (m <sup>2</sup> )	No. of Varieties	No. of Plants Harvested	No. of Ears Harvested	Corn Yield (Kg)	Cobs Yield (Kg)	Stalks, Leaves & Husks Yield (Kg)	Total Corn Stover (Kg)
Rep 1	135	18	663	615	68.4	16.3	111.23	127.53
Rep 2	135	18	676	593	66.7	16.7	109.52	126.22
Rep 3	135	18	695	643	70.8	17.1	115.43	132.53
Rep 4	135	18	698	609	68.2	16.9	111.75	128.65
Total			2732	2460	274.1	67	447.93	514.93



**Field dried matter yield per maize plant**

The total number of maize plants cultivated was 3,456, out of which 2,732 were harvested. These 2,732 maize plants represented a plant population of 40,474 per hectare and yielded 2,460 maize ears (cob with grain covered by the husk). The average number of maize plants and ears harvested per rep were 683 and 615 respectively. Thus, the average number of ears per maize plant was 0.9 which is approximately 1.0. The total field dried matter yield of corn, cobs and stalks, leaves and husks obtained per the 675 m<sup>2</sup> field were 274.10, 67.00 and 447.93 Kg respectively. The total field dried matter was 789.03 Kg. The field dried matter yield per maize plant is calculated to be 0.29 Kg (290 g). This means that on average, each plant contributed 290 g of the field dried matter.

**Field dried weight distribution of the maize plant**

The weight distribution according to the different maize plant fraction (corn or grain, cob, stalk, leaf, and husk) on field dried weight basis is shown below (Figure 4.1).

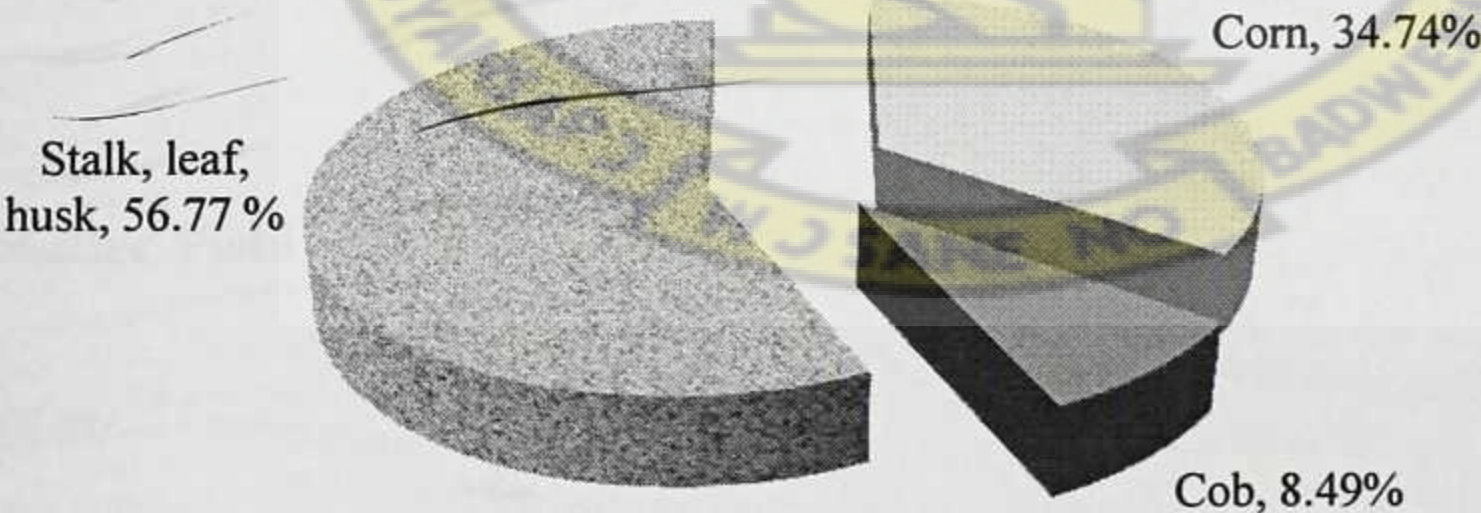


Figure 4.1: Field Dried Weight Distribution of Maize Plant



The weight of the maize plant is more concentrated at the stalk, leaf and husk (56.77 %) than the corn (34.74 %). The weight is less distributed in the cob (8.49 %). However, this weight distribution is biased as the moisture content in the stalk, leaf and husk may be high compared to the corn and cob.

#### ***Field weight distribution of corn stover***

The corn stover constituted the cobs, stalks, leaves and husks. The total field dried weight of the corn stover was 514.93 Kg which comprised of 65.26 % of the total field dried matter. The cobs constituted 13.01 % of the field weight of corn stover. For simplicity, the stalks, leaves and husks were combined in this work. The stalks, leaves and husks combined constituted 86.99 % of the field weight of the corn stover. This shows that about 87 % of the corn stover (field dried basis) is left on the field. Shinnars and Binversie, (2007), obtained a similar results; the field weight distribution after grain harvest were 15 % for cob and 85 % for stalks, leaves, and husks (ie. 56 %, 21 % and 8 % respectively). Sokhansanj et al, (2002), also obtained the field weight distribution to be 15 % for cob and 86 % for stalks, leaves, and husks (i.e. 50 %, 22 %, and 14 % respectively).

#### **4.1.2 Dried Matter Yield**

##### ***Moisture Content***

The moisture content was used to correct the field dried weight to give the actual weight of biomass, i.e. dried matter yield (Table 4.2). The moisture contents of the cobs and stalks, leaves and husks were 40.77 % and 58.35 % respectively, which compared with 50.03 % moisture



content of corn stover recorded by Pordesimo et al, (2005). The moisture content for the corn at each plot differed from each other, so the actual weight of grain was calculated using the respective moisture contents. The average moisture content of the corn was 28.01 which compares closely with 30 % recorded by Shinnars and Binversie, (2007).

Table 4.2: Dried Matter Yield of the Maize Cultivation

Maize Plant Fraction	Yield (Field dried) (Kg)	Moisture (%)	Dried Matter (Kg)	Dried Matter (MT ha <sup>-1</sup> )	Plant fraction Ratio	Corn stover to Corn Ratio
Corn (grain)	274.10	28.01	195.15	2.89	0.46	
Cobs	67.00	40.77	39.68	0.59	0.09	0.20
Stalk, leaf, husk	447.93	58.35	186.59	2.76	0.44	0.96
<b>Total</b>	<b>789.03</b>		<b>421.42</b>	<b>6.24</b>	<b>0.99 ≈ 1</b>	

MT ha<sup>-1</sup> = metric tonnes per hectare

#### Dried matter yield per maize plant

The dried matter yield of corn, cobs and stalks, leaves and husks obtained per the 675 m<sup>2</sup> field were 195.15 Kg (2.89 MT ha<sup>-1</sup>), 39.68 Kg (0.59 MT ha<sup>-1</sup>) and 186.59 Kg (2.76 MT ha<sup>-1</sup>) respectively. The total dried matter yield obtained was 421.42 Kg (6.24 MT ha<sup>-1</sup>) and the total number of maize plants harvested was 2,732. The dried corn stover yield per hectare was calculated to be 3.35 MT. The dried matter yield per maize plant was also calculated to be 0.154 Kg (154 g). This means that, each maize plant contributed 154 g of the total dried matter. When considering only the corn stover, the total dried matter was 226.27 Kg, thus, the dried matter per maize plant is calculated to be 0.083 Kg (83 g). This means that, each maize plant produced about 83 g of corn stover.



### *Dried matter weight distribution of the maize plant*

Corn is the edible part of the maize plant. The field dried weight obtained was 274.10 Kg with moisture content of 28.01 %. The moisture content of the corn represented the lowest compared to that of cobs (40.77 %) and stalks, leaves and husks (58.35 %). The corn represents 46.30 % of the maize plant on dried weight basis (Figure 4.2). Pordesimo et al (2005) also found out the corn fraction of the maize plant (Pioneer 32K61 and 32K64 maize varieties) to be 45.90 %. Comparatively, they harvested after 118 days as against 111 days reported in the present work. Also, Pordesimo et al (2005) harvested their plants 15.20 cm above ground while the present work considered 5 cm above ground. The 5 cm above ground cutting of stalk considered in this work was because this study aimed to determine the total corn stover collectable after harvest of grain and assumes that, that represents 100 % corn stover available.

The corn stover is non-edible and represents the lignocellulosic biomass part of the maize plant. The corn stover constitutes the cobs, stalks, leaves and husks. The cobs represented 9.40 % and the stalks, leaves and husks combined represented 44.30 % of the maize plant on dried weight basis (Figure 4.2). In comparison, Pordesimo et al (2005), investigated the weight distribution and found cobs to be 8.2 %, and stalk, leaf and husk combined to be 45.9 % (i.e. stalk 27.5 %, leaf 11.4 % and husk 7.0 %).

In summary, Pordesimo et al (2005) found 46 % corn and 54 % cobs, stalks, leaves and husks as against the 46.30 % grain and 53.7 % cobs, stalks, leaves and husks found in this work. Corn stover constitutes a higher percentage of the maize plant than the corn on dried weight basis.



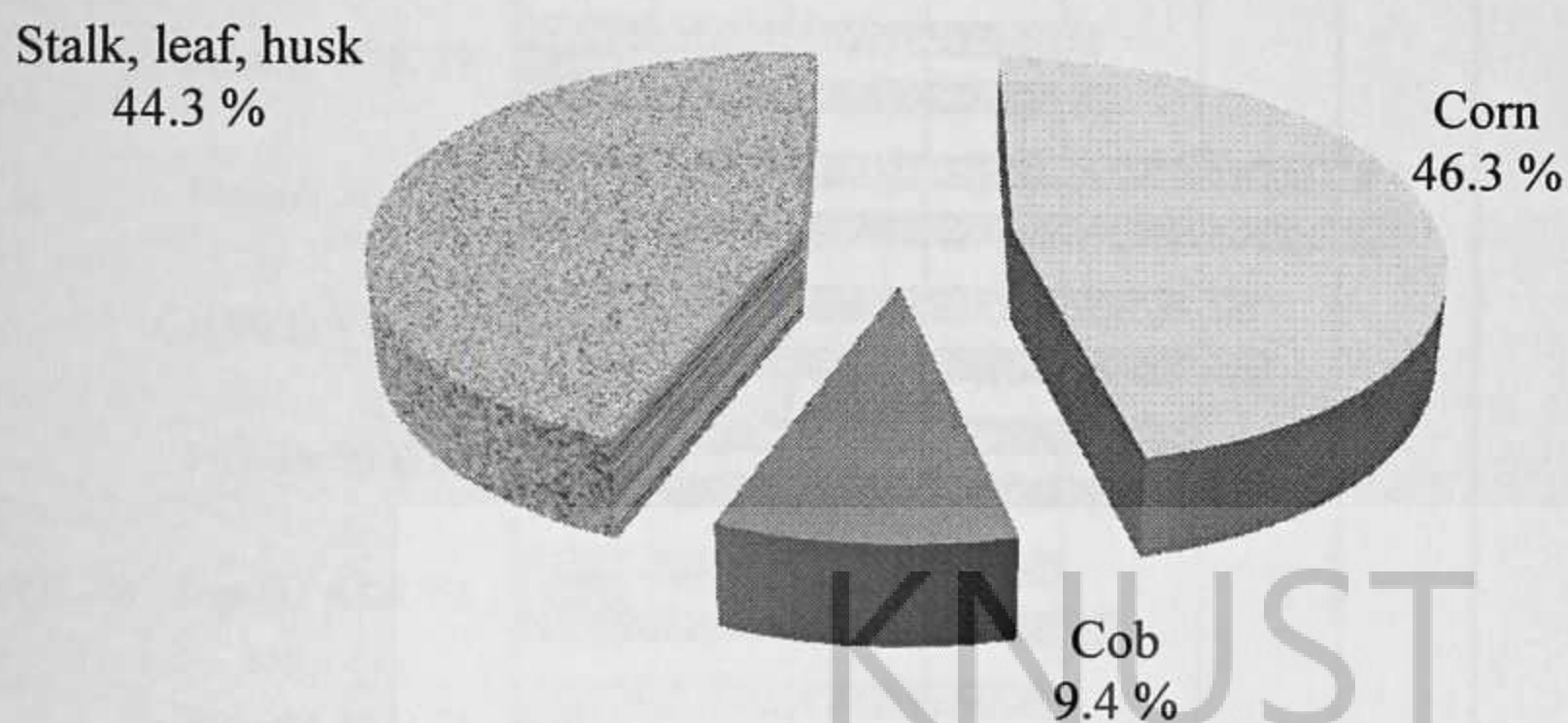


Figure 4.2: Dried Matter Weight Distribution of the Maize Plant

#### ***Dried matter yield per maize variety***

The dried matter yield per maize variety varied depending on the type of maize variety. Some maize varieties have been developed to possess strong stalk in order to withstand all weather conditions, but may yield low amount of corn or grain. Likewise, some have been also developed to yield more grain but may possess weak stalk and maybe vulnerable to the weather conditions. Thus, the dried matter yield in terms of the dry weight of corn, cobs and stover (stalks, leaves and husks) were plotted against the 18 maize varieties (Figure 4.3).

The average dried matter per maize variety was calculated to be 23.41 Kg. This means that each maize variety contributed on average 23.41 Kg of the total dried matter. From Figure 4.3, *Aburohema* recorded the highest yield of dried matter followed by *Dorke SR* and *Pioneer 30 F 96*. *Pioneer 30 F 32*, *Pioneer 30 Y 87*, *TZE-Y-Pop DTSTR C4*, *Pioneer 30 D 55* and *Pioneer 30 W 40* followed accordingly.



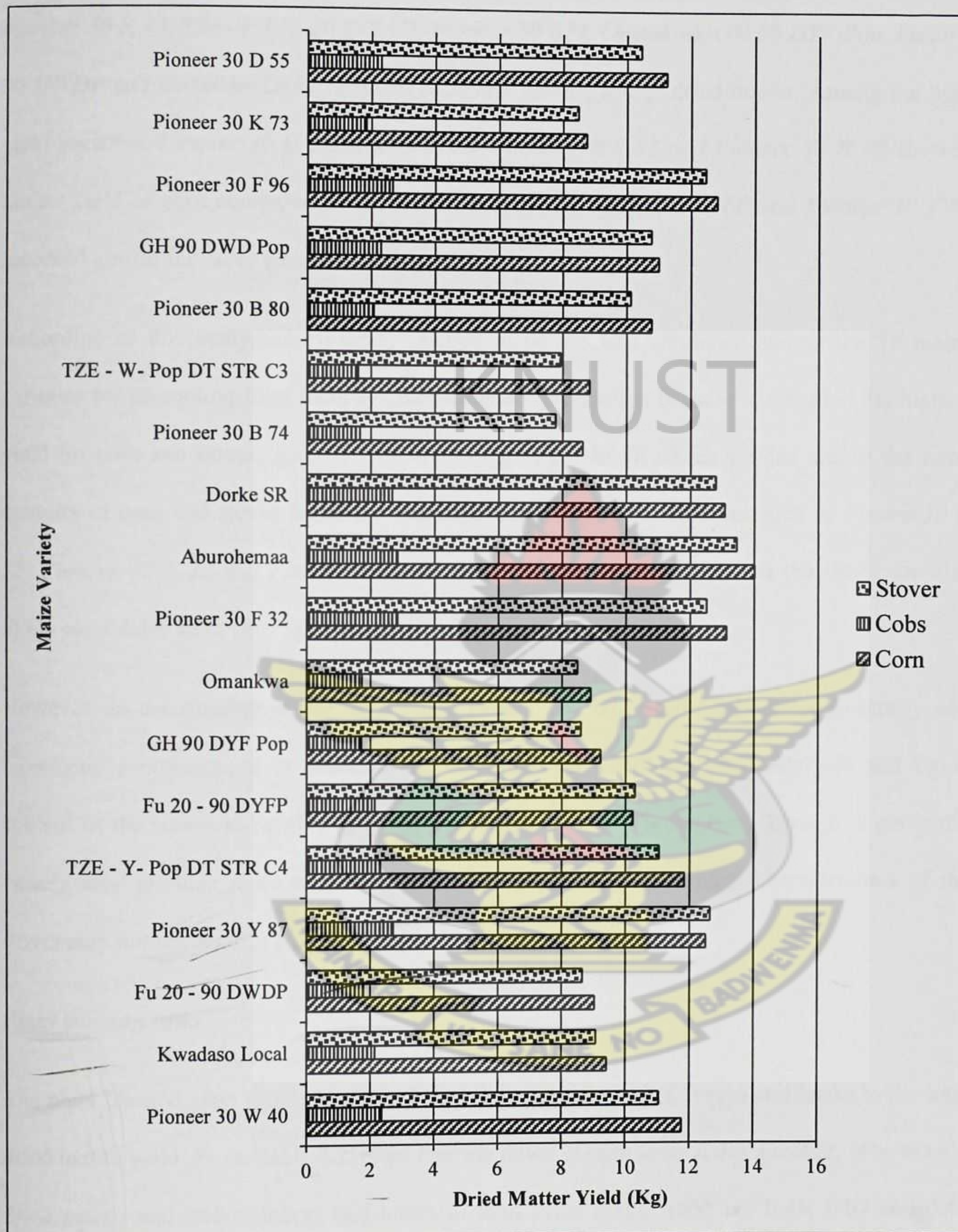


Figure 4.3: Dried Matter Yield of the 18 Local Maize Varieties in Ghana



*Pioneer 30 K 73, TZE-W-Pop DTSTR C3, Pioneer 30 B74, Omankwa, GH 90 DYF Pop, Fu 20 – 90 DWDP and Kwadaso Local* recorded relatively lower yield of dried matter. Among the high yield varieties, *Pioneer 30 D 55, Aburohema, Pioneer 30 F 32 and Pioneer 30 W 40* showed higher yield of corn compared to the stover. *Pioneer 30 F 96, Dorke SR and Pioneer 30 Y 87* recorded almost the same weight for corn and stover.

According to this study, *Aburohema* seemed to be the best candidate among the 18 maize varieties for promoting food security and bioethanol production because it recorded the highest yield for corn and stover. Also, the varieties such as *Dorke SR* which yielded almost the same quantity of corn and stover are likely best candidates. Again the varieties such as *Pioneer 30 D 55, Pioneer 30 F 32 and Pioneer 30 W 40* which yielded higher corn over the stover are also likely candidates since they may promote food security.

However, in determining of the best maize variety that will promote both food security and bioethanol production, it is necessary to determine the cellulose, hemicelluloses and lignin content of the stover to be able to judge which maize variety is the best. Though, a particular variety may produce more corn and stover, the physical and chemical characteristics of the stover may not necessarily be very suitable for bioethanol production.

#### ***Plant fraction ratio***

The plant fraction ratio gives the ratio of the corn, cobs and stalks, leaves and husks to the total dried matter yield. From Table 4.2, plant fraction ratios of corn to total dried matter, cobs to total dried matter and stalks, leaves and husks to total dried matter ratio are 0.46, 0.09 and 0.44 respectively. The 0.46 ratio for corn to total dried matter agreed with 0.45 to 0.55 found by



Sokhansanj et al, (2002). Also, the corn stover to total dried matter was found to be 0.54 which falls in the range 0.43 – 0.59 reported by Pordesimo et al, (2005).

#### ***Corn Stover to Corn Ratio (or residue to product ratio)***

The corn stover to corn ratio (or residue to product ratio) gives an indication of how much of the corn stover that will be generated per production of any given quantity of food product (corn). The residue to product ratio for cob to corn (maize) reported by Sefa-Bonsu, (1997) was 1.00 and stalk to corn ratio was given by Duku et al, (2011) to be 1.50. Kim and Dale (2004) also reported stover to corn ratio to be 1.00. Pordesimo et al (2005) also reported a range of 0.66 – 0.86. However, the stover to corn ratio obtained in this work is 1.16 which is 0.16 higher than the value reported by Kim and Dale (2004).

#### ***Correlation between total corn stover and corn yield***

The correlation between the corn stover and corn yield on dry weight basis was determined in all the four land representatives (reps) (Figure 4.4). The  $R^2$  values were in the range of 0.914 – 0.988 which means that the corn stover and the corn yield in a given maize plantation connects systematically, that is, compares closely. The equations of the straight lines in the graphs can, however, be used for the estimation of corn stover availability when the corn production is known.



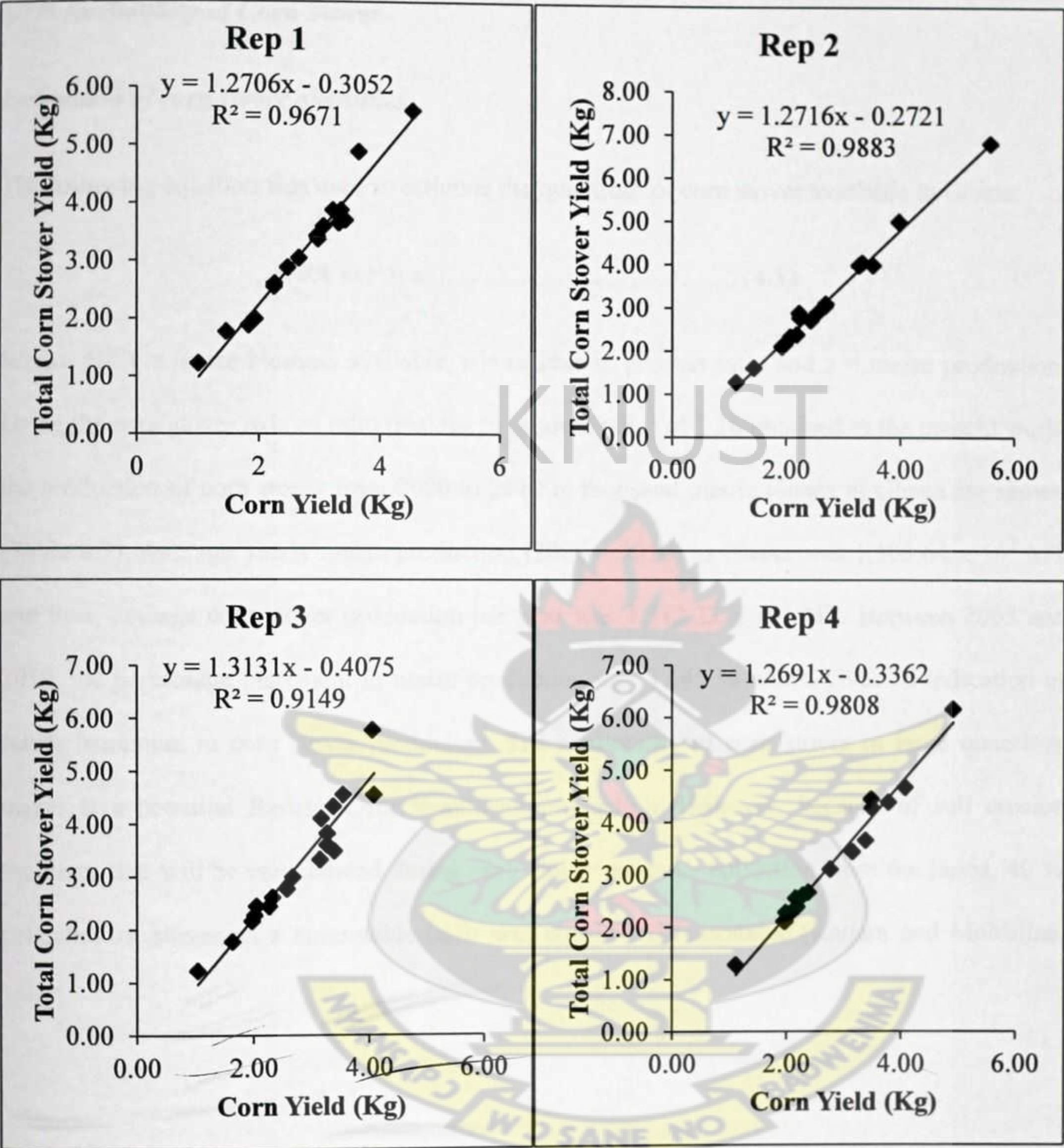


Figure 4.4: Correlation between Total Corn Stover and Corn Yield in each Land Representative



4.1.3 Availability of Corn Stover

Estimation of corn stover quantities

The following equation was used to estimate the quantities of corn stover available in Ghana:

MBA = r × z ..... (4.1)

Where *MBA* = maize biomass available, *r* = residue to product ratio and *z* = maize production. Using the corn stover to corn ratio (residue to product ratio) of 1.16 obtained in the present work, the production of corn stover from 2000 to 2010 in thousand metric tonnes in Ghana are shown (Table 4.3). Average yearly maize production (2000 – 2010) in Ghana was 1,303.64 x 10<sup>3</sup> MT and thus, average corn stover production per year was 1,512.22 x 10<sup>3</sup> MT. Between 2005 and 2010, the percentage increment in maize production was 37.45 % which gives an indication of future increment in corn stover production. The availability of corn stover in large quantities makes it a potential feedstock for bioethanol production. However, because of soil erosion problems that will be encountered during corn stover removal/ collection from the farms, 40 % collection of stover on a sustainable basis was considered reasonable (Kadam and McMillan, 2003).



Table 4.3: Production of Corn Stover in Thousand Metric Tonnes (10<sup>3</sup> MT) in Ghana

Year	Maize Production	Corn Stover Production	40 % Stover Collection/Removal
2000	1,013	1175	470
2001	938	1088	435
2002	1,400	1624	650
2003	1,289	1495	598
2004	1,158	1343	537
2005	1,171	1358	543
2006	1,189	1379	552
2007	1,220	1415	566
2008	1,470	1705	682
2009	1,620	1879	752
2010	1,872	2172	869

MT = 1000 Kg

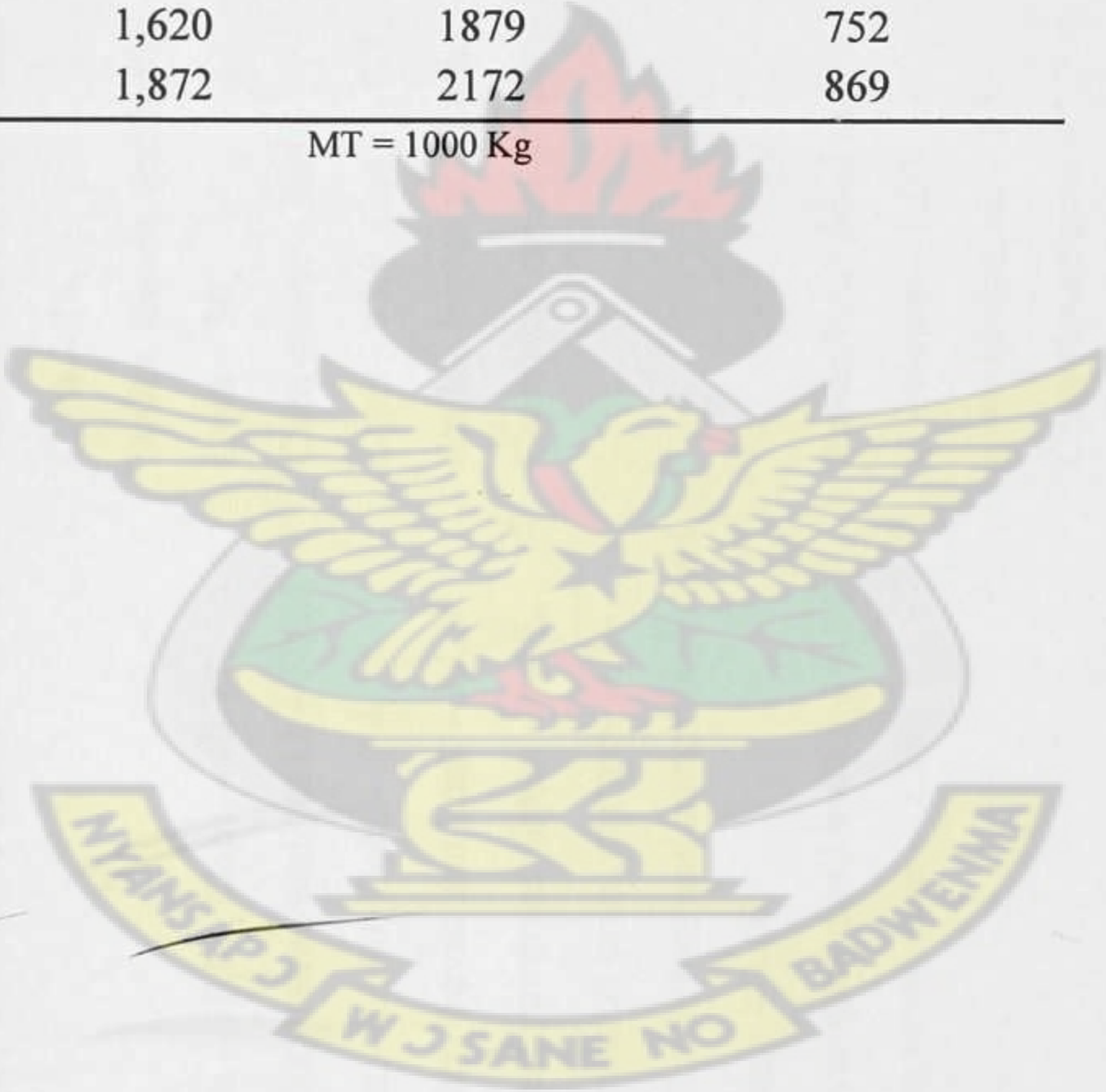




Table 4.4: Summary of Results of Estimation of Quantities of Corn Stover in Ghana

Description	Present Work	Literature	Reference
No. of maize varieties used	18	4	Shinners and Binversie, 2007
Field weight distribution (%)	Cobs – 13.01, Stalks, leaves and husks – 86.99	2 Cobs – 15, Stalks, leaves, and husks – 85	Pordesimo et al, 2005 Shinners and Binversie, 2007
Moisture Content (%)	Corn – 28.01, Cobs – 40.77, Stalks, leaves and husks – 58.55	Cobs – 14, Stalks, leaves, and husks – 86 Corn – 30, Corn stover – 50.03	Sokhansanj et al, 2002 Shinners and Binversie, 2007, Pordesimo et al, 2005
Dried weight distribution (%)	Corn – 46.30, Cobs – 9.4, Stalks, leaves and husk – 44.30	Corn – 45.9, Cobs – 8.2, Stalks, leaves and husks – 45.9	Pordesimo et al, 2005
Plant Fraction Ratio	Corn/total dried matter – 0.46, Cobs/total dried matter – 0.09 , Stalks, leaves and husks/total dried matter – 0.44	Corn/total dried matter – 0.45 – 0.59	Sokhansanj et al, 2002
Total Corn Stover to Corn Ratio	Stover/total dried matter – 0.54 Corn stover/corn – 1.16	Stover/total dried matter 0.43 – 0.59 Cobs/corn – 1.00 Corn stalk/corn – 1.5 Corn stover/corn – 1.0 Corn stover/corn – 0.66 – 0.86	Pordesimo et al, 2005 Sefa-Bonsu, 1997 Duku et al, 2011 Kim and Dale, 2004 Pordesimo et al, 2005



4.2 CHARACTERISTICS OF CORN STOVER

The corn stover characteristics determined in the present work are total solid, moisture content, ash content, extractives, lignin and cellulose. Table 4.5 shows the results of the characterization of corn stover from a local maize variety *Pioneer 30 W 40*. The 18 different local maize varieties including *Pioneer 30 W 40* were cultivated together on the same piece of land for the estimation of the corn stover available quantities. Thus, the characteristics of the corn stover from the *Pioneer 30 W 40* determined may slightly be different (due to the effect of cross pollination) if it were cultivated separately as a mono-crop.

Table 4.5: Characteristics of Corn Stover from *Pioneer 30 W 40* Maize Variety

Corn stover fraction	Total Solid (%)	Moisture (%)	Ash (%)	Extractives (%)	Lignin (AIL) (%)	Cellulose (%)	Hemicellulose (%)
<i>Cobs</i>	95.65	4.36	2.23	3.67	29.61	35.58	28.91
<i>Stalk</i>	93.39	6.61	7.03	8.16	30.62	42.41	11.78
<i>Husk</i>	93.89	6.11	2.96	3.76	30.54	31.17	31.57
<i>Leaves</i>	90.31	9.69	7.93	9.65	30.96	41.26	10.20
<b><i>Whole Stover</i></b>	<b>93.31</b>	<b>6.69</b>	<b>5.03</b>	<b>6.31</b>	<b>30.50</b>	<b>37.72</b>	<b>20.62</b>

Standard deviation: 0.06 – 3.66

4.2.1 Total Solid and Moisture Content

The total solids and moisture contents of the corn husks, stalks, cobs and leaves after 4 months and 21 days of storage under ordinary conditions were determined to be 93.89 % (moisture 6.61 %), 93.39 % (moisture 6.61 %), 95.64 % (moisture 4.36 %), and 90.32 % (moisture 9.69 %) of the air dried matter respectively. These values were solely affected by the environmental



conditions the samples were stored. It appeared that the leaves were more hygroscopic, followed by the cobs, husks and stalks.

#### 4.2.2 Ash Content

The ash content is a measure of the mineral content and other inorganic matter in biomass. The ash remaining after dry oxidation (ashing at 575 °C in a Muffle furnace) of the cobs, stalks, husks and leaves constituted 2.23 %, 7.03 %, 2.96 % and 7.93 % of the biomass at 105 °C oven dried weight respectively. Li et al, (2012), reported ash content for stalk rind, stalk pith and leaf to be 9.9 %, 10 % and 11.3 % respectively. The leaves and the stalks contained the highest percentage of ash. This may be due to the fact that the leaves and stalks are more exposed to the environment where inorganic materials can be adsorbed on them compared to the cobs and husks which are enclosed. Combining the ash contents of the various plant parts, the percentage of the whole corn stover, i.e. the overall ash content became 5.03 %. Aden et al, (2002), and Capunitan and Capareda, (2012), determined ash content of their corn stover sample to be 5.2 % and  $6.62 \pm 0.27$  % respectively.

#### 4.2.3 Extractives

Extractives are non-structural materials in the biomass sample. Non-structural components include sucrose, nitrate/nitrites, protein, ash, chlorophyll, and waxes. The percentage extractives of the cobs, stalks, husks and leaves found were 3.68 %, 8.15 %, 3.76 % and 9.64 % respectively. The stalks and leaves gave the highest percentage extractives. The overall percentage extractive for the overall corn stover was 6.31 %. Li et al, (2012), reported 2.5 %, 6.6



% and 4.5 % of extractives for stalk rind, stalk pith, and leaf respectively. Aden et al, (2002), and Cherubini and Ulgiati, (2010), also reported 4.7 % and 4.78 % of extractives for corn stover. Capunitan and Capareda, (2012), reported 18.1 % of extractives for corn stover which is higher compared to the above reported works.

#### 4.2.4 Lignin

Lignin is a complex phenolic polymer mostly insoluble in mineral acids. The total lignin of a biomass is the sum of the acid-insoluble lignin (AIL) and acid-soluble lignin (ASL). The acid-insoluble lignin is the residue remaining after extensive acid hydrolysis of the biomass corrected for its ash content. Acid-soluble lignin refers to a small portion of the lignin that solubilized during the hydrolysis process.

The acid-insoluble lignin (AIL) obtained for corn cobs, stalks, husks, and leaves are 29.61, 30.62, 30.54, and 30.96 % respectively. Considering the overall corn stover, the acid-insoluble lignin (AIL) was found to be 30.50 %. The acid-soluble lignin obtained for corn cobs, stalks, husks, and leaves are 4.89, 2.01, 5.68, and 2.40 % respectively. The acid-soluble lignin for the overall corn stover was 3.75 %. The lignin content of corn cobs have been reported in the range 11.70 – 22.6 % (Chen et al, 2010, Torre et al, 2008). The lignin content of corn stalk and leaves combined was reported as 20.20 % (Cherubini and Ulgiati, 2010). The lignin content of the overall corn stover was reported to be the range 14.3 – 26.0 % (Pordesimo et al, 2005, Weiss et al, 2010, Chen et al, 2009, Zhao et al, 2008). Comparing these results with the ones obtained in the present work, the present results seemed quite high. However, Dongen et al, (2011), reported lignin content of corn stover to be 30.9 % (AIL = 29.0 % and ASL = 1.9 %) and Eylen et al,



(2011), also determined lignin content of corn stover to be 29.0 %. These results are comparable to the 30.50 % obtained for corn stover in this work.

The lignin contents reported above vary for corn stover since they are influenced by so many factors such as plant variety, plant maturity, biomass impurities (such as soil, dust, etc.), origin of plant, experimental factors such as the possibility of incomplete hydrolysis of the carbohydrate portion of the biomass, etc. Hence, it is necessary to develop an alternative method to the traditional laborious  $H_2SO_4$  method of determination of lignin in order to accurately determine lignin. Foyle et al, (2007), reported the total lignin of straw and paper as the percentage of the residue remaining after acid hydrolysis of the carbohydrate i.e. the acid-insoluble lignin (AIL) and not as the sum of the AIL and ASL. This according to the writer was because the residue remaining after extraction of the sugars contained most of the lignin and also, there is the possibility of some acid insoluble extractives such as waxes and lipids remaining in the residue. Zhao et al, (2008), also did not include the ASL in the total lignin of crofton weed because they observed that there was the possibility of other compounds apart from lignin having their maximum absorption in the 205 nm region of the UV radiation, therefore, the ASL was inaccurate for the weed. However, most publication reported the total lignin of biomass as the sum of the AIL and ASL. The present work reports the acid-insoluble lignin (AIL) and the acid-soluble lignin (ASL) of the corn stover separately.

#### 4.2.5 Carbohydrate

The main carbohydrates in the corn stover are cellulose and hemicellulose. The cellulose is mainly composed of glucose and the hemicellulose is mainly composed of xylose. The cellulose



content of the corn cobs, stalks, husks and leaves were found to be 35.58, 42.41, 31.17, and 41.26 %. The corn stalks recorded the highest cellulose content followed by the corn leaves and the least was the corn husks. The overall cellulose content obtained was 37.72 %.

The cellulose content of the corn cobs determined by Chen et al, (2010) and Torre et al, (2008), were 40.67 % and 31 % respectively which compared closely with the 35.58 % found in this work. The cellulose content of 42.41 % found for the corn stalk was similar to the 39.4 % found by Li et al, (2012). The cellulose content of 41.26 % found for the corn leaves differed by 9.96 % from the 31.3 % reported by Li et al, (2012). Cherubini and Ulgiati, (2010), and Chang et al, (2012), reported cellulose content of the overall corn stover to be 38.1 and 41.30 % respectively which are similar to the 37.72 % reported in this work.

The hemicellulose contents were calculated to be 28.91, 11.78, 31.57 and 10.20 % for corn cobs, stalks, husks and leaves respectively. The hemicellulose contents for the cobs and husk were found to be in the range 18.5 – 31.10 % reported by Chen et al, (2010), Torre et al, (2008) and Weiss et al, (2010).

The method used in the determination of cellulose was the nitric acid – acetic acid – ethanol method published by TAPPI. It is considered to be an approximate method because Wright and Wallis, (1998), analyzed the cellulose extracted with the method with High Performance Anion-Exchange Chromatography with pulsed amperometric detector (HPAE-PAD) and found the noncellulosic impurity present to be 5.5 %. Since the cellulose extracted with this method contains some percentage of hemicelluloses (> 5.5), it is considered a better option for assessing the total carbohydrate in a particular biomass. Weiss et al, (2010), also used Near – Infrared Spectroscopic technique and found the glucan content of corn stover to be within 32.4 – 35.5 %.



Table 4.6: Summary of Results of Characterization of Corn Stover

Description	Present Work	Literature	Reference
Ash Content (%)	5.03	5.20	Aden et al, 2002
Extractives (%)	6.31	6.62	Capunitan and Capareda, 2012
		4.70	Aden et al, 2002
		4.78	Cherubini and Ulgiati, 2010
Lignin (%)	30.50	18.10	Capunitan and Capareda, 2012
		30.90	Dongen et al, 2011
		29.0	Eylen et al, 2011
Cellulose (%)	37.72	14.30 – 26.00	Pordesimo et al, 2005, Weiss et al, 2010,
			Chen et al, 2009, Zhao et al, 2008
		38.10	Cherubini and Ulgiati, 2010
Hemicellulose (%)	20.62	41.30	Chang et al, 2012
		18.5 – 31.10	Chen et al, 2010, Torre et al, 2008,
			Weiss et al, 2010



### 4.3 PRETREATMENT AND HYDROLYSIS OF CORN STOVER

To extract the carbohydrate component of the lignocellulosic biomass in a form suitable for enzymatic hydrolysis, pretreatment processes are usually employed. Alkaline pretreatment appears to increase lignin solubilization, cellulose swelling and the porosity of the biomass (Nlewem and Thrash, 2010). The alkaline catalyst cleaves the ester bonds connecting the lignin and hemicellulose, thereby removing the lignin and some part of the hemicellulose into solution. Sodium hydroxide is one of the alkaline catalysts most studied for pretreatment of various biomasses. The main factors considered in sodium hydroxide pretreatment are sodium hydroxide load or concentration (% NaOH), biomass load or solid to liquid ratio (BL %), time (t min.) and temperature (T °C).

The sodium hydroxide load or concentration was found to be the most sensitive parameter in the pretreatment process. In the present work, the sodium hydroxide concentration was varied i.e. 0.5 % (0.125 M), 1.0 % (0.25 M), 2.0 % (0.5 M), 3.0 % (0.75 M), 4.0 % (1.0 M) at autoclaving periods of 30, 60, 90, and 120 minutes. The biomass load was kept constant at 5.0 % to ensure effective mass transfer. The temperature was also kept constant at 121 °C because according to Zhao et al, (2009), the effect of temperature was insignificant above 80 °C since they observed no significant improvement in the yield of reducing sugars when pretreating bagasse with sodium hydroxide. The pretreatment was done using an autoclave for creating the required condition which was similar to the sodium hydroxide pretreatment done by Zhao et al, (2009) to pretreat bagasse, Xu et al, (2009) and Sun et al, (2011) to pretreat corn stover.

In evaluating the sodium hydroxide pretreatment of the whole corn stover, the above pretreatment conditions were applied to the corn cobs and the most effective pretreatment



conditions arrived at were applied to the corn stalks, husks and leaves. Each experiment was done in triplicate and the mean presented on Table 4.7 below. The effectiveness of each pretreatment condition was assessed by determining the solid yield (%), cellulose content (%) and lignin content (%) of the solid residue remaining after the pretreatment period. The most effective pretreatment process is expected to produce a biomass with a low level of lignin and a high level of cellulose or carbohydrates.

Table 4.7: Evaluation of Sodium Hydroxide Pretreatment of Corn Cobs

Pretreatment conditions			Results			
<i>NaOH load (%)</i>	<i>Solid load (%)</i>	<i>Autoclaving (121 °C) period</i>	<i>Solid Yield (%)</i>	<i>Cellulose (%)</i>	<i>Lignin (%)</i>	<i>Delignification (%)</i>
0.50%	5.00%	30min	64.97	52.19	22.14	29.73
1.00%	5.00%	30min	59.1	60.19	17.48	44.5
2.00%	5.00%	30min	54.66	66.09	17.48	44.52
3.00%	5.00%	30min	47.99	67.81	18.75	40.47
4.00%	5.00%	30min	45.65	72.76	14.69	53.38
0.50%	5.00%	60min	61.55	52.7	21.11	32.98
1.00%	5.00%	60min	58.08	59.52	18.42	41.53
2.00%	5.00%	60min	53.42	63.62	17.37	44.86
3.00%	5.00%	60min	48.84	69.25	15.99	49.35
4.00%	5.00%	60min	45.29	73.29	12.76	59.5
0.50%	5.00%	90min	64.32	56.08	21.46	31.89
1.00%	5.00%	90min	57.89	59.18	13.83	56.1
2.00%	5.00%	90min	52.95	64.78	11.67	62.96
3.00%	5.00%	90min	49.48	68.14	11.5	63.49
4.00%	5.00%	90min	45.53	74.3	9.8	68.9
0.50%	5.00%	120min	63.34	56.48	14.61	53.61
1.00%	5.00%	120min	59.15	62.57	20.61	34.57
2.00%	5.00%	120min	54.81	67.78	18.11	42.5
3.00%	5.00%	120min	51.74	72.24	14.9	52.63
4.00%	5.00%	120min	46.38	78.85	14.07	55.33

NB: Standard deviations: solid yield = 0.28 – 1.21, cellulose = 0.41 – 3.06, lignin (or delignification) = 0.05 – 5.76



#### 4.3.1 Assessment of Pretreatment Processes

##### *Solid Yield (%)*

The solid yield (%) is the weight ratio of residue solid remaining after the pretreatment process to the initial solid. This is referred to as the pretreated biomass. The main reasons for the pretreatment process were to remove the lignin component of the corn cobs in order to increase its cellulose content and to reduce the crystallinity of the cellulose for enzymatic hydrolysis. Since the major components constituting the overall weight of the corn cobs are cellulose, hemicellulose and lignin, removal of the lignin portion reduces the total weight.

In the process of the lignin removal with NaOH, some portions of the hemicellulose are removed because its structure is amorphous and also reduces the weight of the corn cobs. Thus, the weight of corn cobs remaining after pretreatment constituted mainly of cellulose and some portions of hemicellulose and lignin which were not removed by the action of the NaOH. Most works reported solid yield (%) as the basis of assessing pretreating processes (Zhao et al, 2008). Also some works such Chen et al, (2009), reported weight loss (%) instead of the solid yield or weight remaining (%).

The highest solid yield obtained in pretreating corn cobs was 64.97 % at 0.50 % NaOH load and 30 minutes autoclaving period and the least was 45.29 % at 4.0 % NaOH load and 60 minutes (Table 4.7). About 35 – 55 % of the initial weights of corn cobs after the pretreatment were lost. It appeared that the solid yield was much affected by the sodium hydroxide load. Chen et al, (2009), reported weight loss (%) after pretreating corn stover with sodium hydroxide as 41.4 % representing solid yield of 58.6 %.



Cellulose Content (%)

The cellulose content tells the cellulose composition of the pretreated biomass. Since the cellulose is crystalline, compared to the hemicellulose and the lignin which are amorphous, the sodium hydroxide had little or no effect on it, thus, the pretreated corn cobs consisted mainly of cellulose.

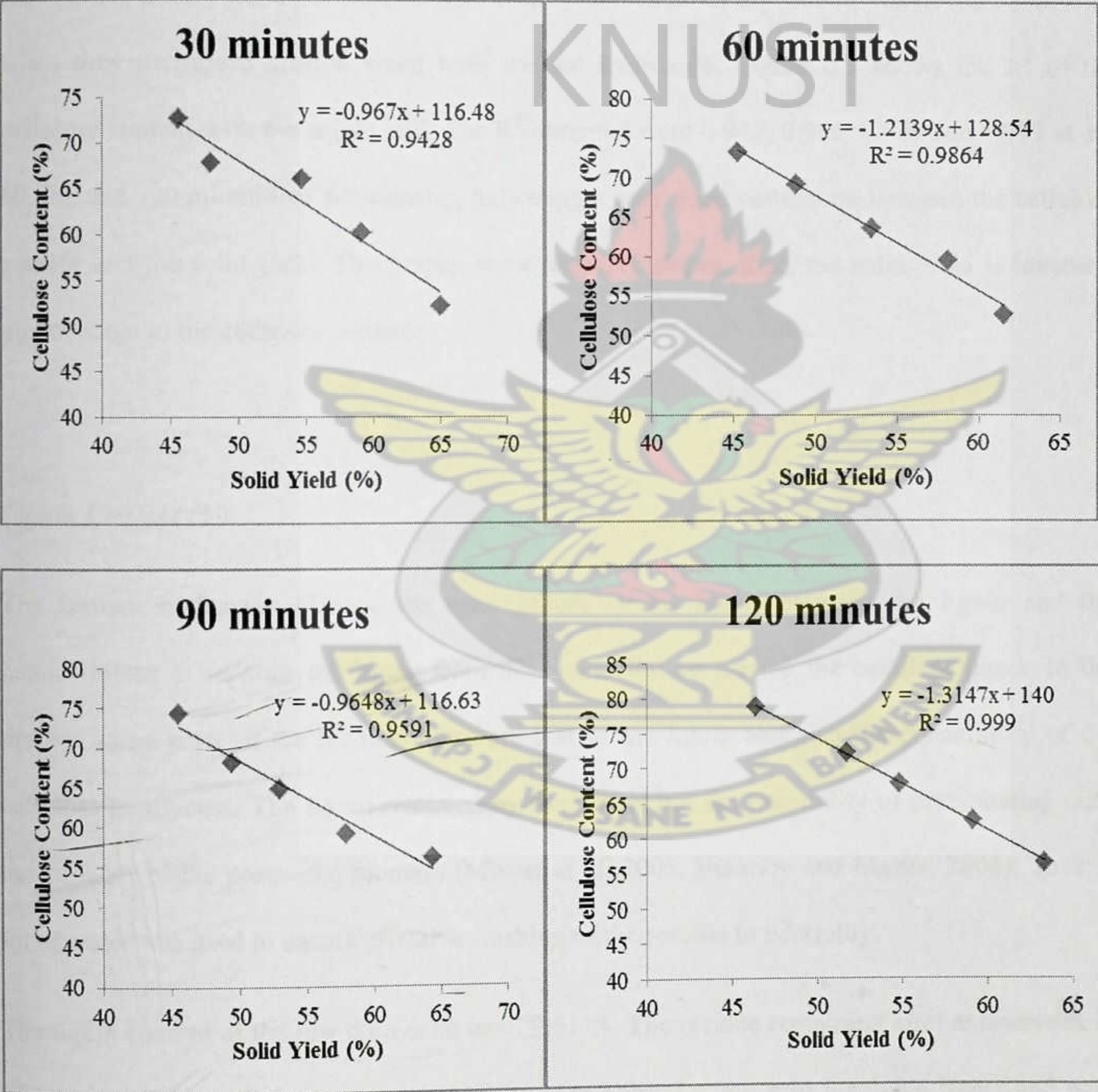


Figure 4.5: Correlation between Cellulose Content and Solid Yield



The highest cellulose content obtained in pretreating the corn cobs was 78.85 % at 4.0 % NaOH load and 120 minutes autoclaving period and the least was 52.19 % at 0.5 % NaOH load and 30 minutes. About 52 – 79 % of the pretreated corn cobs were cellulose. Chen et al, (2009), also obtained cellulose content of 64.1 % of sodium hydroxide pretreated corn stover.

The cellulose content of sodium hydroxide pretreated biomass according to Wright and Wallis, (1998), has a very good correlation with solid yield. Zhao et al, (2008), found the  $R^2$  of 0.89 when they pretreated crofton weed with sodium hydroxide. Figure 4.5 shows the  $R^2$  of the cellulose content with the solid yield. The  $R^2$  obtained were 0.942, 0.986, 0.959 and 0.999 at 30, 60, 90, and 120 minutes of autoclaving, indicating a very good correlation between the cellulose content and the solid yield. The graphs show negative slopes, thus, the solid yield is inversely proportional to the cellulose content.

### ***Lignin Content (%)***

The sodium hydroxide cleaves the ester (alkali labile) bonds between the lignin and the hemicellulose to separate the lignin from the hemicellulose leaving the cellulose intact. In the process some parts of the hemicellulose are lost to the lignin and also the crystallinity of the cellulose is affected. The lignin removed by the NaOH has the possibility of precipitating onto the surfaces of the pretreated biomass (Mosier et al, 2005, Buranov and Mazza, 2008). Thus, a lot of water was used to ensure effective washing of the residue to neutrality.

The lignin content of the raw corn cobs was 29.61 %. The residue remaining after extensive acid hydrolysis of the carbohydrate portion of the corn cobs (i.e. the acid insoluble lignin and acid insoluble ash) was used as the basis of the delignification assessment because it contains the



most part of the lignin separated from the carbohydrates. The acid soluble lignin (ASL) portion of the total lignin was not used because Nlewen and Thrash, (2010), found that ASL was in insignificant amount when assessing the lignin content of a pretreated biomass. The highest delignification obtained in pretreating the corn cobs was 68.9 % at 4.0 % NaOH load and 90 minutes autoclaving period and the least was 29.73 % at 0.5 % NaOH load and 30 minutes autoclaving. Chen et al, (2009), reported the extent of delignification of corn stover pretreated with NaOH to be 73.9 %.

The  $R^2$  values of 0.58, 0.94, 0.87 and 0.136 were obtained when the delignification was plotted against the solid yield at pretreatment periods of 30, 60, 90 and 120 minutes (Figure 4.6). According to Sun et al, (2011), the rate of delignification of corn stover occurs very rapidly such that most part of the lignin is removed at the initial stages of the alkali pretreatment because corn stover is porous compared to wood. Thus, the inconsistency of the correlation values ( $R^2$ ) observed was as a result of the possibility of precipitation of the lignin onto the surfaces of the pretreated biomass. Figure 4.6 shows the correlation between the delignification (%) and the solid yield (%). Nlewen and Thrash, (2010), observed switch grass pretreated with 0.5 % NaOH with high residual lignin to give the highest yield of sugar, which according to them is counter intuitive since the presence of high amount of lignin is known to inhibit cellulase enzyme.



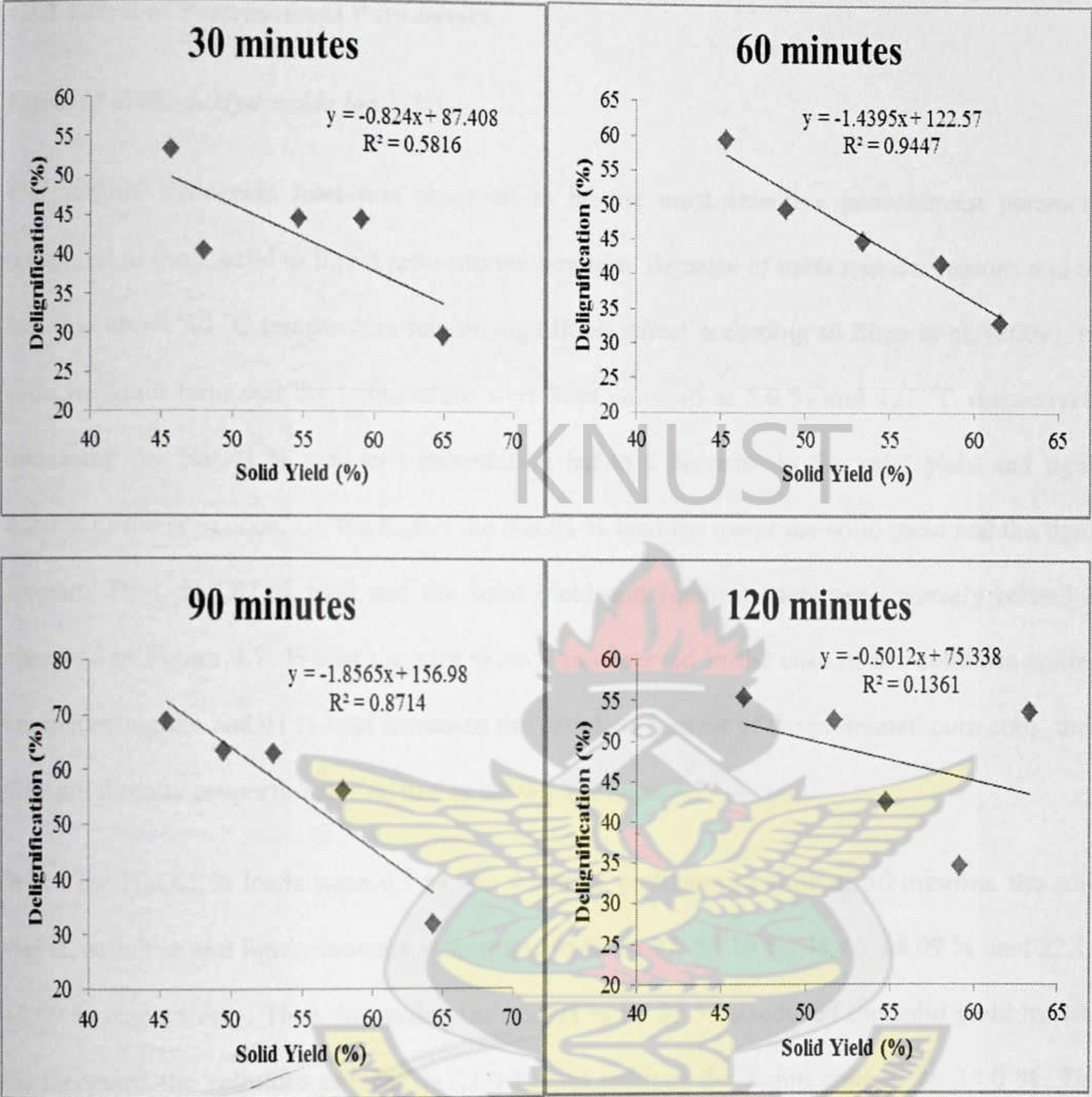


Figure 4.6: Correlation between Delignification and Solid Yield



### 4.3.2 Effect of Pretreatment Parameters

#### *Effect of Sodium Hydroxide load (%)*

The sodium hydroxide load was observed to be the most sensitive pretreatment parameter compared to time, solid to liquid ratio and temperature. Because of mass transfer reasons and the fact that above 80 °C temperature had no significant effect according to Zhao et al, (2009), the solid to liquid ratio and the temperature were kept constant at 5.0 % and 121 °C respectively. Increasing the NaOH % load or concentration led to a decrease in the solid yield and lignin content of the corn cobs, i.e. the higher the NaOH % load the lower the solid yield and the lignin content. Thus, NaOH % load and the solid yield and lignin content are inversely related as observed in Figure 4.7. Whilst the vice versa was observed in the case of the cellulose content i.e. increasing the NaOH % load increased the cellulose content of the pretreated corn cobs, thus, they are directly proportionally related as shown in Figure 4.7.

When the NaOH % loads were 0.5 % and 4.0 % at pretreatment time of 30 minutes, the solid yields, cellulose and lignin contents were found to be 64.97, 52.19 %; 45.46, 66.09 % and 22.14, 14.69 % respectively. Thus, increasing the NaOH % by 87.5 % reduced the solid yield by 30.0 %, increased the cellulose content by 21.0 % and reduced the lignin content by 34.0 %. This observation is in accordance to Sun et al, (2011), that the kinetics of delignification of corn stover at high temperatures (>100) follows a first order reaction since the progress of the reaction depended only on the concentration of the alkali.



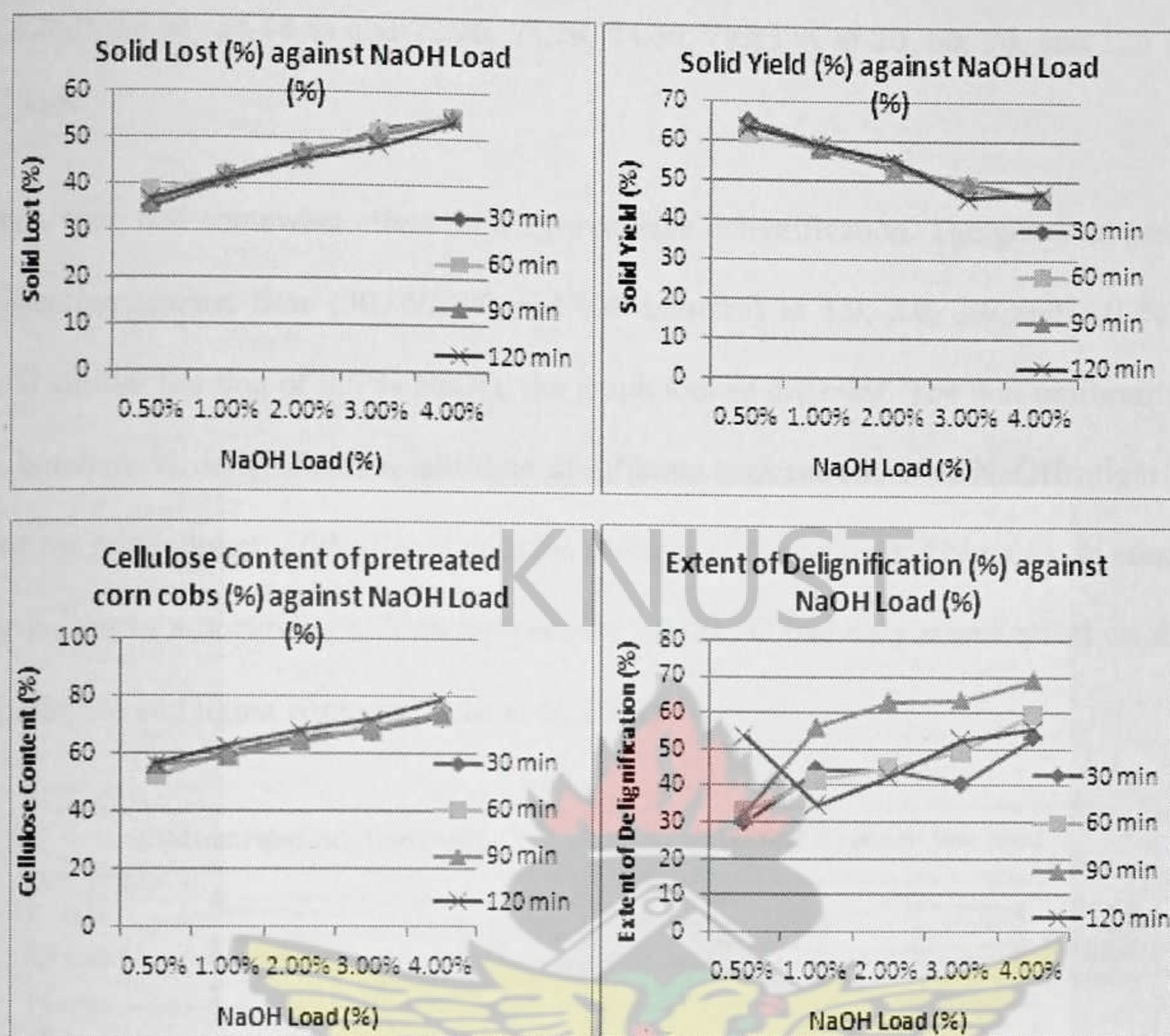


Figure 4.7: Effect of NaOH Load (%) on Solid Yield, Cellulose Content and Delignification

### Effect of Time

The effect of time i.e. the period of autoclaving at 121 °C is shown in Figure 4.8. It appeared that the time had only slight effect on the solid lost and solid yield of the biomass. At 0.5 % and 4.0 % NaOH, the solid yields were 64.97, 61.55, 64.32, 63.34 % and 45.65, 45.29, 45.53, 46.38 % at 30, 60, 90, and 120 minutes respectively. It also appeared that time of autoclaving to some extent had minor effect on the cellulose content. At 0.5 % and 4.0 % NaOH, the cellulose contents were



52.19, 52.70, 56.08, 56.48 % and 72.76, 73.29, 74.30, 78.85 % at 30, 60, 90, and 120 minutes respectively.

However, time had somewhat effect on the percentage delignification. The graph of percentage delignification against time (30, 60, 90 and 120 minutes) at 1.0, 2.0, 3.0 and 4.0 % NaOH appeared similar but that of 0.5 % NaOH, the graph looked different. The non-uniformity of the graphs between % delignification and time at different concentrations of NaOH might be as a result of the precipitation of the lignin onto the surfaces of the pretreated biomass. In conclusion, time or period of autoclaving at high temperature of 121 °C had only minor effect on the solid yield, cellulose and lignin contents (Zhao et al, 2009).

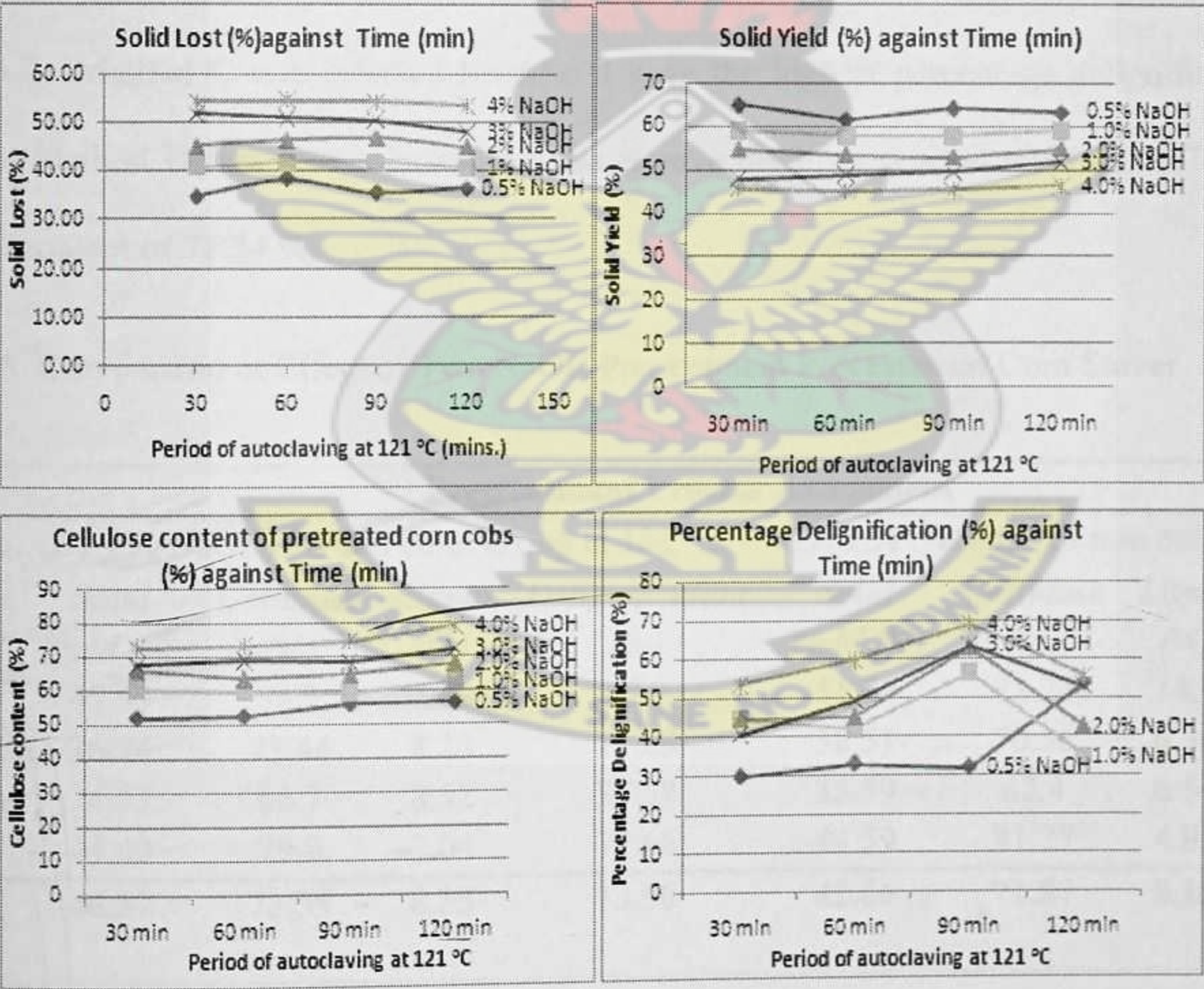


Figure 4.8: Effect of time on solid yield, cellulose content and delignification



4.3.3 Optimal Pretreatment Processes

It was realized that the NaOH % or concentration was the most sensitive parameter in the pretreatment process and that time had only minor effect at high temperatures (121 °C). The pretreatment processes observed to be the most effective methods for pretreating the corn cobs were (A) 4.0 % NaOH / 30 min / 5.0 % solid to liquid ratio, (B) 4.0 % NaOH / 60 min / 5.0 % solid to liquid ratio, (C) 4.0 % NaOH / 90 min / 5.0 % solid to liquid ratio, (D) 3.0 % NaOH / 120 min / 5.0 % solid to liquid ratio, and (E) 4.0 % NaOH / 120 min / 5.0 % solid to liquid ratio. The solid yields and cellulose contents were 45.65, 45.29, 45.53, 51.74, 46.38 % and 72.76, 73.29, 74.30, 72.24, 78.85 % for methods A, B, C, D, and E respectively. Out of these optimal pretreatment conditions, C and D were selected and applied to the corn stalk, leaves and husks (Table 4.8). Method C was selected because it gave the highest percentage delignification of 68.90 %. Method D was also selected because it gave the highest solid yield of 51.74 % and cellulose content of 72.24 %.

Table 4.8: Comparison of Effect of Two NaOH Pretreatment Processes on Corn Stover

Pretreatment Process Assessment								
Corn stover fraction	(C) 4 % NaOH, 90 min autoclaving at 121 °C				(D) 3 % NaOH, 120 min autoclaving at 121 °C			
	Solid Yield (%)	Cellulose (%)	Lignin (%)	Delignification (%)	Solid Yield (%)	Cellulose (%)	Lignin (%)	Delignification (%)
Cobs	45.53	74.3	9.8	68.9	51.74	72.24	14.9	52.63
Husks	36.74	73.44	8.23	73.88	38.51	70.56	6.36	79.82
Leaves	34.77	66.7	8.32	73.58	35.79	67.4	6.54	79.25
Stalks	44.42	79.9	7.04	77.66	44.59	81.27	4.83	84.67
Whole stover	40.37	73.58	8.35	73.50	42.66	72.87	8.16	74.09

Comparing the effect of the pretreatment method D on corn cobs, husks, leaves and stalk, the highest solid yield of 51.74 % was obtained for corn cobs and the lowest of 35.79 % was



obtained for corn leaves. This observation may be associated to the percentage of extractives contained in each biomass which was removed alongside with the lignin during the pretreatment. Corn cobs had the least extractives of 3.67 % and recorded the highest solid yield whilst corn leaves had the highest extractives of 9.65 % and recorded the lowest solid yield. Corn stalk recorded the highest cellulose content of 81.27 % and the lowest was 67.40 % for corn leaves. Corn stalk recorded the highest percentage of delignification of 84.67 % and the lowest of 52.63 was recorded for corn cobs. Similar observation was made under pretreatment method C (Table 4.8).

But, it appeared that pretreatment method D, (i.e. 3.0 % NaOH / 120 min / 5.0 % solid to liquid ratio) recorded the highest cellulose content of 81.27 and the highest delignification of 84.67 % (for corn stalk) compared to pretreatment C, (i.e. 4.0 % NaOH / 90 min / 5.0 % solid to liquid ratio) which recorded the highest cellulose content of 79.9 % and the highest delignification of 77.66 % (for corn stalk). Corn stalk was the most affected by pretreatment methods C and D, followed by corn husks, corn leaves and then corn cobs. Both pretreatment methods were effective but method D gave the optimal cellulose content and percentage delignification.

However, the use of JMP 10 statistical analysis software to predict the optimal cellulose content, predicted pretreatment method A (i.e. 4.0 % NaOH / 30 min / 5.0 % solid to liquid ratio) to be the optimal pretreatment condition. Pretreatment method A, gave solid yield, cellulose content and delignification of corn cobs to be 45.65, 72.76, and 53.38 % respectively which was quite similar to the results obtained for pretreatment method D except that method D had solid yield of 51.74 % (refer Table 4.7).



#### 4.3.4 FT-IR Analysis of Raw and Pretreated Corn Stover

The effect of the pretreated method D (3.0 % NaOH / 120 min / 5.0 % solid to liquid ratio) on corn stover was also analyzed by obtaining the FT-IR spectra for the raw and pretreated corn cobs (Figure 4.9).

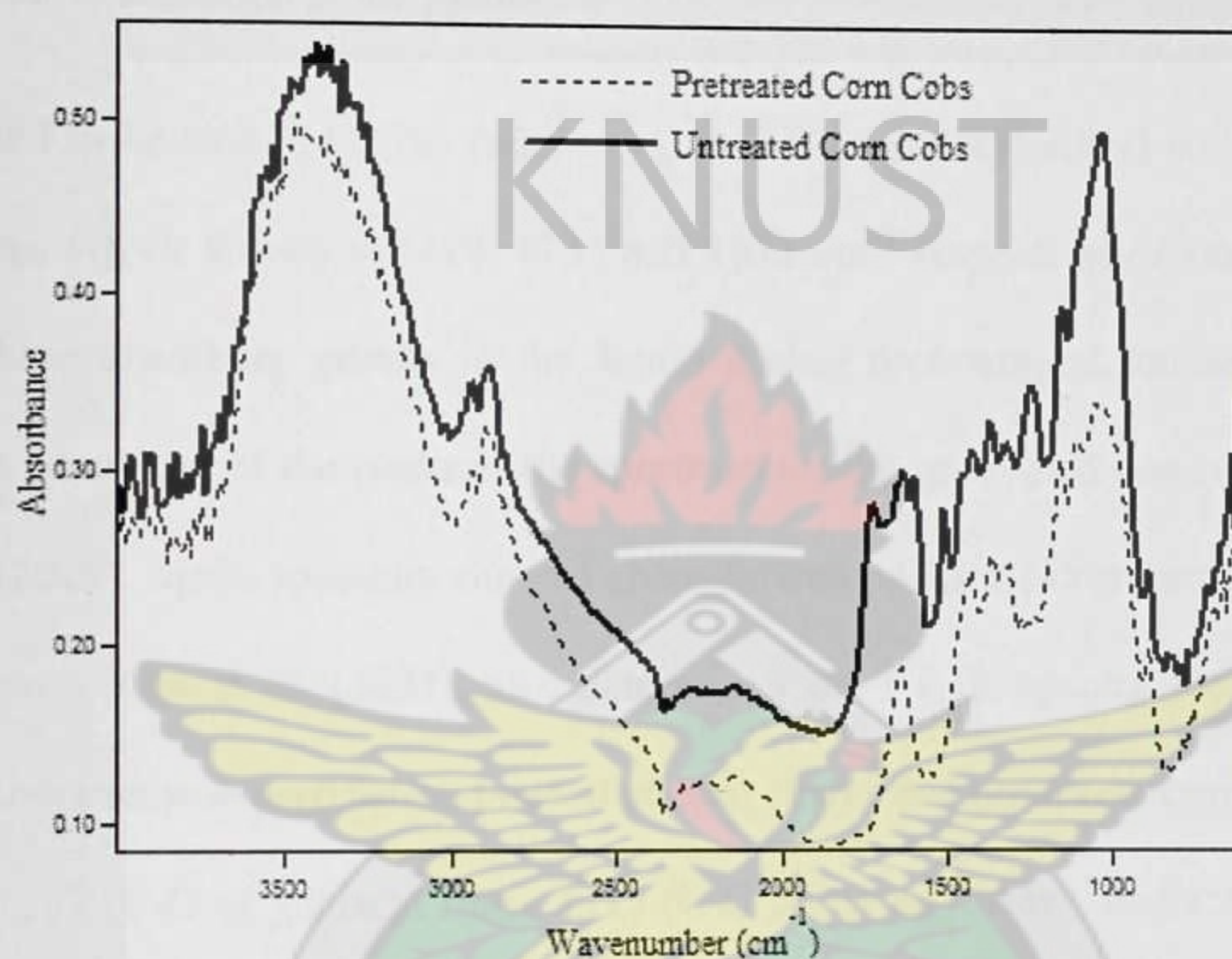


Figure 4.9: FT-IR Spectra for Raw and Pretreated Corn Cobs

The raw corn cobs and the pretreated corn cobs showed similar spectra of FT-IR. Both spectra had significant peaks at 3500 - 3000  $\text{cm}^{-1}$  representing OH stretching, peaks at 3000 - 2500  $\text{cm}^{-1}$  representing C-H<sub>n</sub> stretching, and peaks at 2000 - 1000  $\text{cm}^{-1}$  which are characteristic of the cellulose, hemicellulose and lignin since they all consist of C=O (1510 - 1560  $\text{cm}^{-1}$ ), C-O-C (1232  $\text{cm}^{-1}$ ) and C-O-(H) (~1050  $\text{cm}^{-1}$ ) according to Yang et al, (2007). However, the spectrum



for the raw corn cobs is of higher peak intensities than the spectrum for the pretreated corn cobs. The reduction in peaks intensities was significant in the region of  $2000 - 1000 \text{ cm}^{-1}$ . This was as a result of the reduced lignin content in the pretreated corn cobs. The pretreated corn cobs was rich in cellulose of 72.24 %, and poor in hemicellulose (12.86 %) and lignin (14.9 %) while the raw corn cobs consisted 35.58 % cellulose, 28.91 % hemicellulose and 29.61 % lignin, which indicated about 50 % reduction in the lignin.

Lignin is reported to be rich in  $\text{O-CH}_3$  (methoxy),  $\text{C-O-C}$  (aryl-alkyl ether) and  $\text{C=C}$  (benzene stretching) groups which absorb at 1470, 1232 and  $1632 \text{ cm}^{-1}$  respectively (Yang et al, 2007). Reduction of these absorbing groups in the lignin during pretreatment, consequently led to reduction in the intensities of the peaks in the spectrum for the pretreated corn cobs. According to Adapa et al, (2009), lignin spectrum showed characteristic peaks at wavenumbers 1697, 1603, 1514, and  $837 \text{ cm}^{-1}$ . Kim et al, (2003), also determined the FT-IR spectra for untreated corn stover and ammonia recycle percolation pretreated corn stover and observed lignin characteristic peaks at 1218, 1268 ( $\text{C-O}$  of guaiacyl ring), 1315 ( $\text{C-O}$  of syringyl ring), and  $1502 - 1600 \text{ cm}^{-1}$  (aromatic skeletal vibration). Sun et al, (2011), also determined FT-IR spectra of corn stover, and found bands at 1630, 1140 and  $835 \text{ cm}^{-1}$  which according to them were characteristic of grass lignin. Hence the difference in the spectra of the raw and pretreated corn cobs indicated the reduction in the lignin content of the corn cobs when treated with sodium hydroxide.



4.3.5 Enzymatic Hydrolysis and Fermentation of Pretreated Corn Stover

The Figure 4.10 below shows the comparison of the results obtained for the hydrolysis and simultaneous saccharification and fermentation (SSF) of the various fractions of the corn stover. The highest concentration of reducing sugars of 157.96 mg/mL was obtained within 24 hours of enzymatic hydrolysis of corn cobs representing 60.29 % of conversion. Corn stalks hydrolysis also yielded 128.74 mg/mL of reducing sugars within 48 hours of reaction representing 56.30 % conversion. Corn leaves yielded relatively low concentration of reducing sugars of 28.05 mg/mL within 24 hours representing 38.25 % of conversion.

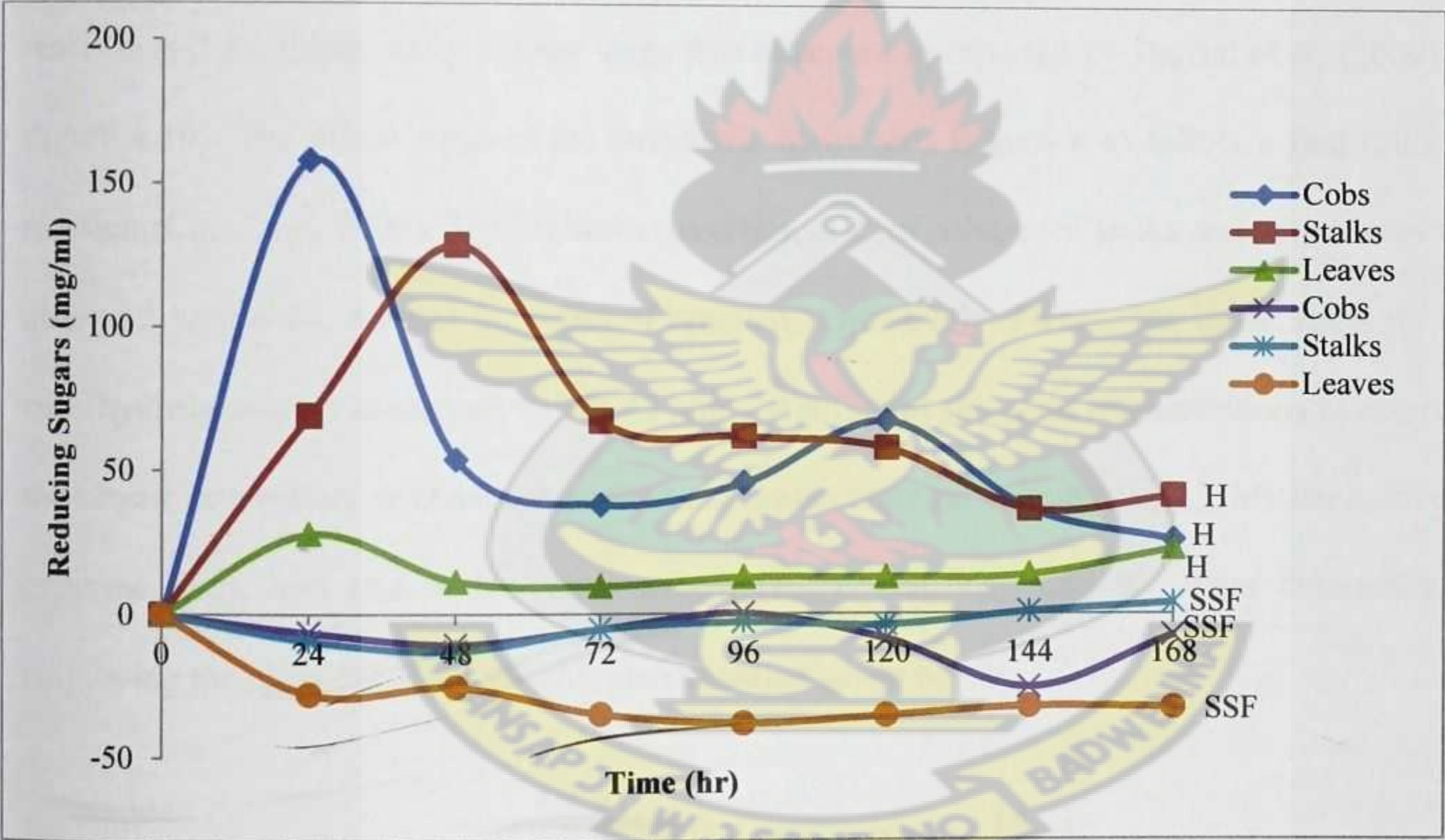


Fig. 4.10: Levels of reducing sugars produced by the hydrolysis and SSF of corn stover (H: Hydrolysis, SSF: Simultaneous Saccharification and Fermentation)

Sarkar et al, (2012), obtained similar sugar concentration of 86 mg/mL (glucose 56.7 mg/mL, xylose 23.6 mg/mL and arabinose 5.7 mg/mL) when they performed enzymatic hydrolysis of 2



% NaOH pretreated corn straw (at 80 °C, 1 hour) with cellulase of *Trichoderma reesei* ZU-02 and cellobiose of *Aspergillus niger* ZU-07. However, the lines indicating the levels of sugars available in the SSF systems run into the negative side of the graph, implying that the sugars produced in those systems were simultaneously converted to ethanol by the yeast (*Saccharomyces Cerevisie*).

#### 4.3.6 Kinetic Study of Hydrolysis of Corn Cobs

The fact that the rate of enzymatic hydrolysis of the biomass occur rapidly at the initial stage of reaction and falls drastically at later stage was observed as reported by Bansal et al, (2009) (see Figure 4.10). The initial stage of the enzymatic hydrolysis is known to follow a first order rate reaction (Copeland, 2000). The highest conversion of corn cobs, corn stalks and corn leaves were observed within 24, 48 and 24 hours of reaction respectively. Hence, the initial rates for corn cobs hydrolysis were measured within 48 hours at different substrate concentrations to determine the kinetic parameters or constants such as maximum reaction velocity ( $V_{max}$ ), Michaelis-Menten constant ( $K_m$ ), and first order rate constant ( $K_{cat}$ ). The  $V_{max}$  and  $K_m$  were determined by employing the classical Michaelis-Menten equation given below:

$$v = \frac{V_{max}[S]}{K_m + [S]} \dots \dots \dots (4.2)$$

Where  $v$  is the reaction velocity and  $[S]$  is the substrate concentration. Table 4.9 shows the initial velocities at various substrate concentrations, the intercept and  $R^2$  of the line of best fit. The increasing  $R^2$  values (in Table 4.9) shows that the initial enzymatic reaction assumes a first order rate kinetics and the drop to a lower  $R^2$  value after the  $V_{max}$  was attained shows a change in the



order of the reaction. First order rate of reactions always give a linear dependence as the rate depends on only one reactant.

K<sub>m</sub> is defined as the substrate concentration that provides a reaction velocity that is half of the maximum velocity obtained under saturating substrate conditions. K<sub>cat</sub> is given by:

$$K_{cat} = \frac{V_{max}}{[E]} \dots \dots \dots (4.3)$$

Where [E] is the enzyme concentration. K<sub>cat</sub> defines the number of catalytic turnover events that occur per unit time. Catalytic efficiency of an enzyme is defined by the ratio of the kinetic constants, K<sub>cat</sub>/K<sub>m</sub> (Copeland, 2000). Lineweaver-Burk and Eadie-Hoftee methods for linear transformation of enzyme kinetic data were used in addition to Michaelis-Menten to determine the V<sub>max</sub>, K<sub>m</sub>, and K<sub>cat</sub>. Given that,

$$\frac{1}{V} = \left( \frac{K_m}{V_{max}} \frac{1}{[S]} \right) + \frac{1}{V_{max}} \dots \dots \dots (4.4)$$

$$v = V_{max} - K_m \left( \frac{v}{[S]} \right) \dots \dots \dots (4.5)$$

A plot of 1/V against 1/[S] gives the Lineweaver-Burk plot (equation 3) whilst V against V/[S] gives the Eadie-Hoftee plot (equation 4).

Table 4.9: Initial velocities of hydrolysis of corn cobs

Substrate Conc. (mg/ml)	Velocity or Rate (mg/ml*hr)	Intercept (mg/ml)	R <sup>2</sup>
11.454	0.1398	134.09	0.2061
22.908	0.2823	143.82	0.2556
34.362	0.8207	172.05	0.5652
45.816	1.0066	180.36	0.5576
57.27	0.5786	197.62	0.2188



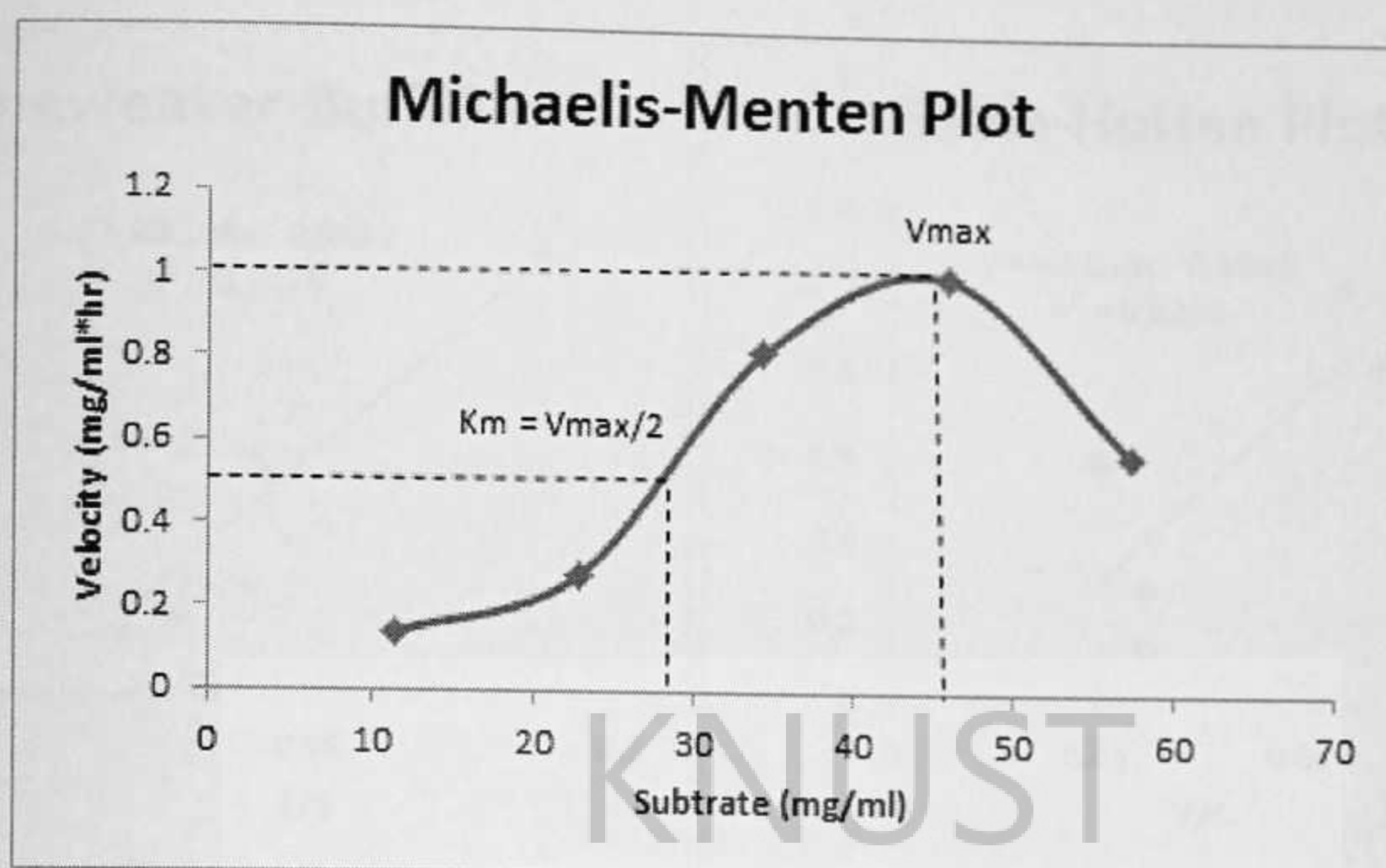


Figure 4.11: Michaelis-Menten plot for enzymatic hydrolysis of corn cobs

The maximum velocity,  $V_{max}$ ,  $K_m$  and  $K_{cat}$  obtained in the Michaelis-Menten plot for the hydrolysis of corn cobs was 1.01 mg/mL\*hr, 28 mg/mL and 0.017 hr<sup>-1</sup> (Figure 4.11). Lineweaver-Burk and Eadie-Hofstee plots gave negative values for  $V_{max}$ ,  $K_m$  and  $K_{cat}$  (Figure 4.12 and Table 4.10). The reason for the differing values from the plots may be to the fact that Michaelis-Menten kinetics is valid for homogeneous systems and not for heterogeneous systems such as the corn stover hydrolysis (Bansal et al, 2009). This classical enzyme kinetics was applied because the hydrolysis of the cellulose occurs first of all in a heterogeneous phase and later in a homogeneous phase. The breakdown of the biomass into cellobiose or oligomers occurs in a heterogeneous phase and the conversion of the cellobiose or oligomers to monomeric sugars occur in a homogeneous phase.



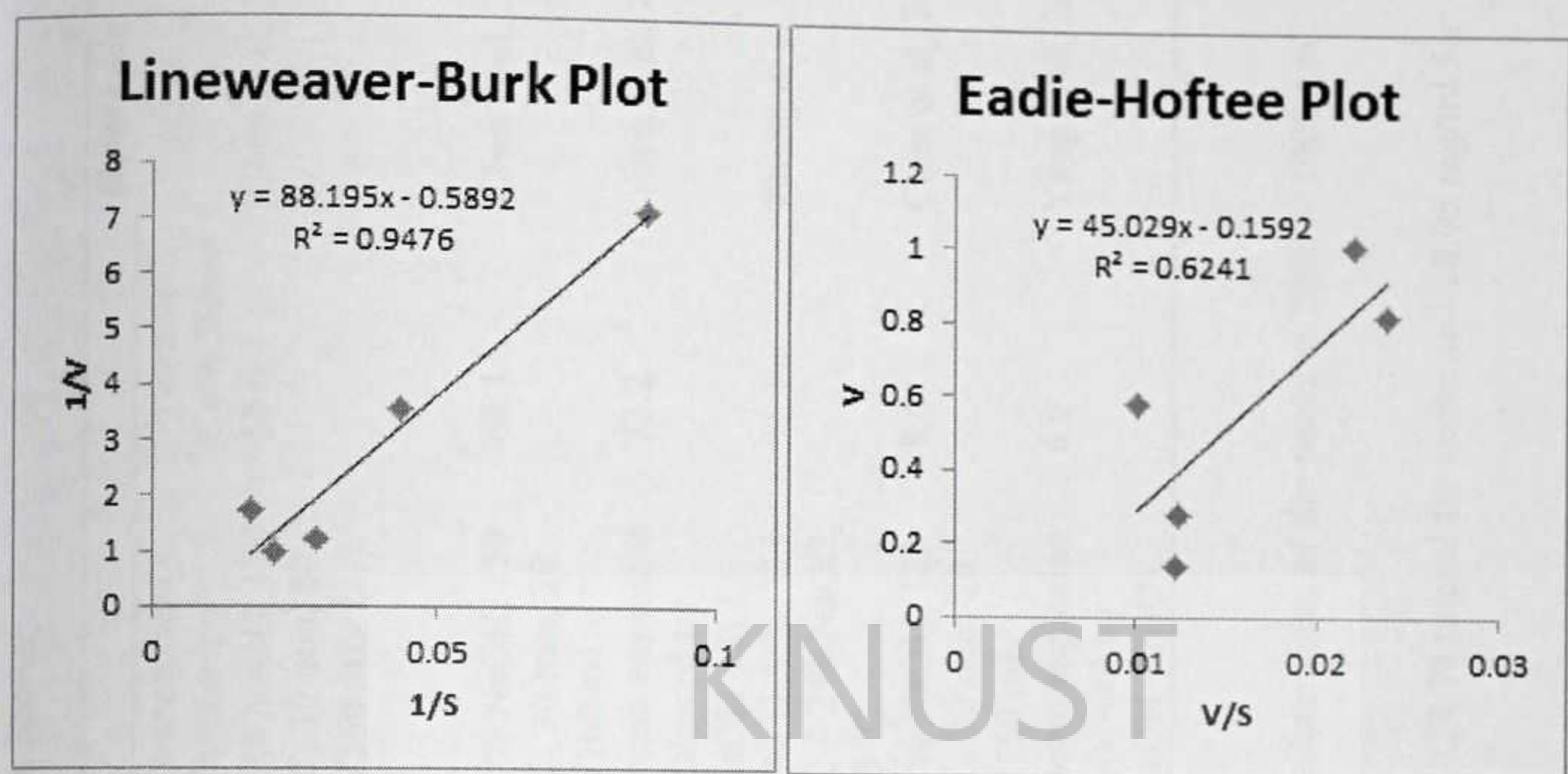


Figure 4.12: Lineweaver-Burk and Eadie-Hofstee plots for enzymatic hydrolysis of corn cobs

Table 4.10: Kinetic constants for enzymatic hydrolysis of corn cobs

Plots	Vmax	Km	Kcat	R <sup>2</sup>
Michaelis-Menten	1.01	28.00	0.017	
Lineweaver-Burk	-1.70	-149.68	-0.029	0.95
Eadie-Hofstee	-0.16	-45.03	-0.003	0.62



Table 4.11: Summary of Results of Pretreatment and Enzymatic Hydrolysis of Corn Stover

Description	Present Work		Literature		Reference
	Untreated Corn Stover	Pretreatment Parameters	Pretreated Corn Stover	Untreated Corn Stover	
Solid Yield (%)	100	3% NaOH, 120 min of autoclaving, 121°C, 5 % Solid-Liquid	Cob – 51.74 Husk– 38.51 Leaf – 35.79 Stalk– 44.59	100	Chen et al, 2009
				2 % NaOH, 120 °C, 30 min, 20 g/160 mL	
Cellulose (%)	Cob – 35.58 Husk– 31.17 Leaf – 41.26 Stalk– 42.41	3% NaOH, 120 min of autoclaving, 121°C, 5 % Solid-Liquid	Cob – 72.24 Husk– 70.56 Leaf – 67.4 Stalk– 81.27	38.70	Chen et al, 2009
				2 % NaOH, 120 °C, 30 min, 20 g/160 mL	
Lignin (%)	Cob – 29.61 Husk– 30.54 Leaf – 30.96 Stalk– 30.62	3% NaOH, 120 min of autoclaving 121°C, 5 % Solid-Liquid	Cob – 14.9 Husk– 6.36 Leaf – 6.54 Stalk– 4.83	31.0	Yang et al, 2010
				Steam explosion followed by NaOH – H <sub>2</sub> O <sub>2</sub>	
				2 % H <sub>2</sub> SO <sub>4</sub> , 121 °C – 2 % NaOH, 80 °C	Zhang et al, 2010
				2 % NaOH, 120 °C, 30 min, 20 g/160 mL	Chen et al, 2009
				Steam explosion followed by NaOH – H <sub>2</sub> O <sub>2</sub>	Yang et al, 2010

1. The FT-IR spectra of the pretreated corn stover showed a major reduction in the intensities of the peaks at 2000 – 1000 cm<sup>-1</sup> indicating reduction in the lignin content.
2. The concentration of total reducing sugars obtained were 157.96 mg/ml for cobs, 128.74 mg/ml for stalks and 28.05 mg/ml for leaves compares with the 86.00 mg/ml obtained by Sarkar et al, (2012).



4.4 THEORETICAL ETHANOL YIELD

The theoretical conversion factor for converting cellulose to ethanol by *S. cerevisiae* is reported to be 0.568. Thus, 1 g of cellulose yields theoretically 0.568 g ethanol (1 g cellulose yields 1.11 g of glucose, 1 g glucose yields 0.511 g ethanol) (Zhang et al, 2010). According to Janick and Whipkey, (2002), 100 g of glucose theoretically yields 51.4 g ethanol and 48.8 g CO<sub>2</sub>. In calculation of the theoretical ethanol yield of corn stover, Janick and Whipkey, (2002), assumed cellulose conversion and recovery as 0.76, ethanol stoichiometric yield from glucose as 0.51, glucose fermentation efficiency as 0.75, hemicellulose conversion and recovery as 0.90, ethanol stoichiometric yield from xylose as 0.51 and xylose fermentation efficiency as 0.50.

Table 4.12: Theoretical Ethanol Yield of the Various Fractions of Corn Stover

Ethanol Yield	Corn cobs	Corn stalks	Corn husks	Corn leaves
<b>Ethanol from Cellulose</b>				
Dry residue (Kg)	1000	1000	1000	1000
Cellulose content	0.3558	0.4241	0.3117	0.4126
Cellulose conversion and recovery efficiency	0.76	0.76	0.76	0.76
Ethanol stoichiometric yield	0.51	0.51	0.51	0.51
Glucose fermentation efficiency	0.75	0.75	0.75	0.75
Ethanol yield from glucose (Kg)	103.43	123.29	90.61	119.94
<b>Ethanol from Hemicellulose</b>				
Dry residue (Kg)	1000	1000	1000	1000
Hemicellulose content	0.2891	0.1178	0.3157	0.102
Hemicellulose conversion and recovery efficiency	0.9	0.9	0.9	0.9
Ethanol stoichiometric yield	0.51	0.51	0.51	0.51
Xylose fermentation efficiency	0.5	0.5	0.5	0.5
Ethanol yield from xylose (Kg)	66.35	27.04	72.45	23.41
<b>Ethanol yield per tonne (1000 Kg)</b>				
Total Ethanol Yield (Kg)	169.78	150.32	163.06	143.35
Density of Ethanol (Kg/L)	0.789	0.789	0.789	0.789
Total Ethanol Yield (L)	215.18	190.52	206.67	181.69

NB: Conversion and recovery efficiency, fermentation efficiency and ethanol stoichiometric yield were obtained from Janick and Whipkey, (2002) and Zhang et al, (2010).



#### 4.4.1 Theoretical Ethanol Yield of Raw and Pretreated Corn Stover

Tables 4.12, 4.13 and 4.14 were derived using the results of the characterization of the corn stover and assumptions of Janick and Whipkey, (2002). Based on the assumptions, corn cobs yielded the highest ethanol of 215.18 L, followed by corn husks 206.67 L, corn stalks 190.52 L and 181.69 L per one dry tonne (1000 Kg) of corn stove (Table 4.12). The overall corn stover yielded 198.95 L ethanol per one dry tonne of corn stover (Table 4.13). The theoretical ethanol obtained per 1000 Kg of dry pretreated corn stover (cellulose content 72.87 – 73.58 %) was between 268.48 – 271.099 L (Table 4.14). These yields compare with the theoretical ethanol yield of 227 L and 290 L ethanol per 1000 Kg dry corn stover reported by Janick and Whipkey, (2002) and Kim and Dale, (2004) respectively. However, according to EERE (U.S. DoE) a tonne of dry corn stover could yield theoretically about 124.56 gallons (472.09 L) of ethanol (EERE Theoretical Ethanol Calculator).

#### 4.4.2 Percentage of Gasoline Consumption Replaceable with Bioethanol

The national target on biofuels as described in the bioenergy policy is to substitute the national petroleum fuels consumption with biofuel by 10 % by 2020 and 20 % by 2030 (Energy Commission, 2010). The average annual gasoline consumption from 2000 to 2010 is 569.38 Kilotonnes ( $569.38 \times 10^6$  Kg). Replacing 10 % by 2020 and 20 % by 2030 of the gasoline (or petrol) consumed with bioethanol will require only 56.94 ( $56.94 \times 10^6$  Kg) and 113.88 ( $113.88 \times 10^6$  Kg) Kilotonnes of bioethanol respectively.



Table 4.13: Theoretical Ethanol Yield of Overall Corn Stover

Ethanol Yield	
<i>Ethanol from Cellulose</i>	
Dry stover	1 tonne (1000 Kg)
Cellulose content	× 0.3772
Cellulose conversion and recovery efficiency	× 0.76
Ethanol stoichiometric yield	× 0.51
Glucose fermentation efficiency	× 0.75
Ethanol yield from glucose	109.652 Kg (138.976 L)
<i>Ethanol from Hemicellulose</i>	
Dry stover	1 tonne (1000 Kg)
Hemicellulose content	× 0.2062
Hemicellulose conversion and recovery efficiency	× 0.9
Ethanol stoichiometric yield	× 0.51
Xylose fermentation efficiency	× 0.5
Ethanol yield from xylose	47.3229 Kg (59.978 L)
<i>Ethanol yield per 1 tonne (1000 Kg) of corn stover</i>	
Total ethanol yield in kilograms	156.9749 Kg
Density of ethanol	0.789 Kg/L
Total ethanol yield in liters	198.95 L

NB: Conversion and recovery efficiency, fermentation efficiency and ethanol stoichiometric yield were obtained from Janick and Whipkey, (2002) and Zhang et al, (2010).

Table 4.14: Theoretical Ethanol Yield of Pretreated Corn Stover

Ethanol Yield	Pretreatment	
	3% NaOH/120min/121°C	4%NaOH/120min/121°C
<i>Ethanol from Cellulose</i>		
Dry pretreated stover	1 tonne (1000 Kg)	1 tonne (1000 Kg)
Cellulose content	× 0.7287	× 0.7358
Cellulose conversion and recovery efficiency	× 0.76	× 0.76
Ethanol stoichiometric yield	× 0.51	× 0.51
Glucose fermentation efficiency	× 0.75	× 0.75
Ethanol yield from glucose	211.8331 Kg	213.8971 Kg
Density of ethanol	0.789 Kg/L	0.789 Kg/L
Ethanol yield in liters	268.48 L	271.099 L

NB: Conversion and recovery efficiency, fermentation efficiency and ethanol stoichiometric yield were obtained from Janick and Whipkey, (2002) and Zhang et al, (2010).



Table 4.15 shows the 40 % corn stover removal (sustainable approach) and the percentage of gasoline replaceable by bioethanol from the year 2000 to 2010 in Ghana. The highest percentage of gasoline replaceable by bioethanol produced from corn stover is 19.64 % in 2008 and the lowest 12.76 % in 2001. If only 40 % of the average 1,512.09 thousand metric tonnes (1,512.09 Kilotonnes =  $1,512.09 \times 10^6$  Kg) corn stover (2000 – 2010) was collectable as a measure to control soil erosion, then an average of 604.84 thousand metric tonnes ( $604.84 \times 10^6$  Kg) of corn stover were available for bioethanol production. Based on the carbohydrate content of the raw corn stover determined in this work, the theoretical ethanol yield per dry tonne (1000 Kg) of corn stover is 198.95 L (156.9749 Kg) (refer Table 4.13).

Table 4.15: Percentage of Gasoline Replaceable with Bioethanol produced from Corn Stover

Year	Corn Stover -40% Removal (10 <sup>3</sup> MT)	Bioethanol potential (Kilotonnes)	Bioethanol potential (ML)	Gasoline consumption (Kilotonnes)	% Gasoline Replaceable with Bioethanol
2000	470	73.78	93.51	524.4	14.07
2001	435	68.28	86.55	535.1	12.76
2002	650	102.03	129.32	570.2	17.89
2003	598	93.87	118.97	479.8	19.56
2004	537	84.30	106.84	575.6	14.64
2005	543	85.24	108.03	537.8	15.85
2006	552	86.65	109.82	511.9	16.93
2007	566	88.85	112.61	544.2	16.33
2008	682	107.06	135.69	545	19.64
2009	752	118.05	149.61	701.4	16.83
2010	869	136.41	172.89	737.8	18.49

MT = metric tonnes (1000 Kg), 1 kilotonne =  $10^6$  Kg, Density of ethanol =0.789 Kg/L ML = theoretical ethanol yield in million liters. Source: National Energy Statistics, Ghana, (2000 – 2010)

Thus, the 604.84 thousand metric tonnes ( $604.84 \times 10^6$  Kg) of dry corn stover have the potential of yielding  $94.945 \times 10^6$  Kg ( $120.33 \times 10^6$  L) of ethanol able to replace 16.67 % of the average



annual gasoline consumption of 569.38 Kilotonnes. That is, the bioethanol that can be produced from only 40 % of the corn stover produced annually in Ghana will be able to meet the national target of 10 % by 2020 and 20 % by 2030, since it is perceived that maize production will increase with consequent increment in corn stover production in the coming years because there are much focus of government in improving agricultural productivity.

4.5 PRACTICAL ETHANOL YIELD

The results of the simultaneous saccharification and fermentation (SSF) of the corn cobs, corn stalks and corn leaves are shown in Figure 4.13. The graph shows that the concentration of ethanol obtained were within 0 – 2.0 mg/ml. The highest ethanol yield obtained for corn cobs, stalks and leaves were 1.79, 0.94, and 0.36 mg/ml respectively.

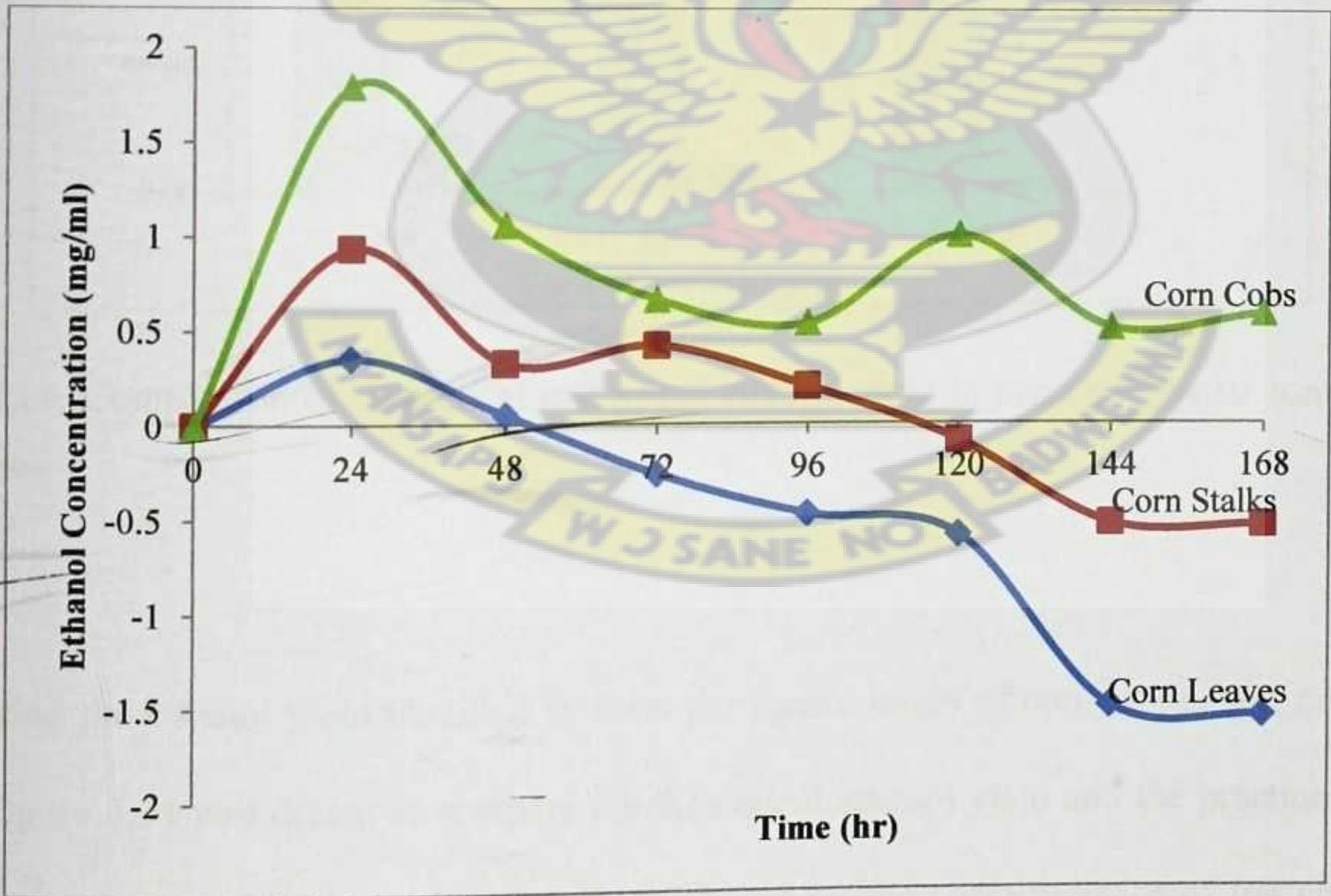


Figure 4.13: Ethanol yield of the simultaneous saccharification and fermentation (SSF) of corn stover

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The ethanol concentration dropped drastically after 48 hours of fermentation. Absolutely, no amount of ethanol was determined in the SSF system of the corn stalks and corn leaves after 120 and 48 hours of fermentation respectively, whereas, ethanol concentration of 0.59 mg/ml was determined in fermentation of corn cobs even at 168 hours. This may be as a result of the nature of the different fractions of the corn stover and also the possibility of inhibition of the fermentative microorganisms. Also conversion of the ethanol to acetic acid is also possible and explains why the concentration of ethanol dropped drastically after 48 hours of fermentation.

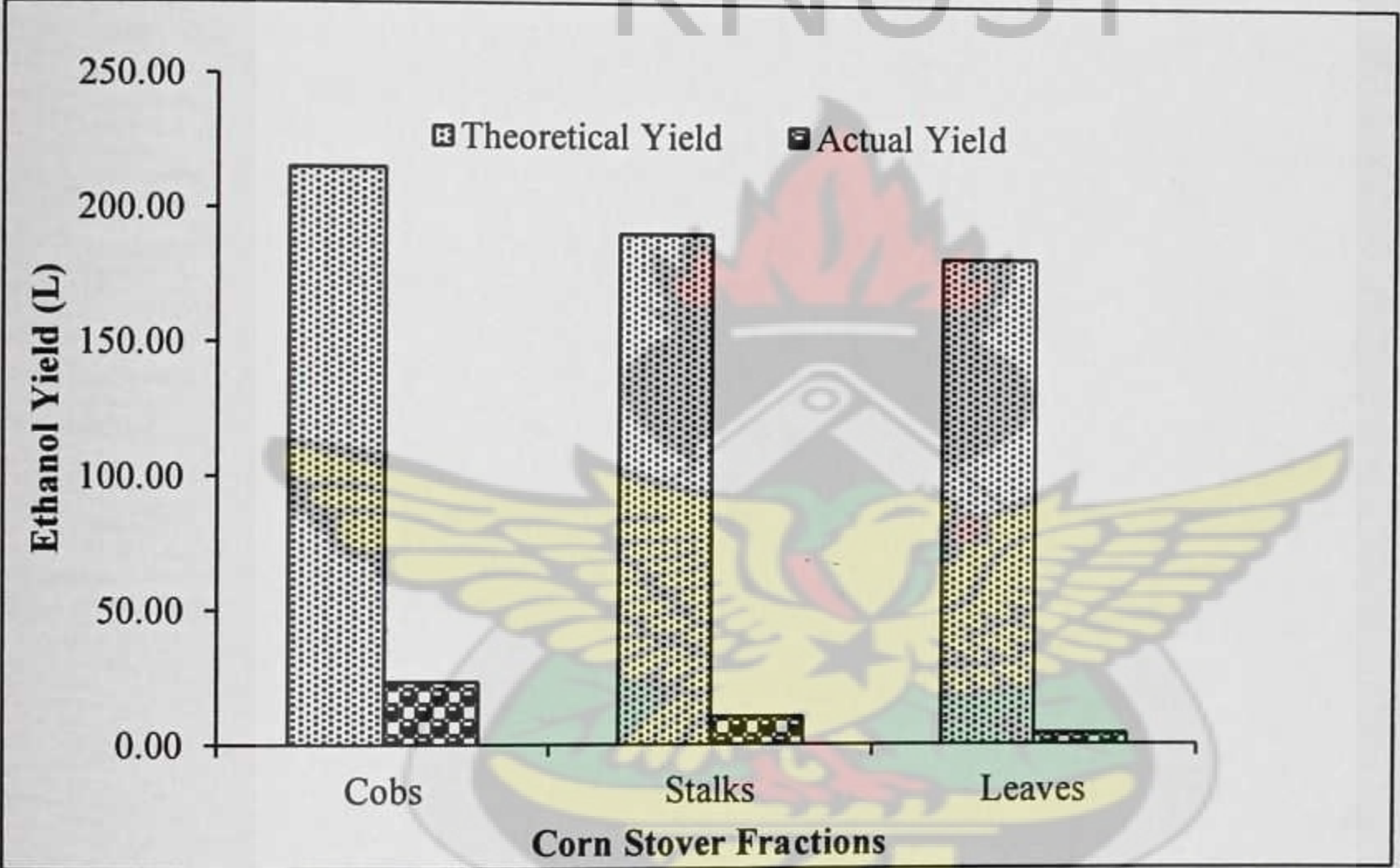


Figure 4.14: Comparison of theoretical and actual ethanol yield in liters per metric tonne of dry corn stover

Considering the ethanol yield obtained in liters per metric tonne of corn stover (on dry weight basis), Figure 4.14 was drawn to compare the theoretical ethanol yield and the practical ethanol yield. The theoretical yield of ethanol expected based on the characteristics of the corn cobs, corn stalks and corn leaves were 215.18, 190.52, and 181.69 L/metric tonne and the practical



ethanol yield obtained were 23.52, 10.58 and 1.07 L/metric tonne representing 10.9, 5.4 and 2.3 % of the theoretical respectively. The actual ethanol yields obtained in  $\text{LKg}^{-1}$  of dry corn stover were 0.0235, 0.0106, and 0.0011  $\text{LKg}^{-1}$  of corn cobs, stalks and leaves respectively. The ethanol yields obtained were very low compared to the 0.29  $\text{LKg}^{-1}$  and 25 g/L reported by Kim and Dale (2004) and Ohgren et al, (2006).

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## 4.6 SUMMARY OF MAIN FINDINGS

### Estimation of Corn Stover Quantities in Ghana

1. The dried corn stover (cobs, stalks, leaves and husks) yield per hectare was calculated to be 3.35 metric tonnes.
2. Among the 18 local maize varieties assessed, *Aburohema* produced the highest yield of maize and corn stover whilst *Pioneer 30 B 74* produced the lowest yield of maize and corn stover.
3. The corn, cobs and the stalks, leaves and husks represented 46.30 %, 9.40 % and 44.30 % respectively of the maize plant on dried weight basis.
4. Plant fraction ratios of corn to total dried matter, cobs to total dried matter and stalks, leaves and husks to total dried matter ratio were found to be 0.46, 0.09 and 0.44 respectively.
5. The stover to corn (i.e. crop residue to product) ratio obtained in this work was found to be 1.16
6. Average corn stover production per year in Ghana was estimated to be  $1,512.22 \times 10^3$  metric tonnes.
7. A simple model (or equation) was derived based on the stover to corn ratio to calculate the quantities of Maize Biomass (corn stover) Available (MBA) given the maize production (z):

$$MBA = r \times z$$

The  $r$  is the residue to product ratio (which was found to be 1.16) and  $z$  is the maize production.



### Characterization of Corn Stover

8. The chemical characteristics of the corn stover fractions from *Pioneer 30 W 40* local maize variety are as follows: cellulose 35.58, 42.41, 31.17, and 41.26 %, hemicellulose 28.91, 11.78, 31.57 and 10.20 %, and lignin 29.61, 30.62, 30.54 and 30.96 % for corn cobs, stalks, husks and leaves respectively
9. The overall chemical characteristics of the corn stover are as follows: total solids 93.31 %, moisture 6.69 %, extractives 6.31 %, lignin 30.50 %, cellulose 37.72 % and hemicellulose 20.62 %.

### Pretreatment and Enzymatic Hydrolysis of Corn Stover

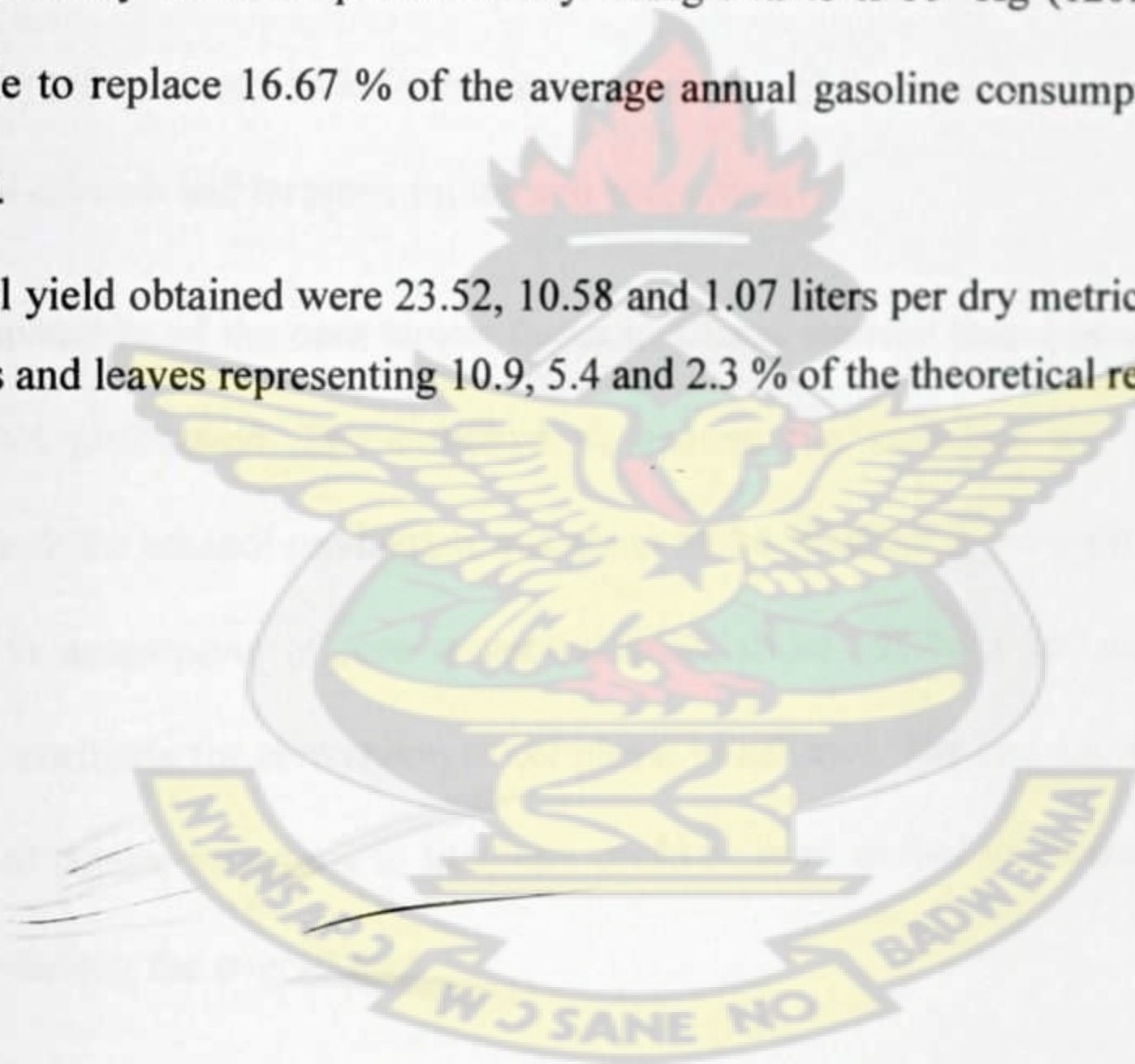
10. Autoclaving of corn stover with 3.0 % NaOH for 120 min at 5 % solid load was the optimal pretreatment method obtained and it gave the highest cellulose content of 81.27 and delignification of 84.67 %.
11. The sodium hydroxide load or concentration in the pretreatment process was observed to be the most sensitive pretreatment parameter compared to time, solid to liquid ratio and temperature.
12. The pretreated corn stalks recorded the highest cellulose content of 81.27 % and the highest delignification of 84.67 %. Pretreated corn cobs, leaves and husks recorded their highest cellulose contents of 78.85, 67.40, 73.44 % and their highest delignification of 68.9, 79.25, 79.82 % respectively. Thus, the pretreated corn stover was rich in cellulose and has high level of glucose for fermentation to produce bioethanol.
13. The FT-IR spectra of the pretreated corn stover showed a major reduction in the intensities of the peaks at  $2000 - 1000 \text{ cm}^{-1}$  indicating reduction in the lignin content.



14. The total reducing sugars concentration obtained were 157.96, 128.75, and 28.05 mg/mL for corn cobs, stalks and leaves respectively

#### Theoretical and Practical Ethanol Yield

15. Based on the carbohydrate content of the overall corn stover, the average theoretical ethanol yield per one dry tonne (1000 Kg) of corn stover is 198.95 L
16. 40 % (i.e. 604.84 thousand metric tonnes =  $604.84 \times 10^6$  Kg) of the average 1,512.09 thousand metric tonnes (1,512.09 Kilotonnes =  $1,512.09 \times 10^6$  Kg) of dry corn stover produced annually have the potential of yielding  $94.945 \times 10^6$  Kg ( $120.33 \times 10^6$  L) of ethanol able to replace 16.67 % of the average annual gasoline consumption of 569.38 Kilotonnes.
17. The ethanol yield obtained were 23.52, 10.58 and 1.07 liters per dry metric tonne of corn cobs, stalks and leaves representing 10.9, 5.4 and 2.3 % of the theoretical respectively.





## 5.0 CONCLUSIONS AND RECOMMENDATIONS

### 5.1 CONCLUSIONS

Corn stover has been shown in this work to be a promising feedstock useful for bioethanol production in Ghana. About  $1,512.22 \times 10^3$  metric tonnes of dry corn stover was estimated to be available per year in Ghana. However, only 40 % of the quantity produced (i.e.  $604.84 \times 10^3$  metric tonnes) was assumed to be available for conversion into cellulosic bioethanol. The reason for this assumption was the accessibility and soil erosion factors. The corn stover is scattered in farms and collection of it will present an economic cost which will add to the overall cost of production. And also, it is necessary to leave some percentage of the corn stover produced on the farm to prevent soil erosion and improve on the soil properties.

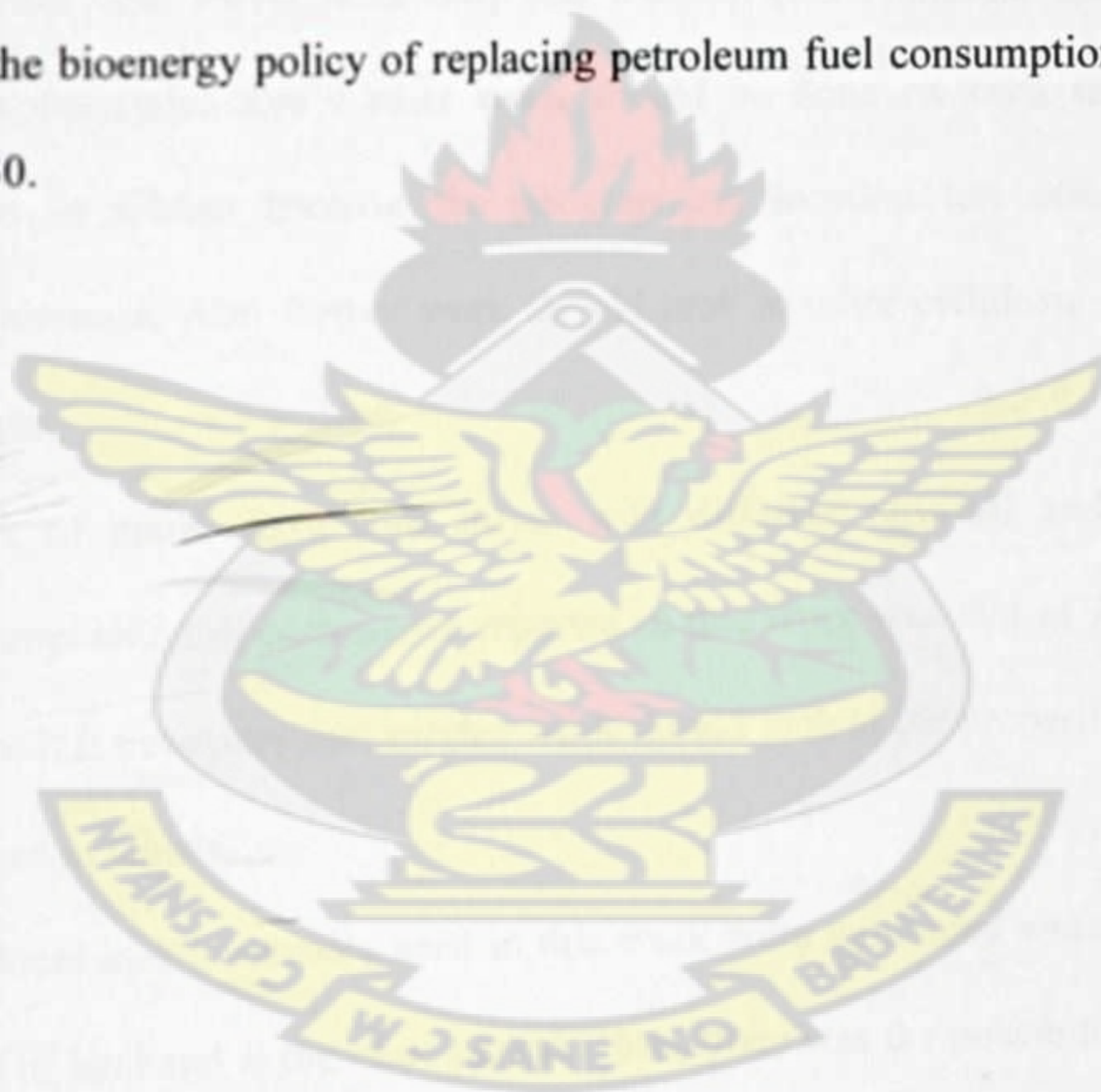
The chemical composition of the corn stover found in Ghana showed that they are suitable for cellulosic bioethanol production. The carbohydrate content (i.e. cellulose and hemicellulose) which is the feedstock for ethanol production was about 58.34 % of the dry weight. This revealed that, given the 40 % assumption of corn stover available, about  $352.86 \times 10^3$  metric tonnes of carbohydrate were available for conversion to cellulosic bioethanol. The rest i.e. 41.66 % which comprised mainly of lignin, according to literature could be used as fuel to produce heat for the process, thereby, reducing the overall cost.

Pretreating the corn stover with 3.0 % NaOH for 120 minutes at 5 % solid load and 121 °C yielded a substrate rich in cellulose of about 81.27 % and low lignin of 4.83 %. The enzymatic hydrolysis of the cellulose rich substrate yielded maximum reducing sugar concentration of 157.96 mg/mL. The maximum percentage conversion was 60.29 % and the minimum was 38.25 % which indicated that most parts of the cellulose were broken down to fermentable sugars.



Theoretically, it was possible to obtain about 198.95 liters of ethanol per metric tonne of dry corn stover, however, practically only about 10.9 % of the theoretical yield was achieved.

According to this work, about 16.67 % of the mean annual gasoline consumption can be replaced with bioethanol produced *only* from the quantities of corn stover produced in Ghana. Hence, this work sets the platform of bridging the research gaps related to cellulosic bioethanol production and attempts to achieve the bioenergy policy of replacing petroleum fuel consumption by 10 % by 2020 and 20 % by 2030.





## 5.2 RECOMMENDATIONS

This work was intended to determine the potential of producing bioethanol from corn stover in larger quantities and blending with gasoline; however, this objective was not fully met. The following recommendations are given to inform future work in order to achieve this objective:

1. This work used only corn stover from only one location (i.e. Fumesua, Kumasi) and therefore it is recommended that further work should be done on corn stover from different locations in Ghana because the geographical location has effect on the properties of the biomass. Also further work should look at other cellulosic bioethanol feedstocks in Ghana.
2. Different varieties of maize have corn stover with different physical and chemical properties. The composition of corn stover reported in this work was that of *Pioneer 30 W 40* and therefore it is necessary that another work should look at the properties of other local maize varieties in Ghana.
3. The 18 different local maize varieties used in this work were cultivated simultaneously on the same piece of land and at the same period. Thus, there was the possibility of cross pollination among the different maize varieties and that may have affected the properties of the corn stover produced. Therefore, it is recommended that further work should be done to look at cultivating the different maize varieties on different piece of land to reduce the effect of cross pollination.
4. Corn stover in this work was assumed to be cheap and substantially available in Ghana. However, no work was done to evaluate the cost of collection and transporting to the cellulosic bioethanol industry site. Collection of corn stover from the farms will be a difficult job since about 90 % of farm holdings are less than 2 hectares. Therefore, further



work should be done to develop models of collection of corn stover in Ghana and also to estimate the cost of collection and transport.

5. In this work, the pretreatment process utilized only one type of catalyst (i.e. sodium hydroxide) because of its cheapness. It is therefore necessary to look at other relatively cheap catalysts and also catalysts that are locally available in Ghana (e.g. potash from cocoa pod husks or plantain peels) for pretreating corn stover.
6. Also, this work used only one type of cellulase enzyme donated by Novozymes, (Denmark) for breaking down the corn stover to sugars. It is therefore expedient to use other cellulase from different microorganisms and also from different enzyme producing companies. This will aim at testing the activities of different enzyme combinations on corn stover produced in Ghana in order to select which is efficient on the local feedstock.
7. It is also necessary to develop cellulase enzymes from local microorganisms which can be incorporated into the cellulosic bioethanol industry in Ghana in order to reduce the overall cost of production.
8. This work used common baker's yeast for conversion of the sugars to ethanol. However, baker's yeast is *Saccharomyces Cerevesie* microorganisms which only ferment glucose to ethanol. It is therefore recommended that further work should be done to look at other microorganisms (in addition to *Saccharomyces Cerevesie*) able to ferment especially xylose sugar to ethanol which was available in relatively large quantities in the fermentation broth.
9. This work used approximate methods in the determination of sugars and alcohols. It is therefore recommended that further work should be done using Chromatographic



methods or other methods which separates the different sugars and alcohols available in the fermentation broth.

10. Finally, since economic factors are important in determining the feasibility of a project, it is therefore recommended that, further work should look at the socio-economic advantages and disadvantages of cellulosic bioethanol production in Ghana using corn stover as feedstock.

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## APPENDICES

### Appendix 1: Fuel properties comparison for bioethanol and gasoline

Property	Bioethanol	Gasoline
Chemical Formula	C <sub>2</sub> H <sub>5</sub> OH	C <sub>4</sub> to C <sub>12</sub>
Molecular Weight	46.07	100–105
Carbon	52.2	85–88
Hydrogen	13.1	12–15
Oxygen	34.7	0
Specific gravity, 60° F/60° F	0.796	0.72–0.78
Density, lb/gal @ 60° F	6.61	6.0–6.5
Boiling temperature, °F	172	80–437
Reid vapor pressure, psi	2.3	8–15
Research octane no.	108	90–100
Motor octane no.	92	81–90
(R + M)/2	100	86–94
Cetane no.(1)	--	5–20
Fuel in water, volume %	100	Negligible
Water in fuel, volume %	100	Negligible
Freezing point, °F	-173.2	-40
Centipoise @ 60° F	1.19	0.37–0.44 <sup>b</sup>
Flash point, closed cup, °F	55	-45
Auto ignition temperature, °F	793	495
Lower	4.3	1.4
Higher	19	7.6
Btu/gal @ 60° F	2,378	≈900
Btu/lb @ 60° F	396	≈150
Btu/lb air for stoichiometric mixture @ 60° F	44	≈10
Higher (liquid fuel-liquid water) Btu/lb	12,800	18,800–20,400
Lower (liquid fuel-water vapor) Btu/lb	11,500	18,000–19,000
Higher (liquid fuel-liquid water) Btu/gal	84,100	124,800
Lower (liquid fuel-water vapor) Btu/gal @ 60° F	76,000 <sup>b</sup>	115,000
Mixture in vapor state, Btu/cubic foot @ 68° F	92.9	95.2
Fuel in liquid state, Btu/lb or air	1,280	1,290
Specific heat, Btu/lb °F	0.57	0.48
Stoichiometric air/fuel, weight	9	14.7 <sup>b</sup>
Volume % fuel in vaporized stoichiometric mixture	6.5	2

<sup>a</sup>Pour Point, ASTM D 97, <sup>b</sup>Calculated, <sup>c</sup>Based on Cetane, Source: U.S. Department of Energy, Office of Energy

Efficiency and Renewable Energy, Alternative Fuels Data Center,



## Appendix 2: Biofuels Targets and Blending Mandate

Country	Biofuel Targets	Blending Mandates
Australia	350 million liters of biofuels by 2010	E2 in New South Wales, increasing to E10 by 2011; E5 in Queensland by 2010
Argentina		E5 and B5 by 2010
Bolivia		B2.5 by 2007 and B20 by 2015
Brazil		E22 – E25 existing (slight variation over time); B3 by 2008 and B5 by 2013
Canada		E5 by 2010 and B2 by 2012; E7.5 in Saskatchewan and Manitoba; E5 by 2007 in Ontario
Chile		E5 and B5 by 2008 (voluntary)
China	12 million metric tonnes of biodiesel by the year 2020	E10 in 9 provinces
Colombia		E10 and B10 existing
Dominican Republic		E15 and B2 by 2015
Germany	5.75% share of biofuels in transport by 2010; 10% by 2020	E5.25 and B5.25 in 2009; E6.25 and B6.25 from 2010 through 2014
India		E5 by 2008 and E20 by 2018; E10 in 13 states/territories
Italy	5.75% share of biofuels in transport by 2010; 10% by 2020	E1 and B1
Jamaica		E10 by 2009
Japan	20% of total oil demand met with biofuels by 2030; 500 million liters by 2010	
Korea		B3 by 2012
Malaysia		B5 by 2008
New Zealand	3.4% total biofuels by 2012	
Paraguay		B1 by 2007, B3 by 2008 and B5 by 2009, E18 or higher (existing)
Peru		B2 in 2009; B5 by 2011; E7.8 by 2010
Philippines		B1 and E5 by 2008; B2 and E10 by 2011
South Africa		E8 – E10 and B2 – B5 (proposed)
Thailand	3% of biodiesel share by 2011; 8.5 million liters of biodiesel production by 2012	E10 by 2007 and B10 by 2012
United Kingdom		E2.5/B2.5 by 2008 ; E5/B5 by 2020
United States	130 billion liters/year of biofuels nationally by 2022; 3.4 billion liters/year by 2017 pennsylvanis	E10 in Iowa, Hawaii, Missouri, and Montana; E20 in Minnesota; B5 in New Mexico; E2 and B2 in Louisiana and Washington State
Uruguay		E5 by 2014; B2 from 2008 – 2011 and B5 by 2012

E= Ethanol, B= Biodiesel (Source: Timilsina and Shrestha, 2011)



### Appendix 3: Cellulosic Bioethanol Pilot and Demonstration Plants

Company	Location	Feedstock	Capacity or feed rate	Start date
<b>Pilot plants</b>				
Iogen	Ottawa, Canada	Wood chips	$9.0 \times 10^2 \text{ kgday}^{-1}$	1985
Iogen	Ottawa, Canada	Wheat straw	$9.0 \times 10^2 \text{ kgday}^{-1}$	1993
Masada/TVA	Muscle Shoals, AL	Wood	NA	1193
SunOpta	Norval, Canada	Various (non-wood)	$4.5 \times 10^2 \text{ kgh}^{-1}$	1995
Arkenol	Orange, CA	Various	$9.0 \times 10^2 \text{ kgday}^{-1}$	1995
Bioengineering Resources	Fayetteville, AR	Softwood & bark	NA	1998
NREL/DOE	Golden, CO	Corn stover, others	$9.0 \times 10^2 \text{ kgday}^{-1}$	2001
Pearson Technologies	Aberdeen, MS	Wood residues, rice straw	$27 \text{ Mgday}^{-1}$	2001
NEDO	Izumi, Japan	Wood chips	$3.0 \times 10^2 \text{ lday}^{-1}$	2002
Dedini	Pirassununga, Brazil	Bagasse	$1600 \text{ m}^3 \text{ year}^{-1}$	2002
Tsukishima Kikai Co.	Ichikawa, Chiba, Japan	Wood residues	$9.0 \times 10^2 \text{ kgday}^{-1}$	2003
Etek Ethanol Teknik	Ornskoldsvik, Sweden	Spruce sawdust	$5.0 \times 10^2 \text{ lday}^{-1}$	2004
Pure Vision	Ft. Lupton, CO	Corn stover, bagasse	$9.0 \times 10^2 \text{ kgday}^{-1}$	2004
Universal Entech	Phoenix, AZ	Municipal garbage	$1.0 \times 10^2 \text{ lday}^{-1}$	2004
Sicco A/S	Odense, Denmark	Wheat straw	$1.0 \times 10^2 \text{ kgh}^{-1}$	2005
Abengoa Bioenergy	York, NE	Corn stover (co-located with grain ethanol plant)	$2000 \text{ m}^3 \text{ year}^{-1}$	2006
<b>Demonstration plants</b>				
Iogen	Ottawa, Canada	Wheat, oat and barley straw	$3000 \text{ m}^3 \text{ year}^{-1}$	2004
ClearFuels Technology	Kauai, HI	Bagasse and wood residues	$11,400 \text{ m}^3 \text{ year}^{-1}$	2007
Celunol	Jennings, LA	Bagasse, rice hulls (co-located with grain ethanol plant)	$5000 \text{ m}^3 \text{ year}^{-1}$	2007
Etek Etanol Teknik	Sweden	Softwood residues (spruce and pine)	$30,000 \text{ m}^3 \text{ year}^{-1}$	2009

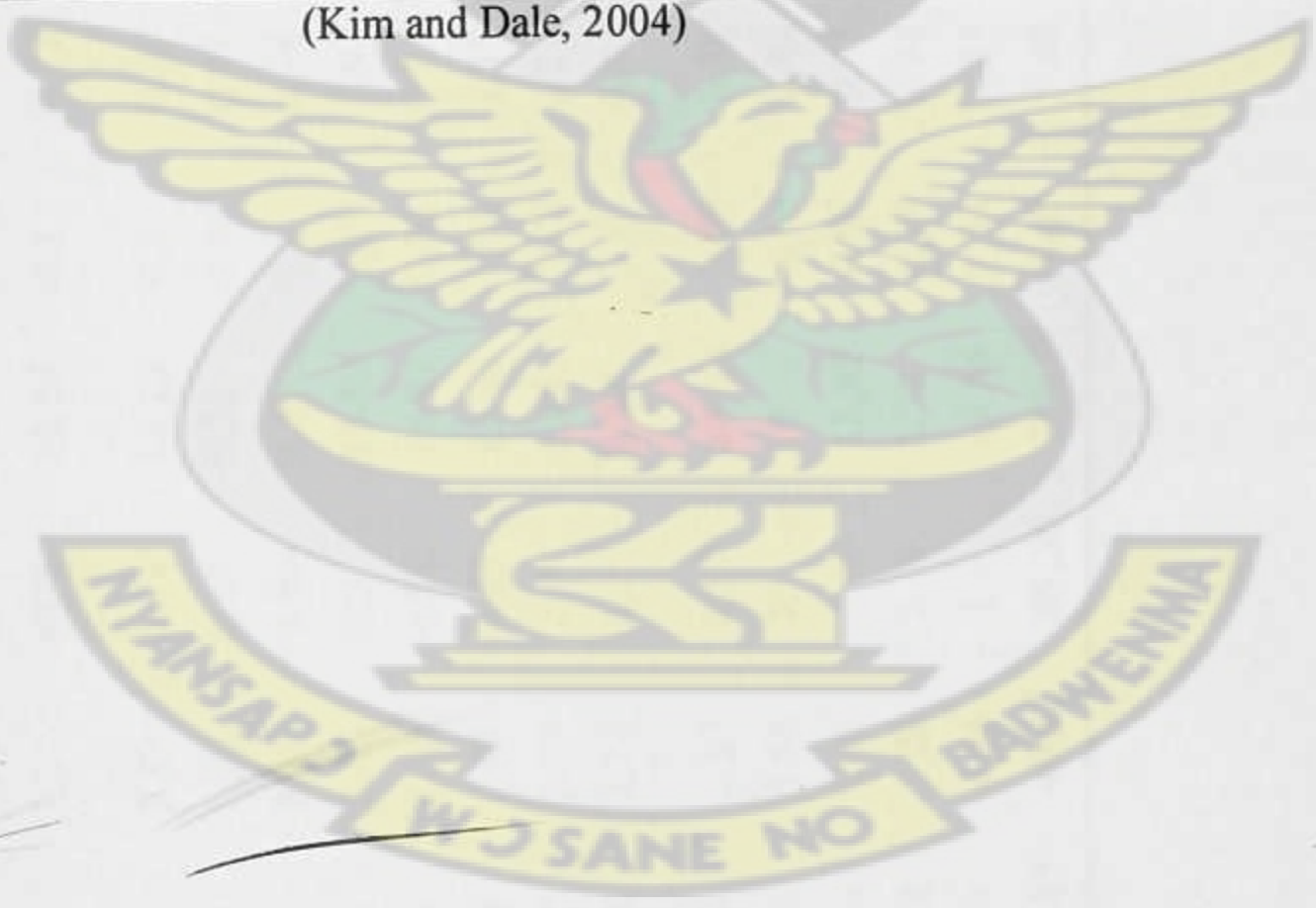
Source: Solomon et al, 2007



**Appendix 4: Ethanol yield of some common crops and their residues**

Crop	Residue/crop ratio	Dry matter (%)	Lignin (%)	Carbohydrates (%)	Ethanol yield (L kg <sup>-1</sup> of dry biomass)
Barley	1.2	88.7	2.90	67.10	0.41
Barley Straw		81.0	9.00	70.00	0.31
Corn	1	86.2	0.60	73.70	0.46
Corn stover		78.5	18.69	58.29	0.29
Oat	1.3	89.1	4.00	65.60	0.41
Oat straw		90.1	13.75	59.10	0.26
Rice	1.4	88.6		87.50	0.48
Rice straw		88.0	7.13	49.33	0.28
Sorghum	1.3	89.0	1.40	71.60	0.44
Sorghum straw		88.0	15.00	61.00	0.27
Wheat	1.3	89.1		35.85	0.40
Wheat straw		90.1	16.00	54.00	0.29
Sugarcane		26.0		67.00	0.50
Bagasse	0.6	71.0	14.50	67.15	0.28

(Kim and Dale, 2004)



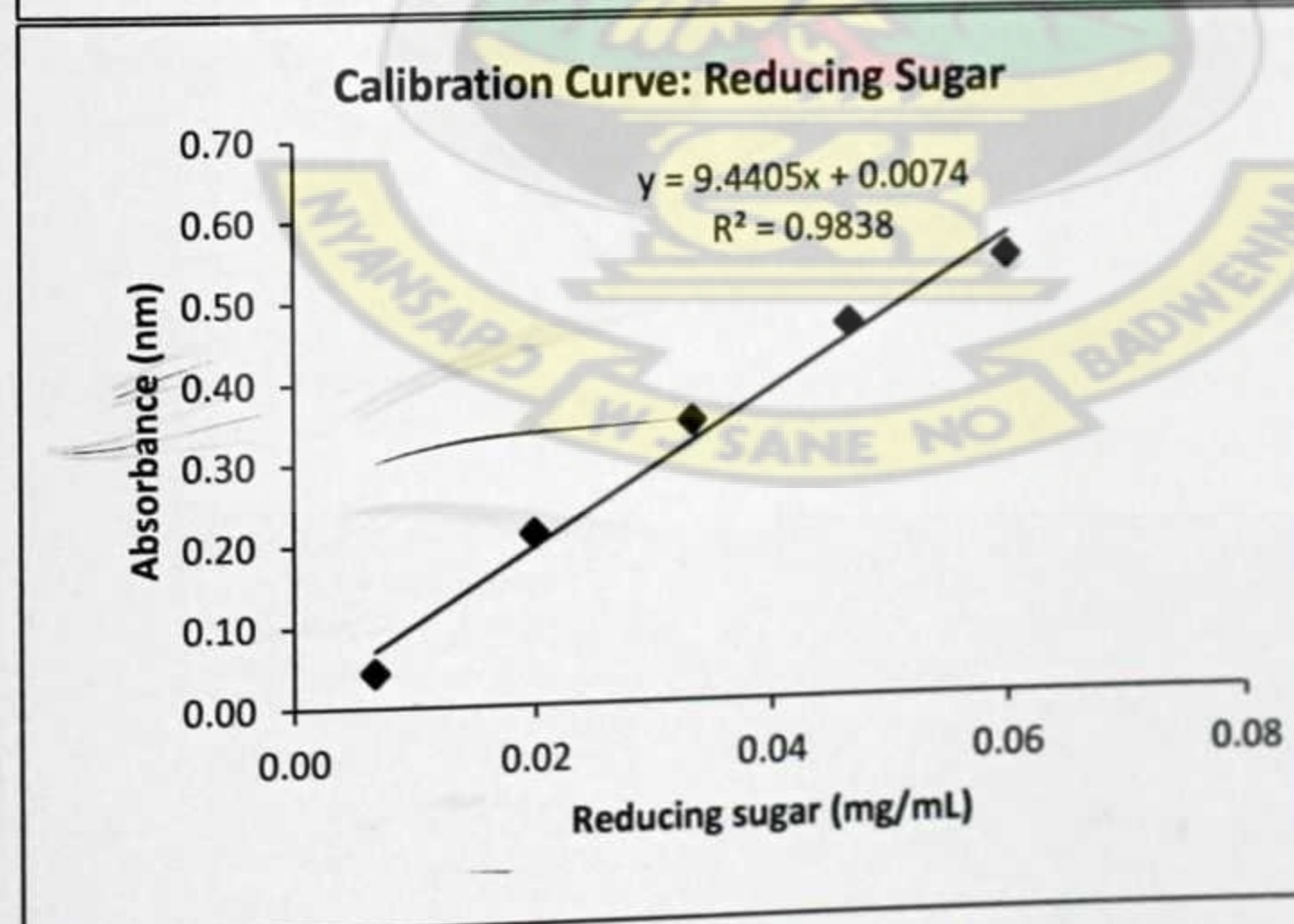
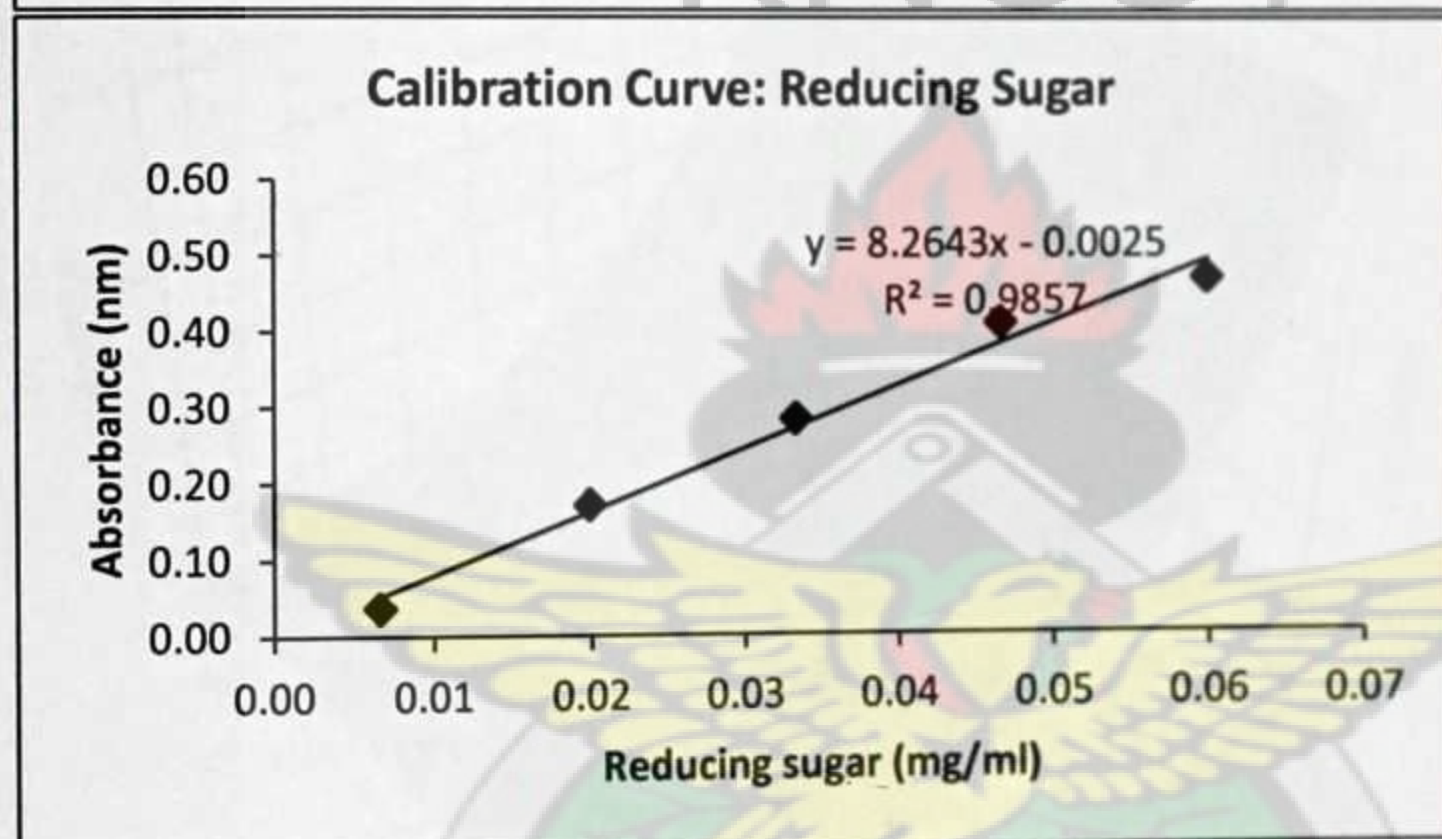
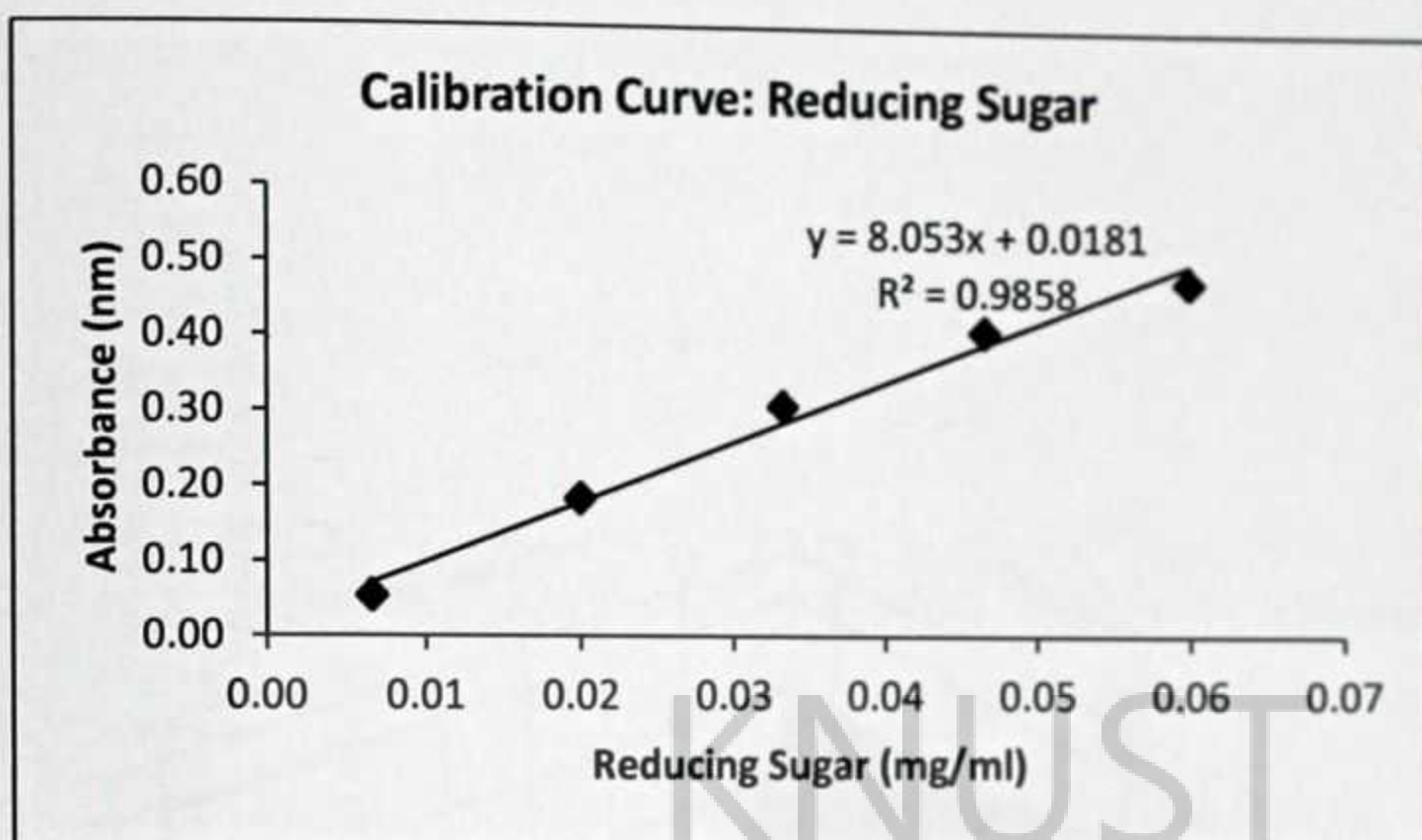


Appendix 5: Yield of biomass on dry weight (Kg)

Maize Variety	Grain Yield				Cobs Yield				Stalk, leaves & husks Yield (Kg)						
	REP	REP	REP	Total	REP	REP	REP	Total	REP	REP	REP	REP	REP	REP	Total
	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3
Pioneer 30 W 40	4.55	2.19	1.64	3.39	1.07	0.41	0.3	0.59	4.54	1.97	1.48	3.06	11.05		
Kwadaso Local	3.65	1.44	2.33	1.99	1.01	0.3	0.47	0.36	3.88	1.31	2.13	1.75	9.07		
Fu 20 - 90 DWDP	3.23	2.7	1.98	1.12	0.65	0.53	0.36	0.24	3.23	2.57	1.81	1.04	8.65		
Pioneer 30 Y 87	3.43	2.25	4.06	2.77	0.59	0.47	1.13	0.53	3.12	2.3	4.65	2.57	12.64		
TZE - Y- Pop DT	1.94	2.55	3.27	4.08	0.3	0.53	0.59	0.89	1.7	2.35	3.23	3.77	11.05		
STR C4															
Fu 20 - 90 DYFP	1.47	2.23	3.37	3.16	0.3	0.53	0.77	0.53	1.48	2.35	3.56	2.9	10.29		
GH 90 DYF Pop	2.97	2.19	2.02	2.03	0.53	0.41	0.41	0.36	2.9	1.97	1.86	1.86	8.59		
Omarkwa	1.83	1.93	3.17	1.96	0.3	0.36	0.71	0.36	1.59	1.75	3.39	1.75	8.48		
Pioneer 30 F 32	2.26	3.98	3.4	3.5	0.47	1.07	0.53	0.77	2.13	3.94	2.95	3.5	12.52		
Aburohemaa	2.98	3.53	2.58	4.92	0.53	0.71	0.47	1.13	2.84	3.28	2.3	5.03	13.45		
Dorke SR	2.66	5.58	1.05	3.79	0.53	1.13	0.24	0.77	2.52	5.69	0.98	3.61	12.8		
Pioneer 30 B 74	2.26	1.13	3.15	2.09	0.47	0.24	0.53	0.41	2.08	1.04	2.79	1.86	7.77		
TZE - W- Pop DT	1.93	2.44	2.27	2.2	0.3	0.47	0.41	0.41	1.7	2.24	2.02	1.97	7.93		
STR C3															
Pioneer 30 B 80	3.36	1.98	3.27	2.17	0.65	0.36	0.59	0.47	3.23	1.81	3.01	2.08	10.13		
GH 90 DWD Pop	3.07	2.08	3.55	2.3	0.59	0.41	0.83	0.47	3.01	1.91	3.72	2.13	10.77		
Pioneer 30 F 96	3.32	3.34	2.67	3.49	0.59	0.71	0.53	0.83	3.06	3.34	2.46	3.61	12.47		
Pioneer 30 K 73	1.01	3.28	2.06	2.39	0.24	0.71	0.41	0.47	0.98	3.28	2.02	2.19	8.47		
Pioneer 30 D 55	2.48	2.69	4.08	2	0.53	0.53	0.83	0.41	2.35	2.52	3.72	1.86	10.45		
Total:	48.4	47.51	49.92	49.35	195.18	9.65	9.88	10.11	10.00	46.34	48.08	46.54	186.58		

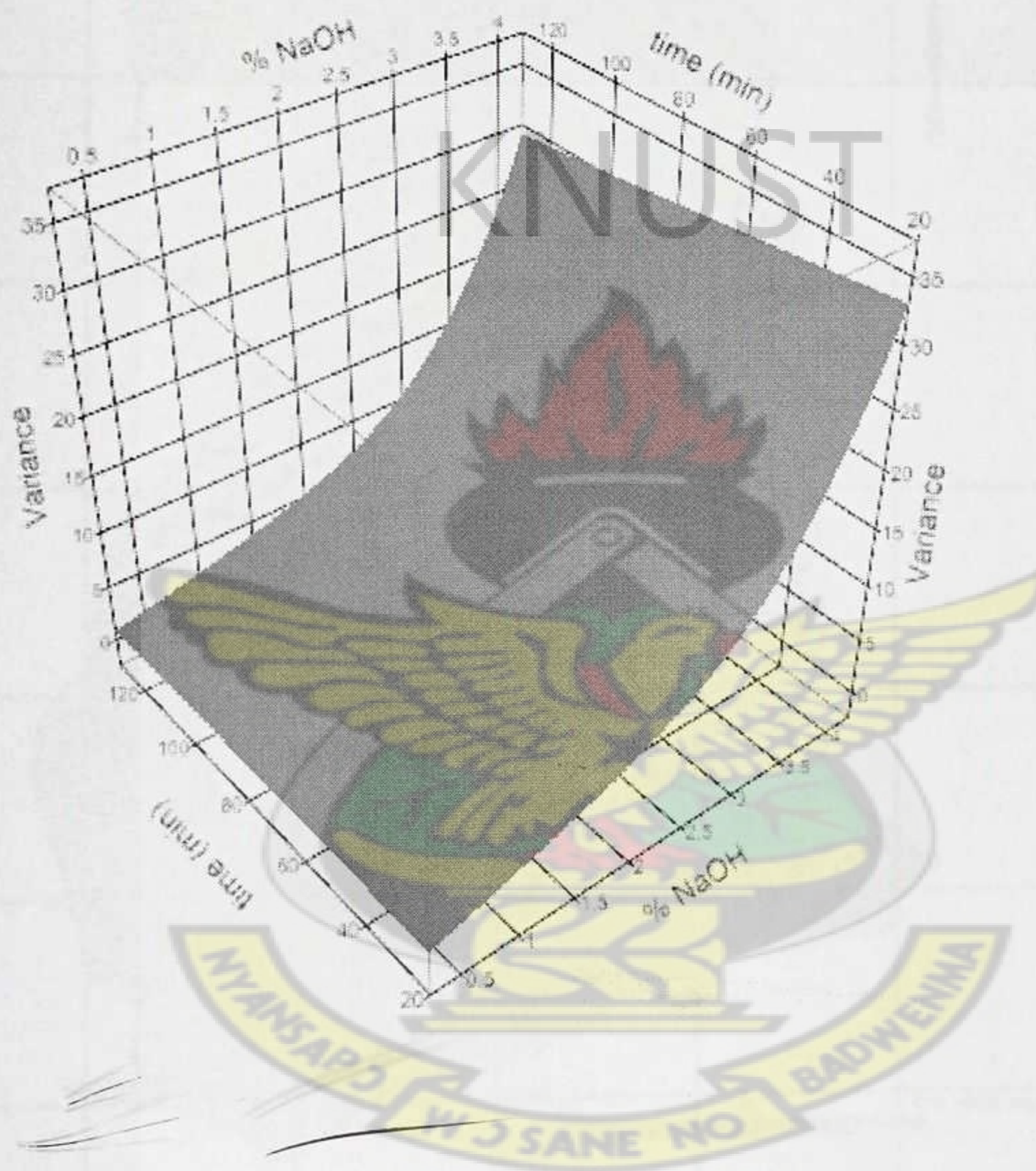


## Appendix 6: Calibration Curves



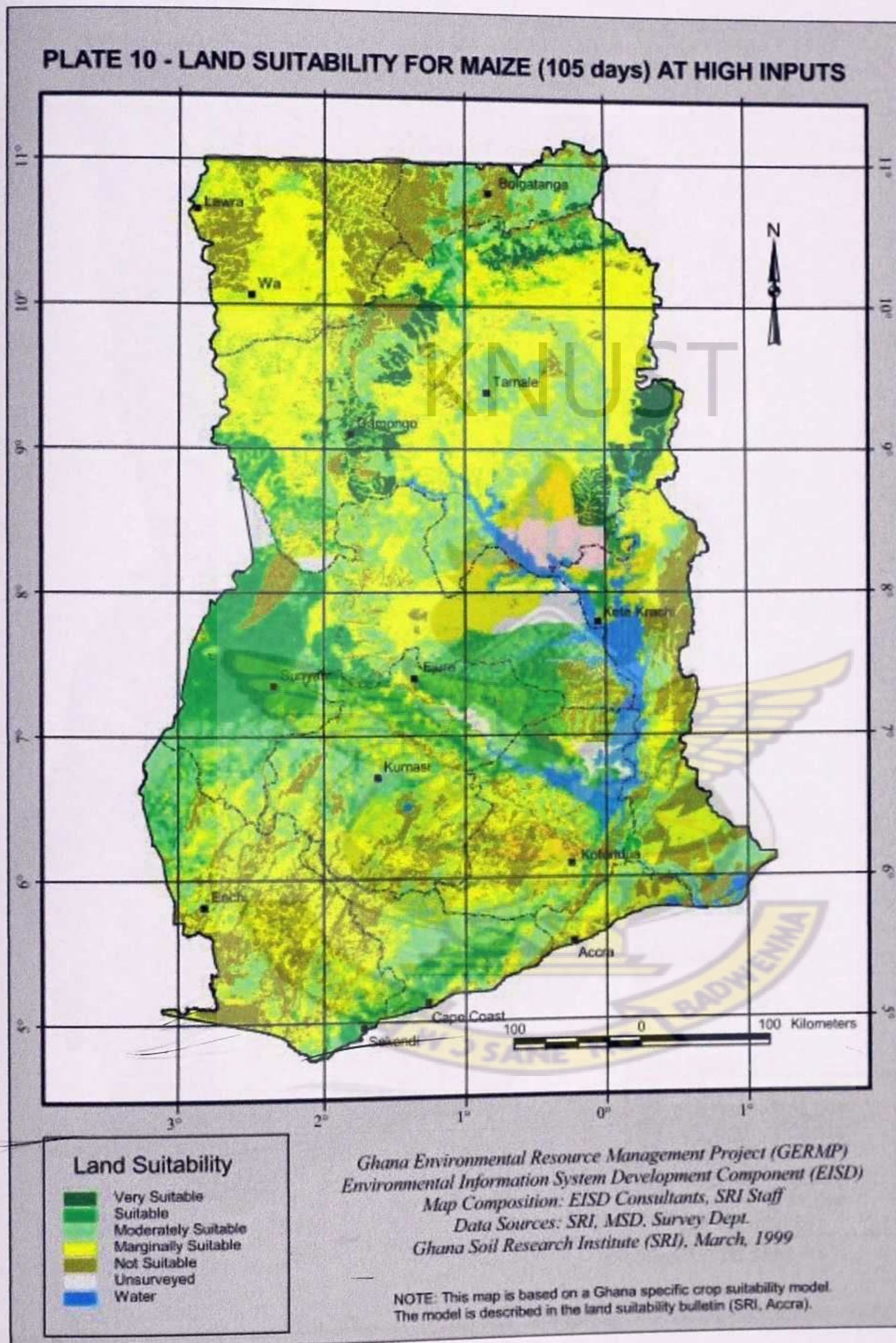


**Appendix 7: Effect of NaOH load and Time on the Cellulose Content**



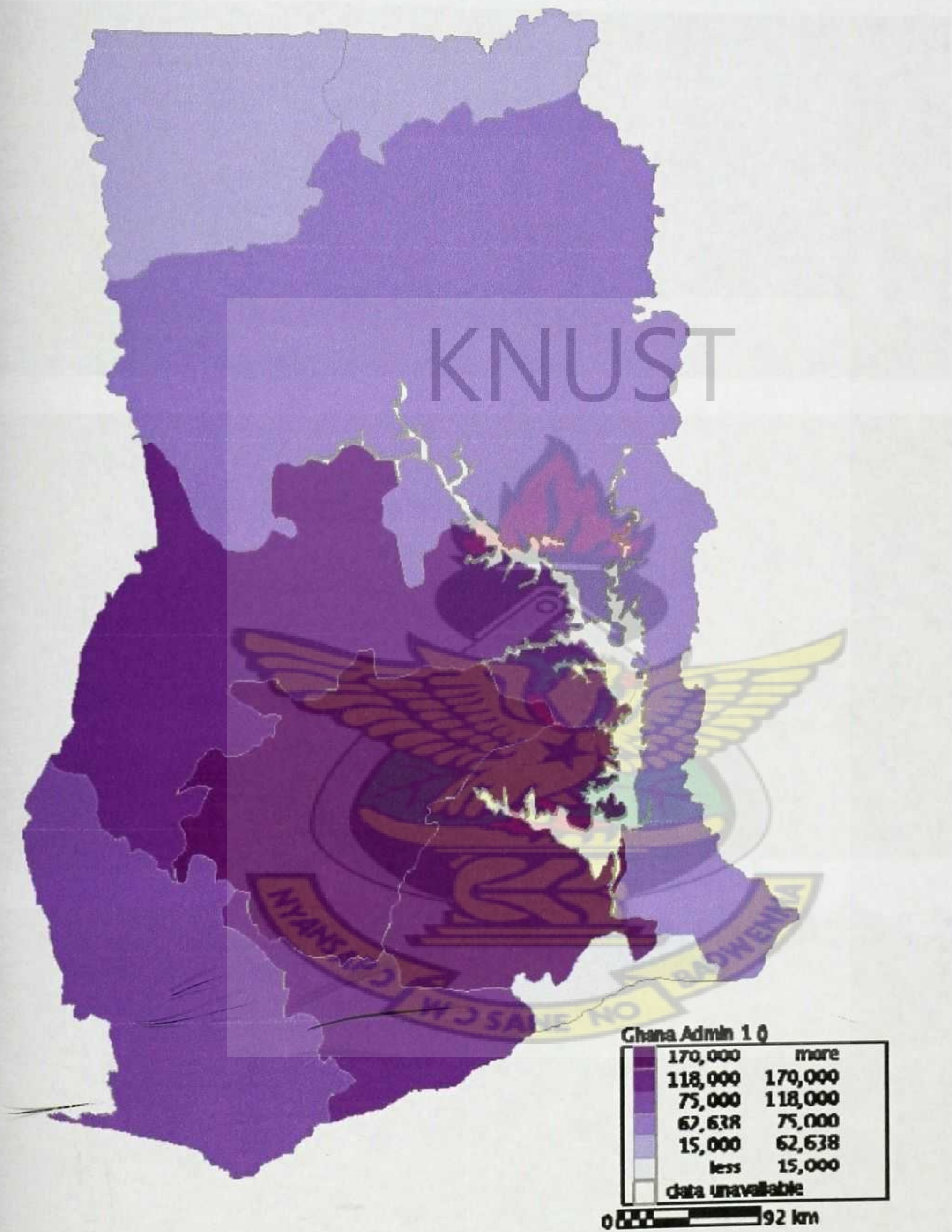


## Appendix 8 (a): Maize Land Suitability





Appendix 8 (b): Maize Production Map



Source: Maize, Soya and Rice Production and Processing, Millennium Development Authority (MiDA)



Appendix 9: Sampling Photographs





