OPTIMIZATION OF SUGAR PRODUCTION FROM SWEET SORGHUM STALKS (BAGASSE) FOR BIOETHANOL-PRODUCTION

BY

ALEKE, ANN UKAMAKA

B.Sc (UNN) (MSC/SCIEN/06645/06-07)

A RESEARCH THESIS SUBMITTED TO THE POSTGRADUATE SCHOOL, AHMADU BELLO UNIVERSITY, ZARIA, NIGERIA IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF MASTER DEGREE (M.Sc) IN BIOCHEMISTRY

DEPARTMENT OF BIOCHEMISTRY,
FACULTY OF SCIENCE,
AHMADU BELLO UNIVERSITY,
ZARIA, NIGERIA

DECEMBER, 2011

DECLARATION

The research work presented in this thesis titled **"Optimization of Sugar Production from Sweet Sorghum stalks (bagasse) for Bioethanol-Production"** has been performed by me in the Department of Biochemistry under the supervision of **Professor, H.C. Nzelibe and Dr E. Onyike.** The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this thesis was previously presented for another degree or diploma at any university.

Aleke, Ann Ukamaka **----------** **----------**

MSC/SCIEN/06645/2006-2007 **Signature** **Date**

CERTIFICATION

This research thesis entitled **"Optimization of Sugar Production from Sweet Sorghum stalks (bagasse) for Bioethanol-Production" by Aleke, Ann Ukamaka** meets the regulations governing the award of degree of Master of Science (M.Sc.) of Ahmadu Bello University, Zaria, and is approved for its contributions to knowledge and literary presentation.

---------- ----------

Prof. H.C. Nzelibe **Date**

(Chairman, Supervisory Committee)

---------- ----------

Dr. E. Onyike **Date**

(Member, Supervisory Committee)

---------- ----------

Dr. H. M. Inuwa **Date**

(Head, Department of Biochemistry)

---------- ----------

Prof. A. A. Joshua **Date**

(Dean, School of Postgraduate Studies)

DEDICATION

I dedicate this thesis to Almighty God for all His mercies, grace, and the divine strength which he provided that pulled me through.

ACKNOWLEDGEMENTS

I thank God for his super abundant mercies, protection, provisions, grace and guidance in all my endeavours especially at the period of my research work. If not for him, I would have been forgotten.

I highly appreciate the contributions, suggestions and care of my supervisors, Prof. H.C Nzelibe and Dr. E. Onyike towards the success of this work and for creating time out of their busy schedule to attend to me anytime I needed assistance. I also appreciate the Head of Department, Dr. H.M. Inuwa for her all round support and the Department for giving me the opportunity to use her facilities.

I also appreciate the kindness of Prof. S. E. Atawodi and Dr. S. Ibrahim who in many occasions have asked about the level of progress in my work. I thank Dr. D. B. James and Dr. Balarabe Salau for their encouragement. I appreciate the contributions, assistance, advice and suggestions of Mr. G.C. Njoku, Mr. Samuel Ndidi Uche, Mr. Idowu Amiola and Mr. Nathan Habila and other lecturers in the Department of Biochemistry Ahmadu Bello University Zaria. I will not fail to appreciate the following laboratory staff; Mr. Kolawole Enok (Chief Laboratory technologist), Mr. S. Jok, Mr. John Shaffa, Miss Jobin Amara, Mr. Yakubu Apeh and Mansir Aliyu for their assistance.

I also appreciate the following post graduate students of Department of Biochemistry; Melvin Emeka Ugwu, Azubuike Okafor, Jamiul Oritola, Abdulkarim Zubairu, Otuu Olumide, Mary Ann Abbas, Shuaibu Bala, Mal. Aminu Yusuf, Ernest Penn Awa, Charity Odoh , Godwin Ebiloma and others for their assistance and encouragement, may God reward you all. My profound

gratitude goes especially to Dr. Elijah Ella, Mrs. Eno Adayi, Mr. Pius Awodi, Mr. Onu John, and Mal. Ibrahim Shittu of department of microbiology, Mal. Jumare Ibrahim of department of Plant Science and Mr. Edward Adegbe of department of Food Science and Technology, Institute for Agricultural Research Ahmadu Bello Zaria for their support and provisions. I thank Mr. Celestine Ochibo of the Department of Chemistry, Mal. Adamu of Department of Pharmacognosy, Mrs. Augustina Ugwoke of Faculty of Veterinary medicine all in Ahmadu Bello University Zaria, for their assistance and support and the following students of Microbiology and Chemical Engineering; Grace Abakpa, Blessing Obasi, Dr. Jerry Yabefa and James for their assistance.

I dearly appreciate my lovely husband Dr. H.M. Ijeomah for his understanding, encouragement, moral and financial support throughout the period of my study.

I cannot forget the sacrifice, encouragement, moral supports, assistance and contributions of my dear parents; Mr. Elias and Ezinne Roseline Aleke and the prayers, encouragement and moral supports of my siblings; Sister Nneka, Brother Emeka Mclouis, Chinwe, Engr. Chinedu, Uche and Engr. Chibuike; my relations especially Aleke Okwudilichukwu John (O.J); and my friends especially Ezugwu Lebechukwu and Onyesi Chika; my inlaws Mrs. A. Ijeomah and Uche Ijeomah, I extend my gratitude to you all and I pray that God should reward all your good works in Jesus name.

I appreciate my employer Nasarawa State University (NSUK) for giving me an opportunity to study for my Master of Science degree in Biochemistry. The patience and efforts of the academic staff of Biochemistry and Molecular Biology Department, NSUK during the period of my study is commendable.

Abstract

Periodic field sampling for reducing sugar content of 14 Sweet Sorghum varieties at 5, 7 and 9 weeks of age were investigated using Dinitro Salicylic acid (DNS) method. Eight sweet sorghum varieties which comprise 3 Indian: E 36-1, ICSR 93034, SPV 422; 3 Nigerian: SWSV 2006-3, SWSV 2006-5-2, SWSV 2007-1; and 2 Indian crosses: ICSA 89002 X SPV 422 and ICSA X NTJ -2 were selected out of fourteen based on the order of increasing reducing sugar content. At ninth week, ICSA 89002 X SPV 422 had the reducing sugar value of 149.38 ± 1.53mg/ml which was significantly higher than the other varieties at $p<0.05$. Two sweet sorghum varieties: SWSV 2006-3 and SPV 422 were subsequently selected from the eight based on field performance, susceptibility to hydrolysis, % bagasse yield, cellulose, hemicelluloses and lignin content. Two pretreatment options which include: HCl and H_2O_2 were then investigated for effective optimization of glucose yield from the bagasse of two sweet sorghum varieties using glucose oxidase kit. The results obtained showed that the final optimal concentration and ratio of sample to pretreatment solution for acid pretreatment are 2%HCl concentrations and 1:50 (ratio of sample to 2%HCl) respectively for both varieties. At these conditions (2%HCl and 1:50), the temperature; 121^oC released the highest % glucose yield of 15.36 ± 0.51 (from SWSV 2006-3) which is significantly higher than 10.36 ± 0.51 (of same variety) at 138^oC at $p<0.05$. These results also revealed that for acid pretreatment, the sweet sorghum variety; SWSV 2006-3 performed better than SPV422 at the temperatures investigated. The optimal concentration and ratio of sample to pretreatment solution for peroxide pretreatment are 3%H_2O_2 concentrations and 1:200 (ratio of sample to 3% H_2O_2) respectively for both varieties. At these conditions (3%H_2O_2 and 1:200), the temperature; 121^oC also released the highest % glucose yield of 87.14 ± 2.02 (from SPV 422) which is not significantly higher than 85.71 ±0.00 (of same variety) at 138^oC ($p<0.05$). These results also revealed that for peroxide pretreatment, the sweet sorghum variety; SPV422 performed better than SWSV 2006-3 at the temperatures investigated. H_2O_2 is a better pretreatment solution compared to HCl and NaOH. The % glucose yield released from H_2O_2 pretreated bagasse showed that 3% H_2O_2 together with the optimized condition can degrade cellulose significantly.

TABLE OF CONTENTS

Title Page i

Declaration ii

Certification iii

Dedication iv

Acknowledgment v

Abstract vii

Table of Contents viii

List of Figures xiii

List of Tables xiv

List of Appendices xv

Chapter One: Introduction 1

1.1 Sweet Sorghum Plant 1

1.2 Statement of Research Problem 3

1.3 Justification 3

1.4 Aim 4

1.5 Specific Objectives 4

Chapter Two: Literature Review.....5

2.1 Origin and production of Sweet Sorghum.....5

2.2 Taxonomy and varieties.....6

2.3 Chemical constituent of the juice and bagasse of Sweet Sorghum.....9

2.4 Uses of Sweet Sorghum.....9

2.4.1 As Food.....10

2.4.2 Local non alcoholic and alcoholic beverages.....11

2.4.3 As feed for livestock.....12

2.4.4 As Fodder and Forage Material.....13

2.4.5 Industrial feed stock.....13

2.4.5.1 Housing and packaging.....14

2.4.5.2 In brewing.....14

2.4.5.3 Adjunct.....14

2.4.6 Raw material in sugar and syrup production.....15

2.4.7 Sweet Sorghum, as an energy crop and direct raw material for fermentation.....15

2.5.0 Bioethanol/history.....19

2.5.1 Brief Chemistry of ethanol 19

2.6.0 Type/source/raw material for bioethanol.....20

2.6.1 From Sugar and Starch.....20

2.6.2 From lignocellulosic feedstock.....20

2.7.0 Major Factor that militate against efficient use of lignocellulose as bioethanol resource.....21

2.8.0 Uses of bioethanol.....24

2.8.1 Bioethanol as an alternative and environmentally friendly fuel.....24

2.8.2 Alcohol as a Solvent.....26

2.9.0 Environmental benefit of bioethanol use.....27

2.10.0 Processes involved in the production of Bioethanol.....28

2.10.1 Preatreatment 28

2.10.2 Hydrolysis (Saccharification) of Lignocellulosic Material.....34

2.10.3 Fermentation.....35

2.11.0 The global Bioethanol production capacity.....38

2.12.0 Challenges of bioethanol production.....39

2.13.0 Availability of Promising Sweet Sorghum Varieties in Nigeria.....40

2.14.0 Sustainability of bioethanol project in Nigeria.....41

Chapter Three: Materials and Methods.....42

3.1 Material.....42

3.1.1 Sample Collection and Identification.....42

3.1.2 Chemicals and Reagents.....42

3.1.3 Instruments.....43

3.2 Methods.....43

3.2.1 Preparation of Samples.....43

3.2.2 Determination of cellulose, hemicellulose and lignin content of the stem bagasse of eight sweet sorghum varieties.....44

3.2.3 Sample Pre-treatment.....47

3.2.3.1 Susceptibility of the bagasse to pretreatment solution.....47

3.2.3.2 Acid pretreatment.....47

3.2.3.3 Peroxide preatreatment……47

3.2.4 Optimization of pre-treatment conditions.....48

3.2.5 Chemical analysis.....48

3.2.5.1 Reducing Sugar Determination.....48

3.2.5.2 Glucose determination.....49

3.2.6 Determination of Moisture content.....49

3.2.7 Drying of bagasse.....50

3.2.8 Yield calculations.....51

3.2.8.1 Percentage Glucose and reducing sugar Yield.....51

3.2.9 Statistical Analysis.....51

Chapter Four: Results.....52

4.1 Periodic assessment for reducing sugar concentration and moisture content of fourteen Sweet Sorghum varieties.....52

4.2 Cellulose, hemicellulose, lignin content and harvest yield.....57

4.3 The Percentage (%) Glucose and reducing sugar yield (w/w) of Eight Sweet sorghum varieties.....60

4.4 Effect of pretreating the bagasse of two sweet sorghum varieties with different concentrations of HCl and H_2O_2 on the percentage (%) yield of glucose (w/w).....63

4.5 Effect of ratio of bagasse to pretreatment solutions: HCl and H_2O_2 on the percentage (%)yield of glucose (w/w) from the bagasse of two Sweet Sorghum varieties.....65

4.6 Effect of temperature on the percentage (%) yield of glucose and reducing sugar (w/w) from the bagasse of two Sweet sorghum varieties.....67

Chapter Five: Discussion.....69

5.1 The percentage moisture content 69

5.2 The reducing sugar concentration.....70

5.3 Cellulose, hemicelluloses and lignin content of bagasse from eight sweet sorghum varieties 70

5.4 Percentage bagasse yield 71

5.5 Susceptibility of the pith bagasse to hydrolysis.....71

5.6 Selection of two sweet sorghum varieties used for subsequent studies.....71

5.7 Effect of different concentration of the pretreatment Solution on the amount glucose released from the bagasse of two sweet sorghum varieties.....73

5.8 Effect of ratio of sample to the pretreatment solutions on the amount of glucose released from the bagasse of two sweet sorghum varieties.....74

5.9 Effect of temperature on the amount of glucose and reducing sugars released from two sweet sorghum varieties 75

Chapter Six: Conclusion and Recommendation.....76

6.1 Conclusion.....76

6.2 Recommendation.....76

References.....77

Appendices.....86

LIST OF FIGURES

Figure 1.1. Sweet Sorghum at 7 weeks.....2

Figure 2.1 Arrangement of cellulose in the plant cell wall.....24

Figure 2.2. Cellulose Chain 24

Figure 4.1 Percentage (%) moisture content of bagasse from the stalks of fourteen Sweet Sorghum varieties.....54

Figure 4.2. Reducing sugar concentrations (mg/g) of the stem juice from growing stalks of 14 Sweet Sorghum varieties at 5 weeks 55

Figure 4.3 Reducing sugar concentrations (mg/g) of the stem juice from growing stalks of 8 Sweet Sorghum varieties at 5, 7 and 9 weeks 56

Figure 4.4 Cellulose, Hemicellulose and lignin content of bagasse from the stalks of eight Sweet Sorghum varieties harvested on 30th October, 2010.....58

Figure 4.5 The percentage (%) bagasse yield of eight sweet sorghum varieties after harvest.....59

LIST OF TABLES

Table 2.1 Comparative value advantage of sweet sorghum over sugarcane and maize.....18

Table 2.1 Other advantages of sweet sorghum.....19

Table 4.1 Percentage Glucose yield of eight Sweet Sorghum varieties pretreated with 1.0% HCl and 1.0% H_2O_2.....61

Table 4.2 Percentage Reducing sugar yield (w/w) released from the bagasse of eight Sweet sorghum varieties pretreated with 1.0% HCl and 1.0% H_2O_2.....62

Table 4.3. Effect of pretreating the bagasse of two sweet sorghum varieties with different concentrations of HCl and H_2O_2 on the %yield of glucose (w/w) 64

Table 4.4 Effect of ratio of bagasse to pretreatment solutions on the Percentage yield of glucose (w/w) released from the bagasse of two Sweet sorghum varieties.....66

Table 4:5. Effect of temperature on the percentage yield of glucose (w/w) released from the bagasse of two Sweet Sorghum variety.....68

LIST OF APPENDICES

Appendix 1: Percentage moisture content of bagasse from the stalks of fourteen sweet sorghum varieties……86

Appendix 2: Reducing sugar concentrations (mg/g) of the stem from growing stalks of 14 Sweet Sorghum varieties at 5 weeks……87

Appendix 3: Reducing sugar concentrations (mg/g) of the stem from growing stalks of 8 Sweet Sorghum varieties at 5, 7 and 9 weeks……88

Appendix 4: Cellulose, Hemicellulose and lignin content of bagasse from the stalks of eight sweet sorghum varieties harvested on 30th 0ctober 2010……89

Appendix 5: The percentage bagasse yield of eight sweet sorghum varieties after harvest.....90

Appendix 6: Field performance and Physical characteristics of the Sweet sorghum varieties during growth periods at week 5, week 7 and week 9.....91

CHAPTER ONE

INTRODUCTION

Bioethanol production from Biomass has received considerable global attention in recent times. Brazil and the United States of America (US) are currently producing and consuming bioethanol from sugarcane and corn respectively (Hahn-Hägerdal *et al.,* 2006). United States of America has about 29 states that produce bioethanol with over one hundred and eighty plants (Anonymous, 2010). Majority of these plants run their production using purified enzymes. In Canada, Iogen incorporation is currently running a commercial production of bioethanol using commercial enzymes. In other parts of the world, bioethanol is being produced in laboratory, pilot and commercial scale from wheat, sugar beets, wastes from sugar refineries, wheat-starch, potato waste, cheese whey, milo (a species of grain sorghum), sweet sorghum juice, and beverage waste (Hinkova and Bubnik, 2001; Davenport, 2006). They make use of the sugar and starch in the feedstock. Current researches are focusing on how to efficiently utilize the rich and abundant carbon resources in the cell wall polysaccharides of agro wastes for bioethanol production.

1.1 Sweet sorghum plant

Sweet sorghum is the common name for an erect and corn-like grass that is a native of Africa and Asia. It is also an important crop in Latin America. The shoot of sorghum consists of stem, leaves, nodes and internodes during vegetative stage. The general appearance of sorghum is like that of corn. The stem consists of many alternating nodes and internodes. The leaf is born at each node with leaf sheath, an arrangement much like that of corn. The stem ranges from slender to very strong and thick stem of 0.5 to 5cm in diameter near the base and 0.5 to 4m tall depending

on the variety. The picture of a typical sweet sorghum plant is shown in Figure 1.1 below.



Figure 1.1: Sweet Sorghum at 7 weeks

A mature stem has seed grains which vary in colour from white, brown, yellow, red to cream. They are primarily raised for food (grain and syrup), production of alcoholic beverage from the grain, or for fodder, livestock feed and currently for biofuel production. They thrive in dry and hot environments across the globe. The term 'Sweet sorghum' is used to describe subspecie of sorghum (*Sorghum bicolor*) plants that has a high concentration of soluble sugars in the plant sap

or juice. This crop is attractive because of the easy accessibility of readily fermentable sugars combined with very high yields of green biomass. In all varieties, the primary carbohydrate in stem sap/juice is sucrose, with variable amounts of reducing

sugars and starch. Similar to sugarcane, the sap of sweet sorghum is extracted by milling and pressing. Once extracted, the sugars from sweet sorghum can be easily fermented to produce ethanol usually at local distilleries. Other products from Sweet sorghum include syrup, molasses, and crystal sugar as noted by Vermerris *et al.,* (2008).

1.2 Statement of Research Problem

In view of the declining fossil reserve, unrenewable nature of fossil fuels, increasingly constant demand for fossil fuel and increasing fossil fuel price, research is being intensified in the area of bioethanol production from sugar-containing energy crops and lignocellulosic feedstocks/biomass as an alternative renewable source of fuel.

Combustion of fossil fuel leads to emission of various types of gases into the atmosphere. These gaseous emissions from fossil fuel combustion have negative impact on the environment. The gases such as carbon IV oxide, carbon monoxide, methane, nitrous oxide e.t.c leads to atmospheric air pollution and global warming with carbon IV oxide being the major contributor to the global warming.

The nature of plant cell wall polysaccharide has posed great difficulty in utilization of this feedstock for ethanol production, thus optimization of bagasse (Sweet sorghum) pretreatment conditions then becomes imperative.

1.3 Justification

Bioethanol will serve as a renewable and alternative energy for automobile and other energy consumption purposes. It is a fuel that has better combustion quality with high octane rating when compared to fossil fuel (Reddy, 2007). Utilization of bioethanol as an alternative energy will help in reduction of green house gas emission, hence reduction in atmospheric air pollution, eventual reduction of global warming and global climate change (Sanchez and Cardona, 2008). The renewable and abundant nature of biomass material (raw material), will guarantee renewable and sustainable bioethanol production.

The short life span/generation time of Sweet sorghum plant and relatively low production cost is also considered an advantage in bioethanol production because, turnover will be high.

Integration of the sugars from sweet sorghum bagasse (cellulosic residue after extraction of sweet sorghum stem juice) with the sugar derived from the Sweet sorghum stem juice will further increase bioethanol yield. Utilisation of the bagasse from sweet sorghum for bioethanol production could lead to product yield maximization.

1.4 Aim

To investigate two chemical pre-treatment options (HCl and H_2O_2) and find out the most effective in the optimization of glucose yield from sweet sorghum bagasse for bioethanol production.

1.5 Specific Objectives

- To screen 14 sweet sorghum varieties for reducing sugar at 5, 7 and 9weeks.

- To determine the percentage composition of cellulose, hemicelluloses and lignin of eight selected sweet sorghum varieties at harvest.
- To determine the percentage bagasse yield at harvest.
- To determine the effect of pre-treatment conditions: concentration of the pretreatment solutions (HCl and H_2O_2), the ratio of sample to pre-treatment solution and temperature on the yield of glucose from sweet sorghum bagasse.

CHAPTER TWO

LITERATURE REVIEW

2.1 Origin and production of Sweet sorghum

Sweet sorghum is a grass of old world origin. It is a drought resistant heat tolerant, member of grass family of Africa origin (Undersander *et al.,* 1990) where they grow on savanna land (Grassi, 2004). Sorghum was grown in India before recorded history and in Assyria as early as 700BC. It reached China in the 13th century and Western hemisphere much later (Undersander *et al.,* 1990). It was introduced into America in 1853 by the United States (US) patent officer and since has been cultivated for sugar syrup production. It is grown extensively for this purpose in the South Eastern United States of America; Alabama, Arkansas, Georgia, Kentucky, Iowa, Mississippi and also in the Gulf States including Texas, North to Winsconsin, Northern Tennessee, West Kansas and Minnesota. Kentucky and Tennessee lead other States of the United States in syrup production. US recorded the peak of syrup production of 24million gallons per year in the 1880s. Currently, less than 1 million gallons of syrup is produced annually in US. However, some sweet sorghum has been grown in Winsconsin and Minnesota for either production of syrup or silage. By 1950s, 90% of the acreage of Sweet sorghum in the US was for forage (Undersander *et al.,* 1990) and silage and also for cattle feed concentrate in Texas, Kansas, and Nebraska where insufficient rainfall and high temperature makes corn production unprofitable.

According to the information provided by Food and Agriculture Organization of the United Nations (FAOSTAT) in 2008, United States was enlisted as the World largest producer of sweet sorghum. In Africa, Nigeria ranks highest with the capability of producing 9.3 million tonnes of

Sweet sorghum per year which is about 14.2% of the world total of 65.5 million tonnes (FAOSTAT, 2008).

Sweet sorghum differs from the grain sorghum mainly because the grain yield is lower and its stalk is taller and juicier with higher sugar content. Sweet sorghum is an annual, warm-season crop (Kangama and Rumei, 2005) that matures earlier under high temperature and short days. It has a short generation time of three (3) to four (4) months which implies that sweet sorghum matures in 100 to 140 days after planting. It tolerates drought and high-temperature stress better than many crops (Mask and Morris, 1991). With good soil drainage, sweet sorghum can adapt in all soil type (Kangama and Rumei, 2005). Under moist soil condition, the optimum temperature range for germination is 25 to 30^{o}C and can germinate in three (3) to five (5) days after sowing the shoot.

2.2 Taxonomy and varieties

Sorghum makes up the genus *Sorghum* in the class of *Liliopsida*, the order of *Andropogoneae*, family of *Poaceae* (previously known as *Gramineae*), genus of sorghum and specie of bicolor hence botanically known as *Sorghum bicolor (L.)* Moench (Doggett, 1988) as cited by Mamoudou *et al.,* (2006).

There are about 30 species. Sorghum has many varieties which are placed into four (4) groups namely; the grain sorghum (also called milo), grass or forage sorghums (for pasture and hay), sweet sorghums (formerly called “Guinea corn", develop a sweet juice in their stalks and are grown for syrup production and currently for biofuels), and broom corn (for brooms and brushes), (Kimber, 2000). Their names indicate what they are majorly used for. The grain sorghum is basically grown for their grains which are round and starchy seeds. They are used for

human food and livestock feed. The grass sorghum is grown for green feed (forage) and for making silage and hay (dried fodder). Sweet sorghum has a sweet juicy stem and is grown for making ancient sorghum syrup (a sweetener used in food industry in the US). Sweet sorghum stalk and leaves can also be useful in making silage and hay. The broom corn sorghum is grown for the branches of the seed cluster which are used for making brooms.

Integrated Taxonomic Information System, (ITIS, 2006) and Ritter *et al.* (2007) documented that Sweet sorghums are classified as *Sorghum bicolor sp bicolor* (L.) *moench* with many other cultivars of which grain sorghum is part of. Other common names under this class are; Black amber, broom-corn, broomcorn, chicken corn, common wild sorghum, Drummond broomcorn, durra, Egyptian millet, feterita, forage sorghum , great millet, guinea corn, jowar, Kaffir-corn, Kaffircorn, milo, shallu, shatter cane, shattercane, sorghum, Sudan Grass, wild cane (ITIS, 2006). All sorghum species are locally called “Dawa” plant in Hausa language of Northern Nigeria, a name adopted by many regions in the Country. It is called Jowar in India, sorgo in Brazil, sorghu hirse in Germany, sorgo in Spain, Italy and Porugal.

Many varieties of Sorghum bicolor seeds, range from white through red to brown in colour.

Taxonomic classification:
Sorghum bicolor (L.) Moench common name sweet sorghum

Kingdom	*Plantae* – Plants
Subkingdom	*Tracheobionta* – Vascular plants
Superdivision	*Spermatophyta* – Seed plants
Division	*Magnoliophyta* – Flowering plants
Class	*Liliopsida* – Monocotyledons
Subclass	*Commelinidae*
Order	*Cyperales*
Family	*Poaceae* – Grass family
Genus	*Sorghum* Moench – sorghum
Species	*Sorghum bicolor* (L.) Moench – sorghum

Culled from USDA, Natural resources conservation services plant database.
http://plants.usda.gov/java/profile?symbol=SOBI2

Subspecies	*Sorghum bicolor* ssp. Bicolor (L.) Moench

Ritter *et al*., (2007)

Subspecies	*Sorghum bicolor* (L.) Moench ssp. Bicolor

USDA, ARS, National Genetic Resources Program. Germplasm Resources Information Network - (GRIN) [Online Database]. National Germplasm Resources Laboratory, Beltsville, Maryland. URL: http://www.ars-grin.gov/cgi-bin/npgs/html/taxon.pl?35169 (04 June 2011)

2.3 Chemical constituent of the juice and bagasse of sweet sorghum

Though sweet sorghum is used for other processes, it is considered promising in bioethanol production due to its high biomass and carbohydrate content (Mehmood *et al*., 2008). The stem juice contains variable amounts of reducing sugar. The work of Mehmood *et al.*, (2009) also revealed that the bagasse of the stem contain 35.01±0.71%, 24.40±1.06, and 19.00±2.07% of cellulose, hemicelluloses and lignin respectively. The increased glucan (cellulose) and decreased lignin content of sweet sorghum bagasse may provide a good source of fermentable sugar after chemical hydrolysis and saccharification. The field experiment conducted in China in 1989 on five varieties of sweet sorghum by Food and Agricultural Organisation revealed that the juice of sweet sorghums sampled contained protein, non essential and essential amino acids (with the exception of tryptophan), some minerals such as; potassium, sodium, calcium, magnesium, iron, and manganese in addition to sugar; glucose, fructose and sucrose; with sucrose ranking highest among the sugars (FAO, 1994).

2.4. Uses of Sweet sorghum

Sweet sorghum is an important food crop in Africa, Central America and South Asia, and is the "fifth most important cereal crop grown in the world" (Zohary and Hopf, 2000)**.** Emphasis here is made on its use as food, feed, forage and as biofuel raw material. Sweet sorghum is an extraordinarily promising multifunctional crop, not only for its high economic value (to the wide range of its products: grains, sugar, lignocellulosics) but also for its capacity to provide a very wide range of renewable energy products, industrial commodities, food (grains and sugar) and animal feed products and thus has been described as one of the best world food feed energy crop (Grassi, 2004).

Sweet sorghum can be considered thus a crop of universal value**,** because it can be grown in all continents, in tropical, sub- tropical, temperate regions as well as in poor quality soils and in semi-arid regions. It is therefore possible to obtain two plantations per year reaching full maturity and a large production. Other interesting issues relevant to sweet sorghum are: Its low water requirements 1/3 of sugar cane, 1/2 of corn, 1/4 of short rotation forestry. Its high capacity to absorb CO_2 (~45 tonnes CO2/ha per cycle), 3 times more than short rotation forestry (Grassi *et al*., 2005).

Sweet sorghum leaves can be used as fodder for livestocks and the grain for animal feed production. Like sugarcane stem, sweet sorghum stem also has high sugar content. For this reason, the sugar derivable from Sweet sorghum stem is currently being used in bioethanol production Vermerris *et al*., 2008; Agriculturist, 2008).

2.4.1 As Food

Sorghum is well suited for human use as food, snack, feedstocks for baking and brewing industries (FAO, 2002). Similar to the grain sorghum variety, sweet sorghum grain is utilized in making various food products like couscous, flour, porridge, unleavened bread, cookies, cakes and malted beverages are made from this versatile grain (Carter and Capenter, 1981). For most traditional food preparations, the whole grain or the decorticated grains are milled into flour. The flours are then used for varieties of product for example in northern part of Nigeria where it is mostly cultivated, it serve as the second most important cereal after corn and like the grain sorghum can be consumed in different processed form such as: a thick porridge popularly called "*tuwon dawa*" in Hausa made from sorghum flour and served with spicy vegetable soup/sauce called "miya"; and light/thin porridge called pap , "*Koko*" and "*Ogi*" in English, Hausa and

Yoruba respectively made from wet sorghum starch. Pop corn-like local snack is also made from the grains of sorghum. Fermented breads called '*masa*' and unleavened bread called '*waina*' are also consumed in Ghana and Nigeria (USDA, 2005). In South Africa, sorghum meal is cooked into stiff porridge much like pap and served with soured milk or boiled vegetable (Rooney *et al.,* 1986).

In Arab regions, unmilled grain is often cooked to make couscous, porridge, soups and cakes. They are also used in combination with flours or starches for bread making (Murty and Subramanian, 1982; FAO, 1995). In India, sorghum is used in making unleavened bread called *Bhaki* or *Jolade rotti* in Northern Karnataka. In Eastern Karnataka and Andhra Pradesh, *Roti* (*Jonna rotti*) made from Sorghum is a staple food.

In the diet of United States citizens, sorghum syrup is used as a sweet condiment, usually for biscuits, corn bread, pancake, hot cereal or baked beans and previously used as Maple syrup.

2.4.2 Local non alcoholic and alcoholic beverages

In Nigeria, the grains are also utilized in making opaque beverage and alcoholic beer locally called: "*kunu*" and "*burukutu*" respectively. The by-product from these preparations is used for feeding livestock. The local alcoholic beer for which sorghum serves as a source of malt and adjunct is also referred to as African sorghum beer. It is a popular drink among the black communities in Africa and traditional drink among the Zulus of southern Africa. It is called *pombe* in East Africa and *bil-bil* in Cameroon. This beer is also consumed in Zimbabwe, Burundi, Mali, Burkina Faso and Ghana. African sorghum undergoes lactic acid as well as alcoholic fermentation and is sold in a microbiologically active state, with a shelf life of only 5-7 days (Daiber and Taylor, 1995). The main attributes of this product are short shelf life of about

one week, low alcohol content, acidic nature, suspended solids and a characteristic taste and colour (Chitsika and Mudimbu, 1992). Among the grains, white is used in preference to red and brown varieties because of low polyphenol content. Red sorghum imparts a pinkish-brown colour to the beer. High tannin and polyphenol Sorghum is not desirable for beer production.

In China, Sorghum serves as an important ingredient for the production of distilled beverages known as *maotai* and *kaoliang*.

In southern Africa, it is used in beer production including the local Guinness and has been useful in recent years as a substitute for other grains in production of gluten free beer for those with celiac disease; though the African beer are not gluten free.

USDA has classified certified Food Sorghum as sorghum varieties that provide for the highest and most consistent quality standards allowed. These varieties can be used for breads, cereals, salty snack foods, and pastas and popped snacks (USDA, 2005).

2.4.3 As feed for livestock

Sorghum serves as important animal feed used in countries like the United States, Mexico, South America, and Australia. In the US, sorghum is used primarily as a feed grain for livestock. It has similar feed value with corn but lesser in vitamin A and greater in protein and fat content than corn. The nutritional value of good-quality sorghum has an average feeding value of 96 to 98% that of corn (Okrah, 2008). Sorghum can be processed to further improve its feed value using techniques such as grinding; crushing, steaming, steam flaking, popping, and extruding. The products are then fed to beef or dairy cattle, poultry including egg laying hens, pigs, and pets. The grains are highly palatable to livestock, and intake seldom limits livestock productivity unlike some sorghum varieties and hybrids (which were developed to deter birds) are less

palatable due to tannins and phenolics. Such varieties should be cracked or rolled before feeding to cattle; to improve the portion digested (Carter and Capenter, 1981).

Cattle and sheep can also graze on field of harvested grain where they feed on the forage. However, the danger of prussic acid (HCN) poisoning could result from stubble of secondary growth and therefore must be pastured carefully (Okrah, 2008).

Certified sorghum Feed includes sorghum varieties that benefit the animal feed industry. Sorghum feed represents one of the largest market segments in the US for example. Growth areas for these varieties include poultry and dairy feeds (USDA, 2005).

Certified Pet Food sorghum is used in both dog and cat foods. Mycotoxins like aflotoxin and vomitoxin do not readily occur in grain sorghum while they do occur in other grain sources. This eliminates a potential health risk for the family pet (USDA, 2005).

A very valuable co-product created from the processing of grain sorghum for ethanol is dry distiller's grain. This high protein animal feed supplement generates additional revenues for the ethanol processing plant (Okrah, 2008).

2.4.4 As Fodder and Forage material

Sweet sorghum leaves are mainly used as livestock fodder: its high rate of photosynthesis produces leafy stalks up to 5 metres tall that make excellent silage (FAO, 2002). Grain sorghum may be used as whole-plant silage; however, sweet sorghum was developed as a silage crop. Sweet sorghum produces much higher forage yields than grain sorghum (Okrah, 2008).

2.4.5. Industrial feed stock

Sweet sorghum serves as Industrial feed stock in different capacities. The grains, stalks (stem) and stem juice serves as industrial raw materials for housing, packaging, brewing, sugar syrup and bioethanol industries.

2.4.5.1 Housing and packaging

Sorghum straw (stem fibres) is also used in the production of wallboard for the housing industry. Millions of bushels of sorghum are processed into wallboards per year in central US alone (Okrah, 2008). They are also used in the production of biodegradable packaging materials (FAO, 2002) which are especially useful for packaging sensitive electronic equipment as it does not accumulate static electricity (David, 1992; Stroade and Boland, 2011). In addition to wallboard grain sorghum can be processed interchangeably with corn for the production of ethanol (USDA, 2005).

2.4.5.2 In brewing

Certain varieties of red sorghums can be used as malt in brewing lager beer because they contain active amylases at concentrations suitable for certain brewing applications
(Okafor and Aniche, 1980). The amylase content is variable and so cannot be used as a direct replacement for barley malt in commercial beer production and therefore requires supplementary amylolytic and proteolytic enzymes to complete the fermentations
(O'Rouke, 2004).

2.4.5.3 Adjunct

Adjuncts are substances used in beer manufacturing purposefully for improving the outcome of a beer product. They are mostly used in non malted state. The adjuncts are used for the following reasons: enhancement of the physical stability, adjustment of the fermentability of a wort, for their flavour contribution, alteration of the carbohydrate and nitrogen ratio of the wort, for colour adjustment as in the case with dark sugars, enhancement of foam stability due to their glycol-proteins (Bamforth and Barclay, 1993), improvement of colloidal stability as a diluents for haze-forming proteins due to their low protein content, for increasing of brewing capacity, for reducing labour cost, improved hot and cold break, and shorter brewing cycles (Okrah, 2008).

Like corn, rice, rye, oats, barley, wheat, sorghum grain can also be used as a cereal adjunct in brewery either in malted or non-malted form (Okrah, 2008). It is precooked before being added to the main mash because the gelatinization temperature is higher than those required by starch hydrolytic enzymes (O'Rouke, 1996). Although adjuncts are used mainly because they provide extract at a lower cost (a cheaper form of carbohydrate) than is available from malted barley, other definite advantages are also achieved because of their readily availability (O'Rouke, 2004) as mentioned above.

2.4.6. Raw material in sugar and syrup production

The stalks are also rich in sugar, which can be processed into jaggery or fermented to produce ethanol (FAO, 2002). The sweet sorghum hybrid "Madhura" has been specially developed for ethanol, syrup and unrefined sugar production. The syrups are used as sweetner for eating pastries like hot biscuits. It is also used on pancakes, corn meal mush, grits and other hot cereals. Like molasses, it can be used as cooking ingredient (Nebraska grain sorghum, 2011)[2]. Sorghum

syrup is extracted from the plant by crushing the stalk. After crushing, the syrup is cooked down to concentrate the natural sugars and packaged for sale. The syrup is produced primarily in the United States and is used by the food industry as a substitute for sugar. Generally, it takes eight gallons of juice to produce one gallon of sweet sorghum (molasses) syrup (Undersander *et al.*, 1990).

2.4.7 Sweet Sorghum, as an energy crop and direct raw material for fermentation

It will not be out of place to say that the sweet sorghum stem can be consumed just like sugarcane stem, because the stalks are also high in sugar, which can be processed into jaggery or fermented to produce ethanol (FAO, 2002).

Sweet sorghum has been identified as energy crop due to the sugary juice derivable from the stem (Miller and Creelman, 1980, Yu *et al.,* 2009 and Wortmann and Regassa, 2011) and for this reason; the sugar derivable from sweet sorghum stem is currently being used in bioethanol production. Bioethanol production of about 7,000 litres per hectare makes sweet sorghum a potential energy crop; and thus makes sweet sorghum highly attractive for bioethanol production (FAO, 2002).

It is among the most widely adaptable cereal grass potentially useful for bioethanol production. Its soil and climatic adaptability is of great advantage in bioethanol production. Sweet sorghum can be harvested three to four times yearly if proper irrigation is applied. Its short life span/generation time, soil and climatic adaptability and relatively low production cost is considered an advantage in bioethanol production and also a better alternative which can replace sugarcane (Centre for Plant Breeding, 2011).

The use of sweet sorghum as a feedstock for bioethanol production will in no way affect food security. In fact it encourages economy of farm land (space) because same parcel of land used for the cultivation of sweet sorghum will produce both grains (food), feedstock for bioethanol production and fodder for livestock. A hectare of land can produce between two (2) to six (6) tonnes of grain as well as the stock (Reddy, 2007). Apart from the sugary juice which is used as the primary bioethanol resource, the bagasse; a lignocellulosic feedstock could also be considered potential second generation bioethanol resource. It also has more fermentable sugar than sugarcane and, therefore, a better alternative which can replace sugarcane. A comparative cost advantage of sweet sorghum over sugarcane is presented in Table 2.1. Sweet sorghum has a total value of US$1033 (summed over grain and stalk value) against grain Sorghum US$ 913 (Reddy *et al.,* 2006).

There could also be much economic benefit for local farmers. They can earn greater income from sweet sorghum cultivation through the sale of stalks to distilleries and sale of grain, stalk and threshed panicles for food and feed (Reddy *et al.,*2006).

The lignocellulose serves as rich source of fermentable sugar if they are efficiently utilized. In fact, United State energy policy of 2005 made provision that 250 million gallons of renewable fuel to be consumed from 2013 and beyond should be from cellulose ethanol (EPA, 2007). Current interest in bioethanol research is focusing on how to efficiently liberate sugar molecules from lignocellulosic feedstock for increased bioethanol production. Therefore, integration of the sugars from sweet sorghum bagasse (cellulosic residue after cane extraction) with the sugar derived from the stem/cane will further increase ethanol yield and also make bioethanol affordable.

Table 2.1: Comparative value advantage of Sweet sorghum over sugarcane and maize

Parameter	Sweet sorghum[2]	Sugarcane[2]	Maize[3]
Crop duration	4 months	12 months	4 months
Water requirement	4000 m3	36000 m3	8000 m3
Grain yield (t ha-[1])	2.0	-	3.5
Ethanol from grain (l ha-[1])	760	-	1400
Green stalk cane yield (t ha-[1])	35	75	45
Ethanol from stalk cane juice (l ha-[1])	1400	5600	0
Stillage/ stover (t ha-[1]1)	4	13.3	8
Ethanol from residue (l ha-[1])	1000	3325	1816
Total ethanol (l ha-[1])	3160	8925	3216
Corn oil (l ha-1)[4]	-	-	140
Income from corn oil (US$ ha-[1])	-	-	61
Cost of cultivation (US$ ha-[1])	220	995	272
Cost of cultivation (ha-1) after corn oil profit (US$)	220	-	211
Cost of cultivation with irrigation water cost (US$)[5]	238	995	287
Ethanol cost per kilo liter (US$)[6]	69.6	111.5	65.6
Ethanol cost per kilo liter (US$)[7]	75.3	111.5	89.2

1.Processing costs assumed equal and excluded from the estimates; does not take into account water needs and crop duration
2. Sorghum grain ethanol: 380 l t-1; sorghum stalk juice ethanol: 40 l t-1; sorghum or sugarcane stillage ethanol: 250 l t-1 [Ref. Badger (2002) Trends in New Crops and New Uses];
3. Corn (grain) ethanol: 400 l t-1; maize stover ethanol: 227 l t-1 [Ref. Badger (2002) Trends in New Crops and New Uses];
4. Oil produced from corn: 40 l t-1; oil cost of production: Rs 15 l-1; oil sale price: Rs 35 l-1;

5. Sorghum needs two irrigations and maize four each at the cost US$19 ha-1 per irrigation in rainy season
6. Without accounting for water cost;
7.After accounting for water cost

Culled from Reddy *et al,* (2006).

From table 2.1 above, it implies that a total of 9480 litres ethanol per year per hectare of Sweet sorghum which is higher than 8925 litres ethanol per year per hectare that of sugarcane.

Other advantages of sweet sorghum are summarized in table 2.2 below.

Table 2.2: Other advantages of sweet sorghum

As a crop	As process and product (ethanol)	As by-product (stillage)
Shorter gestation period	Eco-friendly process	Higher biological value
Dryland crop	Superior quality	Rich in micronutrients
Greater resilience to abiotic stresses	Less sulphur	Use as feed/ for power cogeneration
Farmer friendly	High octane rating	
Meets fodder/ food needs	Automobile friendly (up to 25%)	

Culled from Reddy *et al.,* (2006).

2.5.0 Bioethanol/history

2.5.1 Brief Chemistry of ethanol

Bioethanol is an alcohol, precisely an ethanol generated from renewable biomass such as sugar, starch and lignocellulosic based substances. It has the formular C_2H_5OH. It is a colourless liquid with a pleasant smell. It has low ignition temperature of 13^oC or 55^oF which means that it does not ignite fire spontaneously. It has a boiling point of 78.5^o C (173.3^oF), and a specific gravity of

0.789 at 20^{o}C (68^{o}F). Ethanol is normally concentrated by distillation of dilute solution. Commercial ethanol contains 95% by volume ethanol and 5% by volume water. Dehydrating agent removes the remaining water and produce absolute ethanol. Ethanol production dates back to ancient time where synthesis was made from sugar through fermentation by yeast or another microorganism. Much of the industrial ethanol and all beverage ethanol used today is being synthesised through this way.

2.6.0 Type/source/raw material for bioethanol

Bioethanol is produced from whole or parts of crops/plant, finished products and wastes of carbohydrate origin.

2.6.1 From Sugar and Starch

Bioethanol is produced currently from potato, sugar cane, sugar beets and other cereals, wastes from sugar refineries, milo, wheat starch, potato waste, cheese whey, sweet sorghum juice, beverage waste, corn and other cereals. This current bioethanol technology uses only parts of these plants namely, the storage sugars like glucose, sucrose and starch.

2.6.2 From lignocellulosic feedstock

The biomass/lignocellulosic feedstock for production of bioethanol are generally classified under the following headings:

a. Agricultural residues which includes straws such as rice and wheat straws, corn stalk and cobs, bagasse, cotton gin trash and palm oil wastes.
b. Crops grown specially for their biomass example includes grass such as bermuda and switch grasses, sweet sorghum, sugarcane, grain corn e.t.c

c. Papers example includes recycled news papers, paper mill sludge, and sorted municipal solid wastes e.t.c

d. Wood wastes examples include pruning, wood chips and sawdust e.t.c

e. Green wastes example includes leaves, grass clippings, vegetable and fruit wastes.

2.7.0 Major Factor that militate against efficient use of lignocellulose as bioethanol resource

- **Complex nature of cell wall polysaccharide (cellulose, hemicelluloses, lignin)**

Lignocellulosics are rich in plant polysaccharides which can be hydrolysed into fermentable sugars for bioethanol production. The polysaccharides include cellulose and the hemicelluloses. Lignocellulosics is a complex molecular aggregate which is chiefly represented by the physico-chemical interaction of: cellulose, pentose sugar polymer; hemicelluloses, a highly branched heteropolymer; and lignin, a very high molecular weight and crosslinked aromatic macromolecule (Bon and Ferrara, 2007). Cellulose is a fibrous, tough, water insoluble substance, found in the cell wall of plants particularly in the stalk, stem, trunk and all woody part of plants (Nelson and Cox, 2004). It is a linear, unbranched chain homopolysaccharide of glucose. According to Bhat (2000) as cited by

Sridevi *et al.*, (2009) cellulose rich plant is one of the sustainable sources of fuel, animal feed and feedstock for chemical synthesis. Cellulose is the most abundant of the polysaccharide on earth and is a highly ordered polymer of cellobiose (D-glucopyranosyl-β-1,4-D-glucopyranose) made up of units of β-D-glucose molecules (Park *et al*., 2010) with the molecular weight of

$(C_6H_{10}O_5)$n where n is the degree of polymerization. The arrangement and chemical structure of cellulose in the cell wall of plant is presented in figure 2.1 and 2.2 below respectively.

The glucose units in cellulose varies from 2000 to 15,000 (Nelson and Cox, 2004; Metzler, 2003). Cellulose materials present crystalline domains separated by less ordered, amorphous region that are potential points for mechanical, chemical and biological attacks (Bon and Ferrara, 2007).

Parallel chains of cellulose are stabilized by hydrogen bonding. The ordered crystalline arrangement is brought about by the involvement of the hydroxyl groups of cellulose in a number of intra and intermolecular hydrogen bonding (Park *et al*., 2010). Thorough reviews of cellulose crystalline allomorphs can be found elsewhere (O'Sullivan, 1997: Pérez and Mazeau, 2005: Zugenmaier, 2008). Cellulose crystals are thought to be imperfect, and thus a significant portion of the cellulose structure is less ordered; this portion is often referred to as amorphous. A parameter termed the crystallinity index (CI) has been used to describe the relative amount of crystalline material in cellulose. The traditional two-phase cellulose model describes cellulose chains as containing both crystalline (ordered) and amorphous (less ordered) regions (Nisizawa, 1973).

The CI of cellulose has been used for more than five decades to interpret changes in cellulose structure after physicochemical and biological treatments. Dasari and Berson (2007) reported that for a good interpretation to be given to enzyme hydrolysis, crystallinity must be coupled with other cellulose properties. During any chemical/mechanical/biological treatment, the CI of cellulose can be changed and then correlated with the measured digestibility. However, differences in observed enzyme hydrolysis kinetics may be governed by other characteristics

such as available surface area, degree of polymerization and particle size. For example, the increased digestibility for finely ground sawdust particles may be due to both decreased CI and increased surface area.

Hemicelluloses are heteropolymers of the xylose, plus glucose, mannose, galactose and arabinose, as well as different sorts of uronic acids. Hemicelluloses are the linking material between cellulose and lignin. The five carbon (C5) and six carbon (C6) sugars, linked through 1→3, 1→6 and 1→4 glycosidic bonds and often acetylated, form a loose, very hydrophilic structure that acts as glue between cellulose and lignin.

Depending on the predominant sugar types, the hemicelluloses are referred to as mannans, xylans or galactans (Bon and Ferrara, 2007). The major hemicelluloses of cereal and hard wood are made up of xylan. Xylan is a polymer consisting of a β1→4-linked D-xylose backbone and can be substituted by different side groups such as L-arabinose, D-galactose, acetyl, feruloyl, p-coumaroyl and glucuronic acid residues with ferulic acid being the most abundant hydroxy cinnamic acid in the cell wall of cereals (Mueller-Harvey and Hartley, 1986).

Currently, cellulose is receiving increased attention due to its potential use in bioenergy production. Most animals cannot use cellulose as fuel source unlike glycogen and starch because they lack enzymes that can hydrolyzes the β 1→4 glycosidic linkage. This makes it available for utilization in bioethanol production. However, utilization of cellulosics for bioethanol production requires cellulase enzymes that can cleave β1→4 glycosidic bonds between constituent glucose sugars. These cellulases represent a system of enzyme that acts synergistically to degrade cellulose. The enzyme system includes endoglucanase

(EC 3.2.1.4), cellobiohydrolase (EC3.2.1.11), β glucosidase (EC 3.2.1.21). Other enzyme activities required for utilization of lignocellulosics include ligninase (feruloyl esterase (EC 3.1.1.73), laccase, manganese peroxidase), hemicellulase (xylanase (EC 3.2.1.8). The major problem of efficient lignocellulose utilization is the accessibility of cellulose which is affected by parameters such as cellulose crystallinity, lignin/hemicellulose contents and distribution, porosity, and particle size (Park *et al.,* 2010).

Pretreatment of biomass becomes imperative before glucose can be effectively released.

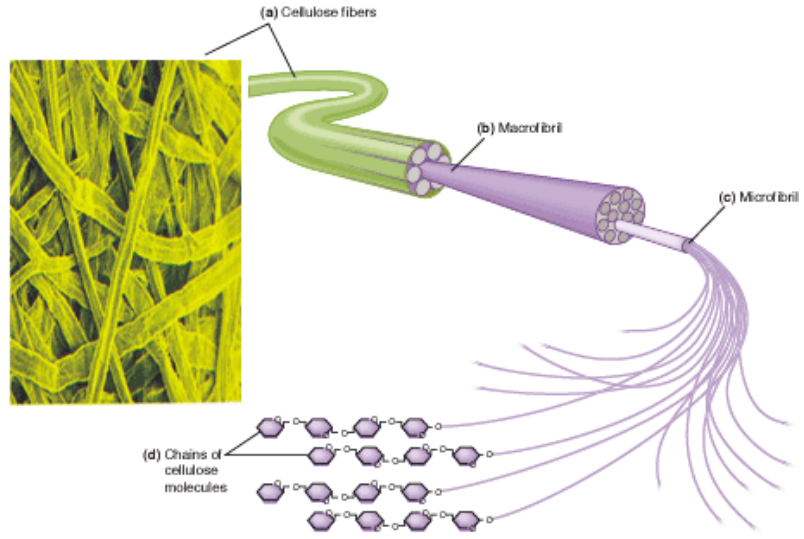


Figure 2.1: Arrangement of cellulose in the plant cell wall
Culled from: http://nutrition.jbpub.com/resources/images/images/fiber.gif

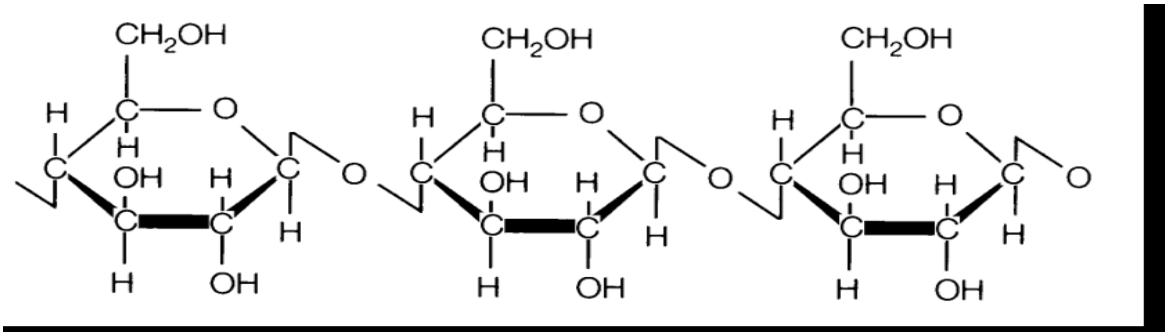


Figure 2.2: Cellulose Chain

Culled from: http://www.greenspirit.org.uk/resources/cellulose.gif

2.8.0 Uses of bioethanol

Ethanol has found use as solvent, in making alcoholic beverages, in the manufacture of other chemicals, and presently as fuel.

2.8.3 Bioethanol as an alternative and environmentally friendly fuel

Presently, there is a serious efforts made in the laboratory for large scale production and global commercialization of bioethanol as fuel for automobile, lightening, heating, power supply and other industrial and domestic purposes. In view of the depleting fossil reserves across the globe and the advocacy for a safe and better environment free of gaseous emission, the issue of alternative energy has become even more necessary. Bioethanol fuel is used partly as fuel in form of ethanol:gasoline mix/blend called Gasohol. The blends are denoted by E-x. Where x is the percentage proportion of ethanol in the blend. The various known ethanol blends are E-10, E-85 and they are used elsewhere in the world. Places like Brazil are currently running automobiles that use alcohol blend fuels. It can also be used wholly as fuel in what is termed E-100.

Brazilian cars presently run on either a gasoline blend containing 22-24% ethanol or 100% ethanol. It has been forecasted that bioethanol consumption will increase as the number of "flex cars" with engines that can run on both gasoline blend or bioethanol alone increases from 4 million to 15 million in 2013 (Bon and Ferrera, 2007).

Bioethanol is termed a cleaner fuel. It burns cleaner than petroleum fuel because it is made up of organic compounds, which are less complex chemically than gasoline and diesel fuels as a result, burns more completely and leaves no residue that can cause pollution. The percentage

composition of oxygen (35%) may be the reason why it would combust more completely in bioethanol than in fossil fuel (Chandel *et al.,* 2007).

The use of fossil derived fuel has posed serious danger to our environment with the attendant environmental consequences. The gaseous emissions as a result of fossil fuel combustion are currently creating serious environmental problems across the globe. As a result, research has identified bioethanol as a substitute because it burns cleaner than its fossil counterpart. The use of fossil fuel releases carbon dioxide, methane and significant quantity of nitrous oxide (Chandel *et al.,* 2007) and sulphur dioxide into the environment, most of which are formed as a result of incomplete combustion of fossil fuel. These products of incomplete combustion of fossil fuel are the primary cause of atmospheric pollution.

At present, 85% of the CO_2 emitted annually into the atmosphere originates from fossil combustion (Kangama and Rumei, 2005). Other advantage of using bioethanol as fuel is that it is more biodegradable than gasoline in case spillage occurs.

Bioethanol blend also proves a cleaner fuel than fossil fuels. The use of even low level blends of alcohol has a better environmental benefit than gasoline itself. It produces lesser CO, CO_2, SO_2 and NO_2 species than gasoline. Higher blends like E-85 or E 100 burns with less of virtually all the pollutants mentioned.

2.8.2 Alcohol as a Solvent

It is used as diluents or thinning solvent for variety of substances such as lacquers, enamels, furniture and metal polishes, lotions and liniments, solvent for dye, nitrocellulose, celluloid elements, paste and vanishes, photographic films. It is a solvent for oil in the manufacture of

vanilla, lemon, pepper mint, jasmine, solvent in manufacture of liquid soap, it is also a solvent in the manufacture of chemicals like dinitrobenzol and oxalic acid. It is used in purification process for casein and milk products.

Ethanol is used in drug manufacturing and also in the manufacture of ethyl halides e.g. ethyl bromide and ethyl iodide.

Ethanol is also one of the reactant in the production of ethylethanoate.

It is a raw material for production of ether and ethylene through dehydration (Prescott and Dunn, 1959).

According to Laskin (1977), ethanol has been demonstrated to be useful in single cell protein production from yeast and bacteria. Biological specimens are preserved using ethanol.

Ethanol is combined with water to act as antifreeze in automobile radiator, in insulin and glycerophosphate manufacturing, ethanol is used as a precipitating agent.

2.9.0 Environmental benefit of bioethanol use

Bioethanol production process from sweet sorghum is less polluting than sugarcane and the resulting bioethanol has clean burning quality with high octane rating (Reddy, 2007). With bioethanol-derived fuel from sweet sorghum, there will be no net emission of CO_2 into the atmosphere unlike fossil fuel. El Bassam (1998) observed that carbon four (C4) plants have better CO_2 reduction rate and also less fertilizer, water, chemical need among other qualities. C4 carbon fixation is one of the biochemical mechanisms, along with carbon three (C3) photosynthesis, used in carbon fixation. It is called C4 carbon fixation because of the 4-carbon

molecule present in the first product of carbon fixation in these plants, in contrast to the 3-carbon molecule products in the more common C3 carbon fixation plants. It is believed to have evolved more recently. C4 plants overcome the tendency of the enzyme ribulose 1,5 bisphosphate carboxylase/oxygenase (RuBisCO) to wastefully fix oxygen rather than carbon dioxide in what is called photorespiration. This is achieved by using a more efficient enzyme to fix CO_2 in mesophyll cells and shuttling this fixed carbon via malate or asparate to bundle-sheath cells where RuBisCO is isolated from atmospheric oxygen and saturated with the CO_2 released by decarboxylation of the malate or oxaloacetate (Boag and Jenkins, 1986; Raghavendra and Sage, 2011; Varaprasad, 1997).

Sweet sorghum like other C4 plants is highly efficient in CO_2 utilization more than other direct CO_2 metabolizing plant known as C3 plants which fix CO_2 directly into Calvin cycle. The ability of Sweet sorghum to salvage much of CO_2 (a substrate for carbohydrate synthesis in plant via Calvin cycle) and reducing photorespiration is in the cellular location of the Calvin pathway enzyme ribulose biphosphate carboxylase oxygenase (RuBisCO) at the bundle sheath cell instead of the mesophyll cells. Atmospheric O_2 cannot diffuse easily into the bundle sheath, therefore photorespiration by RuBisCO is reduced to favour anabolic work of photosynthesis through calvin cycle. With the Sweet sorghum plant being well adapted for CO_2 delivery into the bundle sheath through a shunt pathway, calvin cycle is favoured more than photorespiration. The consequences of CO_2 utilization efficiency being reduction in net CO_2 circulating in the environment and increased biomass production (CO_2 utilized in biomass production).

2.15.0 Processes involved in the production of bioethanol.

Bioethanol production involves many processing steps. The steps employed vary depending on the nature of raw materials used whether it is sugar, starch or lignocellulosic materials. A sugar based raw materials is immediately fermented after extraction while starchy materials requires a starch hydrolysis step to convert the polysaccharides into sugars which are subsequently fermented. Lignocellulosic materials are more difficult to handle and requires several major steps which are pretreatment, hydrolysis and fermentation.

2.15.1 Preatreatment

In bioethanol production, lignocellulosic biomass has remained significantly untapped source of fermentable sugars. As a result of the strength and resistance posed by lignocellulosic biomass to biological, physical and chemical attacks, pretreatment becomes imperative. Pretreatment removes the complexity in the structure of lignocellulosic biomass by altering the organization and chemical interactions among the cellulose, the hemicellulose and the lignin thereby rendering cellulose fibre more digestible as it is exposed to the activity of cellulolytic enzymes which converts the carbohydrate polymer into fermentable sugars (Mosier *et al.,* 2005). Pretreatment is also necessary for cellulase producing microorganisms.

The various pretreatment methods can be classified into physical, physical-chemical, chemical and biological pretreatment or combination of any of the pretreatment methods mentioned above.

A. Physical pretreatment

Physical method involves physical processing coupled with manipulation of temperature and pressure examples include mechanical and pyrolysis.

Mechanical method is the physical reduction in size of lignocellulosic biomass to reduce cellulose crystallinity. It involves clipping, grinding and milling e.t.c. This is unattractive

because of high energy and capital cost. It also includes pyrolysis (use of high temperature). Pyrolysis involves the use of high temperature with subsequent cooling and condensation. Cellulose rapidly decomposes when treated with high temperature.

B. **Physicochemical Method**

Physicochemical treatment is more effective than physical pretreatment. They include the following: Wet Oxidation, Stem Explosion and Ammonia Fibre Explosion (AFEX).

i. Steam explosion

This involves the use of saturated steam at high pressure leading to autohydrolysis of hemicelluloses and lignin. Kaur *et al.,* (1998) used steam pressure with alkali treatment to pretreat rice straws. A two-step steam pretreatment of softwood by dilute acid impregnation which includes cellulose hydrolysis at second step is very promising. Pretreatment using steam explosion or mild acid treatment renders hemicelluloses soluble with the production of soluble oligomers, pentose sugars which can be extracted and separately fermented.

Steam pretreatment can be adopted in substances such as soft wood. Addition of catalyst such H_2SO_4 or SO_2 is a prerequisite for high sugar yield. Different materials however have different pretreatment procedure most appropriate for it. Unlike SO_2 impregnation during steam pretreatment of spruce chip, dilute acid pretreatment results in a harder material to ferment because of generation of inhibitory compounds (Söderström *et al.,* 2005;
Hahn-Hägerdal *et al.,* 2006). Acid catalysed steam pretreatment is especially suitable for agro waste and forestry residues. Palmarola-Adrados *et al.,* (2005) optimized pentose and hexose release from barley husk using steam pretreatment with varying concentrations of H_2SO_4.

Closest to commercialization is the steam pretreatment with the addition of a catalyst.

Method such as Ammonia fibre explosion (AFEX), wet oxidation and liquid hot water (LHW) pretreatments are more successful for agricultural residues.

ii. Liquid Hot Water (LHW)

This is also called thermohydrolysis. This is recognised to be cost effective for hardwood and agricultural residue but less efficient for softwood. A variant of steam explosion pretreatment for soft wood is being designed. Liquid Hot Water (LHW) thermo hydrolysis makes use of pressurized hot water, a pressure of >5MPA, temperature of 170-230^{o}C, for 1 to 46 minutes. Advantages of this process is the elevated recovery rates of Pentose and does not generate inhibitors (Ogier *et al.,* 1999).

iii. Ammonia Fibre Explosion (AFEX)

AFEX is similar to steam explosion and CO_2 explosion but ammonia, instead of steam and CO_2 are used in each process respectively. Lia *et al.*,(2010) optimized pretreatment of forage and sweet sorghum bagasse with AFEX and achieved an increase in both glucan and xylan conversion up to 90% at 1% glucan loading by supplementing xylanase with cellulase during enzymatic hydrolysis. They also recorded a maximum final bioethanol concentration in the fermentation broth as 30.9 g/L (forage sorghum) and 42.3 g/L (sweet sorghum bagasse).

C. Chemical Method

This involves the use of chemical agents such as ozone, acid, alkalis, peroxide, Na_2CO_3 and organic solvent. They include oxonolysis, dilute acid hydrolysis, concentrated acid hydrolysis, alkaline hydrolysis, oxidative delignification, wet oxidation, Organosolvent process.

i. Wet oxidation

This type of pretreatment is carried out at high temperature and pressure with the addition of a chemical substance. Petersson *et al*., (2007) used wet oxidation process using Na_2CO_3 at 195^oC, 15min, 2gl-^{1} in pretreating three lignocellulosic biomass; winter rye, oilseed rape and faba bean which gave a theoretical bioethanol yield of 66%, 70% and 52% respectively after enzymatic hydrolysis. Wet oxidation of these lignocellusics yielded more than 85% glucose, 60% mannose recovery and more than 60% hemicelluloses sugar recovery (Petersson *et al.,* 2007). However the use of atmospheric air and hydrogen peroxide in wet oxidation has also been documented. Sørensena *et al.*, (2008) reported a yield of 94.9% and 61.3%, for xylose and glucose respectively when atmospheric air was used as oxidant while hydrogen peroxide gave the yield of 82.4% and 63.7% for xylose and glucose respectively in a wet oxidation pretreatment of Miscanthus.

ii. Dilute Acid Hydrolysis

Dilute acid pretreatment is usually conducted at a lower temperature ranging from 120^oC – 220^oC for a short period of time (Chandel *et al.,* 2007) using either of the following acids; HNO_3, HCL and H_2SO_4. The target of the pretreatment solution at the condition specified above is majorly the hemicellulosic fraction though cellulose may also be mildly affected. The depolymerisation of the hemicellulose fraction yields xylose and other sugars and improves cellulose digestibility by further chemical hydrolysis or enzymatic saccharification. However, the process is not without any limitation. The works of Olsson and Hahn-Hagerdal (1996); Larsson *et al*., (1999); Klinke *et al*., (2004) independently observed that the production of fermentation inhibitors like furfurals, carboxylic acids and phenolic compounds at high temperature and

prolonged time is the major drawback of this process. This is because the inhibitors affect the fermenting organism and causes premature termination of the fermentation process. Hydrolysis of biomass at a lower temperature allows avoidance of sugar degradation to furfural and hydroxymethyl furfural though sugar yield will be affected. The National Renewable Energy Laboratory (NREL) who currently produces ethanol from biomass has preferred dilute acid pretreatment. Some researchers have pointed out that higher sugar recovery can be achieved with dilute acid hydrolysis. This recovery is about 80% for hard wood (Lynd, 1996). However, the yield of fermentable sugars varies from one lignocellulosic biomass to the other. Hernandez-Salas *et al.,* (2009) reported a yield of 37.21% and 35.37% reducing sugar for both depithed and pit bagasse from sugarcane treated with mild acid of 1.2%v/v HCl at 121^{o}C autoclave temperature.

iv. Dilute Alkaline Pretreatment

Alkali pretreatment mainly attacks part of the lignin. The use of dilute alkali leads to increase of internal surface area by swelling, decrease of polymerization degree and crystallinity, destruction of links between cellulose and other polymers and breakdown of lignin. The effectiveness depends on lignin content (Sun and Cheng, 2002). For hemicellulose to be removed after alkali pretreatment, hemicellulases need to be added (Hahn-Hägerdal *et al*, 2006).

The use of bases such as sodium hydroxide; organic solvents such as methanol and ethanol allows dissolution of lignin but the cost is high and is not competitive for large scale plants (Lynd *et al.,* 1999). Robert *et al.,*(2005), in the Dutch sponsored program on economy, ecology and technology, used mild temperature lime ($Ca(OH)_2$) hydrolysis to pretreat wheat straw. Lime

pretreatment leads to high enzymatic degradability and fermentability of lignocellulose, without significant delignification or xylan degradation.

d. Biological Method

Biological pretreatment involves the use of enzymes or whole organism such as fungi in pretreating raw materials for bioethanol production.

White rot fungi which decolourizes decaying woods has been found to be useful in degrading lignin in lignocellulosic materials. They are utilized in ligninase production for lignocellulosic biomass degradation. One major disadvantage of this process is the slow rate of conversion which actually limits its industrial application. The advantage of using biological method is that it has low energy requirement and mild environmental condition.

Zhang *et al.* (2006) developed a fusant by way of protoplast fusion of phanerochaete chrysosporium, a white rot fungi and *Geotrichum candidium* for corn stover fermentation. This fusant has both lignin degradation activity and protein accumulation ability which is gotten from Phanerochaete chrysosporium and Geotrichum candidium respectively.

The efficacy of pretreatment can be accessed through enzymatic hydrolysis (Saccharification) for its digestibility and fermentation of pretreated sample to assess the effect of inhibitors towards the fermenting microorganism.

2.10.2 Hydrolysis (Saccharification) of Lignocellulosic Material

For effective conversion of lignocellulosic material to bioethanol, cellulose should be degraded to glucose using acid or enzymes. In acid hydrolysis, both concentrated and dilute acids can be used in a one stage or a two stage process.

A. One-stage process

If dilute acids (H_2SO_4 or HCl) are employed, high temperature; 200-240^oC at 1.5% acid concentrations are required to hydrolyze the crystalline cellulose. The major disadvantage of this process is the degradation of glucose and xylose into hydroxymethyl furfural and furfural respectively with other non-desired products which can inhibit microbial fermentation.

B. Two-stage process

Acid hydrolysis can be done also in two stages. The first employs a milder condition of 190^oC, 0.7%acid for 3minutes. Hydrolysis of about 80% of hemicelluloses and 29% cellulose is achieved in the first reactor (Chandel *et al.*, 2007). Further incubation of the hydrolysate for two hours at lower temperature brings about hydrolysis of oligosaccharides to monosaccharides. A separated solid material is sent to second stage reactor.

The second stage employs a harsher condition of 215^oC, 0.4% acid for 3minutes. The former is useful in recovery of pentoses while the later recovers hexoses. The Two stage process can yield up to 50% glucose (Hamelinck *et al.,* 2005). Another variant of acid hydrolysis is the auto-hydrolysis which employs very low acid concentration at high temperature.

C. Concentrated Acid Hydrolysis

Concentrated acid hydrolysis employs 30-70%H_2SO_4 for a time range of 10-12h at a very low temperature. This could achieve a yield of 90% glucose but the amount of used acid is a critical economic factor. About 97% acid recovery is possible with continuous ion exchange (Hamelinck *et al.,* 2005). The use of low temperature minimizes sugar degradation (Chandel *et. al.,* 2007). There is a high potential for high sugar recovery efficiency and about 90% of hemicelluloses and Cellulose fraction could be depolymerised.

D. Enzymatic hydrolysis

Enzymatic cellulose hydrolysis is currently being used in cellulose hydrolysis. It has demonstrated better results for subsequent fermentation though the process is slower. It has been proven to have eliminated the possibility of glucose degradation into hydroxyl methyl furfural and possibility of wasting glucose.

In enzyme hydrolysis, the various cellulolytic enzymes play a role in degradation of cellulose into glucose. Cellobiohydrolases causes a gradual decrease in the degree of polymerization while endoglucanases causes the rupture of cellulose into smaller chains leading to further decrease in degree of polymerization and β-glucosidase breaks down cellulose into two molecules of glucose.

2.10.3 Fermentation

Fermentation is the breakdown of glucose into ethanol. In bioethanol production, two fermentation processes can be employed. The separate hydrolysis and fermentation (SHF) and an alternative variant called simultaneous saccharification and fermentation (SSF). SHF is a

classical fermentation process where hydrolysis and fermentation are carried out on a separate unit. While in SSF hydrolysis and fermentation are done in a single unit.

Saccharomyces cerevisiae is the most frequently used microorganism. It converts hexose sugar and not pentose sugars to ethanol. Some bacterial strains for example, *Zymomonas mobilis, Zymobacter palmae* and *Clostridium cellulyticum* can produce ethanol but cannot convert and utilize lignocellulosic biomass substrates to produce fermentable sugar. *Zymomonas mobilis* naturally has a better efficiency in converting starch to glucose and ultimately alcohol than Saccharomyces cerevisiae.

Separate Hydrolysis and Fermentation (SHF).

The main feature of SHF is that hydrolysis and fermentation can be performed in its optimum operation conditions. Important factors to be taken into account are reaction time, temperature, pH, enzyme dosage and substrate concentration.

A. Simultaneous Saccharification and Fermentation (SSF).

SSF is more attractive than SHF because of higher bioethanol yield and lesser energy consumption.

In simultaneous saccharification and fermentation, both hydrolysis and fermentation are carried out in one process unit giving room for immediate conversion of glucose produced from cellulose to ethanol by the fermenting organism. This eliminates inhibition effect on cellulases introduced by accumulated sugars and encourages higher ethanol yield with less energy being consumed in the process. The process operates at non optimal conditions for the hydrolysis and requires higher enzyme dosage, which positively influences substrate conversion, but negatively

affect process cost with the aim of reducing overall process cost in mind (Sanchez and Cardona, 2008).

Alkasrawi *et al.,* (2003) as cited by (Sanchez and Cardona, 2008) showed that the addition of non-ionic surfactant Tween-20 to a steam exploded wood during batch SSF using S.cerevisiae led to 8% increase in ethanol yield; 50% reduction in cellulose dosage (from 44FPU/g cellulose to 22FPU/g cellulose); increase in enzyme activity at the end of the process and decrease in the time required for reaching the highest ethanol concentration. The Surfactant Tween-20 reduces a non useful adsorption of cellulase to lignin. Saha *et al.,*(2005) reported a marginal increase of 3.5% in saccharification of rice hull using 2.5g/L of Tween-20.

Many researchers have revealed different conditions optimised for some biomass resources. Hydrolysis and fermentation conditions need to be optimised when utilising sweet sorghum for bioethanol production. Harikrishna *et al.* (1998) as cited by (Sanchez and Cardona. 2008) evaluated the optimal conditions of the SSF of sugar cane leaves and they reported 40^{o}C and pH of 5.1 as the best condition for a 3 day cultivation achieving 31g/L of ethanol from an initial substrate load of 15%. This was achieved with enzyme dosage of 100FPU/g cellulose. A non-isothermal process of Varga *et al.* (2004) achieved 78% Bioethanol. Cellulose was added at 50^{o}C and later at 30^{o}C though the final concentration of solid hydrolysate was brought to 17%. Another organism that can yield ethanol at 40^{o}C higher temperature is the yeast called Kluveromyce Marxianus.

B. Pentose Fermentation

Pentose obtained during hemicelluloses hydrolysis especially xylose cannot be naturally assimilated by *Saccharomyces cerevisiae*. Pentose fermenting organism has been identified and such organisms are *Pichia stipitis, Candida shehatae and Pachysolen tannophilus* (Sanchez and Cardona, 2008) though their bioethanol production rate is 2-4 times lower than S.cerevisiae. However, Purdue yeast a genetically modified *Saccharomyces cerevisiae* developed at Purdue University now has the ability to simultaneously ferment pentoses and hexoses namely: xylose, arabinose mannose, galactose, glucose into bioethanol (Bera *et al*., 2010). This has increased the efficiency of fermentation making sure that more bioethanol is generated from agricultural wastes.

Most of the microorganisms employed in pentose fermentation are inhibited by substrate concentration. However, their culture requires oxygen and ethanol tolerance is 2-4 times lower (Claassen *et al.,* 1999). For efficient use of this organisms, note should be taken on preference of glucose followed by galactose and xylose and arabinose, Gong *et al.,*(1999) explained the catabolic repression that glucose exerted on xylose and other pentose. To offset this effect, Chandrakart and Bisaria (1998) reported a sequential fermentation using S.cerevisiae on the first day to utilize hexoses and later with yeast that utilizes xylose for complete conversion of pentose and hexoses to ethanol. Bioethanol formed after fermentation is distilled out.

2.11.0 The global Bioethanol production capacity:

For many decades now, Brazil has been the world largest producer and consumer of bioethanol. However, in 2006 and 2007, United States of America (USA) surpassed Brazil. About 60% of ethanol production comes from sugar crops, 30% from grains, 7% from synthetic ethanol and 3% from other raw materials.

The annual world production of bioethanol is 65 billion litres (Delft, 2009). According to another article, the world total production of bioethanol is 39 million tonnes, Brazil and USA accounts for 88% of this production from sugar cane and corn respectively. Bioethanol is equally consumed in large quantity in these two countries. About 20.4 million tonnes of bioethanol was consumed in USA as transportation fuel in 2007 while 6.3% was imported. The Brazilian bioethanol fuel consumption in 2006 amounted to 10.2 million tonnes which is 14.69% of total fuel consumption.

In 2006, European Union (EU) produced bioethanol that is equivalent to 90% of its fuel consumption. Consumption of bioethanol for transportation is largest in Germany, Sweden, France and Spain. Their total consumption in 2007 was 1.8 million metric tonnes (Litch, 2008). Bioethanol production has also been aggressively pursued in a number of African countries currently producing sugar. They are Kenya, Malawi, South Africa and Zimbabwe. Others with great potential include Mauritius, Swaziland and Zambia. Nigeria is one of the leading producers of sorghum and could be a major source of bioethanol within the African continents because there is already a bioethanol plant situated in its South-west geopolitical zone. Other sorghum producing countries including India, China, Mexico, Sudan and Argentina.

2.12.0 Challenges of bioethanol production

Utilization of lignocellulosic feedstock for bioethanol production using enzymatic/microbiological methods has become the recent focus of many researches on bioethanol production. However, the complex crystalline structure of native lignocellulosic materials as a result of intimate association of cellulose and hemicelluloses with lignin in the plant cell wall (Ballerini *et al.,* 1994) makes them resistant to enzymatic attack which poses a

major problem in achieving this process**.** It is always not very easy to efficiently break the recalcitrant in lignocellulose structure to release fermentable sugars for bioethanol production. The aim of pretreatment is to change these properties in order to prepare the materials for enzymatic degradation. Since lignocellulosic materials are very complicated, their pretreatment is not simple either. However, with adequate pretreatment option, microbial and commercial enzymes are able to efficiently liberate sugars from the complex crystalline structure of lignocellulosic material. A major challenge in bioethanol production from lignocellulosic biomass is the development of an efficient process to convert these lignocellulosics to simple sugar. With careful manipulation of the various pre treatment options alongside enzymatic/microbial hydrolysis, high yield of fermentable sugars is hoped to be achieved. The best method and conditions of pretreatment depend greatly on the type of lignocelluloses. Therefore an adequately identified pretreatment method has the ability to change these properties in order to prepare the materials for efficient enzymatic degradation.

For instance, pretreatment of bark from poplar trees or corn leaf with a dilute-acid process seems to be promising, but this method is not effective for treating the bark from sweet sorghum or corn stalks (Mohammad and Keikhosro, 2008). Since the best method and conditions of pretreatment depend greatly on the type of lignocelluloses, the aim of this work is to investigate different pretreatment methods and determine the effectiveness of the best pretreatment method for sweet sorghum with respect to fermentable sugar yield after pretreatment. So far, no comparative investigative study on the pre treatment of sweet sorghum bagasse using mild concentration of HCl and H_2O_2 has been documented.

2.13.0 Availability of Promising Sweet Sorghum Varieties in Nigeria

There are sweet sorghum lines in Nigeria though the most cultivated sorghum is the grain type.

Good ICRISAT sweet sorghum varieties are now available in the Department of Plant Science, Institute for Agricultural Research, Ahmadu Bello University Zaria. They are currently conducting field trials for some of the varieties to determine their performance. This is where the sweet sorghum used for this research was obtained from.

2.14.0 Sustainability of bioethanol project in Nigeria

Current interest in bioethanol research is focusing on how to efficiently liberate sugar molecules from lignocellulosic feedstock for increased bioethanol production. Though grain sorghum is the predominant sorghum specie produced and cultivated in Nigeria, sweet sorghum ethanol project has also been introduced due to the growing interest of the world in green fuel. In July 2008, Global biofuels announced the investment of US$750 million for bioethanol production from sweet sorghum in Nigeria. The first series of ethanol plants which utilizes sugar from sweet sorghum stem juice as feedstock will be constructed in Ekiti State and six other states (Daily Biofuel digest, 2008). The Global fuel is working in collaboration with the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT). For more than a decade now, International Crop Research Institute for Semi Arid Tropics (ICRISAT) has been committed to identification of promising sweet sorghum varieties and development of hybrids for bioethanol production. Among their early discoveries were sweet sorghum progeny from Nigeria and Zimbabwe lines with other advanced breeding progeny

(Reddy *et al.*, 2006). ICRISAT has identified many high-sugar yielding sweet sorghum varieties that can grow with less water in the dry lands of the world. This ensures that there will be no environmental limitation in its cultivation anywhere in the world. ICRISAT is also trying to develop hybrids varieties which are being tested for alongside others for their grain and sugar (for bioethanol) production capability.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Materials

3.1.1 Sample Collection and Identification

Mature plant of *Sorghum bicolor L. moench* (Sweet sorghum) already identified at Department of Plant Science, Institute for Agricultural Research, Ahmadu Bello University (ABU) were harvested at periodic intervals of two weeks (5, 7 and 9weeks) from the experimental farm of the Department of Plant Science, Ahmadu Bello University located in Shika Zaria.

List of Sweet sorghum varieties used and their origin

Nigerian Varieties	Indian Varieties	Indian varieties crosses in IAR Zaria
SWSV 2006-3	SPV 422	ICSA 89002 X SPV 422
SWSV 2005-5-1	ICSV 700	ICSA 95 X NTJ -2
SWSV 2006-5-2	NTJ -2	
SWSV 2007-1	E 36-1	
SWSV 2007-3.	ICSR 93034	
	ICSV 93046	
	ENT64 DTN	

3.1.2 Chemicals and Reagents

The chemicals and reagents used for the study were Dinitrosalicylic acid (Sigma chemicals, St Louis U.S.A), Glucose oxidase kit (Teco diagnostics), Sodium Potassium Tartrate (BDH, Poole,England). Sodium Chlorite, Acetic acid, Acetone, Diethyl ether, Potassium hydroxide, Industrial spirit, Glacial acetic acid, diethyl ether, Concentrated Sulphuric acid.

3.1.3 Instruments

The instruments used for the study were: measuring balance, colorimeter (Jenway, UK), laboratory mill model 4 (Thomas wiley), shaker, water bath, oven, incubator, Aluminum pans, and autoclave. Desiccators containing desiccants.

3.2 Methods

3.2.1 Preparation of Samples

Stage 1

For periodic reducing sugar determination

Fourteen different Sweet sorghum plants varieties were harvested at 5, 7 and 9weeks. One stem per variety (Sweet sorghum) was randomly cut from the base. The bark of the stem were peeled off to expose the pith and one gram (1g) wet weight of the pith were pounded in a laboratory mortar to a fine paste, extracted with 20mls of distilled water in a shaker for three (3) hours and filtered with Whatman No. 1 filter paper. The reducing sugar content of the filtrate was determined according to the Dinitrosalicylic acid (DNS) method of Miller (1959). See section 3.2.5.1 for reducing sugar determination procedure.

Eight sweet sorghum varieties were selected at the end of the periodic sampling based on the increasing order of reducing sugar content.

Stage 2

Bagasse preparation

Eight selected Sweet Sorghum varieties were harvested at the ninth week. Their barks were peeled off to expose the pith. The pith was crushed, extracted with water at the ratio of 1:20

[stem (g): water (ml)] in a shaker for 24hr, and filtered with Whatman No. 1 filter paper. The residues were washed with water several times to produce bagasse. The resulting bagasse was dried to a constant weight at 45 ± 3^{o}C according to the method of Hames *et al*., (2008). The dried bagasse were ground with a laboratory mill (Wiley) of a mesh size of 0.5mm to form bagasse powder (ground bagasse) and stored in an air tight container at +4^{o}C. The percentage bagasse powder yield was calculated as follows:

$$\% \text{ Bagasse powder yield} = \frac{\text{Weight of ground Sweet sorghum bagasse}}{\text{Weight of unpeeled Sweet sorghum stem}} \times 100$$

3.2.2 Determination of cellulose, hemicellulose and lignin content of the stem bagasse of eight sweet sorghum varieties

The processed bagasse powders were subjected to compositional analysis to determine non-soluble carbohydrate content. The cellulose, hemicellulose and lignin content of the eight selected sweet sorghum stem were qualitatively determined according to the method outlined in Chemical Analysis of Ecological Materials (Allen *et al.,* 1974).

-Determination of Cellulose content of the bagasse

One (1)g of the bagasse powder of eight Sweet sorghum varieties were weighed into a conical flask. Thirty (30) ml of water, 2ml of 10% acetic acid and 0.6g Sodium chlorite were added into the flask, mixed and incubated at 75^{o}C in water bath. Ten (10) % acetic acid and Sodium chlorite was added at hourly intervals and swirled intermittently . The flasks were removed after 4hours and cooled immediately in ice cold water. The contents were weighed through No 2 Pyrex sintered crucibles. The residues were washed about 10 times with ice cold water and washed 3 or 4 times with acetone and finally once with ether. The ether was allowed to evaporate in oven at

105^{o}C for 30minutes, cooled in a desiccator and weighed. The weighed samples represents the holocellulose.

The ash and N contents (multiplied by 6.25 to give a correction for crude protein) on sub-samples of holocellulose were determined and these values subtracted before calculating the % holocellulose.

$$\text{holocellulose (\%)} = \frac{\text{corrected holocellulose(g)} \times 10^2}{\text{Sample weight}}$$

The holocellulose were weighed into a 50ml conical flask. Twenty (20) ml of 24%KOH was added into the each flask, stopper with rubber bung and placed in water bath at 20^{o}C for 2hours. The flasks were swirled at intervals. There were later filtered through a weighed No. 2 glass sintered crucibles, washed with water until washings were free of alkali. Five (5) ml of 5% acetic acid each was added to the residues and swirled around in crucible. They were washed again with water, acetone and finally ether. The ether was allowed to evaporate. The residues were dried in an oven at 105^{o}C for 30minutes, cooled in desiccators and weighed as α-cellulose.

$$\text{Cellulose (\%)} = \frac{\text{Corrected α-cellulose (g)} \times \text{total uncorrected holocellulose (g)} \times 10^2}{\text{Holocellulose sub-sample (g)}}$$

-Determination of hemicellulose content of the bagasse

The procedure for α-cellulose determination is followed until the filtering stage is reached. The bagasse samples were filtered through No. 2 pyrex sinter crucibles into a reservoirs containing 8ml of glacial acetic acid. The residues were washed with both water to remove all alkali and 5ml of 5% acetic acid. They were again washed with water, acetone and finally ether. The pH of filtrates was adjusted to 4.0 with 5% acetic acid and industrial spirit was added to about 3.5 times

the volume of the filtrates and left to stand overnight. They were filtered through a weighed No. 2 sinter crucible, washed with industrial spirit, acetone and ether. The ether was allowed to evaporate. The residues were dried in the oven at 105^{o}C for 30minutes, cooled in desiccators and weighed.

$$\text{Hemicellulose}(\%) = \frac{\text{Weight of hemicelluloses (g) X total uncorrected holocellulose (g) X } 10^2}{\text{Holocellulose sub-sample (g) X sample weight}}$$

-Determination of lignin content of the bagasse

One (1)g of dried bagasse from eight Sweet sorghum varieties were weighed into glass fibre papers and tied into a bundle with terylene thread. Ether extraction was carried out in a soxhlet for about 6hours. The ether was allowed to evaporate and the bundle was dried for 30minutes at 105^{o}C in an oven. They were cooled and weighed after removing the thread.

About 0.8g from ether extracted bagasse was weighed into a 600ml tall pyrex beakers and about 400ml of water was added. The contents were boiled gently for 3hours and the volume maintained at 400ml. Twenty two (22) ml of 10% Sulphuric acid was added and boiled for extra one hour. The contents were allowed to settle. The supernatant liquid was removed with No. 2 sintered filter sticks leaving the bagasse as dry as possible. Fifteen (15)ml of 72% Sulphuric acid was added to the bagasse residues at 15^{o}C and stirred with the filter sticks. The beakers were left in a water bath for 2hours at 20^{o}C and stirred continuously. The acid strength was reduced to 3% by addition of 560ml of water. The sinter sticks were washed into the beakers and removed. The contents were boiled gently for 4hours and were topped when necessary. The contents were allowed to settle and filtered immediately through a weighed No.2 sintered glass crucible and the residues was washed until it is acid free with hot water. The sinters and contents were dried for 3 hours at 150^{o}C, cooled and weighed.

Ash and N-content were determined as in cellulose determinations which were subtracted before calculating for % lignin.

$$\text{Lignin (\%)} = \frac{\text{Corrected lignin (g) X total ether extracted sample (g) X } 10^2}{\text{Weight taken for water extraction (g) X sample weight (g)}}$$

3.2.3 Sample Pre-treatment

3.2.3.1 Susceptibility of the bagasse to pretreatment solutions

The bagasses powder of eight Sweet sorghum varieties were each treated with 1% V/V concentrations of HCl and H_2O_2 respectively according to the acid and peroxide pre-treatment methods outlined in sections 3,2,3,2 and 3,2,3,3 below. Two sweet sorghum varieties; one each from Nigerian and Indian varieties were selected from the eight for further studies based on the glucose yield after this initial pre-treatment. Other factors considered are: stem size; % bagasse powder yield; overall carbohydrate content (cellulose and hemicelluloses content) and lignin content; yield in glucose after 1.0% HCl and 1.0%H_2O_2; availability and level of pest infestation.

3.2.3.2 Acid pretreatment

Bagasse powders were pre-treated with acid according to the method of Arthe *et al.,* (2008) with a little modification. Ten (10) ml of dilute Hydrochloric acid (of known concentrations) were dispensed into 250 ml conical flasks that contained 0.2g of each sweet sorghum variety (bagasse

powder) and autoclaved at 121^{o}C for 15 minutes. The insoluble residues in the flasks were filtered. The filtrates were used for glucose and reducing sugar determination.

3.2.3.3 Peroxide Pretreatment

Alkaline peroxide pre-treatment was carried out according to the method of Sridevi *et al.,* (2009) with some modifications. One fifth (0.2)g each of the bagasse powders of the sweet sorghum varieties were treated with 10ml Hydrogen peroxide in 250 ml conical flask for 15 minutes at autoclave temperature of 121^{o}C. The insoluble residues in the flasks were filtered, and the filtrates were used for glucose and reducing sugar determination.

3.2.4 Optimization of pre-treatment conditions:

Optimization of sugar-release from the bagasse powder of two selected Sweet Sorghum varieties was carried out by applying the following conditions below to the acid and peroxide pre-treatment methods already outlined in sections 3.2.3.2 and 3.2.3.3 above respectively.

The conditions are:

- Effect of concentrations ranging from 0%, 0.5%, 1%, 1.5%, 2%, 2.5%, 3% of the pre-treatment solutions: HCl and H_2O_2 on glucose yield was determined using glucose assay kit procedure (described below).
- Effect ratio of the sample to pretreatment solution on glucose yield was determined for the following ratios: 1:50, 1:100, 1:150 and 1:200 (g of sample /ml of pretreatment solution).

- Effect of temperature on glucose yield was determined at two different temperatures (121 ^{o}C and 138 ^{o}C) in an autoclave.

3.2.5 Chemical analysis

3.2.5.1 Reducing Sugar (RS) Determination

Quantitative determination of reducing sugar of the filtrates from the sweet sorghum varieties used for periodic RS determination and filtrates from bagasse hydrolysates was carried out using dinitrosalicylic acid (DNS) method (Miller, 1959). DNS reagent was added to the filtrates at the ratio of 3:1(ml) in a test tube and placed in a boiling water bath for 5 minutes. The samples were allowed to cool under running water bath. The colour intensity produced from the reduction of 3, 5-dinitrosalicylic acid (DNS) to 3-amino, 5-nitrosalicylic acid was determined by taking absorbance at 540 nm. Glucose was used as a standard.

3.2.5.2 Glucose determination

Glucose concentrations of the filtrates from pre-treated sweet sorghum samples were determined using glucose oxidase reagent kit. Two (2.0)mls of the reagent were placed in a test tube and pre-incubated in a water bath at 37^{o}C for 5minutes. Ten microlitre (10µL or 0.01ml) of filtrate from the hydrolysate were added into the respective tubes, mixed and incubated at 37^{o}C for 10minute. Absorbance reading was taken in a spectrophotometer at 520 nm. Concentration of glucose was expressed as

$$= \frac{\text{Absorbance of the unknown}}{\text{Absorbance of the standard}} \times \text{concentration of glucose standard (mg/ml)}$$

3.2.6 Determination of Moisture content

This was carried out according to the method of Sluiter et al., (2008). Aluminum dishes were pre-dried by placing them in a drying oven set at 105 ± 3ºC for a minimum of four hours. They were cooled in desiccators and weighed (W1) to the nearest 0.1 mg.

One (1.0)g of Sweet sorghum pith were each weighed into the Aluminum dishes (W2) to the nearest 0.1 mg and dried overnight to a constant weight in an oven set at 105 ± 3^{o}C.

The samples were removed from the oven and allowed to cool to room temperature in desiccators. The dishes containing the oven-dried samples were weighed to the nearest 0.1mg and the weight recorded. This procedure was repeated for a minimum of four hours and re-weighed until a constant weight (W3) is achieved. Constant weight is defined as ± 0.1% change in the weight percent solids upon one hour of re-heating the sample.

$$\% \text{ Moisture content} = 100 - \left[\frac{\text{Weight}_{\text{dry pan plus dry sample}} - \text{Weight}_{\text{drypan}}}{\text{Weight}_{\text{sample as received}}} \times 100\right]$$

$$\% \text{ Moisture content} = 100 - \left[\frac{W3 - W1}{W2 - W1} \times 100\right]$$

3.2.7 Drying of bagasse

This method was described by Hames *et al*., (2008). The bagasse of eight Sweet sorghum varieties were first air-dried before been oven-dried. Air drying method is suitable for the preparation of large quantities (>20 g) of field-collected samples into a form appropriate for compositional analysis. This method is suitable for drying materials where ambient humidity allows the sample to air-dry to moisture content below 10% as measured using LAP "Determination of Total Solids in Biomass and Total Dissolved Solids in Liquid Process Samples" of Sluiter et al., (2008). The bagasse of eight Sweet sorghum varieties were thinly spread out on a suitable surface and allowed to air-dry. They were turned at least once per two hour to avoid microbial growth and ensure even drying of the samples. The % moisture content of the bagasse of eight Sweet sorghum varieties was determined once every 24 hours using the formular below.

$$\% \text{ Moisture content} = 100 - \left[\frac{\text{Weight dry bagasse}}{\text{Weight wet bagasse}} \times 100\right]$$

The bagasse was considered dried when the moisture content was less than 10% by weight and the change in weight is less than 1% in 24h.

The bagasses were also repeatedly oven dried at 45± 3°C for intervals of 1h and placed in desiccators to cool to room temperature. An aluminum containers were dried for 3h at 45± 3°C and dessicated. The weight of dried container (W1), dried container plus bagasse (W2) and dried container plus dried bagasse (W3) to the nearest 0.1g were noted. Drying of Bagasse repeated until the change in the mass of the biomass is less than 1% in one hour.

$$\% \text{ Moisture content} = 100 - \left[\frac{W3 - W1}{W2 - W1} \times 100\right]$$

3.2.8 Yield calculations

3.2.8.1 Percentage glucose (G) and reducing sugar (RS) yield

This was determined as follows:

$$\% \text{ saccarification or } \% \text{ Glucose yield} = \frac{\text{Weight of Glucose after pretreatment}}{\text{Weight of raw sample}} \times 100$$

$$\% \text{ reducing sugar yield} = \frac{\text{Weight of Glucose after pretreatment}}{\text{Weight of raw sample}} \times 100$$

3.2.9 Statistical Analysis

Experiments were performed in triplicates and duplicates. Analysis of Variance (ANOVA) was used to compare mean of each treatment. The results were presented as mean ± standard deviation of glucose/reducing sugar concentration for each treatment. The difference between the mean of treatments were tested using the Duncan Multiple Range test at $P<0.05$.

CHAPTER FOUR

RESULTS

4.1: Periodic assessment for reducing sugar concentration and moisture content of fourteen Sweet sorghum varieties

Figure 4.1 Shows results of percentage (%) moisture content of fourteen sweet sorghum (S.S.) varieties at week 5, 7 and 9. Results revealed that the percentage moisture content varied among the weeks tested. A continuous decrease in % moisture content from week 5 through 7 to 9 weeks was noticed in exactly 50% of the total S.S varieties with 80% of Nigerian varieties exhibiting that trend. However, 42% of the sweet sorghum varieties showed an increase from week 5 to 7 but decreased at week 9. Approximately 67% of Indian varieties showed that trend. The only S.S. variety that showed fluctuation in pattern of decrease from week 5 to 7 and increase in its percentage moisture content at week 9 was SWSV 2007-1.

Results on reducing sugar determination of the fourteen Sweet sorghum varieties at week 5 of plant age are presented in Figure 4.2. The Nigerian variety, SWSV 2006-3 had the highest overall concentration of 83.08 ± 5.05mg/g which was not significantly different ($P>0.05$) from the reducing sugar value observed for ICSA 95 X NTJ -2 (82.24 ± 1.65mg/g)(see Appendix 2). The Sweet sorghum variety with the lowest value of reducing sugar at week 5 was ICSV 700 with reducing sugar concentration of 27.83 ± 0.91mg/g. This value was significantly ($P<0.05$) lower than the Nigerian variety SWSV 2007-3. The Indian cross-varieties: ICSA 89002 X SPV 422 and ICSA 95 X NTJ-2, Nigerian varieties: SWSV2006-3, SWSV2006-5-2 SWSV2007-1, and the Indian varieties: SPV422, E 36-1, ICSR 93034, ENT 64 DTN were the best in reducing sugar yielding capability.

Results on comparison of reducing sugar concentration of eight S.S. varieties at week five (5), seven (7) and nine (9) are presented in Figure 4.3. The reducing sugar concentrations of the Sweet sorghum varieties increased progressively from week 5 through 7 to 9 in about 87.5% of the Sweet sorghum varieties used. The only variety that fluctuated in pattern was E 36-1.

The reducing sugar concentration observed at week 9 is significantly ($p<0.05$) higher than values observed at weeks 5 and 7.

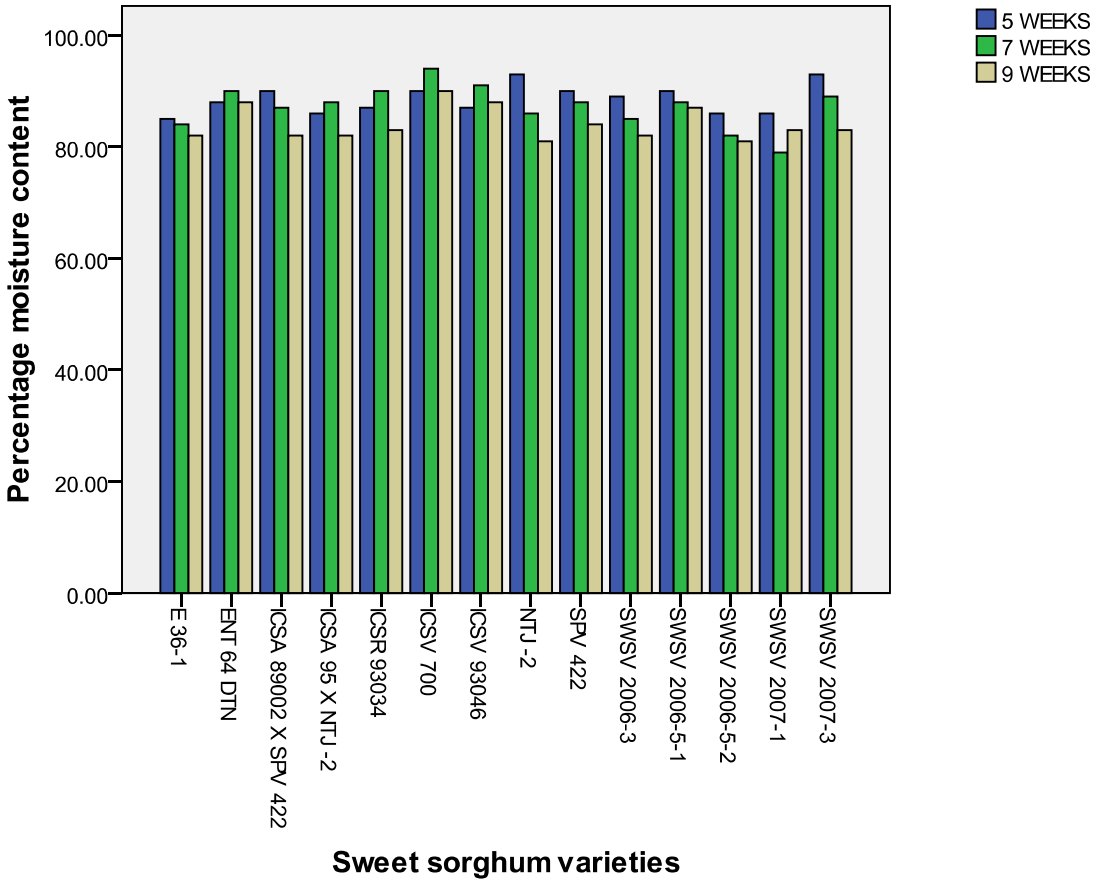


Figure 4.1: Percentage moisture content of bagasse from the stalks of fourteen sweet sorghum varieties

Nigerian Varieties	**Indian Varieties**	**Indian varieties crossed in IAR Zaria**
SWSV 2006-3	SPV 422	ICSA 89002 X SPV 422
SWSV 2005-5-1	ICSV 700	ICSA 95 X NTJ -2
SWSV 2006-5-2	NTJ -2	
SWSV 2007-1	E 36-1	
SWSV 2007-3.	ICSR 93034	
	ICSV 93046	
	ENT64 DTN	

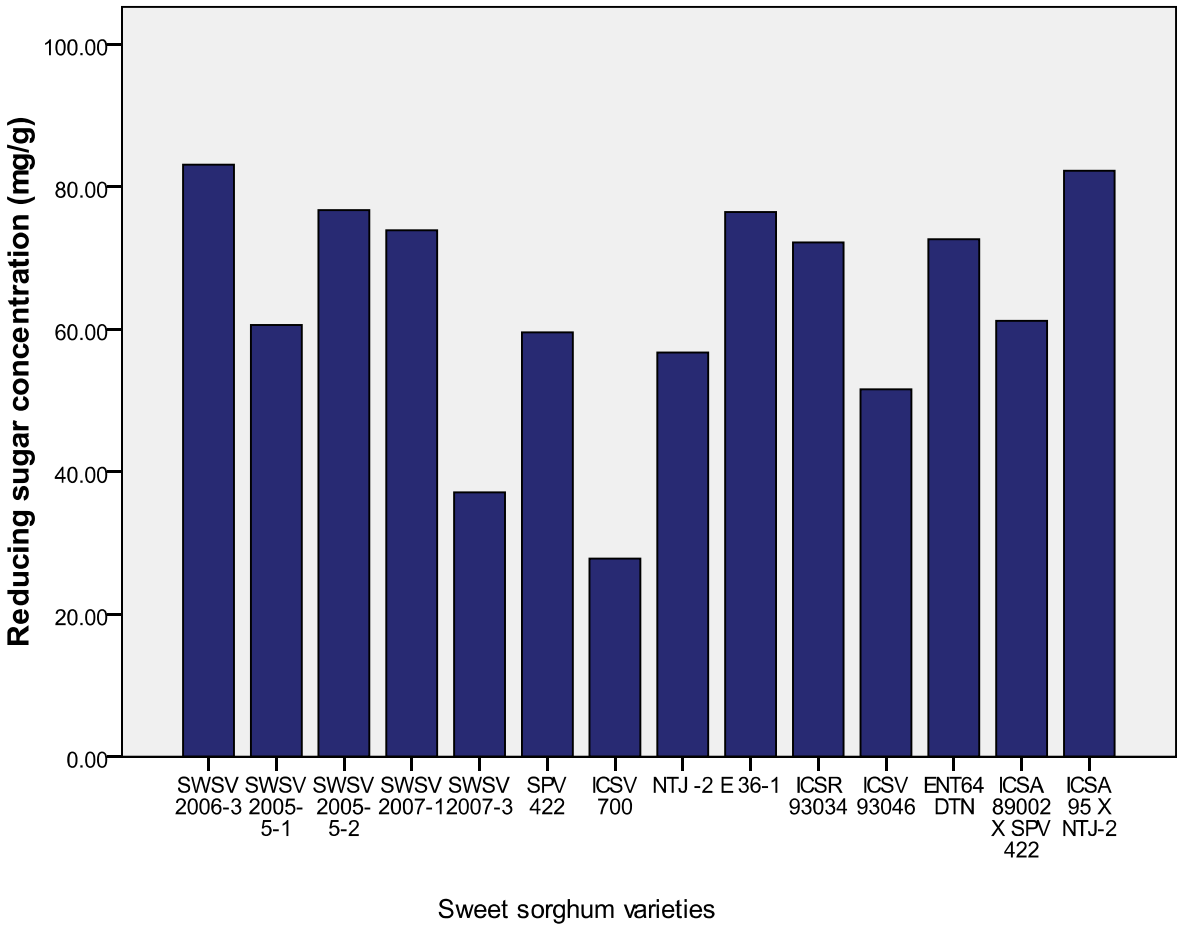


Figure 4.2: Reducing sugar concentrations (mg/g) of the stem from growing stalks of 14 Sweet Sorghum varieties at 5 weeks

Nigerian Varieties	**Indian Varieties**	**Indian varieties crossed in IAR Zaria**
SWSV 2006-3	SPV 422	ICSA 89002 X SPV 422
SWSV 2005-5-1	ICSV 700	ICSA 95 X NTJ -2
SWSV 2006-5-2	NTJ -2	
SWSV 2007-1	E 36-1	
SWSV 2007-3.	ICSR 93034	
	ICSV 93046	
	ENT64 DTN	

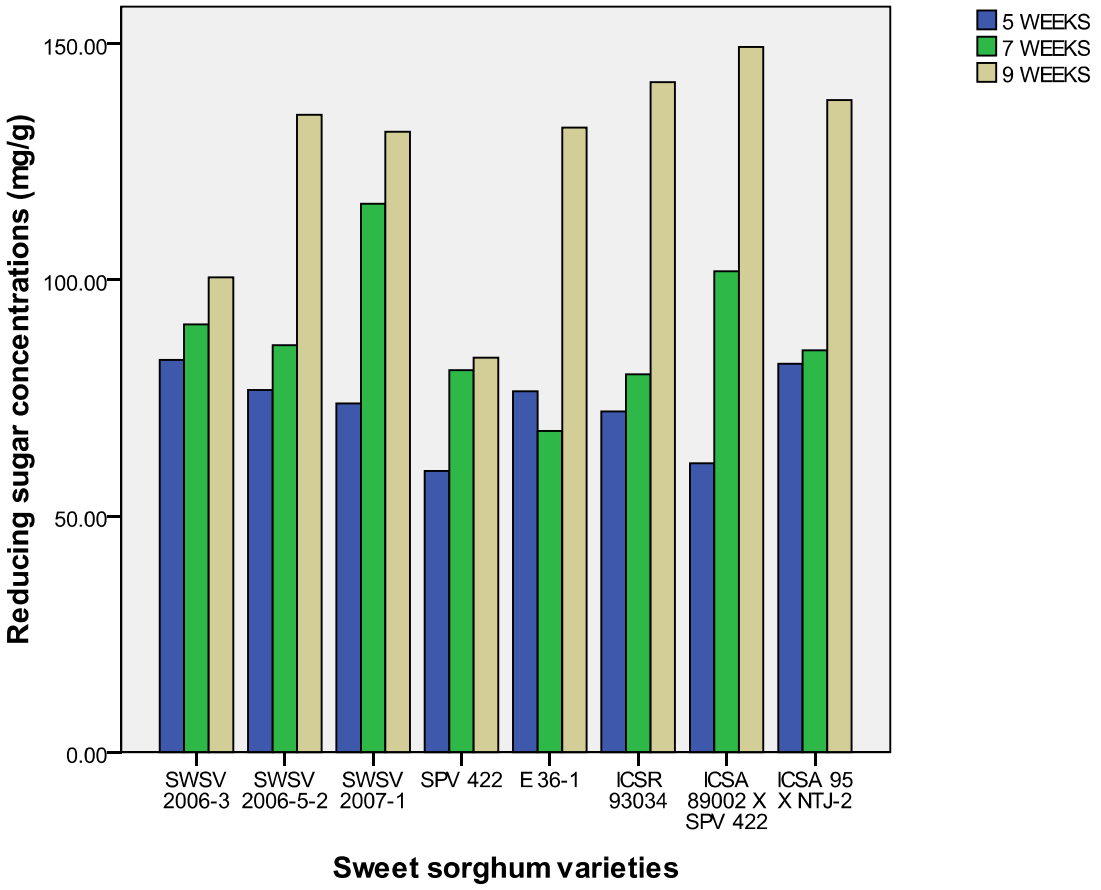


Figure 4.3: Reducing sugar concentrations (mg/g) of the stem from growing stalks of 8 Sweet Sorghum varieties at 5, 7 and 9 weeks.

Nigerian Varieties	Indian Varieties	Indian varieties crossed in IAR Zaria
SWSV 2006-3	SPV 422	ICSA 89002 X SPV 422
SWSV 2006-5-2	E 36-1	ICSA 95 X NTJ -2
SWSV 2007-1	ICSR 93034	

4.2: Cellulose, Hemicellulose, lignin content and Harvest Yield

The results in Figure 4.4 showed a wide variation in the percentage composition of cellulose, hemicellulose and lignin of the bagasse of the three categories of the sweet sorghum varieties analysed. The Nigerian S.S. Variety; SWSV 2007-1 had the highest cellulose percentage of 36.64±0.02 while the Indian Variety, ICSA 95 X NTJ-2 was lowest in cellulose content with percentage cellulose of 30.60 ± 0.02. The percentage hemicellulose composition was also highest in SWSV 2007-1 and lowest in E 36-1 with values of 20.40 ± 0.01 and 16.74 ± 0.04 respectively. However, the highest percentage lignin content of 22.18 ± 0.02 was recorded in ICSA 89002 X SPV422, a value which was not significantly higher than 22.16 ± 0.02 for ICSA 93034 while the lowest lignin value of 18.76 ± 0.02 was recorded in S.S. variety with tags ICSA 95 X NTJ-2.

The Nigerian varieties were observed to have higher cellulose composition than the other two groups. The variations in cellulose, hemicelluose and lignin content of the Sweet sorghum are lesser among varieties in the same category; less variation was observed among sorghum varieties of Nigerian origin than between Nigerian and Indian. The hybrids of Indian varieties were much closer to the Indian varieties in composition than the Nigerian varieties.

Figure 4.5 shows that ICSA 89002 X SPV 422 had a better yield of 2.71% bagasse (fibre) powder after harvest, a value very close to 2.42% for S.S. variety, ICSA X NTJ -2 while SWSV 2006-5-2 recorded the lowest value of 1.48%.

The percentage sweet sorghum bagasse yields were generally very low. However, ICSR 93034 and all the Nigerian varieties had relatively lower values when compared with all the Indian and Indian hybrid varieties.

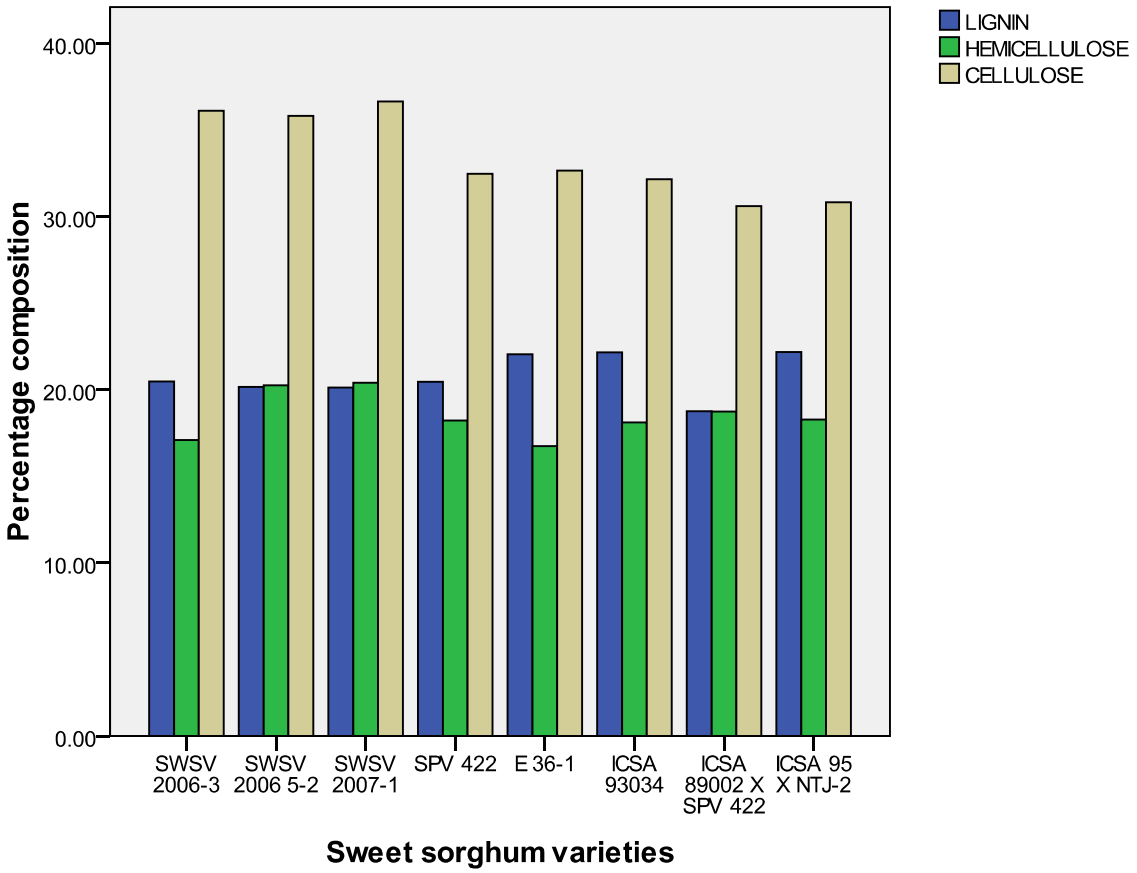


Figure 4.4: Cellulose, Hemicellulose and lignin content of bagasse from the stalks of eight sweet sorghum varieties harvested on 30th 0ctober 2010

Nigerian Varieties	Indian Varieties	Indian varieties crossed in IAR Zaria
SWSV 2006-3	SPV 422	ICSA 89002 X SPV 422
SWSV 2006-5-2	E 36-1	ICSA 95 X NTJ -2
SWSV 2007-1	ICSR 93034	

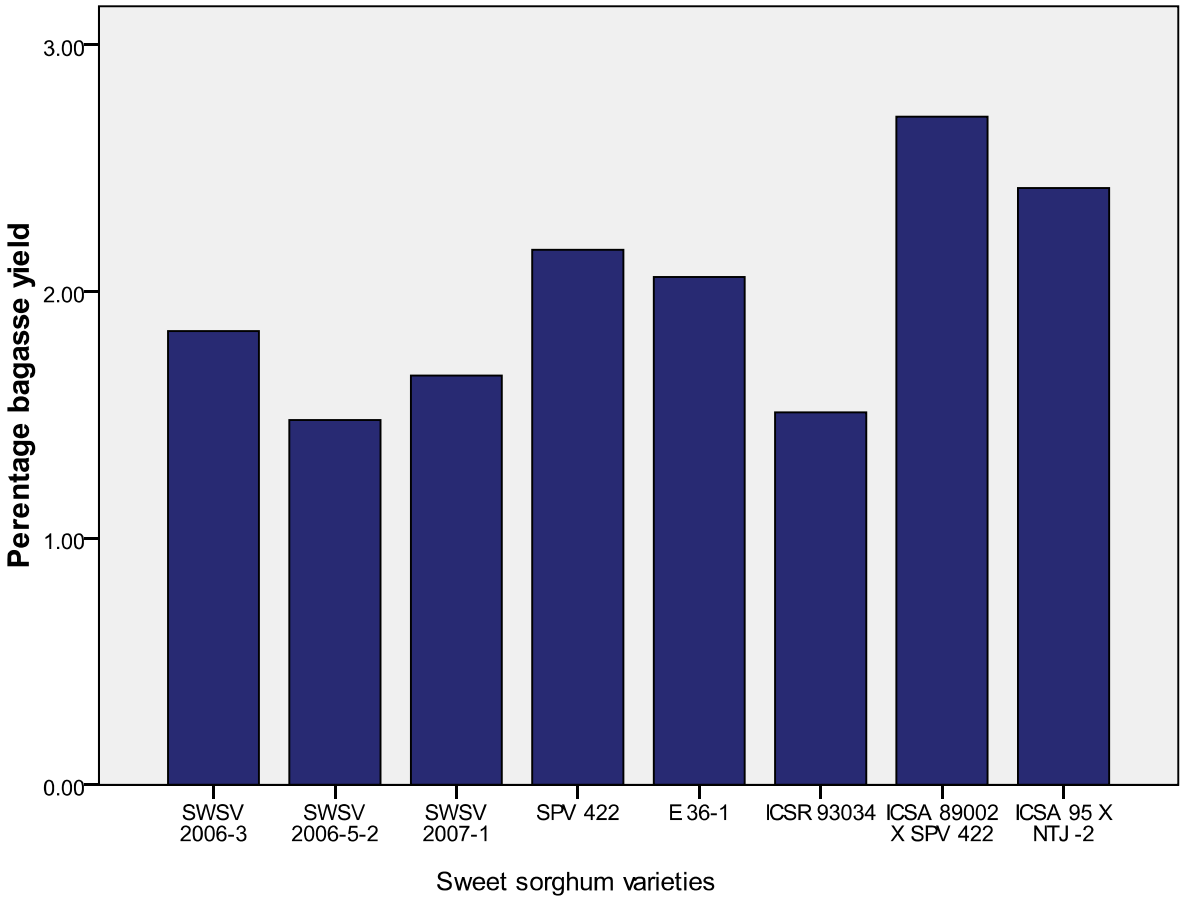


Figure 4.5: Percentage bagasse yield of eight sweet sorghum varieties after harvest

Nigerian Varieties	**Indian Varieties**	**Indian varieties crossed in IAR Zaria**
SWSV 2006-3	SPV 422	ICSA 89002 X SPV 422
SWSV 2006-5-2	E 36-1	ICSA 95 X NTJ -2
SWSV 2007-1	ICSR 93034	

4.3: The percentage glucose and reducing sugar yield (w/w) of eight sweet sorghum varieties

The initial 1.0%HCl and 1.0%H_2O_2 pretreatment was carried out to determine how susceptible the sweet sorghum varieties are to hydrolysis. Table 4.1 presents the percentage glucose yield from eight Sweet sorghum varieties after the pretreatment. Among the Nigerian varieties, SWSV 2006 5-2 was more susceptible to 1.0%HCl hydrolysis in terms of glucose yield. For the Indian S.S. varieties treated with 1.0%HCl, the value of 13.24 ± 0.00% was observed for SPV 422, a value which is significantly ($p<0.05$) higher than others. This is also 41.11% greater than the closest variety E 36-1 in the amount of glucose released. The lowest % glucose yield of 2.50 ± 0.21% was recorded in both ICSA95 X NTJ-2 and ICSR 93034. This value was not significantly different ($p>0.05$) from 2.94 ± 0.00% and 3.24 ± 0.00% for SWSV2007-1 and SWSV2006-3 respectively.

For hydrogen peroxide (1.0%H_2O_2) pretreated S.S. varieties, the highest % glucose yield of 8.38 ± 0.62% was observed for SWSV2006-3; which is not significantly different ($p>0.05$) from 7.94 ± 1.25%, 7.50 ± 0.21%, 7.35 ± 0.00% observed for ICSR93034, SWSV2007-1 and E36-1 respectively. The lowest glucose concentration of 3.97 ± 0.21% was obtained in ICSA89002XSPV422.

Table 4.2 shows the percentage reducing sugar yield of eight sweet sorghum varieties.

SPV422 had the highest reducing sugar concentration of 52.72 ± 0.00% after the pretreatment with 1.0%HCl. The lowest reducing sugar value of 44.95 ± 0.69% was recorded in SWSV2007-1. The highest reducing sugar concentration of 3.69 ± 0.034% was also obtained from E36-1 for

S.S. varieties treated with 1.0 %H_2O_2. This value is not significantly different ($p>0.05$) from the value for SPV422 variety (3.67 ± 0.69%). The lowest value of 1.12 ± 0.03% was obtained in ICSR93034.

Table 4.1: Percentage Glucose yield (w/w) of eight sweet sorghum varieties pretreated with 1.0% HCl and 1.0% H_2O_2

Sweet sorghum varieties	Pretreatment solutions		
	Control (distilled water)	1.0% HCl	1.0% H_2O_2
SWSV2006-3	0.44 ± 0.21^{ab}	3.24 ± 0.00^{ab}	8.38 ± 0.62^{d}
SWSV2006-5-2	0.59 ± 0.00^{ab}	8.38 ± 1.04^{c}	6.18 ± 0.83^{bc}
SWSV2007-1	0.74 ± 0.21^{b}	2.94 ±0.00^{ab}	7.50 ± 0.21^{cd}
SPV422	0.59 ± 0.0^{ab}	13.24 ± 0.00^{d}	5.44 ± 0.62^{ab}
E36-1	0.59 ± 0.00^{ab}	7.79 ± 0.21^{c}	7.35 ± 0.00^{cd}
ICSR93034	0.59 ± 0.00^{ab}	2.50 ± 0.21^{a}	7.94 ± 1.25^{d}
ICSA89002 X SPV422	0.29 ± 0.00^{a}	3.53 ± 0.00^{b}	3.97 ± 0.21^{a}
ICSA95 X NTJ-2	0.44 ± 0.21^{ab}	2.50 ± 0.21^{a}	6.76 ± 0.42^{bc}

Values are Mean ± SD of duplicate determinations.
Means with different superscript down the column are significantly different at $P<0.05$.

Nigerian Varieties	**Indian Varieties**	**Indian varieties crossed in IAR Zaria**
SWSV 2006-3	SPV 422	ICSA 89002 X SPV 422
SWSV 2006-5-2	E 36-1	ICSA 95 X NTJ -2

Table 4.2: Percentage Reducing sugar yield (w/w) released from the bagasse of eight sweet sorghum varieties pretreated with 1.0% HCl and 1.0% H_2O_2

Sweet sorghum varieties	Pretreatment solutions		
	Control (distilled water)	1.0% HCl	1.0% H_2O_2
SWSV2006-3	1.36 ± 0.10^{b}	50.05 ± 0.34^{c}	1.32 ± 0.03^{b}
SWSV2006-5-2	3.23 ± 0.07^{e}	48.59 ± 1.72^{bc}	3.35 ± 0.03^{f}
SWSV2007-1	3.69 ± 0.03^{f}	44.95 ± 0.69^{a}	2.77 ± 0.03^{e}
SPV422	2.65 ± 0.21^{d}	52.72 ± 0.00^{d}	3.67 ± 0.07^{g}
E36-1	2.92 ± 0.10^{de}	50.05 ± 0.34^{c}	3.69 ± 0.03^{g}
ICSR93034	0.78 ± 0.03^{a}	50.29 ± 0.69^{c}	1.12 ± 0.03^{a}
ICSA89002 X SPV422	2.31 ± 0.27^{c}	47.14 ± 0.34^{b}	2.60 ± 0.07^{d}
ICSA95XNTJ-2	1.00 ± 0.07^{a}	50.29 ± 0.69^{c}	1.51 ± 0.03^{c}

Values are Mean ± SD of duplicate determinations.
Means with different superscript down the column are significantly different at $P<0.05$.

Nigerian Varieties	**Indian Varieties**	**Indian varieties crossed in IAR Zaria**
SWSV 2006-3	SPV 422	ICSA 89002 X SPV 422
SWSV 2006-5-2	E 36-1	ICSA 95 X NTJ -2
SWSV 2007-1	ICSR 93034	

4.4: Effect of pretreating the bagasse of two sweet sorghum varieties with different concentrations of HCl and H_2O_2 on the percentage (%) yield of glucose (w/w)

Table 4.3 shows the effect of changing concentrations of HCl and H_2O_2 on the amount of glucose released from the bagasse of two S.S. varieties. The result showed an increasing trend in the value of glucose released as the concentration of pretreatment solution increases for both HCl and H_2O_2 treated S.S. bagasse. For pretreatment with HCL the highest % glucose yield was observed at the concentration of 2.5% for SPV 422 and the value was not significantly different ($p>0.05$) from the glucose concentration released at 2.0% and 1.5% of HCl. Almost the same trend was observed for HCl pretreated S.S. variety SWSV2006-3, which released the highest % glucose yield at 2.5 and 3% of HCl ; acid concentrations which gave numerically the same % glucose yield and are not significantly higher ($p>0.05$) than the glucose values released at 1.5% and 2% HCl.

For S.S variety SPV 422 pretreated with different concentrations of H_2O_2, the highest % glucose yield was recorded at 3.0%H_2O_2 which was not significantly different ($p>0.05$) from glucose released at 2.5%H_2O_2. For SWSV 2006-3, the highest % glucose yield was at 3.0% of H_2O_2. The lowest % glucose yield was observed at 0.50%. No glucose yield was observed for the control in all the groups. Two (2.0) % for HCl and 3.0% for H_2O_2 was chosen and used in the subsequent optimization steps.

Table 4.3: Effect of pretreating the bagasse of two sweet sorghum varieties with different concentrations of HCl and H_2O_2 on the %yield of glucose (w/w)

% concentration	Pretreatment solutions			
	HCl Treated		H_2O_2 Treated	
	SPV 422	SWSV2006-3	SPV 422	SWSV2006-3
0.00 (Distilled water)	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
0.50	5.42 ± 0.59^{b}	1.67 ± 0.00^{b}	1.25 ± 0.00^{a}	5.00 ± 0.00^{b}
1.00	9.79 ± 1.47^{c}	3.33 ± 0.00^{c}	6.46 ± 0.88^{b}	10.63 ± 0.29^{c}
1.50	14.17 ± 0.59^{de}	3.75 ± 0.59^{c}	11.88 ± 0.29^{c}	21.04 ± 2.06^{d}
2.00	14.38 ± 0.88^{de}	3.75 ± 0.59^{c}	13.33 ± 0.00^{c}	31.04 ± 0.88^{e}
2.50	16.04 ± 3.83^{e}	4.17 ± 0.59^{c}	23.96 ± 0.88^{d}	31.25 ± 3.54^{e}
3.00	10.42 ± 0.00^{cd}	4.17 ± 0.59^{c}	24.17 ± 4.71^{d}	46.46 ± 3.24^{f}

Values are Mean ± SD of duplicate determinations.
Means with different superscript down the column are significantly different at $P<0.05$

Nigerian Variety **Indian Variety**
SWSV 2006-3 SPV 422

4.5 Effect of ratio of bagasse to pretreatment solutions (HCl and H_2O_2) on the percentage (%) yield of glucose (w/w) from the bagasse of two sweet sorghum varieties

Table 4.4 shows the effect of ratio of bagasse to pretreatment solutions on the amount of glucose released from the bagasse of two S.S. varieties. The highest % glucose yield for HCl pretreated SPV422 was obtained as 28.57 ± 1.01% at the ratio of 1:50 which is significantly different ($p<0.05$) from other ratios. However, the lowest % glucose yield of 9.64 ± 1.52% was obtained at the ratio of 1:150. Similarly, the highest % glucose yield for HCl pretreated SWSV2006-3 was 25.71 ± 2.02% at the ratio of 1:50, which is significantly different from other ratios. The lowest % glucose yield of 17.14 ± 0.00% was recorded at the ratio of 1:150. There was no significant difference between ($p>0.05$) the glucose yield observed for S.S. varieties; SPV 422 and SWSV2006-3 after NaOH treatment.

The highest % glucose yield for H_2O_2 pretreated SPV422 was recorded as 84.29 ± 2.02% at the ratio of 1:200. This value was significantly different ($p<0.05$) from other ratios. The H_2O_2 pretreated SWSV2006-3 had the highest % yield of 81.43 ± 2.02%, also at the ratio of 1:150 which was significantly different ($p>0.05$) from other values. The lowest value of 26.07 ± 2.53% was recorded for 1:50 ratio. No glucose was released from the control groups.

Table 4.4: Effect of ratio of bagasse to pretreatment solutions on the percentage yield of glucose (w/w) released from the bagasse of the two sweet sorghum varieties

Ratio of sample to pretreatment Solutions	Pretreatment solutions					
	Control (distilled water)		2.0%HCL Treated		3.0% H_2O_2 Treated	
	SPV422	SWSV 2006-3	SPV422	SWSV 2006-3	SPV422	SWSV 2006-3
1:50	0.00±0.00	0.00±0.00	28.57±1.01^{d}	25.71±2.02^{c}	25.36±0.51^{a}	26.07±2.53^{a}
1:100	0.00±0.00	0.00±0.00	25.71±0.00^{c}	21.43±0.00^{b}	46.43±5.05^{b}	45.00±5.05^{b}
1:150	0.00±0.00	0.00±0.00	9.64±1.52^{a}	17.14±0.00^{a}	64.29±0.00^{c}	62.14±0.00^{c}
1:200	0.00±0.00	0.00±0.00	14.29 ± 0.00^{b}	22.86±0.00^{b}	84.29±2.02^{d}	81.43±2.02^{d}

Values are Mean ± SD of duplicate determinations.

Means with different superscript down the column are significantly at $P<0.05$.

Nigerian Variety **Indian Variety**
SWSV 2006-3 SPV 422

4.6 Effect of temperature on the percentage (%) yield of glucose and reducing sugar (w/w) released from the bagasse of two Sweet sorghum varieties

Table 4.5 shows the effect of temperature on the % yield of glucose released from the bagasse of two sweet sorghum varieties. The highest % glucose yield of 87.14 ± 2.02% was released at the temperature of 121^{o}C from S.S. variety SPV422 pretreated with 3.0% H_2O_2 at the ratio of 1:200. This value is significantly different ($p<0.05$) from values obtained from SPV422 pretreated at the ratio of 1:50 with both 3.0%NaOH and 2.0% HCl at this temperature.

A similar trend was also recorded in SPV422 pretreated at the temperature of 138^{o}C. The highest % glucose yield of 85.71 ± 0.00% was observed when this variety was pretreated with 3.0%H_2O_2 at 121^{o}C. The lowest % glucose yield of 0.00 ± 0.00% was obtained from 3.0%NaOH pretreatment, which yielded no glucose similar to the controls.

Also at the temperature of 121^{o}C, the highest % glucose yield of 84.29 ± 2.02% was obtained from S.S. variety SWSV2006-3 at the ratio of 1:200 of sample to 3% H_2O_2. This value is significantly different ($p<0.05$) from values obtained from 3.0%NaOH 1:50 and 2.0% HCl at 1:50. Similarly, the highest % glucose yield of 82.86 ± 0.00% was observed for SWSV2006-3 at the ratio of 1:200 with 3% H_2O_2 at the temperature of 138^{o}C. This result is significantly different ($p<0.05$) from values obtained for S.S. variety SWSV2006-3 at the ratio of 1:50 with 3.0%NaOH 1:50 and 2.0%HCl.

.The control had no value for glucose. The lowest % glucose yield of 0.71 ± 0.00% of SWSV2006-3 was released when treated with 3.0%NaOH (1:50). The S.S. variety SWSV2006-3 pretreated at the temperature of 121^{o}C gave a better glucose yield than at 138^{o}C.

Table 4:5: Effect of temperature on the percentage yield of glucose (w/w) released from the baggase of two Sweet Sorghum varieties.

Ratio of sample to pretreat-ment solutions	Temperature			
	121^{o}C		138^{o}C	
	SPV422	SWSV2006-3	SPV422	SWSV2006-3
1:50 distilled water	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
1:200 distilled water	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
1:50 2% HCl	3.93 ± 1.52^{a}	15.36 ± 0.5^{c}	4.64 ± 0.51^{a}	10.36 ± 0.51^{b}
1:200 3%H_2O_2	87.14 ± 2.02^{b}	84.29 ± 2.02^{ab}	85.71 ± 0.00^{ab}	82.86 ± 0.00^{a}

Values are Mean ± SD of duplicate determinations.

Means with different superscripts across the row are significantly at P<0.05.

Nigerian Variety **Indian Variety**
SWSV 2006-3 SPV 422

CHAPTER FIVE

DISCUSSION

5.1 The percentage moisture content

The variation in trend of percentage moisture content of the Sweet sorghum varieties can be attributed to changes in season and microclimatic conditions surrounding each variety. The varieties that showed an increase in percentage moisture from week 5 to 7 and a decrease in week 9 had relatively thicker stems and wider leave diameter which shaded the soil especially the rhizosphere from direct impact of heat from the sun. This resultant microclimatic condition led to reduction in the evaporation of water from the soil, thus the plants can absorb available water from the soil.

Conversely, those that experienced a progressive decrease had slender stems and relatively thinner/ narrower leaves surface area (see appendix 6) which could hardly shade the rhizosphere of the roots from direct impact of heat from the sun. Consequently, the soil had less available water for the plants to absorb as most of the soil water is lost through evaporation when the soil temperature increased and relative humidity of the micro environment became highly reduced. As dry season approaches, the microclimatic condition around the plant including the relative humidity, temperature, light intensity and soil moisture gradually changes. Evaporation of water from the soil and the rate of transpiration increases because of less water in the atmosphere. Consequently, the percentage moisture content of the plant decreases. This agrees with the findings of Billia *et al.* (1997), who documented a percentage moisture content of 70% for a certain variety of sweet sorghum; this value is much lower than the ones gotten for the varieties tested in the present study. Season and period of growth could affect the amount of water (moisture) the pith contains.

5.2 The reducing sugar concentration

Sweet sorghum just like any other plant synthesizes and accumulates sugar the product of photosynthesis in the form of starch in the grain and juice together with sucrose, fructose glucose and others sugars. Sweet sorghum as a C-4 plant has the extra capability to efficiently utilize carbon dioxide (CO_2) for carbohydrate synthesis. The reducing sugar concentration of sweet sorghum could drop after heading because much sugar will be diverted for storage as starch in the seeds. This implies that for optimum bioethanol production, the industry can harvest at this stage except if grain is desired otherwise, fibre (bagasse) and foliage can be gotten from such harvest.

5.3 Cellulose, hemicelluloses and lignin content of bagasse from eight sweet sorghum varieties

The percentage of cellulose was higher than that of hemicelluloses and lignin in the eight Sweet sorghum varieties. This indicates that Sweet sorghum is a good raw material for bioethanol production. The percentages of cellulose in the three selected Nigerian varieties were relatively higher (Figure 4.4) as they were well adapted to the environment. The fact that the crossbred varieties had the lowest percentages of cellulose can be attributed to the fact that the purpose of crossing was not to increase their percentage cellulose yield. Though the Nigerian varieties had higher cellulose percentage when compared with others, most of them have small stem diameter, relatively low survival rate and higher rate of pest infestation (see Appendix 6). The wide variation among all the sweet sorghum varieties could be as a result of origin and genetic

differences. This also shows that similar environments, though in different regions of the globe can support growth of a species in as much as the environmental requirements for the survival of such species are intact. This agrees with Mehmood *et al*. (2009) who reported the % composition of a sweet sorghum variety YSS-9 which had % cellulose, % hemicellulose and %lignin closer to that of Nigerian varieties.

5.4 Percentage bagasse yield

The highest percentage yield observed in the hybrid variety ICSA 89002 X SPV 422 can be attributed to the fact that they have bigger stem diameter, fewer rots and taller stem than other varieties (see Appendix 6). Some of these characteristics could be the reason for crossing different Indian varieties to develop them.

5.5 Susceptibility of the pith bagasse to hydrolysis

The association between cellulose, hemicelluloses and lignin in the Indian variety, SPV 422 was in such a way that allowed much glucose to be released after bagasse pretreatment with HCl. The same reason can be attributed to the highest yield obtained from SWSV 2006-3 when treated with H_2O_2.

5.6 Selection of two sweet sorghum varieties used for subsequent studies

Among the Nigerian sweet sorghum varieties tested, SWSV 2006-5-2 had the highest stem juice reducing sugar concentration at week 9 as shown in Figure 4.2 and is more susceptible to 1.0%HCl hydrolysis (Table 4.1) than the other two, a quality which is highly desirable in the

bioethanol industry. However, this variety (SWSV 2006 5-2) had slender tall stem that are prone to pest (maggot-like larva of a pest possibly stem borer) infestation. The leaves were also attacked. The pest attacked and destroyed majority of the stems such that it drastically reduced the percentage bagasse/fibre (powdered) yield at the time of harvest. SWSV 2006 5-2 variety had the overall lowest percentage bagasse yield. Similarly, SWSV 2007-1 is characterised also by tiny slender stems though a little bigger than SWSV 2006 5-2. The leaves were attacked by the same type of pest which destroyed the pith and also affected the quality of the pith of the survived ones. This led to a relatively poor bagasse yield. Although a high stem juice reducing sugar concentration was observed in this variety, its least susceptibility to 1.0% HCl hydrolysis is also a disadvantage. This implies that both SWSV 2006 5-2 and SWSV 2007-1 may not serve as a good variety for bioethanol production except if genetic modification of these promising line (high reducing sugar-yielding varieties) could introduce enhancement in stem size and a pest resistance factor.

However, SWSV 2006-3 had better stem characteristics than the other two varieties in terms of size and height with the stem being moderately attacked by the pest. The bagasse yield was also low but better than the other two. It had the highest % glucose yield when treated with 1.0%H_2O_2, highest reducing sugar with 1.0%HCl and lowest reducing sugar concentration with 1.0%H_2O_2 (Tables 4.1 and 4.2 respectively. For this reason, SWSV 2006-3 was selected among the three.

For the Indian and the hybrid varieties, SPV 422 was more susceptible to 1.0%HCl as presented in table 4.5 with E 36-1 ranking second while ICSR 93034 was more susceptible to 1.0% H_2O_2 than E 36-1, ICSA 95 X NTJ-2 and SPV422 which ranked second, third and fourth respectively in the % glucose yield released after hydrolysis. E 36-1 had value that is not statistically different

from the value observed for ICSR 93034 while value SPV 422 was also not significantly different from that of ICSA 95 X NTJ-2. Treating the bagasse with 1.0%HCl gave the highest reducing sugar concentration with SPV 422 which is not significantly different from the value obtained from E 36-1. E 36-1 had the highest concentration of reducing sugar released after treatment with 1.0%NaOH and 1.0%H_2O_2 value which was not significantly different from that of SPV 422. It was observed from tables 4.1 and 4.2 that SPV 422 is generally more susceptible to HCl than E 36-1 while E 36-1 and ICSR 93034 are more susceptible to H_2O_2 hydrolysis. Though ICSR 93034 in Figure 4.3 showed a carbohydrate (Cellulose + Hemicellulose) content comparable to the value recorded for SPV422, its lignin concentration is significantly higher than that of SPV 422. However, the stems of ICSR 93034 were also attacked by pest during growth and this also affected its % bagasse yield at the time of harvest.

E 36-1 had a mixture of slender and medium sized stem. E 36-1 had equal or close to equal likelihood to be selected based on the facts discussed above but other factors such as the composition of overall carbohydrate in the fibre was considered. E 36-1 had much lower carbohydrate content than SPV 422, higher lignin content and also lower % bagasse yield than SPV 422. It also had a mixture of medium and slender stems unlike SPV 422 which had relatively bigger stems with about 100% seed survival rate. This was why SPV 422 was preferentially chosen for further studies.

5.7 Effect of different concentration of the pretreatment solution on the amount glucose released from the bagasse of two sweet sorghum varieties

HCl pretreatment solution at concentration of 1.5%, 2.0% and 2.5% gave highest yield of glucose from bagasse of SPV 422. Though numerically, the yields differ but the differences were

not significant. This implies that either concentrations of 1.5%, 2.0% and 2.5% can be used to obtain highest glucose yield from bagasse. For SWSV 2006-3, concentrations of 1.0%, 1.5%, 2.0%, 2.5% and 3.0% gave values that are not significantly different ($p>0.05$) from the numerically highest yield; which also implies that any of the concentrations can be used. The HCl concentration of 1.5% is far from 2.5% which gave the numerically highest yield in the case of SPV 422 while 2.0% is closer to 2.5% and also gave highest yield that is not significantly different ($p>0.05$) from that obtained with 3.0% concentration. More so, apart from the fact that the yield is not significantly different from the numerically highest yield (4.17±0.59), it is closer to it. For H_2O_2 pretreatment at 2.5% and 3.0% gave maximum yield of 23.96 ± 0.88 and 24.17 ± 4.71 respectively which were not significantly different ($p>0.05$) and therefore implies that any of them can be used to obtain the highest result. However, 3.0% concentration is preferable since numerically, it gave the highest yield in both SPV 22 and SWSV 2006-3. This trend of result is different from that obtained when cotton fibre is treated with acids. Arthe *et al*. (2008) reported that an increase in acid severity in terms of concentration from 1% to 3% resulted in a higher sugar release from cotton fibre treated with dilute sulphuric acid.

5.8 Effect of ratio of sample to the pretreatment solutions on the amount of glucose released from the bagasse of two sweet sorghum varieties

The ratio of sample to pretreatment solution, 1:50 gave the maximum yield of 28.57 ± 1.01and 25.71 ± 2.02 for SPV 422 and SWSV 2006-3 respectively (Table 4.4). Increase in the proportion of pretreatment solution to 1:100 and 1:150 resulted in continuous decrease in glucose yield. This can be attributed to the fact that 1:50 is the ratio of sample to pretreatment solution that can maximally release glucose from hemicellulose. As the proportion of the pretreatment solution increases, the glucose released becomes degraded until a point is reached as in 1:200 (Table 4.4)

when the acid start attacking the cellulose portion of the bagasse (hemicellulose) which will result in further release of glucose. Contrarily, increase in the proportion of the pretreatment solution of H_2O_2 from 1:50 to 1:200 gave a progressively increasing yield of glucose. Further increase may continue to yield glucose at an increasing rate until a maximum ratio is reached when further increase begins to give reduced yield due to degradation of glucose as a result of increased ratio.

5.9 Effect of temperature on the amount of glucose and reducing sugars released from two sweet sorghum varieties

The effect of temperature on bagasse for all selected best conditions (which are concentration of pretreatment solutions and the ratio of sample to pretreatment solution) that released the highest glucose concentration showed that the temperature of 121^{o}C was the best for the two varieties. This could be attributed to the fact that 121^{o}C is the optimum temperature for glucose release for the selected condition. Any further increase in temperature will likely cause the degradation of glucose. However, the yield obtained at 138^{o}C, though numerically different, is still not significantly different ($p>0.05$) from those obtained at temperature of 121^{o}C.

CHAPTER SIX

CONCLUSION AND RECOMMENDATION

6.1 Conclusion

The initial field reducing sugar sampling provided information on the promising sweet sorghum varieties based on the amount of reducing sugar they can accumulate and the following: ICSA 89002 X SPV 422, ICSR 93034, ICSA X NTJ -2, E 36-1, SPV422, SWSV 2006-5-2, SWSV 2006-3, SWSV 2007-1 were selected.

The optimal conditions discovered for HCl treatment are 2.0%HCl concentrations, 1:50 ratio of sample to 2.0%HCl, at 138^{o}C for SPV 422 and 2.0%HCL concentrations, 1:50 ratio of sample to 2.0%HCl at 121^{o}C for SWSV 2006-3.

For H_2O_2 optimal conditions of 3.0% H_2O_2 concentrations, 1:200 ratio of sample to 3% H_2O_2, at 121^{o}C for both SPV 422 and SWSV 2006-3.

The glucose concentration released from the two sweet sorghum varieties suggests that 3.0% H_2O_2 together with the optimized condition can degrade cellulose effectively. The pith of sweet sorghum may be more susceptible to hydrolysis than the whole stem. Therefore the difficulty and cost of hydrolysis can be reduced if the pith alone is used.

6.2 Recommendations

The following recommendations are made:

-Genetic modification of the reducing sugar rich sweet sorghum is required in other to harness their usefulness, by introduction of a factor that enhances stem size and pest resistance.

- Further enzymatic hydrolysis to validate the effect of treatment conditions should be optimized

REFERENCES

Alkasrawi, M., Eriksson, T., Borjesson, J., Wingren, A., Galbe, M., Tjerneld, F., Zacchi, G. (2003). The effect of Tween-20 on simultaneous saccharification and fermentation of softwood to ethanol. *Enzyme and Microbial Technology,* **33**: 71–78.

Allen S.E. Grimshaw, H.M., Parkinson, J.A., Evans, C.C. and Quarmby, C. (1974). *Chemical analysis of ecological materials*. Allen S.E. (ed). Blackwell Scientific. Oxford, London. 565pp.

Anonymous. (2010). Ethanol facilities capacity by State and plant official Nebraska government. http://www.neo.ne.gov/statshtml/122.htm

Arthe, R., Rajesh, R., Rajesh, E.M., Rajendran, R. and Jayechandran, S. (2008). Production of Bio-ethanol from cellulosic cotton waste through microbial extracellular enzymatic hydrolysis and fermentation. *Electronic Journal of Environmental, Agriculture and Food Chemistry,* **7** (6):2984-2992.

Ballerini, D., Desmarquest, J.P., Pourquie, J. (1994). Ethanol production from lignocellulosics: Large scale experimentation and economics. *Bioresource Technology,* **50**: 17-23.

Bamforth, C.W. and Barclay, A.H.P. (1993). Malting technology and the uses of malt. *In: Barley: Chemistry and Technology*, MacGregor A.W. and Bhatty, R.S. (eds). American Association of Cereal Chemists, St. Paul, pp.615-698.

Bera, A. K., Sedlak, M., Khan, A., Ho,N.W.Y. (2010). Establishment of l-arabinose fermentation in glucose/xylose co-fermenting recombinant Saccharomyces cerevisiae 424A (LNH-ST) by genetic engineering. *Applied Microbiology and Biotechnology*. **87**(5):1803-1811.

Bhat, M.K. (2000). Cellulases and related Enzymes in Biotechnology. *Biotechnology Advances*, **18**: 355-383.

Billa, E., Koullas, D.P., Monties, B. and Koukios, E.G. (1997). Structure and composition of Sweet Sorghum stalk components. *Industrial Crops and Products*, **6:**297-302.

Boag, S. And Jenkins, C.L.D. (1986). Involvement of Aspartate and Glutamate in the Decarboxylation of Malate by isolated bundle sheath chloroplast from *Zea mays. Plant Physiology*. **80**:115-119.

Bon Elba, P.S. and Ferrara Maria, A. (2007). Bioethanol production via enzymatic hydrolysis of cellulosic biomass. Seminar presented "*The Role of Agricultural Biotechnology for production of Bioenergy in Developing Countries*" and FAO. http.//www.fao.org/ biotech/seminar Oct 2007.htm

www.anfavea.com.br. Accessed 10/06/2009

Carter, E.G.A. and Carpenter, K.J. (1981). Bound niacin in sorghum and its availability. *Journal of Nutrition and Research*, **1**:571 – 579.

Centre for plant breeding. (2011). Sweet sorghum Production Technology. Department of Millet, Centre for Plant Breeding and Genetics, Tamil Nadu Agricultural University, India. p.2.

Chandel, A.K., Chan, E.S., Rudravaram, R., Narasu, M.L., Rao, V.L. and Ravindra, P. (2007). Economics and environmental impact of bioethanol production technologies: an appraisal. *Biotechnology and Molecular Biology Review,* **2** (1):014-032.

Chandrakant, P., and Bisaria, V.S. (1998). Simultaneous bioconversion of cellulose and hemicellulose to ethanol. *Critical Reviews in Biotechnology,* **18** (4): 295–331.

Chitsika, J.M. and Mudimbu, M.W. (1992). Quality criteria for opaque beer in Zimbabwe. In Gomez, M.l., House, L R., Rooney, L W. and Dendy, D.A V. (eds). *Utilization of sorghum and millets*. ICRISAT. Patancheru, India. pp. 151 – 155.

Claassen, P.A.M., van Lier, J.B., Lopez Contreras, A.M., van Niel, E.W.J., Sijtsma, L., Stams, A.J.M., De Vries, S.S., Weusthuis, R.A. (1999). Utilisation of biomass for the supply of energy carriers. *Applied Microbiology and Biotechnology,* **52:** 741–755.

Daiber, K.H. and Taylor, J.R.N. (1995). Opaque beers. In *Sorghum and millets: Chemistry and Technology*. Dendy, D.A.V. (ed). American Association of Cereal Chemists. St. Paul, USA. pp. 299-323.

Daily Biofuel digest. (2008). Global Biofuels announces $750 million investment in sweet sorghum ethanol project in Nigeria. *E-newsletter*. www.biofuelsdigest.com/blog2/tag/nigeria/

Dasari, R. and Berson, R. (2007). The effect of particle size on hydrolysis reaction rates and rheological properties in cellulosic slurries. *Applied Biochemistry and Biotechnology*,**137**:289–299.

Davenport, R. (2006). Safe and sustainable chemicals report: Raw material for biofuels. Delft University of Technology. "Adding one single gene to yeast dramatically improves Bioethanol production from Agricultural wastes" *Science Daily*.

David, W. (1992). *Energy, Plant and Man*. Oxygraphics limited. Brighton, East Sussex. 277pp.

Delft University of Technology. (2009). Adding one single gene to yeast dramatically

improves bioethanol production from agricultural waste. Delft University of Technology. *ScienceDaily*.
http://www.sciencedaily.com /releases/2009/11/091120084617.htm

Doggett, H. (1988). *Sorghum*. 2nd Ed, Longman Scientific and Technical, London.

El Bassam, N. (1998). C3 and C4 plant species as energy sources and their potential impact on environment and climate. *Renewable Energy*, **15**(1-4):205-210.

EPA. (2007-2008). Regulatory Impact analysis: Renewable Fuel Standard Program. EPA 20th April 2007 – EPA 8th March to April 2008.

FAO. (1994). Integrated energy systems in china - the cold North-eastern region experience. Nan, L., Best, G. And Neto, C.C.D. (Eds). Natural Resources Management and Environment Department, FAO corporate document repository. Rome.
http://www.fao.org/docrep/T4470E/t4470e07.htm. Accessed 20/04/2011

FAO. (1995). Sorghum and Millet in human nutrition. FAO Corporate Document Repository.
http://www.fao.org/docrep/T0818E/T0818E00.htm

FAO. (2002). Sweet Sorghum in China. Agriculture and consumer protection,Food agricultural organization of United Nations, Department.

FAOSTAT (2008). Food and Agriculture Organization of the United Nations.
http://faostat.fao.org/site/567/default.aspx#ancor. Accessed 20/04/2011

Fukushima, R. S., and Hatfield, R. D. (2001). Extraction and isolation of lignin for utilization as a standard to determine lignin concentration using the acetyl bromide spectrophotometric method. *Journal of Agriculture and Food Chemistry*, **49**:3133–3139.

Fukushima, R.S., and Hatfield, R.D. (2004). Comparison of the acetyl bromide spectrophotometeric method with other analytical lignin methods for determining lignin concentration in forage samples. *Journal of Agriculture and Food Chemistry*, **52**:3713–3720.

Gong, C.S., Cao, N.J., Du, J., Tsao, G.T. (1999). Ethanol production from renewable resources. *Advances in Biochemical Engineering/Biotechnology,* **65**: 207–241.

Grassi, G. (2004). Sweet sorghum, one of the world best food-feed-energy crop. 7th Latin America Thematics network (LAMNET) workshop on Bioenergy in Beijin.

Grassi, G., Nardi, A. and Vivarelli, S. (2005). Low cost production of Bioethanol in the E.U. through integrated full processing of Sweet sorghum crops, 14th European Biomass conference. Paris, France.

Hahn-Hägerdal, B., Galbe, M., Gorwa-Grauslund, M. F., Lidén, G. and Zacchi, G. (2006). Bioethanol-the fuel of tomorrow from the residue of today. *Trends in Biotechnology,* **24** (12):449-556.

Hames, B., Ruiz, R., Scarlata, C., Sluiter, A., Sluiter, J. and Templeton, D. (2008). Preparation of Samples for Compositional Analysis, Laboratory Analytical Procedure of the National Renewable energy laboratory, A National Laboratory of United State Department of energy, Office Efficiency and Renewable Energy.

Hatfield, R.D., Jung, H.G., Ralph, J., Buxton, D.R. and Weimer, P.J. (1994). A comparison of the insoluble residues produced by the Klason lignin and acid detergent lignin procedures. *Journal of Science and Food Agriculture,* **65**:51–58.

HariKrishna, S., Prasanthi, K., Chowdary, G., Ayyanna, C. (1998). Simultaneous saccharification and fermentation of pretreated sugarcane leaves to ethanol. *Process Biochemistry,* **33** (8): 825–830.

Herna´ndez-Salas, J.M., Villa-Ramı´rez, M.S., Veloz-Rendo´ n, J.S., Rivera-Herna´ndez, K.N., Gonza´lez-Ce´sar, R.A., Plascencia-Espinosa, M.A. and Trejo-Estrada, S.R. (2009). Comparative hydrolysis and fermentation of sugarcane and agave bagasse. *Bioresource Technology*, **100**: 1238–1245.

Hinkova, A. and Bubnik, Z. (2001). Sugar Beets as Raw material for Bioethanol Production. *Czech Journal of Food Scieence,* **19**(6) 224-234.

ITIS. (2006). Sorghum Taxonomy. Integrated Taxonomic Information System online database. www.itis.usda.gov Accessed 20/12/2011 http://www.gramene.org/species/sorghum/sorghum_taxonomy.html Accessed 20/04/2011

Kangama, C.O. and Rumei, X. (2005). Production of crystal sugar and Alcohol from sweet Sorghum. *African journal of Food Agriculture Nutrition development*, **5**(2). www.ajfand.net . Accessed 07/08/2011

Kaur, P.P., Arneja, J.S. and Singh, J. (1998). Enzymatic hydrolysis of Rice straws by crude cellulose from Trichoderma. *Bioresource technology,* **66:**267-269.

Kimber, C.T. (2000). Origins of domesticated sorghum and its early diffusion into India and China. In *Sorghum*: Origin, History, Technology and Production, Wayne-Smith, C. and Frederiksen, R.A. (eds), John Wiley and Sons, New york, pp: 3-98.

Klinke, H.B., Thomsen, A.B., Ahring, B.K. (2004). Inhibition of ethanol-producing yeast and

bacteria by degradation products produced during pre-treatment of biomass. *Applied Microbiology and Biotechnology*, **66**(1): 10–26.

Laskin, A.I. (1977). Single cell protein. *Ann. Reports on Fermentation Processes,* **1:**151-180 Perlman, D. Ed. Academic Press, New York.

Larsson, S., Palmqvist, E., Hahn-Hägerdal, B., Tengborg, C., Stenberg, K., Zacchi, G., Nilvebrant, N-O. (1999). The generation of fermentation inhibitors during dilute acid hydrolysis of softwood. *Enzyme Microbial Technology*. **24**:151–159.

Lia, B., Balanb, V., Yuana, Y. and Daleb, B.E. (2010). Process optimization to convert forage and sweet sorghum bagasse to ethanol based on ammonia fiber expansion (AFEX) pretreatment. *Bioresource Technology*, **101**(4):1285-1292.

Licht, F.O. (2008). World Ethanol and Biofuel Report. **6**(17), May 8, 2008. http://www.agra-net.com/portal2/puboptions.jsp?Option=archive&pubid=ag072

Lynd, L.R., (1996). Overview and Evaluation of Fuel Ethanol from Cellulosic Biomass: Technology, Economics, the Environment, and Policy. *Annual Review of Energy and the Environment,* **21**:403–465.

Lynd, L.R., Wyman, C.E., Gerngross, T.U. (1999). Biocommodity Engineering. *Biotechnology Progress,* **15** (5): 777–793.

Mamoudou, H. D., Harry, G., Alfred, S. T., Alphons, G. J. V. and Willem, J. H. Van, B. (2006). Sorghum grain as human food in Africa: relevance of content of starch and amylase activities, a Review. *African Journal of Biotechnology,* **5** (5):384-395.

Mask, P.L amd Morris, W.C. (1991). Sweet Sorghum Culture and Syrup Production. Alabama Cooperative Extension System, Alabama A$E and Auburn Universities. http://www.aces.edu/pubs/docs/A/ANR-0625/

Mehmood, S., Haq, I., Khan, K.S., Gulfraz, M. (2008). Comparision of regional varieties of sorghum for ethanol, proximate and metal ions concentration. *Canadian Jounal of Pure Applied Science* **2**(1): 133-137.

Mehmood, S., Gulfraz, M., Rana, N.F., Ahmad, A., Ahring, N.M., Malik, M.F. (2009). Ethanol Production from Sorghum bicolour using both separate and simultaneous saccharification and fermentation in batch and fed batch systems. *African Journal of Biotechnology*, **8**: 2857-2865.

Metzler, D.E. (2003). Biochemistry, The chemical reaction of living cells. 2nd edn. Vol. 1 and 2. Academic press, Iowa, USA. 1973pp.

Miller, G. L. (1959). Use of Dinitrosalicylic acid reagent for determination of reducing sugar. *Analytical Chemistry*, **31:** 426-428.

Miller, F.R. and Creelman, R.A. (1980). Sorghum-A New Fuel. Pp. 219-232. *In* Loden, H.D. and Wilkinson, D. (eds.) *Proceeding of 35th Annual Corn Sorghum Res. Conference of American Seed Trade Association*, Chicago IL.

Mohammad, J. T. and Keikhosro, K. (2008). Pretreatment of Lignocellulosic Wastes to Improve ethanol and biogas production: a review. *International Journal of Molecular Sciences*. **9**: 1621-1651.

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

Mueller –Harvey, I. and Hartley, R. D. (1986). Linkage of L-Coumaroyl and Feruloyl groups to cell wall polysaccharide of Barley straw. *Carbohydrate Resource*, **148:** 71-85.

Murty, D.S. and Subramanian, V. (1982). Sorghum Roti 1, Traditional methods of consumption and standard procedures for evaluation. In Rooney, L.W. and Murty, D.S. (eds) Proceedings of the international symposium on sorghum grain quality. Hyderabad, India. 28-31 October, 1981, pp.73-78. ICRISAT, Patancheru, India.

Nebraska grain soghum. (2011). Celebrate sorghum-the versatile, gluten free whole grain. United Sorghum check off programme, Nebraska, USA.

Nelson, D.L. and Cox, M.M. (2004). *Lehninger Principles of Biochemistry.* 4th ed. W.H. Freeman and company , p. 248.

Nisizawa K. (1973). Mode of action of cellulases. *Journal of Fermentation Technology*, **51**:267–304.

Ogier, J.C., Ballerini, D., Leygue, J.P., Rigal, L., Pourquie, J. (1999). Ethanol production From Lignocellulosic Biomass. *Oil Gas Sci. Technology*, **54**: 67-94.

Olsson, L. and Hahn-Hägerdal, B. (1996). Fermentation of lignocellulosic hydrolysates for ethanol production. *Enzyme Microbial Technology*, **18**:312-331.

Okafor, N. and Aniche, G.N. (1980), Brewing a lager beer from Nigerian Sorghum. *Journal Brewing and distilling international,* **10**:32-35.

Okrah S.G. (2008). Screening Of Six Local Sorghum Varieties for Their Malting and Brewing Qualities. Thesis. Department of Biochemistry and Biotechnology. Faculty of Science, Kwame Nkrumah University of Science and Technology.

O'Rouke, T. (1996). 'Brewing', in Godfrey, T. and West, S. (eds). *Industrial Enzymology*, 2nd Edition, Macmillan, London, pp:105-031.

O'Rouke, T. (2004). Malting of barley. *Journal of the Institute of Brewing,* **109**: 21-23.

O'Sullivan A. (1997). Cellulose: the structure slowly unravel. *Cellulose*, **4**:173–207.

Palmarola-Adrados, B., Galbe, M., Zacchi, G. (2005). Pretreatment of barley husk for bioethanol production. *Journal of chemical technology and biotechnology,* **80** (1):85-91. ISSN 0268-2575.

Park, S., Baker, J.O., Himmel, M.E., Parilla, P.A. and Johnson, D.K. (2010). Cellulose crystallinity index: measurement techniques and their impact on interpreting cellulase performance. *Biotechnology Biofuel,* **3**:10.

Pérez, S. and Mazeau, K. (2005). Conformations, structures, and morphologies of celluloses. In: *Polysaccharides*: Structural diversity and functional versatility 2. Dumitriu, S. (ed). Marcel Dekker Inc, New York.

Petersson A, Thomsen M, Hauggaardnielsen H, Thomsen A. (2007). Potential bioethanol and biogas production using lignocellulosic biomass from winter rye, oilseed rape and faba bean. Biomass Bioenergy, **31**: (11–12):812–819.

Prescott, S.C. and Dunn, C.G. (1959). *Industrial Microbiology*. McGraw-Hill, New York.

Raghavandra, A.S. and Sage, R.F. (2011). C_4 Photosynthesis and related CO_2 concentrating mechanisms. *Advances in Photosynthesis and Respiration* . vol. **32.** Springer. Dordrecht, Netherlands. Books.google.com.ng/books?id=oijxe1mu_dwC&pg=PA364&da=CO_2+released+by+decarboxylation+of+malate+or+oxaloacetate&f=false accessed 20/12/2011

Reddy B.V.S., Ramesh, S., Reddy, S.P., Kumar, A.A., Sharma , K.K., Chetty, K.S.M. and Palaniswamy, A.R. (2006). *Sweet Sorghum: Food, Feed, Fodder and Fuel Crop*, p. 8. The International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, Andhra-Pradesh, India.

Reddy, BVS. (2007). *Sweet Sorghum, A water saving bio-energy crop for the Philippines*. Sweet sorghum flyer. International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) Philippines.

Ritter, K.B., McIntyre, C.L., Godwin, I.D., Jordan, D.R. and Chapman, S.C. (2007) An assessment of the genetic relationship between sweet and grain sorghums, within *Sorghum bicolor ssp. bicolor (L.) Moench*, using AFLP markers. *Euphytica*, **157** (1-2): 161-176.

Robert, R. B., Maas, R.H.W., Kabel, M.A., Schols, H.A., Weusthuis, R.A. and Jong, E.D. (2005). Hydrolysis and Fermentation of Lime-pretreated Biomass. Research conducted by:Agrotechnology & Food Innovations. Wageningen, the Netherlands and Laboratory of Food Chemistry, Wageningen University, the Netherlands. www.agrotechnologyandfood.wur.nl. Accessed 21/12/2009

Rooney, L.W., Kirleis, A.W., Murty, O.S. (1986). Traditional Foods from Sorghum: Their Production Evaluation and Nutritive value. In Pomeranz, Y. (ed) *Advances in cereal science technology*. St Paul, MN. Am. Assoc. Cereal Chemist. pp.317-353.

Saha, B.C., Iten, L.B., Cotta, M.A., Wu, Y.V., (2005). Dilute acid pretreatment, enzymatic saccharification, and fermentation of rice hulls to ethanol. *Biotechnology Progress*, **21**: 816–822.

Sanchez, O. J. and Cardona, C. A. (2008). Trends in Biotechnological Production of fuel ethanol from different Feedstock, a review. *Bioresource Technology.*, **99**: 5270-5295.

Seema, P., Onkarappa, R., Gurumurthy, S. (2009). Pretreatment studies on wheat straw and rice straw. *The Internet Journal of Microbiology* **7** (1).

Sluiter, A., Hames, B., Hyman, D., Payne, C., Ruiz, R., Scarlata, C., Sluiter, J. Templeton, D. and Wolfe, J.(2008). Determination of Total Solids in Biomass and Total Dissolved Solids in Liquid Process Samples. *Laboratory Analytical Procedure (LAP) of National Renewable Energy Laboratory*, U.S. Department of Energy Office of Energy Efficiency and Renewable Energy. Midwest Research Institute. pp. 6.

Söderström, J., Galbe, M., Zacchi, G. (2005). Separate versus Simultaneous Saccharification and Fermentation of two-step steam pretreated softwood for ethanol production. *Journal of Wood Chemical Technology*, 25 (3): 187–202.

Sørensena, A., Tellera, P.J., Hilstrøma, T. and Ahring, B. K. (2008). Hydrolysis of Miscanthus for bioethanol production using dilute acid presoaking combined with wet explosion pre-treatment and enzymatic treatment. *Bioresource Technology*, **99**(14): 6602-6607.

Sridevi, A., Narasimha, G. and Rajasekhar, B.R. (2009). Production of cellulase by *Aspergillus niger* on natural and pretreated lignocellulosic wastes. *The internet journal of Microbiology,* **7**(1). 9pp.

Stroade, J. and Boland, M. (2011). *Sorghum Profile*. Agricultural Marketing resource centre, Iowa State University. USA. www.agmrc.org/commodities_products/grains_oilseeds/sorghum/sorghum_profile.cfm. accessed 21/12/2011.

Sun, Y. and Cheng, J. (2002). Hydrolysis of lignocellulosic materials for ethanol production: A review. *Bioresource Technology,* **83**(1):1-11.

TAPPI. (1985). Acid-soluble lignin in wood and pulp. Useful method 246 TAPPI, Atlanta, US

Undersander, D.J., Lueschen ,W. E., Smith, L.H., Kaminski, A.R., Doll, J.D., Kelling, K.A., and Oplinger, E.S. (1990). *Sorghum—for Syrup. Alternative field crop manual*. University of winscosin Extension, Corporate Extension and University of Minnesota, centre for Alternative plant and Animal products and Minnesota Extension service.

(USDA). (2005). Composition of foods: cereal grains and pasta. United States Department of Agriculture/Human Nutrition Information Service Agriculture Handbook No. 8-20. Washington, DC.

Vara Prasad, M.N. (1997). *Plant Physiology*. John Wiley & Sons Inc, New York. 542pp.

Varga, E., Klinkle, H.B., Reczey, K., Thomsen, A.B. (2004). High solid simultaneous saccharification and fermentation of wet oxidized corn stover to ethanol. *Biotechnology and Bioengineering,* **88** (5): 567–574.

Vermerris, W., Rainbolt, C., Wright, D. and Newman Y. (2008). Production of Biofuel Crops in Florida: *Sweet Sorghum*. University of Florida IFAS Extension Publication No. SS AGR293.pp.1-7. http://edis.ifas.ufl.edu/ag298. Accessed 21/12/2011

Wortmann, C.S. and Regassa, T. (2011). *Sweet Sorghum as a Bioenergy Crop for the US Great Plains.* Department of Agronomy and Horticulture, University of Nebraska-Lincoln, Lincoln,USA. 16pp.

Yoshihara, K., Kobayashi, T., Fujii, T. and Akamatsu, I. (1984). A novel modification of Klason lignin quantitative method. *Journal of Japan Tappi,* **38**:86–95.

Yu, J., Zhang, X. and Tan, T. (2009). Optimization of media conditions for the production of ethanol from sweet sorghum juice by immobilized Saccharomyces cerevisiae. Biomass and Bioenergy, **33**(3):521-526.

Zhang, Y.H.P., Himmel, M.E., Mielenz, J.R. (2006). Outlook for cellulase improvement: screening and selection strategies. *Biotechnology Advances*, **24**(5): 452-481.

Zohary, D. and Hopf, M. (2000). *Domestication of plants in the Old World* (3rd edn) Oxford: University Press, p.89.

Zugenmaier, P. (2008). Crystalline cellulose and cellulose derivatives: characterization and structures. Springer Series in Wood Science. Berlin, Heidelberg: Springer-Verlag. *Cellulose* :101–174.

Appendices

Appendix 1: Percentage moisture content of bagasse from the stalks of fourteen sweet sorghum varieties

Sweet sorghum varieties	Percentage (%) moisture content		
	5 week	7 weeks	9 weeks
SWSV 2006-3	89	85	82
SWSV 2006-5-1	90	88	87
SWSV 2006-5-2	86	82	81
SWSV 2007-1	86	79	83
SWSV 2007-3	93	89	83
SPV 422	90	88	84
ICSV 700	90	94	90
NTJ -2	93	86	81
E 36-1	85	84	82
ICSR 93034	87	90	83
ICSV 93046	87	91	88
ENT 64 DTN	88	90	88
ICSA 89002 X SPV 422	90	87	82
ICSA 95 X NTJ -2	86	88	82

Nigerian Varieties	**Indian Varieties**	**Indian varieties crossed in IAR Zaria**
SWSV 2006-3	SPV 422	ICSA 89002 X SPV 422
SWSV 2005-5-1	ICSV 700	ICSA 95 X NTJ -2
SWSV 2006-5-2	NTJ -2	
SWSV 2007-1	E 36-1	
SWSV 2007-3.	ICSR 93034	
	ICSV 93046	
	ENT64 DTN	

Appendix 2: Reducing sugar concentrations (mg/g) of the stem from growing stalks of 14 Sweet Sorghum varieties at 5 weeks

Sweet Sorghum varieties	Reducing sugar
	5 weeks
SWSV 2006-3	83.08 ± 5.05^{f}
SWSV 2005-5-1	60.56 ± 1.74^{d}
SWSV 2006-5-2	76.71 ± 0.91^{e}
SWSV 2007-1	73.88 ± 2.92^{e}
SWSV 2007-3	37.09 ± 4.00^{b}
SPV 422	59.57 ± 3.18^{d}
ICSV 700	27.83 ± 0.91^{a}
NTJ -2	56.75 ± 2.64^{cd}
E 36-1	76.47 ± 2.56^{e}
ICSR 93034	72.20 ± 7.14^{e}
ICSV 93046	51.58 ± 0.79^{c}
ENT64 DTN	72.62 ± 2.49^{e}
ICSA 89002 X SPV 422	61.20 ± 2.03^{d}
ICSA 95 X NTJ-2	82.24 ± 1.65^{f}

Values are mean ± SD of triplicate determinations.
Means with different superscripts down the column are significantly different at $P<0.05$

Nigerian Varieties	**Indian Varieties**	**Indian varieties crossed in IAR Zaria**
SWSV 2006-3	SPV 422	ICSA 89002 X SPV 422
SWSV 2005-5-1	ICSV 700	ICSA 95 X NTJ -2
SWSV 2006-5-2	NTJ -2	
SWSV 2007-1	E 36-1	
SWSV 2007-3.	ICSR 93034	
	ICSV 93046	
	ENT64 DTN	

Appendix 3: Reducing sugar concentrations (mg/g) of the stem from growing stalks of 8 Sweet Sorghum varieties at 5, 7 and 9 weeks

Sweet Sorghum varieties	Reducing sugar		
	5 week	7 week	9 week
SWSV 2006-3	83.08 ± 5.05^{a}	90.60 ± 2.51^{b}	100.57 ± 0.63^{c}
SWSV 2006-5-2	76.71 ± 0.91^{a}	86.20 ± 1.22^{b}	134.96 ± 0.21^{c}
SWSV 2007-1	73.88 ± 2.92^{a}	116.13 ± 1.86^{b}	131.41 ± 1.40^{c}
SPV 422	59.58 ± 3.18^{a}	80.93 ± 0.64^{b}	83.56 ± 1.40^{b}
E 36-1	76.46 ± 2.56^{b}	68.07 ± 0.70^{a}	132.31 ± 1.47^{c}
ICSR 93034	72.20 ± 7.14^{a}	80.00 ± 1.91^{a}	141.93 ± 0.82^{b}
ICSA 89002 X SPV 422	61.20 ± 2.03^{a}	101.87 ± 1.94^{b}	149.38 ± 1.54^{c}
ICSA 95 X NTJ -2	82.24 ± 1.66^{a}	85.13 ± 0.12^{b}	138.08 ± 0.68^{c}

Values are mean ± SD of triplicate determinations.
Means with different superscripts across the rows are significantly different at $P<0.05$

Nigerian Varieties	**Indian Varieties**	**Indian varieties crossed in IAR Zaria**
SWSV 2006-3	SPV 422	ICSA 89002 X SPV 422
SWSV 2006-5-2	E 36-1	ICSA 95 X NTJ -2
SWSV 2007-1	ICSR 93034	

Appendix 4: Cellulose, Hemicellulose and lignin content of bagasse from the stalks of eight sweet sorghum varieties harvested on 30th 0ctober 2010

Sweet sorghum varieties	Percentage (%) composition		
	Cellulose	Hemicellulose	Lignin
SWSV 2006-3	36.12 ± 0.01^{g}	17.10 ± 0.02^{b}	20.48 ± 0.05^{c}
SWSV 2006 5-2	35.82 ± 0.01^{f}	20.26 ± 0.02^{f}	20.16 ± 0.01^{b}
SWSV 2007-1	36.64 ± 0.02^{h}	20.40 ± 0.01^{g}	20.12 ± 0.02^{b}
SPV 422	32.48 ± 0.02^{d}	18.22 ± 0.12^{d}	20.46 ± 0.04^{c}
E 36-1	32.66 ± 0.01^{e}	16.74 ± 0.04^{a}	22.04 ± 0.01^{d}
ICSA 93034	32.16 ± 0.02^{c}	18.12 ± 0.02^{c}	22.16 ± 0.02^{e}
ICSA 89002 X SPV 422	30.82 ± 0.02^{b}	18.28 ± 0.02^{d}	22.18 ± 0.02^{e}
ICSA 95 X NTJ-2	30.60 ± 0.02^{a}	18.74 ± 0.04^{e}	18.76 ± 0.02^{a}

Values are Mean ± SD of triplicate determinations.
Means with different superscripts down the column are significantly different at $P<0.05$

Nigerian Varieties	**Indian Varieties**	**Indian varieties crossed in IAR Zaria**
SWSV 2006-3	SPV 422	ICSA 89002 X SPV 422
SWSV 2006-5-2	E 36-1	ICSA 95 X NTJ -2
SWSV 2007-1	ICSR 93034	

Appendix 5: The percentage bagasse yield of eight sweet sorghum varieties after harvest.

Sweet Sorghum varieties	% pith yield
SWSV 2006-3	1.84
SWSV 2006-5-2	1.48
SWSV 2007-1	1.66
SPV 422	2.17
E 36-1	2.06
ICSR 93034	1.51
ICSA 89002 X SPV 422	2.71
ICSA 95 X NTJ -2	2.42

Nigerian Varieties	**Indian Varieties**	**Indian varieties crossed in IAR Zaria**
SWSV 2006-3	SPV 422	ICSA 89002 X SPV 422
SWSV 2006-5-2	E 36-1	ICSA 95 X NTJ -2
SWSV 2007-1	ICSR 93034	

Appendix 6: Field performance and Physical characteristics of the Sweet sorghum varieties during growth periods at week 5, week 7 and week 9

Sample Tag	Survival rate	Height description	Description of stem diameter	Level of pest infestation
SWSV 2006-3		medium height	big to medium stem diameter	rot present
SWSV 2006-5-2	80% survival	slender tall stems	small stem diameter	rot attacked almost all the stems and also the leaves
SWSV 2007-1	low %survival	few tall stems	mixture of slender and medium stem	rot spoilt majority of the stem
SPV 422	100% survival	moderately tall	medium to big fleshy stem diameter	rot present
E 36-1	high survival	slender to Medium height	medium stem diameter	rot present
ICSA 93034	high survival	taller than E 36-1	medium stem diameter	stem highly attacked by rot
ICSA 89002 X SPV 422	100% survival	tall stems	medium stem diameter	had good stems but Rot present
ICSA 95 X NTJ-2	100% survival	very tall stems. taller than ICSA 89002 X SPV 422	medium to big stem diameter. fleshy stems	few rot present