Microwave assisted pretreatment of sweet sorghum bagasse for bioethanol production

Busiswa Ndaba (B.Sc. Hons)

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Supervisor: Prof. S. Marx

Co-Supervisor: Dr. I. Chiyanzu

ABSTRACT

The growing demand for energy in the world, the implications of climate change, the increasing damages to our environment and the diminishing fossil fuel reserves have created the appropriate conditions for renewable energy development. Biofuels such as bioethanol can be produced by breaking down the lignocellulosic structure of plant materials to release fermentable sugars. Sweet sorghum bagasse has been shown to be an important lignocellulosic crop residue and is potentially a significant feedstock for bioethanol production. The aim of this study was to investigate suitable microwave assisted pretreatment conditions of sweet sorghum bagasse for bioethanol production. A chemical pretreatment process of sweet sorghum bagasse, using different concentrations (1 to 7 wt%) of sulphuric acid (H_2SO_4) and calcium hydroxide ($Ca(OH)_2$) was applied to break up the lignocellulosic matrix of sweet sorghum bagasse. The pretreated broth, which contained pentose and hexose sugars, was fermented using a combination of *Zymomonas mobilis* ATCC31821 and *Saccharomyces cerevisiae* to produce bioethanol at pH 4.8 and 32^{o}C for 24 hours. The highest reducing sugar yield of 0.82 g/g substrate was obtained with microwave irradiation at 180 W for 20 minutes in a 5 wt% sulphuric acid solution. The highest ethanol yield obtained was 0.5 g/g from 5 wt% H_2SO_4 pretreated bagasse at 180 W using a 10:5% v/v of *Saccharomyces cerevisiae* to *Zymomonas mobilis* ratio, whereas for 3 wt% $Ca(OH)_2$ microwave pretreatment, a sugar yield of 0.27 g/g substrate was obtained at 300 W for 10 minutes. Thereafter, an ethanol yield of 0.13 g/g substrate was obtained after 24 hours of fermentation when using a 10:5% v/v of *Saccharomyces cerevisiae* to *Zymomonas mobilis* ratio. The effect of microwave pretreatment on the bagasse was evaluated using Scanning Electron Microscopy (SEM) and Fourier Transform Infrared Spectroscopy (FTIR) analysis. The reducing sugars formed were quantified using High Performance Liquid Chromatography (HPLC). The results showed that microwave pretreatment using 5 wt% H_2SO_4 is a very effective pretreatment that can be used to obtain sugars from sweet sorghum bagasse. The analytic results also showed physical and functional group changes after microwave pretreatment. This confirms that microwave irradiation is very effective in terms of breaking up the lignocellulose structure and improving fermentable sugar yield for fermentation. Bioethanol yields obtained from microwave pretreatment using different solvents also show that *Saccharomyces cerevisiae* and *Zymomonas mobilis* ATCC31821 is a good combination for producing ethanol from sweet sorghum bagasse.

Sweet sorghum bagasse is clearly a very effective and cheap biomass that can be used to produce bioethanol, since very high yields of fermentable sugars were obtained from the feedstock.

Keywords: Sweet sorghum bagasse, microwave pretreatment, fermentation, *Zymomonas mobilis*, Bioethanol.

OPSOMMING

Die groeiende vraag na energie in die wêreld, die implikasies van klimaatsverandering, toenemende skade aan ons omgewing en die dalende fossielbrandstofreserwes het die toepaslike omstandighede vir die ontwikkeling van hernubare energieontwikkeling geskep. Biobrandstof, soos byvoorbeeld bio-etanol, kan geproduseer word deur die afbreek van die sellulose-struktuur van plantmateriale om fermenteerbare suikers vry te stel. Soetsorghum-bagasse het getoon dat dit 'n belangrike lignosellulose oesres en 'n potensieel belangrike grondstof vir die produksie van bio-etanol is. Die doel van hierdie studie was om die kondisies vir geskikte behandeling van soetsorghum-bagasse vir bio-etanol-produksie met mikrogolf-hulp te ondersoek. 'n Chemiese behandelingsproses van soetsorghum-bagasse deur gebruik te maak van verskillende konsentrasies (1 tot 7 massa%) swaelsuur (H_2SO_4) en kalsiumhidroksied ($Ca(OH)_2$) is toegepas om die sellulose-matriks van soetsorghum-bagasse op te breek. Die voorafbehandelde sap, wat pentose- en heksose-suikers bevat, is gefermenteer met behulp van 'n kombinasie van *Zymomonas mobilis* ATCC31821 en *Saccharomyces cerevisiae* om bio-etanol te produseer by pH 4,8 en 32^{o}C vir 24 uur. Die hoogste reduserende suikeropbrengs van 0.82 g/g substraat is verkry met mikrogolfbestraling by 180 W vir 20 minute in 'n 5 massa% swaelsuur-oplossing. Die hoogste etanol opbrengs behaal, was 0.5 g/g van 5 massa% H_2SO_4 voorbehandelde bagasse by 180 W met 'n 10:05 %v/v *Saccharomyces cerevisiae*- tot *Zymomonas mobilis*-verhouding terwyl, vir 3 massa% $Ca(OH)_2$ mikrogolfbehandeling 'n suikeropbrengs van 0.27 g/g substraat verkry is by 300 W vir 10 minute. Daarna is 'n etanol-opbrengs van 0.13 g/g substraat na 24 uur fermentasie verkry met die gebruik van 'n 10:05 %v/v *Saccharomyces cerevisiae*- na *Zymomonas mobilis*-verhouding. Die effek van die mikrogolfbehandeling op die bagasse is geëvalueer met behulp van skandeer-elektronmikroskopie (SEM) en Fourier-tansform-Infrarooispektroskopie (FTIR)-analise. Die gevormde reduserende suikers is gekwantifiseer met behulp van Hoëwerkverrigting-Vloeistofchromatografie (HPLC). Die resultate het getoon dat die mikrogolfvoorbehandeling met 5 massa% H_2SO_4 'n baie doeltreffende behandeling is wat gebruik kan word om suikers van soetsorghum-bagasse te verkry. Die analitiese resultate het ook fisiese en funksionele groepveranderings getoon na mikrogolfbehandeling. Dit bevestig dat die mikrogolfbestraling baie effektief is vir die afbreek van die lignosellulose-struktuur en die verbetering van die fermenteerbare suiker-opbrengs. Bio-etanol-opbrengste verkry na

mikrogolfbehandeling met gebruik van verskillende oplosmiddels toon ook dat *Saccharomyces cerevisiae* en *Zymomonas mobilis* ATCC31821 'n goeie kombinasie is vir die vervaardiging van etanol uit soetsorghum-bagasse. Soetsorghum-bagasse is duidelik 'n baie doeltreffende en goedkoop biomassa wat gebruik kan word om bio-etanol te produseer, aangesien baie hoë opbrengste fermenteerbare suikers verkry is vanaf die grondstof.

Sleutelwoorde: Soetsorghum-bagasse, mikrogolfbehandeling, fermentasie, *Zymomonas mobilis*, bio-ethanol.

DECLARATION

I, Busiswa Ndaba hereby declare that the work contained in this dissertation is my own, and has not already been submitted to any other university.

.....

Busiswa Ndaba

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"Alternative energy is a future idea whose time is past. Renewable energy is a future idea whose time has come".

Bill Penden

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LIST OF ABBREVIATIONS

DDGs- Dry Distillers Grain's

SSF- Simultaneous Saccharification and Fermentation

SHF- Separate Hydrolysis and Fermentation

HPLC- High Performance Liquid Chromatography

FTIR- Fourier Transform Infrared Spectroscopy

SEM- Scanning Electron Microscope

ATCC- American Type Culture Collection

ICE- Internal Combustion Engine

AFEX- Ammonia Fibre/Freeze Explosion

SSB- Sweet sorghum bagasse

ARC- Agricultural Research Council

USA- United States of America

Z.mobilis- *Zymomonas mobilis*

S.cerevisiae- *Saccharomyces cerevisiae*

E.coli- *Escherichia coli*

W- Watt

v/v- volume per volume

g/g- gram per gram

g/L- gram per litre

wt%- weight percentage

^{O}**C-** degrees Celsius

Kg- Kilogram

S- second

min- minute

hrs- hours

rpm- rotation per minute

nm- nanometre

g- gram

mL- millilitre

mg- milligram

µL- microlitre

Conc. - Concentration

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Chapter 1

GENERAL INTRODUCTION

1.1 Introduction

Each year, fossil energy resources, such as crude oil and coal, which are the major suppliers of transportation fuel and electricity in South Africa and the world, are declining. Therefore, alternative energy should be found for these fossil fuel resources. There are many crops available for producing energy (such as sweet sorghum, maize, sugar cane, cassava, and wheat) which not only produce food (Anglani, 1998), but also energy. Sweet sorghum has been shown to be an alternative crop for producing bioethanol, because of its high resistance to drought conditions, fast growing cycle, high sugar yields and shorter gestation period (Reddy *et al.*, 2005; Woods, 2000).

Bioethanol (ethyl alcohol) as a liquid fuel for cooking and lighting is an attractive alternative to paraffin in developing countries. The use of bioethanol as an alternative motor fuel has been progressively increasing all over the world for many reasons. Domestic production and use of bioethanol for fuel can decrease dependence on foreign oil, reduce trade deficits, create jobs in rural areas, reduce air pollution, reduce global climate change and carbon dioxide build-up. Bioethanol, unlike petrol, is an oxygenated fuel that contains 35% oxygen, which reduces particulate and NO_x emissions from combustion (Dermibas, 2007).

In times of fuel shortages, fermentation ethanol has been commercially manufactured in the United States (US) from cellulosic biomass feedstocks using hydrolysis techniques. However, it is only recently that cost-effective technologies for producing bioethanol-from-cellulose in the US have started to emerge, and now the technologies have developed worldwide. Second generation biomass typically consists of wheat straw, sugarcane bagasse, maize stover, and sweet sorghum bagasse. Lignocellulosic biomass is a sufficiently abundant, renewable source of energy and it is not used as a source of food for humans (Kumar *et al*., 2009). Sweet sorghum bagasse can also be used as a lignocellulosic feedstock for producing bioethanol which is the main aim of this study.

Sweet sorghum (also known as Sorgo) is an indigenous African C4 plant in the grass family belonging to the genus *Sorghum bicolor* (L) Moench which also includes grain and fibre sorghum (FAO, 2002). Sweet sorghum is one of the most promising feedstock, particularly for bioethanol production (Wang and Liu, 2009; Jianliang *et al*., 2008; Yun-long *et al*., 2006). The lignocellulosic part of sweet sorghum can be milled, pretreated, hydrolysed, and then fermented to produce ethanol (Goshadrou and Karimi, 2010).

Sweet sorghum consists of approximately 75% cane, 10% leaves, 5% seeds and 10% roots by weight (Grassi *et al*., 2002). Sweet sorghum bagasse is the part of the sweet sorghum plant which remains on the surface after harvesting the grain and it consists of stalks and husks. Bagasse is regarded as a promising feedstock for the production of cellulosic ethanol because like any other lignocellulosic biomass, it contains cellulose (consisting of C6 sugars) and hemicellulose (consisting of C5 sugars) which can be hydrolyzed into fermentable sugars and produce high yields of bioethanol (Balat *et al*., 2008).

Conversion technologies from starch are currently regarded as uneconomical, since starch competes with food, while lignocellulosic feedstocks are abundant and non-edible to humans. Bioethanol from lignocellulosic sources, such as; wood, grass and bagasse are promising feedstocks. For this reason it is advisable to seek more convenient technologies which will produce even higher yields of bioethanol from the lignocellulosic feedstocks.

1.2 Research aim

The main aim of the study was to investigate an economically feasible method for bioethanol production from sweet sorghum bagasse using microwave irradiation as a pretreatment method to liberate reducing sugars from the biomass. The present work focused on the different parameters (power input, pretreatment time, solvent concentration) that affect the sugar and bioethanol yields from sweet sorghum bagasse.

Research objectives

- To quantify sweet sorghum bagasse components.
- To optimise microwave assisted pretreatment conditions.
- To identify and quantify sugars found in hemicellulose and cellulose.

- To ferment sugars using *S. cerevisiae* and *Z. mobilis* for production of ethanol

1.3 Scope of the study

Chapter 1: An introduction to biofuels, bioethanol and sweet sorghum bagasse used in this study is discussed.

Chapter 2: A review of current literature on microwave irradiation as a pretreatment method for lignocellulosic biomass is given in this Chapter.

Chapter 3: In the desire to assess the effectiveness of sweet sorghum bagasse into bioethanol, there was a need to evaluate different solvent concentrations during microwave pretreatment to see which solvents are best in terms of sugar release. Specific procedure for producing bioethanol from sweet sorghum bagasse and analytical methods applied are discussed.

Chapter 4: The results for microwave acid and alkali pretreated sweet sorghum bagasse are presented in this Chapter.

Chapter 5: The results obtained during fermentation of the sugars from sweet sorghum bagasse to produce bioethanol are discussed.

Chapter 6: An overall conclusion of the results obtained in this study for both microwave pretreatment and fermentation are summarised.

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Chapter 2

LITERATURE REVIEW

2.1 Introduction

This chapter focuses on reviewing the research on sweet sorghum bagasse as an alternative feedstock for bioethanol production. An introduction to biofuels is discussed in Section 2.2, sweet sorghum bagasse as a feedstock is discussed in section 2.4. In Section 2.6, the general conventional methods for bioethanol synthesis from sweet sorghum bagasse are discussed.

2.2 Biofuels

Biofuels i.e. bioethanol and biodiesel have drawn remarkable attention from many researchers from all the corners of the continent. There has been extensive research to develop or improve methods for producing biofuels as an alternative to petroleum-based transportation fuel, namely petrol and diesel (Albert and Robert, 2009). Conventional petroleum-based energy resources are currently used however, due to their depletion, increased oil prices, greenhouse gases emissions, and high energy demand, scientists and engineers have been prompted to develop alternative energy resources with beneficial properties, i.e. renewable, efficient, sustainable, cost-effective and environmentally friendly resources (Chum and Overend, 2001; Chen *et al*., 2011). It is envisaged that in future bioethanol will substitute the conventional fossil fuel to mitigate greenhouse gases emission problems.

It has been reported that countries such as the USA, Brazil, Europe and China have already adopted bioethanol synthesis from renewable resources (Jianliang *et al*., 2008). It is also reported that bioethanol accounts approximately 94% of global biofuels production (Balat, 2011). Biodiesel is mostly produced in the European countries. Renewable resources can be categorised into edible and non-food resources. The former refers to resources consumed by humans, and the latter cannot be consumed. The major bioethanol producing countries, the USA and Brazil produce bioethanol from maize and sugarcane. Other countries such as China and India use mainly sweet sorghum as a feedstock for producing bioethanol (Binod *et al*., 2012).

Stringent food regulations have initiated research into non-edible feedstock as alternative resources for biofuel production.

According to the South African biofuel industrial strategy published in 2007 (ICRISAT, 2008), sugar beet and sugarcane are the main feedstocks that can be used for producing bioethanol in South Africa. Maize was excluded due to the rising concerns of food security, since maize is a staple food in South Africa. Biodiesel feedstocks are sunflower, canola and soybean.

2.3 Bioethanol as biofuel

Albert and Robert (2009) and Balat *et al*. (2008) showed that bioethanol could be produced from many different feedstocks with novel properties as potential alternative fuel. Generally, Bioethanol is an oxygenated fuel that contains 35% oxygen which reduces particulate and NO_x emissions from combustion (Balat *et al*., 2008). Bioethanol has been reported to have a high octane number, high compression ratio and a shorter burn time, and thus have a theoretical efficiency advantage over petrol in an internal combustion engine (ICE). Some bioethanol properties are listed in Table 2.1. The octane number measures the petrol quality and a high octane number is reported to prevent early ignition which leads to cylinder knocks and engine damages. In principle, the high octane number of bioethanol can help prolong engine life (De Oliveria *et al*., 2005). There are some disadvantages concerning the bioethanol properties, these include its lower energy density than petrol, low flame luminosity, lower vapor pressure which makes cold starts difficult, miscibility with water, increase in exhaust emissions of acetaldehyde, and vapor pressure increase when blended with petrol (MacLean and Lave, 2003).

Table 2. 1 Properties of bioethanol fuel (Balat, 2007)

Fuel property	Bioethanol
Cetane number	8
Octane number	107
Auto-ignition temperature (K)	606
Latent heat of vaporization (MJ/kg)	0.91
Lower heating value (MJ/kg)	26.7
Carbon (%)	52.2
Hydrogen (%)	13.1
Oxygen (%)	34.7
Specific gravity (%)	0.794
Density (kg/L)	0.794
Boiling point (^{0}C)	78
Flash point (^{0}C)	12.8
Stoichiometric ratio	9.0

Although there are feedstocks currently used to produce bioethanol, there is still a need to identify an efficient and suitable feedstock for production of bioethanol. These kinds of feedstock have to ensure food security, and also sustainability in terms of water consumption since South Africa is one of the water-stressed countries.

Bioethanol feedstocks are classified into three categories: sucrose-containing feedstock (e.g. sugar beet, sweet sorghum and sugar cane), starchy materials (e.g. wheat, maize and barley), and lignocellulosic biomass (e.g. wood, straw, bagasse and grasses) (see Table 2.2). In the recent

years, lignocellulosic feedstocks have been primarily considered for producing bioethanol, because lignocellulose does not compete with food crops (FAO, 2002).

Table 2.2 Types of feedstock and their potential field (Linoj *et al*., 2006)

Feedstock	Bioethanol production potential (l/ton)
Sugarcane	70
Sugar beet	110
Sweet potato	125
Potato	110
Cassava	180
Maize	360
Rice	430
Barley	250
Wheat	340
Sweet sorghum	60
Bagasse and other cellulose biomass	280

Table 2.2 clarifies the most important feedstocks for bioethanol production, and the amount of each feedstock that is required to produce a litre of bioethanol.

2.4 Sweet sorghum bagasse as a feedstock for bioethanol production

Sweet sorghum is a C4 crop in the grass family of genus *Sorghum bicolor* L. Moench. The genus sorghum belongs to the tribe *Andropogoneae* of the family *Poaceae*. Sweet sorghum has been reported to be a promising alternative crop for fuel bioethanol (see Figure 2.1), because it has a

high photosynthetic activity and can produce non-edible biomass, food as well as fermentable sugar syrup. Sweet sorghum is also known to be resistant to drought and is of particular interest as a potential crop for large volume bioethanol production (Yu *et al*., 2011).

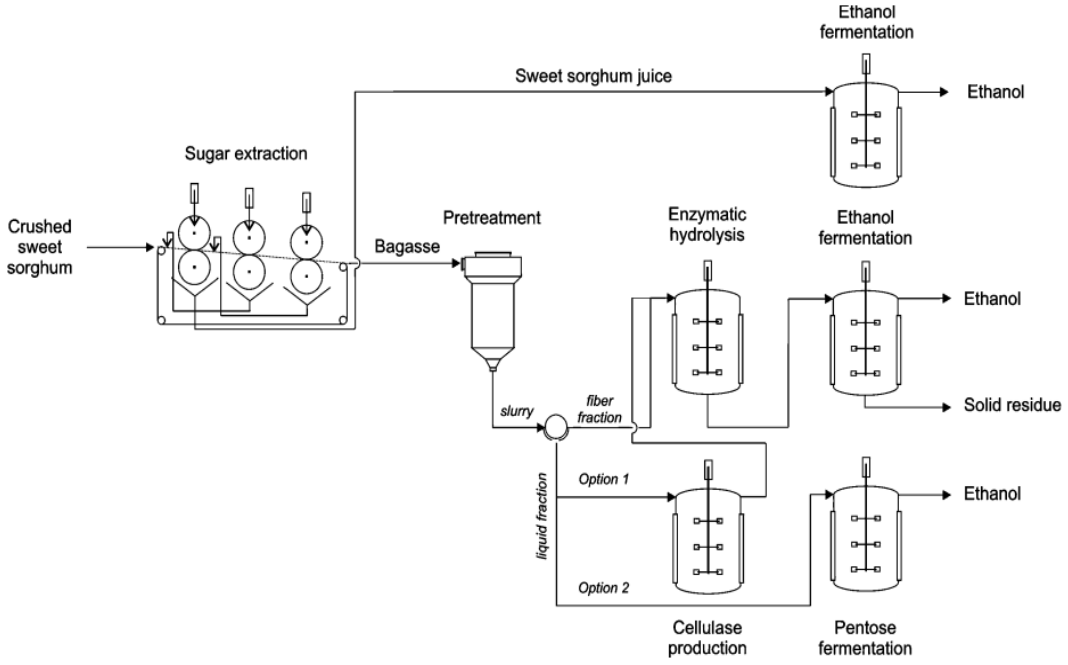

Figure 2.1 Schematic diagram of bioethanol production from sweet sorghum (Bálint *et al*., 2009).

Figure 2.1 shows a two way route that is usually taken in order to produce bioethanol from sweet sorghum plant. The juice obtained from the feedstock is also used for bioethanol production, since it consists of sugars. The bagasse after juice extraction is also pretreated so as to obtain fermentable sugar which can also be fermented to produce bioethanol.

The initial estimated world surface area for sorghum cultivation in 1972 was 40 Mha, with the largest areas being in India (16 Mha) and Africa (10.3), but by the 1980s, sorghum production had spread across the world. Japan and Europe use sweet sorghum for stock feed, while India and Africa use sorghum for human consumption and beer production. Sweet sorghum has been reported to contain fermentable sugars in its bagasse.

The sweet sorghum plant consists of a kernel (7%), stalks (75%) which mostly contains sucrose and cellulose, leaves (10-15%), and the roots (10%). The grain kernel contains approximately 60-65% starch (Gnansounou *et al*., 2005). A sweet sorghum plant is shown in Figure 2.2.

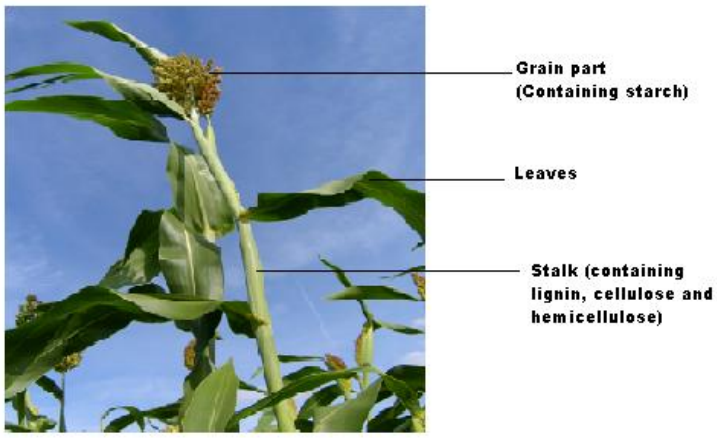

Figure 2.2 A sweet sorghum plant (www.googleimages.co.za)

Sweet sorghum bagasse is the stem part of the sweet sorghum plant left after juice extraction (see Figure 2.3). Sorghum bagasse has been reported to have a very high content of sugars that are attainable from the cellulose and hemicellulose part of the stem (Linoj *et al*., 2006). Bagasse is considered to be a good feedstock for bioethanol production, because it is abundant and cheaper than conventional agricultural feedstock (Jianliang *et al*., 2010).

Figure 2.3 Unmilled sweet sorghum bagasse after juice extraction (Mutepe, 2012)

Many researchers have found sweet sorghum bagasse to be an interesting feedstock for bioethanol production. Liu *et al*. (2007) investigated the fermentation of sweet sorghum stem juice, which contained most of the soluble carbohydrates, using immobilized yeast cells. The

residual bagasse was hydrolyzed with acid or enzyme to soluble oligosaccharides and then fermented to bioethanol. This approach achieved 68.6% of the theoretical yield based on total polysaccharides and exceeded that based on oligosaccharides of sorghum stem by 53.7%.

The pioneering published work by Goshadrou and Karimi (2010) demonstrated the production of bioethanol from sweet sorghum bagasse. They found that the bagasse from sweet sorghum bagasse could be efficiently converted to fermentable sugars, i.e glucose and pentose sugars through pretreatment and hydrolysis. Glucose is the easiest sugar to convert to bioethanol using yeast (*S. cerevisiae*), while the pentose sugars have relied on a bacterial strain *(Z. mobilis*).

2.5 Composition of sweet sorghum bagasse

Sweet sorghum bagasse is mainly composed of three components i.e. lignin, hemicellulose, and cellulose. Cellulose (see Figure 2.6) and hemicellulose (see Figure 2.5) contain fermentable sugars. Lignin (see Figure 2.4) cannot be converted to sugars and it interferes and inhibits the explosion of the sugars from cellulose and hemicellulose in the feedstock (Jianliang *et al*., 2010).

2.5.1 Lignin

Lignin is a very complex molecule constructed of phenyl propane units, and is regarded as a hard wood which is particularly difficult to biodegrade. It usually covers cellulose and hemicellulose. It has been reported that if a plant has a high lignin content, it is very resistant to chemical and enzymatic degradation (Palmqvist and Hahn-Hägerdal, 2000). It is one of the disadvantages of using lignocellulosic materials in fermentation, as it makes lignocellulose resistant to chemical and biological degradation (Taherzadeh, 1999; Palmqvist and Hahn-Hägerdal, 2000).

Berlin *et al*. (2006) discovered that sweet sorghum bagasse contains approximately 4.7-7.1% lignin. The resistance properties of lignin to enzymatic reaction compelled its dissolution under very harsh acidic or alkaline conditions.

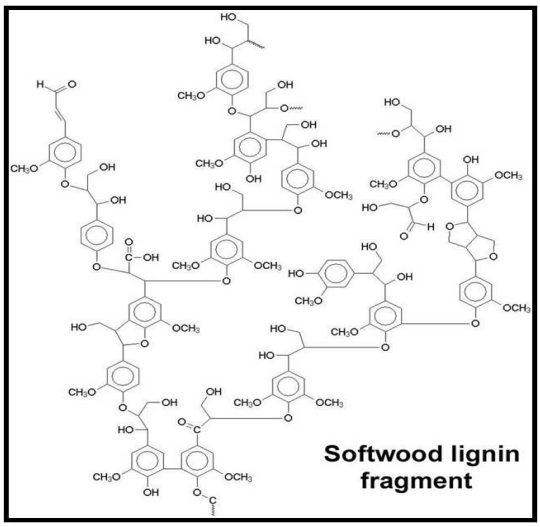

Figure 2.4 Lignin structure in softwood (Klemm *et al*., 2005)

2.5.2 Hemicellulose

Hemicellulose can be hydrolyzed by enzymatic hydrolysis using hemicellulases and the resultant sugars can be fermented to bioethanol using the appropriate fermenting microorganisms e.g. *Z. mobilis,* genetically modified bacteria of *E. coli* or *S. cerevisiae* (Mohan *et al*., 2006). Mohagheghi *et al*. (2002) reported that hemicellulose usually accounts for 28% of the total sugars such as glucose, mannose, galactose, xylose, arabinose, glucuronic acid and galacturonic acid residues.

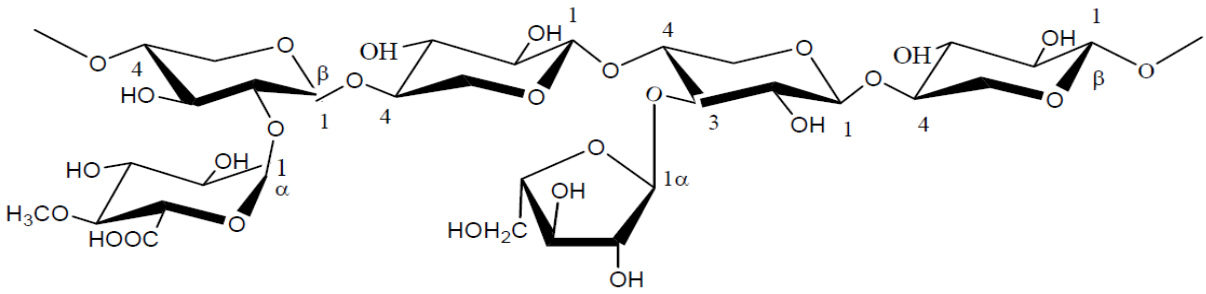

Figure 2.5 Principal structure of hemicellulose in softwood (Mitikka *et al*., 1995).

2.5.3 Cellulose

Cellulose was first discovered in 1838 by French chemist Anselme Payen, who isolated it from plant matter. He found that cellulose contains 44% to 45% carbon, 6 to 6.5% hydrogen and the

rest oxygen (Klemn *et al*., 1998). The cellulose content in sweet sorghum bagasse is approximately 30-40%, which are mainly simple sugars such as glucose (Mitikka *et al*., 1995). The carbon-6 sugars found in cellulose can be fermented to bioethanol by *Saccharomyces cerevisiae*

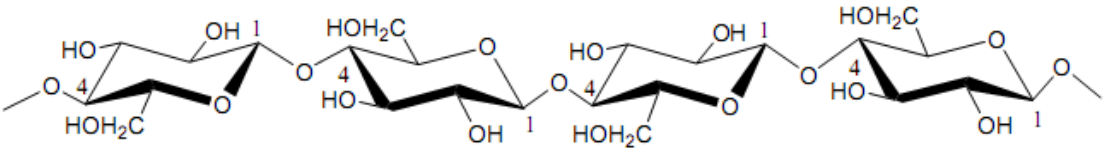

Figure 2.6 Structure of cellulose (Klemn *et al*., 1998).

2.6 Conventional methods for bioethanol synthesis from sweet sorghum bagasse

Bioethanol synthesis from sweet sorghum bagasse is classified into chemical and physicochemical bioconversion of cellulose and hemicellulose to monomeric sugars for bioethanol fermentation. Sugars are fermented to bioethanol under aerobic and anaerobic conditions, producing bioethanol and a variety of other products, including lactic acid. Some of the by-products may be processed further into plastics and other products such as glycerol (Mabee *et al*., 2006). Processing of sweet sorghum bagasse to bioethanol consists of some major unit operations such as: milling, pretreatment, hydrolysis, and fermentation.

2.6.1 Milling

Milling is done to reduce the size of lignocelluloses and to change the degree of crystallinity, and consequently make it more accessible to cellulose. Milling can improve susceptibility to enzymatic hydrolysis, which improves enzymatic degradation of these materials toward bioethanol (Zeng *et al*., 2007).

Among the milling processes, colloid mill, fibrillator and dissolver are suitable only for wet materials, such as wet paper from domestic waste separation or paper pulps, while the extruder, roller mill, cryogenic mill and hammer mill are usually used for dry materials. The ball mill can be used for either dry or wet materials. Grinding with hammer milling of waste paper is a favourable method (Walpot, 1986).

The milling process has been studied prior to and in combination with enzymatic hydrolysis, where mechanical actions, mass transport and enzymatic hydrolysis are performed simultaneously in order to improve the hydrolysis process. The milled bagasse contains the lignin, hemicellulose, cellulose and ash (formed after the milling process) (Maes *et al*., 2002). A milled sweet sorghum bagasse is reported to be very accessible to enzyme attack (see Figure 2.7) (Mosier *et al*., 2005).

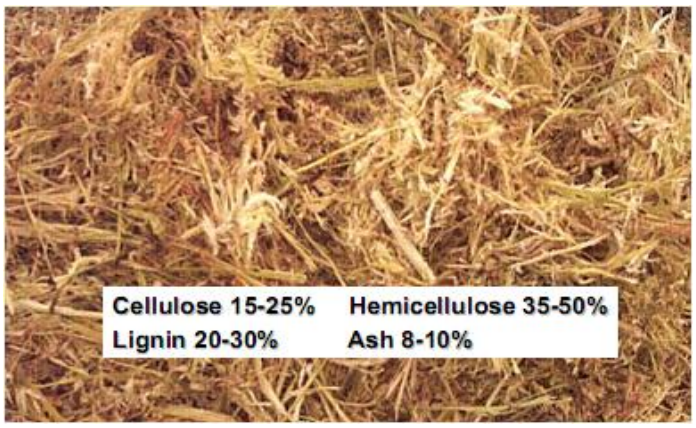

Figure 2.7 Milled sweet sorghum bagasse (Maes *et al*., 2002).

2.6.2 Pretreatment methods

The role of pretreatment is to break the lignin down and expose hemicellulose and cellulose which contain fermentable sugars and it also increases the accessible surface area of cellulose (see Figure 2.8). Different types of physical processes can be used to improve the enzymatic hydrolysis of lignocellulosic waste materials. Pretreatment also increases the yields of fermentable sugars from cellulose or hemicellulose (Mosier *et al*., 2005).

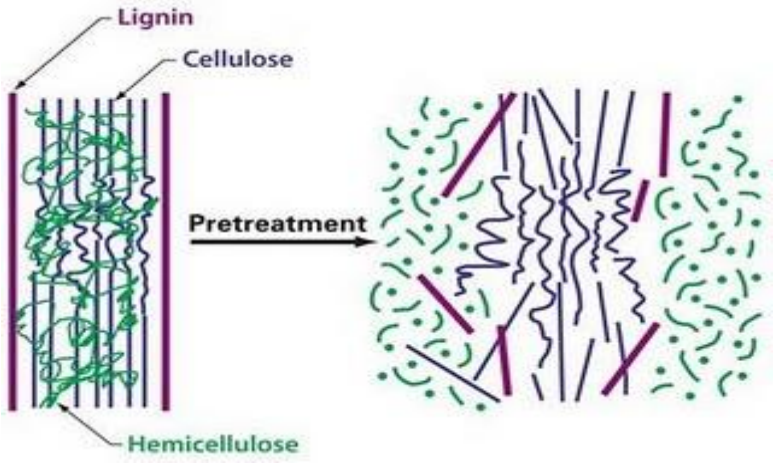

Figure 2.8 Schematic diagram of lignocellulosic biomass pretreatment (Mosier *et al.*, 2005).

2.6.2.1 Steam explosion pretreatment

In steam explosion, the pressure is reduced and makes the materials undergo an explosive decompression. High pressure and high temperature, typically between 160 and 260°C, for a few seconds (e.g. 30 s) to several minutes (e.g. 20 min), were used in steam explosion. The steam explosion process is well documented and has been tested in laboratory and pilot processes by several research groups and companies. Its energy cost is relatively moderate, and it satisfies all the requirements of the pretreatment process (Varga *et al*., 2004).

Steam explosion removes most of the hemicellulose, thus improving the enzymatic digestion. The process of steam explosion was demonstrated on a commercial scale at the Masonite plants. Ruiz *et al*. (2008) studied steam explosion for pretreatment of sunflower stalks before enzymatic hydrolysis at temperature in the range of 180–230°C. The highest glucose yield was obtained in steam-pretreated sunflower stalks at 220°C, while the highest hemicellulose recovery was obtained at 210°C (Ruiz *et al*., 2008).

2.6.2.2 Ammonia fibre/ freeze explosion

Ammonia fibre/freeze explosion (AFEX) pretreatment involves liquid ammonia and steam explosion. The AFEX is a process in which ground, pre-wetted lignocellulosic material at a moisture content of 15–30% is placed in a pressure vessel with liquid ammonia (NH_3) at a loading of about 1–2 kg NH_3/kg dry biomass (Hamelinck *et al*., 2005).

The AFEX process can either modify or effectively reduce the lignin fraction of the lignocellulosic materials, while the hemicellulose and cellulose fractions may remain intact. This system does not liberate any sugars, but it allows the hemicellulose and cellulose to be attacked by the enzymes during hydrolysis. The method is similar to steam explosion, but the pretreatment conditions (30-100°C) are less severe than in steam explosion (Mosier *et al*., 2005).

There are some disadvantages in using the AFEX process compared with some other processes. AFEX is more effective on biomass that contain less lignin, and the AFEX pretreatment does not significantly solubilise hemicellulose compared to other pretreatment processes such as dilute-acid pretreatment (Wyman, 1996).

2.6.2.3 Alkaline pretreatment

Alkali pretreatment processes use lower temperatures and pressures compared with other pre-treatment technologies. The characteristic of alkaline pretreatment is that it can remove the lignin without having large effects on other components. During this process the biomass is incorporated into a solution containing alkaline chemicals such as potassium hydroxide (KOH), calcium hydroxide ($Ca(OH)_2$), or ammonium hydroxide (NH_4OH). Sodium hydroxide (NaOH) treatment causes lignocellulosic biomass to swell, leading to an increase in the internal surface area, a decrease in the degree of crystallinity, and disruption of the lignin structure (Lee, 2005).

Alkali pretreatment reduces the lignin and hemicellulose content in biomass, increases the surface area, allowing penetration of water molecules to the inner layers, and breaks the bonds between hemicellulose and lignin carbohydrate. Dilute NaOH is usually used for alkali pretreatment (Lee, 2005).

Lime (calcium hydroxide) has been used to pretreat wheat straw (358K for 3 h), poplar wood (423K for 6 h), switchgrass (373K for 2 h), and corn stover (373K for 13 h) . Calcium hydroxide, water, and an oxidising agent (air or O_2) are mixed with the biomass at temperatures ranging from 313 to 426K for a period ranging from hours to weeks. The major effect is to remove the lignin from the biomass, thus improving the reactivity of the remaining polysaccharides (Mosier *et al*., 2005).

2.6.2.4 Acid pretreatment

The aim of acid pretreatment is to produce high yields of sugars from lignocellulosic biomass. Treatment of lignocellulosic materials with acid at a high temperature can efficiently improve the enzymatic hydrolysis. The acid is mixed or contacted with the biomass, and the mixture is held at temperatures from 433–493K for periods ranging from minutes to seconds. Sulphuric acid (H_2SO_4) is the most applied acid, while other acids such as hydrochloric acid (HCl) and nitric acid were also reported by Taherzadeh and Karimi (2007). The acid pretreatment can operate either under a high temperature and low acid concentration (dilute acid pretreatment) or under a low temperature and high acid concentration (concentrated acid pretreatment) (Taherzadeh and Karimi, 2007).

In general, higher pretreatment temperatures and shorter reactor residence times result in higher soluble xylose recovery yields and enzymatic cellulose digestibility. Higher temperature dilute acid pretreatment has been shown to increase cellulose digestibility of pretreated residues (Tucker *et al*., 2003).

2.6.2.5 Ultrasonic pretreatment

The effect of ultrasound on lignocellulosic biomass has been employed for extracting hemicelluloses, cellulose and lignin, but less research has been addressed to study the susceptibility of lignocellulosic materials to hydrolysis (Sun and Tomkinson, 2002). High enzymatic hydrolysis yields after ultrasound pretreatment could be explained by cavitation effects caused by the ultrasound field into the enzyme processing solution that enhanced the transport of enzyme macromolecules toward the substrate surface. The maximum effects of cavitation were reported to occur at 50^oC, which is the optimum temperature for many enzymes (Yachmenev *et al*., 2009).

2.6.2.6 Microwave pretreatment

Microwave pretreatment is one of the technologies which have been considered to be very effective in breaking up the lignocellulose parts of woods. Ooshima was the first researcher to introduce this type of pretreatment method for both softwoods and hardwoods in 1984 (Xu *et al*., 2011).

According to Zhu *et al*. (2006), microwave pretreatment has been shown to be the more effective than other pretreatment methods. The enzymatic hydrolysis of pretreated rice straw showed that the pretreatment by microwave had the highest hydrolysis rate and glucose content in the hydrolyzate compared to other methods. Although different kinds of pretreatment processes have been proposed, the major challenge that remains is the development of cost-effective pretreatment technology which can help make cellulosic bioethanol production economically viable (Gabhane *et al*., 2011).

Microwave-based pretreatment can be considered to be a physicochemical process since both thermal and non-thermal effects are often involved. Pretreatment is carried out by immersing the biomass in dilute chemical reagents and exposing the slurry to microwave radiation for residence times ranging from 5 to 20 min (Keshwani, 2009). An evaluation of different alkalis identified sodium hydroxide as the most effective alkali reagent. Gabhane *et al*. (2011) demonstrated that microwave pretreatment processes results clearly indicated that microwave pretreatment could be effective for pretreatment if the temperature is increased to 200^{o}C.

2.6.3 Hydrolysis

After pretreatment, the cellulose and hemicellulose are hydrolysed to sugars which can be fermented to bioethanol. Hydrolysis of lignocellulosic biomass is more difficult than that of pure cellulose, because of the presence of nonglucan components such as lignin and hemicellulose. Hydrolysis is catalysed by dilute acid, concentrated acid or enzymes (Balat *et al*., 2008).

2.6.3.1 Acid hydrolysis

There are two basic types of acid processes i.e. dilute acid and concentrated acid, each with variations. Dilute-acid hydrolysis is probably the most commonly used method among the chemical pretreatment methods. Dilute sulphuric acid is mixed with biomass to hydrolyze hemicellulose to xylose and other sugars. According to Badger (2002), most dilute acid processes can only produce up to 50% sugar. The reason for this is that at least two reactions are part of this process i.e. the first reaction converts the cellulosic materials to sugars and the second one converts the sugars to other chemicals. The disadvantage of dilute acid hydrolysis is its low sugar yield and the advantage is that the reaction is fast.

Concentrated acid hydrolysis rapidly converts cellulose to glucose and hemicelluloses to 5-carbon sugars (xylose and arabinose) with little degradation. Reaction time for the process is much longer than in dilute acid hydrolysis. The concentrated acid process uses 70% sulphuric acid at 313–323K for 2–4 h in a reactor. The main advantage of the concentrated acid process is the potential for producing high sugar yield (Badger, 2002). Cara *et al*. (2007) reported the maximum hemicellulose recovery (83%) of olive tree biomass to be obtained at 170 °C and 1% sulphuric acid concentration, but the enzyme accessibility of the corresponding pretreated solid was not very high.

2.6.3.2 Enzymatic hydrolysis

Enzymes are naturally occurring plant proteins that cause certain chemical reactions to occur. For enzymatic reactions to be effective, some kind of pretreatment process is needed to break the crystalline structure of the lignocellulose and remove the lignin to expose the cellulose and hemicellulose molecules for enzymatic attack. The enzymes responsible for the hydrolysis of cellulose are known as cellulases. Pretreatment, under certain conditions, retains nearly all of the cellulose present in the original material and allows close to theoretical yields upon enzymatic hydrolysis. Enzyme hydrolysis is usually conducted at pH-4.8 and temperature 318-323K. This process produces higher yields of sugar than acid hydrolysis (Zhang and Lynd, 2004).

2.6.4 Types of fermentation

Fermentation for bioethanol is a natural metabolic process that produces bioethanol by breaking down carbohydrates (like sugars) in the absence of oxygen. It is facilitated/catalyzed by the action of enzymes present in microorganisms like yeasts (*S. cerevisiae*). End products formed during fermentation are ethyl alcohol, lactic acid and glycerol (Hamelinck *et al*., 2005).

The hydrolysate obtained from pretreatment and hydrolysis is used for bioethanol fermentation by microorganisms (*S. cerevisiae* or *Z. mobilis*). Lignocellulose hydrolysate contains not only glucose, but also various monosaccharides, such as xylose, mannose, galactose, arabinose, and oligosaccharides Microorganisms should be able to ferment these sugars for the successful industrial production of bioethanol. According to equation (1) and (2), the theoretical maximum yield is 0.51 kg bioethanol and 0.49 kg carbon dioxide per kg of xylose and glucose (Hamelinck *et al*., 2005). There are various types of fermentation that can be used to produce bioethanol.

$3C_5H_{10}O_5 \rightarrow 5C_2H_5OH + 5CO_2$…….. (1) (Xylose fermentation)

$C_6H_{12}O_6 \rightarrow 2C_2H_5OH + 2CO_2$……. (2) (Glucose fermentation)

2.6.4.1 Simultaneous Saccharification and Fermentation

Enzymatic hydrolysis and fermentation can be performed in a combined step known as simultaneous saccharification and fermentation. In SSF, cellulases and xylanases convert the carbohydrate polymers to fermentable sugars. SSF gives higher reported bioethanol yields and requires lower amounts of enzyme because end-product inhibition from cellobiose and glucose formed during enzymatic hydrolysis is relieved by the yeast fermentation. Bioethanol production yields of approximately 5% on a w/w basis can be obtained using SSF (Jeffries and Jin, 2000).

2.6.4.2 Separate Hydrolysis and Fermentation

Enzymatic hydrolysis and fermentation can also be performed separately by separate hydrolysis and fermentation steps. When using separate hydrolysis and fermentation the final bioethanol yield is higher than when using other methods, and less energy is required and production costs are minimized. Iogen Corporation, a major manufacturer of industrial enzymes in Canada, developed an SHF process comprising a dilute-acid-catalyzed steam explosion pretreatment process, and the use of *S. cerevisiae* as a fermenting organism (Cardona and Sanchez, 2007).

2.6.5 Fermenting microorganisms

Fermentation can be done using different microorganisms that are best in processing different types of sugars. Sugars are grouped into two, carbon-5 sugars which are mainly derived from hemicellulose and carbon-6 which mainly obtained from cellulose.

2.6.5.1 Fermentation by S. cerevisiae

S. cerevisiae is one of the most effective bioethanol-producing yeasts, and it is tolerant to bioethanol up to 15% of its concentration in the fermentation broth. The organism is able to grow under highly anaerobic conditions. However, because wild-type strains of this yeast cannot ferment pentoses, such as xylose and arabinose, bioethanol production from a lignocellulose hydrolysate becomes insufficient (Balat *et al*., 2008). There are other microorganisms such as *Z. mobilis, E. coli, K. oxytoca* which have been shown to ferment pentose sugars for high bioethanol yield (Balat *et al*., 2008).

2.6.5.2 Fermentation by Z. mobilis

Z. mobilis is a gram-negative bacterium that has been attracting increasing attention for bioethanol production. It is a bioethanol tolerant bacterium and it has shown higher specific rates of sugar uptake (including pentose sugars) for bioethanol production via the Entner-Doudoroff pathway under anaerobic conditions (Cazetta *et al*., 2007).

Several researchers (Panesar *et al*., 2006; Deanda *et al*., 1996) have shown that *Z. mobilis* can achieve 5% higher yields and up to five-fold higher volumetric productivity when compared with traditional yeast fermentations. *Z. mobilis* has demonstrated bioethanol yields up to 97% of theoretical and bioethanol concentrations up to 12% (w/v) in glucose fermentations (Balat *et al*., 2008). A generalized flow diagram for the conversion of lignocellulosic biomass to bioethanol, based on recombinant *Z. mobilis*, is given in Figure 2.9.

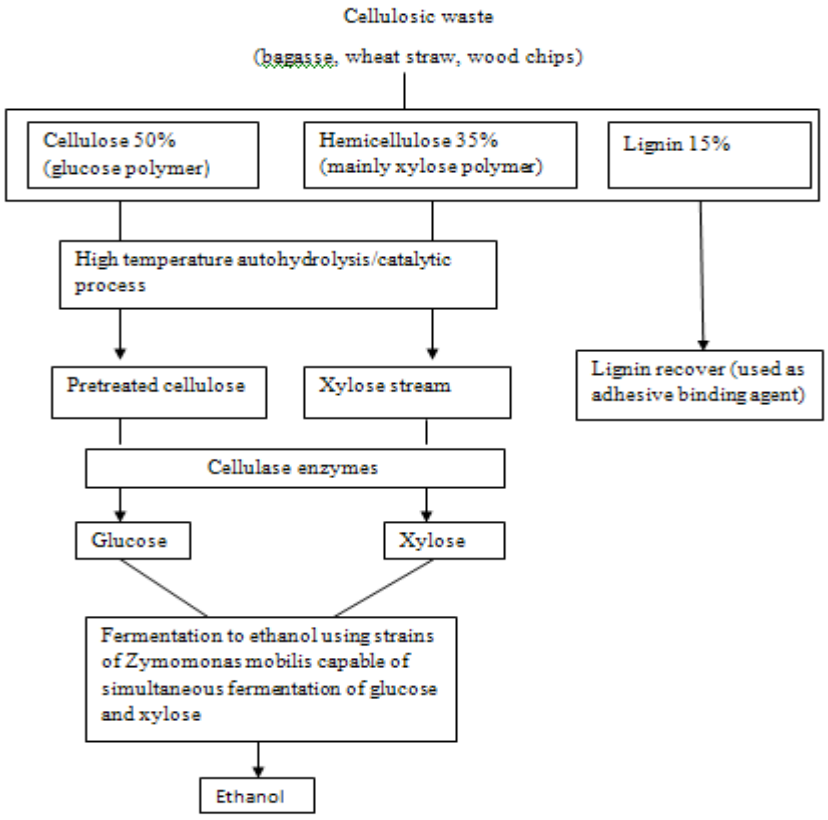

Figure 2.9 *Zymomonas* based process for conversion of lignocellulosic hydrolysates to bioethanol (Balat *et al*., 2008).

2.7 Concluding remarks

It is clear that fuel bioethanol from sweet sorghum is the best choice to be implemented under hot and dry climatic conditions. Sweet sorghum is a renewable energy source. Production of bioethanol is an alternative for reducing the consumption of crude oil, and greenhouse gas emissions. Bioethanol properties allow higher compression ratio and shorter burn time, which lead to theoretical efficiency advantages over gasoline in an ICE. Bioethanol is blended with gasoline to form an E10 blend (10% bioethanol and 90% gasoline), but it can be used in higher concentrations such as E85 or E95.

Several conventional pretreatment methods have been presented in this study to obtain fermentable sugars, but microwave pretreatment is a new and a cheaper and less time consuming method of improving sugar yield for bioethanol production. All these methods should make the lignocelluloses available for enzymatic attack. The most preferable method for the hydrolysis of cellulose to fermentable sugars is the enzymatic route because it produces higher yields and it is conducted in mild conditions. Bioethanol is currently made by large scale fermentation of sugars that are extracted from the biomass. However the fermentation of bioethanol by yeast does not ferment pentose sugars, hence the use of other microorganisms such as *Z. mobilis* to ferment the pentose sugars and to increase bioethanol yield through fermentation. In the end, bioethanol production technologies should be able to reduce air pollution and produce higher concentration of bioethanol.

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Chapter 3

MATERIALS AND METHODS

3.1 Introduction

This chapter is intended to show how this study was conducted using the specific research chemicals and materials presented in Section 3.4. Attention is afforded to the choice of feedstock (see Section 3.2). Section 3.6 entails the procedure used throughout the study. The description of analytical methods used during this study is presented in Section 3.8.3.

3.2 Raw material

Sweet sorghum bagasse (Hunni green) was obtained from sweet sorghum harvested at six months by the Agricol Research Company in Potchefstroom, North West Province, South Africa. The bagasse was obtained after the juice had been pressed from the plants. The bagasse was initially dried to 10% moisture content, milled and screened to a particle size of ±1.5mm. The milled bagasse was packed in air-tight bags and then stored at room temperature for further use.

3.3 Compositional analysis

An initial compositional analysis of the sweet sorghum bagasse used for experiments was done to determine the fermentable sugar potential. The percentages of total ash, acid-soluble lignin, acid insoluble lignin, cellulose, and hemicellulose were determined. The milled sweet sorghum bagasse was analysed at Agricultural Research Council (ARC) - Analytical Services (Pretoria, RSA)

3.4 Microorganism and media

3.4.1 *Z. mobilis*

Z. mobilis ATCC 31821 was obtained from the American Type Culture Collection (ATCC) and maintained as a freeze dried pellet at -80^{o}C till further use. The freeze dried organism was rehydrated with sterile water and inoculated on sucrose broth medium and was grown for two days at 30^{o}C, 120 rpm. Stock cultures were either made up in 15% glycerol for long term storage at 4°C; as it was subcultured on nutrient agar plates for 72 hrs at 32°C, from which an inoculum was prepared (see Figure 3.1) (Fu *et al*., 2009).

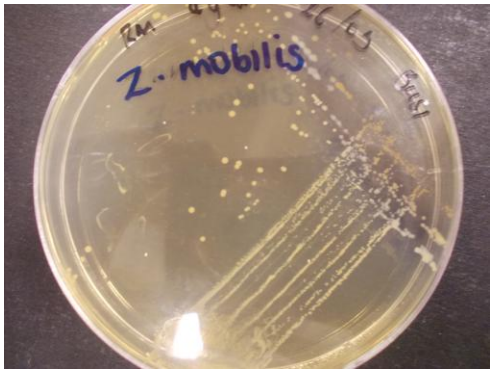

Figure 3.1 *Z.mobilis* grown on agar plate containing growth nutrients

3.4.2 *S.cerevisiae*

Commercial *S. cerevisiae* was used for fermentation. Firstly the dried cells of yeast were revived from the inactive state by using a broth containing nutrients shown in Table 3.1. Prior to fermentation, the broth was incubated at 32^{o}C, for 24 hrs. This was done to reduce lag time of the yeast (Mutepe, 2012).

3.5 Experimental procedure

The experimental process used to produce bioethanol from microwave pretreated sweet sorghum bagasse in this study is shown in Figure 3.2.

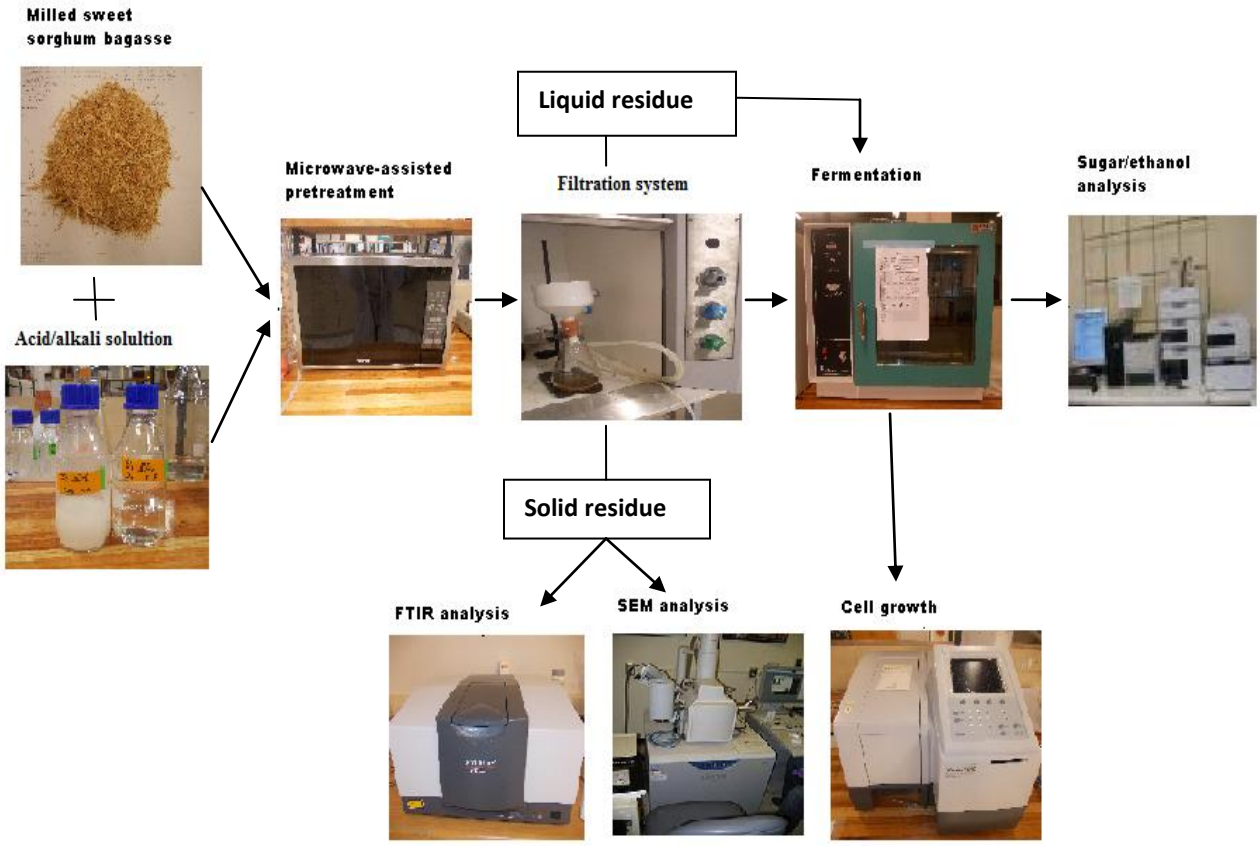

Figure 3.2 flow chart of pretreatment to fermentation of sweet sorghum bagasse

The milled sweet sorghum bagasse (5 wt%) was pretreated with varying concentrations of sulphuric acid and calcium hydroxide (1, 3, 5 and 7 wt %) at varying power settings (100, 180, 300 Watts) for 20 min. Sampling was done at 5 min intervals. The microwave pretreated sweet sorghum bagasse samples with optimal sugars were fermented. The solid residue after pretreatment was analysed using FTIR and SEM. Fermentation was carried out in an incubator shaker at 32°C for 24 hrs at a shaking speed of 120 rpm, at a constant pH of 4.8. *Z. mobilis* (1, 3, 5% v/v) and *S. cerevisiae* (3, 5, 10% v/v) of the batch volume were added. Cell growth during

fermentation was monitored using UV-spectroscopy. Before and after fermentation samples were analysed for sugars and ethanol at set time intervals.

3.6 Microwave experimental setup

Pretreatment experiments were conducted in a Samsung domestic microwave. A photo and a diagram of the experimental setup are shown in Figure 3.3 and 3.4, respectively. The microwave was designed with variable power settings with a minimum setting of 100 W and a maximum setting of 900 W.

Figure 3.3 Samsung microwave oven used in this study

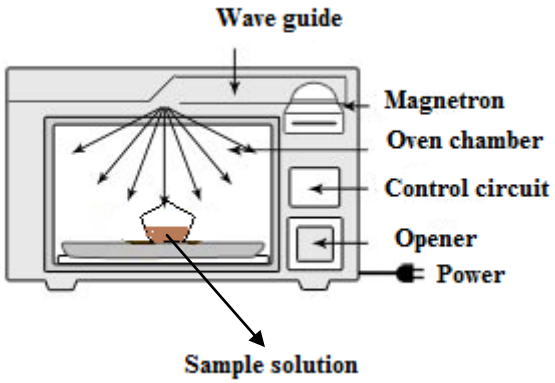

Figure 3.4 Schematic diagram of the microwave oven used in this study

3.7 Experimental methods

3.7.1 Microwave assisted pretreatment methods

The first set of pretreatment experiments was carried out at a power level of 300 W in this study. This power level was chosen since it allowed the required length of pretreatment time without any volumetric losses of the liquid part, which was also required for further use. The higher the power used, the less liquid is obtained from the pretreated biomass. The experiments were done in triplicates. The method used in this study for both microwave- acid and alkali was adapted from Binod *et al*, (2012).

3.7.1.1 Microwave-acid pretreatment

Bagasse samples of 5 g were weighed into 100 mL of sulphuric acid solution, either 1, 3, 5 or 7 wt% in a 500 mL Duran bottle. Bottles were placed in a microwave maintained at different powers for 20 min. Three microwave treatment wattages were selected: 100, 180, or 300 W. Samples (2mL) were taken out of the mixture at time interval of 5 min for analysis of reducing sugars. After pretreatment, the remaining samples were neutralised with sodium hydroxide and filtered. The filtrates were used for fermentation and available sugars were quantified using High performance liquid chromatography (HPLC). The insoluble residues were dried at 50^{o}C for 24 hrs for Scanning electron microscopy (SEM), Fourier transform infrared spectroscopy (FTIR).

3.7.1.2 Microwave-alkali pretreatment

Bagasse samples of 5 g were mixed with 100 mL, of either 1, 3, 5 or 7 wt% sodium hydroxide solutions in Duran bottles. The bottle was placed in a microwave oven at varying wattage (100, 180 and 300 W) for a time period of 20 minutes. Samples (2 mL) were taken from the mixture at time intervals of 5 min for analysis of reducing sugars. Sugar monomers including glucose, arabinose, galactose, mannose and xylose present in the filtrate were quantified using High performance liquid chromatography (HPLC) system. After treatment the liquids to be used in fermentation were collected by filtration, and neutralized with sulphuric acid. The residues were washed with deionised water until pH 6 was reached for SEM and FTIR analyses.

3.7.2 Fermentation

The microwave pretreated sweet sorghum bagasse samples with optimal sugars were fermented. Fermentation was carried out in 250 mL Duran bottles. The amount dispersed into the bottles varied, due to liquid loss during microwave pretreatment. The pH was kept constant at 4.8. *Z. mobilis* and *S. cerevisiae*; 1 and 3% v/v, 3 and 5% v/v, and 5 and 10% v/v of batch, respectively were added and fermentation was carried out in a shaker incubator at 32°C for 24 hrs at a shaking speed of 120 rpm. The medium for *Z. mobilis* and *S. cerevisiae* is given in Table 3.1. Samples were collected and analysed for cell growth, sugars and ethanol at set time intervals. The method of inoculation into the fermentation broth was derived from Song *et al*, (2005).

Table 3.1 Medium for *Z. mobilis* and *S. cerevisiae*

Nutrients	***Z. mobilis* (g/L)**	***S. cerevisae* (g/L)**
Yeast extract	10	-
peptone	5	0.5
$(NH_4)SO_4$	1	1
K_2HPO_4	2	0.1
$MgSO_4$.7H2O	0.5	0.5
K_2HPO_4	0.5	0.5

3.7.3 Analytical methods

3.7.3.1 High Performance Liquid Chromatography *(HPLC)*

All sugar and ethanol analyses were done using high performance liquid chromatography (HPLC). An Agilent series 1200 HPLC fitted with a Shodex SP0810 column and IR detector was used. The samples were filtered through a 0.2 μm micro pore size syringe filter after microwave pretreatment and fermentation for analyses. The different sugars (glucose, sucrose, xylose, mannose, arabinose, galactose), and ethanol were quantified using a set of standard calibration

curves (see Appendix A). The mobile phase used was water at a flow rate of 1 ml.min^{-1} at 80^{o}C with an injection volume of 5 μL for 20 min using an IR detector temperature of 55^{o}C.

3.7.3.2 Scanning Electron Microscope (*SEM*)

A Scanning Electron Microscope (SEM, FEI Quanta 250 FEG) in high vacuum mode was used for observing the changes in the morphological structure of sweet sorghum bagasse before and after pretreatment (Goshadrou and Karimi, 2011).

3.7.3.2 Fourier Transform Infrared Spectroscopy *(FTIR)*

A spectrometer (SHIMADZU) with resolution at 4 cm^{-1} and 25 scans per sample was used to analyse bagasse for the presence of functional groups for cellulose, hemicellulose and lignin. Disc preparation was 3 mg dried bagasse with 300 mg of potassium bromide (KBr). The analysis was done before and after pretreatment. The powder of bagasse was analysed in transmittance mode within the range of 400-4 000 cm^{-1} (Binod *et al*., 2012).

3.7.3.3 Ultraviolet spectroscopy

Spectrometer (SHIMADZU) at a wavelength of 600 nm was used to quantify cell growth during fermentation. The absorbances for each sample at different fermentation times were recorded (Kurian *et al*., 2010).

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Chapter 4

MICROWAVE ASSISTED PRETREATMENT RESULTS

4.1 Introduction

In this Chapter the results which were obtained during characterization (see Section 4.2) and microwave assisted pretreatment of sweet sorghum bagasse are provided. The experimental method used for microwave pretreatment is presented in Chapter 3. Preliminary experiments were conducted to determine the feasibility of using acids or alkali for microwave assisted pretreatment. Microwave assisted pretreatment was done using different concentrations of sulphuric acid (H_2SO_4) and calcium hydroxide ($Ca(OH)_2$), followed by optimisation of different microwave powers. Microwave assisted pretreatment breaks down the lignocellulose so as to expose fermentable sugars from cellulose and hemicellulose. The literature on microwave pretreatment was reviewed (see Chapter 2) and it was proven to have a tremendous effect in liberating sugars, which can be later fermented into bioethanol.

4.2 Compositional analysis of raw sweet sorghum bagasse

Compositional analysis of untreated raw sweet sorghum bagasse (SSB) was done by the Agricultural Research Council of South Africa. Table 4.1 shows the initial composition of sweet sorghum bagasse harvested at 6 months in the Potchefstroom area which contained cellulose (36.6%), hemicellulose (22.96%), and lignin (5.90%). Cellulose and hemicelluloses were the major components and from Table 4.1 it can be seen that a maximum theoretical total sugar yield of 59.56 g/100 g dry sweet sorghum bagasse can be obtained from the cellulose and hemicellulose contained in the bagasse used in this study. Similar compositional analyses were found in literature (Binod *et al*., 2012; Wu *et al*., 2011; Goshadrou and Karimi, 2010).

Table 4.1 Composition of raw sweet sorghum bagasse used in this study

Content	Composition (%)
Cellulose	36.60
Hemicellulose	22.96
ADF	42.50
NDF	65.46
ADL (Lignin)	5.90
Moisture	10.85
Ash	3.07

The initial sugar yields from the juice contained in the sweet sorghum bagasse before pretreatment were quantified. The results are presented in Table 4.3.

Table 4.2 Initial sugar composition (g/g) from the juice that was still remaining in the sweet sorghum bagasse

Sugars	Yield (g/g)
Sucrose	0.15
Glucose	0.06
Fructose	0.04

Sucrose, glucose and fructose were present in different concentrations in the juice that was still remaining in the bagasse after extraction. The sucrose yield was very high in the initial biomass, but during pretreatment its concentration decreased as it was decomposed to glucose and fructose. The total theoretical sugar yield that can be obtained from the bagasse and the residual sugar monomer in the juice still present in the bagasse can be calculated to be 84.56 g/100 g bagasse solids.

4.3 Microwave assisted pretreatment

In the present study, a cost-effective pretreatment method for SSB was carried out using a domestic microwave oven with power settings from 100 to 900 W. Pretreatment experiments in this study were carried out at a power level of 100 to 300 W. These power settings were chosen such that they afforded the required length of pretreatment time without any volumetric losses of the pretreatment solvent. At a power setting higher of than 300 W losses of the liquid in the pretreatment broth were observed, which affected the fermentation process negatively.

4.3.1 Microwave-acid pretreatment

Microwave pretreatment was conducted using different concentrations (1-7 wt%) of sulphuric acid (H_2SO_4) at a fixed irradiation power (300 W). Thereafter, an optimal H_2SO_4 concentration of 5 wt% was used to pretreat sweet sorghum bagasse using different irradiation powers of 100-300 W.

4.3.1.1 Effect of dilute acid concentrations on reducing sugar yields

The development of microwave technology based on the treatment of biomass with acid has been successfully applied to sugarcane bagasse (Chen *et al*., 2011). The production of glucose and hemicellulose sugars such as xylose under conventional conduction heating is time and energy consuming to achieve high sugar yields and the impact of this on processing costs can be reduced by using microwave irradiation as energy source. Acid pretreatment of sweet sorghum bagasse on sugar yield were investigated using dilute sulphuric acid (H_2SO_4) concentrations (1, 3, 5 and 7 wt%) at 300 W for 20 min. The total soluble sugar yield from sweet sorghum bagasse at different time intervals is shown in Figure 4.1.

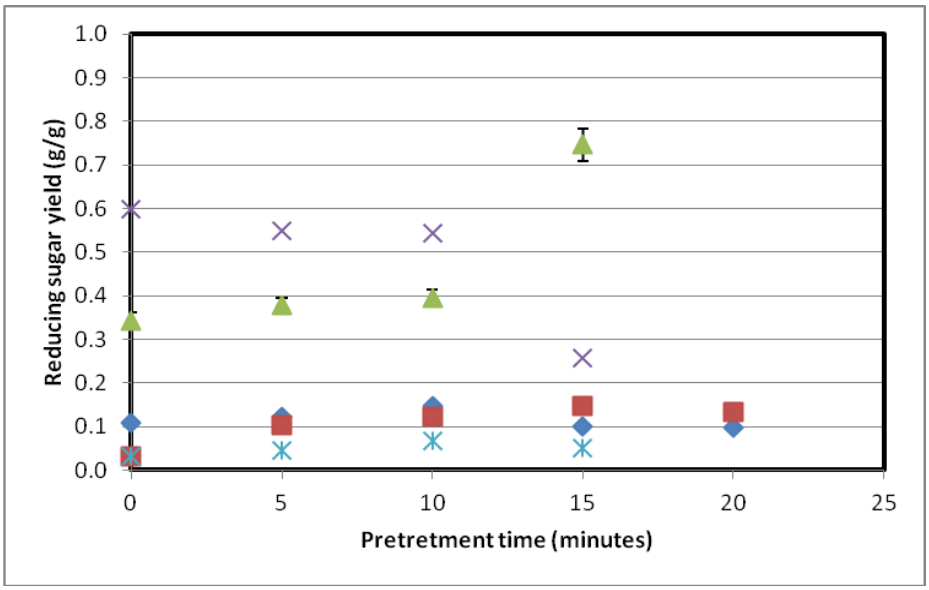

Figure 4.1 Total reducing sugar yields at different H_2SO_4 concentrations at 300 W power

(♦ -1% H_2SO_4, ■- 3% H_2SO_4, ▲- 5% H_2SO_4, × - 7% H_2SO_4, Ж- Control)

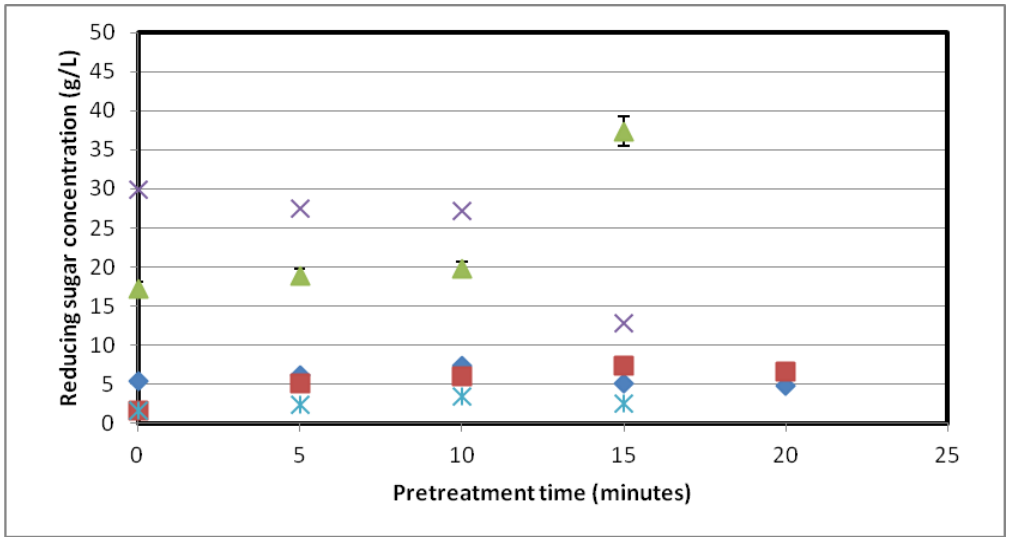

Figure 4.2 Total reducing sugar concentration at different H_2SO_4 concentrations at 300 W power

(♦ -1% H_2SO_4, ■- 3% H_2SO_4, ▲- 5% H_2SO_4, × - 7% H_2SO_4, Ж- Control)

From Figure 4.1 and 4.2 it can be seen that the total amount of soluble sugars liberated from SSB increases with an increase in pretreatment time up to approximately 15 min. All sugar yields and concentrations (in the case of Figure 4.2) from dilute acid treated bagasse are higher than the control value, which clearly indicates that dilute sulphuric acid has a significant influence in

liberating sugars from SSB. Figure 4.2 depicts the same pattern as Figure 4.1, the only difference are the units in which they are expressed. There was an increase in total sugar yield as H_2SO_4 concentration was increased from 1 to 5 wt%, but at 7 wt% total sugars were decreasing. This means that the optimal acid concentration of SSB pretreatment in a microwave is 5 wt%, since the highest yields (0.75 g/g of biomass) in 15 min was found at that concentration. The increase in reaction time to 20 min resulted in loss of sugars to 0.15 g/g of raw material. At 7 wt% H_2SO_4, the concentration was too high to release sugars, instead the hydrogen bonding of the cellulose chains was disrupted, converting the cellulose from a crystalline state to an amorphous state with little sugar liberation. However, 5 wt% acid concentration allows the cellulose chains to form a gel with the acid allowing contact for the pretreatment reaction. When the acid gel is combined with water it causes the oligomer mixture to resolve into an aqueous glucose solution for fermentation (Orozco and Ahmad, 2007). The 5 wt% H_2SO_4 was repeated three time (see appendix B) and the results were expressed with percentage error bars (see Figure 4.17). Higher sugar yields were obtained during microwave acid pretreatment compared with what has been reported by Binod *et al*. (2012) in which more than 70% of the total monomeric sugars were released during microwave pretreatment (H_2SO_4, 30 min, and microwave power 180 W) after enzymatic hydrolysis. These results show a tremendous improvement in the pretreatment of biomass and suggest that a one-step pretreatment with 5 wt% H_2SO_4 at 300 W is sufficient to release most soluble sugars from lignocellulosic feedstock such as sweet sorghum bagasse without the need of enzymatic hydrolysis.

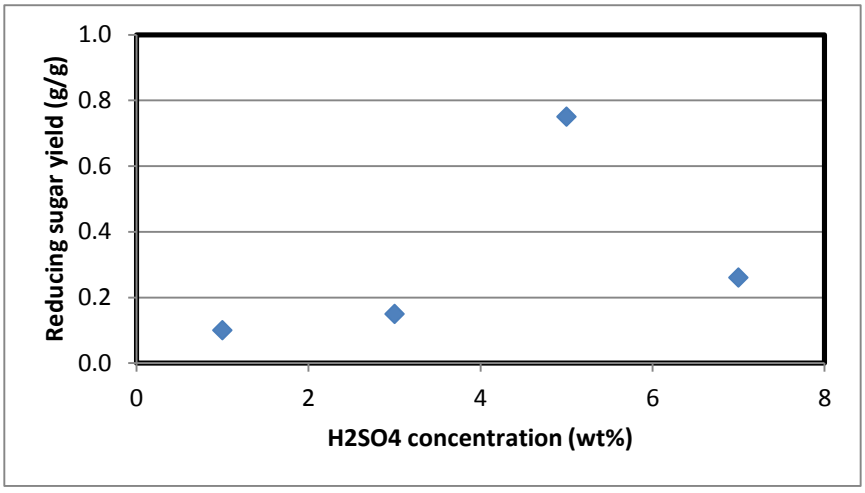

Figure 4.3 Total reducing sugar yield at 15 min microwave-acid pretreatment time at 300 W

Figure 4.3 shows the total reducing sugars that were obtained at 15 min microwave-acid pretreatment time. It was observed that at 1 and 3 wt% sulphuric acid, the total sugar yields were low, since the concentration of acid was not high enough to liberate sugars from the bagasse. A 5 wt% acid concentration was suitable for releasing sugars from sweet sorghum bagasse, because the oligomer mixture during the interaction between hydrogen molecules from water and acid were converted into glucose solution. The hydronium ions in 5 wt% acid are high enough to breakdown or attack intermolecular and intramolecular bonds in cellulose. The results show that the 1 and 3 wt% have less ions than 5 wt% to react with the cellulose structure of sweet sorghum bagasse. Hence the wide difference of sugar composition observed between 3 and 5 wt% acid concentration. A lower sugar yield was obtained at 7 wt% acid than 5 wt%. Although at 7 wt% acid sugars were lower than 5 wt%, they were higher than 1 and 3 wt% sulphuric acid. 5 wt% of sulphuric acid was the maximum concentration that could be used in order to get the highest fermentable sugars. Figure 4.4 depicts the same data presented in Figure 4.3, but in terms of concentration (g/L).

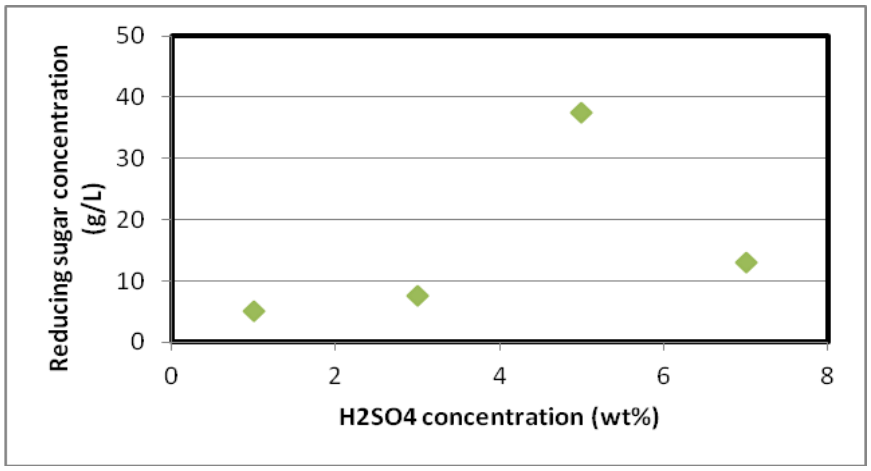

Figure 4.4 Total reducing sugar concentration at 15 min microwave-acid pretreatment time at 300 W.

4.3.1.2 Effect of irradiation power on total sugar yield from sweet sorghum bagasse in acid

The effect of irradiation power on the total reducing sugar yield from SSB was investigated at a constant dilute acid concentration of 5 wt% and pretreatment time of 15 min. The microwave irradiation power was varied from 100 to 300 W. The highest sugar yield (0.82 g/g of substrate) was obtained during microwave pretreatment of bagasse using 5 wt% H_2SO_4 for 20 min at 180

W. The improvement in the total sugar yields with increasing irradiation power can be attributed to disruption of the crystalline structure of sweet sorghum bagasse because higher power causes higher temperatures and leads to cell wall disruption of SSB. Breakage in the parenchyma cells was also observed, thus leading to high sugar release from cellulose (See Figure 4.7 for SEM and Figure 4.8 for FTIR).

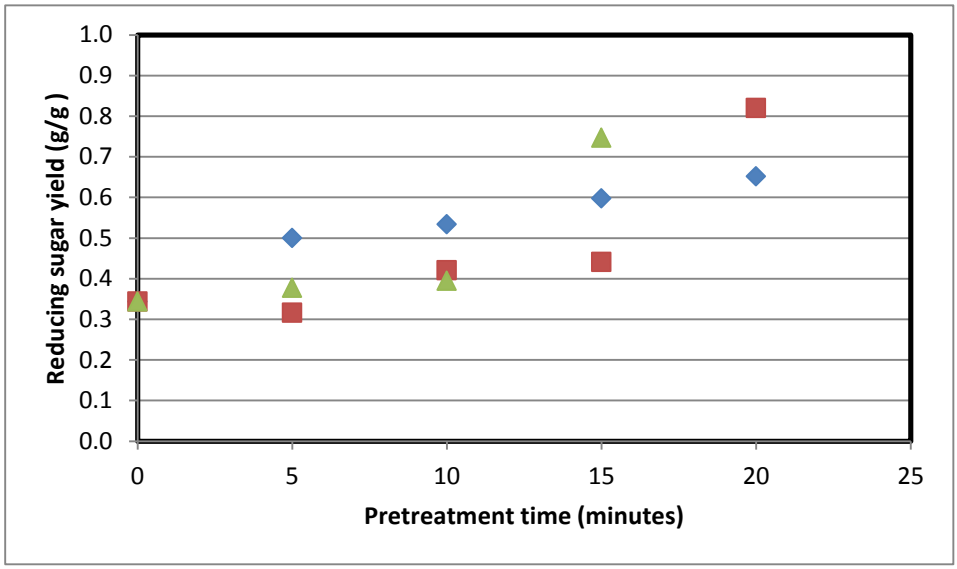

Figure 4.5 Effect of microwave irradiation power on total sugar yield (♦-100 W, ■- 180 W, ▲- 300 W) using 5 wt% H_2SO_4.

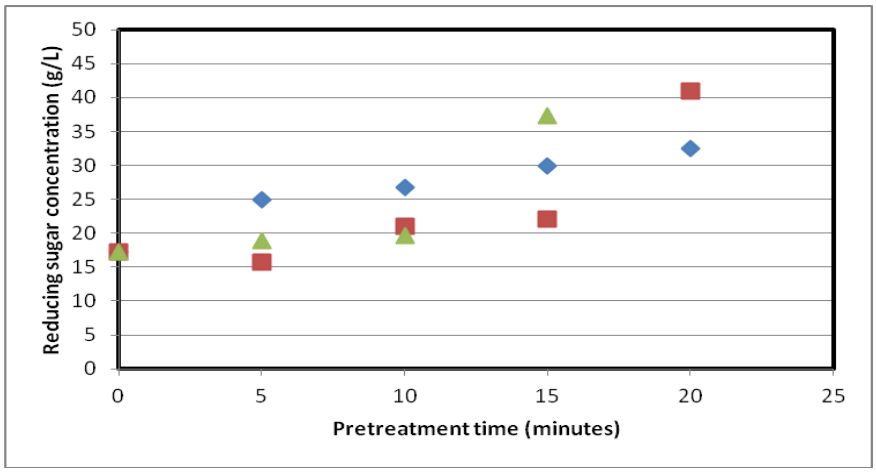

Figure 4.6 Effect of microwave irradiation power on total sugar concentration (♦-100 W, ■- 180 W, ▲- 300 W) using 5 wt% H_2SO_4

When the irradiation power was increased 180 W there was a decrease in total sugar yields. The decrease in total sugar yield was observed with increasing the power from 180 to 300 W. It was noted that whenever power higher than 180 W was applied, the pretreatment time needed to be shortened in order to preserve the sugar structure and liquid from disintegration. At 300 W the highest yield was found at 15 min, thereafter there was no liquid left for HPLC analysis, while at 180 W the highest total sugar yield of 0.82 g/g was obtained at 20 min. Pretreatment at 100 W yielded a high total sugar yield 0.65 g/g at 20 min; this shows that 100 W requires a longer time to get to higher sugar yield, because the intensity of the microwave is too low to speed up the reaction to yield a high total sugar yield in short time.

4.3.1.3 Effect of 180 W irradiation power on sugars from cellulose and hemicellulose in acid

In this section total sugars from cellulose and hemicellulose are presented separately (see Table 4.3). This was done to quantify obtainable carbon-5 and carbon-6 sugars from hemicellulose and cellulose, respectively. The results are presented on the effect of 180 W microwave irradiation power on total sugars from cellulose and hemicellulose for 5 wt% H_2SO_4 pretreated sweet sorghum bagasse.

Table 4.3 Total sugars from hemicellulose and cellulose at microwave power of 180 W using 5 wt% H_2SO_4

Time (min)	**Cellulose sugars (g/g)**	**Hemicellulose sugars (g/g)**
0	0.33	0.01
5	0.31	0
10	0.40	0.02
15	0.43	0.02
20	0.82	0.02

In these results high sugar yields from cellulose were observed at 180 W. The highest glucose and pentose yields were detected at 20 min. The literature also shows that H_2SO_4 is able to liberate more sugars from cellulose than hemicellulose. The results were validated by the use of SEM which indicated the disruption of the bagasse physical structure which led to cellulose exposure. FTIR spectra also indicated the disappearance of C-H stretching associated with cellulose peak after pretreatment (see Figure 4.8). The morphological changes in SSB were investigated by method of scanning electron microscope (SEM) images which were analysed using an FEI Quanta 250 FEG. Figure 4.7 shows the microstructure of untreated and microwave acid pretreated sweet sorghum bagasse.

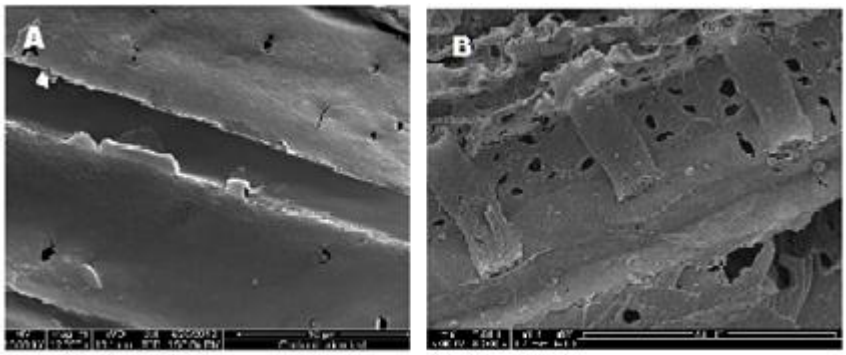

Figure 4.7 Scanning Electron Microscope images for untreated SSB (A) and microwave-acid pretreated SSB at 180 W (B)

The untreated bagasse (see Figure 4.7 A) showed a very smooth surface layer of the biomass. After the SSB had been pretreated in H_2SO_4 (see Figure 4.7 B) in a microwave at 180 W, the smooth surface disappeared and showed a physical disruption on the primary (containing cellulose) and secondary (containing lignin) cell wall of the bagasse. This indicated that the acid completely removed the fibres of the biomass, which are needed to obtain more sugars. The hollow areas allowed water to break down the inner parts of the cells. The round coiled structures in the pretreated bagasse are known as parenchyma cells, usually found in the secondary layer of the cell wall. Hence the primary cell wall was disrupted and the acid exposed the secondary layer of the bagasse. This proves that acid pretreatment is an effective method for disrupting the cell wall of the bagasse to release sugars.

The FTIR spectroscopy technique was done to investigate changes in the functional groups of the biomass before and after microwave pretreatment. The FTIR spectra shows distinctive characteristics of untreated (see Figure 4.8 A) and acid pretreated (see Figure 4.7 B) bagasse in the measured wavelength range of 4000 cm^{-1} to 500 cm^{-1}.

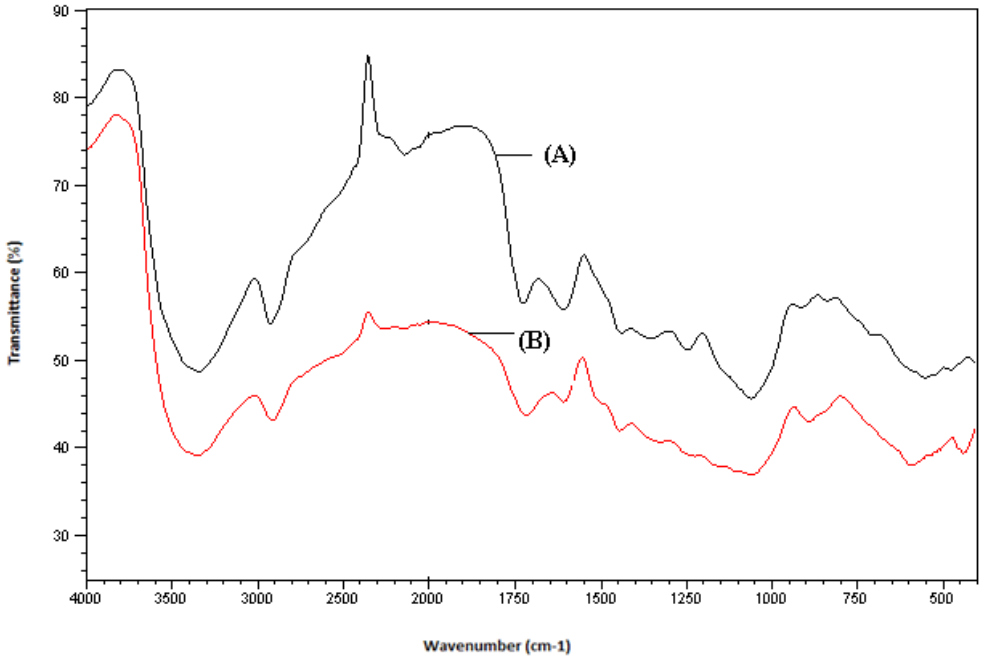

Figure 4.8 FTIR spectra for untreated SSB (A) and microwave-acid pretreated SSB (B) at 180 W

The broad absorption at 3350-3500cm^{-1} is the O-H stretching band of hydroxyl group associated with cellulose. Similar results were also observed by Binod *et al*. (2012). The bands at 2800-3000cm^{-1} showed the C-H stretching associated with lignin which decreased for acid pretreated bagasse. The peak at 2300-2500 is due to CH_2 stretching which is also sharp for the untreated bagasse and slightly disappears after alkali pretreatment. The broad peak from 1400-1500 cm^{-1} is associated with aromatic skeletal vibrations that were caused by microwave-alkali during pretreatment. The signals at 1050- 1150 are glycosidic (C-O-C) stretching associated with lignin vibrations during pretreatment. The peak observed between 800- 900cm^{-1} for alkali pretreated bagasse is a characteristic of carbohydrates. For untreated and microwave treated bagasse, peaks were similar; changes observed did not have a wide variety of peak formations. Although more

sugars were obtained during microwave-acid pretreatment, H_2SO_4 is not as aggressive as $Ca(OH)_2$.

4.3.1.4 Composition of microwave-acid pretreated sweet sorghum bagasse

After pretreatment the dried solid residue was analysed for compositional analysis at ARC, to quantify cellulose and hemicellulose from sweet sorghum bagasse after microwave-acid pretreatment (see Appendix D, Table D 21 and 22 for calculations and composition). Table 4.4 shows the composition of cellulose, hemicellulose and lignin after microwave-acid pretreatment of sweet sorghum bagasse

Table 4.4 Composition of cellulose, hemicellulose and lignin after microwave-acid pretreatment of sweet sorghum bagasse

Content	**Composition (%)**
Cellulose	35.75
Hemicellulose	11.92
ADL (Lignin)	6.66

A total cellulose content of 35.75% was obtained after microwave acid pretreatment, whereas, the total amount of hemicellulose was 11.92% in the pretreated bagasse. The amount of lignin was found to be 6.66% in the pretreated sweet sorghum bagasse.

4.3.2 Microwave-alkali pretreatment

Microwave pretreatment was conducted using different concentrations (1-7 wt%) of calcium hydroxide ($Ca(OH)_2$ at a fixed irradiation power (300 W). An optimal $Ca(OH)_2$ concentration of 3 wt% was used to pretreat sweet sorghum bagasse using different irradiation powers of 100-300 W.

4.3.2.1 Effect of dilute alkali concentrations on reducing sugar yields

The influence of calcium hydroxide $Ca(OH)_2$ concentrations (1,3,5 and 7 wt%) on the reducing sugar yield from pretreatment of SSB was studied at a 5 wt% biomass loading and 300 W microwave power. As shown in Figure 4.2, the release of sugars for all alkali concentrations increased with an increase in irradiation power. Sugar yields at 100 and 180 W were lower compared with sugars at 300 W pretreatment. However, an increase above 3 wt% $Ca(OH)_2$ concentration reduced the yield of sugars. This was due to high concentration of the alkali such that instead of sugars being released there are salts (e.g acetate) formed during $Ca(OH)_2$ pretreatment. The maximum yield of total sugars (0.27 g/g of SSB) was detected at 3% $Ca(OH)_2$, microwave setting of 300 W and 10 min residence time. This means a yield of 27% with respect to the cellulose and hemicellulose content of the untreated sweet sorghum bagasse was obtained. These results indicate that the addition of $Ca(OH)_2$ was effective in treating the SSB during microwave pretreatment, because it can break the recalcitrant structure of lignocellulose (see Figure 4.15) and promoted the degradation of macromolecules and carbohydrates. The control (water only, without addition of Ca $(OH)_2$) was also monitored at each time interval.

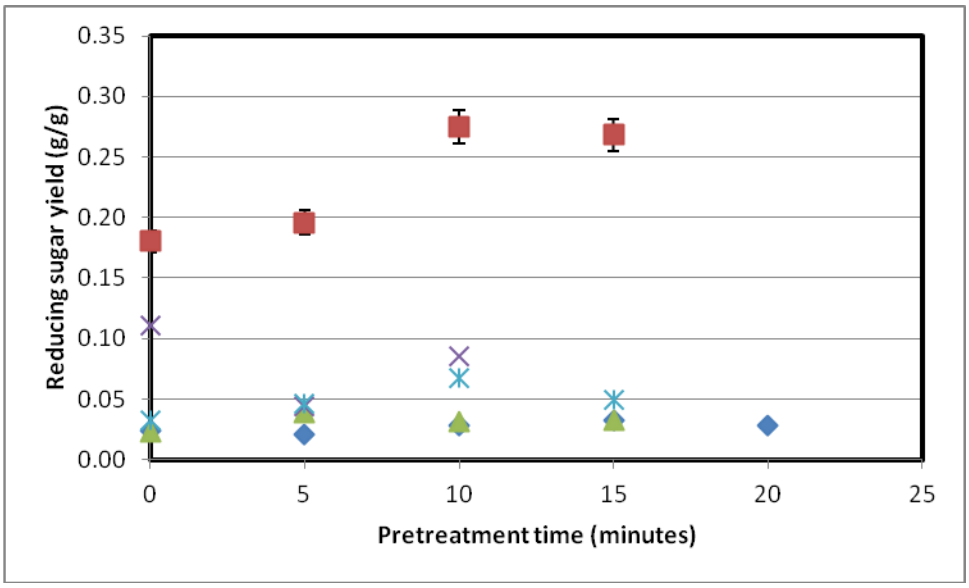

Figure 4.9 Total sugars yields (g sugar/g sweet sorghum bagasse) at different $Ca(OH)_2$ concentrations at 300 W (♦ -1% $Ca(OH)_2$, ■- 3% $Ca(OH)_2$, ▲- 5% $Ca(OH)_2$, × - 7% $Ca(OH)_2$, Ж- Control)

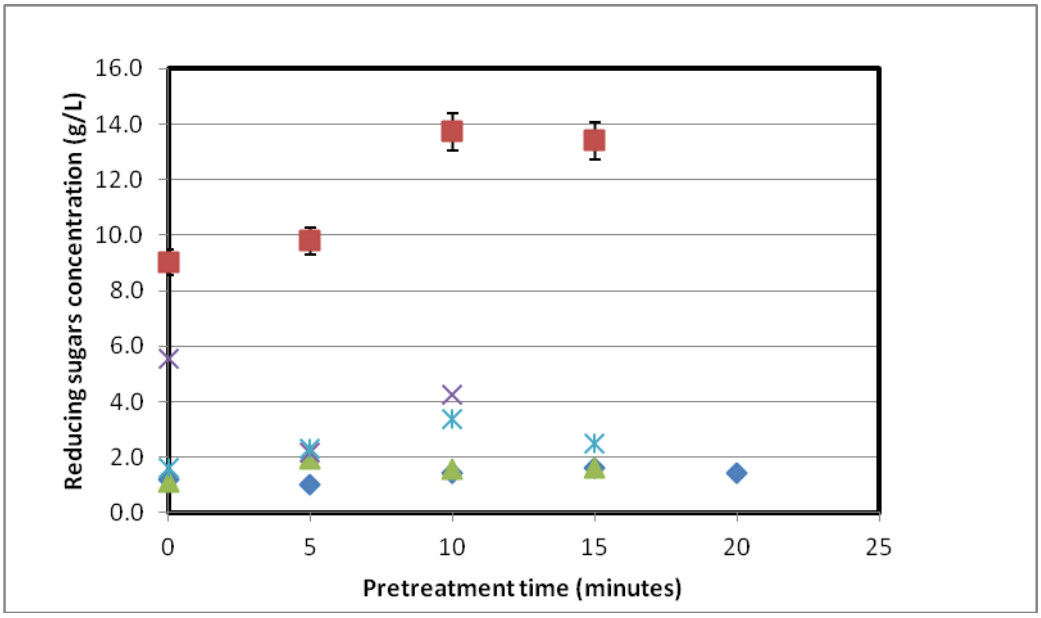

Figure 4.10 Total sugars concentration (g/L) of microwave assisted pretreatment of sweet sorghum bagasse in different concentrations of $Ca(OH)_2$ at 300 W (♦ -1% $Ca(OH)_2$, ■- 3% $Ca(OH)_2$, ▲- 5% $Ca(OH)_2$, × - 7% $Ca(OH)_2$, Ж- Control)

At 7 wt% concentration of Ca $(OH)_2$, pretreatment time had a negative impact since reducing sugars decreased with pretreatment time. Although it appeared that the total sugar yield was high at 0 minute for 7 wt%, this gives an explanation that high yields of sugars can be obtained from 7 wt% without exposing the bagasse to the microwave. The results showed that concentrations of $Ca(OH)_2$ higher than 5 wt% degraded the sugars in the bagasse. Concentrations of 1 wt%, 3 wt%, and the control had very low content of sugars than 3 wt% (0.27 g/g). This shows that 3 wt% increases the sugars up to 10 min. Based on the fact that alkaline can effectively remove the hemicellulose and lignin and small amount of cellulose (see figure 4.15), that could be the reason why the yields might be lower compared to acid conditions as described in Section 4.3.1.1.

Figure 4.11 (g/g) and 4.12 (g/L) show the highest yield obtained at 10 min for different alkali concentrations. The results only give the total sugar yield at one point of pretreatment time.

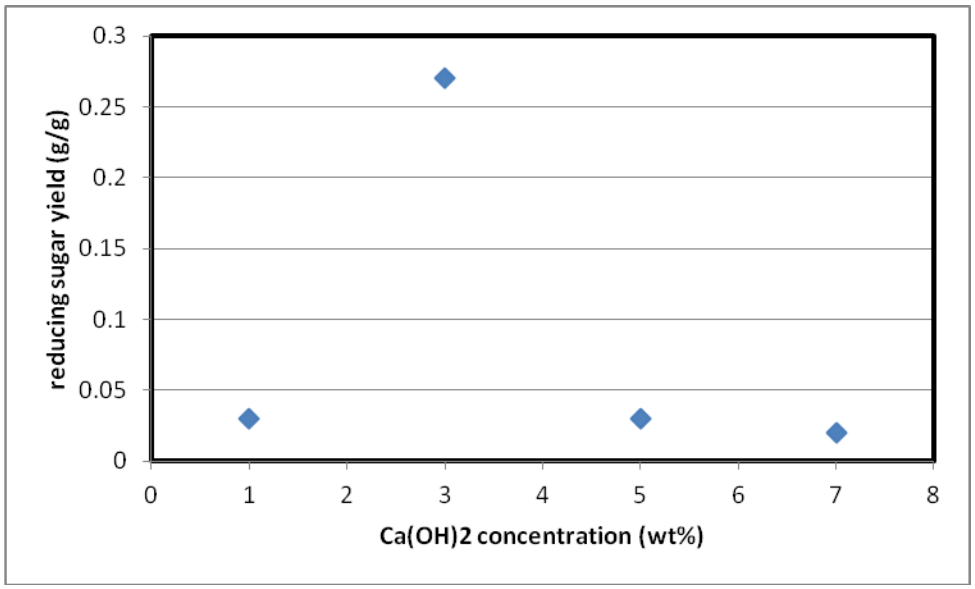

Figure 4.11 Total reducing sugar yield at 10 min microwave alkali-pretreatment time.

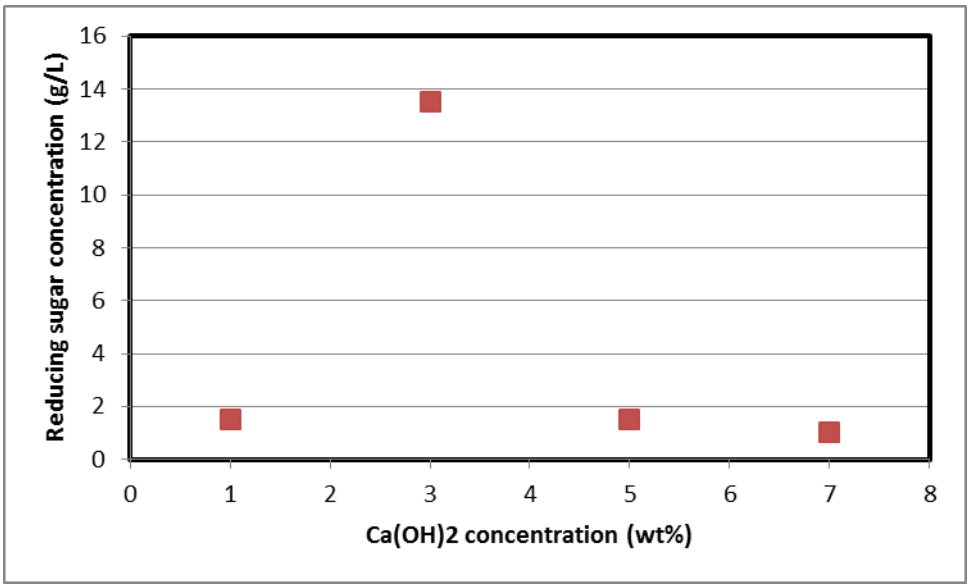

Figure 4.12 Total reducing sugar concentration at 10 min microwave-alkali pretreatment time.

The optimal concentration of 3 wt% $Ca(OH)_2$ was repeated three times and results are presented with percentage error bars in Figure 4.17. Sugar concentrations were very low for 1, 5, and 7 wt% at 10 minutes, the reason being that 1 wt% is a very low concentration of $Ca(OH)_2$ and unable to break the structure of the bagasse so as to release sugars. While 7 wt% was a very

concentrated solution, such that it degraded sugars with time, 5 wt% seemed not to have an effect on the bagasse since sugar contents were neither decreasing nor increasing.

4.3.2.2 Effect of irradiation power on total sugar yield from sweet sorghum bagasse in alkali

The effect of irradiation power on the reducing sugar yield in dilute alkali solution was investigated at power settings of 100 to 300 W at a concentration of 3 wt% $Ca(OH)_2$ and 10 min pretreatment time. As shown in Figure 4.13, the highest sugar yield (0.27 g/g of substrate) was obtained at 300 W.

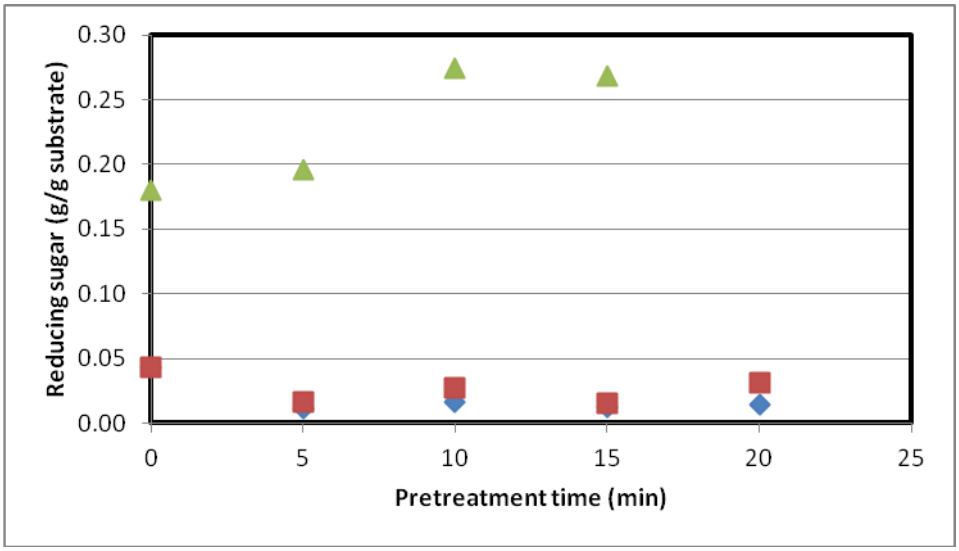

Figure 4.13 Effect of microwave irradiation power on total sugar yield (♦-100 W, ■- 180 W, ▲- 300 W)

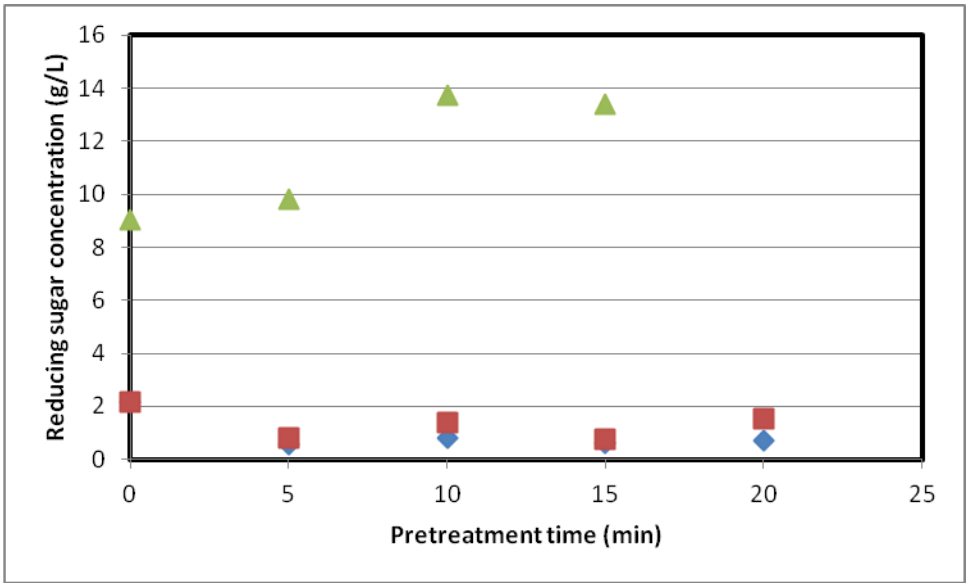

Figure 4.14 Effect of microwave irradiation power on total sugar concentration (♦-100 W, ■- 180 W, ▲- 300 W)

At low irradiation power settings (100 and 180 W) the total sugar yield was low and remained at constant level with an increase in pretreatment time, such that very low or no sugar yields were obtained. A lower sugar release can be attributed to temperatures too low to break the C-O-C bonds in the lignin-carbohydrate matrix. Interestingly, in the case of H_2SO_4 pretreatment under similar conditions the total sugar contents were much higher than with $Ca(OH)_2$ pretreatment (see Section 4.3.2). The reason for achieving higher release of sugars in H_2SO_4 is because hemicelluloses are easier to break down in acid pretreatment even at lower power intensities unlike under basic conditions (Wang *et al.*, 2010). Contrary to acid catalysed pretreatment, the alkaline catalysts have several limitations, such as being converted to irrecoverable salts or assimilated as salts into the biomass at low temperatures. This phenomenon has previously been reported when lime was used in the pretreatment of various biomass feedstocks (Mosier *et al.*, 2005).

4.3.2.3 Effect of 300 W irradiation power on sugars from cellulose and hemicellulose in alkali

In this section total sugars from cellulose and hemicellulose were studied separately (see Table 4.5). This was done to quantify attainable carbon-5 and carbon-6 sugars from hemicellulose and cellulose, respectively. The results are presented in Table 4.5 and show the effect of 300 W microwave irradiation power on total sugars from cellulose and hemicellulose for 3 wt% $Ca(OH)_2$ pretreated sweet sorghum bagasse at 300 W only.

Table 4.5 Total sugars from hemicellulose and cellulose at microwave power of 300 W using 3 wt% $Ca(OH)_2$

Time (minutes)	**Cellulose sugars (g/g)**	**Hemicellulose sugars (g/g)**
0	0.15	0.03
5	0.16	0.04
10	0.17	0.10
15	0.15	0.12
20 (ND)		

Note : ND=Not determined

Table 4.6 shows the production of total reducing sugars from cellulose and hemicellulose on pretreated SSB for 10 min at $Ca(OH)_2$ concentration of 3wt%. Sugar yields for hemicellulose increased with an increase in pretreatment time, while cellulosic sugar increased up to 10 min and decreased thereafter. This shows that $Ca(OH)_2$ is more effective in solubilising sugars from hemicellulose than H_2SO_4 since hemicellulosic sugars were very low in H_2SO_4 pretreatment. Due to salt formation during microwave-alkali pretreatment (see Figure 4.15), much lower sugar yields were obtained, than in microwave-acid pretreatment. These results are similar to the work

that was studied by Keshwani, (2009), where the highest sugar yield was obtained at 10 min pretreatment time for 3 wt% $Ca(OH)_2$.

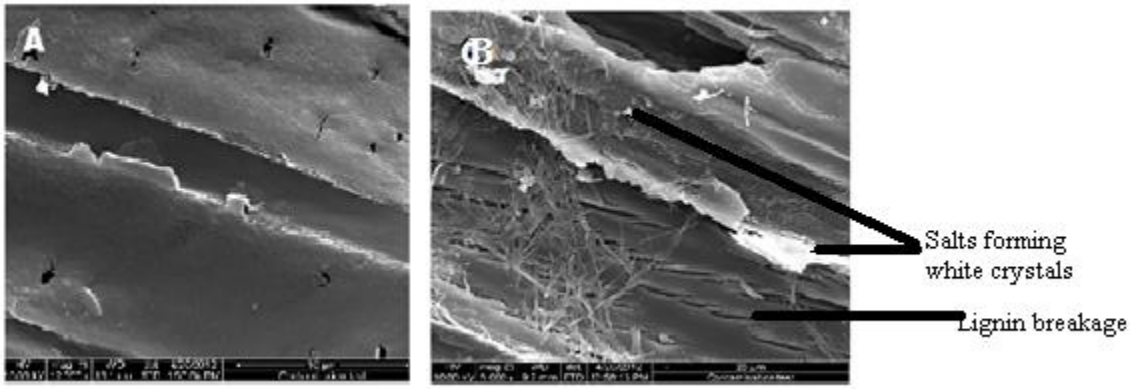

Figure 4.15 Scanning Electron Microscope (SEM) photos for untreated SSB (A) and microwave alkali pretreated SSB (B) at 300 W

Physical structural changes were observed for microwave-alkali pretreated bagasse (see Figure 4.15 B). Cellulose microfibrils containing sugars were exposed from the secondary layer of the lignocellulose biomass. Physical disruption of the rigid part (lignin) of the bagasse was observed; this led to release of sugars that were found in cellulose and hemicellulose. The results demonstrate the benefits of microwave-alkali pretreatment which facilitates the disruption of biomass and enhances the release of monomeric sugars and lignin precipitation.

A plausible explanation of lignin breakdown during microwave-alkali pretreatment is the ability of calcium ions to form linkages with the biomass structure via ionic interactions with negatively charged functional groups (see Figure 4.16) present under alkaline conditions (Xu *et al*., 2008). These interactions allow calcium ions to disrupt lignin in alkaline environments (Sundin and Hartler, 2000).

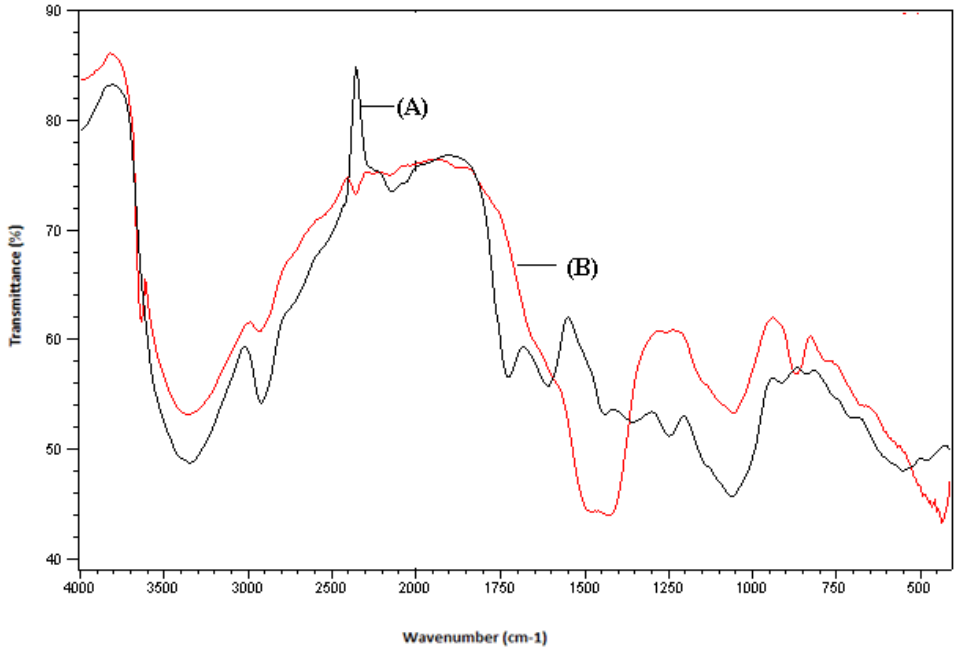

Figure 4.16 FTIR spectra of untreated SSB (A), microwave alkali pretreated SSB (B) at 300 W

The most representative bands can be summarised as follows: the broad absorption at 3350-3500cm^{-1}, which is the O-H stretching band of hydroxyl group associated with cellulose Li *et al*. (2012); Binod *et al*. (2012), was weak after microwave-alkali pretreatment (Figure 4.16 B). The bands at 2800-3000cm^{-1} show the C-H stretching bands associated with lignin, for the untreated bagasse, the stretching bands were clearly visible, because lignin was still intact before pretreatment, and after microwave-alkali the stretching band decreased, suggesting that pretreatment might have removed the lignin. The sharp peak at 2300-2500 cm^{-1} is due to CH_2 stretching which for the untreated bagasse and almost disappears after alkali pretreatment. The peak at 1500- 1700 cm^{-1} for the untreated bagasse is associated with hemicellulose, indicating that there was elimination of hemicellulose for alkali pretreatment, because the peak disappeared after microwave alkali pretreatment (Dergisi, 2011). The broad peak from 1400-1500 cm^{-1} is associated with aromatic skeletal vibrations of lignin degradation during alkali pretreatment. The peak is also more visible due to absorbed water molecules via hydrogen bonding in the amorphous region of cellulose macromolecules, that has been caused by alkali solution during microwave-alkali pretreatment (Abidi *et al*., 2007). The signals at 1050- 1150 cm^{-1} are glycosidic

(C-O-C) stretching bands. The peak observed between 800-900 cm^{-1} for alkali pretreated bagasse is a characteristic of carbohydrates. The removal of lignin during pretreatment is vital because it interferes with biological treatment such as enzyme hydrolysis and fermentation.

4.3.2.4 Composition of microwave-alkali pretreated sweet sorghum bagasse

After microwave-alkali pretreatment the dried solid residue was analysed for compositional analysis at ARC to determine the actual composition of cellulose and hemicellulose from alkali pretreated sweet sorghum bagasse (see Appendix D, Tables D 21 and 22 for calculations and composition). Table 4.6 shows the composition of cellulose, hemicellulose and lignin after microwave-alkali pretreatment.

Table 4.6 Cellulose, hemicellulose and lignin after microwave alkali pretreatment of sweet sorghum bagasse

Content	**Composition (%)**
Cellulose	39.87
Hemicellulose	12.83
ADL (Lignin)	6.19

A total cellulose composition of 39.87% was obtained after microwave-alkali pretreatment whereas, the total amount of hemicellulose was 12.83%. The amount of lignin was 6.19% for alkali pretreated sweet sorghum bagasse.

4.3.3. Comparison of H_2SO_4 and $Ca(OH)_2$ as pretreatment catalysts

Total sugar yields obtained through acid and alkali pretreatment of SSB with microwave were compared at different irradiation power settings (see Figure 4.17). The experimental error associated with this study was determined to be 3.9% at a 95% confidence level and indicated in Figure 4.17 with vertical error bars. Standard deviation calculations are presented in Appendix B.

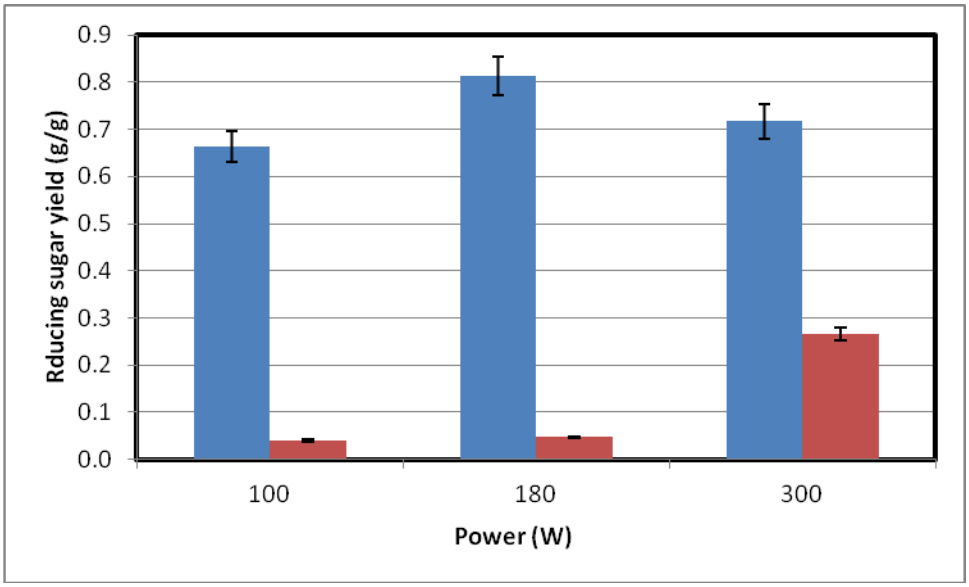

Figure 4.17 Effect of microwave irradiation power on reducing sugar yield (■- Microwave-acid pretreatment, ■- Microwave-alkali pretreatment).

From Figure 4.17 it can be seen that dilute acid consistently liberated more sugars than the alkali. The highest total sugar was 0.82 g/g with acid pretreatment at 180 W. The efficiency of microwave acid pretreatment was found to be 0.96, which of microwave alkali pretreatment was found to be 0.32. The high total sugar yields under acid conditions prove that acid is an effective catalyst for microwave pretreatment of sweet sorghum bagasse. The covalent bonds, hydrogen bonds and van der Waals forces in the biomass structure can easily release sugars without requiring higher temperatures for microwave-acid pretreatment; hence the highest yield at 180 W. The alkali acts by removal of lignin-carbohydrate bond which is much stronger and requires additional heat energy; hence the highest sugar yield for microwave-alkali pretreatment was found at 300 W.

4.4 Concluding remarks

This work showed that microwave assisted pretreatment substantially improved the recovery of fermentable sugars from sweet sorghum bagasse in a single step. Microwave irradiation in the presence of (H_2SO_4) and $Ca(OH)_2$ facilitated the disruption of the sweet sorghum bagasse structure to liberate monomeric sugars. Compared with conventional two-step pretreatment methods, microwave irradiation yields obtained here are extremely promising. For example, overall yields of 0.82 g/g dry biomass fermentable sugars were obtained at a microwave power setting of 180 W for 20 min pretreatment time in H_2SO_4. Whereas, with microwave-alkali ($Ca(OH)_2$), the reducing sugar yield was 0.27 g/g dry biomass at microwave power of 300 W with 10 min pretreatment time. The untreated and microwave pretreated sweet sorghum bagasse showed a physical difference in the disruption of the cell walls of microwave pretreated bagasse. Microwave-alkali pretreated bagasse was more damaged than the microwave-acid pretreated bagasse, hence it has been reported that an alkali solution is more effective in damaging the lignin of the bagasse. The FTIR spectra showed the stretching of hydrogen bonds of pretreated sweet sorghum bagasse arose at higher number which indicates the structural formation of new peaks and disappearance of lignin after microwave pretreatment.

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Chapter 5

FERMENTATION RESULTS

5.1 Introduction

This Chapter is intended to show results obtained during the biological conversion of sugars to bioethanol. Section 5.2 represents the results obtained during fermentation using *Z. mobilis* and *S.cerevisiae* to test the activity of each organism separately and when combined. Fermentation of mixed sugars from microwave-acid and alkali pretreated sweet sorghum bagasse is discussed in Section 5.3.

5.2 Optimisation of *S. cerevisiae* and *Z. mobilis*

Prior to fermentation of microwave-acid/alkali pretreated sweet sorghum bagasse each of the microorganisms was used to ferment glucose and xylose sugars contained in a sterilised broth to observe the time each organism took to efficiently convert the total sugars to bioethanol in 48 hrs before they were combined as mixed cultures. Media containing both xylose (20 g/L) and glucose (20 g/L) and other nutrients presented in Table 3.4 were prepared, thereafter *Z. mobilis* and *S. cerevisiae* were firstly used separately to ferment the sugars contained in the separate broths, and later combined in one broth. The Duran bottles were kept at 32^{o}C in a rotary shaker at120 rpm.

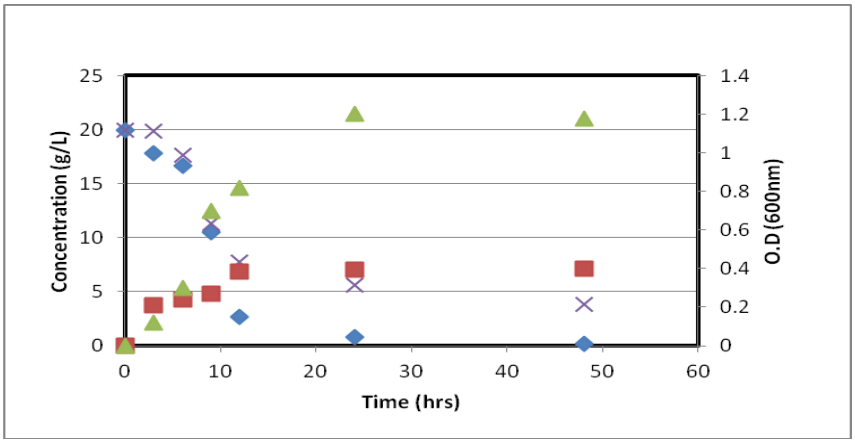

Figure 5.1 Fermentation curve of *Z. mobilis* on broth containing 20 g/L xylose and glucose (♦- Glucose, ×- Xylose, ■- Ethanol, ▲- O.D (600nm).

Figure 5.1 shows the growth curve for *Z.mobilis* as well as sugar uptake curves. The time between 3 to 24 hrs depicts the exponential phase. The maximum growth in the results was observed after 24 hrs of fermentation time, after which a stationery phase was observed (24- 48 hrs). While ethanol and cell growth were increasing, there was a decrease in xylose and glucose to 3.87 and 0.21g/L after 48 hrs, respectively. The results show a faster glucose uptake rate than the uptake rate of xylose. These results (Figure 5.1) also demonstrated that while the available glucose was completely depleted after only 24 hrs of fermentation time, there was still residual xylose of approximately 3.87 g/L even after 48 hrs of fermentation. Figure 5.2 shows the activity of *S. cerevisiae* to ferment glucose and xylose sugars in the broth.

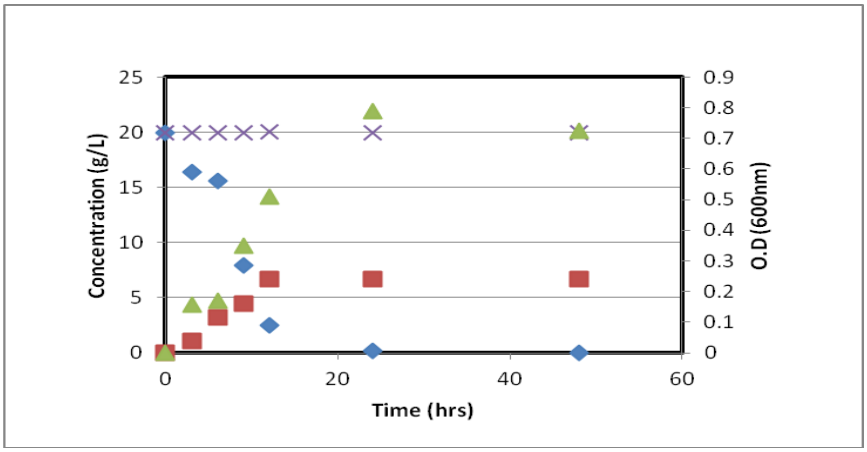

Figure 5.2 Fermentation curve of *S. cerevisiae* on broth containing 20 g/L xylose and glucose (♦- Glucose, ×- Xylose, ■- Ethanol, ▲- O.D (600nm).

Fermentation by *S.cerevisiae* is not effective for xylose sugar fermentation; hence the concentrration of xylose was constant throughout the fermentation process. This means that there was a need to use a strain that would be able to ferment both xylose and glucose. The concentration of ethanol produced during the course of fermentation of the broth by *Z. mobilis* was higher (7.15 g/L) than that produced by *S.cerevisiae* (6.7 g/L).

After fermentation of sugars to ethanol using the different microorganisms (*Z. mobilis* and *S.cerevisiae*) separately, a co-culture fermetation was done using a broth containing xylose and glucose sugars (see Figure 5.3).

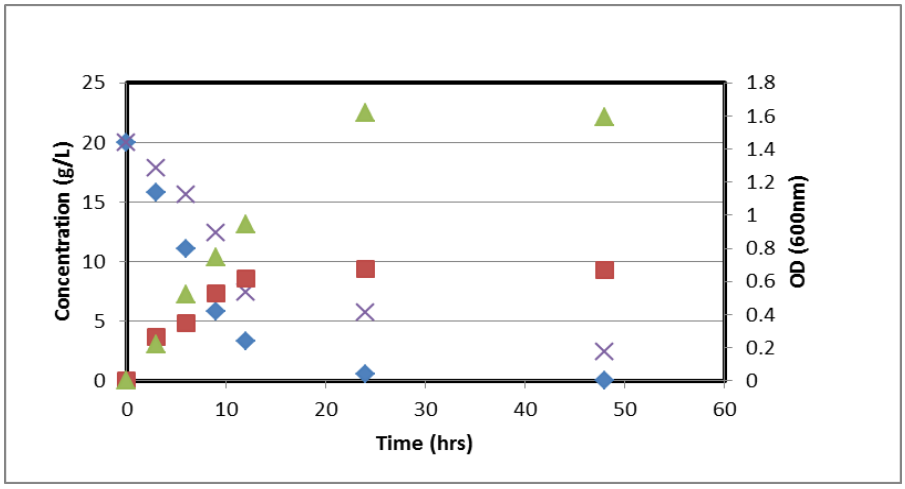

Figure 5.3 Fermentation curve of *S. cerevisiae* and *Z. mobilis* on broth containing 20 g/L xylose and glucose (♦-Glucose, ■- Xylose, ▲- Ethanol, ×-O.D (600nm).

A combination of the two microorganisms used in this study was also investigated. It was found that *S. cerevisiae* and *Z. mobilis* worked better when they were used in the same broth, since an even higher ethanol concentration of 9.3 g/L was obtained. Glucose was thoroughly fermented to ethanol while the xylose yield was very low after 48 hrs. The increase in ethanol concentration might be because *S. cerevisiae* and *Z.mobilis* were competing for the same source of substrate, this led to a very rapid conversition of sugars to ethanol.

5.3 Fermentation of microwave-pretreated sweet sorghum bagasse

The microbial co-cultures of *Z. mobilis* 31821 (xylose fermenting strain) and *S. cerevisiae* (yeast) were used for efficient bioconversion of carbon-5 sugars from hemicellulose and carbon-6 sugars from cellulose into bioethanol. Before the fermentation of hemicellulose acid and alkali hydrolysate, both the monocultures and varying ratios of 1:3, 3:5, 5:10% v/v of *Z. mobilis* to *S. cerevisiae* were grown on synthetic medium (presented in Chapter 3, Table 3.4).

The organisms were used as mixed cultures because of the results obtained during optimisation (see Section 5.2). Fu *et al*. (2009) and Amutha and Gunasekaran (2001) reported that the combination of *Z. mobilis* and *S. cerevisiae* worked better in obtaining higher bioethanol yields. Biological conversion of sugars to produce ethanol has been studied for many years. This discussion was focused mainly on the fermentation, which was done only for the optimum sugar yields that were obtained from pretreatment with different levels of microwave power

irradiation, and different concentrations of both yeast and *Z. mobilis* in each fermentation batch. In the entire fermentation batch, yeast concentration was higher than that of *Z. mobilis* due to the higher 6-carbon sugar concentration than the 5-carbon sugar concentration in the broth.

5.3.1 Results for mixed fermentation cultures of *S. cerevisiae* and *Z. mobilis*

After the pretreatment with diluted acid and dilute base of the sweet sorghum bagasse, the fermentation of the hydrolysates produced, was investigated at various ratios (3:1, 5:3, and 10:5% v/v) of *S. cerevisea* to *Z. mobilis*. The efficiency of the mixed fermentation cultures was optimised in this study and conversion was expressed in terms g bioethanol/g of sugars. The results in this Section show all the different combinations of varying amount of *S. cerevisiae* with those of *Z. mobilis* in batch fermentations.

5.3.1.1 Effect of 3% v/v of *S. cerevisiae* and 1% v/v of *Z. mobilis* on ethanol and sugar yield for microwave-acid pretreated bagasse at 180 W.

To find the optimum ratio of *S. cerevisiae* and *Z. mobilis* that would yield the highest amount of bioethanol, the aforementioned microorganisms were added to the media in the ratio 3:1, respectively, and fermentation was carried out for 24 hrs. Samples were collected at intervals of 3 hrs during fermentation, and ethanol, residual sugar concentration, and optical cell density were recorded for each time interval. The ethanol yield, residual sugars and cell growth for fermentation with 3% v/v of *S. cerevisiae* and 1% v/v of *Z. mobilis* is shown in Figure 5.4.

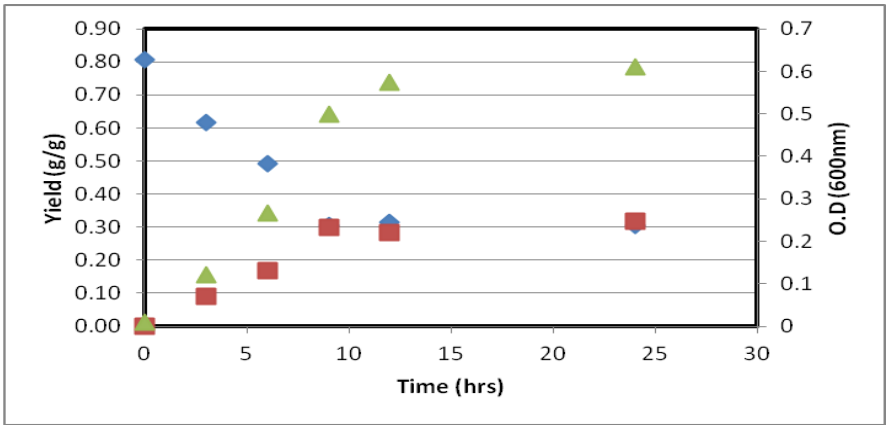

Figure 5.4 Effect of 3% v/v *S. cerevisiae* and 1% v/v *Z. mobilis* concentration on ethanol for sweet sorghum bagasse pretreated at 180 W using 5% H_2SO_4 (♦- Reducing sugars, ■- Ethanol, ▲- O.D (600nm).

The concentration of initial sugar (0.82 g/g) decreased rapidly to 0.31 g/g while producing the highest amount of ethanol (0.32 g/g) within 24 hrs. The fermentation process was incomplete since there was no complete conversion of the sugar substrate to ethanol. This was mainly caused by the short fermentation time which made it very difficult for all the sugars to be assimilated by *S. cerevisiae* and *Z. mobilis* to ethanol within 24 hrs of fermentation. Cells were also growing with shorter lag phase. Cell growth is an indicator that ethanol was still being produced after 24 hrs. It was reported that both *S. cerevisiae* and *Z. mobilis* worked best at pH 4.8 and a temperature between 30-32^{o}C; hence these conditions were applied in the study during fermentation.

5.3.1.2 Effect of 10% v/v of *S. cerevisiae* and 5% v/v of *Z. mobilis* on ethanol and sugar yield for microwave-acid pretreated bagasse at 180 W.

The concentration of *S. cerevisiae* and *Z .mobilis* were increased to 10 and 5% v/v, respectively, in this fermentation batch. Environmental conditions such as pH, temperature and time were kept constant. This was done to observe the effectiveness and the time consumed by the organisms when they were added in large quantities to the fermentation batch. The results in Figure 4.9 also show the effect of 10% v/v of *S. cerevisiae* and 5% v/v of *Z. mobilis* on ethanol and sugar yield for microwave-acid pretreated bagasse at 180 W.

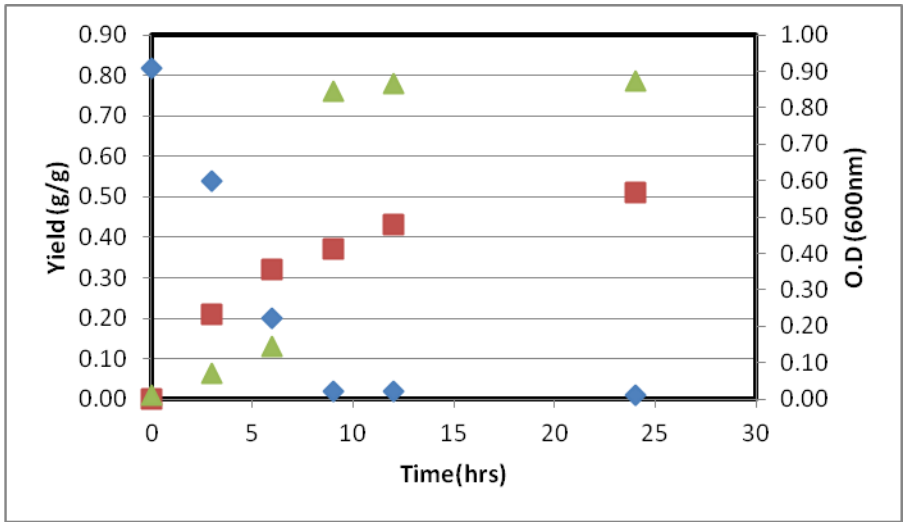

Figure 5.5 Effect of 10% v/v *S. cerevisiae* and 5% v/v *Z. mobilis* concentration on ethanol for sweet sorghum bagasse pretreated at 180 W using acid (♦- Reducing sugars, ■- Ethanol, ▲- O.D (600nm).

Figure 5.5 shows a rapid increase of ethanol and a decrease in reducing sugars, with an increase in cell growth as expected. The initial reducing sugar concentration was 0.82 g/g, and was completely converted to ethanol in just 12 hrs. On the other hand, an overall ethanol yield (0.50 g/g) after 24 hrs was obtained and it was closer to the theoretical yield of 0.51 g/g. Cell growth was even higher during this fermentation batch. The higher ethanol yields in this study led to the conclusion that 10:5 ratio of *Z. mobilis* to *S. cerevisiae* was very effective in converting all reducing sugars to ethanol.

5.3.1.3 Effect of 3% v/v of *S. cerevisiae* and 1% v/v of *Z. mobilis* on ethanol and sugar yield for microwave-alkali pretreated bagasse at 300 W.

Hydrolysate from SSB pretreated at 300 W was chosen for fermentation since the irradiation power had given the highest sugar yield during microwave pretreatment. This sugar solution was exposed to different concentrations of *S. cerevisiae* and *Z. mobilis* for 24 hours as previously discussed in Section 5.3.1 for microwave-acid pretreated bagasse. As described previously, 3% v/v of *S. cerevisiae* and 1%v/v of *Z. mobilis* were added in order to observe their effect of mixed fermentation culture on ethanol yields and sugar utilisation. (see Figure 5.6).

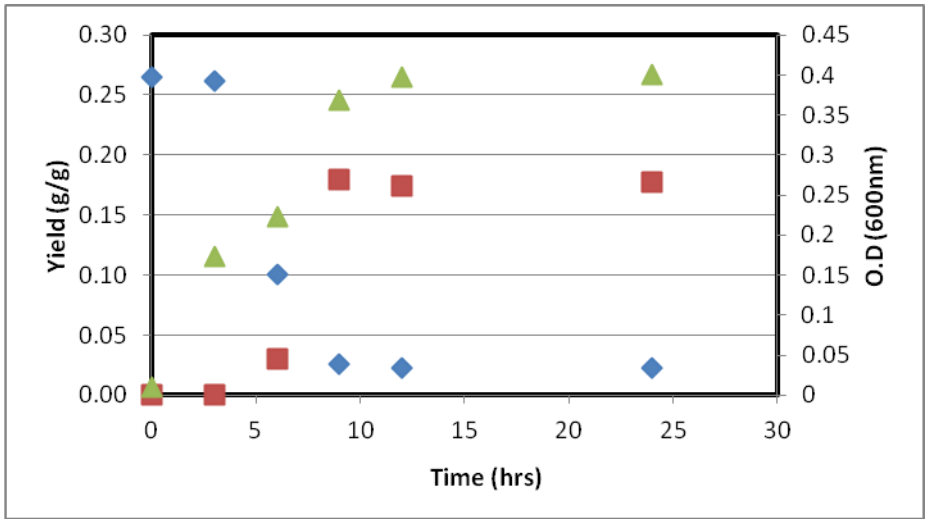

Figure 5.6 Effect of 3% v/v *S.s cerevisiae* and 1% v/v *Z. mobilis* concentration on ethanol for sweet sorghum bagasse pretreated at 300 W using 3% $Ca(OH)_2$ (♦- Reducing sugars, ■- Ethanol, ▲- O.D (600nm).

A similar trend as for microwave-acid pretreated bagasse was observed during the fermentation of hydrolysate from microwave-alkali pretreatment. The initial reducing sugar concentration was 0.27 g/g, and a gradual drop was observed after 3 hrs. Ethanol was produced after 6 hrs. This was due to a low concentration of *S. cerevisiae* and *Z. mobilis* which led to slow ethanol production. A maximum ethanol yield (0.18 g/g) was produced after 24 hrs of fermentation, despite the fact that not all sugars were converted to ethanol. However, the low yields of ethanol in this experiment were expected, since the hydrolysate had a low total sugar concentration during alkaline pretreatment. Another possible explanation is the formation of inhibitors during fermentation, which may have inhibited ethanol production by forming acetic acid instead of ethanol.

5.3.1.4 Effect of 10% v/v of *S. cerevisiae* and 5% v/v of *Z. mobilis* on ethanol and sugar yield for microwave-alkali pretreated bagasse at 300 W.

In this fermentation, maximum concentrations of 10% v/v of *S. cerevisiae* and 5% v/v of *Z. mobilis* were used to observe: ethanol production, utilisation of the total sugars and growth of cells (see Figure 5.7). The fermentation conditions were kept constant at 32^{o}C, pH 4.8 at 120 rpm. The aim of this experiment was to determine the effect of higher *S. cerevisiae* and *Z. mobilis* in the fermentation broth.

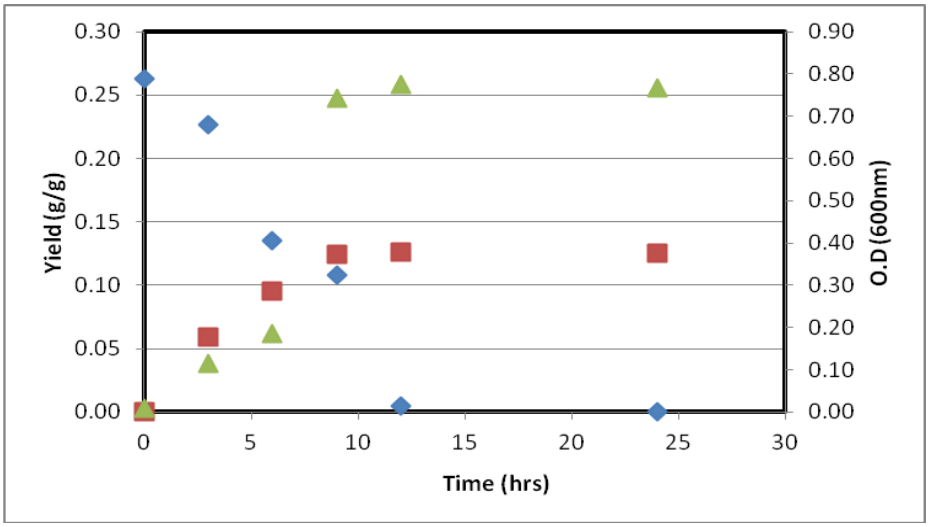

Figure 5.7 Effect of 10% v/v *S. cerevisiae* and 5 % v/v *Z.mobilis* concentration on ethanol for sweet sorghum bagasse pretreated at 300 W using 3% Ca $(OH)_2$ (♦- Reducing sugars, ■- Ethanol, ▲- O.D (600nm).

Figure 5.7 shows the increase in ethanol, while sugars were depleted. The highest yield of ethanol produced was 0.13 g/g with initial sugar of 0.27 g/g. Surprisingly, the ethanol yield was lower compared with that produced when using low concentrations of microorganism loading. Lu *et al*., 2011; Sasaki *et al*., 2011, confirmed that during fermentation, a high concentration of yeast may cause alkali pretreated bagasse to form acetic acid which usually interferes with the production of ethanol, thus leading to less ethanol produced. This means that low ethanol yields may be caused by high concentrations of by-products that might have been formed during fermentation of microwave-alkali pretreated bagasse.

5.3.1.5 Effect of ethanol concentrations on microwave-acid and alkali pretreated SSB

Table 5.1 shows ethanol yield for sweet sorghum bagasse that was pretreated using dilute H_2SO_4 (5 wt%) at 180 W and bagasse that was pretreated with dilute alkali ($Ca(OH)_2$ (3 wt%) at 300 W.

Table 5.1 Ethanol yield for microwave-acid and alkali pretreated SSB at different concentrations of *S. cerevisiae* and *Z. mobilis*

***Z. mobilis* to *S. cerevisiae* ratio (%v/v)**	**Acid pretreatment at 180 W (g/g)**	**Alkali pretreatment at 300 W (g/g)**
1:3	0.32	0.18
3:5	0.33	0.23
5:10	0.50	0.13

Table 5.1 shows the optimal bioethanol yield of 0.50 g/g at 5:10% v/v of *Z. mobilis* to *S. cerevisiae* ratio, this was because of high total reducing sugar yield that was obtained for acid pretreated bagasse. High sugar yields may lead to high ethanol yield provided the appropriate amount of the fermenting strain is added. Microwave-alkali pretreated bagasse gave an optimal ethanol concentration of 0. 23 g/g at 3:5% v/v of *Z. mobilis* to *S. cerevisiae* ratio.

5.4 Concluding remarks

This work showed that microwave-assisted pretreatment substantially improved the recovery of fermentable sugars from sweet sorghum bagasse in a single step. Fermentation was conducted only for optimal sugars obtained from microwave pretreatment with different microwave powers. The concentration of *S. cerevisiae* and *Z. mobilis* was varied to observe the acceleration of ethanol production within 24 hours of fermentation. The study revealed the highest ethanol yield of 0.5 g/g from sweet sorghum bagasse that was pretreated using microwave-acid pretreatment, with concentration of 10% v/v *S. cerevisiae* and 5% v/v *Z. mobilis* during fermentation. An ethanol yield of approximately 0.18 g/g of substrate was obtained from sweet sorghum bagasse pretreated using microwave-alkali pretreatment, with concentration of 10% v/v *S. cerevisiae* and 5% v/v *Z. mobilis* during fermentation. *Zymomonas mobilis* showed a potential in fermenting xylose sugars that were mainly obtained during microwave-alkali pretreatment. The results also show that *S. cerevisiae* and *Z. mobilis* are a good combination for producing ethanol from sweet sorghum bagasse. This also proves that sweet sorghum bagasse is a very good feedstock for producing bioethanol in South Africa that could have an impact in integrated food and energy systems.

5.5 References

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Chapter 6

CONCLUSIONS

6.1 Overview

The key results found in this study and recommendations for future research are summarised in this Chapter. The effect of time, acid, and alkali concentrations showed a good evidence of increase in reducing sugar yield. Lignocellulosic materials are viewed as promising alternative feedstocks for bioethanol production. However, the recalcitrant nature of these materials requires a pretreatment step to improve the yield of fermentable sugars. The overall goal of this dissertation was to evaluate the conditions and change in acid or alkali concentrations for the microwave-based pretreatment of sweet sorghum bagasse. The microwave pretreated sweet sorghum bagasse was evaluated and optimum conditions for fermentation were identified.

6.2 Conclusions

- The optimum reducing sugar yield for microwave pretreatment using dilute sulphuric acid was found to be 0.82 g/g of biomass at 180 W irradiation powers with 15 min pretreatment time.
- With microwave-alkali (calcium hydroxide) pretreatment an overall sugar yield of 0.27 g/g of biomass was obtained at 300 W irradiation powers with 10 min pretreatment time.
- SEM analysis showed structural changes and biomass disruption after microwave pretreatment for both acid and alkali.
- The FTIR spectra showed the stretching of hydrogen bonds of pretreated sweet sorghum bagasse and also indicated structural changes after microwave treatment.
- The use of calcium hydroxide pretreatment resulted in the production of higher xylose yields than sulphuric acid pretreatment.

- An ethanol yield of 0.5 and 0.32 g/g was achieved during fermentation of sweet sorghum bagasse using 5:10 and 1:3% v/v of *Z. mobilis* to *S. cerevisiae*, respectively for acid pretreated bagasse.
- An ethanol yield of 0.13 and 0.18 g/g was achieved during fermentation of sweet sorghum bagasse using 5:10 and 1:3% v/v of *Z. mobilis* to *S. cerevisiae*, respectively for alkali pretreated bagasse.
- The results obtained from this study gave an indication that sweet sorghum bagasse can be used for bioethanol production through microwave-based pretreatment and fermentation with mixed cultures.

6.3 Recommendations

The present study showed the feasibility of microwave-based pretreatment of lignocellulosic biomass to improve sugar yields for bioethanol production. However, the research was conducted using a domestic microwave oven. Therefore, the next step is to verify the effectiveness of microwave pretreatment using laboratory scale continuous microwave system. This will be of much help in identifying the appropriate parameters that will be effective in the microwave-based pretreatment process. It is also necessary to evaluate the effect of temperature during microwave pretreatment. During fermentation, it is necessary to do cell counts, rather than measuring the optical density only. This will help to know the exact amount of initial cells before fermentation and cells left after fermentation.

Appendix A

STANDARD CALIBRATION CURVES

A1 Introduction

In order to use high performance liquid chromatography for analysis, calibration curves for all the sugars and ethanol to be identified during the study had to be constructed. Standards of pure sugars and ethanol were prepared by combining a certain mass of sugars with distilled water.

A2 HPLC Sugar Analysis

The concentration of each sugar and ethanol (g/L) was determined by high performance liquid chromatography. The standardized sugars were sucrose, fructose, glucose, galactose, mannose, arabinose, and xylose. The sugar samples were serially diluted and the concentrations were: 24, 12,6,3,1.5, and 0.75g/L for each of the sugars. After the HPLC had been done, a straight line was fitted to the data and a constant (k) was obtained which was used in the calculation of the sugar and ethanol yields.

Table A1 Retention times for sugars and ethanol analysed by HPLC

Sugars and ethanol	Retention times (min)
Sucrose	10.9- 11.1
Fructose	17.9- 22-4
Glucose	12.4-12.5
Galactose	14.2-14.3
Mannose	18.3- 20.7
Arabinose	16.1-16.5
Xylose	13.3-13.4
Ethanol	11.4-12.1

Peaks for sugar calibration standards were also recorded form the HPLC results (see Table A2)

Table A.2: Peak areas obtained for each sugar concentration using HPLC

Concentration (g/L)	Sucrose	Fructose	Xylose	Glucose	Arabinose	Galactose	Mannose
1.5	4.55E+04	6.79E+04	5.06E+04	3.83E+04	2.95E+04	3.71E+04	3.44E+04
3	7.76E+04	9.90E+04	7.95E+04	-	5.16E+04	6.79E+04	1.57E+05
6	1.67E+05	1.61E+05	1.81E+05	1.90E+05	1.44E+05	1.38E+05	7.51E+04
12	3.50E+05	3.14E+05	1.80E+05	3.69E+05	3.12E+05	3.05E+05	3.59E+05
24	7.05E+05	6.84E+05	5.08E+05	7.54E+05	5.95E+05	6.59E+05	7.02E+05

Table A3 Peak areas obtained for ethanol concentration using HPLC

Concentration (g/L)	Ethanol area
1.23	9.04E+03
2.47	1.91E+04
4.93	4.18E+04
9.86	8.63E+04
19.73	1.85E+05
39.45	3.53E+05
78.9	7.29E+05

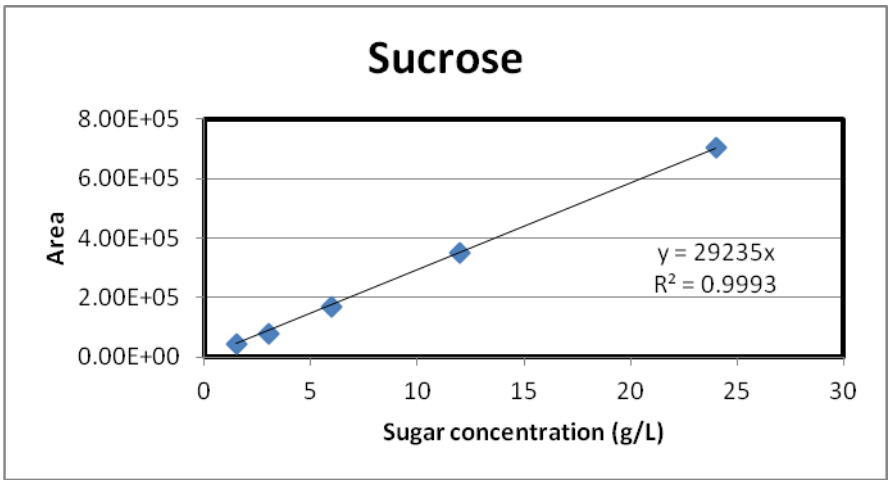

Figure A1 Sucrose calibration curve

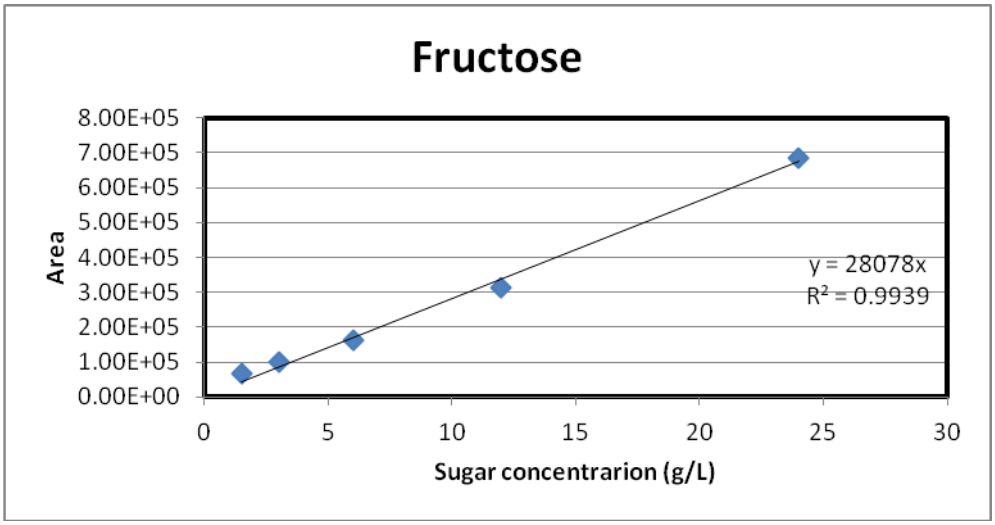

Figure A2 Fructose calibration curve

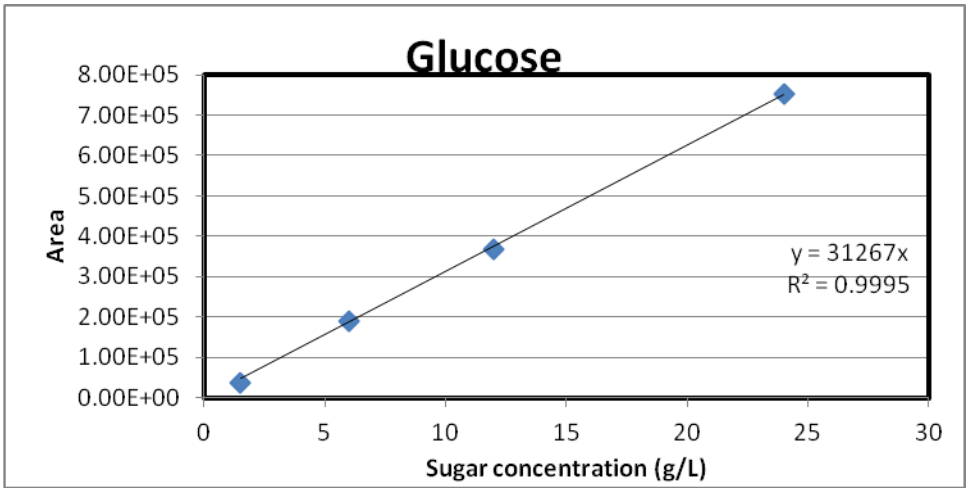

Figure A3 Glucose calibration curve

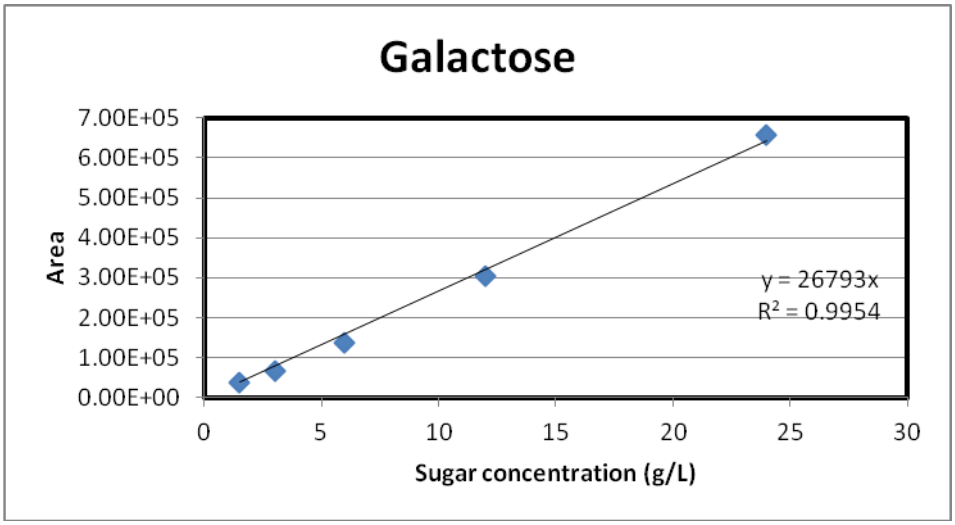

Figure A4 Galactose calibration curve

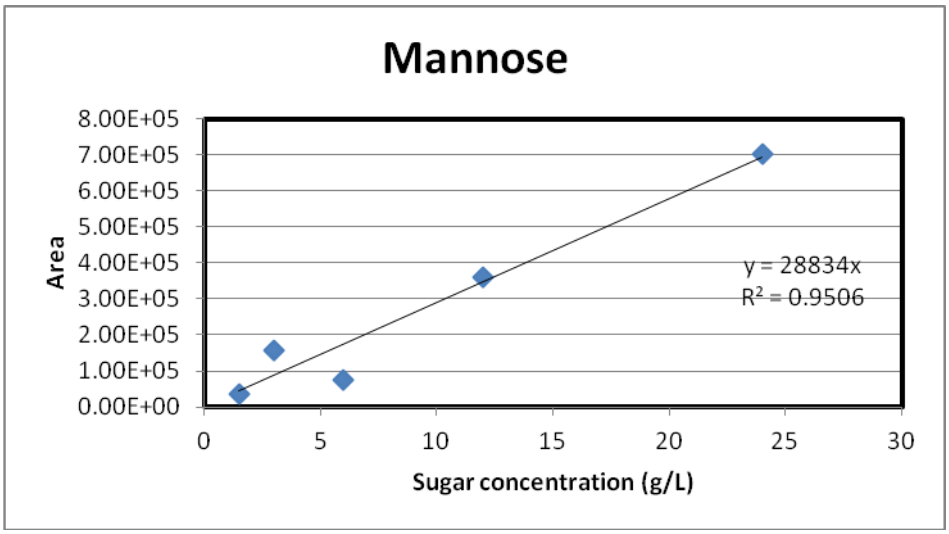

Figure A5 Mannose calibration curve

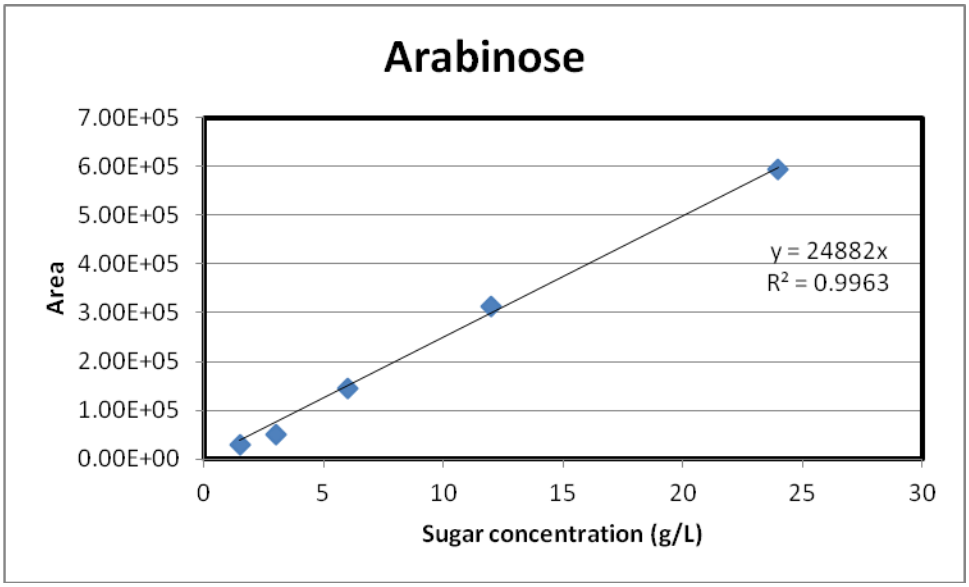

Figure A6 Arabinose calibration curve

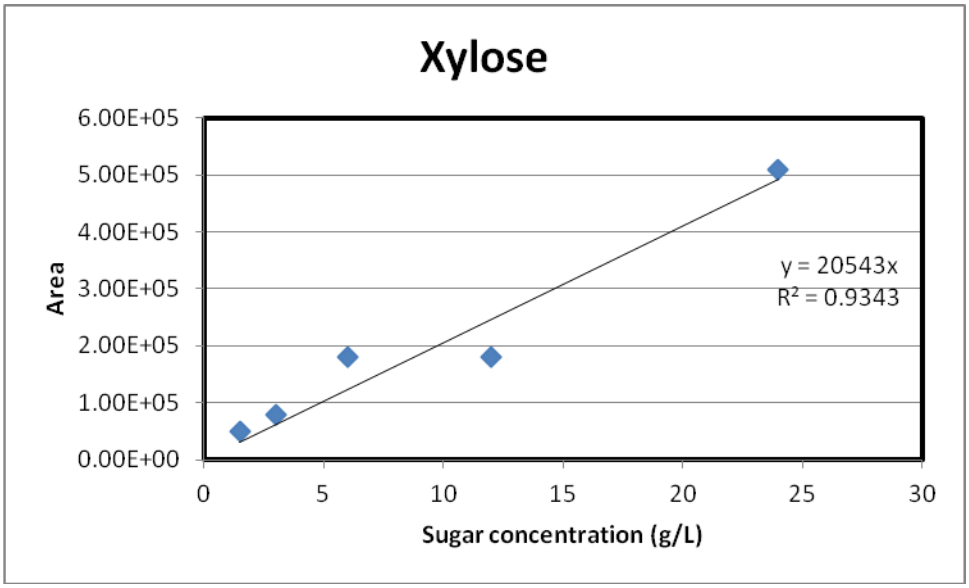

Figure A7 Xylose calibration curve

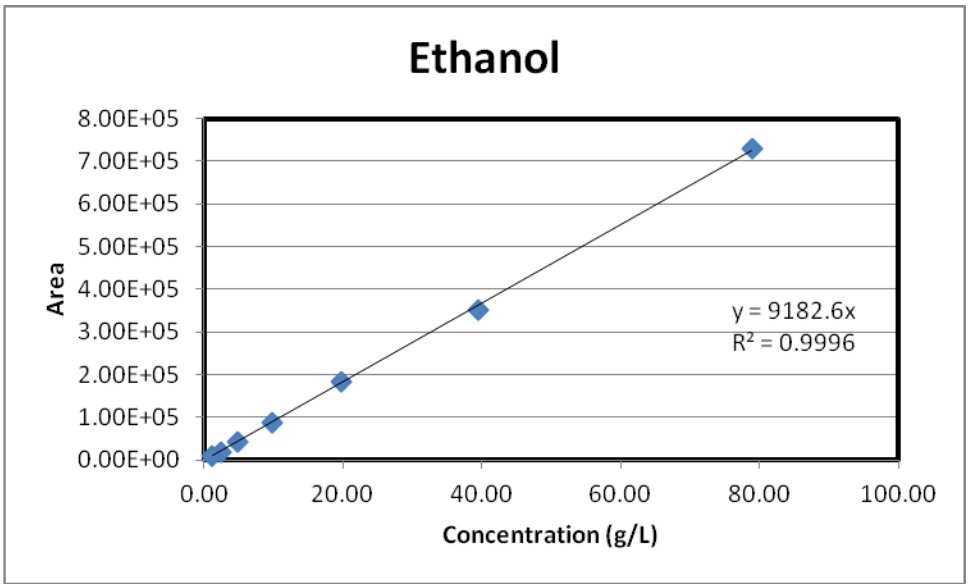

Figure A8 Ethanol calibration curve

Appendix B

EXPERIMENTAL ERROR AND CALCULATIONS

B1 Introduction

These Sections give the experimental errors associated with the parameters investigated in this study. Section B2 shows the calculation of experimental error calculations, while Section B3 gives the experimental error obtained for each parameter investigated.

B2 Experimental error calculations

The experimental error was calculated and determined using Equation B.1

(Mutepe, 2011)

$$\% \text{ Error} = \frac{\text{Confidence limit}}{\bar{x}} * 100 \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \text{B. 1}$$

Where x is the average

Confidence limit is calculated using the following equation:

$$\overline{x} \pm 1.96\left(\frac{\sigma}{\sqrt{n}}\right) \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\

B3 Calculations for sugar concentrations in the biomass

Cs $_{(g/L)}$ = (Area of sugar/Slope of sugar calibration)* dilution factor……..**1**

Cs was used in equation 2

Cs $_{(g/g)}$ = Cs * total volume (L)/ biomass weight……..**2**

Where:

Cs- Concentration of sugars

The same Equations were also used for ethanol calculations

B4 Experimental error calculations

Table B 1 Statistical analysis used to calculate experimental error for the effect of sugar yield for microwave- acid pretreated bagasse at 100 W

	Reducing sugar yield (g/g)
Experiment 1	0.66
Experiment 2	0.68
Experiment 3	0.65
Average	0.66
Standard deviation ($x^{\cdot}$**)**	0.02
Confidence limit (σ)	0.02
Error %	2.30

Table B 2 Statistical analysis used to calculate experimental error for the effect of sugar yield for microwave-acid pretreated bagasse at 180 W

	Reducing sugar yield (g/g)
Experiment 1	0.81
Experiment 2	0.82
Experiment 3	0.82
Average	0.82
Standard deviation ($\bar{x}$)	0.01
Confidence limit (σ)	0.01
Error %	0.71

Table B 3 Statistical analysis used to calculate experimental error for the effect of sugar yield for microwave-acid pretreated bagasse at 300 W

	Reducing sugar yield (g/g)
Experiment 1	0.70
Experiment 2	0.70
Experiment 3	0.75
Average	0.72
Standard deviation ($\bar{x}$)	0.03
Confidence limit (σ)	0.03
Error %	4.03

Table B 4 Statistical analysis used to calculate experimental error for the effect of sugar yield for microwave- alkali pretreated bagasse at 100 W

	Reducing sugar yield (g/g)
Experiment 1	0.04
Experiment 2	0.04
Experiment 3	0.04
Average	0.04
Standard deviation ($\bar{x}$)	0
Confidence limit (σ)	0
Error %	0

Table B 5 Statistical analysis used to calculate experimental error for the effect of sugar yield for microwave-alkali pretreated bagasse at 100 W

	Reducing sugar yield (g/g)
Experiment 1	0.04
Experiment 2	0.04
Experiment 3	0.04
Average	0.04
Standard deviation ($\bar{x}$)	0
Confidence limit (σ)	0
Error %	0

Table B 6 Statistical analysis used to calculate experimental error for the effect of sugar yield for microwave- alkali pretreated bagasse at 180W

	Reducing sugar yield (g/g)
Experiment 1	0.05
Experiment 2	0.05
Experiment 3	0.05
Average	0.05
Standard deviation ($x^{\cdot}$**)**	0.01
Confidence limit (σ)	0.01
Error %	0

Table B 6 Statistical analysis used to calculate experimental error for the effect of sugar yield for microwave- alkali pretreated bagasse at 300 W

	Reducing sugar yield (g/g)
Experiment 1	0.26
Experiment 2	0.28
Experiment 3	0.26
Average	0.27
Standard deviation ($x^{\cdot}$**)**	0.01
Confidence limit (σ)	0.01
Error %	4.33

Appendix C

MICROWAVE PRETREATMENT EXPERIMENTAL DATA

C1 Introduction

This section covers the experimental data for microwave pretreatment and fermentation. The uptake of different sugars during the parameters investigated is also given. Table C1 shows the sugar codes that were used to shows the presence of each sugar during microwave pretreatment and fermentation

Table C1 Sugar codes and k-values used for determination of each sugar present in the sample

Sugar codes	**Sugars**	**K-value**
x2	Fructose	280780
x3	Sucrose	292349
x4	Glucose	312666
x5	Xylose	205434
x6	Galactose	267929
x7	Arabinose	248818
x8	Mannose	288342

C2 Sugar yield for microwave-acid pretreated bagasse with different acid concentrations

Table C2 Yield and area for different sugars obtained during pretreatment with 1% sulphuric acid at 300 W at different time intervals

Time (min)	Sugar codes	Area	Sugar (g/g)
0	x2	1.21E+04	0.01
	x3	9.37E+04	0.06
	x4	2.57E+04	0.02
	x5	1.50E+04	0.01
	x8	5.48E+03	0.00
5	x2	2.63E+04	0.02
	x3	9.12E+04	0.06
	x4	3.43E+04	0.02
	x5	1.98E+04	0.02
	x8	5.26E+02	0.00
10	x2	4.54E+04	0.03
	x3	9.71E+04	0.07
	x4	4.73E+04	0.03
	x5	1.80E+04	0.02
	x8	2.34E+02	0.00
15	x2	2.35E+04	0.02
	x3	9.41E+04	0.06
	x4	2.21E+04	0.01
	x5	5.85E+03	0.01
	x8	2.33E+02	0.00
20	x2	5.14E+04	0.04
	x4	2.46E+04	0.02
	x5	3.75E+04	0.04
	x8	1.41E+04	0.01

Table C3 Yield and area for different sugars obtained during pretreatment with 3% sulphuric acid at 300 W at different time intervals

Time (min)	Sugar codes	Area	sugar (g/g)
0	x2	1.26E+04	0.01
	x3	1.84E+04	0.01
	x4	9.28E+03	0.01
	x5	2.12E+03	0.00
	x8	4.67E+03	0.00
5	x2	1.97E+04	0.01
	x3	2.38E+03	0.00
	x4	1.11E+05	0.07
	x5	1.23E+04	0.01
	x8	5.87E+03	0.00
10	x2	2.39E+04	0.02
	x4	1.34E+05	0.09
	x5	1.38E+04	0.01
	x8	6.96E+03	0.00
15	x2	2.76E+04	0.02
	x4	1.68E+05	0.11
	x5	1.36E+04	0.01
	x8	8.32E+03	0.01
20	x2	4.42E+04	0.03
	x4	1.37E+05	0.09
	x5	1.33E+04	0.01
	x8	1.51E+03	0.00

Table C4 Yield and area for different sugars obtained during pretreatment with 5% sulphuric acid at 300 W at different time intervals

Time (min)	Sugar codes	Area	Sugar (g/g)
0	x2	1.84E+04	0.01
	x4	4.95E+05	0.32
	x8	2.05E+04	0.01
5	x2	2.73E+04	0.02
	x4	5.35E+05	0.34
	x8	2.19E+04	0.02
10	x2	4.74E+04	0.03
	x4	5.36E+05	0.34
	x8	2.59E+04	0.02
15	x2	5.24E+04	0.04
	x4	1.07E+06	0.68
	x8	3.91E+04	0.03
20	Not determined		

Table C5 Yield and area for different sugars obtained during pretreatment with 7% sulphuric acid at 300 W at different time intervals

Time	Sugar codes	Area	Sugar (g/g)
0	x2	3.57E+04	0.03
	x4	8.87E+05	0.57
	x8	9.37E+03	0.01
5	x2	4.46E+04	0.03
	x4	8.10E+05	0.52
	x8	3.14E+00	0.00
10	x2	2.68E+04	0.02
	x4	8.12E+05	0.52
	x8	8.75E+03	0.01
15	x2	3.19E+04	0.02
	x4	3.44E+05	0.22
	x8	2.00E+04	0.01
20	Not determined		

C3 Sugar yield for microwave-acid pretreated bagasse with different alkali concentrations

Table C6 Yield and area for different sugars obtained during pretreatment with 1% calcium Hydroxide at 300 W at different time intervals

Time (min)	Sugar codes	Area	Sugar (g/g)
0	x2	3.65E+03	0.00
	x3	1.33E+04	0.01
	x4	1.28E+04	0.01
	x5	2.70E+03	0.00
	x8	1.80E+03	0.00
5	x2	1.16E+03	0.00
	x3	1.47E+04	0.01
	x4	1.37E+04	0.01
	x5	5.83E+02	0.00
	x8	4.49E+02	0.00
10	x2	1.55E+03	0.00
	x3	2.21E+04	0.02
	x4	1.75E+04	0.01
	x5	4.79E+02	0.00
	x8	1.21E+03	0.00
15	x2	4.21E+03	0.00
	x3	2.29E+04	0.02
	x4	1.80E+04	0.01
	x5	1.62E+03	0.00
	x8	7.98E+02	0.00
20	x2	3.55E+03	0.00
	x3	2.83E+04	0.02
	x4	4.14E+03	0.00
	x5	3.11E+03	0.00
	x8	1.05E+03	0.00

Table C7 Yield and area for different sugars obtained during pretreatment with 3% calcium Hydroxide at 300 W at different time intervals

Time (min)	Sugar codes	Area	sugar (g/g)
0	x3	2.61E+05	0.18
	x5	6.53E+02	0.00
	x8	1.29E+03	0.00
5	x2	6.60E+03	0.00
	x3	4.41E+04	0.03
	x4	2.45E+05	0.16
	x5	3.70E+03	0.00
	x8	1.27E+03	0.00
10	x2	7.66E+03	0.01
	x4	4.17E+05	0.27
	x5	1.54E+03	0.00
	x8	1.52E+03	0.00
15	x2	1.94E+04	0.01
	x4	3.96E+05	0.25
	x5	1.10E+03	0.00
	x8	5.36E+02	0.00
20	Not determined		

Table C8 Yield and area for different sugars obtained during pretreatment with 5% calcium Hydroxide at 300 W at different time intervals

Time (min)	Sugar codes	Area	Sugar (g/g)
0	x2	1.47E+04	0.01
	x3	6.97E+03	0.00
	x4	3.74E+03	0.00
	x8	7.04E+03	0.00
5	x2	1.16E+04	0.01
	x3	1.97E+03	0.00
	x4	3.77E+04	0.02
	x8	7.48E+03	0.01
10	x2	6.14E+03	0.00
	x3	1.58E+03	0.00
	x4	3.80E+04	0.02
	x8	2.98E+03	0.00
15	x2	7.70E+03	0.01
	x4	3.68E+04	0.02
	x8	4.98E+03	0.00
20	Not determined		

Table C9 Yield and area for different sugars obtained during pretreatment with 7% calcium Hydroxide at 300 W at different time intervals

Time (min)	Sugar codes	Area	Sugar (g/g)
0	x2	2.18E+04	0.02
	x4	1.47E+04	0.01
	x8	1.24E+05	0.09
5	x2	1.97E+03	0.00
	x4	1.82E+04	0.01
	x8	4.44E+04	0.03
10	x2	3.36E+04	0.02
	x4	2.20E+04	0.01
	x8	6.79E+04	0.05
15	Not determined		
20	Not determined		

C4 Sugar yield for microwave-acid pretreated bagasse without acid/alkali concentration

Table C10 Yield and area for different sugars obtained during microwave pretreatment without a catalyst at 300 W

Time (min)	Sugar	Area	sugar (g/g)
0	x2	2.15E+04	0.02
	x3	1.58E+04	0.01
	x4	8.22E+03	0.01
	x8	1.03E+03	0.00
5	x2	2.35E+04	0.02
	x3	2.58E+04	0.02
	x4	1.55E+04	0.01
	x8	2.78E+03	0.00
10	x2	4.83E+04	0.03
	x3	2.69E+04	0.02
	x4	2.18E+04	0.01
	x8	1.17E+03	0.00
15	x2	1.52E+04	0.01
	x3	3.03E+04	0.02
	x4	2.60E+04	0.02
	x8	2.25E+03	0.00
20	Not determined		

C5 Sugar yield for microwave-acid pretreated bagasse without 5% sulphuric acid at different microwave powers

Table C11 Yield and area for different sugars obtained during microwave pretreatment with 5% sulphuric acid at 100 W

Time (min)	Sugar codes	Area	Sugar (g/g)
0	x2	1.84E+04	0.01
	x4	4.95E+05	0.32
	x8	2.05E+04	0.01
5	x2	1.22E+05	0.09
	x4	6.31E+05	0.40
	x8	1.36E+04	0.01
10	x2	1.76E+05	0.13
	x4	6.22E+05	0.40
	x8	1.52E+04	0.01
15	x2	1.62E+05	0.12
	x4	7.41E+05	0.47
	x8	1.19E+04	0.01
20	x2	1.55E+05	0.11
	x4	8.32E+05	0.53
	x8	1.25E+04	0.01

Table C12 Yield and area for different sugars obtained during microwave pretreatment with 5% sulphuric acid at 180 W

Time (min)	Sugar codes	Area	Sugar (g/g)
0	x2	1.84E+04	0.01
	x4	4.95E+05	0.32
	x8	2.05E+04	0.01
5	x2	9.62E+04	0.07
	x4	3.80E+05	0.24
	x8	6330.671	0.00
10	x2	4.48E+04	0.03
	x4	5.81E+05	0.37
	x8	2.47E+04	0.02
15	x2	1.13E+05	0.08
	x4	5.51E+05	0.35
	x8	1.14E+04	0.01
20	x2	2.44E+04	0.02
	x4	1.24E+06	0.79
	x8	3.22E+04	0.02

C6 Sugar yield for microwave-alkali pretreated bagasse without 5% sulphuric acid at different irradiation powers

Table C13 Yield and area for different sugars obtained during microwave pretreatment with 3% calcium Hydroxide at 100 W

Time (min)	Sugar codes	Area	Sugar (g/g)
0	x2	2.17E+04	0.02
	x4	4.26E+04	0.03
	x8	1.28E+03	0.00
5	x2	2.29E+03	0.00
	x4	1.32E+04	0.01
	x8	2.30E+03	0.00
10	x2	1.54E+03	0.00
	x4	1.96E+04	0.01
	x8	3.16E+03	0.00
15	x2	3.09E+03	0.00
	x4	1.39E+04	0.01
	x8	1.94E+03	0.00
20	x2	2.94E+03	0.00
	x4	1.76E+04	0.01
	x8	1.28E+03	0.00

Table C14 Yield and area for different sugars obtained during microwave pretreatment with 3% calcium Hydroxide at 180 W

Time (min)	Sugar codes	Area	Sugar (g/g)
0	x2	2.17E+04	0.02
	x4	4.26E+04	0.03
	x8	1.28E+03	0.00
5	x2	3.55E+03	0.00
	x4	1.92E+04	0.01
	x8	1.67E+03	0.00
10	x2	9.49E+03	0.01
	x4	2.47E+04	0.02
	x8	7.21E+03	0.00
15	x2	9.91E+02	0.00
	x4	2.19E+04	0.01
	x8	1.18E+03	0.00
20	x2	9.13E+03	0.01
	x4	3.64E+04	0.02
	x8	1.37E+03	0.00

Appendix D

FERMENTATION EXPERIMENTAL DATA

D1 Introduction

This section deals with data obtained during fermentation with different concentrations of *S. cerevisiae* and *Z. mobilis* for microwave pretreated sweet sorghum bagasse. D4 presents data of converted hemicellulose, cellulose and lignin that was found after microwave acid and alkali pretreatment.

D2 Effect of 3% *S. cerevisiae* and 1% *Z. mobilis* on different sugar and ethanol, and cell growth during fermentation for bagasse pretreated at different microwave powers.

Table D1 Effect of 3% *S. cerevisiae* and 1% *Z. mobilis* on different sugars and ethanol, and cell growth during fermentation for bagasse pretreated with 5% sulphuric acid at 100 W

Ferm. time	Sugar codes	Area	Sugar (g/g)
0h	x2	1.14E+05	0.08
	x4	8.78E+05	0.56
	x8	2.89E+04	0.02
3h	x1	1.25E+03	0.03
	x4	7.60E+05	0.49
	x2	5.46E+04	0.04
	x8	1.59E+04	0.01
6h	x1	9.34E+03	0.20
	x4	6.95E+05	0.44
9h	x1	1.21E+04	0.26
	x4	6.78E+05	0.43
12h	x1	1.28E+04	0.28
	x4	4.91E+05	0.31
24h	x1	1.30E+04	0.28
	x4	3.70E+05	0.24

Where X1= Ethanol

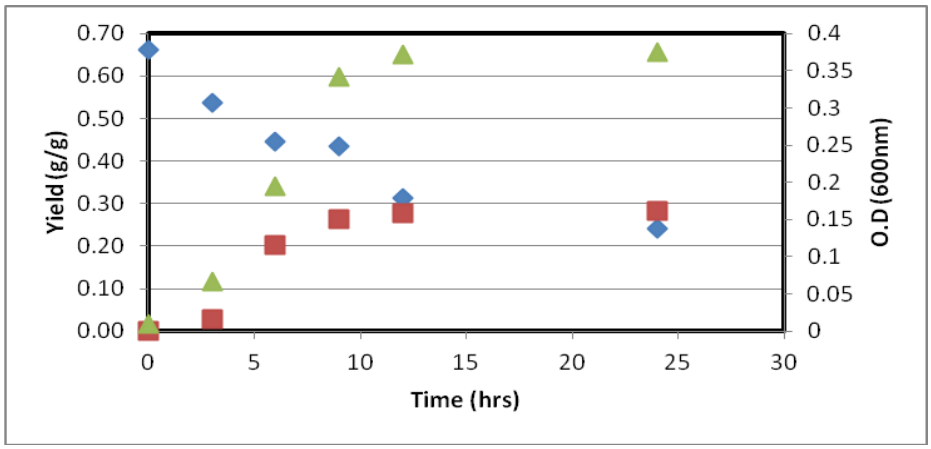

Figure D1 Effect of 3% *S. cerevisiae* and 1% *Z. mobilis* on different sugar and ethanol, and cell growth during fermentation for bagasse pretreated with 5% sulphuric acid at 100 W

(♦- reducing sugars, ■- Ethanol, ▲- O.D (600nm).

Table D2 Effect of 3% *S. cerevisiae* and 1% *Z. mobilis* on different sugars and ethanol, and cell growth during fermentation for bagasse pretreated with 5% sulphuric acid at 180 W

Ferm time	Sugar codes	Area	Sugar (g/g)
0h	x2	2.20E+05	0.16
	x4	1.00E+06	0.64
	x8	1.39E+04	0.01
3h	x1	4.22E+03	0.09
	x4	9.35E+05	0.60
	x2	2.46E+04	0.02
	x8	9.97E+01	0.00
6h	x1	7.67E+03	0.17
	x4	7.68E+05	0.49
9h	x1	1.37E+04	0.30
	x4	4.79E+05	0.31
12h	x1	1.30E+04	0.28
	x4	4.91E+05	0.31
24h	x1	1.45E+04	0.32
	x4	4.80E+05	0.31

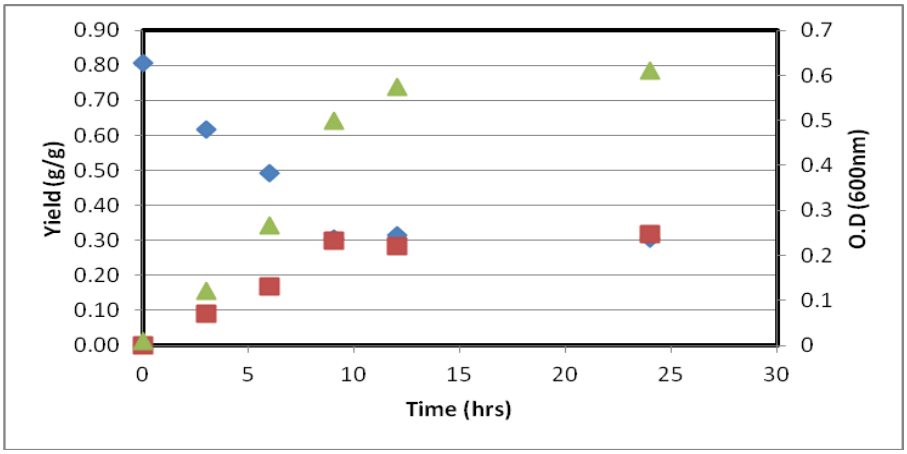

Figure D2 Effect of 3% *S. cerevisiae* and 1% *Z. mobilis* on different sugar and ethanol, and cell growth during fermentation for bagasse pretreated with 5% sulphuric acid at 180 W

(♦- reducing sugars, ■- Ethanol, ▲- O.D (600nm).

Table D3 Effect of 3% *S. cerevisiae* and 1% *Z. mobilis* on different sugars and ethanol, and cell growth during fermentation for bagasse pretreated with 5% sulphuric acid at 300 W

Ferm time	Sugar codes	Area	Sugar (g/g)
0h	x2	8.22E+04	0.06
	x4	9.96E+05	0.64
	x8	1.32E+04	0.01
3h	x1	4.71E+03	0.10
	x4	8.71E+05	0.56
	x2	2.32E+04	0.02
	x8	1.00E+03	0.00
6h	x1	8.11E+03	0.18
	x4	6.99E+05	0.45
9h	x1	1.29E+04	0.28
	x4	5.67E+05	0.36
12h	x1	1.30E+04	0.28
	x4	4.91E+05	0.31
24h	x1	1.22E+04	0.27
	x4	4.90E+05	0.31

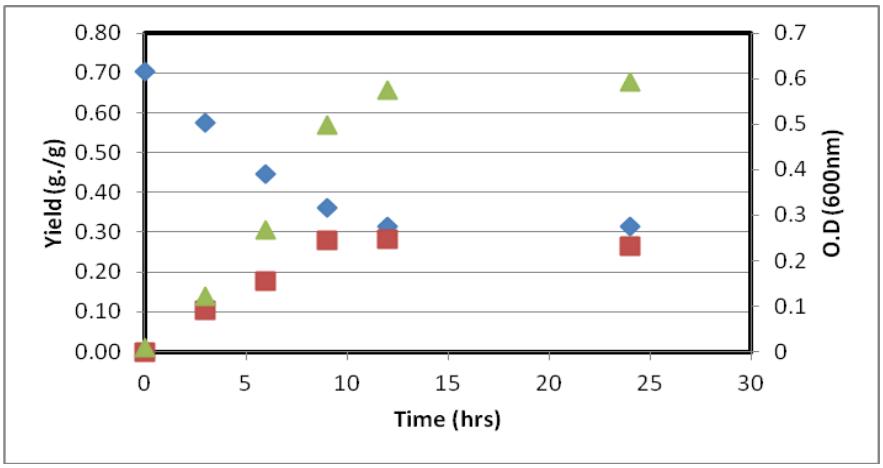

Figure D3 Effect of 3% *S.cerevisiae* and 1% *Z. mobilis* on different sugar and ethanol, and cell growth during fermentation for bagasse pretreated with 5% sulphuric acid at 300 W

(♦- reducing sugars, ■- Ethanol, ▲- O.D (600nm).

Table D4 Effect of 3% *S. cerevisiae* and 1% *Z. mobilis* on different sugars and ethanol, and cell growth during fermentation for bagasse pretreated with 3% calcium hydroxide at 100 W

Ferm time	Sugar codes	Area	Sugar (g/g)
0h	x2	1.85E+04	0.01
	x4	4.31E+04	0.03
	x8	2.29E+03	0.00
3h	x1	1.12E+03	0.02
	x4	4.10E+04	0.03
	x2	1.51E+02	0.00
	x8	2.26E+01	0.00
6h	x1	1.13E+03	0.02
	x4	3.42E+04	0.02
9h	x1	1.11E+03	0.02
	x4	1.25E+04	0.01
12h	x1	1.14E+03	0.02
	x4	1.27E+04	0.01
24h	x1	1.13E+03	0.02
	x4	1.31E+04	0.01

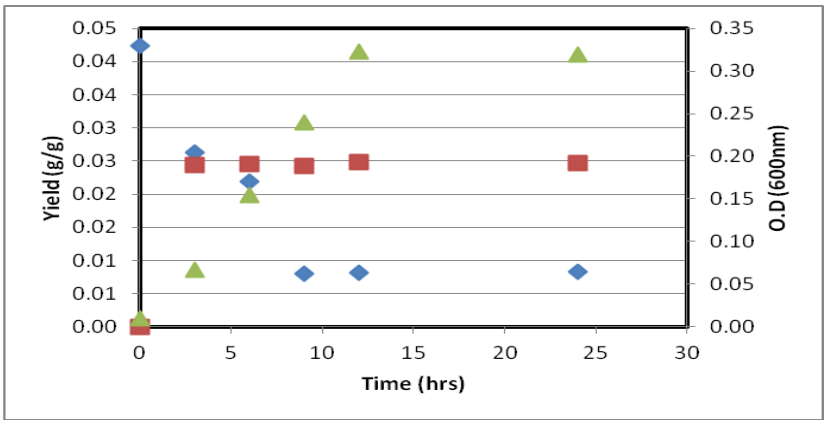

Figure D4 Effect of 3% *S. cerevisiae* and 1% *Z. mobilis* on different sugar and ethanol, and cell growth during fermentation for bagasse pretreated with 3% calcium hydroxide at 100 W (♦- reducing sugars, ■- Ethanol, ▲- O.D (600nm).

Table D5 Effect of 3% *S. cerevisiae* and 1% *Z. mobilis* on different sugars and ethanol, and cell growth during fermentation for bagasse pretreated with 3% calcium hydroxide at 300 W.

Ferm time	Sugar codes	Area	Sugar (g/g)
0h	x2	5.69E+04	0.04
	x4	3.50E+05	0.22
	x8	4.85E+02	0.00
3h	x1	1.70E+02	0.00
	x4	3.42E+05	0.22
	x2	5.99E+04	0.04
	x8	4.76E+02	0.00
6h	x1	1.41E+03	0.03
	x4	1.58E+05	0.10
9h	x1	8.24E+03	0.18
	x4	3.98E+04	0.03
12h	x1	7.99E+03	0.17
	x4	3.57E+04	0.02
24h	x1	8.13E+03	0.18
	x4	3.60E+04	0.02

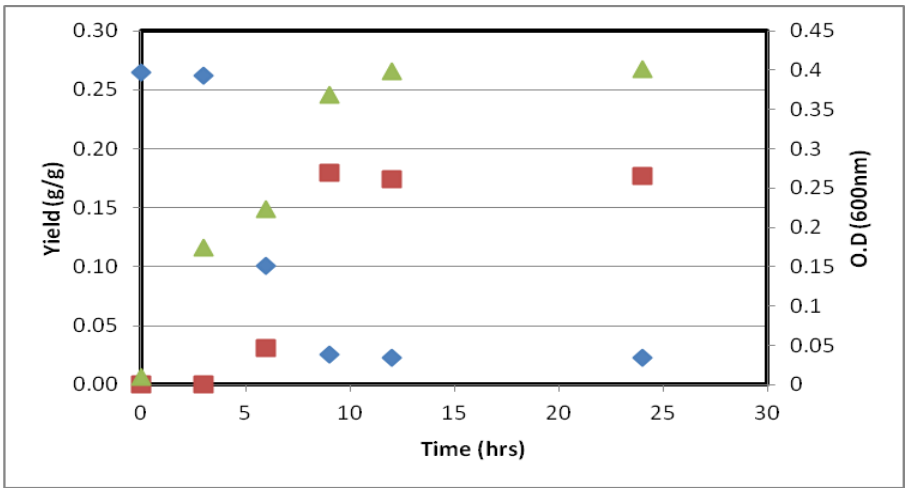

Figure D5 Effect of 3% *S.cerevisiae* and 1% *Z. mobilis* on different sugars and ethanol, and cell growth during fermentation for bagasse pretreated with 3% Calcium hydroxide at 300 W (♦- reducing sugars, ■- Ethanol, ▲- O.D (600nm).

D3 Effect of 5% *S. cerevisiae* and 3% *Z. mobilis* on different sugar and ethanol, and cell growth during fermentation for bagasse pretreated at different microwave powers.

Table D6 Effect of 5% *S. cerevisiae* and 3% *Z. mobilis* on different sugars and ethanol, and cell growth during fermentation for bagasse pretreated with 5% sulphuric acid at 100 W

Ferm time	Sugar codes	Area	Sugar (g/g)
0h	x2	1.65E+05	0.12
	x4	8.49E+05	0.54
	x8	2.76E+04	0.02
3h	x1	1.14E+03	0.02
	x4	7.52E+05	0.48
	x2	6.54E+04	0.05
	x8	2.35E+04	0.02
6h	x1	8.47E+03	0.18
	x4	5.62E+05	0.36
9h	x1	1.07E+04	0.23
	x4	4.97E+05	0.32
12h	x1	1.35E+04	0.29
	x4	3.78E+05	0.24
24h	x1	1.66E+04	0.36
	x4	2.51E+05	0.16

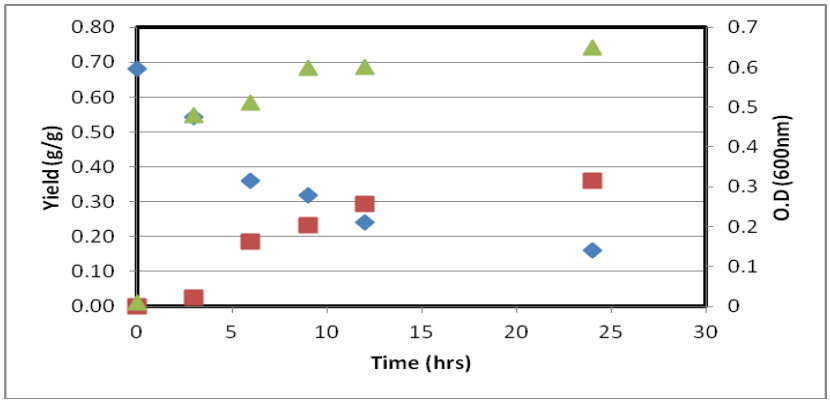

Figure D6 Effect of 5% *S. cerevisiae* and 3% *Z. mobilis* on different sugars and ethanol, and cell growth during fermentation for bagasse pretreated with 5% sulphuric acid at 100 W

(♦- reducing sugars, ■- Ethanol, ▲- O.D (600nm).

Table D7 Effect of 5% *S. cerevisiae* and 3% *Z. mobilis* on different sugars and ethanol, and cell growth during fermentation for bagasse pretreated with 5% sulphuric acid at 180 W

Ferm time	Sugar codes	Area	Sugar (g/g)
0h	x2	1.95E+05	0.14
	x4	1.03E+06	0.66
	x8	1.60E+04	0.01
3h	x1	1.31E+03	0.03
	x4	8.66E+05	0.55
	x2	6.49E+04	0.05
	x8	1.47E+04	0.01
6h	x1	3.34E+03	0.07
	x4	7.36E+05	0.47
9h	x1	9.66E+03	0.21
	x4	4.72E+05	0.30
12h	x1	1.80E+04	0.39
	x4	3.59E+05	0.23
24h	x1	1.78E+04	0.39
	x4	3.55E+05	0.23

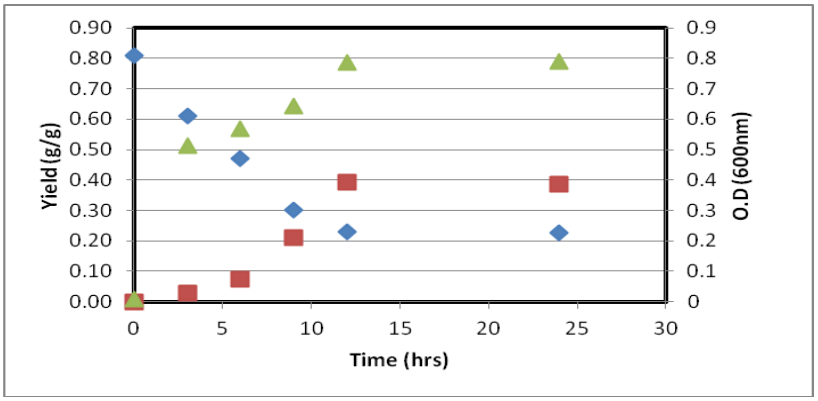

Figure D7 Effect of 5% *S. cerevisiae* and 3% *Z. mobilis* on different sugars and ethanol, and cell growth during fermentation for bagasse pretreated with 5% sulphuric acid at 180 W

(♦- reducing sugars, ■- Ethanol, ▲- O.D (600nm).

Table D8 Effect of 5% *S. cerevisiae* and 3% *Z. mobilis* on different sugars and ethanol, and cell growth during fermentation for bagasse pretreated with 5% sulphuric acid at 300 W

Ferm time	Sugar codes	Area	Sugar (g/g)
0h	x2	7.55E+04	0.05
	x4	1.00E+06	0.64
	x8	1.14E+04	0.01
3h	x1	2.45E+03	0.05
	x4	7.96E+05	0.51
	x2	4.27E+04	0.03
	x8	1.76E+03	0.00
6h	x1	3.27E+03	0.07
	x4	6.55E+05	0.42
9h	x1	8.67E+03	0.19
	x4	5.66E+05	0.36
12h	x1	1.56E+04	0.34
	x4	3.59E+05	0.23
24h	x1	1.50E+04	0.33
	x4	3.28E+05	0.21

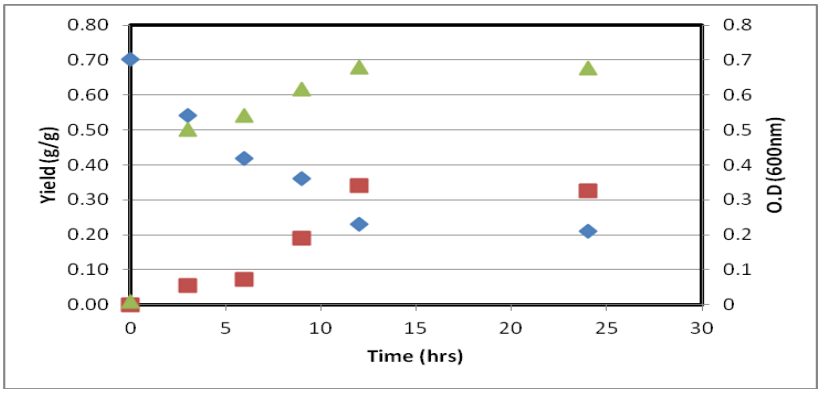

Figure D8 Effect of 5% *S. cerevisiae* and 3% *Z. mobilis* on different sugars and ethanol, and cell growth during fermentation for bagasse pretreated with 5% sulphuric acid at 300 W

(♦- reducing sugars, ■- Ethanol, ▲- O.D (600nm).

Table D9 Effect of 5% *S. cerevisiae* and 3% *Z.s mobilis* on different sugars and ethanol, and cell growth during fermentation for bagasse pretreated with 3% calcium hydroxide at 100 W

Ferm time	Sugar codes	Area	Sugar (g/g)
0h	x2	1.85E+04	0.01
	x4	4.31E+04	0.03
	x8	2.29E+03	0.00
3h	x1	1.63E+02	0.00
	x4	4.28E+04	0.03
	x2	1.50E+04	0.01
	x8	3.71E+03	0.00
6h	x1	1.15E+03	0.02
	x4	3.42E+04	0.02
9h	x1	1.12E+03	0.02
	x4	1.25E+04	0.01
12h	x1	1.14E+03	0.02
	x4	8.98E+01	0.00
24h	x1	1.15E+03	0.03
	x4	5.63E+01	0.00

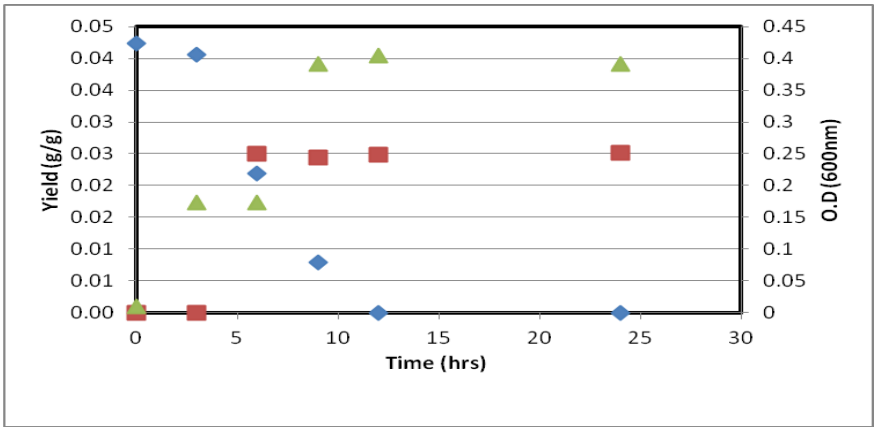

Figure D9 Effect of 5% *S. cerevisiae* and 3% *Z. mobilis* on different sugars and ethanol, and cell growth during fermentation for bagasse pretreated with 3% calcium hydroxide at 100 W (♦- reducing sugars, ■- Ethanol, ▲- O.D (600nm).

Table D10 Effect of 5% *S. cerevisiae* and 3% *Z. mobilis* on different sugars and ethanol, and cell growth during fermentation for bagasse pretreated with 3% calcium hydroxide at 180 W

Ferm time	Sugar codes	Area	Sugar (g/g)
0h	x2	2.33E+04	0.02
	x4	4.26E+04	0.03
	x8	5.44E+03	0.00
3h	x1	1.00E+03	0.02
	x4	4.31E+04	0.03
	x2	1.37E+03	0.00
	x8	2.28E+01	0.00
6h	x1	1.16E+03	0.03
	x4	3.21E+04	0.02
9h	x1	1.16E+03	0.03
	x4	1.16E+03	0.00
12h	x1	1.16E+03	0.03
	x4	9.93E+01	0.00
24h	x1	1.10E+03	0.02
	x4	6.80E+01	0.00

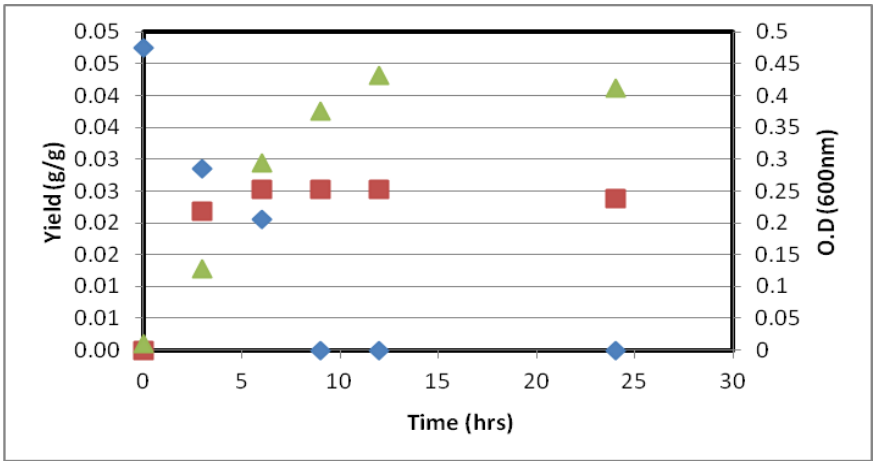

Figure D10 Effect of 5% *S. cerevisiae* and 3% *Z.mobilis* on different sugars and ethanol, and cell growth during fermentation for bagasse pretreated with 3% calcium hydroxide at 180 W (♦- reducing sugars, ■- Ethanol, ▲- O.D (600nm)

Table D11 Effect of 5% *S. cerevisiae* and 3% *Z. mobilis* on different sugars and ethanol, and cell growth during fermentation for bagasse pretreated with 3% calcium hydroxide at 300 W

Ferm time	Sugar codes	Area	Sugar (g/g)
0h	x2	6.19E+04	0.04
	x4	3.68E+05	0.24
	x8	4.85E+02	0.00
3h	x1	1.70E+02	0.00
	x4	3.42E+05	0.22
	x2	5.99E+04	0.04
	x8	4.76E+02	0.00
6h	x1	1.41E+03	0.03
	x4	1.58E+05	0.10
9h	x1	8.24E+03	0.18
	x4	3.98E+04	0.03
12h	x1	9.68E+03	0.21
	x4	3.57E+04	0.02
24h	x1	1.17E+04	0.25
	x4	5.79E+03	0.00

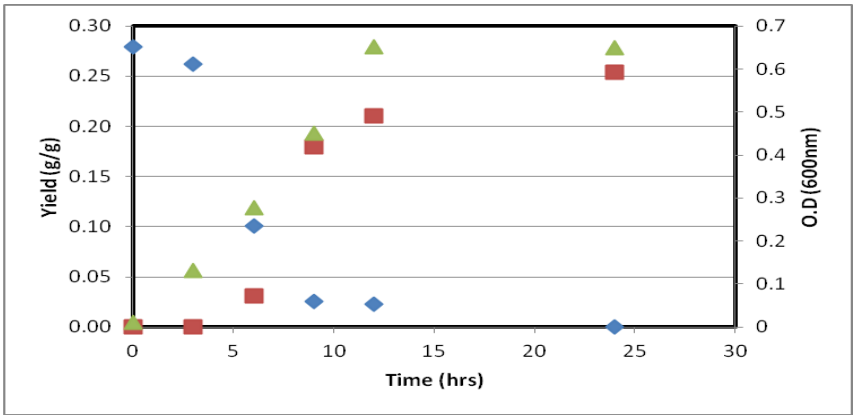

Figure D11 Effect of 5% *S.cerevisiae* and 3% *Z. mobilis* on different sugars and ethanol, and cell growth during fermentation for bagasse pretreated with 3% calcium hydroxide at 300 W (♦- reducing sugars, ■- Ethanol, ▲- O.D (600nm).

Table D12 Effect of 10% *S. cerevisiae* and 5% *Z. mobilis* on different sugars and ethanol, and cell growth during fermentation for bagasse pretreated with 5% Sulphuric acid at 100 W

Ferm. time	Sugar codes	Area	Sugar (g/g)
0h	x2	1.55E+05	0.11
	x4	8.32E+05	0.53
	x8	1.25E+04	0.01
3h	x1	6.35E+03	0.14
	x4	7.55E+05	0.48
	x2	1.34E+04	0.01
	x8	2.39E+02	0.00
6h	x1	9.86E+03	0.21
	x4	5.62E+05	0.36
9h	x1	1.07E+04	0.23
	x4	3.63E+04	0.02
12h	x1	1.77E+04	0.39
	x4	2.03E+04	0.01
24h	x1	1.80E+04	0.39
	x4	1.78E+03	0.00

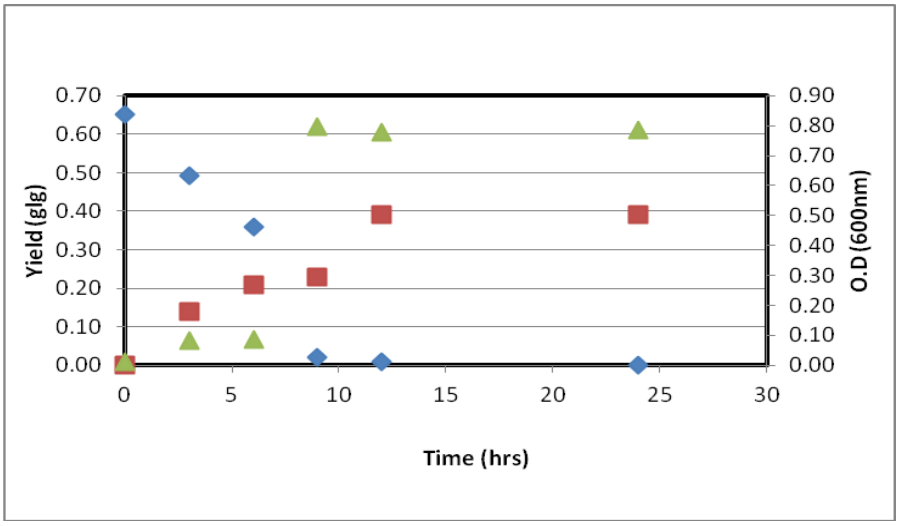

Figure D12 Effect of 10% *S. cerevisiae* and 5% *Z. mobilis* on different sugars and ethanol, and cell growth during fermentation for bagasse pretreated with 5% Sulphuric acid at 100 W (♦- reducing sugars, ■- Ethanol, ▲- O.D (600nm).

Table D13 Effect of 10% *Saccharomyces cerevisiae* and 5% *Zymomonas mobilis* on different sugars and ethanol, and cell growth during fermentation for bagasse pretreated with 5% Sulphuric acid at 180 W

Ferm time	Sugar codes	Area	Sugar (g/g)
0h	x2	7.08E+04	0.05
	x4	1.19E+06	0.76
	x8	9.21E+03	0.01
3h	x1	9.55E+03	0.21
	x2	4.77E+04	0.03
	x4	7.89E+05	0.50
6h	x1	1.46E+04	0.32
	x4	3.19E+05	0.20
9h	x1	1.70E+04	0.37
	x4	2.75E+04	0.02
12h	x1	1.99E+04	0.43
	x4	2.48E+04	0.02
24h	x1	2.32E+04	0.51
	x4	2.00E+04	0.01

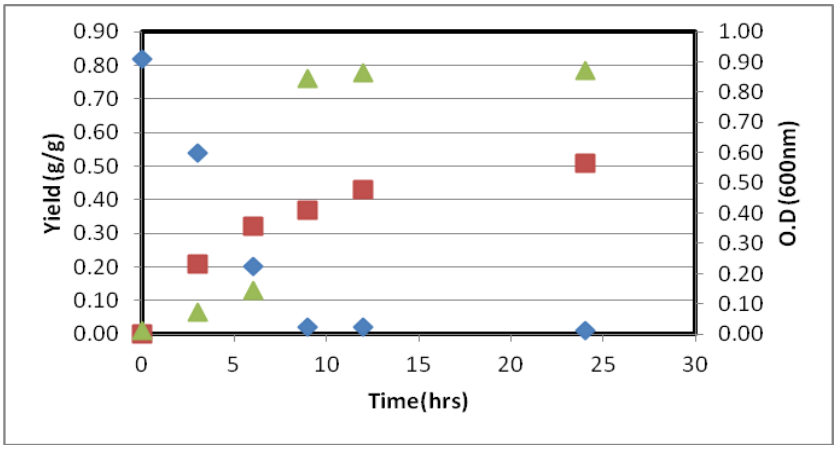

Figure D13 Effect of 10% *S. cerevisiae* and 5% *Z. mobilis* on different sugars and ethanol, and cell growth during fermentation for bagasse pretreated with 5% Sulphuric acid at 180 W (♦- reducing sugars, ■- Ethanol, ▲- O.D (600nm).

Table D14 Effect of 10% *S. cerevisiae* and 5% *Z. mobilis* on different sugars and ethanol, and cell growth during fermentation for bagasse pretreated with 5% Sulphuric acid at 300 W

Ferm. time	Sugar codes	Area	Sugar (g/g)
0h	x2	5.24E+04	0.04
	x4	1.07E+06	0.68
	x8	3.91E+04	0.03
3h	x1	4.79E+03	0.10
	x4	7.27E+05	0.43
6h	x1	4.99E+03	0.11
	x4	6.75E+05	0.43
9h	x1	1.45E+04	0.32
	x4	6.96E+04	0.04
12h	x1	1.55E+04	0.34
	x4	4.58E+04	0.03
24h	x1	2.23E+04	0.48
	x4	2.34E+04	0.01

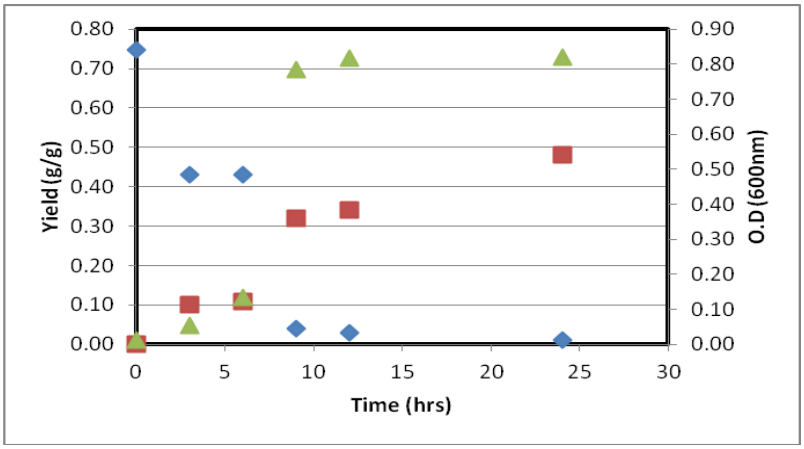

Figure D14 Effect of 10% *S. cerevisiae* and 5% *Z. mobilis* on different sugars and ethanol, and cell growth during fermentation for bagasse pretreated with 5% Sulphuric acid at 300 W (♦- reducing sugars, ■- Ethanol, ▲- O.D (600nm).

Table D15 Effect of 10% *S. cerevisiae* and 5% *Z. mobilis* on different sugars and ethanol, and cell growth during fermentation for bagasse pretreated with 3% calcium hydroxide at 100 W

Ferm time	Sugar codes	Area	Sugar (g/g)
0h	x2	1.85E+04	0.01
	x4	4.31E+04	0.03
	x8	1.28E+03	0.00
3h	x1	2.96E+02	0.01
	x4	3.51E+04	0.02
	x2	9.93E+03	0.01
	x8	1.21E+03	0.00
6h	x1	1.12E+03	0.02
	x4	3.38E+03	0.00
9h	x1	1.11E+03	0.02
	x4	1.84E+02	0.00
12h	x1	1.06E+03	0.02
	x4	2.45E+03	0.00
24h	x1	1.11E+03	0.02
	x4	1.73E+03	0.00

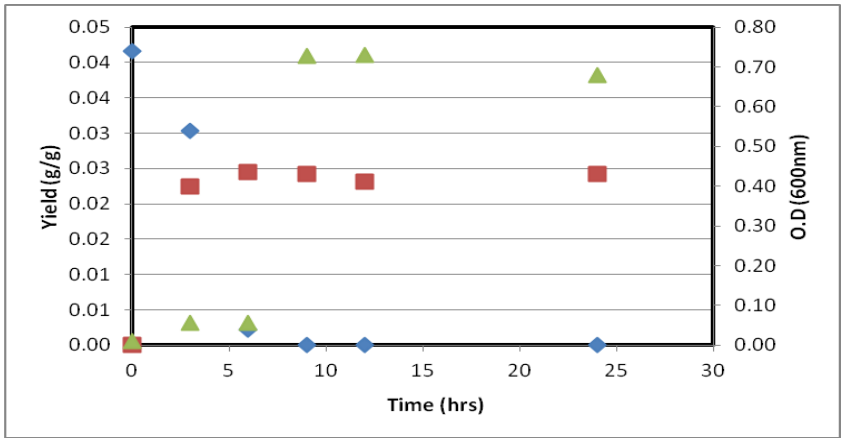

Figure D15 Effect of 10% *S.ccharomyces cerevisiae* and 5% *Z. mobilis* on different sugars and ethanol, and cell growth during fermentation for bagasse pretreated with 3% calcium hydroxide at 100 W (♦- reducing sugars, ■- Ethanol, ▲- O.D (600nm).

Table D16 Effect of 10% *S. cerevisiae* and 5% *Z. mobilis* on different sugars and ethanol, and cell growth during fermentation for bagasse pretreated with 3% calcium hydroxide at 180 W

Ferm time	Sugar codes	Area	Sugar (g/g)
0h	x2	2.17E+04	0.02
	x4	4.26E+04	0.03
	x8	1.28E+03	0.00
3h	x1	2.78E+02	0.01
	x4	3.30E+04	0.02
	x2	1.31E+03	0.00
	x8	1.21E+03	0.00
6h	x1	1.22E+03	0.03
	x4	2.25E+01	0.00
9h	x1	1.12E+03	0.02
	x4	8.35E+01	0.00
12h	x1	1.01E+03	0.02
	x4	8.96E+02	0.00
24h	x1	1.09E+03	0.02
	x4	1.80E+03	0.00

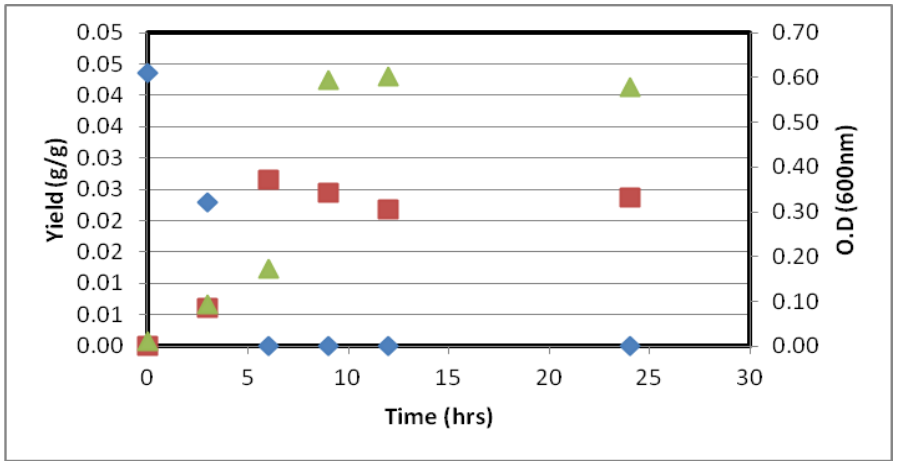

Figure D16 Effect of 10% *S.cerevisiae* and 5% *Z. mobilis* on different sugars and ethanol, and cell growth during fermentation for bagasse pretreated with 3% calcium hydroxide at 180 W (♦- reducing sugars, ■- Ethanol, ▲- O.D (600nm).

Table D17 Effect of 10% *S. cerevisiae* and 5% *Z. mobilis* on different sugars and ethanol, and cell growth during fermentation for bagasse pretreated with 3% calcium hydroxide at 300 W

Ferm time	Sugar codes	Area	Sugar (g/g)
0h	x2	7.66E+03	0.01
	x4	4.01E+05	0.26
	x8	1.52E+03	0.00
3h	x1	2.70E+03	0.06
	x4	3.54E+05	0.23
	x2	3.40E+01	0.00
	x8	9.69E+01	0.00
6h	x1	4.38E+03	0.10
	x4	2.12E+05	0.14
9h	x1	5.72E+03	0.12
	x4	1.69E+05	0.11
12h	x1	5.80E+03	0.13
	x4	7.67E+03	0.00
24h	x1	5.76E+03	0.13
	x4	3.38E+01	0.00

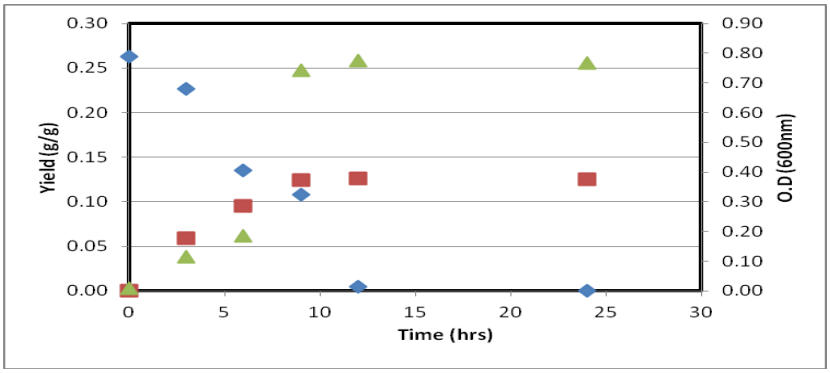

Figure D17 Effect of 10% *S. cerevisiae* and 5% *Z. mobilis* on different sugars and ethanol, and cell growth during fermentation for bagasse pretreated with 3% calcium hydroxide at 300 W (♦- reducing sugars, ■- Ethanol, ▲- O.D (600nm).

D4 Determination of initial, cellulose, hemicelluloses and lignin

Compositional analysis of sweet sorghum bagasse was done and calculated. Table D 17 shows the NDF, ADF and ADL compositional analysis done on sweet sorghum bagasse by the ARC laboratory, Irene.

Table D 18 Compositional analysis of unpretreated sweet sorghum bagasse

Analysis	Accreditation number	Composition (%)
NDF	ASM 060	65.46
ADF	Not SANAs accredited	42.50
ADL	Not SANAs accredited	5.90

NDF – Neutral Detergent Fibre

ADF – Acid Detergent Fibre

ADL – Acid Detergent Lignin

Cellulose = ADF –ADL

Hemicellulose = NDF- ADF

Lignin= ADL

Table D 19 Calculated cellulose, hemicellulose and lignin content

	Composition (%)
Cellulose	36.6
Hemicellulose	22.96
Lignin	5.90

Table D 20 Compositional analysis after different pretreatment methods

	NDF	**ADF**	**ADL**
Unpretreated	65.46	42.50	5.90
Microwave-acid treated	54.33	42.41	6.66
Microwave-alkali treated	58.89	46.06	6.19

Table D 21 Hemicellulose and cellulose composition after different pretreatment methods

	Unpretreated	**Microwave-acid treated**	**Microwave-alkali treated**
	wt%	wt%	wt%
Cellulose	36.6	35.75	39.87
Hemicellulose	22.96	11.92	12.83
Lignin	5.90	6.66	6.19

The initial composition (g) of cellulose, hemicellulose and lignin content was calculated as follows:

Using values in Table D 21:

Cellulose (g) = Cellulose (wt%)/ 100* Initial mass of bagasse (g)

Calculations for the mass left after different pretreatment methods were calculated as follows:
Cellulose (g) = Cellulose (wt%)/100* Mass left per pretreatment method (g)

Initial bagasse was 5g
Bagasse left after acid pretreatment was 3.97g
Bagasse left after alkali pretreatment was 4.03g

Table D 22 Bagasse mass (g) left after different pretreatment methods

	Unpretreated	**Acid pretreated**	**Alkali pretreated**
Cellulose	1.830	1.419	1.606
Hemicellulose	1.148	0.473	0.517
Lignin	0.295	0.264	0.249

Appendix E

CHEMICALS AND MATERIALS USED

E 1 Chemicals and materials used

Table E 1 describes the sugar standards that were used to determine the presence of different sugars in the biomass (sweet sorghum bagasse). The sugar standards were calculated and plotted (see Appendix A).

Table E1 List of sugar standards used in this study

Sugars	Purpose	Supplier	Purity (%)
Glucose	Standard calibration curve	Sigma Aldrich	99.9
Fructose	Standard calibration curve	Sigma Aldrich	99
Sucrose	Standard calibration curve	Sigma Aldrich	99
Mannose	Standard calibration curve	Sigma Aldrich	99
Xylose	Standard calibration curve	Sigma Aldrich	98
Arabinose	Standard calibration curve	Sigma Aldrich	99
Galactose	Standard calibration curve	Sigma Aldrich	98

Table E 2 shows all the chemicals and their suppliers that were used during the implementation and practice of the study.

Table E 2 List of chemicals used in this study

Chemicals	**Purpose**	**Supplier**	**Purity (%)**
Ethanol	Standard calibration curve	Rochelle chemicals	99.9
Glycerol	Stock culture storage	ACE PTY.LTD	98
Calcium Hydroxide	Pretreatment	Saarchem (Merck)	95
Sulphuric acid	Pretreatment	Labchem	98
Yeast extract	Growth media nutrient	Sigma	N/A
Peptone	Growth media nutrient	Fluka Analytical	N/A
Ammonium sulphate	Growth media nutrient	Sigma	99
Magnesium sulphate	Growth media nutrient	Sigma	98
Potassium hydrogen phosphate	Growth media nutrient	Sigma	98
Nutrient agar	Growth media	Merck	N/A

Materials used from pretreatment to fermentation are described in Table E 3. Each material was used for a specific purpose during the experiments.

Table E 3 List of equipment used in this study

Materials	Purpose	Supplier and model
Hammer mill	Milling of bagasse	Trapp-TRF 70
Domestic Microwave	Pretreatment	Samsung
Incubator shaker	Fermentation	Labcon, FSIE-SPO 8-35
pH meter	Monitoring pH	Hanna- HI 9025
Duran bottles (250mL)	Pretreatment and fermentation	GL 45
HPLC	Sugar/ ethanol analysis	1200 series
FTIR	Functional group determination	SHIMADZU IR-Affinity-1
SEM	Physical determination	SHIMADZU
UV Spectroscopy	Cell growth measurement	SHIMADZU UV-mini 1240
Sample vials	Analysis	Agilent (1.5mL)
Petri plates	Microbial growth	Merck
Bunsen burner	Sterilization	
Inoculating loops	Plate streaking	Merck
Microorganisms		
S. cerevisiae	Fermentation	GOLD-STAR
Z. mobilis	Fermentation	American Type Culture Collection (ATCC)