OPTIMIZATION OF ANAEROBIC FERMENTATION CONDITIONS FOR BIOETHANOL PRODUCTION FROM BANANA PEELS USING YEAST IN A STILL REACTOR

GEORGE OGUTA NYANDIGA

B.Eng. (Hons), Grad. Eng. ERB

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DECLARATION

Declaration by the Candidate

This thesis is my original work and has not been presented for a degree in any other University. No part of this thesis may be reproduced without the prior written permission of the author and/or Moi University.

Signature: …….…… ……..……

George OgutaNyandiga Date

TEC/PGMP/03/11

Declaration by Supervisors

This thesis has been submitted for examination with our approval as University Supervisors.

Signature: …….…… ……..……

Dr. Zachary O. Siagi, PhD Date

Department of Mechanical & Production Engineering,

School of Engineering,

Moi University.

Signature: …….…… ……..……

Dr. Augustine Makokha, PhD Date

Department of Mechanical & Production Engineering,

School of Engineering,

Moi University.

DEDICATION

This work is dedicated to my father,Mr. Samuel Nyandiga Mac'Ondiwa and my three mothers.

ABSTRACT

Limited scientific information on optimal biofuels production conditions to both the small and medium scale enterprises has led to non-economical and inefficient processes hence uncompetitive low-grade biofuels. Fermentation process behavior prediction and optimization is very crucial especially while using relatively low fermentable sugars substrates in simple and sustainable bioreactors such as the still batch anaerobic systems. In the current work, banana peels derived from three comparative cultivars were dried, ground into a fine powder to pass through a 1 mm screen, and then hydrolyzed using 60% concentrated H_2SO_4 at 50^oC. Bioethanol was produced by anaerobic fermentation of these hydrolysates using *Saccharomyces cerevisiae*. Sterilized Erlenmeyer flasks fitted with non-return air valves were used as laboratory scale still reactors. Fermentation systems were subjected to various conditions based on half factorial Central Composite Rotatable Design (CCRD). Total Reducing Sugars (TRS) concentrations and bioethanol yields analyses were done by the Dubois and Gas Chromatography methods respectively. Various mathematical models estimating the behavior of these simple fermentation systems were developed, analysed, and statistically revised for future predictions and enhancement of bioethanol yield. Dried banana peels powder derived from the three banana cultivars under study presented average TRS contents of 15.80% w/w and 36.21% w/w before and after concentrated acidic hydrolysis respectively. Ash rangedbetween 5.67% and 7.85% w/w in various banana cultivars used.Optimum bioethanol yields of 13.93 ml/L, 12.59 ml/L, and 13.09 ml/L from *Sialamule*, *Uganda Green*, and *Ngombe* respectively were obtained at 180 g/L Substrate Concentration, 35^oC Fermentation Temperature, 5.5 Initial medium pH, 2 g/L Yeast Concentration, and 120 hours Incubation Period for all the three banana cultivars under study. These corresponded to TRS degradations of 31.57 g/L, 29.88 g/L, and 30.30 g/L in *Sialamule*, *Uganda Green*, and *Ngombe* respectively. Lowest bioethanol yields of 1.51 ml/L, 1.33 ml/L, and 1.44 ml/L from *Sialamule*, *Uganda Green*, and *Ngombe* respectively were obtained at 84.86 g/L Substrate Concentration, 35^oC Fermentation Temperature, 5.5 Initial medium pH, 2 g/L Yeast Concentration, and 120 hours Incubation Period for all the three banana cultivars under study. These similarly corresponded to TRS degradations of 3.28 g/L, 2.85 g/L, and 2.85 g/L in *Sialamule*, *Uganda Green*, and *Ngombe* respectively. From the Analysis of Variance (ANOVA) and Correlation Coefficients (R^2), there were strong indications that the set of mathematical models predicting bioethanol yields from the three different cultivars were the same and could be used alternatively. The student test proved a significant interaction between both the substrate concentration and incubation temperature across all the mathematical models developed. Other statistical features such as the $R^2$and f-test of various regression models developed also showed that they were significant in estimating both bioethanol yields and TRS degradations associated with this simple fermentation process.Concentrated acidic hydrolysis raised TRS concentrations in these peels powder by more than twice the free TRS.Bioethanol yields closely coincided with TRS degradation in various experimental runs and showed that optimal fermentation conditions is important to achieve higher yields.This study recommends quantitative measurement and elimination of various inhibitors contained in banana peels hydrolysates thus enhancing bioethanol yields. Further, economical and cost analysis and implementation of a pilot plant for bioethanol production from banana peels in the country and dissemination of results from this to small and medium scale bioethanol manufacturers to help them better their yields in such fermentation processes.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

ACRONYMS

ACFC:	Agro Chemical and Food Corporation
AGRA:	Alliance for a Green Revolution in Africa
ANOVA:	Analysis of Variance
AR:	Analytical Grade Reagent
ATP:	Adenosine Tri-Phosphate
BGAK:	Banana Growers Association of Kenya
CCD:	Central Composite Design
CCRD:	Central Composite Rotatable Design
DAP:	Dilute Acid Pre-treatment
DM:	Dry Matter
DM-0:	Dry Milling
DoE:	Design of Experiment
EtOH:	Bioethanol
F:	Fischer test
GC:	Gas Chromatography
GHG:	Green House Gases
HCDA:	Horticultural Crop Development Authority
HPLC:	High Performance Liquid Chromatography
ICE:	Internal Combustion Engines
ITDG:	International Technology Development Group
KACE:	Kenya Agricultural Commodity Exchange Limited
KARI:	Kenya Agricultural Research Institute
KSI:	Kenya Sugar Industry
MATLAB:	Matrix Laboratory

MoE:	Ministry of Energy
MT:	Metric Tones
NBC:	National Biofuels Committee
NGO:	Non-Governmental Organisation
NREL:	National Renewable Energy Laboratory
OD:	Optical Density
PABA:	*para* Amino Benzoic Acid
PAC:	Practical Aid Consulting
PSA:	Phenol Sulphuric Acid Analysis
RSM:	Response Surface Methodology
SHF:	Separate Hydrolysis and Fermentation
SSF:	Simultaneous Saccharification and Fermentation
TLC:	Thin Layer Chromatography
TRS:	Total Reducing Sugars
WB:	Water Bath
WM-0:	Wet Milling
WM-HW:	Wet Milling and Hot Water
WM-TW:	Wet Milling and Tap Water

LIST OF SYMBOLS AND NOMENCLATURE

$\bar{x_i}$: Real value of the independent variable at the central point

Δx_j: Step change

$F_{\propto,r,n-r-1}$: Critical F-value

F_o: Observed F-value

H_1: Valid Hypothesis

H_o: Null hypothesis

MSS_E: Error Mean Sum of Squares

MSS_R: Regression Mean Sum of Squares

M_d: Percentage moisture content (dry basis)

M_w: Percentage moisture content (wet basis)

R^2: Co-efficient of determination

R^2_{adj}: Adjusted co-efficient of determination

SS_E: Error Sum of Squares

SS_R: Regression Sum of Squares

SS_y: Total Sum of Squares

W_f: Weight of the residue after combustion

X_i: Real value of the independent variable

Y_{EtOHA}: Predicted bioethanol yield in *Sialamule* fermentation (g/L)

Y_{EtOHB}: Predicted bioethanol yield in *Uganda Green* fermentation (g/L)

Y_{EtOHC}: Predicted bioethanol yield in *Ngombe* fermentation (g/L)

Y_{TRSA}: Predicted TRS degradation in *Sialamule* fermentation (g/L)

Y_{TRSB}: Predicted TRS degradation in *Uganda Green* fermentation (g/L)

Y_{TRSC}: Predicted TRS degradation in *Ngombe* fermentation (g/L)

n_o: Central point experimental repetitions

x_i: Dimensionless values of the independent variables

y_{trsA}: TRS yield in *Sialamule* hydrolysis (% w/w)

y_{trsB}: TRS yield in *Uganda Green* hydrolysis (% w/w)

y_{trsC}: TRS yield in *Ngombe* hydrolysis (% w/w)

β_{ij}: Regression co-efficient

ε_{EtOHA}: Random error associated with bioethanol yield prediction in *Sialamule* fermentation

ε_{EtOHB}: Random error associated with bioethanol yield prediction in *Uganda Green* fermentation

ε_{EtOHC}: Random error associated with bioethanol yield prediction in *Ngombe* fermentation

ε_{trsA}: Random error associated with TRS degradation prediction in *Sialamule* fermentation

ε_{trsB}: Random error associated with TRS degradation prediction in *Uganda Green* fermentation

ε_{trsC}: Random error associated with TRS degradation prediction in *Ngombe* fermentation

$\propto$: Distance of axial points

Ash: Percentage ash content of the substrate

D: Dry weight

F: F-Value

N: Total experimental combinations

W: Wet weight

i: Linear co-efficient

j: Quadratic co-efficient

k: Number of independent factors

r : Regression degrees of freedom

x: Sulphuric acid concentration (% v/v)

y: Predicated response

z : x is a function of z

β: Regression co-efficient

ε: Random error

LIST OF APPENDIXES

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CHAPTER ONE: INTRODUCTION

1.1. BACKGROUND AND MOTIVATION

Biofuels technology has been present for years though it diminished due to the discoveries of enormous petroleum deposits. Ever escalating costs of fossil fuels, their forecasted depletion, Green House Gas (GHG) emissions,and other associated challenges havere-ignited research in alternative approaches to energy supply. Currently, biofuels technologies are being supported through governments' subsidies especially in research.For instance, the government of Kenya has developed various policies to spearhead the biofuels sector in the country (Diaby, 2011). Ethanol, wood fuel, and methane are some of the most explored renewable energy products. Biofuels technologies are nowadays regaining their lost popularity.

Ethanol, synonymously known as ethyl alcohol, is a clear biodegradable colorless liquid. It is a high octane fuel and burns to produce carbon dioxide and water. These properties make ethanola suitable energy resource. It has been employed in various industrial applications as a clean source of energy. In the transport sector, engines have been crafted to consume ethanol-gasoline mixtures e.g. E10 and E85. Ethanol has also been used as a cooking fuel in rural homesteads since it is less polluting as compared to conventional fossil fuels. Other than the fuel value of ethanol, itis also applied in industrial setups to produce a wide range of products.

Figure 1.1: A lady preparing a meal on a bioethanol stove (Ebelechukwu, 2013)

Figure 1.1 shows the domestic use of bioethanol as a cooking fuel in rural homes. A local Non-Governmental Organisation (NGO), Practical Aid Consulting (PAC) which was formerly known as International Technology Development Group (ITDG) has been carrying out field trials of these bioethanol stoves in some Kenyan rural homes (Francis, 2012).

In some parts of the world, ethanol has been widely used as fuel in the automotive industry (Liao, 2010). This started by its application in internal combustion engines (ICE) developed in 1897 by Nicholas Otto (Mustafa et al, 2008). The technology has been practiced by blending gasoline with ethanol which oxygenates the fuel mixture and causes it to burn more exhaustively thereby reducing associated polluting emissions. E10 is the most common blend consisting of 10% ethanol and 90% gasoline.

Gasoline engine requires no modification to run on this E10 and the engine warranties are also unaffected. Flexible engines can run on E85 (85% ethanol and 15% gasoline) (Liao, 2010).Most automobiles crafted in the United States since 1998 are fitted with systems that allow then operate on E85 (Monster, 2013).

Ethanol derived from biological fermentation of carbonaceous feedstocks e.g. banana peels, molasses, and sorghum amongst others is referred to as bioethanol.It is a renewable energy resource which can ensure greater fuel security to the world's economy and can be a better alternative during energy crises if extensively harnessed.The use of bioethanol as an energy resource can boost the rural economy by offering employment to people engaged in its massive production enterprises.

Main feedstocks for the Kenyan bioethanol industry have been planned to mainly comprise of the sweet sorghum and sugarcane. Elsewhere, it has been established through research that about 10% of *Musa Spp.* is comprised of wastes including stems, skins or peels, and leaves (Mazlan, 2013). All banana plant parts including stems, fruits, pulps, and peelings have been researched on as a bioethanol production feedstock (Manikandan et al, 2008; Kumar, 2011). Banana peels have relatively viable sugar contents which can be harnessed in bioethanol production rather than being discarded as witnessed in Kenya and other parts of the world. They have enormous potential in the bioethanol industry and stand as a cheaper source of feedstock in bioethanol production.

1.2. POLICIES REGARDING BIOFUELS PRODUCTION IN KENYA

The government of Kenya has developed various policies to spearhead its biofuels sector(Diaby, 2011) as well as environmental management during the previous regimes. These include the National Energy Policy, the National Biofuels Committee (NBC), and the Energy Act of 2006. Other legislation concerning biofuels production in Kenya include the Agricultural Act of 2002, the Forest Act of 2005, the Trade and Industry Act of 2002, and the Water Act of 2002.

1.2.1 The National Energy Policy

This policy was developed by Kenya to seek wider support for the implementation of renewable energy techniques. Its overall objective is "…… *to ensure affordable, sustainable and reliable supply to meet national and county development needs, while protecting and conserving the environment*"(Ministry of Energy, 2012).Initially, the petroleum energy products imports into Kenya increased by 12% to 4,677 metric tons in 2009 from 2,972 metric tons in the year 2005 (Peter et al, n.d.). The National Energy Policy majorly aims at reducingthe dependence on imported petroleum fuel products by 25% in the year 2030.

a. The National Energy Policy on Biofuels

Some policies and strategies in this draft related to biofuels production in Kenya include:

i. "*Support RD&D for the cultivation of high yielding and fast maturing feedstock so as to enhance the production and use of liquid bio-fuels*" (Ministry of Energy, 2012).
ii. "*Collaborate with other stakeholders to ensure efficient use of land resource to minimize the adverse effects arising from competition for land use between liquid bio-fuel feedstock and food production*" (Ministry of Energy, 2012).
iii. "*Create awareness on the importance and viability of growing bio-fuel feedstock among the public*" (Ministry of Energy, 2012).
iv. "*Encourage small scale initiatives on the production and use of bio-fuels around the country*" (Ministry of Energy, 2012).
v. "*Invest in research on the production chain and sustainability of biofuels particularly biodiesel*" (Ministry of Energy, 2012).

As part of the short term plans to be carried out between the year 2012 and 2016, the government of Kenya planned to initiate comprehensive research on the viability of various biofuel resources and map their feedstocks production areas across the entire country. It further planned to identify and set aside land for biofuels pilot projects. Under its medium plans to be achieved between the year 2012 and 2022, both the national and various county governments were planned to allocated pieces of land to be used in biofuels feedstocks production (Ministry of Energy, 2012).

Main feedstocks for Kenya bioethanol industry have been planned to mainly comprise sweet, sorghum and sugarcane (Ministry of Energy, 2012; Peteret al, n.d.). There are plans underway to discourage illegal use of ethanol and exportation which will be achieved by taxation regime harmonization. It was planned that facilities used in

ethanol and gasoline blending be established in Kisumu, Eldoret, and Nakuru (Ministry of Energy, 2012).

The National Energy Policy draft proposed that all the government and public transport vehicles should use E10 engines by 2017 and all vehicles to use E10 by 2022. This draft further proposed that all vehicles in Kenya should use E30 by 2030 (Ministry of Energy, 2012)

Like other nations in the world, the need for alternative energy to fossil fuels in Kenya sparked off in 1970s (Mustafa et al, 2008; Francis, 2012; Stephen, n.d.). The Agro Chemical and Food Corporation (ACFC) was set up in 1978 with the main aim of using excess molasses from sugar manufacturing industriesbased in western regions of the country to produce bioethanol. A capacity of 60,000 liters of bioethanol per day was established and it operated at 45,000 liters per day before collapsing in 1990s. In the year 2001, Kisumu Ethanol Plant was revived with Energen owning 55% of the plant and it produced 60,000 liters per day of industrial ethanol (Stephen, n.d.). In the year 2003, the ownership was transferred to Spectre International Inc. (Francis, 2012).

Currently, bioethanol plants operating in the country include Spectre International (formerly the Kisumu Ethanol Plant), Agro Chemical and Food Corporation, and Mumias Sugar.In the year 2008, the two major players in the bioethanol industry, Spectre International and the Agro Chemical and Food Corporation produced 22 million and 18 million liters of ethanol per year respectively (Francis, 2012). The Kenya Sugar Industry (KSI), in its strategic plan 2010 to 2014, also set aside 15.3 billion Kenya Shillings for the initiation of bioethanol projects (KSI, 2010).

Table 1.1: Current and Planned Ethanol production in Kenya (GTZ, 2008)

Production (L/day)	**ACFC**	**Spectre International**	**Mumias Sugar**	**Total**
Current Capacity	60,000	65,000	0	125,000
Current Production	27,400	30,000	0	57,400
Current and Planned Capacity	60,000	230,000	~ 50,000	340,000

Table 1.1 shows the various capacities of the three ethanol producing plants in Kenya. Due to the scarcity of molasses from sugar millers the production of bioethanol has been currently deteriorating (Ramenya et al, 2014).

b. The National Energy Policy on Municipal Wastes

Municipal wastes include "*.....food scraps....., yard wastes .....*, *from homes, institutions and business, wastes generated by manufacturing, agriculture,.....*". "*With appropriate waste-to-energy technologies, municipalcan be used to provide energy while helping to clean the environment*"(Ministry of Energy, 2012).With this view of "Waste to Energy" technology as an avenue of managing municipal wastes in the country, there is still an impediment of *"……inadequate data and information on potential of municipalwaste*" (Ministry of Energy, 2012). To this effect, the National Energy Policy has laid down some policies and strategies to this promising "Waste to Energy" technology including to "*Acquire adequate data and information on potential of municipal waste*" (Ministry of Energy, 2012).

This National Energy Policy,therefore, offers a favourable environment to foster more development and application of bioethanol as an energy resource in the region.

1.3. BIOETHANOL

Humans have been familiar with bioethanol since the beginning of history (Muhammad, 2011). Initially, it was produced through spontaneous fermentation. Several research works have been started to control the process of bioethanol fermentation (Muhammad, 2011). Bioethanol is an important commodity as far as fuel market is concerned. It burns to produce heat energy alongside other products of its combustions as shown in equation 1.1.

$$C_2H_5OH + 3O_2 \rightarrow 2CO_2 + H_2O - 1371\ \frac{kJ}{mol} \quad (1.1)$$

Due to the oil embargo of 1970s, bioethanol was established as an alternative to fossil fuels and has been considered from 1980s to date. Back in 1970, only 76 x 10^6 liters was produced through fermentation as compared to the 7.95 x 10^6 liters by synthesis (Mustafa et al, 2008). In 1975, the market for bioethanol was still below 1,000 x10^6 liters. It then shot to over 39,000 x10^6 liters in 2006. It is projected to be 100,000 x10^6 liters come the year 2015 (Litch, 2006). Considering equation 1.1 above together with these enormous volumes of this biofuel presents a very promising renewable energy resource. Using bioethanol as a fuel, therefore, has a high potential of sustainability than the conventional fossil energy resources (Leah, 2005).

Fermentation is a key process in bioethanol production. To facilitate proper activity of microorganisms (e.g. *Saccharomyces cerevisiae*), optimum conditions are set for various fermentation media. Bioethanol is produced using two major categories of bio-resources, i.e. starchy materials and sugar substances. Competition between these starchy materials and sugar substance for the production of bioethanol is very stiff.

Share for sugar substances use in biofuel production dropped to 47% in the year 2006 from the initial 60% at the beginning of 2000 with grains taking 53% in 2006 (Litch, 2006). This shows the need to develop alternative feedstocks which don't compete with human food resources and employ superior techniques in bioethanol production processes.

1.3.1 Bioethanol fuel properties

Bioethanol is a highly oxygenated fuel. It contains 35% O_2 which ensures stoichiometric supply of oxygen during combustion,therefore, ensuring complete combustion leading to the reduction of hydrocarbons, particulate matter, and NO_x emissions in the exhaust fumes. (Mustafa et al, 2008). Some of the bioethanol fuel properties are shown in the table 1.2.

Table 1.2: Some fuel properties of bioethanol (Mustafa et al, 2008)

Fuel property	**Value**
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

Table 1.2 shows some fuel properties of bioethanol. These fuel properties permit short fuel burn time, leaner burn engine, and high compression ratio thus leading to great theoretical efficiency advantages over a comparable gasoline in an internal combustion engine (ICE). A high octane number fuel like bioethanol can be used in providing antiknock advantage in an ICE.

1.3.2 Benefits of bioethanol as a biofuel

Advantages of using bioethanol in energy applications are as follows:

a. It is biodegradable
b. It can be easily integrated into the current transport systems
c. It is a renewable source of energy
d. Bioethanol can provide a sustainable and reliable energy resource
e. Using bioethanol can reduce Green House Gas (GHG) emissions as compared to fossil fuels
f. Using bioethanol can ensures greater fuel security
g. It can reduce fossil fuels dependence
h. Engagement in bioethanol production and use can boostthe rural economy
i. Venture in bioethanol enterprises can create jobs

1.3.3 Disadvantages of bioethanol as a biofuel

Inasmuch as bioethanol is very important as a biofuel, it presents some few disadvantages (Mustafa et al, 2008) which include.

a. Corrosiveness
b. Lower energy density compared to gasoline.
c. Difficult cold starts due to lower vapor pressure.
d. Miscibility with water.
e. Low flame luminosity.
f. Toxicity to ecosystems.

1.4. STATEMENT OF THE PROBLEM

There has been escalating costs of fossil fuels making their use as energy resources very expensive and unsustainable. The Green House Gas (GHG) emissions which are majorly associated with fossil fuels are also destroying the ozone layermorerapidly as years roll by. Moreover, the world will soon run out of its petroleum and coal deposits. Most researchersargue that oil, natural gas, and coal will run out by 53, 54, and 110 year respectively(Oil, 2015). Therefore, there isa seriousneed to investigate possible alternative energy resources.

Bioethanol which is one of the alternatives to fossil fuels has a very narrow profit marginacross its production chain. This has lowered interest in exhaustive exploitation of this promising renewable energy resource. With feedstock's cost taking the biggest share in the fermentation process, "Waste to Energy"technologies have now been incorporated to take care of thisproblem. However, even with these cheap alternative fermentative feedstocks, there still exist problems of inefficient biofuel production processes. This has led to very low bioethanol yieldsfrom these agro-residues thereby killing interest in "Waste to Energy" technologies. The main aim of this study, therefore, is to determine optimal anaerobic fermentation conditions for bioethanol production from banana peelings using yeast in a still batch reactor through both experimental and statistical approaches.

1.5. RESEARCH OBJECTIVES

1.5.1 The main research objective

To determine optimal anaerobic fermentation conditions for bioethanol production from banana peelings using yeast in a still batch reactor through both experimental and statistical approaches.

1.5.2 Specific research objectives

1) To quantify the composition of banana peelings.
2) To produce bioethanol through anaerobic fermentation of banana peelings under varying operational parameters using yeast in a still batch reactor.
3) To determine optimal conditions (parameter settings) using Central Composite Rotatable Design (CCRD) and Response Surface Methodology (RSM).

1.6. SCOPE OF THE STUDY

This study is limited to determining optimum substrate concentration, incubation temperature, initial medium pH, yeast concentration, and incubation period in a still batch anaerobic fermentation of *Sialamule*, *Uganda Green*, and *Ngombe* concentrated sulphuric acid peels hydrolysatesand development of associated mathematical models.

1.7. JUSTIFICATION AND SIGNIFICANCE OF THE STUDY

Disseminationof sufficient scientific information on “Waste to Energy”production techniques to both the small and medium scale industrialists will lure most of them to get involved in thesebusinesses.Thiswill mean ready and cheaper alternative biofuelto the overburdened, unreliable, unsustainable, and environmental polluting fossil

fuels.The rural dwellers will, therefore, be able to substitute their paraffin cooking stoves with the environmental friendly bioethanol stoves. Motorists and industrialists as well will enjoy the use of superior engine ranges like the E10 and the E85 which will ensure a cleaner environment and cheaper production costs among other benefits.

This bioethanol production will increase the raw material's demand,therefore, triggering the supply of banana peelings as the major raw material in this clean venture. High demand of banana wastes will translate to more banana farmingby both the small scale and large scale farmers. Banana plants can be grown on lands that have over the seasons been degraded through farming. Increased demand for banana wastes will, therefore, lead to reclamation of these lands.The pick-up of this "Waste to Energy" enterprisewill,therefore,improve food supply through extensive banana farming.

Increased demand for banana peelings due to efficient and improved bioethanol production processes will translate into productive waste management. This will thus create acceptable and economic enterprises using banana wastes in "Waste to Energy"techniques. People involved in these activities: banana farming, banana peels collection,fermentation, and trading will have employment to help them raise their living standards.

Due to improved energy reliability, food security, employment, and economical waste management, there will be expectations of high poverty reduction levels among the community members thus improving their livelihoods. Kenya and other countries practicing similar ventureswill thus be viewed internationally as producers of bioethanol from food wastes which in turn will promote international trade.

1.8. OUTLINE OF THE RESEARCH

This research comprises of five chapters. It covers the introduction, literature review, materials and methodology, results and discussion, and summary and conclusions. Chapter one gives the background and motivation to carry out this study, policies related to bioethanol and other biofuels in Kenya, and bioethanol with regard to its fuel characteristics, advantages, and disadvantages. It further points out the research objectives, scope, and the justification and significance of this study.Chapter two surveys the existing literature on bioethanol and its production processes, banana and other fermentative feedstocks, lignocellulosic biomass hydrolysis, microorganisms in fermentation, fermentation parameters analysis, process optimization and statistical analysis, and economics of fermentation. This review tries to assess the extent of research in similar “Waste to Energy” techniques.Chapter three reports various research stations, equipment, chemicals/biochemicals, and all experimental and statistical procedures employed.Chapter four presents results, analyses, and discussions of various research findings. Finally, chapter five gives the overall conclusions and recommendations to this study.

CHAPTER TWO: LITERATURE REVIEW

2.1 INTRODUCTION

In this chapter, bioethanol and its production processes, banana and other fermentative feedstocks, lignocellulosic biomass hydrolysis, microorganisms in fermentation, fermentation parameters analysis, process optimization and statistical analysis, and economics of fermentation are reviewed to assess the extent of research in similar "Waste to Energy" techniques.

2.2 BIOETHANOL PRODUCTION

A basic schematic representation of bioethanol production is shown in figure 2.1:

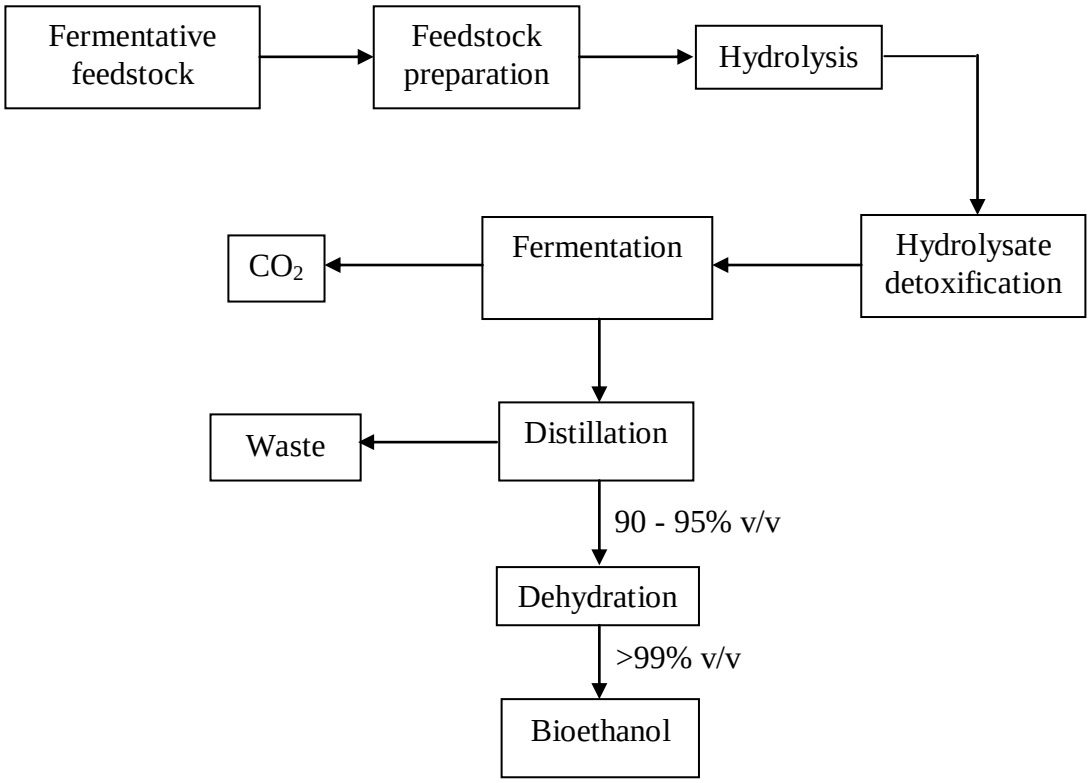


Figure2.1: Bioethanol production chart (Taherzadeh et al, 2007)

Portable or hydrous bioethanol is 96.5% v/v while anhydrous bioethanol is 99.5% v/v. Portable bioethanol can be used as a gasoline substitute in car engines which are

currently not available in Kenya (Patriciaet al, 2007). In anhydrous bioethanol, water is eliminated by the use of either a molecular sieve or chemical absorption. Various materials, processes, and products depicted in figure 2.1 above for bioethanol production are discussed in subsequent sections.

2.3 FERMENTATIVE FEEDSTOCKS

All substances containing sugars can be degraded or fermented to bioethanol by suitable microorganisms such as *Saccharomyces cerevisiae*. Fermentative feedstocks take the largest proportion of the whole fermentation cost. Therefore, cheap materials which can be fermented with high efficiency to the desired economic products and by-products such as bioethanol can be of high value in industrial scale biofuel and other manufacturing processes (Muhammad, 2011). Several potential substrates used in industrial scale bioethanol fermentation establishments have been researched on and reported under various studies (Muhammad, 2011). Bioethanol has been produced from corn, sugarcane, Jerusalem artichoke derived juice, barley, cassava, and cellulose (Muhammad, 2011). The table 2.1 reports the realized of various fermentative feedstocks in bioethanol production.

Table 2.1: Bioethanol realized from some fermentative feedstocks (Mustafa et al, 2008)

Feedstock	**Bioethanol realized (l/ton)**
Sugar cane	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 biomasses	280

Table 2.1 shows a comparative bioethanol productionpotential of some fermentative feedstocks. Major problems associated with fermentative feedstocks include their price, food usage competition, and their supply.Someof them are also seasonal crops.Since feedstocks take more than two thirds in bioethanol production cost, ensuring maximum bioethanol yield from a given substrate is very crucial (Mustafa et al, 2008). Other studies have investigated employment of banana peels and other food wastes in bioethanol and other biofuels production (Manikandan et al, 2008) in the currently evolving “Waste to Energy” technologies.

Feedstocks containing high hexoses, as well as disaccharides values, are preferred in industrial scale fermentation processes. Major categories of fermentative feedstocks include starchy materials, sucrose containing feedstocks, and lignocellulosic feedstocks (Mustafa et al, 2008).

2.3.1 Starchy materials

These feedstocks e.g. wheat and corn are mostly used in Europe and North America in the production of bioethanol. They mainly contain starch which is a homopolymer i.e. it only contains one monomer, the D-glucose. Prior to a fermentation process, these chains of carbohydrates in starch are first broken down to glucose which can further be fermented by yeast to produce bioethanol (Mustafa et al, 2008).

2.3.2 Sucrose containing feedstocks

Examples include sugar beet and sugar cane. Transformation of five and six carbon sugars contained in these feedstocks is much easier compared to both the lignocellulosic and starchy biomasses thusmaking them most preferred in bioethanol production processes (Mustafa et al, 2008).

2.3.3 Lignocellulosic feedstocks

Lignocellulosic feedstocks are more promising compared to both the sucrose containing and starchy biomasses due to their low cost and high availability. However, they are not yet implemented in bioethanol production processes due to the associated high production costs (Mustafa et al, 2008).They mainly contain carbohydrate polymers, lignin, and ashes. Carbohydrate polymers are classified as either cellulosic or hemicelluloses. Holocellulose refers to total carbohydrate found in a microbial or plant cell. Carbohydrates in lignocellulosic materials are called holocellulose. Hemicelluloses in a lignocellulosic biomass range from 10% to 37% of its dry weight. These feedstocks are majorly prepared through acidic hydrolysis of hemicelluloses into their respective monomers: mannose, xylose, glucose, arabinose, galactose, rhamnose, methyl

glucuronic acid, glucuronic acid, and galacturonic acid during fermentation processes (Taherzadeh et al, 2007).

2.3.4 Fermentative nutrients

There are various components of a suitable fermentative feedstock as discussed below:

a. Nitrogen Source

Nitrogen is a very crucial unit of nucleic acid, proteins, and other cellular components essential to life (Kahl, 2004). Its sources include urea, ammonium sulphate, peptone, and casamino acids among others (Muhammad, 2011).

b. Phosphorous Source

Phosphorous is very crucial in yeast and other microorganisms metabolism and its sources include KH_2PO_4 and K_2HPO_4 among others.

c. Minerals Requirement

Banana peels contain various minerals, some of which are important to the metabolic activities of yeast in various quantities.

Table 2.2: Banana peels ash minerals content (Olabanji et al, 2012)

Element	Composition (%)
Ca	17.50
Mg	7.36
K	41.45
Na	0.72
Mn	5.41
Fe	22.58
Cu	0.38
Zn	4.50
Pb	0.005
Cr	0.009
Cd	0.00
Pd	0.01
Ni	0.011
Ag	0.005
B	0.04
Al	0.044

Table 2.2 shows mineral composition of banana peels. Mineral nutrients required in yeast cell growth (Srishail, 2010) include:

i) **Potassium ions**: Assists in ATP storage inside the microorganism cells.

ii) **Calcium ions**: Stimulates permeability of cell wall as well as assisting in cell growth.

iii) **Magnesium ions**: Assists in insulating microorganism cells against stress factors such as alcohol levels, osmotic pressure, and temperatures.

iv) **Copper ions**: Assists in the cell internal enzymes production.

v) **Zinc ions**: Helps in fermentation enzymes production. Its absence, therefore, slows fermentation and may lead to poor bioethanol yields.

It has been established that yeasts need various inorganic ions in small concentrations (micro and millimolar) in order to achieve both optimum growth and optimum fermentative activities (Colin et al, 1987). The presence of these elements in appropriate concentrations either accelerates yeast growth or increases biomass yields or both of these two effects. This ultimately results to higher substrate - biomass conversion. Imbalances in these inorganic ions concentrations can lead to complex and subtle alterations of metabolic patterns as well as growth characteristics i.e. the morphology and yeast tolerance to the growth medium (Colin et al, 1987).

These inorganic ions play both enzymatic and structural roles (Rodney et al, 1984).They can either be stabilizers or activators of enzyme functions. Inorganic ions also regulate antagonism between various activators and respective deactivators. Cu^{2+}, Zn^{2+}, Co^{2+},and Mn^{2+}act as catalytic centers, Mg^{2+} is one of the most common enzymatic activities activators, and K^{+} acts as a metal co-enzyme (Michael et al, 1978).

A yeast cell is relatively impermeable to bivalent ions and hardly any leakage of this group of cations occurs when yeast cells are left suspended in distilled water. There exist rapid and reversible binding of bivalent cations (exogenous) by yeast cells (Colin et al, 1987). There is a system in baker's yeast which transports these ions into the yeast cell, i.e. into a non-exchangeable pool (virtual pool), a system in which there is no surface attachment by fixed (-ve) groups on a yeast cell (Colin et al, 1987). This uptake of bivalent cations is the same under both aerobic and anaerobic conditions. This implies that energy of their transport can be supplied by fermentative reactions (Fuhrmann et al, 1968).

Yeast cells take up Co^{2+}, Zn^{2+}, and Ni^{2+} into this virtually non-exchangeable pool through the same system which transports Mn^{2+} and Mg^{2+} in the order of Mg^{2+}, Co^{2+},

Zn^{2+}, Mn^{2+}, Ni^{2+}, Ca^{2+}, and finally Sr^{2+} (Colin et al, 1987). These bivalent cations uptake reduces at low pH because a hydrogen ion (H^{+}) exchange system in the yeast cell is not being involved. Instead of this, two K^{+} are secreted as a result of absorption of each bivalent cation (Colin et al, 1987).

2.3.5 Fermentative feedstocks preparation methods

Prior to hydrolysis and subsequent fermentation procedures, feedstocks are always pre-treated to make them suitable in bioethanol productions processes. There are four possible ways of preparing substrate samples (Phatcharaporn et al, 2009).

a. **Dry Milling (DM-0)**: Here the feedstocks are dried in an oven overnight, and then ground to pass through a 1 mm screen using a cyclone mill.
b. **Wet Milling (WM-0)**: In this preparation method, the feedstock is blended with water in the ratio of 1 to 5 and then sieved through a 1 mm screen.
c. **Wet Milling and Tap Water (WM-TW)**: In WM-TW method (b) above is carried out using tap water for 5 minutes.
d. **Wet Milling and Hot Water (WM-HW)**: In WM-HW method (b) above is carried out using hot water at 95^{o}C for 5 minutes.

The milling process may have a significant manipulation on the production processes by altering substrate nutritional content. Dry milling is always preferred because it enhances concentration of sugars, other fermentative nutrients, and minerals in the samples. Materials prepared by this method can also be easily stored, awaiting use, without denaturing the substrate. On the other hand, hot water washing leads to high loss of soluble feedstock components due to the leaching effect of hot water (Phatcharaporn et al, 2009).

2.4 BANANA AS A FERMENTATIVE FEEDSTOCK SOURCE

Banana plant is composed of a succulent and cylindrical pseudo stem. Its leaves have a cylindrical petiole of a height ranging between 6 m and 7.5 m. These leaves arise from a corm of fleshy rhizome (HCDA, 2013) and around the main plant springs up suckers. This main plant is replaced by the eldest of all suckers when it bears fruits and dies, therefore building an indefinite succession process. Its inflorescence shoots out of the heart which is in the stem tip. Its leaves are elliptic or oblong, tender, smooth, and fleshy. These leaves are spirally arranged and are about 60cm wide and 270 m long (HCDA, 2013).

(a) (b)

Plate 2.1: (a) Mature banana and (b) Green harvested Banana bunches (KARI-Kisii)

Plate 2.1 shows (a) a manure banana and (b) green harvested banana bunches. The crop is vulnerable to sigatoka virus, panama disease, nematodes, and weevils. Infections by diseases and pests, combined with primitive farming practices, reduce banana productivity. Primitive banana farming practices which include propagation through

suckers can reduce yields by up to 90%. The reduction of banana production per acre from 12.8 million MT to 9.9 million MT between the year 1992 and the year 1994 (Njuguna et al, 2010) could be attributed to the above factors.

2.4.1 The Origin of Banana and its Distribution in Kenya

Banana is believed to have originated from South Asia to the Northern Australia. Its cultivation is popular with most communities in Kenya (HCDA, 2013) and spread across the Kenyan counties. Agro-ecological zones under which banana are grown range from lower highlands to coastal lands. However, 1.8 km above the sea level is highly recommended for banana farming.

Banana does well with 1000-2500mm annual rainfall. Best yields require annual rainfall distribution of at least 1400mm without prolonged dry seasons (HCDA, 2013). Warm and humid climate with average temperatures ranging between 20^{o}C and 30^{o}C is essential for banana's optimal growth. At average temperatures less the 20^{o}C, banana plant growth would be retarded (HCDA, 2013). Amongst other banana cultivars, *Valery* and *Lacatan* can withstand cold weathers. Higher altitudes (usually cold) retards plant growth and this may lead to failure of the inflorescence to emerge. Banana should be planted on deeply cultivated soil free from bushes and trees. Plant spacing vary with cultivars, i.e., 250 x 300 cm for short varieties (*Giant Cavendish* and *DwarfCavendish*), 300 x 400 cm for medium varieties (*Williams* and *Valery*), and 400 x 400 cm for tall varieties (*Lacatan* and *Poyo*).

Table 2.3: Banana Distribution in Kenya (2004) (Njuguna et al, 2010)

Province	Area (ha)	Production (MT)	Avg Yield (MT /ha)	Area share (%)	Production share (%)
Nyanza	34,401	532,886	15.5	42.1	51.5
Central	15,520	164,171	10.6	19.0	15.8
Western	11,753	146,036	12.4	14.4	14.1
Eastern	9,238	88,442	9.3	11.7	8.5
Coast	7,124	54,237	7.6	8.7	5.2
Rift Valley	2,861	44,291	15.5	3.5	4.3
N/Eastern	422	5,721	13.6	0.5	0.6
Nairobi	54	354	6.6	0.1	<0.05
Total	**81,073**	**1,030,138**	**12.7**	**100.0**	**100.0**

MT: Metric tons

Table 2.3 gives statistical data on banana production and distribution across Kenyan provinces in the year 2004. The crop thrives well in fertile (humus rich) and well-drained areas, i.e. light to medium loam soil and pH range between 5.5 and 6.5 is the best. Banana withstands short flooding periods as long as there is adequate soil aeration (HCDA, 2013).In Kenya, banana is grown in Kisii, Bungoma, Kakamega, Murang'a, Nyeri, Meru, Embu, Kericho, Kerio Valley, Kirinyaga, Kitui, Makueni, Baringo, and Coastal regions (HCDA, 2013).

2.4.2 Banana Production and Uses in Kenya

Global production of Banana in the year 2003 was approximately 102 million MT, 32% of which was plantains and 68% classified as banana (FAO, 2003). In Kenya, Horticultural Crops Development Authority (HCDA) cited fifteen different varieties of banana (HCDA, 2013) which are grown in different parts of the country. These include *Uganda Green*, *Apple*, *Kampala*, *Lacatan*, *Williams*, *Sukari*, *Paz*, *Valery*, *Kiganda*, *Poyo*, *Dwarf Cavendish*, *Mararu*, *Gross Michel*, *Grand Nain*, and *Giant Cavendish* (HCDA, 2013).

Table 2.4: The Trend of Banana Production in Kenya (Njuguna et al, 2010)

Year	Area (ha)	Production (MT)	Yield (MT/ha)
1992	76,917[a]	985,982	12.8
1993	79,591[a]	817,508	10.3
1994	49,575[a]	489,537	9.9
1995	44,434[a]	445,733	10.0
1996	45,269[a]	500,627	11.1
1997	75,131[a]	1,057,586	14.1
1998	75,502[a]	1,128,297	14.9
1999	75,286[a]	1,097,673	14.6
2000	74,308[a]	1,027,768	13.8
2001	77,576[a]	1,084,312	14.0
2002	78,154[a]	1,073,001	13.7
2003	79,598[a]	1,019,377	12.8
2004	81,673[a]	1,036,138	12.7
2006	82,000[ant]	-	-
2011	86,183[pro]	1,374,049	15.94
2016	90,580[pro]	1,720,567[s1]	19.00
		2,024,644[s2]	22.35

a: Actual; *ant*: Anticipated; *pro*: Projected
s1: 25% total banana under TC banana; *s2*: 40% total banana under TC banana

Table 2.4 gives the trend of banana production in Kenya from 1992 (actual) to 2016 (prediction).Most of the banana crop grown in Kenya is eaten as either cooked or ripe (dessert) locally. A very minute percentage is exported to foreign markets. AGRA has committed itself to improving banana seeds, developing disease resistant and high quality banana cultivars, intensifying the use of local crops, connecting farmers with local and foreign markets, and assisting them to creategroups (AGRA, 2012).

2.4.3 Use of banana peels as a biofuel

Banana peels are common food wastes, usually, discarded because people feel they lack economic value. Banana peels have been investigated on their potential to produce bioethanol (Manikandan et al, 2008).

Table 2.5: Banana Peels Composition (% dry matter, DM) (Srishail, 2010)

Parameters	**Dry Matter (%)**
Moisture	78.9
Dry matter	14.3
Carbohydrates	60.2

Table 2.5 depicts some of the most important banana peels characteristics with regards to bioethanol production. Through Dilute H_2SO_4 Acid Pretreatment (DAP) and subsequent enzymatic hydrolysis, banana peels have yielded maximum total reducing sugars (TRS) of 36.67% w/w (Arumugam et al, 2011). About one kilogram of banana wastes including skins, stems, and leaves can be produced from ten kilograms of a mature banana plant. Elsewhere in Columbia, 0.85 million MT of surplus banana fruits is produced annually with at least 1.15 million MT of associated biomass produced annually (Mazlan, 2013). These wastes can be processed into glucose through processes such as hydrolysis to create appropriate fermentation feedstocks.

Just like other substrates, the use of banana peels in bioethanol production requires high biomass yields, inexpensive and efficient biomass recovery, and reduced ecological contamination.

2.5 FERMENTATIVE FEEDSTOCKS HYDROLYSIS

This is the cleaving or separation of cellulosic and hemicellulosic polymers to producetheir respective monomers. The major reason for feedstock hydrolysis is to

increase fermentative enzymes accessibility thus improving digestibility of cellulose (Alvira et al, 2010). In hydrolysis process, temperature, acid concentration, and time are very crucial factors to be considered (Mussatto et al, 2010). Complete cellulose hydrolysis gives glucose whereas hemicellulose hydrolysis results to several hexoses and pentoses. There are two major classes of hydrolysis: chemical and enzymatic hydrolysis.

2.5.1 Chemical hydrolysis

In this process, lignocellulosic substrate is exposed to a given chemical at predetermined conditions: time, temperature, and concentration. These factors influence glucose yield in acidic hydrolysis (Ajani et al, 2011). This process results into various sugar monomers derived from polymers in the substrate. The use of Sulphuric Acid in hydrolysis has been investigated (Harris et al, 1945) although other acids like hydrochloric acid have also been employed (Hashem et al, 1993) in the same process. Acidic hydrolysis can be classified as either concentrated acid hydrolysis or dilute acid hydrolysis. Table 2.6 gives some comparison between both the concentrated acidic and dilute acidic hydrolysis techniques (Taherzadeh et al, 2007).

Table 2.6: Comparison between acidic hydrolysis techniques

Technique	Merits	Demerits
Concentrated acid hydrolysis	• Yields higher sugar levels. • Can be carried out at lower temperatures.	• Acid consumption is higher. • Can cause corrosion of hydrolysis equipment. • Acid recovery requires higher energy. • Reaction time is longer.
Dilute acid hydrolysis	• Hydrolysis residence time is shorter. • Acid consumption is	• Yields lower sugar levels. • Corrosion of equipment • Higher temperature

	lower.	• Undesirable by-products are formed

2.5.2 Enzymatic hydrolysis

This method involves the use of enzymes instead of acids in substrate hydrolysis. It is not very popular since it is very expensive and is still at initial stages of research and development.

2.5.3 Lignocellulosic Biomass Hydrolysis Parameters

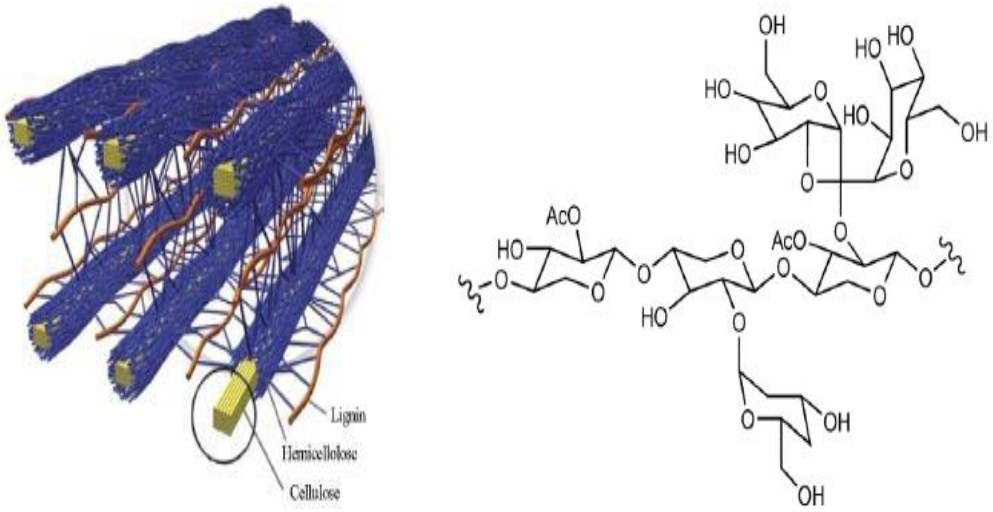


Figure 2.2: Lignocellulosic biomass structure (Galletti, 2011)

Figure 2.2 is a schematic representation of a lignocellulosic biomass structure. This biomass is majorly composed of three polymeric fractions: hemicellulose, cellulose, and lignin.

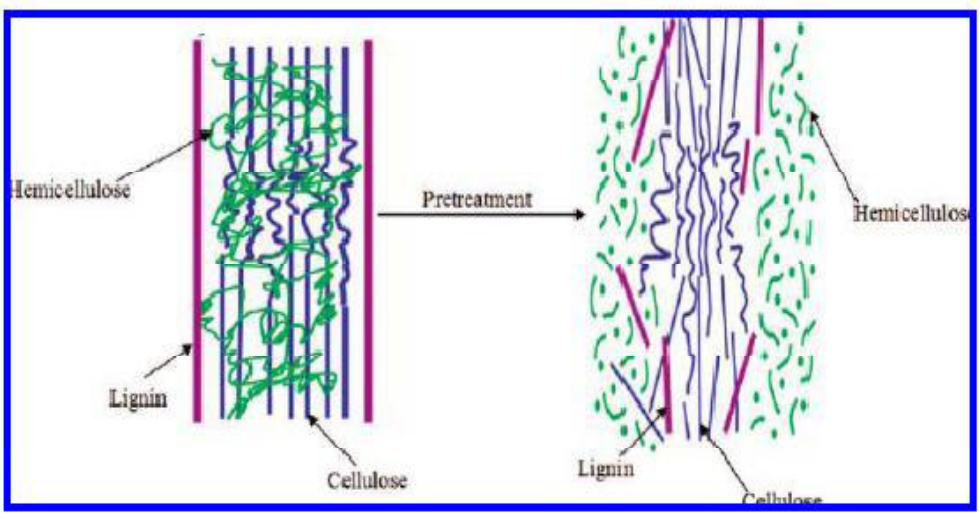


Figure 2.3: Effect of pretreatment on lignocellulosic biomass (Galletti, 2011)

Figure 2.3 is a schematic representation of the effect of pre-treatment on a lignocellulosic biomass. Hydrolysis of lignocellulosic material is a very complex process and biomass themselves are complicated as well (Taherzadeh et al, 2007). Effective parameters normally considered include substrate properties, hydrolysis system acidity, and the rate of decomposition of hydrolysis products.

a. Substrate properties

Biomass properties such as feedstock composition, particles size, neutralizing capacity, hydrolysis rate, ratio of hydrolysable to non-hydrolysable polymers, molecules length, cellulose degree of polymerization, and cellulose chain configuration can influence a hydrolysis process. Cellulose degree of polymerization depends on the plant type and is estimated to be between 2,000 and 27,000 glucan units (Taherzadeh et al, 2007).

Large material's surface area is enhanced by its fineness. It presentsmany sites for reactions during hydrolysis. Lignocellulosic biomasses mainly contain carbohydrate polymers, lignin, and ashes. Carbohydrate polymers are classified as either cellulosic or hemicellulosic. Holocellulose refers to the total carbohydrate found in a microbial or plant cell. Carbohydrates in lignocellulosic materials are called holocellulose. Hemicelluloses in a lignocellulosic biomass range from 10% to 37% of its dry weight. Decomposition rate of hydrolysis products also depends on sugars concentration and medium acidity, both of which are attributed to the feedstock nature.

Particle size is one of the key factors in substrate hydrolysis (Taherzadeh et al, 2007). Finer particle sizes reduce biomass crystallinity thus enhancing sugars yield during hydrolysis. Finer particle sizes also elevate sugar recovery and ensures energy saving.

19% sugar recovery was achieved using a 149 µm screen in banana wastes hydrolysis (Mohammadet al, 2011). Porous feedstocks also allow penetration of the hydrolyzing agent during sugars recovery processes.

b. Hydrolysis system acidity

System acidity depends on the acid type used, acid concentration, amount of acid used, and quantity of acid released from the substrate during hydrolysis such as acetic acid, liquid to solid ratio, substrate neutralizing capacity, and hydrolysis medium movement during heating. Using dilute acids in a continuous reactor minimizes residence time in a dilute acid hydrolysis process. Penetration of an acid catalyst and dispersion in hydrolysis system can greatly influence the process as well as performance of the reactor. Sulphuric acid diffusivity relies on lignocellulosic biomass nature and it has been proven that it is better in agricultural wastes than hardwood (Sung-Bae et al, 2002).Agricultural wastes are loosely packed as compared to hardwood rendering them higher porosity.

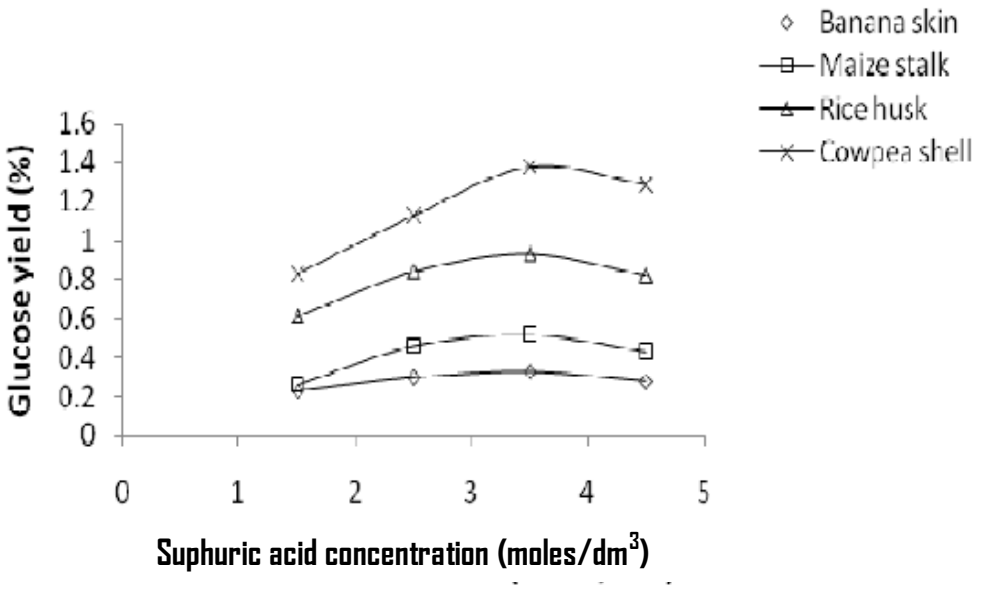


Figure 2.4: Effect of acid concentration on glucose yield (Ajani et al, 2011)

Figure 2.4 depicts the effect of sulphuric concentration on glucose yield of some lignocellulosic feedstocks during acidic hydrolysis. Percentage cellulose yields from maize stalks, cowpea shells, and banana skin were 56.7%, 37.2%, and 28.4% respectively. 60.12% had also been obtained from wheat straws (Ajani et al, 2011). Manikandan used 2 ml/g of 67% H_2SO_4 in the hydrolysis of banana peels substrates while steaming for 60 minutes and achieved viable fermentable sugars to produce maximum bioethanol production of 9 g/L in yeast fermentation (Manikandan et al, 2008).

c. Decomposition rate of hydrolysis products

This majorly depends on sugars concentration, medium acidity, reaction time, and hydrolysis temperature.

2.5.4 Detoxification of a Lignocellulosic Hydrolysate

Both the feedstock pretreatment procedures and hydrolysis create inhibitory substances (South, 2010). These toxic substances affect fermentation of the derived hydrolysates. Concentration of various toxic compounds depends on the material used, pretreatment processes and pretreatment severity. Inhibitory compounds present in lignocellulosic hydrolysate can possibly be bio-transformed.

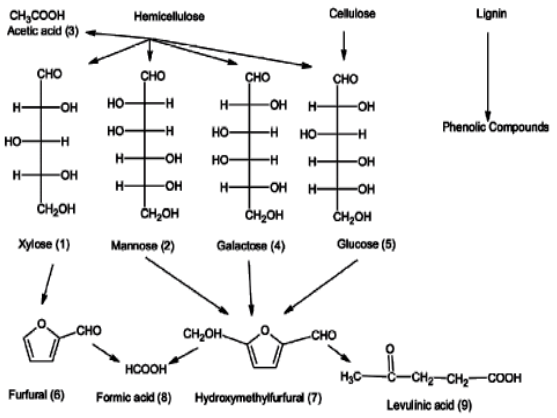


Figure 2.5: Lignocellulosic derived inhibitors to fermentation (Chandel, 2011)

Figure 2.5 depicts lignocellulosic derived inhibitors to fermentation processes which can wholly be metabolized by *Saccharomyces cerevisiae* in some cases. This transformation is possible for several phenolic compounds, carboxylic acids, and even furans during lignocellulosic hydrolysates fermentation.

2.6 MICROORGANISMS IN FERMENTATION

Microorganisms used in fermentation are categorized as either prokaryotic cells or eukaryotic cells. Prokaryotic cells include unicellular (e.g. bacteria and cyanobacteria) and multicellular (e.g. cyanobacteria) whereas eukaryotic cells include unicellular (e.g. yeast and algae) and multicellular (e.g. fungi and algae). Micro-fauna and unicellular are rarely employed in fermentation processes whereas isolated cells from multicellular animals are mostly cultured (Srishail, 2010).

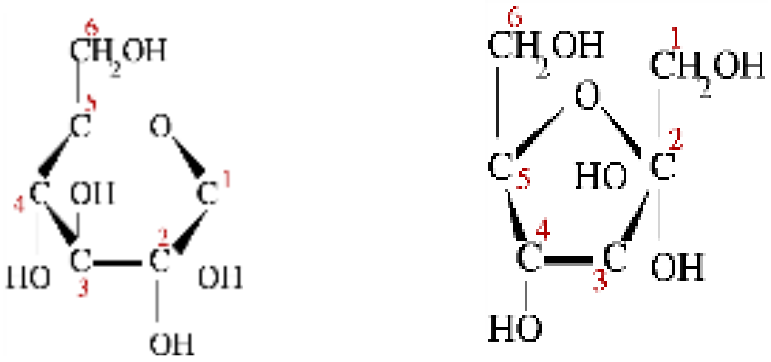
2.6.1 Yeasts

Yeasts are eukaryotic microorganisms belonging to the kingdom of Fungi. Yeasts are some of the most demanded and utilized unicellular microbes. Over 1500 species of yeasts have been described (Cletus et al, 2006) in various studies involving their microbiology. Primitive man used yeasts unknowingly in the preparation of alcoholic beverages. Fermentation by *Saccharomyces cerevisiae* species converts carbohydrates into CO_2 and alcohols. This concept has been applied over the years in baking and production of alcoholic beverages (Legras et al, 2002).

Bioethanol fuel production by conversion of sugars using yeast into ethanol has been constantly investigated in biotechnology studies. Yeasts contain enzyme, invertase or sucrase (intra/extra cellular), which acts as acatalyst and helps in the conversion of sucrose sugars into glucose and fructose as shown in equation 2.1.

$$C_{12}H_{22}O_{11_{Sucrose}} + H_2O \xrightarrow{Invertasecatalyst} 2C_6H_{12}O_{6_{Glucose+\ Fructose}} \quad (2.1)$$

Maximum invertase production of 16.10U/ml has been achieved at 30°C incubation temperature (Muhammad, 2011). Microbiology of yeast cells considers several functions such as enzyme formation rate, enzyme production rate, biomass production rate, and substrate consumption rate among others. Studies have shown that both the enzyme synthesis and substrate consumption rates are affected by fermentation temperatures (Muhammad, 2011). This confirms the importance of incubation temperature in yeast fermentation processes.



(a) (b)

Figure 2.6: (a) D-Glucose, (b) D-Fructose (Bloemen et al, 2010)

Figure 2.6 shows the arrangement of both the (a) D-Glucose molecule and the (b) D-Fructose molecule. The glucose and fructose are degraded to produce bioethanol with the aid of another enzyme, zymase, also contained in yeasts as shown in equation 2.2.

$$C_6H_{12}O_{6_{Glucose+\ Fructose}} \xrightarrow{Zymasecatalyst} 2C_2H_5OH + 2CO_2 \quad (2.2)$$

2.6.2 Baker's Yeast Fermentation

Table 2.7: Composition of a fresh baker's yeast (Argyro et al, 2006)

Component	**Approx. composition (% w/w)**
Dry materials	30 - 33
Nitrogen	6.5 - 9.3
Protein	40.6 - 58.0
Carbohydrates	35.0 - 45.0
Lipids	5.0 - 7.5
Vitamins	Dependent on growth conditions

Table 2.7 highlights the composition of a fresh baker's yeast. This yeast is commercially available in various forms as liquid, creamy or in compressed forms, and the active dry yeast (Argyro et al, 2006). A special strain of *Saccharomyces cerevisiae* can be utilized in the production of various yeast products: instant dry yeasts or active dry yeasts. Instant dry yeasts are available as fine particles and do not need rehydration prior to their usage whereas active dry yeasts are available as beads or grains of live dried yeast cells which have the leavening power. Inactive dry yeast is another product which does not have the leavening characteristics. It is majorly used in the development of a characteristic flavor as well as conditioning dough properties in the baking process. Most of these yeast products are available on-shelf in various retail outlets.

2.6.3 Yeast adaptation

Inoculation of a growth medium with a microorganism such as yeast means introducing them to a condition at which they have to cope with in order to survive and enhance their productivity. Microorganisms' growth undergoes series of phases: lag, growth, deceleration, and stationary phases as shown in the figure.

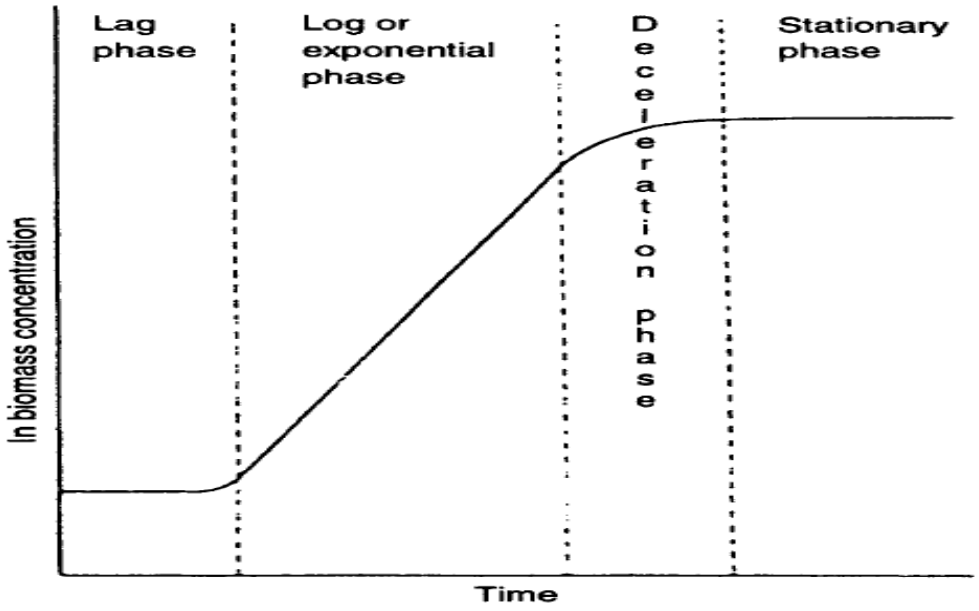


Figure 2.7: Kinetics and phases of yeast growth (Peter, 1995)

Figure 2.7 presents both the kinetics and phases of yeast growth. Major yeast growth phases include the lag, the log / exponential, the deceleration, and the stationary / steady phase.

a. Lag Phase

When a microorganism is introduced to a growth medium, its growth does not occur immediately. This is due to adaptation of the microorganism to the new conditions.

b. Growth / Log / Exponential Phase

Immediately after the lag phase, growth of a microorganism is rapidly increased for some time known as the growth phase.

c. Deceleration Phase

After a given duration, due to factors such as the accumulation of inhibitory substances, the growth rate starts decelerating.

d. Stationary / Steady Phase

Finally, cultures stop growing and biomass attains a constant concentration.

2.6.4 Inoculum

Improved initial population of cells enhances better substrates' (sugars) consumption as well as products' formation in a bioethanol fermentation process. Information on yeast responses in industrial scale bioethanol production is limited because the associated media stresses have not been widely studied (Muhammad, 2011).

2.7 FERMENTATION

Basically, fermentation involves feeding a solution of sugars, other nutrients, and minerals to yeast cells which in turn produces alcohol and carbon dioxide. Other than the common bioethanol production application, fermentation is also applied in food preservation due to its energy efficiency and low cost (Aidoo, 2011).The process of sugar fermentation progresses until either the sugar is fully depleted or the yeast can no longer tolerate the broth conditions which highly depends on the yeast strain used (Aidoo, 2011). Equation 2.3 shows both the reactants and products of a glucose degradation process.

$$C_6H_{12}C_6 + 2ATP + 2ADP + 4NADH \rightarrow 2C_2H_5OH + 2CO_2 + 4ATP + 2H_2O + 4NAD^+ \quad (2.3)$$

In this energy generation process (fermentation), organic compounds act both as electron acceptors as well as donors. This implies that fermentation is a non-oxygen (anaerobic) consuming process.

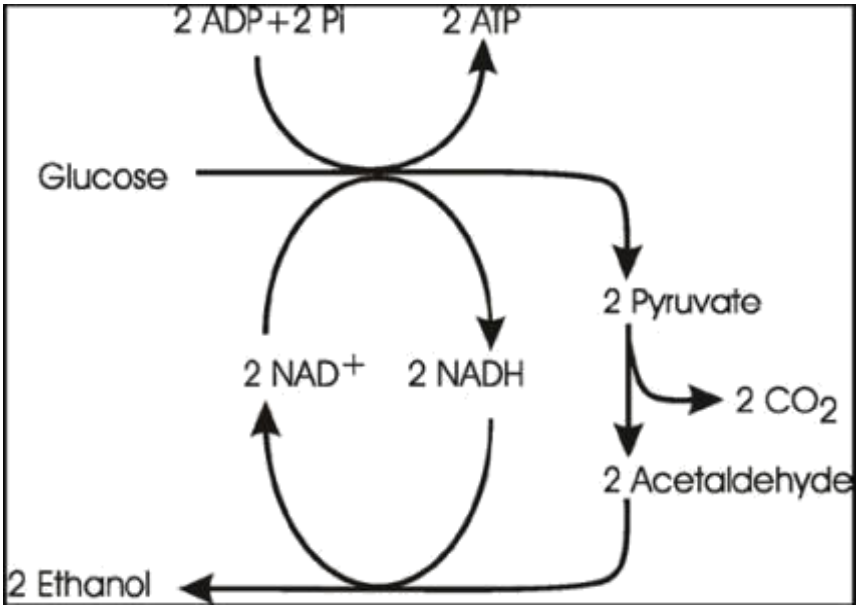


Figure 2.8: Fermentation process (Held, 2012)

Figure 2.8 shows a fermentation process, as depicted by equation 2.3 above, in which energy production is without oxygen participation or any other electron acceptor.

2.7.1 Applications of microbial fermentation

Fermentation is categorized into five major applications (Peter, 1995).

a. Production of recombinant products.
b. Production of microbial enzymes.
c. Production of microbial metabolites.
d. Production of cells.
e. Transformation processes i.e. modification of a compound added in a fermentation process.

2.7.2 Some factors Influencing Fermentation Processes

Some of the major factors influencing fermentation results include substrate concentration, fermentation temperature, initial medium pH, yeast concentration, and incubation period.

a. Temperature

Internal broth temperature is very crucial in bioethanol fermentation processes. Temperatures above 60^{o}C kill microorganisms. Yeasts are generally active between 0^{o}C and 50^{o}C and suitable fermentation temperatures always range between 25^{o}C and 30^{o}C (Aidoo, 2011). Biochemical processes during fermentation also generate some residual heat (exothermic) which may offset the ideal temperature. Temperature during fermentation controls yeast growth and products accumulation. Slow fermentation and low temperatures favor volatile compounds accumulation.

Highest achievable enzyme activities correspond to the highest bioethanol yields. This is because the productivity of invertase highly relies on temperature (Muhammad, 2011). Further incubation temperature increase result to low bioethanol productionattributed to the reduction in invertase productivities. High temperatures inactivate yeast culture and therefore not conducive for yeast growth (Muhammad, 2011). Thermo-tolerant strains of yeast are the best at high incubation temperatures.

Banana peels fermentation at 33^{o}C has been tried and produced a maximum of 9 g/L bioethanol (Manikandan et al, 2008). Lin also realizedmaximum bioethanol production from glucose fermentation at temperatures between 30 ^{o}C and 45^{o}C under varying glucose concentrations (Yan et al, 2012).

b. pH

pH, usually, measured using modern digital pH meters, refers to the concentration of H^{+} (hydrogen ions) of an acidic solution or conversely the concentration of OH^{-} (hydroxyl ions) of an alkaline solution. Internal medium pH affects fermentation directly. Most microorganisms including yeasts are active at a slightly neutral pH whereas pH less than 3.5 eliminates or kills them. Lower than required pH may lead to

growth of undesired microorganisms (Aidoo, 2011). Inhibitory effect of pH above the optimum values is attributed to the reduced formation of ATP across metabolic changes in yeast (Muhammad, 2011). Traditionally, pH in fermentation was controlled using ammonium acetate and sodium bicarbonate.

Figure 2.9 shows the effect of pH on bioethanol production with initial glucose concentration of 40 kg/m^3 over an incubation period of one week. This Research realized maximum bioethanol yields at a pH value of 4.0 (Yan et al, 2012). Most yeast and other fungi withstand a pH range of between 3.5 and 5.0 (Mustafa et al, 2008).

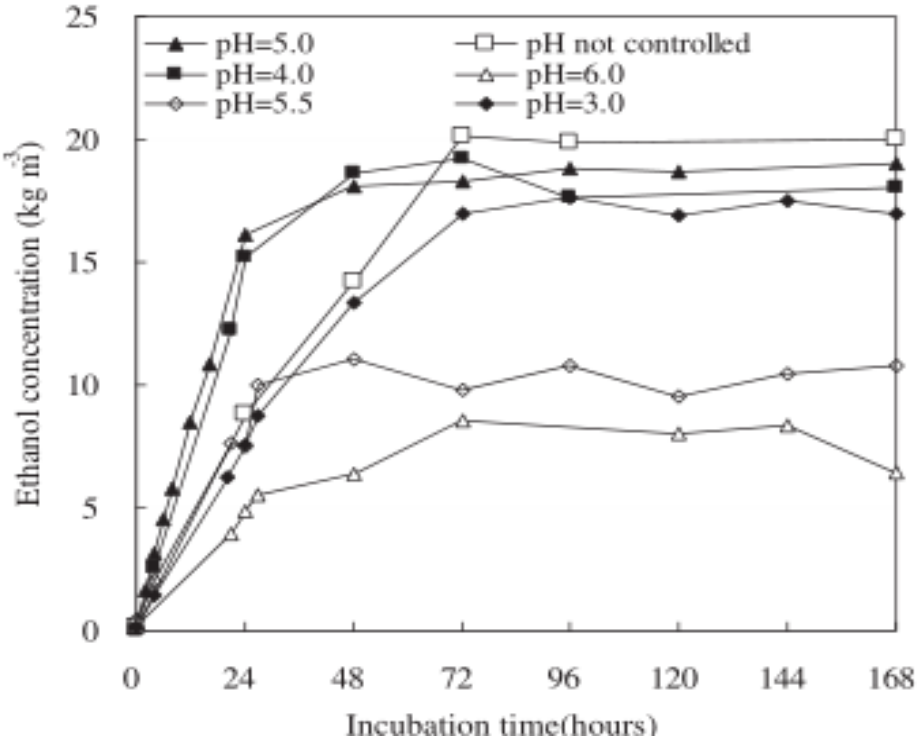


Figure 2.9: Effect of pH on Bioethanol production (Yan et al, 2012)

Banana peels fermentation at a pH of 4.5 also produced a maximum of 9 g/L bioethanol yield (Manikandan et al, 2008).

c. Microorganisms

Microorganisms are identified with their respective fermentative product. Yeast has been known for bioethanol fermentation processes and has been used by several researchers. Yeasts among other similar fungi are found in the air, vineyards, orchards, animals' intestinal tract, and soil amongst other environments. Yeasts can be used in dead or dried form. Recommended yeast dosage ranges between 0.24g/L and 1.00g/L (Aidoo, 2011). Banana peels fermentation with 2% v/v yeast inoculum produced a maximum of 9 g/L bioethanol yield (Manikandan et al, 2008) and ripened red banana and their hydrolyzed peels produced 1.3% and 0.27% v/v respectively with 10% v/v yeast inoculation (Kumar et al, 2011).High residual broth substrate levels have always been attributed to few yeast cells in a fermentative system (Peter, 1995).

d. Substrate Concentration

Sugar is the most common and a major fermentation carbon source even though other nutrients like fats and proteins can also be degraded by other microorganisms in cases of limited sugar (Aidoo, 2011). Fermentative sugars are also used in cell maintenance and ATP generation (Shafaghat et al, 2010).

Despite the importance of sugar in a fermentation process, its high concentrations may inhibit microorganisms' growth. Some yeasts tolerate fairly high sugar concentrations of about 40% w/v. Certain yeast strains (osmophilic) can tolerate sugar concentrations above 40% w/v. Few yeasts withstand sugar concentrations between 65% and 70% w/v (Aidoo, 2011).

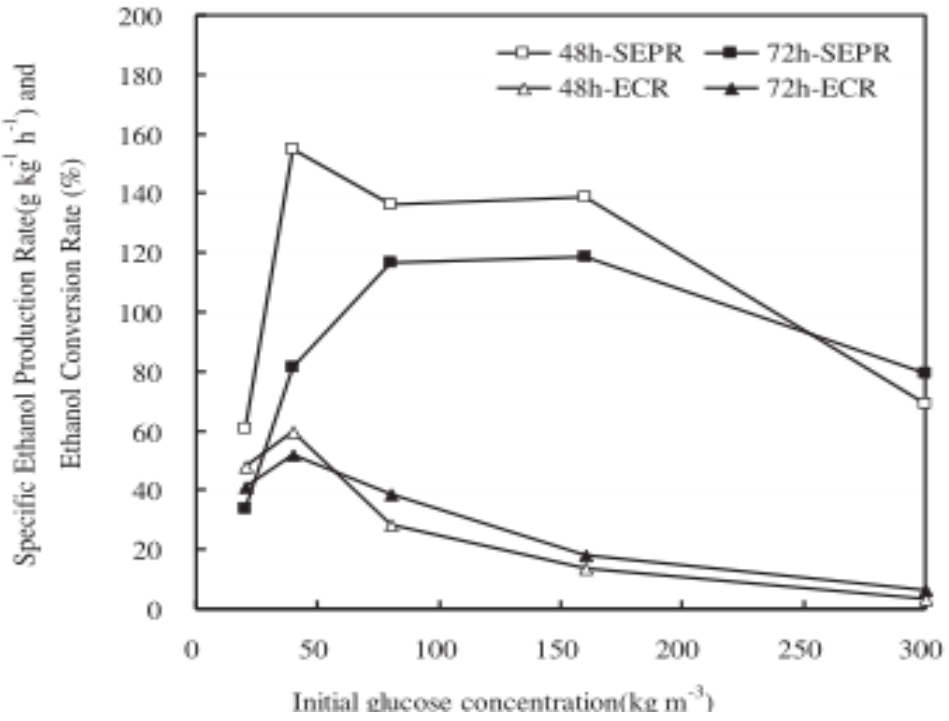


Figure 2.10: Effect of initial glucose concentration on fermentation (Yan et al, 2012)

Figure 2.10 shows specific bioethanol production rates and conversion efficiencies with different initial glucose concentrations after 48 and 72 hours incubation at 30^{o}C (Yan et al, 2012). In a fermentation process, bioethanol concentrations increase with increasing substrate concentrations up to an optimum level. At high substrate sugars, yeast cells overcome osmotic stresses attributed to the increased bioethanol concentrations in the bioreactor (Muhammad, 2011) which tends to stop further yields. Banana peels fermentation at 10% w/v produced a maximum of 9 g/L bioethanol yield (Manikandan et al, 2008) and ripened red banana and their hydrolyzed peels produced 1.3% and 0.27% v/v respectively with 10% w/v substrate concentration (Kumar et al, 2011).

e. Incubation period

Time is a very important factor in fermentation processes.Longer broth retention times ensure complete fermentation whereas shorter retention time leads to high quantities of residual sugars.

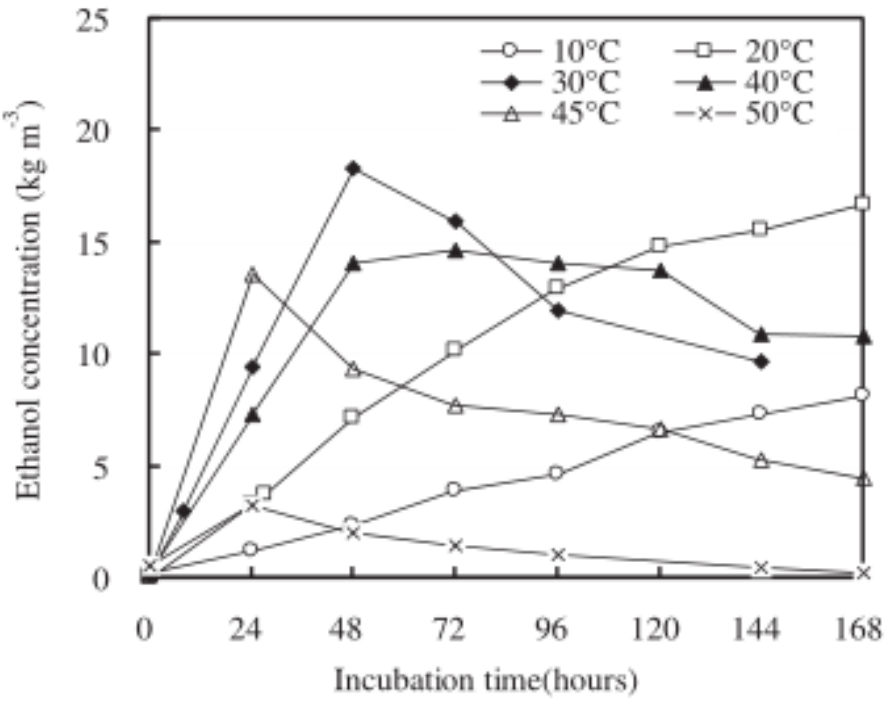


Figure 2.11: Kinetics of bioethanol production (Yan et al, 2012)

Figure 2.11 shows the trend of bioethanol production with initial glucose concentration of 40 kg/m^3 over an incubation period of one week at different fermentation temperatures (Yan et al, 2012).

f. Bioethanol concentrations

High bioethanol levels in a fermentative broth can kill yeasts. Most microorganisms cannot withstand bioethanol concentrations above 10 - 15% w/vwhereas ethanologens produce significantly above 1% w/v of bioethanol (Mustafa et al, 2008).In banana peels fermentation, 2.7 ml/L bioethanol yield was realized by fermenting hydrolyzed red banana peel at 10% substrate concentration (Kumar, 2011), 11.41 ml/L from banana

peels fermentation using mutant strain of *Saccharomyces cerevisiae* (Manikandan et al, 2008), 10.14 ml/L achieved through Simultaneous Saccharification and Fermentation (SSF) process involving the co-culture of both *Saccharomyces Cerevisiae* and *Candida tropicalis* (Nuttiya et al, 2013), and 13.00 ml/L gotten through Separate Hydrolysis and Fermentation (SHF) process involving the same co-culture.

Bioethanol yield of 39.29 ml/L has also been achieved by fermenting banana peels within72 hours incubation period (Vikash et al, 2012), 35.74 ml/L from hydrothermally pre-treated banana peeling has also been attained by optimizing Simultaneous Saccharification and Fermentation (SSF) processes (Harinder et al, 2011), and 19.00 ml/L from banana peels fermentation within 96 hours incubation (Arati et al, 2010) have also been achieved.

These differences are most often attributed to the type of banana cultivars used, fermentation media composition, microorganisms' purity, culturing procedures and majorly fermentation techniques involved alongside other factors influencing fermentation.

2.7.3 Sugars in Fermentation

Different types of sugars can be degraded by various microorganisms e.g. yeasts degrade some sugars to bioethanol when subjected to suitable conditions which can facilitate their growth. Theoretical yields of bioethanol from these sugars can be calculated based on stoichiometric equations regarding conversion of respective substrate content to bioethanol as given by equations 2.4 to 2.7:

a. Pentosan to Pentose

$$\begin{array}{ccccc} nC_5H_8O_4 + & nH_2O & \rightarrow & nC_5H_{10}O_5 & \\ n132_{MWU} & n18_{MWU} & & n150_{MWU} & \\ (1\ gram) & (0.136\ gram) & & (1.136 gram) & \end{array} \qquad (2.4)$$

b. Hexosan to Hexose

$$\begin{array}{lll} nC_6H_{10}O_5 + & nH_2O & \rightarrow \ nC_6H_{12}O_6 \\ n162_{MWU} & n18_{MWU} & n180_{MWU} \\ (1\ gram) & (0.1111\ gram) & (1.111 gram) \end{array} \quad (2.5)$$

c. Pentose and Hexose to Ethanol

Pentose:

$$\begin{array}{lll} 3C_5H_{10}O_5 \ \rightarrow & 5C_6H_{12}O_6 \ + & 5CO_2 \\ 3 * 150_{MWU} & 5 * 46_{MWU} & 5 * 44_{MWU} \\ (1\ gram) & (0.511\ gram) & (0.489\ gram) \end{array} \quad (2.6)$$

Hexose:

$$\begin{array}{lll} C_6H_{12}O_6 \rightarrow & 2C_2H_5OH \ + & 2CO_2 \\ 180_{MWU} & 2 * 46_{MWU} & 2 * 44_{MWU} \\ (1\ gram) & (0.511\ gram) & (0.489\ gram) \end{array} \quad (2.7)$$

Different substrates contain varying proportions of fermentable sugars suitable to a particular microorganism. Equations 2.4to 2.7 above can be used to compute stoichiometric products' yields from various sugar degradation processes. For instance, in a glucose molecule oxidation (respiration), 180 unit mass of glucose would require 192 unit mass of oxygen for a complete oxidation. *Saccharomyces cerevisiae* cells obtain energy for growth by digesting foods containing sugars in its various forms. Sugars popular to yeast fermentation include:

i) **Sucrose** (non-reducing disaccharide) is found in sugarcane or sugar beets.

ii) **Fructose** A (monosaccharide ketopentose) is found in fruits, maple syrup, molasses, and honey.

iii) **Glucose** (monosaccharide aldohexose) is also found in fruits, maple syrup, molasses, and honey as fructose (iii above).

iv) **Maltose** comes from starch in the flour.

2.7.4 Sugar Fermentation Pathways

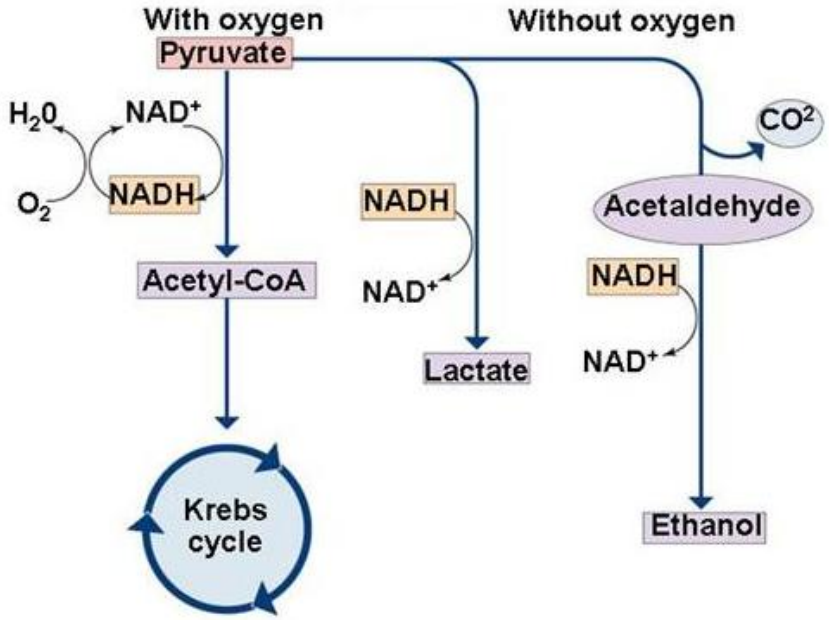


Figure 2.12: Fermentation pathways (Bioserv, 2004)

Figure 2.12 shows both the aerobic and anaerobic fermentation pathways. Yeast obtains energy through glucose oxidation as shown above.

a. Anaerobic alcoholic fermentation

Through this pathway, pyruvate which results from glycolysis of glucose is further decarboxylated to ethanal (acetaldehyde) which is then reduced to ethyl alcohol (ethanol). Only 2 more ATP molecules per glucose molecule are produced on top of the 2 which result from glycolysis.

b. Aerobic respiration

Yeast, usually, prefers this route. Glucose glycolysis results to pyruvate and 2 ATP molecules per glucose molecule. The resulting pyruvate is further oxidized to CO_2 and H_2O through a citric-acid cycle as well as oxidative phosphorylation. Through this pathway, further 36 to 38 ATP molecules per glucose molecule are produced. Aeration is controlled making the pathway a forbidden one.

c. Glyceropyruvic fermentation

8% glucose goes through this pathway. This pathway is very important at the initial stage of alcoholic fermentation at which the enzyme alcohol dehydrogenase (which converts ethanal to ethanol) is limited.

2.7.5 Agitation and Aeration in Aerobic Fermentation

Agitation or stirring maintains even conditions in a fermentation medium whereas aeration supplies microorganisms with the necessary oxygen. Therefore agitated and aerated fermentation is characterized with highly effective and efficient mass transfer within the liquid medium during a microbial degradation process (Hensirisak, 1997). The influence of oxygen on the propagation of yeast, as well as yeast fermentative capability in various cultures, has been extensively studied (Muhammad, 2011).In laboratory scale fermentations, shaker flasks are employed while, in pilot and production scale setups, mechanical agitation and compressed air aeration are, usually, employed. Today, well designed bioreactors fitted with conditions' control systems which achieve high cell growth as well as optimal products formation are used in industrial scale establishments.

2.7.6 Still and Anaerobic fermentation

In both the fed batch and batch fermentation, limited oxygen supply elevates bioethanol formation (Hensirisak, 1997; Muhammad, 2011). However, oxygen supply at initial stages is important to ensure high cell growth in order to satisfy the demand of microorganisms. Low oxygen supply can also lead to the system being considered anaerobe. With anaerobic (no oxygen) fermentation environments, yeast cells do not consume substrate in the production of more cell mass but convert glucose through fermentative processes to bioethanol and carbon dioxide instead (Hensirisak, 1997).

2.7.7 Bioethanol Purification

Yeast fermentation leads to the formation of several undesired compound such as organic acid (e.g. acetic acid), higher alcohols, methanol, and aldehydes among others which are very difficult to separate using a normal distillation procedure (Muhammad, 2011). The presence of these by-products reduces the quality of bioethanol produced. Aeration during fermentation influences the production of higher alcohols (fusels). Production of these higher alcohols in bioethanol fermentation can also be attributed to the yeast strain used (Muhammad, 2011). For instance, in an orange juice fermentation study, *Saccharomyces cerevisiae var. ellipsoideus* yielded a maximum of 0.9038v/v bioethanol while *Saccharomyces cerevisiae* yielded a minimum of 0.8149 v/v bioethanol. *Saccharomyces cerevisiae var. ellipsoideus* yielded 0.0951v/v methanol while *Saccharomyces carlsbergensis* yielded 0.1493 v/v methanol (Muhammad, 2011). Isopropanol yield among fusels was nearly negligible. During this orange juice fermentation, only bioethanol could be desirable leaving the rest to be discarded as unwanted by-products or diverted to other uses. Therefore after a complete fermentation process, there is a need to separate various products for their respective usages. Bioethanol is, usually, separated from the broth through distillation and further dehydration.

a. Bioethanol Distillation

Distillation which is deemed to be among the oldest practices has been employed in the separation of molten and liquid substances. It has also been used in domestic and industrial cleaning operations. This is an energy intensive and very scalable technology but comparatively cheaper than chromatographic approaches of components' separation (Mazlan, 2013). Separation of fermentative products and by-products is very important in bioethanol production. Distillation is always opted for because it is a simple, fast,

and an effective separation and cleaning technique. Fermentate distillation can beemployed to obtain up to 96% v/v bioethanol (U.S., 2009).

b. Bioethanol Dehydration

After the distillation process, further elimination of the water proportion is necessary to enhance bioethanol purity. However, attaining absolutely pure bioethanol has never been practical due to the azeotropic nature of ethanol-water mixture.

2.7.8 Bioreactors used in fermentation

Fermentation can be done in either closed or open bioreactors. It was traditionally performed in tanks made of concrete or large wooden barrels which were susceptible to unwanted wild microorganisms and hectic to clean (Aidoo, 2011). Modern fermentation industries use highly hygienic stainless steel tanks fitted with sophisticated control systems. They are also fixed with high conductivity cooling rings to control fermentation temperature.

2.7.9 Economics of a Fermentation Process

The core expectation of any fermentation process is to get viable amounts of fermentative products which must reciprocate their total production cost as well as giving some profit. A fermentation process just like other manufacturing processes must consider current market conditions like demand and supply. Bioethanol is common in most markets because it has been present and known over the era. However, it is still new to others especially on its span of applications. Kenya, through the National Energy Policy, has been working on plans to harmonize taxes associated with bioethanol and other biofuels with an aim of discouraging their exportation (Ministry of Energy, 2012).

Difficulties in marketing bioethanol in some market segments can be attributed to low demand or limited knowledge on its applications. Most people, who would be users of bioethanol, know of little or no technologies that consume this product. In order for bioethanol, just like other products, to compete fairly in a market system, its benefit per cost should be lower than similar or alternative candidate products. This means that its unit production cost should be as low as possible. The economic state of bioethanol as a fermentative product is associated with both its production costs as well as distribution. These costs include fermentation broth composition cost, human resource charges cost, fermentation media and equipment sterilization cost, bioethanol recovery cost, bioethanol purification cost, waste management cost, and research cost on process development and improvement (Casida, 1989). High broth bioethanol concentration makes distillation process easy and less expensive thus contributing to the economics of the entire bioethanol production process. Decrease in bioethanol yield is very significant and can cause very enormous financial losses. Bioethanol industries have very narrow profit margins and a 1.0% concentration decrease would be quite significant (Muhammad, 2011).

a. Fermentation broth cost

This is the cost associated with the fermentation and microorganism culturing media. Alternative cheap materials are, usually, applied to reduce the production cost because this is where feedstock is accounted for. Fermentation feedstock take the largest proportion of fermentation cost thus cheap alternative materials which can be fermented with high efficiency to the desired economic products such as bioethanol are significant in industrial scale manufacturing processes (Muhammad, 2011).

b. Human resources cost

This bioethanol production cost is associated with labour charges. Both the skilled and non-skilled staffs are required at various manufacturing stages. Major activities in bioethanol manufacturing include microorganism culturing, inoculation, fermentation setup, product recovery processes like distillation and purification, bioethanol packaging, and management and administrative responsibilities. Good planning is, therefore, necessary to maximize work-man-hour per employee and ensuring high return from their services.

c. Fermentation media and equipment sterilization cost

High population of contaminant cells influences the growth of yeast which further affects bioethanol recovery thus leading to huge losses (Muhammad, 2011). To avoid strenuous or wild microorganisms, both the fermentation medium and microorganism's handling equipment be thoroughly sterilized. Sterilization methods include low media pH, heat treatment, and the use of chemicals that inhibit the growth of unwanted microorganisms.

d. Bioethanol recovery cost

This is the most important step as it involves the product's harvesting. Poor recovery will lead to high wastage and severe losses in the entire economic system of bioethanol production.

e. Bioethanol purification cost

High purity elevates the product value. Bioethanol can be purified by multiple distillations as well as chemical dehydration.

f. Waste management cost

This would consider the minimum accepted waste composition by the municipal authorities. This implies that certain waste treatment procedures might need to be performed before they are released to the disposal sites.

g. Research cost

It is important to investigate operation procedures that would translate to highly competitive bioethanol in the market. A research team may sometimes be engaged in investigating an existing production system or a new production initiative.

2.8 FERMENTATION PROCESS ANALYSIS

2.8.1 Qualitative Analysis of Bioethanol

A redox technique can be applied in confirming the presence of bioethanol in an aqueous mixture solution (Kumar et al, 2011). Reacting bioethanol with excess potassium dichromate in the presence of an acid oxidizes it to an ethanoic acid as shown in equation 2.8.

$$Cr_2O_7^{2-} + 16H^+ + 3C_2H_5OH \rightarrow 4Cr^{3+} + 11H_2O + 3CH_3COOH \quad (2.8)$$

The yellow color of potassium dichromate changes to blue which is a positive result for the presence of bioethanol in the mixture. Titration and colorimetric methods can be performed to further find out the quantity of bioethanol in the mixture using calibrated curves.

2.8.2 Chromatography

Indirect physical methods like refractometry, hydrometry, and polarimetry have been used in both monosaccharides and disaccharides determination. Chemical methods which are semi-empirical such as colorimetry, volumetric analysis, and gravimetry

have also been applied as well (Robards et al, 1986). The above chemical methods can be used to detect a class of sugars. Their principles are based on color reactions. Degradation products of these sugars in a strong mineral acid condense into various compounds which affect the color of the sample to be investigated e.g. in the common Bial and Molisch test. Color reactions can as well be affected by cleavage or breakdown of neighboring hydroxyl (-OH) groups. Some rely on carbonyl (-COOH) group's reducing properties e.g. the Fehling's test.

Chromatographic techniques are very effective in the analysis of type and fraction concentrations. There are three major types of chromatographic methods including Gas Chromatography (GC), High Performance Liquid Chromatography (HPLC), and Thin Layer Chromatography (TLC). GC is based on components' selective affinity towards adsorbent materials. First the sample (gas/liquid) is introduced into the GC injection port using a special syringe. The sample is then vaporized at the injection port and consequently moves through the column assisted by a carrier stream which is in a continuous flow i.e. the mobile phase. The vaporized sample is then separated or detected at the GC detection port programmed at a suitable temperature. The result is then viewed using specialized computer softwares e.g. GC Solution, Peak ABC, etc in the form of peaks.

2.8.3 Colorimetry

Colorimetric optic techniques of assay, due to their selectivity, fastness, low cost, and cheap sample preparations, are always preferred (Gerardo et al, nd). Colorimetry in which the reagent color changes or a certain color is formed or generated due to occurring chemical reactions can be used in analyzing complex matrices. A colorimetric assay technique is done by shining a beam of light through the sample

placed in a cuvette. A highly photosensitive element detects light absorption through the sample. OD (Optical Density) is then read out on a calibrated digital scale.

2.8.4 Total Reducing Sugars (TRS) Test

Reducing sugars and polysaccharides analysis using the colorimetric method has been present for quite some time. Analysis of sugars using Phenol-Sulphuric Acid (PSA) technique relies on light absorbance through the sample inside a cuvette at a wavelength of 490 nm (Fournier, 2001). In the absence of a colorimeter, qualitative observation can be done by visual comparison using known concentration colored samples.

Phenol (C_6H_5OH), a volatile white crystalline solid, is an aromatic organic compound. It is also known as carbolic acid. A molecule of phenol is made up of a combination of a phenyl group ($-C_6H_5$) bonded to a hydroxyl group (-OH) as shown in figure 2.13.

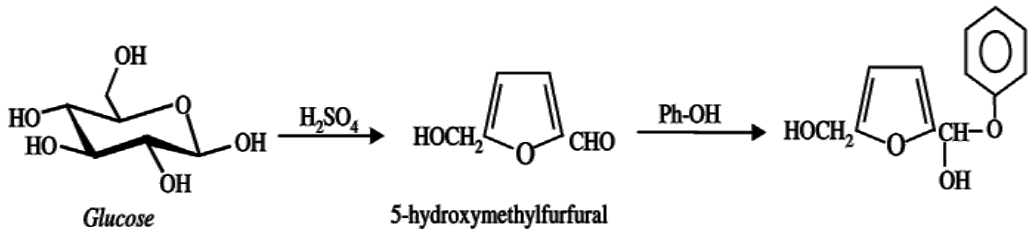


Figure 2.13: PSA method for sugar determination (Panagioto etal, 2005)

Figure 2.13 give the PSA method for sugar determination. C_6H_5OH in the presence of H_2SO_4 is applied in quantitative colorimetric microanalysis of various sugars, methyl derivatives of these sugars, oligosaccharides, and polysaccharides (Dubois, 1956). This method which is dubbed the 'Dubois method' is simple, sensitive, rapid, and is known for giving reproducible results. Reagents required in the Dubois method are relatively cheap and stable. A specific solution only needs one standard curve per sugar being analyzed. Color produced in PSA method is permanent and remains stable for a long

duration. Paying special attention to the method's conditions control isvery necessary (Dubois, 1956).

Reagents required in PSA method(Dubois, 1956) in include:

a. 95.5% v/v H_2SO_4
b. 80% w/v C_6H_5OH

Procedures for PSA method of sugars analysis (Dubois, 1956)are as follows:

a. 2ml solution to be tested is pipetted into a colorimetric tube or a cuvette.
b. 80% w/v C_6H_5OH is then added.
c. 95.5% v/v H_2SO_4 is then added rapidly.
d. The colorimetric tube or cuvette is then allowed 10 minutes to stand.
e. Colorimetric tubes or cuvettes are then shaken and placed in 25^oC to 30^oC water baths for about 10 to 20 minutes.
f. The resultant yellow orange color is measured at 490 nm for hexoses and 480 nm for pentoses and uronic acids.
g. Amount of sugars is then determined using reference standard curves calibrated for a specific sugar.

It is important to take triplicate results in order to minimize errors which could have resulted from contaminations such as the cellulose lint contamination. For difficulties in using micro-pipettes, add phenol as 5% solution in distilled water. Use 1 or 2 ml sample solution, 1 ml of 5% C_6H_5OH in distilled water, 5ml concentrated H_2SO_4, and thereafter proceed with similar steps as shown above(Dubois, 1956).

2.9 PROCESS OPTIMIZATION

2.9.1 Response Surface Methodology (RSM)

Response Surface Methodology generally referred to as RSM comprises of both statistical and mathematical techniques used to generate and analyse models. The main concept is to determine optimal responses (Mazlan, 2013). First, the Design of Experiments (DoE) is applied in organizing experiments in RSM. Product quality in a manufacturing establishment can be improved by integrating Design of Experiments (DoE) in early stages of developments cycle (Huairui et al, 2010).

Central Composite Design (CCD) developed by Box and Wilson in 1951 is one of the techniques used in optimization of experimental conditions. Central Composite Design combines 2^k full factorial or 2^{k-p} fractional experimental runs, 2k star or axial point experimental runs on each k axis of distance $\pm\propto$ away from the central point, and at least one center point experimental run, where *k*, *p*, and α are number of factors in the optimization experiment, the number with which the runs are fractioned, and axial point distance respectively. Different types of Central Composite Designs include the Orthogonal CCD (Box, 1954), rotatable designs (Box et al, 1957), inadequate model robust designs (Box et al, 1959), and outlier robust designs (Box et al, 1975).

a. RSM Goodness of Fit

RSM Goodness of Fit criteria (Wang, 2006) include:

i) Smallest possible difference between observed and predicted responses

ii) Minimum design points

iii) Simplicity of various calculations

iv) Satisfactory information distribution throughout the entire region in which the research is interested.

b. Central Composite Rotatable Design (CCRD)

This method was developed by Box and Hunters (Box et al, 1957). For rotatability of a CCD, the distance of axial points is given by equation 2.9.

$$\propto = (2^k)^{1/4} \tag{2.9}$$

Where

$\propto$ = Distance of axial points

k = Number of independent variables

c. Full factorial design

The total number of experimental combinations for a full factorial design is given by equation 2.10.

$$N = 2^k + 2k + n_o \tag{2.10}$$

Where

N = Total experimental combinations

k = Number of independent factors

n_o= Central point experimental repetitions

The independent variables x_i are coded as shown in equation 2.11.

$$x_i = \frac{X_i - \bar{x_i}}{\Delta x_j}; \qquad i = 1, 2, 3, \ldots\ldots\ldots, k \tag{2.11}$$

Where

x_i = Dimensionless values of the independent variables

X_i = Real value of the independent variable

$\overline{x_i}$= Real value of the independent variable at the central point

Δx_j= Step change

k = Number of independent factors

The predicted response is given by the second order polynomial shown in equation 2.12.

$$y = \beta_o + \sum_{i=1}^{k} \beta_i x_i + \sum_{i=1}^{k} \beta_{ij} {x_i}^2 + \sum_{i_i<j}^{k} \sum_{j}^{k} \beta_{ij} x_i x_j + \varepsilon \quad (2.12)$$

Where

y = Predicated response

β = Regression coefficient

i, j = linear, quadratic co-efficient respectively

k = Number of independent factors

ε = Random error

d. Testing the significance of a regression

In order to determine the presence of a linear relation between a response y and a group of factors, the following hypothesis (Equations 2.13 and 2.14) is employed.

$$H_o: \beta_1 = \beta_2 = \ldots\ldots\ldots = \beta_{r+1} = 0 \quad (2.13)$$

$$H_1: \beta_{ij} \neq 0 \; for \; at \; least \; one \; j \quad (2.14)$$

Where

H_o = Null hypothesis

H_1 = Valid Hypothesis

β_{ij} = Regression co-efficient

This means that the hypothesis H is only valid if at least one of the factors $x_1, x_2, \ldots\ldots, x_k$ contributes significantly to the regression model.

F statistics of $H_o: \beta_1 = \beta_2 = \ldots\ldots\ldots = \beta_{r+1} = 0$ is given by equation 2.15:

$$F = \frac{SS_R/r}{SS_E/n-r-1} = \frac{MSS_R}{MSS_E} \tag{2.15}$$

Where

F = F-Value

MSS_R= Regression Mean Sum of Squares

MSS_E= Error Mean Sum of Squares

SS_R = Regression Sum of Squares

SS_E = Error Sum of Squares

r = Regression degrees of freedom

N = Total Experimental Combinations

H_o is rejected if $F_o > F_{\propto,r,n-r-1}$

Where

H_o= Null hypothesis

F_o= Observed F-value

$F_{\propto,r,n-r-1}$ = Critical F-value

Table 2.8: ANOVA Table

Source	**Sum Sq.**	**d.o.f.**	**Mean Sq.**	**F**	**Prob> F**
Regression	$\mathrm{SS_R}$	r	$\frac{\mathrm{SS_R}}{\mathrm{r}} = \mathrm{MSS_R}$	$\frac{\mathrm{MSS_R}}{\mathrm{MSS_E}}$	$\mathrm{Prob} > F$
Error	$\mathrm{SS_E}$	$\mathrm{N-r-1}$	$\frac{\mathrm{SS_E}}{\mathrm{N-r-1}} = \mathrm{MSS_E}$		
Total	$\mathrm{SS_y}$	$\mathrm{N-1}$			

Table 2.8 shows various elements of a general ANOVA table used in the evaluation of the significance of a regression model.

e. Co-efficient of Determination

This is the measure of reduction in the variability of a response y due to factors $(x_1, x_2, \ldots\ldots, x_k)$.$R^2$is given by equation 2.16.

$$R^2 = \frac{SS_R}{SS_y} = 1 - \frac{SS_R}{SS_y} \qquad (2.16)$$

Where

R^2 = Co-efficient of determination

SS_R = Regression Sum of Squares

SS_y= Total Sum of Squares

Addition of factors affecting a response y will always increase the value of R^2 towards unity. This implies that larger R^2 may not be directly interpreted to mean that the

regression model is finally good. Adjusted co-efficient of determination R^2_{adj} (Equation 2.17) is, therefore, used. With the use of adjusted co-efficient of determination, R^2_{adj} won't rise with the increase in factors. In fact, addition of unnecessary factors will always tend to reduce R^2_{adj}. Equation 2.17 illustrates the computation of R^2_{adj} from R^2.

$$R^2_{adj} = 1 - \frac{SS_E/(N-r-1)}{SS_y/(N-1)} = 1 - \frac{(N-1)}{(N-r-1)}(1-R^2) \quad (2.17)$$

Where

R^2 = Co-efficient of determination

R^2_{adj} = Adjusted co-efficient of determination

SS_y= Total Sum of Squares

SS_E = Error Sum of Squares

r = Regression degrees of freedom

N = Total Experimental Combinations

f. Fractional factorial design

Due to limited resources and time to carry out some research activities, factorial runs can be reduced such that the selected runs represent interaction among other factors leading to 2^{k-p}factorial runs. For half factorial experimental runs, p = 1, as illustrated by equation 2.18.

$$\frac{2^{k-1}}{2^k} = \frac{1}{2} \quad (2.18)$$

Where

k = Number of independent variables

2.9.2 Software application

MATLAB is a language of technical computing and has been used by many researchers in processes design as well as analyses of data. MATLAB Version R2010b has the Design of Experiments, Regression Analysis, Surface Fitting Tool, Surface Plotting, and much more capabilities which can be synchronized in various research designs and statistical analyses.

2.10 CONCLUSIONS TO THE LITERATURE REVIEW

The literature survey realized a number of Kenyan policies enacted in the previous regimes to support biofuels' industries with the major aim of providing alternative cheap fuel to the country's economic growth. "Waste to Energy" techniques are widely under study with the main focus on the optimization of biofuels production from different lignocellulosic residues. In bioethanol production processes, fermentation has remained significant due to its high share in the bioethanol production cost attributed to the cost of feedstocks. Banana peels which are some of the most common food wastes have been tried by various researchers in the production of biofuels like bioethanol. It has been established that Kenya as an agricultural country is forecasted to produce approximately 2 million MT of bananas on about 91 thousand hectares of land come the year 2016.

Most 'Waste to energy' technologies are still in the research stage and have not been implemented. This is because fermenting hydrolysates derives from lignocellulosic biomasses are a bit more difficult and non-economical as compared to the already established bioethanol production substrates like grains and molasses.

Some of the major factors influencing fermentation results include substrate concentration, fermentation temperature, initial medium pH, yeast concentration, and incubation period. Internal broth temperature is very crucial in bioethanol fermentation processes and temperatures above 60^{o}C kill microorganisms. Yeasts are generally active between 0^{o}C and 50^{o}C. Most microorganisms including yeasts are active at a slightly neutral pH whereas pH less than 3.50 eliminates or kills them. Lower than required pH may lead to growth of undesired microorganisms.Yeast has been known for bioethanol fermentation processes and it has been used by several researchers. Recommended yeast dosage ranges between 0.24g/L and 1.00 g/L.Despite the importance of sugar in a fermentation process, its high concentrations may inhibit microorganisms' growth. Most yeast tolerate fairly high sugar concentrations of about 40% w/v, certain yeast strains (osmophilic) can tolerate sugar concentrations above 40% w/v, and some yeasts withstand sugar concentrations between 65% and 70% w/v.Longer broth retention times ensure complete fermentation process whereas shorter retention time leads to high quantities of residual sugars. High bioethanol levels in a fermentative broth can kill yeasts. Most microorganisms cannot withstand bioethanol concentrations above 10 - 15% w/v whereas ethanologens produce significantly above 1% w/v bioethanol.

Both the qualitative and quantitative techniques including colorimetry and chromatography have been used in analyzing experimental results related to biofuels productions. Response Surface Methodology (RSM) has been proven by several researchers to be very efficient, cheap, and fast in the optimization of various processes.

CHAPTER THREE: MATERIALS AND METHODOLOGY

3.1 INTRODUCTION

This chapter presents the research station, research equipment, chemicals/biochemicals, and various experimental and statistical procedures. Banana peelings derived from three different cultivars: *Sialamule*, *Uganda Green*,and *Ngombe*wereused in this research. Bioethanol was produced through anaerobic fermentation of various banana peelings hydrolysates under varying operational parameters using yeast in laboratory scale still batch reactors. Bioethanol yields and TRS degradations were analyzed using the Gas Chromatography and Dubois method respectively.Central Composite Rotatable Design (CCRD) and Response Surface Methodology (RSM) were done on MATLAB Version R2010b (Appendix 1).

3.2 RESEARCH STATION

This study was carried out on a laboratory scale at the Public Health Engineering Laboratory atMoi University in Eldoret - Kenya. Other stations include material preparation at Kenya Agricultural Research Institute (KARI) - Kisii Branch and bioethanol yields analysis at theGovernment Chemist Department (GCD) - Nairobi Laboratories.

3.3 RESEARCH EQUIPMENT

The following equipment were used in this research.

a. Garden panga

This was used in harvesting of various banana cultivars from the farm.

b. Wheel barrow

This was used in transporting harvested banana bunches from the farm to the weighing bay.

c. Cutting knives

These were used in the separation of banana fruits from the stick, peeling of banana fruits and chopping of the peels into 2-3 cm pieces.

d. Agitator

Retsch ER 50 agitator was used in the feedstocks hydrolysis setup. Itsspeed scale range was 0 to 200 rpm with an allowable error of ±10 rpm.

Plate 3.1: Agitator

Plate 3.1 shows an agitator used in the hydrolysis setup.

e. Autoclave

Sanoclav autoclave (Model M-ECZ) was used in media and equipment sterilization. Its temperature scale range was 0 to 160^{o}Cwith an allowable error of ±0.05^{o}C whereas its pressure scale range was -1 to 5 bars with an allowable error of ±0.05 bars.

f. Water bath

A Memmert water bath (Model WB14) with a temperature scale allowable error of±0.05^{o}C was used in feedstocks hydrolysis.

g. Incubator

A Memmert incubator was used in the fermentation process to maintain various incubation temperatures. It had a temperature range of 20^{o}C to 220^{o}C with an allowable error of ±0.5^{o}C

h. Magnetic stirrer

Ikamag REO magnetic stirrer with a speed range of 0 to 1100 rpm and an allowable error of ± 50 rpm was used in samples agitation.

i. Milling machine

Disk Mill (Model FFC-15) coupled to a single phase 1.1 kW a.c. motor with maximum speed of 2850 rpm was used in grinding of the dried feedstocks.

j. Oven

ELE drying ovens with a maximum temperature of 240^{o}C and an allowable error of± 2.5^{o}C were used in feedstocks drying.

k. Refrigerator

Whirlpool refrigerator was used to storevarious materials and samples below 4^{o}C.

l. Non-return air valves

Non-return air valves were used in ensuring one direction flow of gases from the headspace of anaerobic fermentation broths.

m. Ice box

Ice box was used in the transportation of samples from the Public Health Engineering Laboratory at Moi University to the Government Chemist Department (GCD) - Nairobi Laboratories.

n. Laboratory glassware

Various glassware used in experimental studies included Erlenmeyer flasks, schott bottles, volumetric flasks, test tubes, round bottom flasks, burettes, pipets, beakers, glass rods, and droppers.

o. Centrifuge

Hettich EBA III was used in supernatants preparation procedures. It had a speed range of 0 to 6000 rpm with an allowable error ± 750 rpm.

p. Weighing balance

ELE weighing balance with a range of 0 g to 10000 g and an allowable error of ± 0.5 g was used in weighing heavier batches.

q. Sensitive balance

A digital analytical balance (Mettler Toledo AB204) with a range of 10 g to 210 g and an allowable error of ± 0.00005 g was in weighing lighter batches.

r. pH meter

A digital pH meter (Model WTW pH 340) with an allowable error of ±0.005 was used in the determination of pH levels of various media.

s. Vacuum filter

A KNF Neuberger vacuum filter (Model: N810.3 FT 1.18) with a maximum pressure of 1.0 bar was used in samples preparation.

t. Colorimeter

Jenway colorimeter (Model 6051) with an absorbance range of 0.00 to 1.77 and an allowable error of ± 0.005 was used in the determination of total reducing sugars through the Dubois method.

Plate 3.2: Jenway 6051 colorimeter

Plate 3.2 shows a colorimeter used in reading light absorbance across various samples. To use this colorimeter for Total Reducing Sugars (TRS) analysis, it is first connected to an a.c. powers source, absorbance (Abs) mode is selected, wavelength set at 490 nm using the knob on the left side, and then allowed to warm up for 15 minutes to ensure sufficient time for both the electrical and optical systems to stabilize. A blank cuvette containing distilled water is inserted, the lid is then closed, and then absorbance set at zero. The blank cuvette is then removed and new cuvette filled with an unknown sample is inserted. An absorbance reading is then taken from the digital screen as shown above.

u. Gas Chromatograph

A GC-Shimadzu 2010 was used in the quantitative analysis of bioethanol yields from all the fermentation media.

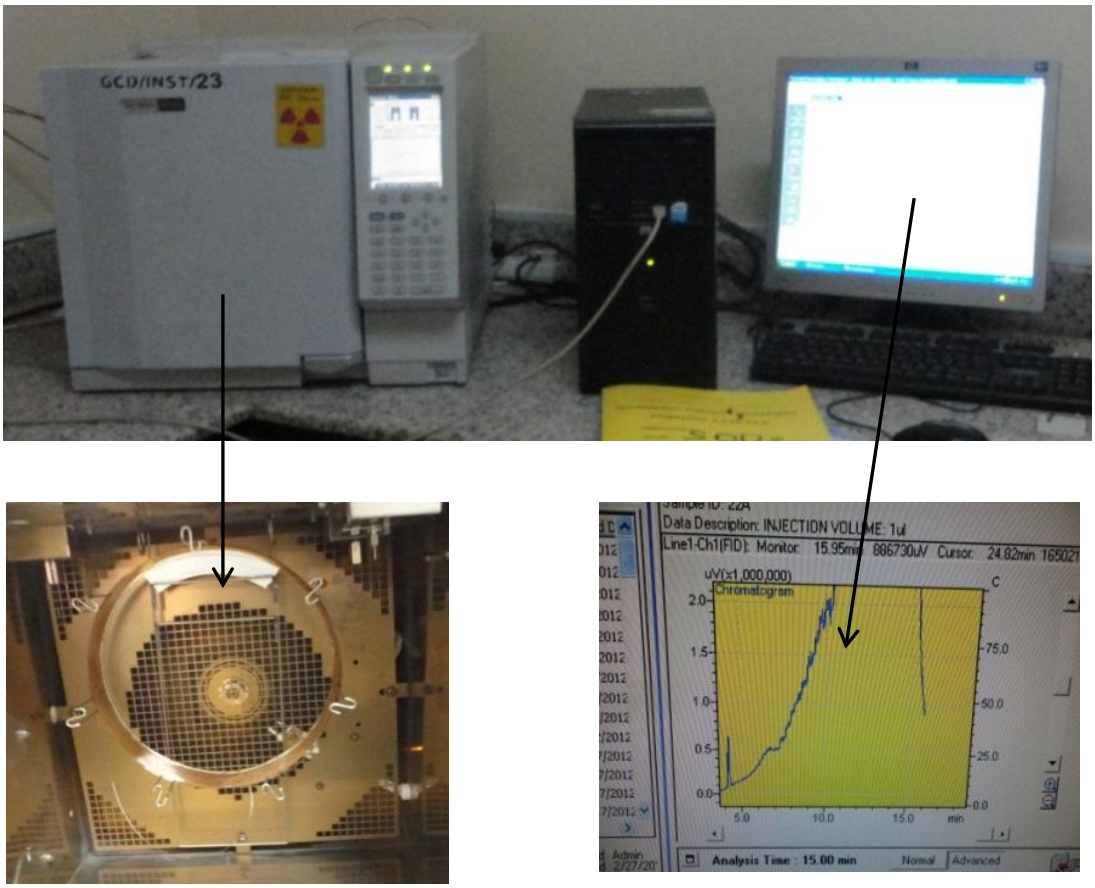


Plate 3.3: Shimadzu GC 2010

Plate 3.3 shows a Shimadzu GC 2010 used in the analysis of bioethanol yields from various fermentation media. Specifications and settings of this Gas Chromatograph (GC) were as shown in the table 3.1.

Table 3.1: GC Analysis Parameters

GC	Shimadzu GC 2010
Injector	Temperature: 150^{o}C; Split ratio: 5.1; Total Flow: 81.2 ml/min; Purge flow: 3 ml/min
Column	ZB-Wax; Length: 30 m; Inner Diameter: 0.32 mm; Film thickness: 0.25 µm
Packing	Polyethylene glycol
Colum	2 min initial hold time, 40^{o}C to 220^{o}C at 20^{o}C/min, 4 min final hold time (Temperature program); Column flow: 12.82 ml/min; Linear velocity: 120.6 cm/sec
Nitrogen (Carrier gas)	Temperature: 150^{o}C; Pressure: 227.9 kPa; Total Flow: 81.2 ml/min; Purge flow: 3 ml/min
Hydrogen (Detector)	Temperature: 280^{o}C; Flow: 80 ml/min; Make up flow: 20 ml/min
Air	Flow: 400 ml/min
Injection volume	1 µL
Detector	Flame Ionization Detector (FID)
Standard	99.8% Ethanol (Sigma-Aldrich)
Software	GC Solution

Table 3.1 shows specifications and setting of a Gas Chromatograph (GC) used in bioethanol yields analysis.

3.4 RESEARCH CHEMICAL / BIOCHEMICALS

Chemicals and biochemicals below were purchased from accredited commercial laboratory chemicals and equipment suppliers. These included Sulphuric Acid(H_2SO_4),Potassium Hydroxide (KOH), Calcium Hydroxide ($Ca(OH)_2$), Phenol Crystals (C_6H_5OH), Glucose ($C_6H_{12}O_6$), Yeast extract, Agar, Peptone, Ammonium Acetate ($NH_4C_2H_3O_2$), Ammonium Chloride(NH_4Cl), Magnesium SulphateHeptahydrate ($MgSO_4.7H_2O$), Manganese Sulphate Monohydrate ($MnSO_4.H_2O$), Dipotassium Phosphate (K_2HPO_4), Monopotassium Phosphate

(KH_2PO_4), p-Amino Benzoic Acid($C_2H_7NO_2$), Ferric (II) SulphateHeptahydrate ($FeSO_4.7H_4O$), Sodium Sulphate (Anhyd) (Na_2SO_4), and Calcium Chloride Hexahydrate($CaCl_2.6H_4O$).

3.5 SUBSTRATE PREPARATIONS

Raw banana peels were used as substrates in this research. Banana plantation at KARI-Kisii was examined for different cultivars grown with the aim of selecting*Sialamule,UgandaGreen*, and *Ngombe* stipulated in the research scope. These banana fruits were washed with tap water and then peeled off separately for individual characterization.The peelswere transported to Moi University in Kesses within 24 hours after harvesting.

(a) (b)

Plate 3.4: Non-edible parts of a banana bunch (a) Banana peels; (b) Banana Stick

Plate 3.4 (a)shows a hip of fresh banana peels. These banana peels, derived from each ofthe three cultivars (*Sialamule, Uganda Green*, and *Ngombe*) under research, were chopped separately.

Plate 3.5: Banana peels chopped into 2-3 cm pieces

Plate 3.5 shows banana peels cut into 2-3 cm sizes. Chopped peels were handled separately. These banana peels were left overnight in open aeration.

Plate 3.6: Sun-drying banana peels

Plate 3.6 shows the sun-drying process. Banana peels were sun-dried for 10 consecutive days.Each of the three cultivars was sundried separately.

Plate 3.7: Oven drying of banana peels

Plate 3.7 shows oven drying. After sun-drying, banana peels were then completely dried in ovens at 60^{o}C for 24 hours.Each of the three cultivars was oven-dried separately.

Plate 3.8: Completely sun-dried banana peels

Plate 3.8 shows oven-dried banana peels ready for milling. Materials purity was ensured throughout all these handling processes.

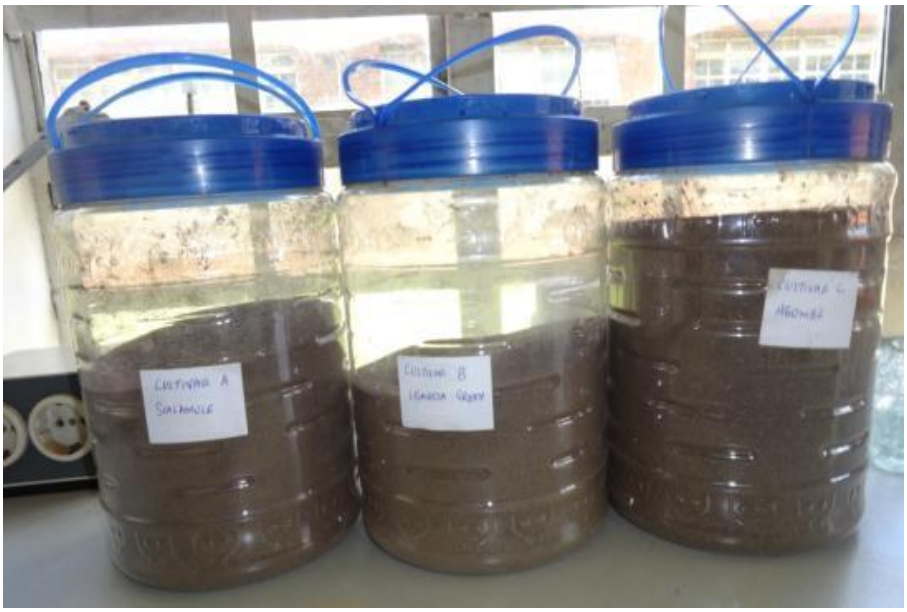


Plate 3.9: Banana peels powder ready for acidic hydrolysis

Plate 3.9 shows ground banana peels powder. The dried banana peels were milled through a 1 mm screen into a fine powder, kept in closed plastic containers, and storedbelow 4^{o}C for the subsequent procedure.

3.6 MEDIA pH CONTROL

Medium pH is one of the factors influencing yeast growth.

Plate 3.10: Digital pH Meter

Plate 3.10 show a digital pH meter used in the determination of various pH levels. Various media pH values were controlled by adding either 1 M KOH or 1 M H_2SO_4 solution drop by drop and reading the resultant pH on a digital screen as shown on the plate above. To raise the pH value, KOH solution was added whereas to lower the pH value, H_2SO_4 solution was added.

3.7 STERILIZATION

To avoid strenuous or wild microorganisms, fermentation media and microorganism's handling equipment were thoroughly sterilized through the heat treatment method.

Plate 3.11: Autoclave

Plate 3.11 shows an autoclave being used in the sterilization process.Various media and yeast handling equipment were, usually, autoclaved at 120^{o}C and 1 bar for 10 minutes, and then allowed to cool to 20^{o}C before use.

3.8 MICROORGANISM USED IN THE RESEARCH

(a) (b)

Plate 3.12: Yeast: (a) Yeast Pack; (b) Yeast nodules

Plate 3.12 shows a high activity and sugar resistant yeast (*Saccharomyces Cerevisiae*) manufactured by Angel. This strain was developed on both the yeast maintenance and inoculation media as discussed in section 3.8.1.

3.8.1 Culture maintenance

Stock culture (20 g of yeast) was maintained in a 100 ml broth of a sterilized yeast maintenance medium (pH set at 5.5) in a 500 ml Erlenmeyer flask and then stored below 4^{o}C.

Table 3.2:Yeast maintenance medium

NH_4Cl	0.3 g/l
KH_2PO_4	0.1 g/l
$MgSO_4.7H_2O$	1.0 g/l
K_2HPO_4	0.06 g/l
Yeast Extract	5 g/l
Glucose	10 g/l
Peptone	10 g/l
Agar	20 g/l

Table 3.2 shows various components of the yeast maintenance medium per litre used in culture maintenance.

3.8.2 Inocula Preparation

Chemicals/Biochemicals in table 3.3 were used in the preparation of the inoculation medium.

Table 3.3: Yeast inoculation medium

KH_2PO_4	0.5 g/l
$MgSO_4.7H_2O$	1.0 g/l
Yeast Extract	5 g/l
NH_4Cl	0.4 g/l
Glucose	0.02 g/l

Table 3.3 above shows various components of an inoculation medium per litre used in both the culture growth and culture adaptation.

Plate 3.13: Inoculum Broth

Plate 3.13 shows an inoculum broth for culture growth and adaptation, set 24 hours prior to banana peels hydrolysates fermentation. The sterilized inocula medium (pH set at 5.5) was inoculated with a 10% v/v colony of yeast from the maintained stock culture. This medium was then incubated at 35^{o}C while being agitated at approximately 120 rpm for 24 hours before the fermentation process.

3.9 HYDROLYSIS

3.9.1 Sulphuric acid concentration in hydrolysis

Optimum Sulphuric Acid concentration for banana peels hydrolysis process was estimated atconstrained substrate particles size, temperature, and time. This was carried out by determining the Total Reducing Sugars (TRS) present in resultant hydrolysates.1 g of banana peels powder was weighed into each of the nine different 100ml conical flasks using a sensitive balance and then impregnated with 0%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, and 80% H_2SO_4in the ratio of 2ml acid to 1gbanana peels powder. 0%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, and 80% Sulphuric Acid concentrations were prepared by mixing 98% AR H_2SO_4 with distilled water in the ratio of 0:100, 10:90, 20:80, 31:69, 41:59, 51:59, 61:39, 71:29, and 82:18 v/v respectively. Each of the nine samples was then maintained at 50.0^{o}C in a water bath for 60 minutes and then cooled to 20^{o}C.Each of these hydrolysates wasthen diluted gradually to form 1000 ml by adding distilled water. 10 ml of each sample was then centrifuged and 2 ml supernatant pipetted into different test-tubes for subsequent TRS analysis using the Dubois Methods as outlined later in the Total Reducing Sugars (TRS) analysis section.

3.9.2 Banana Peels Powder Hydrolysis

This was done at the peak TRS yielding Sulphuric Acid concentration determined above. 576gof each ground banana peels powder was weighed into three different 2000 ml round bottomflasks.

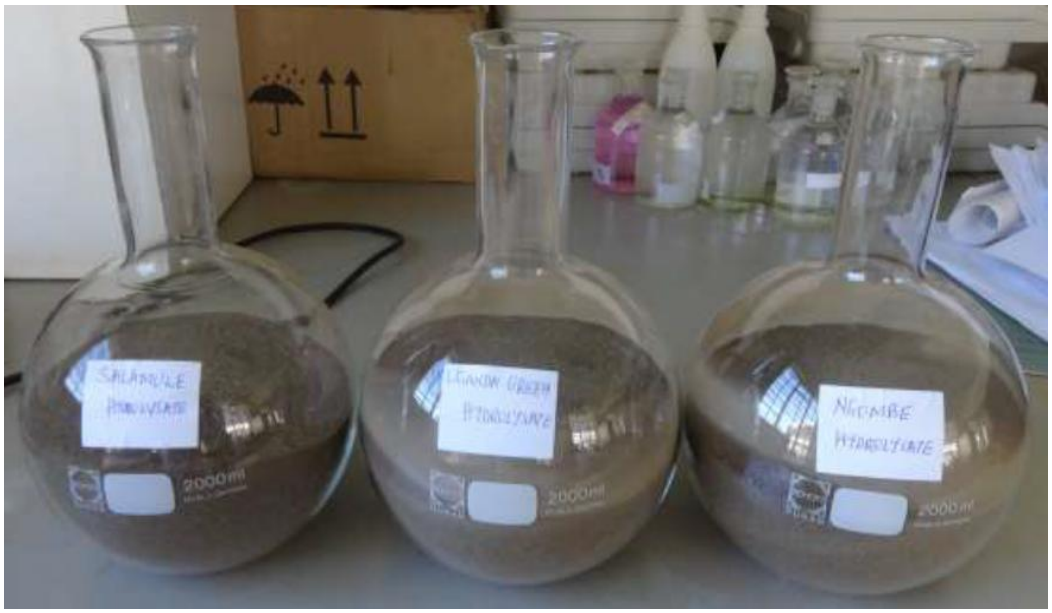


Plate 3.14: Banana peels powder ready for hydrolysis

Plate 3.14 shows various banana peels powder ready for acidic hydrolysis. Each of them was impregnated with 1152 ml of 60% H_2SO_4 after which they were maintained at 50^oC in a water bath while being agitated at 200 rpm for 60 minutes. The resultant hydrolysates were then rinsed with distilled water into respective 3000 ml plastic containers to make 2000 ml hydrolysates and then cooled to 20^oC.

3.10 DETOXIFICATION OF THE HYDROLYSATES

Detoxification for the hydrolysate to present favorable conditions for yeast growth was done by over-liming. Powdered limewas added to the each of the three 2000 ml hydrolysates gradually while being agitated at 200 rpm until the pH was above 8. Detoxified hydrolysates were then cooled and stored below 4^oC for further procedures.

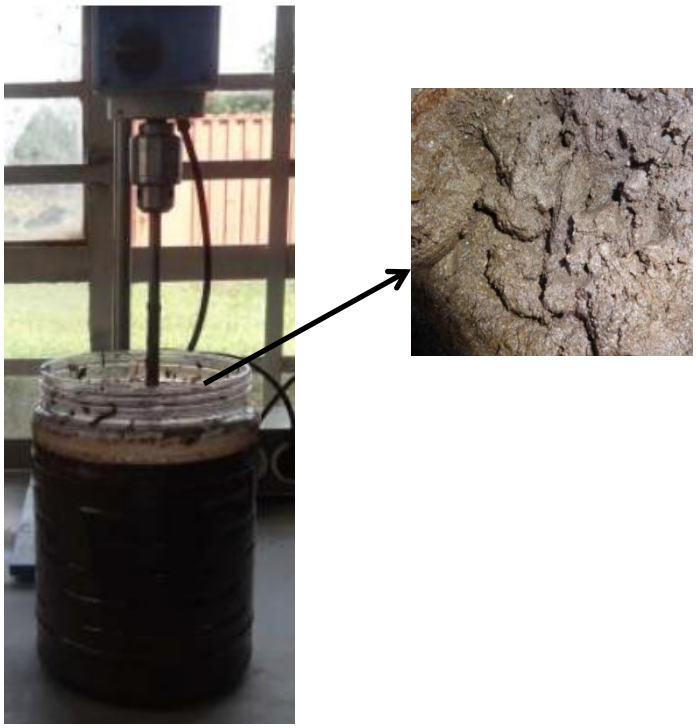


Plate 3.15: Hydrolysate Detoxification

Plate 3.15 shows a hydrolysate detoxification setup.

3.11 ADJUSTMENT OF FERMENTATION CONDITIONS

Table 3.4: Factors description

Factor	**Symbol X_i**	**Axial (-α)**	**Min (-1)**	**Center (0)**	**Max (+1)**	**Axial (+α)**
Substrate concentration (g/L)	X_1	84.863	140.000	180.000	220.000	275.137
Fermentation temperature (^oC)	X_2	23	30	35	40	47
Initial medium pH	X_3	3.12	4.50	5.50	6.50	7.88
Yeast concentration (g/L)	X_4	0.811	1.500	2.000	2.500	3.189
Incubation period (hours)	X_5	62.918	96.000	120.000	144.000	177.082

Table 3.4gives the real values corresponding to -α, -1, 0, +1, and +αfor all the five anaerobic fermentation factors under study. For each cultivar, a total of 31 experimental

runs were carried out based on Central Composited Rotatable Design - CCRD (Appendix 1.3). Ranges for various fermentation factors were chosen based on past research.

3.11.1 Substrate concentration (g/L), X_1

Substrate concentrations: 84.863g/l, 140.000g/l, 180.000g/l, 220.000g/l, and 275.137g/lwere prepared in bulk form to reduce random errors which could be associated with substrate concentrations.Bulk samples were calculated using equation 3.1.

$$Bulk\ Sample = \frac{X_1\ (g) X\ Resultant\ detoxified\ Hydrolysate\ (g)\ X\ Required\ number\ of\ samples}{10\ X\ Hydrolysed\ peels\ powder\ (g)} \quad (3.1)$$

In equation 3.1, actual values for X_1 are 84.863, 140.000, 180.000, 220.000, and 275.137 for -α, -1, 0, +1, and +α levels respectively while the required number of samples are 1, 8, 13, 8, and 1 for -α, -1, 0, +1, and +α levels respectively. Peels powder to be hydrolyzed is 576 for all the three cultivars under study.

Table 3.5: Bulk substrate samples

Cultivar	***Sialamule***	***Uganda Green***	***Ngombe***
Detoxified hydrolysate (g)	3561	3543	3549
Bulk sample for 1(-α) sample (g/100ml)	52	52	52
Bulk sample for 8(-1) samples (g/800ml)	692	689	690
Bulk sample for 13(0) samples (g/1300ml)	1447	1439	1442
Bulk sample for 8(+1) samples (g/800ml)	1088	1082	1084
Bulk sample for 1(+α) sample (g/100ml)	170	169	170

Table 3.5 shows all the 15 bulk substrate samples. Each was filtered through pieces of nylon cloth and further through filter papersusing a vacuum filter. Prepared bulk substrate samples were then stored below 4^{o}C for further procedures.

3.11.2 Fermentation temperature (^{o}C), X_2

Real fermentation temperatures: 23^{o}C, 30^{o}C, 35^{o}C, 40^{o}C, and 47^{o}Cwere controlled using an incubator.

3.11.3 Initial medium pH, X_3

Real initial medium pH settings: 3.12, 4.50, 5.50, 6.50, and 7.88were balanced by adding either 1 M H_2SO_4 or 1M KOH as discussed earlier.

3.11.4 Yeast concentration (g/L), X_4

Real yeast concentrations: 4.06%, 7.5%, 10%, 12.5%, and 15.95% v/v inoculum were added to appropriate fermentation media to achieve 0.811g/l, 1.500g/l, 2.000g/l, 2.500g/l, and 3.189g/l yeast concentrations respectively.

3.11.5 Incubation period (hours), X_5

Real fermentation media incubation periods: 62hrs 55min, 96hrs 00 min, 120hrs 00 min, 144hrs 00 min, and 177hrs 05minwere allowed in an incubator.

3.12 FERMENTATION

100 ml sterelized fermentation media contained banana peels hydrolysates of required concentrations, fermentation medium chemicals/biochemical, and minerals solution in the ratio of 1:1000 v/v.

Table 3.6: Fermentation medium

Banana peels hydrolysate	85 - 275 g/l
Mineral solution	1:1000 v/v
Yeast Extract	3.0 g/l
Ammonium Acetate	3.0 g/l
NH_4Cl	1.6 g/l

$MgSO_4$. $7H_2O$	4.1g/l
K_2HPO_4	0.2 g/l
KH_2PO_4	0.1 g/l
PABA	10 mg/l

Table 3.6 shows the composition of a laboratory scale fermentation media with varying substrate concentrations.

Table 3.7: Mineral solution

$MnSO_4.H_2O$	15 g/l
$FeSO_4.7H_2O$	25 g/l
Na_2SO_4	12 g/l
$CaCl_2.6H_2O$	20 g/l
$MgSO_4.7H_2O$	68 g/l

Table 3.7 shows the composition of a mineral solution used to enhance all the fermentation media.

Fermentation was done using sterilized Erlenmeyer flasks coked with non-return valves to ensure anaerobic fermentation conditions.Each medium was treated depending on the designed experimental run.

Plate 3.16: Fermenting Broths

Plate 3.16 shows laboratory scale experimental set-ups in an incubator. A total of 31experimental runs for each of the three cultivars under study were made according to the Design of Experiments (Appendix 1.3).

3.13 ANALYSES

3.13.1 Substrate moisture content analysis

Dry feedstocks are very convenient to handle, not vulnerable to microbial spoilage, and require simple storage techniques. It is also very costly to eliminate large moisture contents from feedstocks due to the large labour, high energy, and equipment requirements.

10g of banana peels powder was dried to a constant weight at 105^{o}C and then both the dry and wet weightswere calculated using equation 3.2 and equation 3.3 respectively.

a. Dry basis

$$M_d = \frac{W-D}{D} X\ 100 \quad (3.2)$$

b. Wet basis

$$M_w = \frac{W-D}{W} X\ 100 \quad (3.3)$$

Where

M_d = Percentage moisture content (dry basis)

M_w = Percentage moisture content (wet basis)

D = Dry weight (after drying at 105^{o}C to a constant mass)

W= Wet weight (weight of the peels powder used in hydrolysis)

3.13.2 Substrate ash content analysis

Ash contains various minerals in different concentrations required during yeast metabolism.

10 g of banana peels was weighed into a crucible and heated at 550^{o}C for 24 hours. The residue was then weighed and the percentage ash content calculated as follows.

$$Ash\ (\%) = \frac{W_f}{W}\ X\ 100 \qquad (3.4)$$

Where

Ash = Percentage ash content of the substrate

W_f = Weight of the residue after combustion

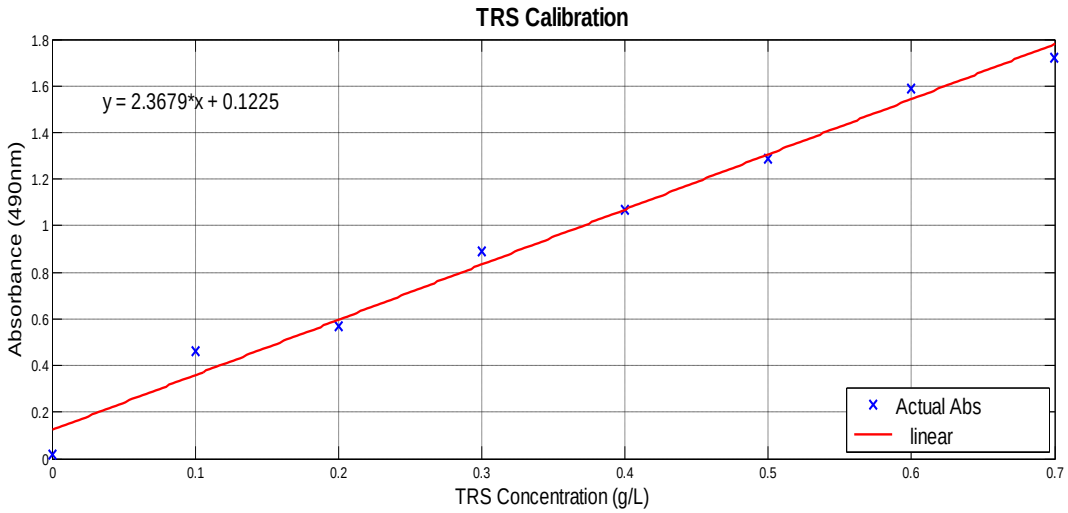
W = Wet weight as received of banana peels powder which was hydrolyzed into simple sugars

3.13.3 Total Reducing Sugars (TRS) Analysis

2 ml of the supernatant to be analysed was pipetted into a test-tube. 0.05 ml of 80% phenol solution was added to the test-tube containing the sample and then hand shaken. 5 ml of 95.5% Sulphuric acid was then dispensed into the mixture at the middle of the test-tube using a burette to facilitate both rapid mixing and reaction rate. 95.5% H_2SO_4 was prepared by mixing 98% AR H_2SO_4 with distilled water in the ratio of 97:3 v/v. The mixture was then allowed to rest for 10 minutes and thereafter hand shaken. It was then put in a 30^{o}C water bath for 20 minutes. Analysis for TRS was done using a colorimeter at 490 nm wavelength as explained earlier in section 3.3 (t).

Total Reducing Sugars (TRS) concentrations calibration

A calibration curve for TRS concentrations was prepared using 0.0, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, and 0.7 g/l TRS concentrations.This was plotted in figure 3.1 to show the relationship between various TRS concentrations and absorbance.



Fi Figure 3.1: TRS Calibration Curve ar

relation below was used in the determination of TRS concentrations of various samples.

$$Absorbance = (2.3679 * TRS\ Conc\ in\ g/L) + 0.1225 \quad (3.5)$$

3.13.4 Bioethanol Yield

A GC-Shimadzu 2010 was used in the quantitative analysis of bioethanol yields from all the fermentation media as explained earlier in section 3.3 (u).

3.14 STATISTICAL ANALYSIS

Response Surface Methodology (RSM) was applied in theoptimization of fermentation process. Designed experimental data were matched with the second order polynomial (Equation 2.12). R^2 - coefficient of determination (Appendix 1.8) and adjusted R^2(Appendix 1.9) wereused to express fit of the developed polynomials.The Fischer (F-test) at 95% confidence level (Appendix 1.6) was used to test statistical significance of the developed polynomials. In order ignore insignificant terms and simplify various

regression models, theStudent's t-test (Appendix 1.7) was employed in testing the significance various polynomial coefficients. The responses under 'no regression modification' and 'with regression modification' were compared. Various statistical analyses were done on MATLAB Version R2010b (Appendix 1). Contour and surface plots (Appendix 1.10) produced on the same software were analyzed to observe the process trends and optimumresults.

CHAPTER FOUR: RESULTS AND DISCUSSION

4.1 INTRODUCTION

This chapter presents results, analyses, and discussions of various findings in the study.

4.2 MATERIALS CHARACTERIZATION

Table 4.1: Harvested Banana Bunches

Cultivar	Plants harvested	Whole weight (kg)	Weight of the Stick (kg)	Weight of peels (kg)
Sialamule	2	17.0	1.5	9.0
Uganda Green	1	18.0	1.5	8.0
Ngombe	1	38.0	2.0	14.5

Table 4.1 shows the weightcharacteristics of harvested banana bunches. Non-edible parts constituted 61.76%w/w, 52.78%w/w, and43.42%w/w of fresh banana harvests in *Sialamule*, *Uganda Green*, and *Ngombe* cultivars respectively. Of particular interest, wet banana peels constituted 58.06%w/w, 48.48%w/w, and 40.28%w/w of the fruits in *Sialamule*, *Uganda Green*, and *Ngombe* cultivars respectively. These closely compared with the range of 30% to 40% w/w reported by Phatcharaporn, Siripan, and Sorada in their study of the effects of banana peels preparations on the properties of banana peels dietary fibre concentrate (Phatcharaporn et al, 2009). This big percentage of food waste always poses a serious wastage handling and disposal problems and therefore it is important to investigate and improve its alternative usages.

Table 4.2: percentage moisture loss in banana peels

Cultivar	Initial weight of peels (g)	Weight loss through sun drying (% w/w)	Weight loss through oven drying (% w/w)
Sialamule	9,000	85.64	0.98
Uganda Green	8,000	85.41	1.15
Ngombe	14,500	85.23	2.84

Table 4.2 shows moisture loss through various peels pretreatment procedures. Considering the entire banana harvests, final ground banana peels powderwas 7.08%w/w, 5.97%w/w, and 4.55%w/w of the whole harvested banana bunches in *Sialamule*, *Uganda Green*, and *Ngombe* cultivars respectively. Based on the entire banana fruits, ground banana peels powder was 7.77%w/w, 6.52%w/w, and 4.80%w/w of banana fruits in *Sialamule*, *Uganda Green*, and *Ngombe* cultivars respectively. Considering the peels, ground banana peels powder was 13.38%w/w, 13.44%w/w, and 11.92%w/w of wet peels in *Sialamule*, *Uganda Green*, and *Ngombe* cultivars respectively. This implies that 86.62% w/w, 86.56% w/w, and 88.08% w/w of wet peels in *Sialamule*, *Uganda Green*, and *Ngombe* cultivars respectively comprised majorly of water. This range compares with 82.47to 86.21% w/w found by Nuttiya and Jirasak(Nuttiya et al, 2013) and 78.9%w/w given by Srishail(Srishail, 2010). This close range of 86.62% to 88.08% w/w shows no significance difference in the moisture contents of various banana cultivars used.

Due to this high moisture content of banana peels, proximity of feedstock handling equipment to the main feedstock sources is very paramount in order to reduce transportation costs. Assuming the maximum forecasted annual banana production estimate of 2,024,644 MT in Kenya by 2016 and that all the banana peels will not discarded, the above analysis presents an annual potential of 261,382 MT of ground banana peels powder in the country from total banana crop land coverage of 90,580

hectares.Banana farming has both the food and energy resource values which reduce the “agricultural land competition” disadvantage evident in other biomass farms.

Table 4.3: Banana Peels Powder Composition

Parameters	***Sialamule***	***Uganda Green***	***Ngombe***
Moisture (% Dry Weight)	10.12	9.34	8.02
Moisture (% Wet Weight)	9.19	8.54	7.42
Total Solids (% w/w)	90.81	91.46	92.58
Ash content (% w/w)	5.67	6.03	7.85
TRS before hydrolysis (% w/w)	16.36	15.52	15.52
TRS after hydrolysis (% w/w)	36.64	35.79	36.21

Figure 4.3 indicates various characteristics of different banana peels powder. Moisture, total solids, ash, and Total Reducing Sugars (TRS) contents were analysed at appropriate stages of materials’ handling and experimental procedures. It was assumed that the analytical procedures used, growing location, harvesting method, season, and stage of harvesting did not cause the differences in the banana peels powder composition since all the above factors were constant for all the three cultivars under investigation.

4.2.1 Ash Content

Ash contains various mineralsin different concentrations required during yeast metabolism. Equation 3.4was employed in ash content analysis. Ash contentsof various ground peels powder were 5.67%w/w, 6.03%w/w, and 7.85%w/w in *Sialamule*, *Uganda Green*, and *Ngombe* cultivars respectively. This range of 5.67% to 7.85% w/w shows slight significance difference in the ash contents of various banana cultivars used. All these ash contentsare higher than the 8.50%w/w found by Srishail in analyzing banana peels nutritional composition (Srishail, 2010).

4.2.2 Moisture Content

Dry feedstocks are very convenient to handle, not vulnerable to microbial spoilage, and require simple storage techniques.It is also very costly to eliminate large moisture contents from feedstocks due to the large labour, high energy, and equipment requirements. Equations 3.2 and 3.3 for dry and wet basis respectively were employed in determining moisture contents of various samples. Residual moisture contents after both sun drying (SD) and oven drying (OD) were 10.12%w/w, 9.34%w/w, and 8.02%w/w on dry basis and 9.19%w/w, 8.54%w/w, and 7.42%w/w on wet basis of peels powder in *Sialamule*, *Uganda Green*, and *Ngombe* cultivars respectively. These ranges show slight differences in the moisture contents of various banana cultivars used. These moisture contents were slightly higher than the 5.00%w/w found by collecting banana peels from a market, few days air drying and two days oven drying at 60^{o}C (Vikash et al, 2012) and the 6.70%w/w found by Srishail in analyzing banana peels nutritional composition (Srishail, 2010).

4.2.3 Total Reducing Sugars (TRS) contents

Viable fermentable sugars compositions make wastes suitable substrates for bioethanol production in the currently prospering "Waste to Energy" technologies. Through concentrated acidic hydrolysis, total reducing sugars contents of ground peels powder were raised from 16.36%w/w to 36.64%w/w, 15.52%w/w to 35.79%w/w, and 15.52%w/w to 36.21%w/w in *Sialamule*, *Uganda Green*, and *Ngombe* cultivars respectively. TRS yields with 0% H_2SO_4 hydrolysis were slightly higher than the 15.2% w/w achieved by distilled water dilution and 30 minutes boiling of banana peels (Vikash et al, 2012).This close range of 15.52% to 16.36% w/w shows no significance difference in the free TRS contents of various banana cultivars used. Similarly, this

range of 35.79% to 36.64% w/w TRS yields shows little difference in the hydrolysates derived from various banana peels used.

4.3 ESTIMATION OF BEST ACID CONCENTRATION IN HYDROLYSIS

Table 4.4: TRS Yield trends of peels hydrolysis

H_4SO_4 Conc. (%)	**TRS Yield (%w/w)**		
	Sialamule	***Uganda Green***	***Ngombe***
0%	16.36	15.52	15.52
10%	18.90	18.90	18.05
20%	25.66	25.23	25.23
30%	29.03	27.77	28.61
40%	34.95	34.10	33.26
50%	34.52	35.37	34.52
60%	36.64	35.79	36.21
70%	34.52	33.26	33.26
80%	29.03	29.03	28.61

Table 4.4 shows the trend of TRS yields(Appendix 5) under various sulphuric acid concentrations hydrolysis. These TRS yields were plotted in MATLAB (Appendix 1.2) as shown in figure 4.1 below against various sulphuric acid concentrations after which peak yields were estimated. Further hydrolysis assumed maximum TRS yields at these peak estimates. System acidity depends on acid type used, acid concentration, amount of acid used, and quantity of released acid from the substrate during hydrolysis, liquid to solid ratio, substrate neutralizing capacity, and hydrolysis medium movement during heating.Penetration of an acid catalyst and dispersion in a hydrolysis system can greatly influence the process as well as the sugars' yields.Sulphuric acid diffusivity relies on lignocellulosic biomass nature and it has been proven that it is better in agricultural wastes than hardwood (Sung-Bae et al, 2002).

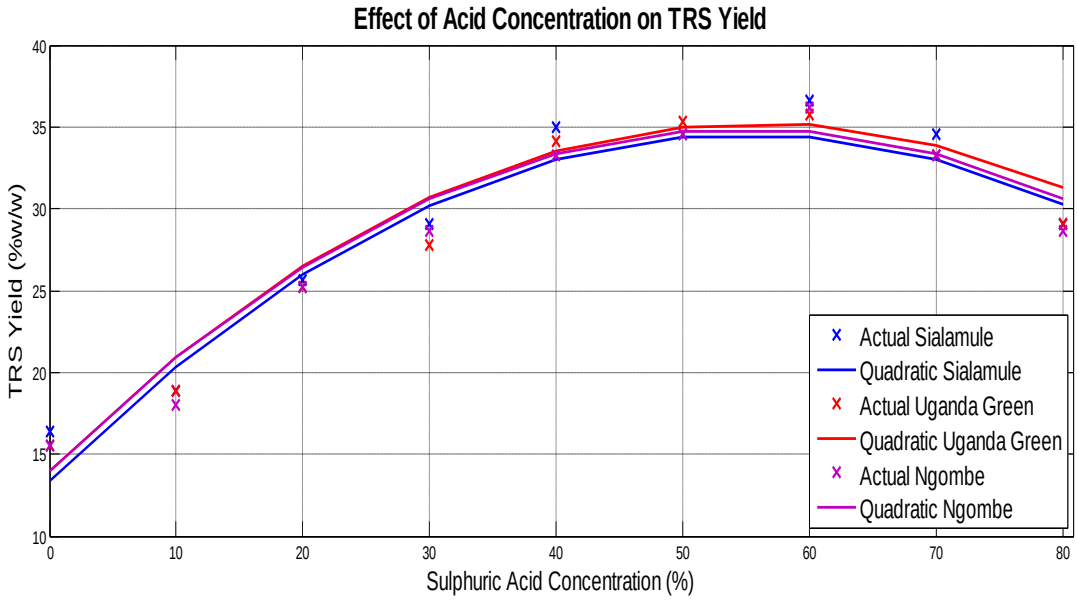


Figure 4.1: Effect of acid concentration on TRS yield

Figure 4.1 shows the trend of TRS yields under various sulphuric acid concentrations hydrolysis. The fitted quadratic functions (Appendix 1.2),relating the sulphuric acid concentration for banana peels hydrolysis and TRS yields,were as shown in equations 4.1 to 4.3below:

$$y_{trsA} = -5.1z^2 + 5.7z + 33 \text{ ; Where } z = {}^{(x-40)}/_{27} \quad (4.1)$$

$$y_{trsB} = -0.0068x^2 + 0.76x + 14 \quad (4.2)$$

$$y_{trsC} = -0.0069x^2 + 0.76x + 13 \quad (4.3)$$

Where

y_{trsA} = TRS yield in *Sialamule*hydrolysis(% w/w)

y_{trsB} = TRS yield in *Uganda Green*hydrolysis(% w/w)

y_{trsC} = TRS yield in *Ngombe*hydrolysis(% w/w)

x = Sulphuric acid concentration (% v/v)

$z = x$ is a function of z

W = Wet weight (weight of the peels powder used in hydrolysis)

Glucose could have been converted to organic acids at sulphuric acid concentrations higher than the above peak levels resulting to these noticeable drops in TRS concentrations curves (Ajani et al, 2011).

4.4 TRS DEGRADATION AND ETHANOL YIELD ANALYSES

Central Composite Rotatable Design (CCRD) was used in the optimization of experimental conditions in this study. However, due to limited resources and time to carry out this bioethanol production research, factorial runs were reduced by half (Equation 2.18) such that only 16 out of the total 32 factorial runs were selected (Appendix 1.3).Further, 10 axial and 5 central experimental runs were also conducted as illustrated by equation 2.10. Both the bioethanol yields and TRS degradations in various experiments are shown in tables 4.5, 4.8,and 4.11.

4.4.1 *Sialamule* Fermentation

Table 4.5: TRS Degradations and Bioethanol Yields in *Sialamule* Fermentation

Run	X_1 (g/L)	X_2 (^oC)	X_3	X_4 (g/L)	X_5 (hrs)	TRS Degradation (g/L)		Bioethanol Yield (ml/L)	
						Actual	Predicted	Actual	Predicted
1.	1	-1	-1	-1	-1	11.72	12.80	5.22	5.72
2.	-1	1	-1	-1	-1	13.83	11.59	6.17	5.17
3.	-1	-1	1	-1	-1	7.49	8.05	3.39	3.62
4.	1	1	1	-1	-1	18.48	17.37	8.24	7.78
5.	-1	-1	-1	1	-1	8.34	7.69	3.77	3.46
6.	1	1	-1	1	-1	17.21	17.01	7.73	7.62
7.	1	-1	1	1	-1	18.06	19.39	8.12	8.66
8.	-1	1	1	1	-1	19.32	18.17	8.62	8.11
9.	-1	-1	-1	-1	1	15.52	10.89	6.97	4.89
10.	1	1	-1	-1	1	15.52	20.21	6.96	9.05
11.	1	-1	1	-1	1	22.28	22.59	9.97	10.09
12.	-1	1	1	-1	1	21.43	21.37	9.57	9.54
13.	1	-1	-1	1	1	22.70	22.22	10.12	9.93
14.	-1	1	-1	1	1	21.44	21.01	9.59	9.38
15.	-1	-1	1	1	1	17.21	17.47	7.70	7.83
16.	1	1	1	1	1	27.35	26.79	12.27	11.99
17.	-2.3784	0	0	0	0	3.28	6.38	1.51	2.90
18.	2.3784	0	0	0	0	21.43	18.91	9.62	8.50
19.	0	-2.3784	0	0	0	8.34	8.87	3.72	3.97
20.	0	2.3784	0	0	0	18.47	18.51	8.24	8.26
21.	0	0	-2.3784	0	0	10.03	10.83	4.50	4.86
22.	0	0	2.3784	0	0	19.32	19.10	8.64	8.55
23.	0	0	0	-2.3784	0	16.36	16.55	7.34	7.41
24.	0	0	0	2.3784	0	23.55	23.94	10.51	10.71
25.	0	0	0	0	-2.3784	7.49	8.09	3.32	3.60
26.	0	0	0	0	2.3784	23.12	23.10	10.31	10.31
27.	0	0	0	0	0	30.72	30.37	13.76	13.53
28.	0	0	0	0	0	31.57	30.37	13.93	13.53
29.	0	0	0	0	0	28.61	30.37	12.75	13.53
30.	0	0	0	0	0	29.45	30.37	13.13	13.53
31.	0	0	0	0	0	31.14	30.37	13.90	13.53

Table 4.5 shows both the TRS degradationsand bioethanol yieldsfrom *Sialamule*fermentationunder various 31 experimental conditions.Coded values used

were generated by MATLAB (Appendix 1.3) and the experimental design used is as shown in appendix 4. Actual values for low, high, central, and axial points for all the five independent variables (X_1, X_2, X_3, X_4, and X_5) used on table 4.5 aboveare shown in table 3.4.From factors settings and actual responses ontable 4.5 above regarding *Sialamule* fermentation, the following 21 terms 2nd order regression polynomials (Equations 4.4 and 4.5) were fitted to predict both the bioethanol yields and TRS degradationsin*Sialamule* fermentation.

2nd Order Regression Polynomial for bioethanol production from *Sialamule*

$$Y_{EtOHA} = 13.5303 + 1.1767x_1 + 0.9021x_2 + 0.7760x_3 + 0.6945x_4 + 1.4101x_5 - 1.3837{x_1}^2 - 0.6469x_1x_2 + 0.3619x_1x_3 + 0.2669x_1x_4 - 0.1169x_1x_5 - 1.31034{x_2}^2 + 0.3219x_2x_3 + 0.1944x_2x_4 - 0.4144x_2x_5 - 1.2060{x_3}^2 - 0.0219X_3x_4 + 0.0244x_3x_5 - 0.7897{x_4}^2 + 0.0619x_4x_5 - 1.1627{x_5}^2 + \varepsilon_{EtOHA} \quad (4.4)$$

Where

Y_{EtOHA} = Predicted bioethanol yield in *Sialamule*fermentation(g/L)

ε_{EtOHA} =Random error associated with bioethanol yield prediction in *Sialamule*fermentation

x_i =Dimensionless values of the independent variables

2nd Order Regression Polynomial for TRS degradation in *Sialamule*

$$Y_{TRSA} = 30.3741 + 2.6327x_1 + 2.0266x_2 + 1.7367x_3 + 1.5546x_4 + 3.1550x_5 - 3.1342{x_1}^2 - 1.4788x_1x_2 + 0.7938x_1x_3 + 0.5800x_1x_4 - 0.2650x_1x_5 - 2.9485{x_2}^2 + 0.7388x_2x_3 + 0.4225x_2X_4 - 0.9500x_2x_5 - 2.7240{x_3}^2 - 0.0525x_3x_4 + 0.0525x_3x_5 - 1.7909{x_4}^2 + 0.1588x_4x_5 - 2.6127{x_5}^2 + \varepsilon_{trsA} \quad (4.5)$$

Where

Y_{TRSA} = Predicted TRS degradation in *Sialamule*fermentation (g/L)

ε_{trsA} = Random error associated with TRS degradation prediction in *Sialamule*fermentation

x_i = Dimensionless values of the independent variables

Table 4.6: *Sialamule*: Test of Significance for Various Regression Coefficients

Term	Coefficient		Se (β_j)		d.o.f.	t_o		$t_{critical}$
	EtOH	TRS	EtOH	TRS		EtOH	TRS	
β_o	13.5303	30.3741	0.3746	0.8418	10	36.1188	36.0824	2.2281
β_1	1.1767	2.6327	0.1618	0.3636	10	7.2721	7.2405	2.2281
β_2	0.9021	2.0266	0.1618	0.3636	10	5.5754	5.5736	2.2281
β_3	0.7760	1.7367	0.1618	0.3636	10	4.7962	4.7763	2.2281
β_4	0.6945	1.5546	0.1618	0.3636	10	4.2922	4.2754	2.2281
β_5	1.4101	3.1550	0.1618	0.3636	10	8.7148	8.6770	2.2281
β_{11}	-1.3837	-3.1342	0.1224	0.2750	10	11.3048	11.3952	2.2281
β_{12}	-0.6469	-1.4788	0.2114	0.4751	10	3.0598	3.1127	2.2281
β_{13}	0.3619	0.7938	0.2114	0.4751	10	1.7117	1.6708	2.2281
β_{14}	0.2669	0.5800	0.2114	0.4751	10	1.2624	1.2209	2.2281
β_{15}	-0.1169	-0.2650	0.2114	0.4751	10	0.5528	0.5578	2.2281
β_{22}	-1.3103	-2.9485	0.1224	0.2750	10	10.7054	10.7203	2.2281
β_{23}	0.3219	0.7388	0.2114	0.4751	10	1.5225	1.5550	2.2281
β_{24}	0.1944	0.4225	0.2114	0.4751	10	0.9194	0.8893	2.2281
β_{25}	-0.4144	-0.9500	0.2114	0.4751	10	1.9601	1.9997	2.2281
β_{33}	-1.2060	-2.7240	0.1224	0.2750	10	9.8533	9.9041	2.2281
β_{34}	-0.0219	-0.0525	0.2114	0.4751	10	0.1035	0.1105	2.2281
β_{35}	0.0244	0.0525	0.2114	0.4751	10	0.1153	0.1105	2.2281
β_{44}	-0.7897	-1.7907	0.1224	0.2750	10	6.4519	6.5105	2.2281
β_{45}	0.0619	0.1588	0.2114	0.4751	10	0.2927	0.3342	2.2281
β_{55}	-1.1627	-2.6127	0.1224	0.2750	10	9.4994	9.4992	2.2281

Table 4.6 shows a double-tailed student (t) test at 95% confidence level (Appendix 1.7) employed in testing the significance of various regression coefficients associated with

bioethanol yields (Equation 4.4) and TRS degradations (Equation 4.5) in *Sialamule* peels fermentation. Regression coefficients associated with interaction terms X_1 (substrate concentration) and X_4 (yeast concentration), X_1 (substrate concentration) and X_5 (hours incubation), X_2 (fermentation temperature) and X_4 (yeast concentration), X_3 (initial medium pH) and X_4 (yeast concentration), X_3 (initial medium pH) and X_5 (hours incubation), and X_4 (yeast concentration) and X_5 (hours incubation) were insignificant and therefore collated with other errors as ε_{EtOHA} in the revised bioethanol yields model (Equation 4.6). Similar level of significance was also present in the TRS degradations model and therefore the coefficients associated with interaction terms were collated as ε_{TRSA} to form a revised regression model (Equation 4.7).

Revised 2nd Order Regression Polynomial for bioethanol production from *Sialamule*

$$Y_{EtOHA} = 13.5303 + 1.1767x_1 + 0.9021x_2 + 0.7760x_3 + 0.6945x_4 + 1.4101x_5 - 1.3837{x_1}^2 - 0.6469x_1x_2 - 1.31034{x_2}^2 - 1.2060{x_3}^2 - 0.7897{x_4}^2 - 1.1627{x_5}^2 + \varepsilon_{EtOHA} \quad (4.6)$$

Where

Y_{EtOHA} = Predicted bioethanol yield in *Sialamule*fermentation (g/L)

ε_{EtOHA} = Random error associated with bioethanol yield prediction in *Sialamule*fermentation

x_i = Dimensionless values of the independent variables

The revised regression model (Equation 4.6) above was plotted on a response surface diagram as shown in figure 4.2 below

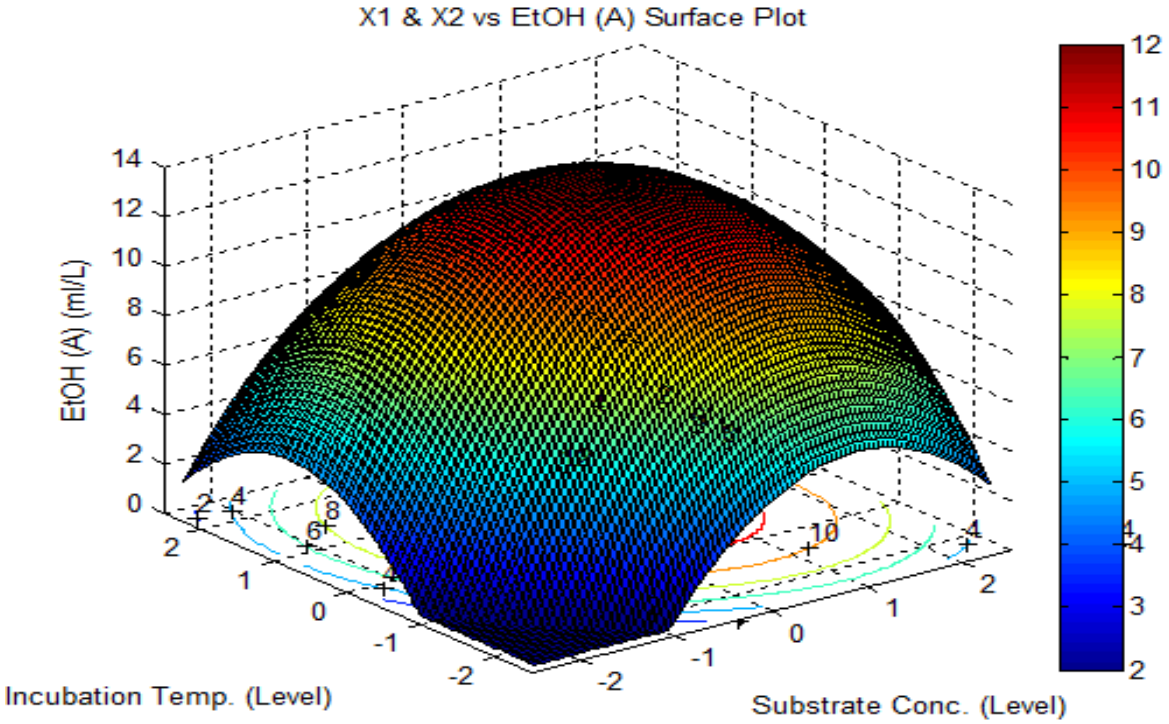


Figure 4.2: *Sialamule*: EtOH (A) vs X_1, X_2: Response Surface

Figure 4.2was plotted on MATLAB Version R2010b (Appendix 1.10) from the revised regression polynomial (Equation 4.6) predicting bioethanol yields from *Sialamule* peels fermentation. It shows a response surface with contours diagram depicting how both the substrate concentrations and incubation temperatures influencedthesebioethanol yields.The smallest ellipse on contours,which corresponds to the surface peak, shows the maximum predicted bioethanol yield of approximately 13.53 ml/L. The bottom regions of the surface plot represent minimal bioethanol yields. Nearly equi-spaced contours show regions that lie on the exponential phase of the fermentation process while the surface peak lies on the stationary stage of invertase activity. Beyond the peak, a significant retardation on the response is noted.High responses lie at a substrate concentration of approximately 0 (180g/L) and an incubation temperature of approximately 0 (35^{o}C), both factors at the central setting. To achieve maximum yield, it is required that both of these two factors be set at these conditions.

Revised 2nd Order Regression Polynomial for TRS degradation in *Sialamule*

$$Y_{TRSA} = 30.3741 + 2.6327x_1 + 2.0266x_2 + 1.7367x_3 + 1.5546x_4 + 3.1550x_5 - 3.1342{x_1}^2 - 1.4788x_1x_2 - 2.9485{x_2}^2 - 2.7240{x_3}^2 - 1.7909{x_4}^2 - 2.6127{x_5}^2 + \varepsilon_{trsA} \quad (4.7)$$

Where

Y_{TRSA} = Predicted TRS degradation in *Sialamule*fermentation (g/L)

ε_{trsA} = Random error associated with TRS degradation prediction in *Sialamule*fermentation

x_i = Dimensionless values of the independent variables

The revised regression model, equation 4.7, above was plotted on a response surface diagram as shown in figure 4.3.

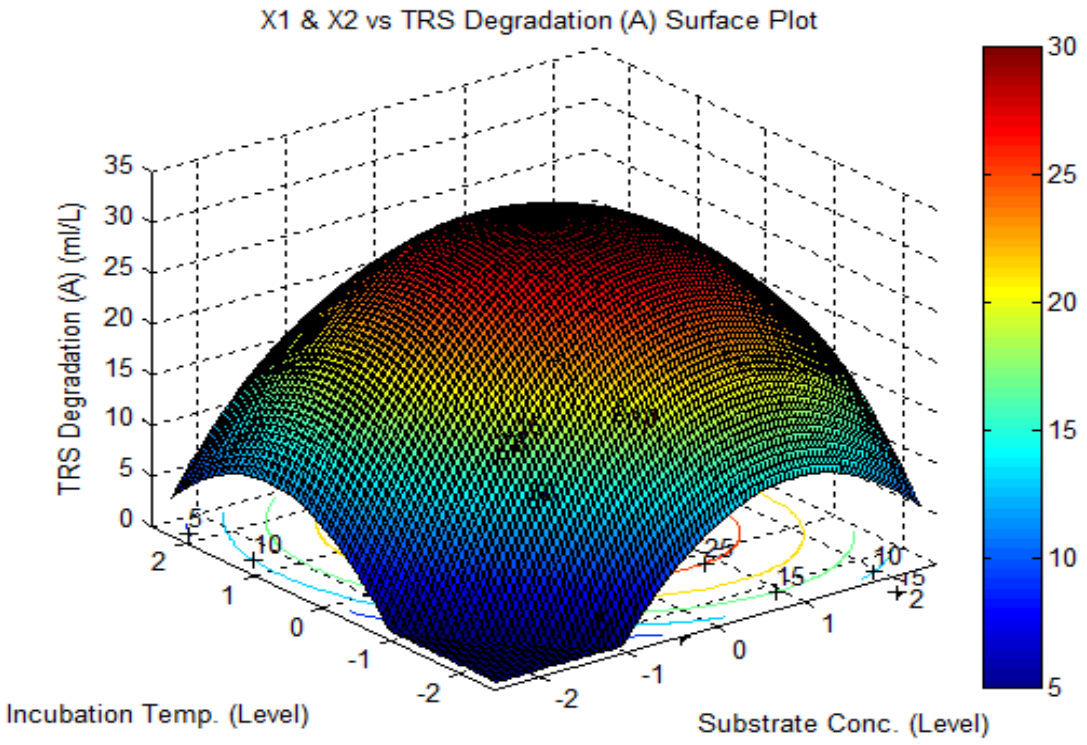


Figure 4.3: *Sialamule*: TRS Degradation (A) vs X_1, X_2: Response Surface

Figure 4.3 was plotted on MATLAB Version R2010b (Appendix 1.10) from the revised regression polynomial (Equation 4.7) predicting TRS degradations from *Sialamule* peels fermentation. It shows a response surface with contours diagram depicting how both the substrate concentrations and incubation temperatures influenced these TRS degradations. The smallest ellipse on contours which correspond to the surface peak shows the maximum predicted TRS degradation of approximately 30.37 g/L. The bottom regions of the surface plot represent minimal TRS degradations. Nearly equi-spaced contours show regions that lie on the exponential phase of the fermentation process while the surface peak lies on the stationary stage of invertase activity. Beyond the peak, a significant retardation on the response is noted. High responses lie at a substrate concentration of approximately 0 (180 g/L) and an incubation temperature of approximately 0 (35^{o}C), both factors at the central setting. The behavior of this surface closely resembles figure 4.2 depicting high correlations between TRS degradations and bioethanol yields in *Sialamule* peels fermentations.

Table 4.7: *Sialamule*: ANOVA for the regressions significance

Source	**Sum of Squares**		**d.o.f.**	**Mean Sum of Squares**		**F-test (observed)**		**F-test (critical)**
	EtOH	**TRS**		**EtOH**	**TRS**	**EtOH**	**TRS**	
Model	334.89	1694.39	20	16.74	84.72	23.42	23.46	2.77
Error	7.15	36.11	10	0.72	3.61			
Total	342.04	1730.51	30					

From the ANOVA Table 4.7, bioethanol yields model F-value of 23.42, higher than the critical F-value of 2.77 at 95% confidence level clearly shows that the developed regression model (Equation 4.6) significantly evaluates experimental data involving bioethanol yields from banana peels hydrolysate derived from *Sialamule* cultivar. The probability of bioethanol yield from banana peels hydrolysate derived from

*Sialamule*cultivar responses occurring due to noise is only 0.00% (negligible) which further asserts that this model is significant. Similarly, the TRS degradations model F-value of 23.46 higher than the critical F-value of 2.77 at 95% confidence level clearly shows that the developed regression model (Equation 4.7) significantly evaluates experimental data involving TRS degradation in *Sialamule* peels hydrolysate fermentation. The chance of these TRS degradation responses occurring due to noise is only 0.00% (negligible) which similarly asserts that this model is significant.Further, from the regression analyses, high coefficient of determination, R^2 value of 0.9768 with an adjusted R^2 of 0.9304 shows that the abovedeveloped2nd order regression polynomial (Equation 4.6) excellently evaluates the experimental data involving bioethanol yields from banana peels hydrolysate derived from *Sialamule* cultivar.

4.4.2 *Uganda Green* Fermentation

Table 4.8: TRS Degradations and Bioethanol Yields in *Uganda Green* Fermentation

Run	X_1 (g/L)	X_2 (^oC)	X_3	X_4 (g/L)	X_5 (hrs)	TRS Degradation (g/L)		Bioethanol Yield (ml/L)	
						Actual	Predicted	Actual	Predicted
1.	1	-1	-1	-1	-1	10.45	11.51	4.72	5.12
2.	-1	1	-1	-1	-1	12.98	10.97	5.85	4.91
3.	-1	-1	1	-1	-1	5.38	6.30	2.38	2.87
4.	1	1	1	-1	-1	15.94	16.13	7.12	7.24
5.	-1	-1	-1	1	-1	7.08	6.30	3.21	2.85
6.	1	1	-1	1	-1	15.10	16.13	6.79	7.21
7.	1	-1	1	1	-1	16.36	17.90	7.30	7.95
8.	-1	1	1	1	-1	18.48	17.36	8.22	7.74
9.	-1	-1	-1	-1	1	12.14	8.81	5.45	3.97
10.	1	1	-1	-1	1	14.68	18.64	6.60	8.34
11.	1	-1	1	-1	1	18.48	20.41	8.27	9.07
12.	-1	1	1	-1	1	19.32	19.87	8.60	8.86
13.	1	-1	-1	1	1	22.28	20.41	9.71	9.05
14.	-1	1	-1	1	1	20.16	19.87	8.97	8.84
15.	-1	-1	1	1	1	16.79	15.19	7.56	6.80
16.	1	1	1	1	1	25.65	25.03	11.43	11.16
17.	-2.3784	0	0	0	0	2.85	6.02	1.33	2.73
18.	2.3784	0	0	0	0	21.44	18.35	9.54	8.17
19.	0	-2.3784	0	0	0	7.50	8.34	3.35	3.71
20.	0	2.3784	0	0	0	20.16	19.39	8.98	8.65
21.	0	0	-2.3784	0	0	8.34	9.22	3.78	4.18
22.	0	0	2.3784	0	0	17.63	16.82	7.93	7.57
23.	0	0	0	-2.3784	0	15.09	13.66	6.75	6.14
24.	0	0	0	2.3784	0	19.74	21.25	8.82	9.47
25.	0	0	0	0	-2.3784	7.92	7.51	3.54	3.39
26.	0	0	0	0	2.3784	20.59	21.07	9.21	9.40
27.	0	0	0	0	0	29.88	27.19	12.59	12.00
28.	0	0	0	0	0	26.50	27.19	11.86	12.00
29.	0	0	0	0	0	26.92	27.19	12.08	12.00
30.	0	0	0	0	0	25.66	27.19	11.43	12.00
31.	0	0	0	0	0	26.92	27.19	12.02	12.00

Table 4.8 shows both the TRS degradations and bioethanol yields from *Uganda Green* fermentation under various 31 experimental conditions. Coded values used were

generated by MATLAB (Appendix 1.3) and the experimental design used is as shown in appendix 4. Actual values for low, high, central, and axial points for all the five independent variables (X_1, X_2, X_3, X_4, and X_5) used on table 4.8 above are shown in table 3.4. From factors settings and actual responses on table 4.8 above regarding *Uganda Green* fermentation, the following 21 terms 2nd order regression polynomials (Equations 4.8 and 4.9) were fitted to predict both the bioethanol yields and TRS degradations in*Uganda Green* fermentation.

2nd Order Regression Polynomial for bioethanol production from *Uganda Green*

$$Y_{EtOHB} = 12.0008 + 1.1433x_1 + 1.0387x_2 + 0.7121x_3 + 0.7001x_4 + 1.2626x_5 - 1.1575{x_1}^2 - 0.6938x_1x_2 + 0.1888x_1x_3 + 0.1775x_1x_4 - 0.0525x_1x_5 - 1.0284{x_2}^2 + 0.2963x_2x_3 + 0.0175x_2x_4 - 0.3600x_2x_5 - 1.0832{x_3}^2 + 0.1300x_3x_4 + 0.0425x_3x_5 - 0.7420{x_4}^2 + 0.2063x_4x_5 - 0.9913{x_5}^2 + \varepsilon_{EtOHB} \quad (4.8)$$

Where

Y_{EtOHB} = Predicted bioethanol yield in *Uganda Green*fermentation (g/L)

ε_{EtOHB} = Random error associated with bioethanol yield prediction in *Uganda Green* fermentation

x_i = Dimensionless values of the independent variables

2nd Order Regression Polynomial for TRS degradation in *Uganda Green*

$$Y_{TRSB} = 27.1862 + 2.5930x_1 + 2.3234x_2 + 1.5972x_3 + 1.5959x_4 + 2.8507x_5 - 2.6521{x_1}^2 - 1.6094x_1x_2 + 0.3944x_1x_3 + 0.4469x_1 - 0.0781x_1x_5 - 2.3542{x_2}^2 + 0.7131x_2x_3 + 0.0256x_2x_4 - 0.8194x_2x_5 - 2.5036{x_3}^2 + 0.2369x_3x_4 + 0.0269x_3x_5 - 1.7204{x_4}^2 + 0.4994x_4x_5 - 2.2791{x_5}^2 + \varepsilon_{trsB} \quad (4.9)$$

Where

Y_{TRSB} = Predicted TRS degradation in *Uganda Green*fermentation (g/L)

ε_{trsB} = Random error associated with TRS degradation prediction in *Uganda Green* fermentation

x_i = Dimensionless values of the independent variables

Table 4.9: *Uganda Green*: Test of Significance for Various Regression Coefficients

Term	Coefficient		Se (β_j)		d.o.f.	t_o		$t_{critical}$
	EtOH	TRS	EtOH	TRS		EtOH	TRS	
β_o	12.0008	27.1862	0.4344	1.0515	10	27.6253	25.8554	2.2281
β_1	1.1433	2.5930	0.1876	0.4542	10	6.0929	5.7094	2.2281
β_2	1.0387	2.3234	0.1876	0.4542	10	5.5356	5.1157	2.2281
β_3	0.7121	1.5972	0.1876	0.4542	10	3.7951	3.5168	2.2281
β_4	0.7001	1.5959	0.1876	0.4542	10	3.7313	3.5139	2.2281
β_5	1.2626	2.8507	0.1876	0.4542	10	6.7288	6.2769	2.2281
β_{11}	-1.1575	-2.6521	0.1419	0.3435	10	8.1547	7.7196	2.2281
β_{12}	-0.6938	-1.6094	0.2452	0.5934	10	2.8298	2.7121	2.2281
β_{13}	0.1888	0.3944	0.2452	0.5934	10	0.7699	0.6646	2.2281
β_{14}	0.1775	0.4469	0.2452	0.5934	10	0.7240	0.7531	2.2281
β_{15}	-0.0525	-0.0781	0.2452	0.5934	10	0.2141	0.1317	2.2281
β_{22}	-1.0284	-2.3542	0.1419	0.3435	10	7.2456	6.8526	2.2281
β_{23}	0.2963	0.7131	0.2452	0.5934	10	1.2084	1.2018	2.2281
β_{24}	0.0175	0.0256	0.2452	0.5934	10	0.0714	0.0432	2.2281
β_{25}	-0.3600	-0.8194	0.2452	0.5934	10	1.4684	1.3808	2.2281
β_{33}	-1.0832	-2.5036	0.1419	0.3435	10	7.6317	7.2874	2.2281
β_{34}	0.1300	0.2369	0.2452	0.5934	10	0.5303	0.3992	2.2281
β_{35}	0.0425	0.0269	0.2452	0.5934	10	0.1734	0.0453	2.2281
β_{44}	-0.7420	-1.7204	0.1419	0.3435	10	5.2279	5.0079	2.2281
β_{45}	0.2063	0.4994	0.2452	0.5934	10	0.2927	0.8416	2.2281
β_{55}	-0.9913	-2.2791	0.1419	0.3435	10	9.4994	6.6339	2.2281

Table 4.9 shows a double-tailed student (t) test at 95% confidence level (Appendix 1.7) employed in testing the significance of various regression coefficients associated with

bioethanol yields, equation 4.8, and TRS degradations, equation 4.9, in*Uganda Green* peels fermentation. Regression coefficients associated with interaction terms X_1(substrate concentration) and X_4 (yeast concentration),X_1(substrate concentration) and X_5 (hours incubation), X_2 (fermentation temperature) and X_4 (yeast concentration), X_3 (initial medium pH) and X_4 (yeast concentration), X_3 (initial medium pH) and X_5 (hours incubation), and X_4 (yeast concentration) and X_5 (hours incubation) were insignificant and therefore collated with other errors as ε_{EtOHB} in the revised bioethanol yields model (4.10). Similar level of significance was also present in the TRS degradations model and therefore the coefficients associated with interaction terms were collated as ε_{TRSB} to form a revised regression model (4.11).

Revised 2nd Order Regression Polynomial for bioethanol production from *Uganda Green*

$$Y_{EtOHB} = 12.0008 + 1.1433X_1 + 1.0387X_2 + 0.7121X_3 + 0.7001X_4 + 1.2626X_5 - 1.1575{X_1}^2 - 0.6938X_1X_2 - 1.0284{X_2}^2 - 1.0832{X_3}^2 - 0.7420{X_4}^2 - 0.9913{X_5}^2 + \varepsilon_{EtOHB} \quad (4.10)$$

Where

Y_{EtOHB} = Predicted bioethanol yield in *Uganda* Greenfermentation (g/L)

ε_{EtOHB} = Random error associated with bioethanol yield prediction in *Uganda Green* fermentation

x_i = Dimensionless values of the independent variables

The revised regression model (Equation 4.10) above was plotted on a response surface diagram as shown in figure 4.4.

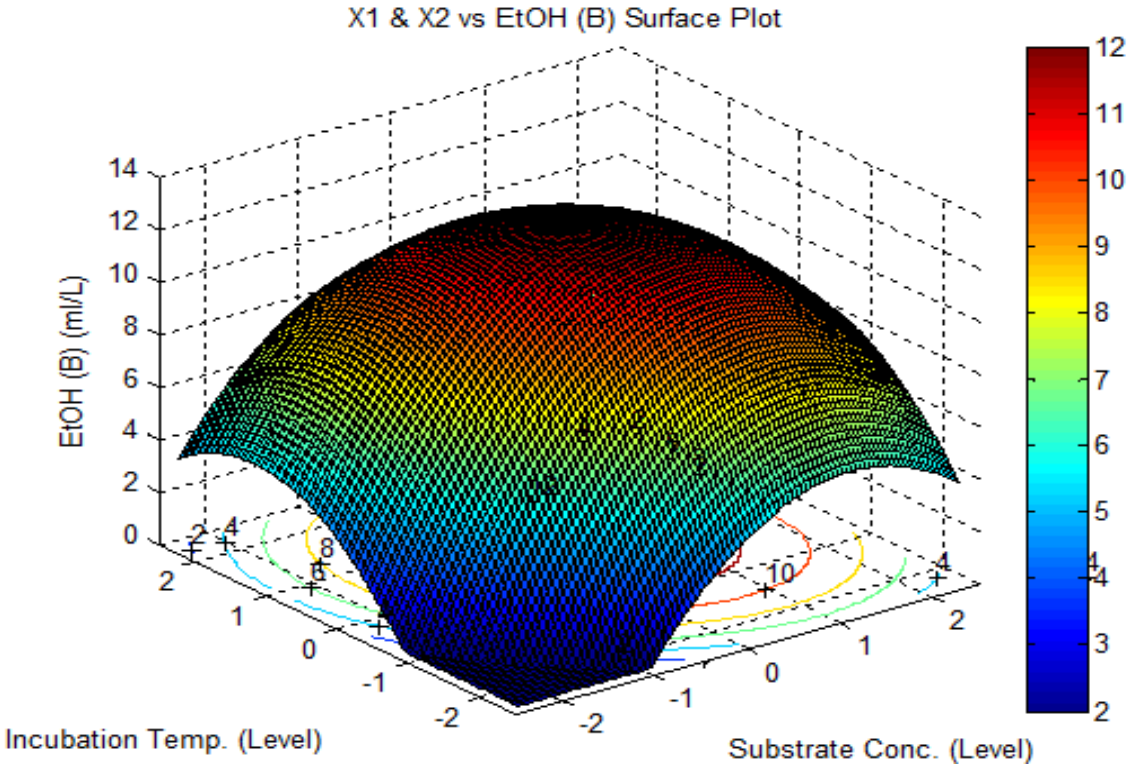


Figure 4.4: Uganda Green: EtOH (B) vs X1, X2: Response Surface

Figure 4.4 was plotted on MATLAB Version R2010b (Appendix 1.10) from the revised regression polynomial (Equation 4.10) predicting bioethanol yields from*Uganda Green* peels fermentation. It shows a response surface with contours diagram depicting how both the substrate concentrations and incubation temperatures influenced these bioethanol yields. The smallest ellipse on contours which correspond to the surface peak shows the maximum predicted bioethanol yield of approximately 12.00 ml/L. The bottom regions of the surface plot represent minimal bioethanol yields. Nearly equi-spaced contours show regions that lie on the exponential phase of the fermentation process while the surface peak lies on the stationary stage of invertase activity. Beyond the peak, a significant retardation on the response is noted. High responses lie at a substrate concentration of approximately 0 (180 g/L) and an incubation temperature of

approximately 0 (35°C), both factors at the central setting. To achieve maximum yield, it is required that both of these two factors be set at these conditions.

Revised 2nd Order Regression Polynomial for TRS degradation in *Uganda Green*

$$Y_{TRSB} = 27.1862 + 2.5930x_1 + 2.3234x_2 + 1.5972x_3 + 1.5959x_4 + 2.8507x_5 - 2.6521{x_1}^2 - 1.6094x_1x_2 - 2.3542{x_2}^2 - 2.5036{x_3}^2 - 1.7204{x_4}^2 - 2.2791{x_5}^2 + \varepsilon_{trsB} \quad (4.11)$$

Where

Y_{TRSB} = Predicted TRS degradation in *Uganda Green*fermentation (g/L)

ε_{trsB} = Random error associated with TRS degradation prediction in *Uganda Green* fermentation

x_i = Dimensionless values of the independent variables

The revised regression model (Equation 4.11) above was plotted on a response surface diagram as shown in figure 4.5.

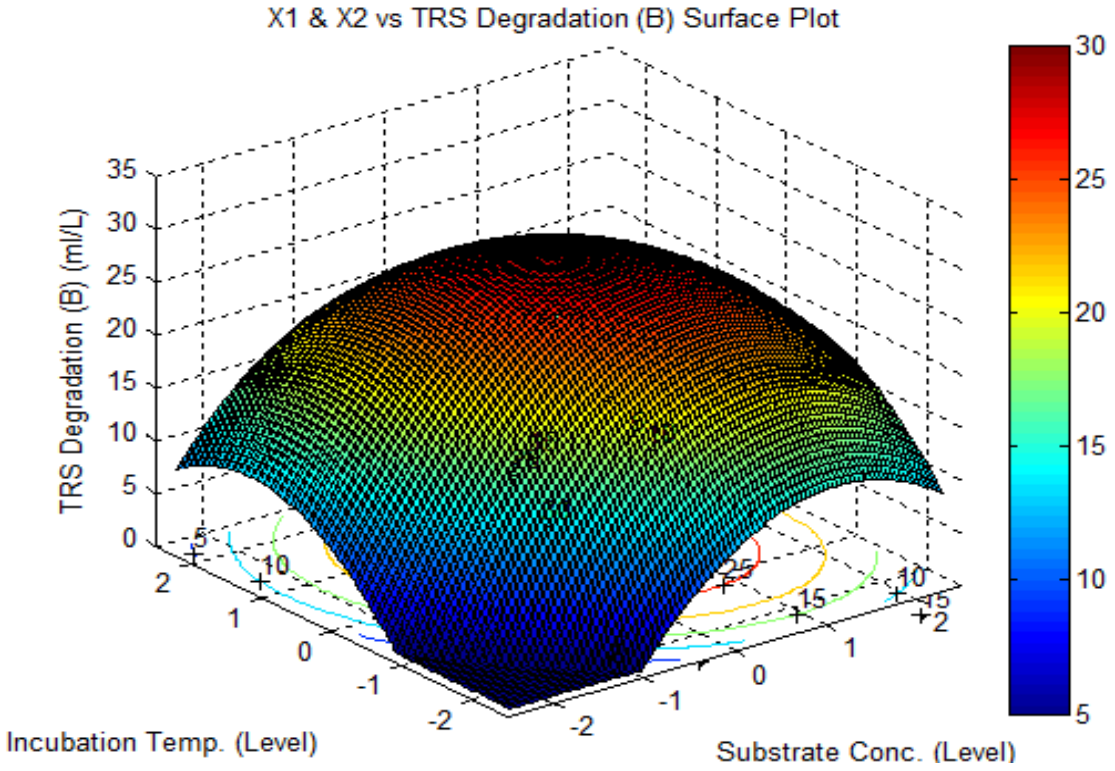


Figure 4.5: *Uganda Green*: TRS Degradation (B) vs X_1, X_2: Response Surface

Figure 4.5 was plotted on MATLAB Version R2010b (Appendix 1.10) from the revised regression polynomial (Equation 4.11) predicting TRS degradations from *Uganda Green* peels fermentation. It shows a response surface with contours diagram depicting how both the substrate concentrations and incubation temperatures influenced these TRS degradations. The smallest ellipse on contours which correspond to the surface peak shows the maximum predicted TRS degradation of approximately 27.19 g/L. The bottom regions of the surface plot represent minimal TRS degradations. Nearly equi-spaced contours show regions that lie on the exponential phase of the fermentation process while the surface peak lies on the stationary stage of invertase activity. Beyond the peak, a significant retardation on the response is noted. High responses lie at a substrate concentration of approximately 0 (180 g/L) and an incubation temperature of approximately 0 (35^{o}C), both factors at the central setting. The behavior of this surface closely resembles figure 4.4 above depicting high correlations between TRS degradations and bioethanol yields in *Uganda Green* peels fermentations.

Table 4.10: *Uganda Green*: ANOVA for the regressions significance

Source	**Sum of Squares**		**d.o.f.**	**Mean Sum of Squares**		**F-test (observed)**		**F-test (critical)**
	EtOH	**TRS**		**EtOH**	**TRS**	**EtOH**	**TRS**	
Model	274.05	1422.74	20	13.70	71.14	14.25	12.63	2.77
Error	9.62	56.34	10	0.96	5.63			
Total	283.66	1479.08	30					

From the ANOVA Table 4.10, bioethanol yields model F-value of 14.25, higher than the critical F-value of 2.77 at 95% confidence level clearly shows that the developed regression model (Equation 4.10) significantly evaluates experimental data involving bioethanol yields from banana peels hydrolysate derived from *Uganda Green* cultivar. The probability of bioethanol yield from banana peels hydrolysate derived from

Uganda Green cultivar responses occurring due to noise is only 0.00% (negligible) which further asserts that this model is significant. Similarly, the TRS degradations model F-value of 12.63 higher than the critical F-value of 2.77 at 95% confidence level clearly shows that the developed regression model (Equation 4.11) significantly evaluates experimental data involving TRS degradation in *Uganda Green* peels hydrolysate fermentation. The chance of these TRS degradation responses occurring due to noise is only 0.00% (negligible) which similarly asserts that this model is significant. Further, from the regression analyses, high co-efficient of determination, R^2 value of 0.9728 with an adjusted R^2 of 0.9184 shows that the above developed 2^{nd} order regression polynomial (Equation 4.10) excellently evaluates the experimental data involving bioethanol yields from banana peels hydrolysate derived from *Uganda Green* cultivar.

4.4.3 *Ngombe* Fermentation

Table 4.11: TRS Degradations and Bioethanol Yields in *Ngombe* Fermentation

Run	X_1 (g/L)	X_2 (^oC)	X_3	X_4 (g/L)	X_5 (hrs)	TRS Degradation (g/L)		Bioethanol Yield (ml/L)	
						Actual	Predicted	Actual	Predicted
1.	1	-1	-1	-1	-1	12.56	12.52	5.65	5.60
2.	-1	1	-1	-1	-1	12.98	11.37	5.82	5.13
3.	-1	-1	1	-1	-1	7.07	8.23	3.17	3.68
4.	1	1	1	-1	-1	19.74	17.44	8.82	7.78
5.	-1	-1	-1	1	-1	8.34	7.79	3.78	3.45
6.	1	1	-1	1	-1	15.52	17.00	6.82	7.54
7.	1	-1	1	1	-1	18.90	19.45	8.48	8.67
8.	-1	1	1	1	-1	17.63	18.31	7.87	8.21
9.	-1	-1	-1	-1	1	13.83	10.21	6.15	4.56
10.	1	1	-1	-1	1	15.52	19.42	6.99	8.65
11.	1	-1	1	-1	1	19.32	21.87	8.64	9.78
12.	-1	1	1	-1	1	20.17	20.72	9.19	9.32
13.	1	-1	-1	1	1	23.12	21.43	10.30	9.55
14.	-1	1	-1	1	1	21.85	20.28	9.74	9.08
15.	-1	-1	1	1	1	17.21	17.14	7.63	7.63
16.	1	1	1	1	1	26.92	26.35	12.01	11.73
17.	-2.3784	0	0	0	0	2.85	4.82	1.44	2.32
18.	2.3784	0	0	0	0	18.90	17.13	8.49	7.74
19.	0	-2.3784	0	0	0	10.03	10.61	4.46	4.74
20.	0	2.3784	0	0	0	20.58	20.20	9.21	9.05
21.	0	0	-2.3784	0	0	9.61	11.03	4.28	4.91
22.	0	0	2.3784	0	0	21.01	19.80	9.35	8.85
23.	0	0	0	-2.3784	0	15.52	15.13	6.89	6.77
24.	0	0	0	2.3784	0	22.27	22.86	9.91	10.15
25.	0	0	0	0	-2.3784	8.34	8.46	3.76	3.82
26.	0	0	0	0	2.3784	21.85	21.93	9.78	9.84
27.	0	0	0	0	0	29.45	29.40	12.92	12.87
28.	0	0	0	0	0	28.61	29.40	12.76	12.87
29.	0	0	0	0	0	30.30	29.40	13.09	12.87
30.	0	0	0	0	0	28.61	29.40	12.80	12.87
31.	0	0	0	0	0	29.88	29.40	12.71	12.87

Table 4.11 shows both the TRS degradations and bioethanol yields from *Ngombe* fermentation under various 31 experimental conditions. Coded values used were

generated by MATLAB (Appendix 1.3) and the experimental design used is as shown in appendix 4. Actual values for low, high, central, and axial points for all the five independent variables (X_1, X_2, X_3, X_4, and X_5) used on table 4.11 above are shown in table 3.4. From factors settings and actual responses on table 4.11 above regarding *Ngombe* fermentation, the following 21 terms 2nd order regression polynomials (Equations 4.12 and 4.13) were fitted to predict both the bioethanol yields and TRS degradations in*Ngombe* fermentation.

2nd Order Regression Polynomial for bioethanol production from *Ngombe*

$$Y_{EtOHC} = 12.8724 + 1.1396x_1 + 0.9064x_2 + 0.8281x_3 + 0.7096x_4 + 1.2652x_5 - 1.3868{x_1}^2 - 0.6450x_1x_2 + 0.3638x_1x_3 + 0.1763x_1x_4 - 0.2438x_1x_5 - 1.0563{x_2}^2 + 0.4050x_2x_3 - 0.0600x_2x_4 - 0.1900x_2x_5 - 1.0598{x_3}^2 - 0.0087x_3x_4 - 0.1238x_3x_5 - 0.7796{x_4}^2 + 0.3263x_4x_5 - 1.0677{x_5}^2 + \varepsilon_{EtOHC} \quad (4.12)$$

Where

Y_{EtOHC} = Predicted bioethanol yield in *Ngombe* fermentation (g/L)

ε_{EtOHC} = Random error associated with bioethanol yield prediction in *Ngombe* fermentation

x_i = Dimensionless values of the independent variables

2nd Order Regression Polynomial for TRS degradation in *Ngombe*

$$Y_{TRSC} = 29.3966 + 2.5882x_1 + 2.0163x_2 + 1.8435x_3 + 1.6239x_4 + 2.8313x_5 - 3.2563{x_1}^2 - 1.3988x_1x_2 + 0.8175x_1x_3 + 0.3963x_1x_4 - 0.5550x_1x_5 - 2.4732{x_2}^2 + 0.8713x_2x_3 - 0.0800x_2x_4 - 0.5012x_2x_5 - 2.4723{x_3}^2 + 0.0262x_3x_4 - 0.2900x_3x_5 - 1.8385{x_4}^2 + 0.7638x_4x_5 - 2.5103{x_5}^2 + \varepsilon_{trsC} \quad (4.13)$$

Where

Y_{TRSC} = Predicted TRS degradation in *Ngombe*fermentation (g/L)

ε_{trsC} = Random error associated with TRS degradation prediction in *Ngombe* fermentation

x_i = Dimensionless values of the independent variables

Table 4.12: *Ngombe*: Test of Significance for Various Regression Coefficients

Term	Coefficient		Se (β_j)		d.o.f.	t_o		$t_{critical}$
	EtOH	TRS	EtOH	TRS		EtOH	TRS	
β_o	12.8724	29.3966	0.2728	0.6557	10	47.1876	44.8353	2.2281
β_1	1.1396	2.5882	0.1178	0.2832	10	9.6720	9.1391	2.2281
β_2	0.9064	2.0163	0.1178	0.2832	10	7.6926	7.1196	2.2281
β_3	0.8281	1.8435	0.1178	0.2832	10	7.0280	6.5096	2.2281
β_4	0.7096	1.6239	0.1178	0.2832	10	6.0226	5.7340	2.2281
β_5	1.2652	2.8313	0.1178	0.2832	10	10.7379	9.9973	2.2281
β_{11}	-1.3868	-3.2563	0.0891	0.2142	10	15.5596	15.2004	2.2281
β_{12}	-0.6450	-1.3988	0.1540	0.3700	10	4.1897	3.7802	2.2281
β_{13}	0.3638	0.8175	0.1540	0.3700	10	2.3628	2.2093	2.2281
β_{14}	0.1763	0.3962	0.1540	0.3700	10	1.1449	1.0709	2.2281
β_{15}	-0.2438	-0.5550	0.1540	0.3700	10	1.5833	1.4999	2.2281
β_{22}	-1.0563	-2.4732	0.0891	0.2142	10	11.8507	11.5448	2.2281
β_{23}	0.4050	0.8713	0.1540	0.3700	10	2.6307	2.3546	2.2281
β_{24}	-0.0600	-0.0800	0.1540	0.3700	10	0.3897	0.2162	2.2281
β_{25}	-0.1900	-0.5012	0.1540	0.3700	10	1.2342	1.3547	2.2281
β_{33}	-1.0598	-2.4723	0.0891	0.2142	10	11.8904	11.5407	2.2281
β_{34}	0.0087	0.0262	0.1540	0.3700	10	0.0568	0.0709	2.2281
β_{35}	-0.1238	-0.2900	0.1540	0.3700	10	0.8038	0.7837	2.2281
β_{44}	-0.7796	-1.8385	0.0891	0.2142	10	8.7467	8.5824	2.2281
β_{45}	0.3263	0.7638	0.1540	0.3700	10	2.1192	2.0641	2.2281
β_{55}	-1.0677	-2.5103	0.0891	0.2142	10	11.9796	11.7181	2.2281

Table 4.12 shows a double-tailed student (t) test at 95% confidence level (Appendix 1.7) employed in testing the significance of various regression coefficients associated

with bioethanol yields (Equation 4.12) and TRS degradations (Equation 4.13) in*Ngombe* peels fermentation. Regression coefficients associated with interaction terms X_1(substrate concentration) and X_4 (yeast concentration),X_1(substrate concentration) and X_5 (hours incubation), X_2 (fermentation temperature) and X_4 (yeast concentration), X_3 (initial medium pH) and X_4 (yeast concentration), X_3 (initial medium pH) and X_5 (hours incubation), and X_4 (yeast concentration) and X_5 (hours incubation) were insignificant and therefore collated with other errors as ε_{EtOHC} in the revised bioethanol yields model (Equation 4.14). Similar level of significance was also present in the TRS degradations model and therefore the coefficients associated with interaction terms were collated as ε_{TRSC} to form a revised regression model (Equation 4.15).

Revised 2nd Order Regression Polynomial for bioethanol production from *Ngombe*

$$Y_{EtOHC} = 12.8724 + 1.1396x_1 + 0.9064x_2 + 0.8281x_3 + 0.7096x_4 + 1.2652x_5 - 1.3868{x_1}^2 - 0.6450x_1x_2 - 1.0563{x_2}^2 - 1.0598{x_3}^2 - 0.7796{x_4}^2 - 1.0677{x_5}^2 + \varepsilon_{EtOHC} \quad (4.14)$$

Where

Y_{EtOHC} = Predicted bioethanol yield in *Ngombe* fermentation (g/L)

ε_{EtOHC} = Random error associated with bioethanol yield prediction in *Ngombe* fermentation

x_i = Dimensionless values of the independent variables

The revised regression model, equation 4.14, was plotted on a response surface diagram as shown in figure 4.6.

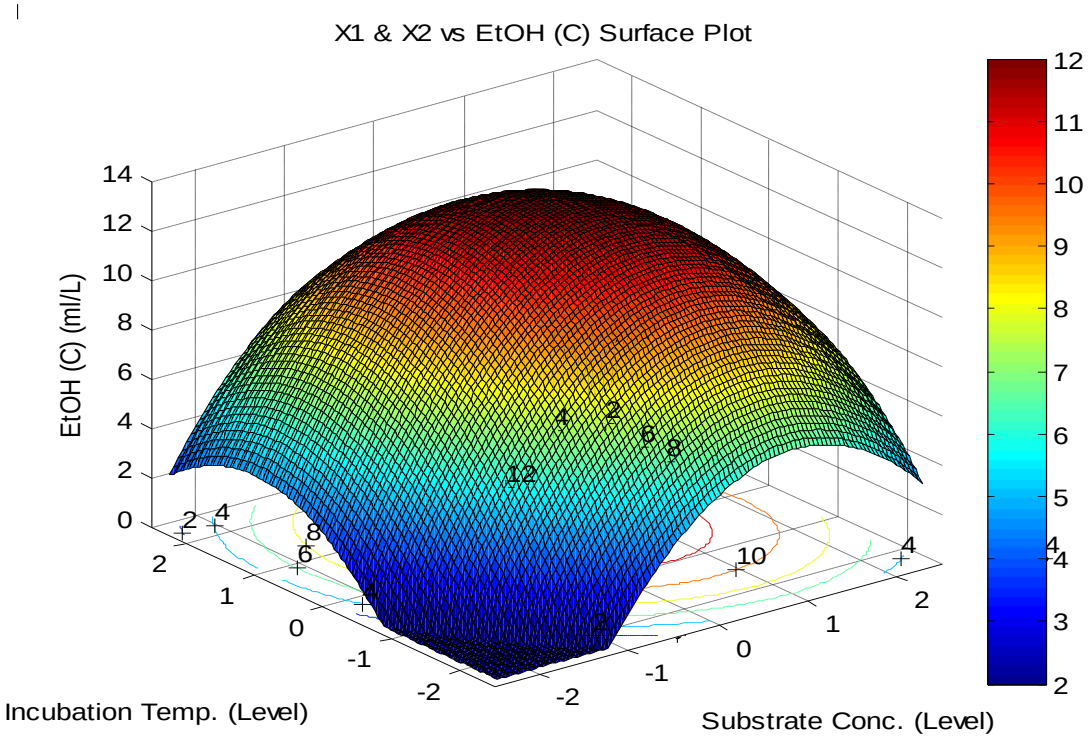


Figure 4.6: *Ngombe*: EtOH (C) vs X_1, X_2: Response Surface

Figure 4.7 was plotted on MATLAB Version R2010b (Appendix 1.10) from the revised regression polynomial (Equation 4.14) predicting bioethanol yields from *Ngombe* peels fermentation. It shows a response surface with contours diagram depicting how both the substrate concentrations and incubation temperatures influenced these bioethanol yields. The smallest ellipse on contours which correspond to the surface peak shows the maximum predicted bioethanol yield of approximately 12.87 ml/L. The bottom regions of the surface plot represent minimal bioethanol yields. Nearly equi-spaced contours shows regions that lie on the exponential phase of the fermentation process while the surface peak lies on the stationary stage of invertase activity. Beyond the peak, a significant retardation on the response is noted. High responses lie at a substrate concentration of approximately 0 (180 g/L) and an incubation temperature of approximately 0 (35^{o}C), both factors at the central setting. To achieve maximum yield, it is required that both of these two factors be set at these conditions.

Revised 2nd Order Regression Polynomial for TRS degradation in *Ngombe*

$$Y_{TRSC} = 29.3966 + 2.5882X_1 + 2.0163X_2 + 1.8435X_3 + 1.6239X_4 + 2.8313X_5 - 3.2563{X_1}^2 - 1.3988X_1X_2 - 2.4732{X_2}^2 - 2.4723{X_3}^2 - 1.8385{X_4}^2 - 2.5103{X_5}^2 + \varepsilon_{trsC} \quad (4.15)$$

Where

Y_{TRSC} = Predicted TRS degradation in *Ngombe*fermentation (g/L)

ε_{trsC} = Random error associated with TRS degradation prediction in *Ngombe* fermentation

x_i = Dimensionless values of the independent variables

The revised regression model, equation 4.15, above was plotted on a response surface diagram as shown in figure 4.7.

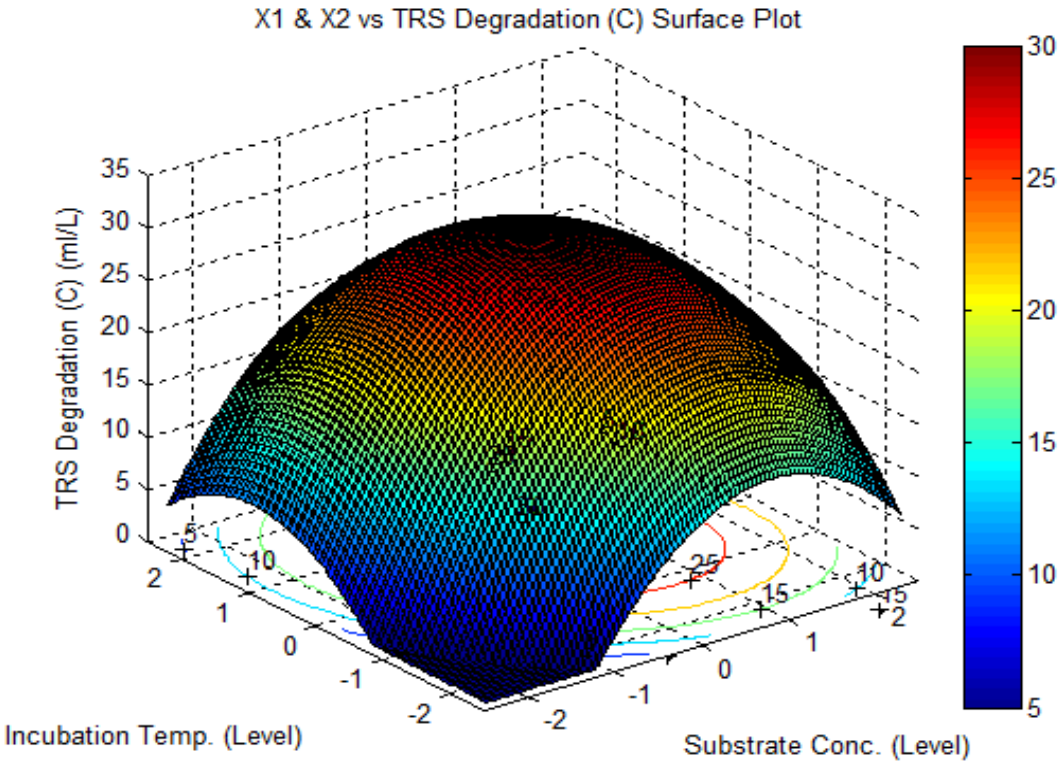


Figure 4.7: *Ngombe*: TRS Degradation (C) vs X_1, X_2: Response Surface

Figure 4.7 was plotted on MATLAB Version R2010b (Appendix 1.10) from the revised regression polynomial (Equation 4.15) predicting TRS degradations from *Ngombe* peels fermentation. It shows a response surface with contours diagram depicting how both the substrate concentrations and incubation temperatures influenced these TRS degradations. The smallest ellipse on contours which correspond to the surface peak shows the maximum predicted TRS degradation of approximately 29.40 g/L.The bottom regions of the surface plot represent minimal TRS degradations. Nearly equi-spaced contours show regions that lie on the exponential phase of the fermentation process while the surface peak lies on the stationary stage of invertase activity. Beyond the peak, a significant retardation on the response is noted. High responses lie at a substrate concentration of approximately 0 (180 g/L) and an incubation temperature of approximately 0 (35^{o}C), both factors at the central setting. The behavior of this surface closely resembles figure 4.6 above depicting high correlations between TRS degradations and bioethanol yields in *Ngombe* peels fermentations.

Table 4.13: *Ngombe*: ANOVA for the regressions significance

Source	**Sum of Squares**		**d.o.f.**	**Mean Sum of Squares**		**F-test (observed)**		**F-test (critical)**
	EtOH	**TRS**		**EtOH**	**TRS**	**EtOH**	**TRS**	
Model	296.91	1564.62	20	14.85	78.23	39.15	35.71	2.77
Error	3.79	21.91	10	0.38	2.19			
Total	300.70	1586.52	30					

From the ANOVA Table 4.13, bioethanol yields model F-value of 23.42, higher than the critical F-value of 2.77 at 95% confidence level clearly shows that the developed regression model (Equation 4.14) significantly evaluates experimental data involving bioethanol yields from banana peels hydrolysate derived from *Ngombe* cultivar. The probability of bioethanol yield from banana peels hydrolysate derived from

*Ngombe*cultivar responses occurring due to noise is only 0.00% (negligible) which further asserts that this model is significant. Similarly, the TRS degradations model F-value of 23.46 higher than the critical F-value of 2.77 at 95% confidence level clearly shows that the developed regression model (Equation 4.15) significantly evaluates experimental data involving TRS degradation in *Ngombe* peels hydrolysate fermentation. The chance of these TRS degradation responses occurring due to noise is only 0.00% (negligible) which similarly asserts that this model is significant. Further, from the regression analyses, high co-efficient of determination, R^2 value of 0.9786 with an adjusted R^2 of 0.9358 shows that the above developed 2^{nd} order regression polynomial (Equation 4.14) excellently evaluates the experimental data involving bioethanol yields from banana peels hydrolysate derived from *Ngombe* cultivar.

4.5 COMPARING REGRESSION MODELS

ANOVA was employed in comparing various regression models(Appendix 1.11).

4.5.1 Bioethanol Yield Regression Models

Equation 4.16 shows three different quadratic models describing bioethanol yields from various banana peels fermentation.

$$
\begin{aligned}
Y_{EtOHA} = {} & 13.5303 + 1.1767x_1 + 0.9021x_2 + 0.7760x_3 \\
& + 0.6945x_4 + 1.4101x_5 - 1.3837{x_1}^2 \\
& - 0.6469x_1x_2 - 1.31034{x_2}^2 - 1.2060{x_3}^2 \\
& - 0.7897{x_4}^2 - 1.1627{x_5}^2 + \varepsilon_{EtOHA} \\
Y_{EtOHB} = {} & 12.0008 + 1.1433x_1 + 1.0387x_2 + 0.7121x_3 \\
& + 0.7001x_4 + 1.2626x_5 - 1.1575{x_1}^2 \\
& - 0.6938x_1x_2 - 1.0284{x_2}^2 - 1.0832{x_3}^2 \\
& - 0.7420{x_4}^2 - 0.9913{x_5}^2 + \varepsilon_{EtOHB} \\
Y_{EtOHC} = {} & 12.8724 + 1.1396x_1 + 0.9064x_2 + 0.8281x_3 \\
& + 0.7096x_4 + 1.2652x_5 - 1.3868{x_1}^2 \\
& - 0.6450x_1x_2 - 1.0563{x_2}^2 - 1.0598{x_3}^2 \\
& - 0.7796{x_4}^2 - 1.0677{x_5}^2 + \varepsilon_{EtOHC}
\end{aligned}
\quad -\infty \le x_i \le +\infty \quad Y_{EtOHA,EtOHB,EtOHC} \ge 0 \qquad (4.16)
$$

Where

Y_{EtOHA} = Predicted bioethanol yield in *Sialamule*fermentation (g/L)

ε_{EtOHA} = Random error associated with bioethanol yield prediction in *Sialamule*fermentation

Y_{EtOHB} = Predicted bioethanol yield in *Uganda Green*fermentation (g/L)

ε_{EtOHB} = Random error associated with bioethanol yield prediction in *Uganda Green* fermentation

Y_{EtOHC} = Predicted bioethanol yield in *Ngombe* fermentation (g/L)

ε_{EtOHC} = Random error associated with bioethanol yield prediction in *Ngombe* fermentation

x_i = Dimensionless values of the independent variables

$\propto$ = Distance of axial points

Table 4.14: ANOVA for comparing Bioethanol Yield Regression Models

Source	**Sum of Squares**	**d.o.f.**	**Mean Sum of Squares**	**F-test (observed)**	**F-test (critical)**
Columns	10.06	2	5.03	0.51	3.10
Error	88.02	90	9.81		
Total	893.08	92			

From ANOVA table 4.14, observed f-value of 0.51 smaller than the critical f-value of 3.10 at 95% confidence levels is a strong indication that the predictions given by the regression models 4.6, 4.10, and 4.14 are the same. The developed set of three regression models, equation 4.16, can therefore be applied alternatively in describing the behavior Bioethanol Yields in similar fermentation broths.

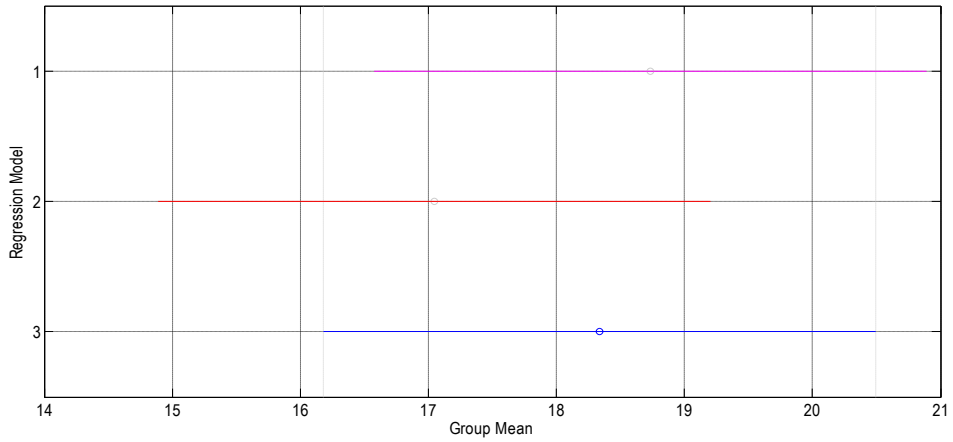


Figure 4.8: Group means from Bioethanol Yield Regression Models

From figure 4.8, it is evident that none of the three models predicts significantly different group means. High correlations co-efficients of 99.33%, 99.42%, and 99.38% between Regression Models (4.6) & (4.10), (4.6) & (4.14), and (4.10) & (4.14) above suggests high coincidence among their predictions. This further asserts that the

developed set of regression models (4.16) above can therefore be applied alternatively in describing the behavior of Bioethanol Yields in similar fermentation broths.

4.5.2 Total Reducing Sugar Models

Equation 4.17 shows three different quadratic models describing TRS degradations in various banana peels fermentation.

$$\left.\begin{aligned}
Y_{TRSA} &= 30.3741 + 2.6327x_1 + 2.0266x_2 + 1.7367x_3 \\
&\quad + 1.5546x_4 + 3.1550x_5 - 3.1342{x_1}^2 \\
&\quad - 1.4788x_1x_2 - 2.9485{x_2}^2 - 2.7240{x_3}^2 \\
&\quad - 1.7909{x_4}^2 - 2.6127{x_5}^2 + \varepsilon_{trsA} \\
Y_{TRSB} &= 27.1862 + 2.5930x_1 + 2.3234x_2 + 1.5972x_3 \\
&\quad + 1.5959x_4 + 2.8507x_5 - 2.6521{x_1}^2 \\
&\quad - 1.6094x_1x_2 - 2.3542{x_2}^2 - 2.5036{x_3}^2 \\
&\quad - 1.7204{x_4}^2 - 2.2791{x_5}^2 + \varepsilon_{trsB} \\
Y_{TRSC} &= 29.3966 + 2.5882x_1 + 2.0163x_2 + 1.8435x_3 \\
&\quad + 1.6239x_4 + 2.8313x_5 - 3.2563{x_1}^2 \\
&\quad - 1.3988x_1x_2 - 2.4732{x_2}^2 - 2.4723{x_3}^2 \\
&\quad - 1.8385{x_4}^2 - 2.5103{x_5}^2 + \varepsilon_{trsC}
\end{aligned}\right\} \quad -\infty \le x_i \le +\infty,\ Y_{TRSA,TRSB,TRSC} \ge 0 \qquad (4.17)$$

Where

Y_{TRSA} = Predicted TRS degradation in *Sialamule*fermentation (g/L)

ε_{trsA} = Random error associated with TRS degradation prediction in *Sialamule*fermentation

Y_{TRSB} = Predicted TRS degradation in *Uganda Green*fermentation (g/L)

ε_{trsB} = Random error associated with TRS degradation prediction in *Uganda Green* fermentation

Y_{TRSC} = Predicted TRS degradation in *Ngombe*fermentation (g/L)

ε_{trsC} = Random error associated with TRS degradation prediction in *Ngombe* fermentation

x_i = Dimensionless values of the independent variables

$\propto$ = Distance of axial points

Table 4.15: ANOVA for comparing TRS Degradation Regression Models

Source	**Sum of Squares**	**d.o.f.**	**Mean Sum of Squares**	**F-test (observed)**	**F-test (critical)**
Columns	48.35	2	24.18	0.48	3.10
Error	4564.92	90	80.72		
Total	4613.27	92			

From ANOVA table 4.15, observed f-value of 0.48 smaller than the critical f-valueof 3.10 at 95% confidence levels is a strong indication that the predictions given by the regression models 4.7, 4.11, and 4.15 are the same. The developed set of three regression models (4.17) above can therefore be applied alternatively in describing the behavior TRS degradations in similar fermentation broths.

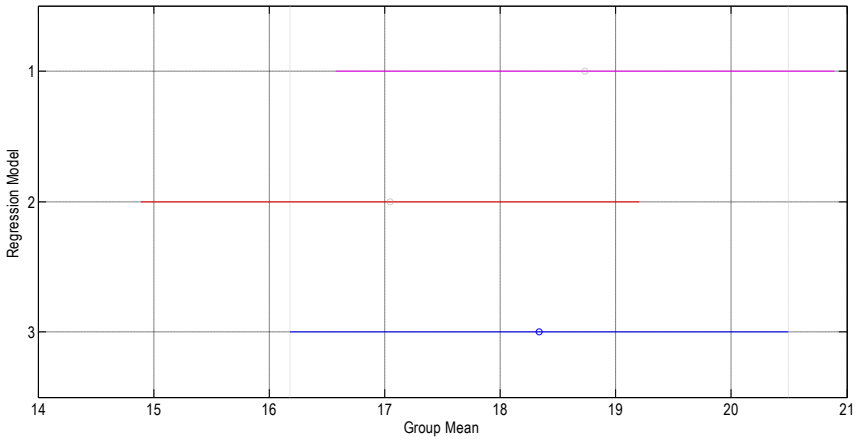


Figure 4.9: Group means from TRS Degradation Regression Models

From figure 4.9, it is evident that none of the three models predicts significantly different group means. High correlations co-efficients of 99.36%, 99.45%, and 99.24% between Regression Models (4.7) & (4.11), (4.7) & (4.15), and (4.11) & (4.15) above suggests high coincidence among their predictions. This further asserts that thedevelopedset of three regression models (4.17) above can therefore be applied alternatively in describing the behavior TRS degradations in similar fermentation broths.

4.6 BIOETHANOL YIELDS

In this study, experiments showed highest actual bioethanol yields at 180 g/L substrate concentration, 35^{o}C fermentation temperature, 5.5 initial medium pH, 2g/L yeast concentration, and 120 hours incubation period for all the three cultivars under study. It similarly shows the lowest bioethanol yields at 84.86g/L substrate concentration, 35^{o}C fermentation temperature, 5.5 initial medium pH, 2 g/L yeast concentration, and 120 hours incubation for all the three cultivars under study. Most actual maximum bioethanol yieldswere higher than 2.7 ml/L found by fermenting hydrolyzed red banana peel at 10% substrate concentration (Kumar, 2011), 11.41 ml/L from banana peels fermentation using mutant strain of *Saccharomyces cerevisiae*(Manikandan et al, 2008), 10.14 ml/L achieved through Simultaneous Saccharification and Fermentation (SSF) process involving the co-culture of both*Saccharomyces Cerevisiae* and *Candida tropicalis*(Nuttiya et al, 2013), and 13.00 ml/L gotten through Separate Hydrolysis and Fermentation (SHF) process involving the same co-culture (Nuttiya et al, 2013).

However, higher bioethanol yields of 39.29 ml/L has been achieved by fermenting banana peels within 72 hours incubation period(Vikash et al, 2012), 35.74 ml/L from hydrothermally pretreated banana peeling has also been attained by optimizing

Simultaneous Saccharification and Fermentation (SSF) processes (Harinder et al, 2011), and 19.00 ml/L from banana peels fermentation within 96 hours incubation (Arati et al, 2010) have also been achieved.These differences could be attributed to the type of banana cultivars used, fermentation media composition, microorganisms' purity, culturing procedures, and majorly fermentation techniques involved alongside other factors influencing fermentation.

In these experiments, bioethanol concentrations increased with increasing substrate concentrations up to an optimum level of 180 grams of various banana peels per litre of the fermentation broth. At high substrate sugars, yeast cells might have overcome osmotic stresses attributed to the increased bioethanol concentrations in the bioreactor (Muhammad, 2011).

The effect of internal broth temperature on bioethanol yields was also significant alongside other main effects. Highest achievable enzyme activities were assumed to correspond to the highest bioethanol yields at 35^{o}C incubation temperature. This is because the productivity of invertase highly relies on temperature (Muhammad, 2011). Further incubation temperature increase resulted to low bioethanol production which was attributed to the reduction in invertase productivities. These higher temperatures inactivate yeast culture and therefore not conducive for yeast growth (Muhammad, 2011). Thermo-tolerant strains of yeast are the best at high incubation temperatures.

The influence of initial fermentation media pH on bioethanol production was similarly considered closely. Some researchers have realized optimum pH of 5.00. Inhibitory effect of pH above the optimum values realized in this research could be due to the reduced formation of ATP across metabolic changes in the yeast (Muhammad, 2011).

Increase in yeast cells population increases bioethanol production. Yeast was used as the main producer of sugar degradation enzymes in this study. Maximum bioethanol yieldscorresponded to an optimum yeast culture loading of 2 g per litre of the fermentation broth. Yeast cells might have probably become inactive and died after 120 hours of incubation. They might have also tolerated maximum bioethanol concentrations beyond which they died thus contributing to these optimum bioethanol yields.

Very evident incubation temperatures effect at rising substrate concentrations were exhibited in the study. Production of invertase enzymes rose and then declined with severity of either factor. The remaining carbon source (sugars) was used in cell maintenance and ATP generation (Shafaghat et al, 2010). There was high substrate concentration in the broth due to fewer yeast cells consuming it (Peter, 1995). Bioethanol yields closely coincided with TRS degradation in various experimental runs.

4.7 CONCLUSION

Analytical procedures, banana growing location, harvesting method, season, and harvesting stage were similar in this study. Characterization of banana peelings, production of bioethanol through anaerobic fermentation of banana peeling under varying operational parameters using yeast in a still batch reactor, determination of bioethanol yields for every set of operational parameters, and determination of optimal conditions (parameter settings) using CCRD and RSM were performed as reported in chapter 3.

CHAPTER FIVE: CONCLUSIONS AND RECOMMENDATIONS

5.1 SUMMARY AND CONCLUSION

The main aim of this study was to determine optimal anaerobic fermentation conditions for bioethanol production from banana peelings using yeast in a still batch reactor through both experimental and statistical approaches.The following conclusions were drawn from the study:

a. A close range of 15.52% to 16.36% w/w was realized in free TRS contents of various banana cultivars used.
b. Ash which contains various minerals in different concentrations required during yeast metabolism rangedbetween 5.67% and 7.85% w/w in various banana cultivars used.
c. Concentrated acidic hydrolysis raised TRS concentrations in these peels powder by more than twice the free TRS.
d. Bioethanol yields closely coincided with TRS degradation in various experimental runs and showed that optimal fermentation conditions is important to achieve higher yields..
e. Optimum bioethanol yields of 13.93ml/L, 12.59ml/L, and 13.09ml/L from *Sialamule*, *Uganda Green*, and *Ngombe* respectively were obtained at 180 g/L Substrate Concentration, 35^{o}C Fermentation Temperature, 5.5 Initial medium pH, 2g/L Yeast Concentration, and 120 hours Incubation Period for all the three banana cultivars under study. These corresponded to TRS degradations of 31.57g/L, 29.88g/L, and 30.30g/L in *Sialamule*, *Uganda Green*, and *Ngombe* respectively.
f. Various mathematical models (equation 4.16) developed could be alternatively applied in estimating the behaviors of similar fermentative broth.

g. There was no significant difference between the results under ‘no modification’ differ from the results ‘with modification.
h. Response Surface Methodology (RSM) compared to ‘One Factor at a Time’ was very efficient, cheap, and fast in the optimization of various anaerobic fermentation factors.

5.2 RECOMMENDATIONS

This study appreciates advances in “Waste to Energy” technologies and recommends the following for further studies:

a. Quantitative measurement and elimination of various inhibitors contained in banana peels hydrolysates thus enhancing bioethanol yields.
b. Amelioration of bioethanol production from banana peels using superior and simpler substrate preparation methods and fermentation techniques.
c. Botheconomicaland cost analysis and further implementation of a pilot plant for bioethanol production from banana peels in the country and further dissemination of results from this pilot project to small and medium brewers to help them better their bioethanol yields in such fermentation processes.

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APPENDIXES

Appendix 1: MATLAB Codes

Appendix 1.1: Plotting of TRS Calibration Curve

```
>>% TRS Calibration Plotting
>>TRS_Cal = xlsread('Banana Peels Fermentation', 3);
>>TRS_Conc=TRS_Cal(:,1);
>> Abs=TRS_Cal(:,2);
>>plot(TRS_Conc, Abs)
```

Appendix 1.2: Plotting of Acid Effect on TRS Yield Curve

```
>>% Acid_effect  Plotting
>>Acid_effect =xlsread('Banana Peels Fermentation', 2);
>>Acid_Conc=Acid_effect(:,1);
>>TRSa_Sialamule=Acid_effect(:,2);TRSt_Sialamule=Acid_effect(:,3);
>> TRSa_Uganda_Green=Acid_effect(:,4);TRSt_Uganda_Green=Acid_effect(:,5);
>>TRSa_Ngombe=Acid_effect(:,6);TRSt_Ngombe=Acid_effect(:,7);
>>plot(Acid_Conc,[TRSa_Sialamule,TRSt_Sialamule,TRSa_Uganda_Green,TRSt_Uganda_Green,TRSa_Ngombe,TRSt_Ngombe])
```

Appendix 1.3: Design of Experiments

```
>>% model based calibration
>>mbcmodel
>>% half factorial experimental runs of five factors
>>generators = fracfactgen('a b c d e',4,5)
>>[dfF,confounding] = fracfact(generators)
```

Appendix 1.4: Defining Variables

```
>>DoE=xlsread('Banana Peels Fermentation', 1, 'B1:F31');
>>D = x2fx(DoE,'quadratic'); % Quadratic Array
>>Responses=xlsread('Banana Peels Fermentation', 1, 'V1:AA31');
>>X1=D(:,2);X2=D(:,3);X3=D(:,4); X4=D(:,5); X5=D(:,6);% Independents Variables
>>EtOHAa=Responses(:,2);% Ethanol Yield in Sialamule Peels Fermentation
>>EtOHBa=Responses(:,4);% Ethanol Yield in Uganda Green Peels Fermentation
>>EtOHCa=Responses(:,6);% Ethanol Yield in Ngombe Peels Fermentation
>>TRSAa=Responses(:,1);% TRS degradation in Sialamule Peels Fermentation
>>TRSBa=Responses(:,3);% TRS degradation in Uganda Green Peels Fermentation
>>TRSCa=Responses(:,5);% TRS degradation in Ngombe Peels Fermentation
```

Appendix 1.5: Regression Co-efficients

```
>>EbA = regress(EtOHAa,D); EbB = regress(EtOHBa,D);EbC = regress(EtOHCa,D);%Regression co-efficients for EtOH
>>TbA = regress(TRSAa,D); TbB = regress(TRSBa,D);TbC = regress(TRSCa,D);%Regression co-efficients for TRS
```

Appendix 1.6: F-Statistics

```
>>fstatEtOHA = regstats(EtOHAa,DoE,'quadratic','fstat'); % f statistics for EtOHA
>>f = fstatEtOHA.fstat;
>>fprintf('\n')
>>fprintf('Regression ANOVA');
>>fprintf('\n\n')
>>fprintf('%6s','Source');
>>fprintf('%10s','df','SS','MS','F','P');
>>fprintf('\n')
>>fprintf('%6s','Regr');
>>fprintf('%10.4f',f.dfr,f.ssr,f.ssr/f.dfr,f.f,f.pval);
>>fprintf('\n')
>>fprintf('%6s','Resid');
>>fprintf('%10.4f',f.dfe,f.sse,f.sse/f.dfe);
>>fprintf('\n')
>>fprintf('%6s','Total');
>>fprintf('%10.4f',f.dfe+f.dfr,f.sse+f.ssr);
>>fprintf('\n')
>>fstatEtOHB = regstats(EtOHBa,DoE,'quadratic','fstat'); % f statistics for EtOHB
>>f = fstatEtOHB.fstat;
>>fprintf('\n')
```

```
>>fprintf('Regression ANOVA');
>>fprintf('\n\n')
>>fprintf('%6s','Source');
>>fprintf('%10s','df','SS','MS','F','P');
>>fprintf('\n')
>>fprintf('%6s','Regr');
>>fprintf('%10.4f',f.dfr,f.ssr,f.ssr/f.dfr,f.f,f.pval);
>>fprintf('\n')
>>fprintf('%6s','Resid');
>>fprintf('%10.4f',f.dfe,f.sse,f.sse/f.dfe);
>>fprintf('\n')
>>fprintf('%6s','Total');
>>fprintf('%10.4f',f.dfe+f.dfr,f.sse+f.ssr);
>>fprintf('\n')
>>fstatEtOHC = regstats(EtOHCa,DoE,'quadratic','fstat'); % f statistics for EtOHC
>>f = fstatEtOHC.fstat;
>>fprintf('\n')
>>fprintf('Regression ANOVA');
>>fprintf('\n\n')
>>fprintf('%6s','Source');
>>fprintf('%10s','df','SS','MS','F','P');
>>fprintf('\n')
>>fprintf('%6s','Regr');
>>fprintf('%10.4f',f.dfr,f.ssr,f.ssr/f.dfr,f.f,f.pval);
>>fprintf('\n')
>>fprintf('%6s','Resid');
>>fprintf('%10.4f',f.dfe,f.sse,f.sse/f.dfe);
>>fprintf('\n')
>>fprintf('%6s','Total');
>>fprintf('%10.4f',f.dfe+f.dfr,f.sse+f.ssr);
>>fprintf('\n')
>>fstatTRSA = regstats(TRSAa,DoE,'quadratic','fstat'); % f statistics for TRSA
>>f = fstatTRSA.fstat;
>>fprintf('\n')
>>fprintf('Regression ANOVA');
>>fprintf('\n\n')
>>fprintf('%6s','Source');
>>fprintf('%10s','df','SS','MS','F','P');
>>fprintf('\n')
>>fprintf('%6s','Regr');
>>fprintf('%10.4f',f.dfr,f.ssr,f.ssr/f.dfr,f.f,f.pval);
>>fprintf('\n')
>>fprintf('%6s','Resid');
>>fprintf('%10.4f',f.dfe,f.sse,f.sse/f.dfe);
>>fprintf('\n')
>>fprintf('%6s','Total');
>>fprintf('%10.4f',f.dfe+f.dfr,f.sse+f.ssr);
>>fprintf('\n')
>>fstatTRSB = regstats(TRSBa,DoE,'quadratic','fstat'); % f statistics for TRSB
>>f = fstatTRSB.fstat;
>>fprintf('\n')
>>fprintf('Regression ANOVA');
>>fprintf('\n\n')
>>fprintf('%6s','Source');
>>fprintf('%10s','df','SS','MS','F','P');
>>fprintf('\n')
>>fprintf('%6s','Regr');
>>fprintf('%10.4f',f.dfr,f.ssr,f.ssr/f.dfr,f.f,f.pval);
>>fprintf('\n')
>>fprintf('%6s','Resid');
>>fprintf('%10.4f',f.dfe,f.sse,f.sse/f.dfe);
>>fprintf('\n')
>>fprintf('%6s','Total');
>>fprintf('%10.4f',f.dfe+f.dfr,f.sse+f.ssr);
>>fprintf('\n')
>>fstatTRSC = regstats(TRSCa,DoE,'quadratic','fstat'); % f statistics for TRSC
f = fstatTRSC.fstat;
```

```
>>fprintf('\n')
>>fprintf('Regression ANOVA');
>>fprintf('\n\n')
>>fprintf('%6s','Source');
>>fprintf('%10s','df','SS','MS','F','P');
>>fprintf('\n')
>>fprintf('%6s','Regr');
>>fprintf('%10.4f',f.dfr,f.ssr,f.ssr/f.dfr,f.f,f.pval);
>>fprintf('\n')
>>fprintf('%6s','Resid');
>>fprintf('%10.4f',f.dfe,f.sse,f.sse/f.dfe);
>>fprintf('\n')
>>fprintf('%6s','Total');
>>fprintf('%10.4f',f.dfe+f.dfr,f.sse+f.ssr);
>>fprintf('\n')
```

Appendix 1.7: t-Statistics

```
>>tstatEtOHA = regstats(EtOHAa,DoE,'quadratic','tstat'); % t statistics for EtOHA
>>tEtOHA = tstatEtOHA.tstat;
>>CoeffTable = dataset({tEtOHA.beta,'Coef'},{ tEtOHA.se,'StdErr'}, ...
>>{ tEtOHA.t,'tStat'},{ tEtOHA.pval,'pVal'})
>>tstatEtOHB = regstats(EtOHBa,DoE,'quadratic','tstat'); % t statistics for EtOHB
>>tEtOHB = tstatEtOHB.tstat;
>>CoeffTable = dataset({tEtOHB.beta,'Coef'},{ tEtOHB.se,'StdErr'}, ...
>>{ tEtOHB.t,'tStat'},{ tEtOHB.pval,'pVal'})
>>tstatEtOHC = regstats(EtOHCa,DoE,'quadratic','tstat'); % t statistics for EtOHC
>>tEtOHC = tstatEtOHC.tstat;
>>CoeffTable = dataset({tEtOHC.beta,'Coef'},{ tEtOHC.se,'StdErr'}, ...
>>{ tEtOHC.t,'tStat'},{ tEtOHC.pval,'pVal'})
>>tstatTRSA = regstats(TRSAa,DoE,'quadratic','tstat'); % t statistics for TRSA
>>tTRSA = tstatTRSA.tstat;
>>CoeffTable = dataset({tTRSA.beta,'Coef'},{ tTRSA.se,'StdErr'}, ...
>>{ tTRSA.t,'tStat'},{ tTRSA.pval,'pVal'})
>>tstatTRSB = regstats(TRSBa,DoE,'quadratic','tstat'); % t statistics for TRSB
>>tTRSA = tstatTRSA.tstat;
>>CoeffTable = dataset({tTRSA.beta,'Coef'},{ tTRSA.se,'StdErr'}, ...
>>{ tTRSA.t,'tStat'},{ tTRSA.pval,'pVal'})
>>tstatTRSC = regstats(TRSCa,DoE,'quadratic','tstat'); % t statistics for TRSC
>>tTRSC = tstatTRSC.tstat;
>>CoeffTable = dataset({tTRSC.beta,'Coef'},{ tTRSC.se,'StdErr'}, ...
>>{ tTRSC.t,'tStat'},{ tTRSC.pval,'pVal'})
```

Appendix 1.8: R2 Statistics

```
>>rsquareEtOHA = regstats(EtOHAa,DoE,'quadratic', 'rsquare'); % rsquare for EtOHA
>>rsquareEtOHB = regstats(EtOHBa,DoE,'quadratic', 'rsquare'); % rsquare for EtOHB
>>rsquareEtOHC = regstats(EtOHCa,DoE,'quadratic', 'rsquare'); % rsquare for EtOHC
>>rsquareTRSA = regstats(TRSAa,DoE,'quadratic', 'rsquare'); % rsquare for TRSA
>>rsquareTRSB = regstats(TRSBa,DoE,'quadratic', 'rsquare'); % rsquare for TRSB
>>rsquareTRSC = regstats(TRSCa,DoE,'quadratic', 'rsquare'); % rsquare for TRSC
```

Appendix 1.9: Adjusted R2 Statistics

```
>>adjrsquareEtOHA = regstats(EtOHAa,DoE,'quadratic', 'adjrsquare'); % adjrsquare for EtOHA
>>adjrsquareEtOHB = regstats(EtOHBa,DoE,'quadratic', 'adjrsquare'); % adjrsquare for EtOHB
>>adjrsquareEtOHC = regstats(EtOHCa,DoE,'quadratic', 'adjrsquare'); % adjrsquare for EtOHC
>>adjrsquareTRSA = regstats(TRSAa,DoE,'quadratic', 'adjrsquare'); % adjrsquare for TRSA
>>adjrsquareTRSB = regstats(TRSBa,DoE,'quadratic', 'adjrsquare'); % adjrsquare for TRSB
>>adjrsquareTRSC = regstats(TRSCa,DoE,'quadratic', 'adjrsquare'); % adjrsquare for TRSC
```

Appendix 1.10: Response Surface Plotting

```
>>xx1 = linspace(min(X1),max(X1),100);xx2 = linspace(min(X2),max(X2),100);
>>[x1,x2] = meshgrid(xx1,xx2);
>>x3=0;,x4=0;,x5=0;
>>% EtOHA regression
>>EtOHA = EbA(1) + EbA(2)*x1 + EbA(3)*x2 + EbA(4)*x3 + EbA(5)*x4 + EbA(6)*x5 + ...
>>EbA(17)*x1.^2 + EbA(7)*x1.*x2 + EbA(8)*x1.*x3 + EbA(9)*x1.*x4 + EbA(10)*x1.*x5 + ...
```

```
>>EbA(18)*x2.^2 + EbA(11)*x2.*x3 + EbA(12)*x2.*x4 + EbA(13)*x2.*x5 + EbA(19)*x3.^2 + ...
>>EbA(14)*x3.*x4 + EbA(15)*x3.*x5 + EbA(20)*x4.^2 + EbA(16)*x4.*x5 + EbA(21)*x5.^2;
>>% EtOHB regression
>>EtOHB = EbB(1) + EbB(2)*x1 + EbB(3)*x2 + EbB(4)*x3 + EbB(5)*x4 + EbB(6)*x5 + ...
>>EbB(17)*x1.^2 + EbB(7)*x1.*x2 + EbB(8)*x1.*x3 + EbB(9)*x1.*x4 + EbB(10)*x1.*x5 + ...
>>EbB(18)*x2.^2 + EbB(11)*x2.*x3 + EbB(12)*x2.*x4 + EbB(13)*x2.*x5 + EbB(19)*x3.^2 + ...
>>EbB(14)*x3.*x4 + EbB(15)*x3.*x5 + EbB(20)*x4.^2 + EbB(16)*x4.*x5 + EbB(21)*x5.^2;
>>% EtOHC regression
>>EtOHC = EbC(1) + EbC(2)*x1 + EbC(3)*x2 + EbC(4)*x3 + EbC(5)*x4 + EbC(6)*x5 + ...
>>EbC(17)*x1.^2 + EbC(7)*x1.*x2 + EbC(8)*x1.*x3 + EbC(9)*x1.*x4 + EbC(10)*x1.*x5 + ...
>>EbC(18)*x2.^2 + EbC(11)*x2.*x3 + EbC(12)*x2.*x4 + EbC(13)*x2.*x5 + EbC(19)*x3.^2 + ...
>>EbC(14)*x3.*x4 + EbC(15)*x3.*x5 + EbC(20)*x4.^2 + EbC(16)*x4.*x5 + EbC(21)*x5.^2;
>>% TRSA regression
>>TRSA = TbA(1) + TbA(2)*x1 + TbA(3)*x2 + TbA(4)*x3 + TbA(5)*x4 + TbA(6)*x5 + ...
>>TbA(17)*x1.^2 + TbA(7)*x1.*x2 + TbA(8)*x1.*x3 + TbA(9)*x1.*x4 + TbA(10)*x1.*x5 + ...
>>TbA(18)*x2.^2 + TbA(11)*x2.*x3 + TbA(12)*x2.*x4 + TbA(13)*x2.*x5 + TbA(19)*x3.^2 + ...
>>TbA(14)*x3.*x4 + TbA(15)*x3.*x5 + TbA(20)*x4.^2 + TbA(16)*x4.*x5 + TbA(21)*x5.^2;
>>% TRSB regression
>>TRSB = TbB(1) + TbB(2)*x1 + TbB(3)*x2 + TbB(4)*x3 + TbB(5)*x4 + TbB(6)*x5 + ...
>>TbB(17)*x1.^2 + TbB(7)*x1.*x2 + TbB(8)*x1.*x3 + TbB(9)*x1.*x4 + TbB(10)*x1.*x5 + ...
>>TbB(18)*x2.^2 + TbB(11)*x2.*x3 + TbB(12)*x2.*x4 + TbB(13)*x2.*x5 + TbB(19)*x3.^2 + ...
>>TbB(14)*x3.*x4 + TbB(15)*x3.*x5 + TbB(20)*x4.^2 + TbB(16)*x4.*x5 + TbB(21)*x5.^2;
>>% TRSC regression
>>TRSC = TbC(1) + TbC(2)*x1 + TbC(3)*x2 + TbC(4)*x3 + TbC(5)*x4 + TbC(6)*x5 + ...
>>TbC(17)*x1.^2 + TbC(7)*x1.*x2 + TbC(8)*x1.*x3 + TbC(9)*x1.*x4 + TbC(10)*x1.*x5 + ...
>>TbC(18)*x2.^2 + TbC(11)*x2.*x3 + TbC(12)*x2.*x4 + TbC(13)*x2.*x5 + TbC(19)*x3.^2 + ...
>>TbC(14)*x3.*x4 + TbC(15)*x3.*x5 + TbC(20)*x4.^2 + TbC(16)*x4.*x5 + TbC(21)*x5.^2;
>>% EtOHA surface plot
>>figure; surf(x1,x2, subplus(EtOHA)), hold on, ECA=contour(x1,x2,subplus(EtOHA));, clabel(ECA)
>>axis([-2.5 2.5 -2.5 2.5 0 14]), xlabel('Substrate Conc. (Level)'), ylabel('Incubation Temp. (Level)'),
>>zlabel('EtOH (A) (ml/L)'), title('X1 & X2 vs EtOH (A) Surface Plot'), bar = colorbar;
>>% EtOHB surface plot
>>figure; surf(x1,x2, subplus(EtOHB)), hold on, ECB=contour(x1,x2,subplus(EtOHA));, clabel(ECB)
>>axis([-2.5 2.5 -2.5 2.5 0 14]), xlabel('Substrate Conc. (Level)'), ylabel('Incubation Temp. (Level)'),
>>zlabel('EtOH (B) (ml/L)'), title('X1 & X2 vs EtOH (B) Surface Plot'), bar = colorbar;
>>% EtOHC surface plot
>>figure; surf(x1,x2, subplus(EtOHC)), hold on, ECC=contour(x1,x2,subplus(EtOHA));, clabel(ECB)
>>axis([-2.5 2.5 -2.5 2.5 0 14]), xlabel('Substrate Conc. (Level)'), ylabel('Incubation Temp. (Level)'),
>>zlabel('EtOH (C) (ml/L)'), title('X1 & X2 vs EtOH (C) Surface Plot'), bar = colorbar;
>>% TRSA surface plot
>>figure; surf(x1,x2, subplus(TRSA)), hold on, TCA=contour(x1,x2,subplus(TRSA));, clabel(TCA)
>>axis([-2.5 2.5 -2.5 2.5 0 35]), xlabel('Substrate Conc. (Level)'), ylabel('Incubation Temp. (Level)'),
>>zlabel('TRS Degradation (A) (ml/L)'), title('X1 & X2 vs TRS Degradation (A) Surface Plot'), bar = colorbar;
>>% TRSB surface plot
>>figure; surf(x1,x2, subplus(TRSB)), hold on, TCB =contour(x1,x2,subplus(TRSA));, clabel(TCB)
>>axis([-2.5 2.5 -2.5 2.5 0 35]), xlabel('Substrate Conc. (Level)'), ylabel('Incubation Temp. (Level)'),
>>zlabel('TRS Degradation (B) (ml/L)'), title('X1 & X2 vs TRS Degradation (B) Surface Plot'), bar = colorbar;
>>% TRSC surface plot
>>figure; surf(x1,x2, subplus(TRSC)), hold on, TCC =contour(x1,x2,subplus(TRSA));, clabel(TCC)
>>axis([-2.5 2.5 -2.5 2.5 0 35]), xlabel('Substrate Conc. (Level)'), ylabel('Incubation Temp. (Level)'),
>>zlabel('TRS Degradation (C) (ml/L)'), title('X1 & X2 vs TRS Degradation (C) Surface Plot'), bar = colorbar;
```

Appendix 1.11: Comparing Regression Models

```
>>EtOHp=xlsread('Banana Peels Fermentation', 4, 'A1:C31');
>>TRSp=xlsread('Banana Peels Fermentation', 4, 'D1:F31');
>> Predictions =xlsread('Banana Peels Fermentation', 4);
>>EtOHAp=Predictions(:,1); EtOHBp=Predictions(:,2); EtOHCp=Predictions(:,3);
>>TRSAp=Predictions(:,4); TRSBp=Predictions(:,5); TRSCp=Predictions(:,6);
>>[p,tbl,EtOHstats] = anova1(EtOHp);[c,m] = multcompare(EtOHstats);
>> [p,tbl,TRSstats] = anova1(TRSp);[c,m] = multcompare(TRSstats);
```

Appendix 2: TRS Concentration Calibration

Table 7.1: TRS Concentration Calibration

TRS Conc. (g/L)	Absorbance (490nm)			
	Trial 1	Trial 2	Trial 3	Average
0.0	0.02	0.02	0.02	0.02
0.1	0.46	0.46	0.45	0.46
0.2	0.57	0.57	0.57	0.57
0.3	0.89	0.89	0.89	0.89
0.4	1.07	1.07	1.06	1.07
0.5	1.29	1.29	1.28	1.29
0.6	1.59	1.59	1.59	1.59
0.7	1.72	1.71	1.72	1.72

Appendix 3: Sample GC Chromatograms

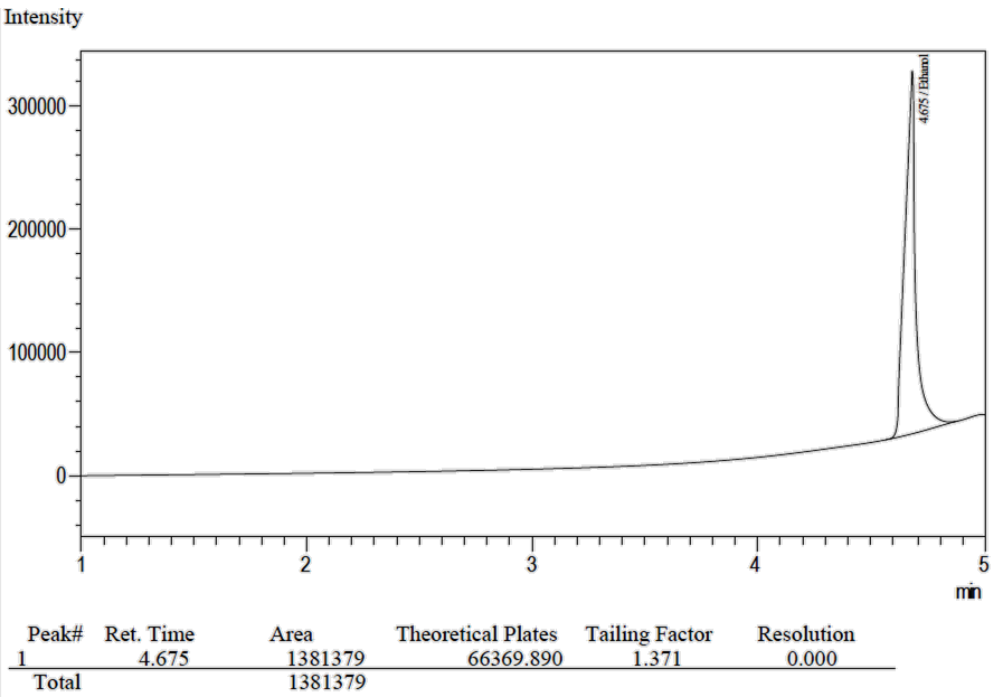
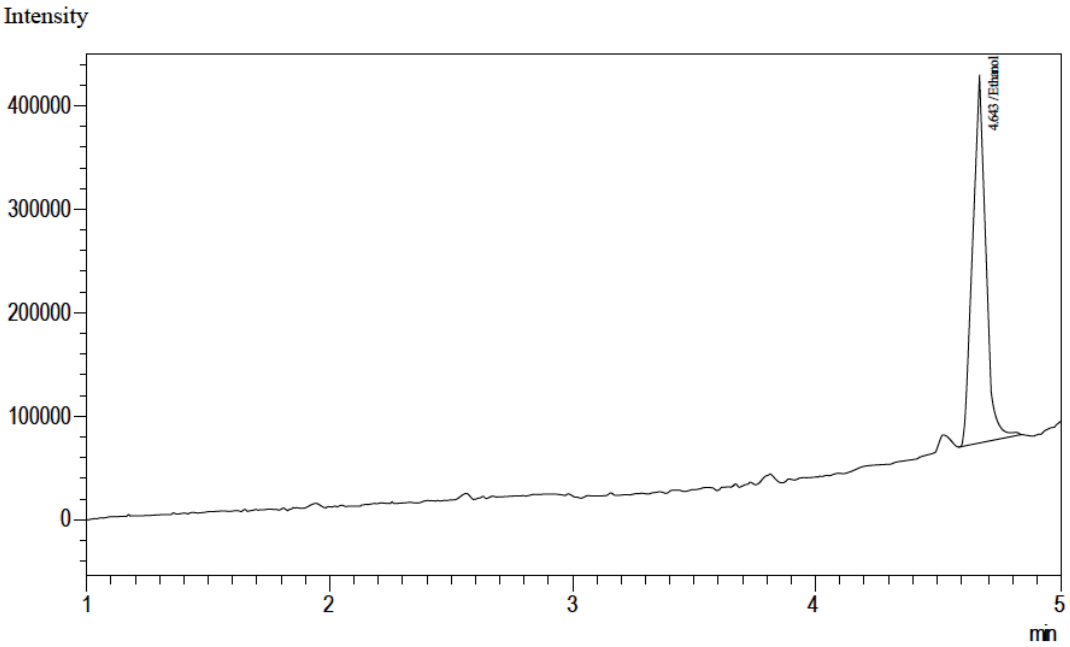
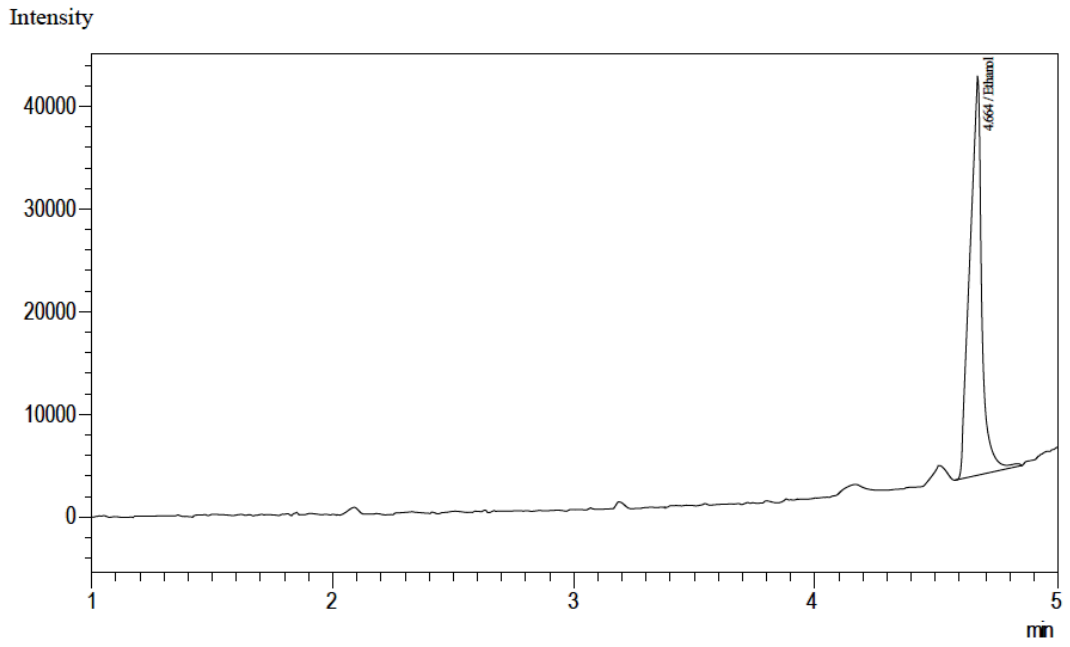


Figure 7.1: Standard (10ml/L EtOH) chromatogram



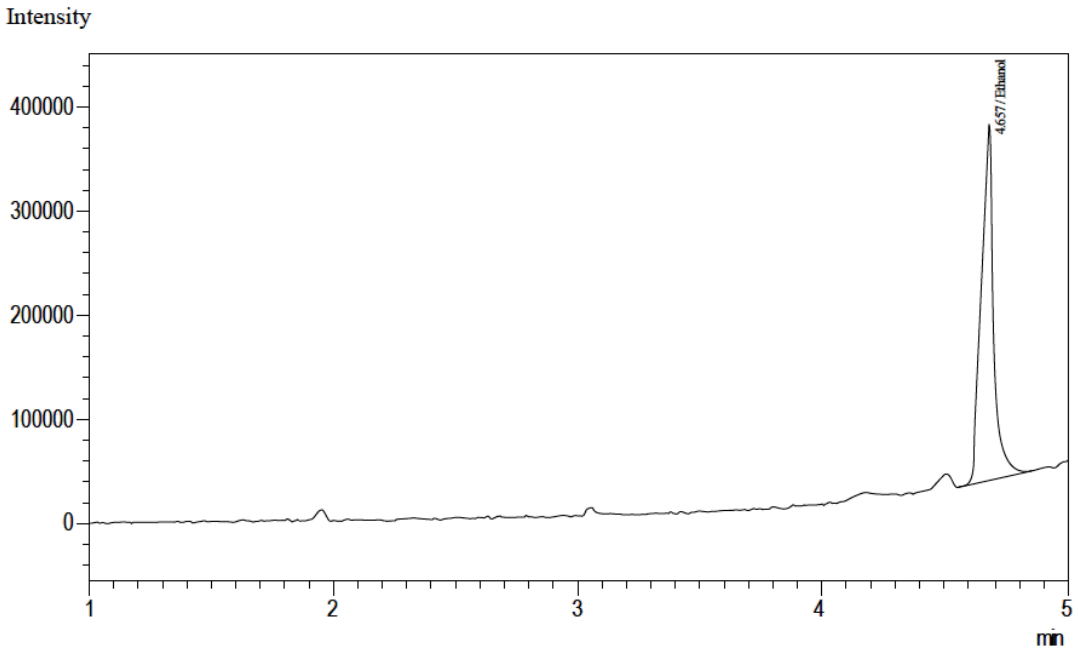
Peak#	Ret. Time	Area	Theoretical Plates	Tailing Factor	Resolution
1	4.643	1923918	66915.538	1.372	0.000
Total		1923918			

Figure 7.2: *Sialamule*: Run 28 chromatogram



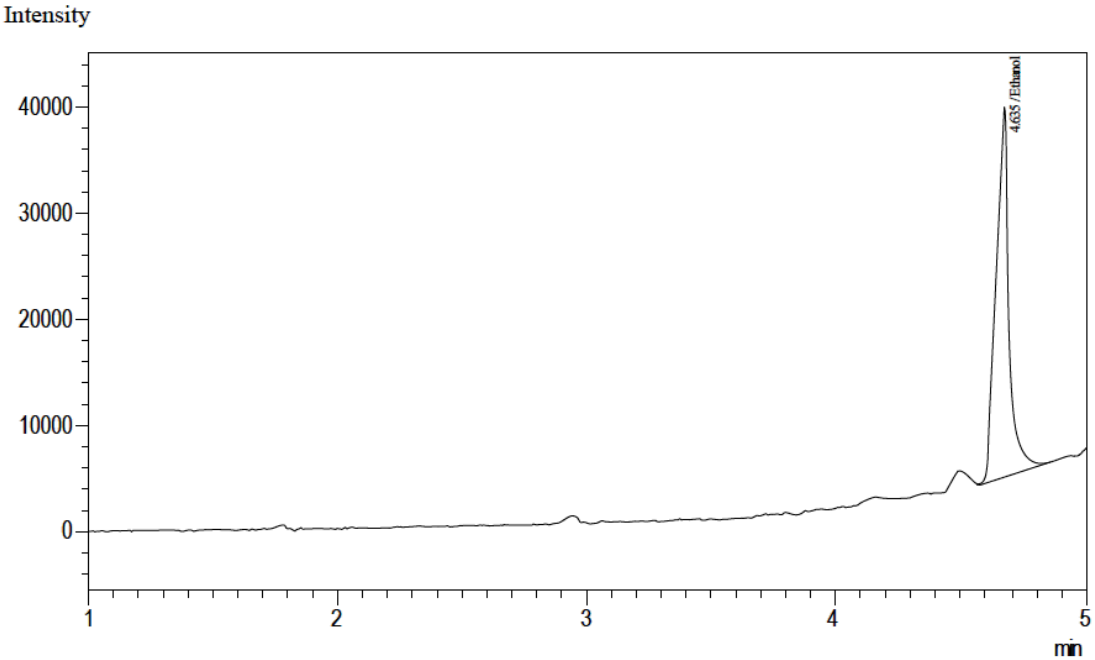
Peak#	Ret. Time	Area	Theoretical Plates	Tailing Factor	Resolution
1	4.664	208763	66973.199	1.374	0.000
Total		208763			

Figure 7.3: *Sialamule*: Run 17 chromatogram



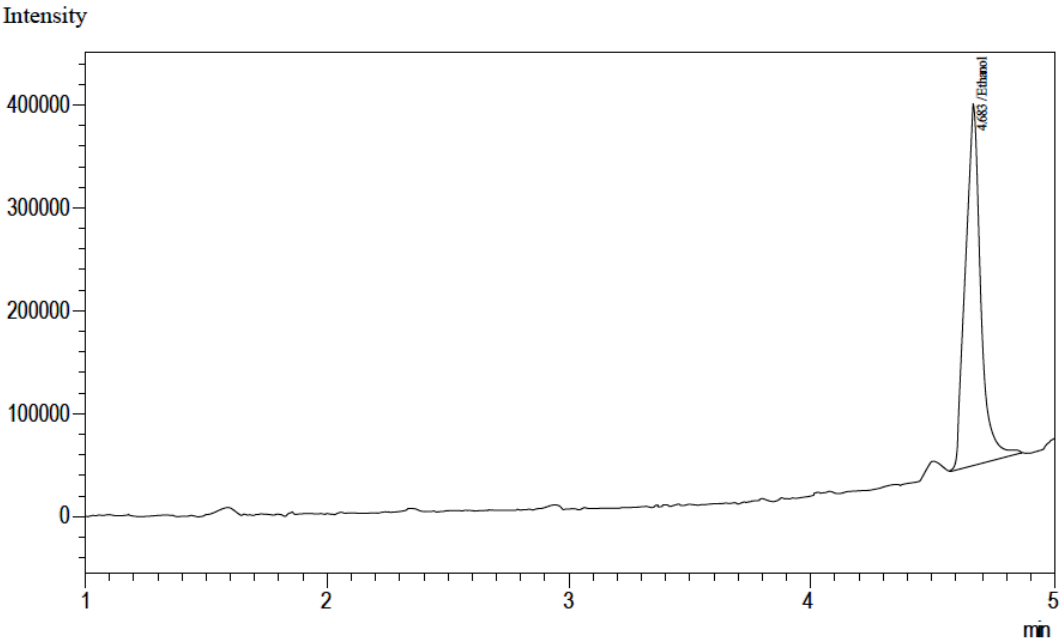
Peak#	Ret. Time	Area	Theoretical Plates	Tailing Factor	Resolution
1	4.657	1739095	67261.877	1.371	0.000
Total		1739095			

Figure 7.4: *Uganda Green*: Run 27 chromatogram



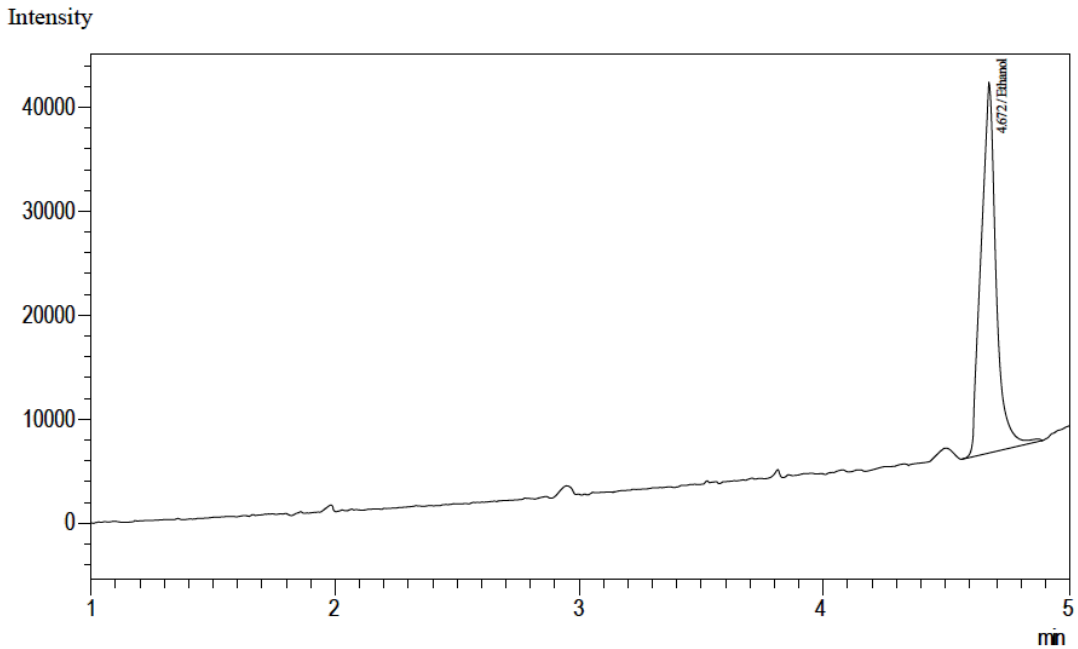
Peak#	Ret. Time	Area	Theoretical Plates	Tailing Factor	Resolution
1	4.635	183548	66685.143	1.370	0.000
Total		183548			

Figure 7.5: *Uganda Green*: Run 17 chromatogram



Peak#	Ret. Time	Area	Theoretical Plates	Tailing Factor	Resolution
1	4.683	1807961	68131.633	1.379	0.000
Total		1807961			

Figure 7.6: *Ngombe*: Run 29 chromatogram



Peak#	Ret. Time	Area	Theoretical Plates	Tailing Factor	Resolution
1	4.672	198723	67841.093	1.382	0.000
Total		198723			

Figure 7.7: *Ngombe*: Run 17 chromatogram

Appendix 4: Experimental Design Table

Table 7.2: Experimental Design Table

Run	(1)	X_1	X_2	X_3	X_4	X_5	X_1^2	X_1X_2	X_1X_3	X_1X_4	X_1X_5	X_2^2	X_2X_3	X_2X_4	X_2X_5	X_3^2	X_3X_4	X_3X_5	X_4^2	X_4X_5	X_5^2
1.	1	1	-1	-1	-1	-1	1	-1	-1	-1	-1	1	1	1	1	1	1	1	1	1	1
2.	1	-1	1	-1	-1	-1	1	-1	1	1	1	1	-1	-1	-1	1	1	1	1	1	1
3.	1	-1	-1	1	-1	-1	1	1	-1	1	1	1	-1	1	1	1	-1	-1	1	1	1
4.	1	1	1	1	-1	-1	1	1	1	-1	-1	1	1	-1	-1	1	-1	-1	1	1	1
5.	1	-1	-1	-1	1	-1	1	1	1	-1	1	1	1	-1	1	1	-1	1	1	-1	1
6.	1	1	1	-1	1	-1	1	1	-1	1	-1	1	-1	1	-1	1	-1	1	1	-1	1
7.	1	1	-1	1	1	-1	1	-1	1	1	-1	1	-1	-1	1	1	1	-1	1	-1	1
8.	1	-1	1	1	1	-1	1	-1	-1	-1	1	1	1	1	-1	1	1	-1	1	-1	1
9.	1	-1	-1	-1	-1	1	1	1	1	1	-1	1	1	1	-1	1	1	-1	1	-1	1
10.	1	1	1	-1	-1	1	1	1	-1	-1	1	1	-1	-1	1	1	1	-1	1	-1	1
11.	1	1	-1	1	-1	1	1	-1	1	-1	1	1	-1	1	-1	1	-1	1	1	-1	1
12.	1	-1	1	1	-1	1	1	-1	-1	1	-1	1	1	-1	1	1	-1	1	1	-1	1
13.	1	1	-1	-1	1	1	1	-1	-1	1	1	1	1	-1	-1	1	-1	-1	1	1	1
14.	1	-1	1	-1	1	1	1	-1	1	-1	-1	1	-1	1	1	1	-1	-1	1	1	1
15.	1	-1	-1	1	1	1	1	1	-1	-1	-1	1	-1	-1	-1	1	1	1	1	1	1
16.	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
17.	1	-2.3784	0	0	0	0	5.6569	0	0	0	0	0	0	0	0	0	0	0	0	0	0
18.	1	2.3784	0	0	0	0	5.6569	0	0	0	0	0	0	0	0	0	0	0	0	0	0
19.	1	0	-2.3784	0	0	0	0	0	0	0	0	5.6569	0	0	0	0	0	0	0	0	0
20.	1	0	2.3784	0	0	0	0	0	0	0	0	5.6569	0	0	0	0	0	0	0	0	0
21.	1	0	0	-2.3784	0	0	0	0	0	0	0	0	0	0	0	5.6569	0	0	0	0	0
22.	1	0	0	2.3784	0	0	0	0	0	0	0	0	0	0	0	5.6569	0	0	0	0	0
23.	1	0	0	0	-2.3784	0	0	0	0	0	0	0	0	0	0	0	0	0	5.6569	0	0
24.	1	0	0	0	2.3784	0	0	0	0	0	0	0	0	0	0	0	0	0	5.6569	0	0
25.	1	0	0	0	0	-2.3784	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5.6569
26.	1	0	0	0	0	2.3784	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5.6569
27.	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
28.	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
29.	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
30.	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
31.	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Appendix 5: Absorbance trend of peels hydrolysis

Table 7.3: Absorbance trend of peels hydrolysis

H_4SO_4 Conc. (%)	Absorbance (490nm)													
	Sialamule					*Uganda Green*					*Ngombe*				
	$TRS_f(Abs_{490nm})$				TRS (%w/w)	$TRS_f(Abs_{490\ nm})$				TRS (%w/w)	$TRS_f(Abs_{490nm})$				TRS (%w/w)
	T1	T2	T3	Avg		T1	T2	T3	Avg		T1	T2	T3	Avg	
0%	0.51	0.51	0.50	0.51	16.36	0.49	0.49	0.49	0.49	15.52	0.49	0.49	0.49	0.49	15.52
10%	0.57	0.57	0.57	0.57	18.90	0.57	0.56	0.57	0.57	18.90	0.55	0.55	0.54	0.55	18.05
20%	0.73	0.73	0.73	0.73	25.66	0.72	0.72	0.71	0.72	25.23	0.72	0.72	0.72	0.72	25.23
30%	0.81	0.81	0.80	0.81	29.03	0.78	0.78	0.78	0.78	27.77	0.80	0.80	0.80	0.80	28.61
40%	0.95	0.95	0.95	0.95	34.95	0.93	0.93	0.92	0.93	34.10	0.91	0.91	0.90	0.91	33.26
50%	0.94	0.94	0.94	0.94	34.52	0.96	0.96	0.96	0.96	35.37	0.94	0.94	0.94	0.94	34.52
60%	0.99	0.99	0.99	0.99	36.64	0.97	0.97	0.96	0.97	35.79	0.98	0.98	0.98	0.98	36.21
70%	0.94	0.94	0.93	0.94	34.52	0.91	0.91	0.91	0.91	33.26	0.91	0.91	0.91	0.91	33.26
80%	0.81	0.81	0.81	0.81	29.03	0.81	0.81	0.81	0.81	29.03	0.80	0.80	0.79	0.80	28.61

Appendix 6: Ethanol Yield

Table 7.4: Actual Ethanol Yields

Run	*SIALAMULE (A)*		*UGANDA GREEN (B)*		*NGOMBE (C)*	
	Peak Area (µVs)	**Actual (ml/L)**	**Peak Area (µVs)**	**Actual (ml/L)**	**Peak Area (µVs)**	**Actual (ml/L)**
1.	721393	5.22	651497	4.72	780481	5.65
2.	852311	6.17	807916	5.85	803716	5.82
3.	468158	3.39	329028	2.38	438213	3.17
4.	1138683	8.24	983636	7.12	1218978	8.82
5.	521396	3.77	443751	3.21	521751	3.78
6.	1067186	7.73	938137	6.79	942487	6.82
7.	1121584	8.12	1008935	7.30	1171893	8.48
8.	1191281	8.62	1135683	8.22	1086459	7.87
9.	962957	6.97	753318	5.45	849146	6.15
10.	961375	6.96	911729	6.60	965137	6.99
11.	1377627	9.97	1142223	8.27	1192913	8.64
12.	1322431	9.57	1187907	8.60	1269804	9.19
13.	1397896	10.12	1341547	9.71	1422657	10.30
14.	1325015	9.59	1238506	8.97	1345758	9.74
15.	1063796	7.70	1043860	7.56	1053928	7.63
16.	1695097	12.27	1579272	11.43	1658781	12.01
17.	208763	1.51	183548	1.33	198723	1.44
18.	1328213	9.62	1317608	9.54	1172683	8.49
19.	514374	3.72	462150	3.35	616570	4.46
20.	1137795	8.24	1241135	8.98	1271804	9.21
21.	621197	4.50	521748	3.78	591871	4.28
22.	1193297	8.64	1095285	7.93	1291284	9.35
23.	1013561	7.34	933038	6.75	951963	6.89
24.	1451151	10.51	1218586	8.82	1368625	9.91
25.	459161	3.32	488549	3.54	519748	3.76
26.	1423729	10.31	1271804	9.21	1351602	9.78
27.	1901035	13.76	1739095	12.59	1785048	12.92
28.	1923918	13.93	1638767	11.86	1762858	12.76
29.	1761905	12.75	1668729	12.08	1807961	13.09
30.	1813572	13.13	1578278	11.43	1767692	12.80
31.	1920017	13.90	1660393	12.02	1755732	12.71

Appendix 7: TRS Degradation in *Sialamule*

Table 7.5: TRS Degradation in *Sialamule*

Run	$TRS_o(Abs_{490nm})$**				TRS_o	$TRS_f(Abs_{490nm})$*				TRS_f	ΔTRS
	T1	T2	T3	Average	(g/L)	T1	T2	T3	Average	(g/L)	(g/L)
1.	0.96	0.96	0.95	0.96	70.74	1.52	1.52	1.51	1.52	59.02	11.72
2.	0.68	0.68	0.68	0.68	47.09	0.92	0.91	0.91	0.91	33.26	13.83
3.	0.69	0.69	0.69	0.69	47.93	1.08	1.08	1.08	1.08	40.44	7.49
4.	0.96	0.96	0.96	0.96	70.74	1.36	1.36	1.36	1.36	52.26	18.48
5.	0.70	0.70	0.69	0.70	48.78	1.08	1.08	1.08	1.08	40.44	8.34
6.	0.98	0.98	0.98	0.98	72.43	1.43	1.43	1.43	1.43	55.22	17.21
7.	0.98	0.98	0.98	0.98	72.43	1.41	1.41	1.41	1.41	54.37	18.06
8.	0.69	0.68	0.69	0.69	47.93	0.81	0.80	0.80	0.80	28.61	19.32
9.	0.69	0.69	0.69	0.69	47.93	0.89	0.89	0.89	0.89	32.41	15.52
10.	0.97	0.97	0.97	0.97	71.58	1.45	1.45	1.45	1.45	56.06	15.52
11.	0.96	0.97	0.96	0.96	70.74	1.27	1.27	1.27	1.27	48.46	22.28
12.	0.68	0.68	0.68	0.68	47.09	0.73	0.73	0.73	0.73	25.66	21.43
13.	0.99	0.99	0.99	0.99	73.27	1.33	1.32	1.32	1.32	50.57	22.70
14.	0.70	0.70	0.70	0.70	48.78	0.77	0.77	0.77	0.77	27.34	21.44
15.	0.70	0.70	0.70	0.70	48.78	0.87	0.87	0.87	0.87	31.57	17.21
16.	0.96	0.96	0.96	0.96	70.74	1.15	1.15	1.15	1.15	43.39	27.35
17.	0.48	0.48	0.48	0.48	30.20	0.77	0.76	0.76	0.76	26.92	3.28
18.	1.19	1.20	1.19	1.19	90.16	1.75	1.75	1.75	1.75	68.73	21.43
19.	0.83	0.83	0.83	0.83	59.76	1.35	1.34	1.34	1.34	51.42	8.34
20.	0.84	0.84	0.84	0.84	60.60	1.12	1.12	1.12	1.12	42.13	18.47
21.	0.87	0.87	0.87	0.87	63.14	1.39	1.38	1.38	1.38	53.11	10.03
22.	0.83	0.83	0.82	0.83	59.76	1.08	1.08	1.08	1.08	40.44	19.32
23.	0.82	0.82	0.82	0.82	58.91	1.13	1.13	1.12	1.13	42.55	16.36
24.	0.83	0.83	0.83	0.83	59.76	0.98	0.98	0.98	0.98	36.21	23.55
25.	0.82	0.82	0.82	0.82	58.91	1.34	1.34	1.34	1.34	51.42	7.49
26.	0.82	0.82	0.81	0.82	58.91	0.97	0.97	0.98	0.97	35.79	23.12
27.	0.82	0.82	0.81	0.82	58.91	0.79	0.79	0.78	0.79	28.19	30.72
28.	0.85	0.84	0.84	0.84	60.60	0.81	0.81	0.81	0.81	29.03	31.57
29.	0.83	0.83	0.82	0.83	59.76	0.86	0.86	0.85	0.86	31.15	28.61
30.	0.82	0.82	0.82	0.82	58.91	0.82	0.82	0.82	0.82	29.46	29.45
31.	0.82	0.82	0.82	0.82	58.91	0.78	0.78	0.78	0.78	27.77	31.14

*Samples were diluted 100 times; **Samples were Diluted 200 times

Appendix 8: TRS Degradation in *Uganda Green*

Table 7.6: TRS Degradation in *Uganda Green*

Run	$TRS_o(Abs_{490nm})$**				TRS_o	$TRS_f(Abs_{490nm})$*				TRS_f	ΔTRS
	T1	T2	T3	Average	(g/L)	T1	T2	T3	Average	(g/L)	(g/L)
1.	0.96	0.96	0.96	0.96	70.74	1.55	1.55	1.55	1.55	60.29	10.45
2.	0.67	0.67	0.67	0.67	46.24	1.91	1.91	1.91	1.91	33.26	12.98
3.	0.69	0.69	0.69	0.69	47.93	1.14	1.13	1.13	1.13	42.55	5.38
4.	0.94	0.93	0.93	0.93	68.20	1.36	1.36	1.36	1.36	52.26	15.94
5.	0.68	0.68	0.68	0.68	47.09	1.07	1.07	1.06	1.07	40.01	7.08
6.	0.97	0.97	0.97	0.97	71.58	1.47	1.46	1.46	1.46	56.48	15.10
7.	0.95	0.95	0.95	0.95	69.89	1.39	1.39	1.39	1.39	53.53	16.36
8.	0.80	0.79	0.79	0.79	56.38	1.02	1.02	1.02	1.02	37.90	18.48
9.	0.66	0.66	0.65	0.66	45.40	0.91	0.91	0.91	0.91	33.26	12.14
10.	0.94	0.94	0.94	0.94	69.05	1.41	1.41	1.40	1.41	54.37	14.68
11.	0.96	0.96	0.96	0.96	70.74	1.36	1.36	1.36	1.36	52.26	18.48
12.	0.66	0.66	0.66	0.66	45.40	0.74	0.74	0.74	0.74	26.08	19.32
13.	0.96	0.96	0.96	0.96	70.74	1.27	1.27	1.27	1.27	48.46	22.28
14.	0.68	0.67	0.67	0.67	46.24	0.74	0.74	0.74	0.74	26.08	20.16
15.	0.68	0.68	0.68	0.68	47.09	0.84	0.84	0.84	0.84	30.30	16.79
16.	0.95	0.95	0.95	0.95	69.89	1.17	1.17	1.17	1.17	44.24	25.65
17.	0.46	0.46	0.45	0.46	28.51	0.73	0.73	0.72	0.73	25.66	2.85
18.	1.18	1.17	1.17	1.17	88.48	1.71	1.71	1.71	1.71	67.04	21.44
19.	0.81	0.81	0.81	0.81	58.07	1.32	1.32	1.32	1.32	50.57	7.50
20.	0.82	0.82	0.82	0.82	58.91	1.04	1.04	1.04	1.04	38.75	20.16
21.	0.84	0.84	0.84	0.84	60.60	1.37	1.36	1.36	1.36	52.26	8.34
22.	0.81	0.81	0.81	0.81	58.07	1.08	1.08	1.07	1.08	40.44	17.63
23.	0.81	0.80	0.80	0.80	57.22	1.12	1.12	1.12	1.12	42.13	15.09
24.	0.82	0.83	0.83	0.83	58.91	1.05	1.05	1.05	1.05	39.17	19.74
25.	0.82	0.82	0.82	0.82	58.91	1.33	1.33	1.33	1.33	50.99	7.92
26.	0.82	0.81	0.81	0.81	58.07	1.01	1.01	1.00	1.01	37.48	20.59
27.	0.80	0.80	0.80	0.80	57.22	0.77	0.77	0.76	0.77	27.34	29.88
28.	0.84	0.84	0.84	0.84	60.60	0.93	0.93	0.93	0.93	34.10	26.50
29.	0.82	0.82	0.82	0.82	58.91	0.89	0.88	0.88	0.88	31.99	26.92
30.	0.79	0.79	0.79	0.79	56.38	0.85	0.85	0.85	0.85	30.72	25.66
31.	0.84	0.84	0.84	0.84	60.60	0.92	0.92	0.92	0.92	33.68	26.92

*Samples were diluted 100 times; **Samples were Diluted 200 times

Appendix 9: TRS Degradation in *Ngombe*

Table 7.7: TRS Degradation in *Ngombe*

Run	$TRS_o(Abs_{490nm})$**				TRS_o	$TRS_f(Abs_{490nm})$*				TRS_f	ΔTRS
	T1	T2	T3	Average	(g/L)	T1	T2	T3	Average	(g/L)	(g/L)
1.	0.95	0.95	0.95	0.95	69.89	1.48	1.48	1.48	1.48	57.33	12.56
2.	0.65	0.65	0.65	0.65	44.55	0.88	0.87	0.87	0.87	31.57	12.98
3.	0.69	0.69	0.69	0.69	47.93	1.09	1.09	1.09	1.09	40.86	7.07
4.	0.93	0.93	0.93	0.93	68.20	1.27	1.27	1.27	1.27	48.46	19.74
5.	0.69	0.69	0.69	0.69	47.93	1.06	1.06	1.06	1.06	39.59	8.34
6.	0.95	0.95	0.95	0.95	69.89	1.41	1.41	1.41	1.41	54.37	15.52
7.	0.96	0.96	0.96	0.96	70.74	1.35	1.35	1.35	1.35	51.84	18.90
8.	0.80	0.80	0.80	0.80	57.22	1.06	1.06	1.06	1.06	39.59	17.63
9.	0.68	0.68	0.68	0.68	47.09	0.91	0.91	0.90	0.91	33.26	13.83
10.	0.95	0.95	0.95	0.95	69.89	1.41	1.41	1.41	1.41	54.37	15.52
11.	0.95	0.94	0.94	0.94	69.05	1.3	1.3	1.3	1.30	49.73	19.32
12.	0.68	0.68	0.68	0.68	47.09	0.76	0.76	0.75	0.76	26.92	20.17
13.	0.96	0.96	0.96	0.96	70.74	1.25	1.25	1.25	1.25	47.62	23.12
14.	0.69	0.69	0.69	0.69	47.93	0.74	0.74	0.74	0.74	26.08	21.85
15.	0.69	0.69	0.69	0.69	47.93	0.86	0.85	0.85	0.85	30.72	17.21
16.	0.95	0.94	0.94	0.94	69.05	1.14	1.12	1.12	1.12	42.13	26.92
17.	0.48	0.47	0.47	0.47	29.35	0.75	0.75	0.75	0.75	26.50	2.85
18.	1.15	1.15	1.14	1.15	86.79	1.73	1.73	1.73	1.73	67.89	18.90
19.	0.82	0.82	0.82	0.82	58.91	1.29	1.28	1.28	1.28	48.88	10.03
20.	0.82	0.82	0.82	0.82	58.91	1.03	1.03	1.02	1.03	38.33	20.58
21.	0.86	0.85	0.85	0.85	61.45	1.36	1.35	1.35	1.35	51.84	9.61
22.	0.82	0.82	0.81	0.82	58.91	1.02	1.02	1.02	1.02	37.90	21.01
23.	0.81	0.81	0.81	0.81	58.07	1.13	1.13	1.13	1.13	42.55	15.52
24.	0.82	0.82	0.82	0.82	58.91	0.99	0.99	0.99	0.99	36.64	22.27
25.	0.82	0.82	0.82	0.82	58.91	1.32	1.32	1.32	1.32	50.57	8.34
26.	0.82	0.82	0.81	0.82	58.91	1.00	1.00	0.99	1.00	37.06	21.85
27.	0.80	0.80	0.80	0.80	57.22	0.78	0.78	0.77	0.78	27.77	29.45
28.	0.84	0.84	0.84	0.84	60.60	0.88	0.88	0.88	0.88	31.99	28.61
29.	0.83	0.83	0.82	0.83	59.76	0.82	0.82	0.81	0.82	29.46	30.30
30.	0.81	0.81	0.81	0.81	58.07	0.82	0.82	0.82	0.82	29.46	28.61
31.	0.81	0.81	0.81	0.81	58.07	0.80	0.79	0.79	0.79	28.19	29.88

*Samples were diluted 100 times; **Samples were Diluted 200 time