OPTIMIZATION OF BIOETHANOL PRODUCTION FROM SELECTED AGRICULTURAL WASTES

BY

OHANUSI, IRENE N. BSc (Uniport), MSc (FUTO)
REG. NO.: 20174079318

A DISSERTATION SUBMITTED TO THE POSTGRADUATE SCHOOL, FEDERAL UNIVERSITY OF TECHNOLOGY, OWERRI

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF DOCTOR OF PHILOSOPHY (PhD) DEGREE IN INDUSTRIAL MICROBIOLOGY

FEBRUARY, 2024.

CERTIFICATION

This is to certify that this research work, **"Optimization of Bioethanol Production from Agricultural Wastes (*Cyperus esculentus, Arachis hypogea, Phaseolus vulgaris*) Owerri, Imo State, Nigeria"**, was carried out by **Ohanusi Irene N. (20174079318)** in partial fulfilment of the requirements for the award of Doctor of Philosophy (Ph.D) in Industrial Microbiology in the Department of Microbiology, School of Biological Sciences, Federal University of Technology, Owerri, Imo State, Nigeria.

-----------------------------	20/02/24
Prof. Wesley Braide (Main Supervisor)	**Date**
-----------------------------	20/02/2024
Prof. I. E. Adieze (Co Supervisor)	**Date**
-----------------------------	20/02/2024
Prof. C. O. Akujobi (Co Supervisor)	**Date**
-----------------------------	20/02/2024
Prof. I. E. Adieze (Head of Department)	**Date**
-----------------------------	20/02/24
Prof. Chinwe Alisi (Dean of SOBS)	**Date**
-----------------------------	--------------------------
Prof. B.O. Esonu (Dean of PGS)	**Date**
-----------------------------	20/02/24
Prof. Tokuibiye Sokari (External Examiner)	**Date**

DEDICATION

Dedicated to God Almighty for His mercies and enablement over the years.

ACKNOWLEDGEMENTS

I am grateful to God for his enablement.

I am also grateful to all those with whom I have had the pleasure to work with during this project. The Iconic supervisors of my Dissertation, Prof. W. Braide, Prof. C.O. Akujobi, and Prof. I.E. Adieze, thank you for providing me extensive professional guidance and teaching me a great deal about scientific research and life in general.

I would especially like to thank Prof. Wesley Braide. As my teacher and mentor, he has taught me more than I could ever give him credit for here. He has shown me, by his example, what a good scientist (and person) should be.

A special appreciation goes to my lecturers and peers at the Department of Microbiology; engaging in stimulating academic exchanges with all of you while maintaining a personal connection has been truly enriching.

I sincerely appreciate Dr. Etinne Chinakwe, Mr. H.U. Anuforo, and Mr. Innocent Eze for their immense support. I won’t fail to acknowledge The Post Graduate School team my amiable Deans, Prof. B.O. Esonu, Prof. Nweke C.O. and Prof. Chinwe Alisi, for their prompt and unwaivering response to my academic needs. Special gratitude to my external examiner, Prof. Tokuibiye Sokari for his professional examination.

Nobody has been more important to me in the pursuit of this project than the members of my family: Okechukwu Samuel Okoroafor. I would like to thank my immediate family, Ugochukwu Ohanusi, whose love and encouragement are with me thus far. Most importantly, I wish to thank my loving and supportive wonderful children, Emmanuel, Daniel, Samuel and Joel, who provided unending inspiration.

TABLE OF CONTENTS

Pages

CHAPTER ONE: INTRODUCTION

CHAPTER TWO: LITERATURE REVIEW

CHAPTER THREE: MATERIALS AND METHODS

CHAPTER FOUR: RESULTS AND DISCUSSION

CHAPTER FIVE: CONCLUSION, CONTRIBUTION AND RECOMMENDATIONS

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

This study investigated the production of bioethanol from agricultural wastes: tigernut (*Cyperus esculentus*) waste, beans (*Phaseolus vulgaris*) husk, and groundnut (*Arachis hypogea*) shell. The use of agricultural wastes in bioethanol production helps in decreasing the reliance on conventional food crops such as corn, sugarcane, cassava etc. *Saccharomyces cerevisiae* and a new strain of mold, *Aspergillus niger isolate HEFAPhR*, were used to ferment tigernut waste, beans husk, groundnut shell to produce bioethanol. The proximate and amino acid analysis of these substrates was carried out to determine the moisture, fat, protein, ash and carbohydrate content of each of the wastes. Effects of substrate weight, inoculum size, pH, fermentation time and temperature were studied; Response surface methodology of Box-Behnken design of five factors at three levels was adopted to determine optimal conditions for improved bioethanol yield. This design gave a total of 46 experimental runs per substrate evaluated. The substrates were independently subjected to physical and biological pretreatments using Cellulase enzyme to hydrolyze the lignocellulose to fermentable sugars. The isolate from food samples and brewers yeast were identified and molecularly characterized. *Aspergillus niger isolate HEFAPhR* and *Saccharomyces cerevisiae* respectively and were able to withstand stress and tolerated exposure to ethanol of different concentration up to 94%. Optimum conditions for brix production were determined and were used to obtain maximum yield of ethanol. A comparative study of bioethanol production between *Saccharomyces cerevisiae* and *Aspergillus niger isolate HEFAPhR* was ascertained. The results showed that the fermentation of tigernut waste with *Saccharomyces cerevisiae* maximum brix of 2.28^{o}Bx and 1.6g/l ethanol was observed at optimum conditions of pH 6.8, temperature 25^{o}C, fermentation time 93h, inoculum size 5 and substrate weight of 11g, brix value of 0.53^{o}Bx and ethanol content of 1.64g/l with actualized volume of 0.2479(ml) were obtained. Fermentation of tigernut waste with *Aspergillus niger isolate HEFAPhR* of observed brix of 8.0^{o}Bx and 4.8g/l ethanol. At optimum conditions of pH 6, temperature of 34^{o}C, time 96h, inoculum size 5 and substrate weight of 11g/l, maximum value of brix of 6.7^{o}Bx and ethanol yield of 5.1g/l with actualized volume of 0.772(ml). Fermentation of beans husk with *Saccharomyces cerevisiae* maximum brix was observed to be 6.7^{o}Bx and 3.0g/l ethanol, at optimum conditions of pH 6, temperature 30^{o}C, fermentation time of 48h, inoculum size of 5.4 and substrate weight of 12g, a maximum brix of 2.8^{o}Bx and 5.47g/l ethanol was obtained with a final volume of 0.829(ml). Fermentation of beans husk with *Aspergillus niger isolate HEFAPhR* resulted in a maximum brix of 3.8^{o}Bx and 2.20g/l ethanol at optimal condition of pH 6, temperature 35^{o}C, time 96h, inoculum size 5, substrate weight of 10g, maximum brix of 2.09^{o}Bx and 2.48g/l ethanol with actualized volume of 0.373(ml). In the fermentation of groundnut shell with *Saccharomyces cerevisiae*, a maximum brix of 4.6^{o}Bx and 2.6g/l ethanol was observed at optimum conditions of pH 6, temperature 35^{o}C, substrate weight of 12g, time 72h, a maximum brix 2.84^{o}Bx and 3.47g/l ethanol with an actualized volume of 0.524(ml) were obtained, while groundnut shell with *Aspergillus niger isolate HEFAPhR* gave a maximum brix of 9.9^{o}Bx and 5.20g/l ethanol at optimum conditions of temperature 35^{o}C, time 96h, substrate weight 15g, pH 6, inoculum, a maximum brix of 10.0^{o}Bx and 23.43g/l with actualized volume of 3.634(ml) indicating that groundnut shell with *Aspergillus niger isolate HEFAPhR* gave the highest production of bioethanol, followed by beans husk with *Saccharomyces cerevisiae*, demostrating that at optimal conditions these agro wastes are best alternative substrates for maximum yield of bioethanol.

Key words: Agricultural wastes, Bioethanol, Brix, Optimization, Response Surface Methodology.

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background Information

The difficult impinge of fossil fuel use upon the society, the high cost and scarcity of fossil fuel resources and its fast exhaustion has rekindled the interest of everyone to reconsider and focus on alternative renewable and sustainable forms of clean energy (Chilakamarry, Sakinah, Zularisam, Sirohi, Khilji, Ahmad & Pandey, 2022). Therefore, the alternative energy supply must confirm with some basic requirements including substantial reduction of greenhouse gas emission, fortifying rural and agricultural economies, increasing sustainability of the world transportations system, and capability of being produced from renewable and sustainable sources. Production of biofuels especially bioethanol from lignocellulosic plant biomass is an interesting replacement for conventional fossil fuels. Bioethanol can be applied in many ways; however, today the major use of ethanol is as an oxygenated fuel additive.

Biofuels are fuels produced from plants, energy crops, or from agricultural, commercial, domestic, and/or industrial wastes (Awogbemi, Kallion, Onuh & Aigbodion, 2021b). These include bioethanol, biodiesel, biogas etc. They are most useful in liquid or gaseous form, it makes them easier to transport, deliver and burn cleanly (Awogbemi et al., 2021a). Atmospheric pollution is a great disadvantage of using petroleum-based fuels; biomass is a better source of energy for bioethanol production (Guo, Wu, Tian, Zang & Liu, 2021). Biomass resources are agricultural and forestry residues, solid wastes from the municipal, industrial wastes, and terrestrial and aquatic crops that are grown just for energy purposes. They can be converted to other usable forms of energy. The most common biomass resources used for generating electricity and power are agricultural and forestry residues, especially residues from paper mills (Baghbanzadeh, Savage, Balde, Sartaj, Vanderzaag & Abdehagh, 2021).

Biofuels are generally compatible with existing liquid transport fuel and this makes them unique among available alternative energy sources. Biofuels are classified into four categories namely the first, second, third and fourth generations of biofuels (Cavelius, Engelhart-Straub, Mehler, Lercher, Awad & Bruk, 2023). First generation biofuels are usually made from carbohydrates, lipids and oils found in food crops. Second generation biofuels are fuels derived from non-food biomass and other agro-industrial wastes. Though bioethanol production from first generation technologies, is estimated to increase to more than 207 billion liters by 2026 (Statista, 2021b), these raw materials compete with food, they are insufficient to meet the increasing demand for fuels, and have negative impact on biodiversity and may even lead to deforestation to gain more farmland (Karthick & Nanthagopal, 2021). The cumulative impact of these concerns have increased the interests in developing ‘‘second generation bioethanol’’ from non-food lignocellulosic materials such as agricultural residues, wood, paper and municipal solid waste, and dedicated energy crops (miscanthus, switchgrass, sweet sorghum, etc.), which constitute the most abundant renewable organic component in the biosphere (Statista, 2022h). Third generations are produced from oil producing microalgae, while fourth generation biofuels produced from engineered microalgae (Kumari & Das, 2019). The production and use of biofuels across the globe has increased in recent years, from 4%-96%, with about 85% of this being bioethanol (JenniferHolmgren, 2021).

Bioethanol is an alcohol produced from organic biomass. It is a biodegradable, non-toxic and proven alternative to fossil fuel that can be blended for use in any petrol-engine car without modification. Bioethanol is a clear colorless liquid that is made by the fermentation of sugar/starch based crops or through hydrolysis and subsequent fermentation of lignocelluloses which is the material that forms the basic structural components of plant dry matter (Favaro, Jansen & van Zyl, 2019). Ethanol fuel, known as ethyl alcohol is mostly used as an additive to biofuel for motor fuel. It burns with a smokeless blue flame that is not always visible in normal light. It is a 2-carbon alcohol with the molecular formula CH_3CH_2OH (Figure 1.1).

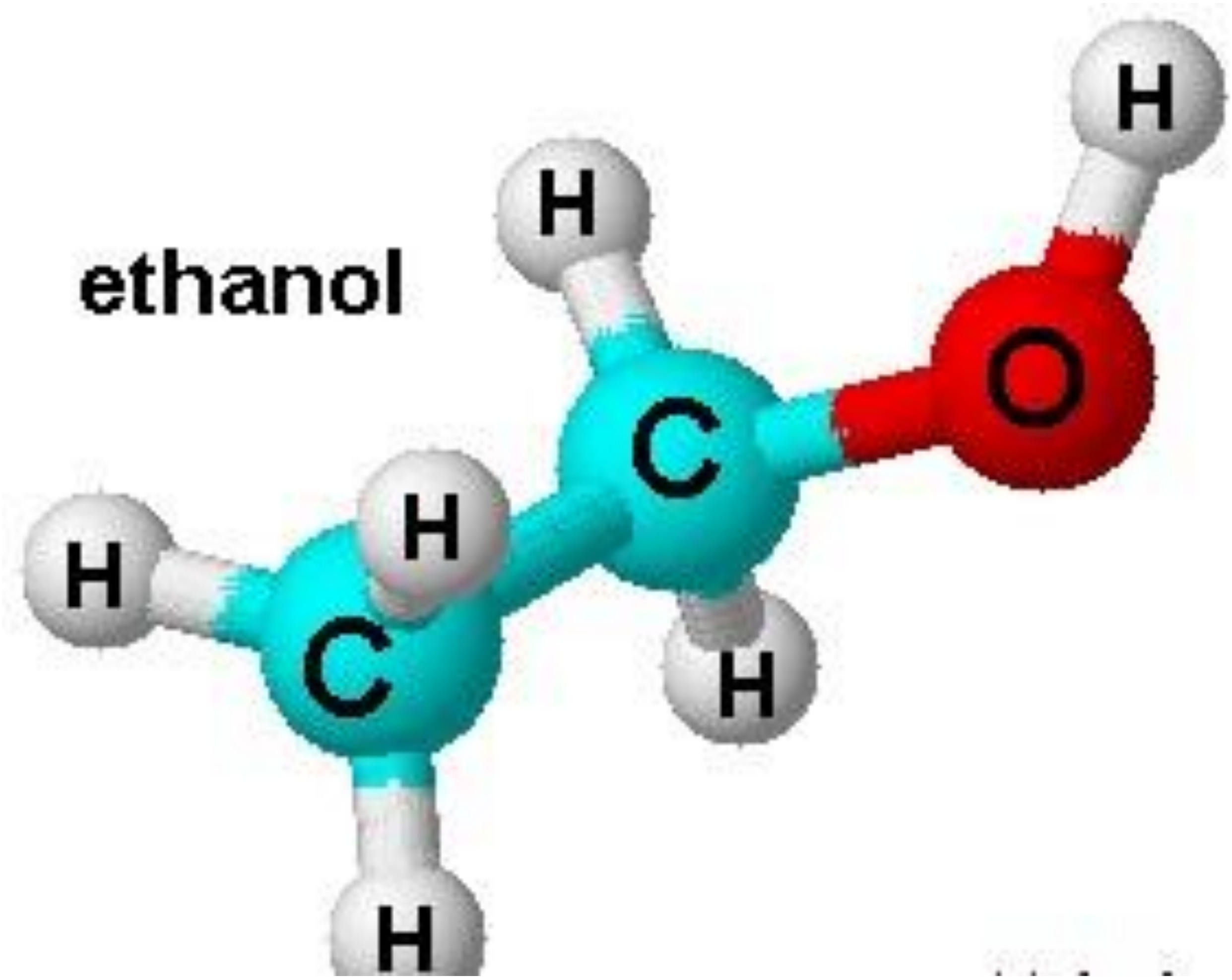


Figure 1.1: Molecular Structure of Ethanol

Source: Kumari & Das (2019)

Bioethanol is a renewable liquid biofuel produced from the fermentation of sugar and starch component of natural materials, usually plants derivatives or agricultural wastes. Bioethanol, or simply ethanol, is the most common liquid biofuel consumed globally. The demand for bio-ethanol has continued to motivate increased production. The global demand for bioethanol which was put at 100.2 billion litres in 2016 has been projected to become 134.5 billion litres by 2024 (Busic, Mardetko, Kundas, Morzak, Belskaya, Santek, 2018).

Ethanol has already been used in cars for over 80 years – Henry Ford regarded it as 'the fuel of the future' and designed his early engine to run on a bioethanol/petrol mix. Greenhouse gas emissions can be reduced through the use of bioethanol, as the crops used to produce bioethanol absorb CO_2 as they grow.

The use of cellulosic materials such as agricultural wastes like corncob, cornstalk, cornhusk, sugarcane bagasse, in the production of bioethanol by converting those to fermentable sugars, has been reported (Zhang, Yuan, Guo, 2020; Braide, Kanu, Oranusi & Adeleye, 2016). The bioethanol can be blended with petrol or used as a pure fuel in certain engines. Depending on the feedstock used, bioethanol can reduce greenhouse gas emissions by approximately 30-85 % compared to gasoline (Stastista, 2022h).

The USA and Brazil are the primary producers of bioethanol across the globe, producing 56.6% and 28 .3 % in 2018-2022 respectively, United States and Brazil manufactured 89.6% and 76.5% of global ethanol production volume as shown Figure 1.2.

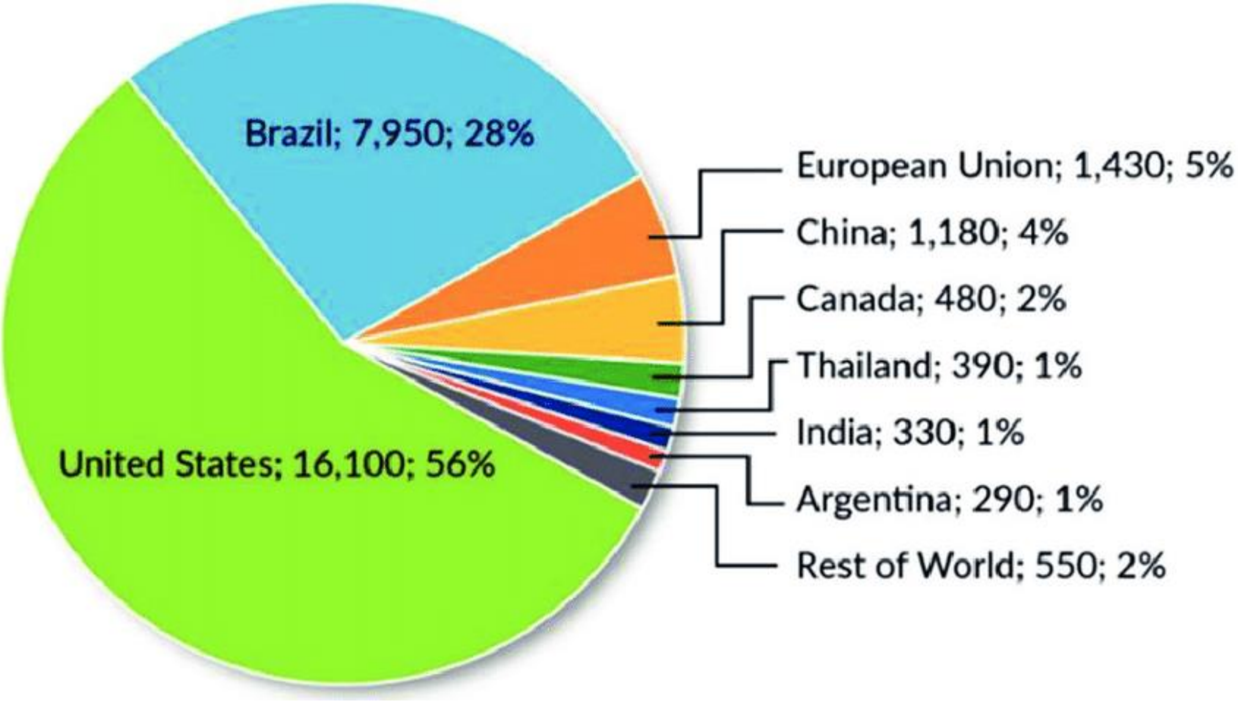


Figure 1.2: Structure of Global Production of Bioethanol by Countries

Source: Bajpai (2021)

The world energy demand for biofuels is set to grow by 41 to 53 billion litters or 28%, over 2021 to 2026 (IEA, 2021). China is nowadays investing heavily in ethanol production and is one of its largest producers (Pattanaik, Pattnaik, Saxena & Naik, 2019). In India, the interest in biofuels is growing so as to substitute oil for achieving energy security and promote agricultural growth. Indian government has planned to achieve a target of 20% blending of fossil fuels with biodiesel and bioethanol by 2025.

It is known that ethanol production using cellulosic materials faces challenges. These problems have been overcome through pre-treatments like acid hydrolysis (Dhyani & Bhaskar, 2018). The production of ethanol is achieved through fermentation processes; also ethanol yield by microorganisms in the fermentation depends on the use of ideal microbial strain, appropriate fermentation substrate and suitable process technology (Dhyani & Bhaskar, 2018; Kumari & Singh, 2018; Zhang et al., 2021). The production of ethanol through bioconversion of cellulosic biomass to fermentable sugar using cellulose degrading microorganisms makes bioethanol production economical, renewable and environmentally friendly (Delrío, Gullon, Rebelo, Romani & Garrote, 2020).

1.2 Statement of Problem

In July, 2023, it was showed by the Sahara Reporters, New York, through the world poverty clock that 133,885,874 plus 4.3 million people in Nigeria which make up 51.5% of Nigeria, population; now live in abject poverty. According to the World Bank, a person can be said to be living in extreme poverty if he/she lives below the poverty line of $1.90 which is equivalent to ₦1,900 per day. This means that more than half of the population of Nigeria lives on less than

$1.90 a day. This high poverty index of Nigeria in relation to the amount of fuel pump price from fossil fuels has reduced the accessibility of energy by many Nigerians.

The decrease in world biofuel generation and CO_2 emission (IEA, 2022) due to the COVID 19-imposed restrictions; the lockdown impeded vehicular movements, social-economic crisis of Russia-Ukraine conflict and industrial activities, has inflated the cost of fuels being consumed. Notwithstanding the disturbing increase in the emissions of CO_2 and other anthropogenic gases, has increased the demand for renewable and environmentally friendly fuels.

Kumar, Yadav, Yadav & Priyanka (2019) documented cellulose as the most abundant biomaterial in the world. Cellulosic materials continuously constitute nuisance to the world because the energy in them is underutilized. Any bioconversion that can access the energy in cellulose such as bioethanol remains a global interest.

Some of the substrates available for bioethanol production globally, compete with our food chain thereby reducing food supply. This work exploited the most abundant material on the earth’s crust and how it can be depolymerised for bioethanol production.

Limited knowledge of advanced optimization and increased yield of bioethanol from biomass.

1.3 Aim and Objectives of the Study

The aim of this research was to optimize the production of bioethanol from selected agricultural wastes.

1.4 Objectives of the Study

The specific objectives are as follows:

To Process tigernut waste, beans husk and groundnut shell using physical and biological methods.

To isolate, identify and characterize molecularly the brewers yeast and the mold from the food samples (irish potatoes).

To determine the chemical compositions of tigernut waste, beans husk and groundnut shell before pre-treatment.

To determine and compare the efficiency of isolated *Saccharomyces cerevisiae* and *Aspergillus niger isolate HEFAPhR* for bioethanol production under different stressors.

To measure brix and estimate bioethanol production by optimizing various operating parameters using five factors and three levels of Box-Behnken design.

To compare bioethanol production between *Aspergillus niger isolate HEFAPhR* and *Saccharomyces cerevisiae*.

1.5 Justification of the Study

Several works have been published on the use of cellulosic materials for bioethanol production using known microorganisms. In this study, a new strain of mold named *Aspergillus niger isolate HEFAPhR* was isolated, identified, the isolate was used as the inoculum for fermentation production of bioethanol. Most of the researches done adopted OVAT (one variable at a time) technique to optimize production process; others employed limited number of variables in the optimization process. However, industrial factors affecting production do not only affect singly but as a result of combination of factors for the production of bioethanol. In this study, a Box-Behnken design was adopted where five factors at three levels were combined to improve production efficiency.

1.6 Scope of the Study

The present study was restricted to use of selected Agro wastes (tigernut, beans husk, groundnut shell) with *Saccharomyces cerevisiae* and a new strain of *Aspergillus niger isolate HEFAPhR* for the production of bioethanol. Both the quantitative and qualitative chemical analysis of the substrates were ascertained. Though there are different types of agro wastes and only TNW, BH, GS were chosen and optimized for the production of brix and ethanol using the refractormeter, at the experimental stage. Other studies were limited to one variable factor at a time. Box–Behnken design of five factors at three levels of Temperature (^{o}C) 25, 30, 35, Time (h) 48, 72, 96, Substrate weight (g) 10, 15, 20, Innoculum size of 0.5 (MacFaland) 5, 6, 7 (ml) and pH 6, 7, 8 was used for modeling the responses and the fermentation of substrates to produce bioethanol.

CHAPTER TWO

LITERATURE REVIEW

The global energy crisis has swept across developing and developed countries. This is caused by the availability of fossil fuels which is very limited and non-renewable. Thus, the need for enhanced and optimized production of bioethanol as the alternative clean energy that is renewable. As reported by the World Energy Outlook, about 82% of the world's energy needs come from petroleum, natural gas and coal (IEA, 2022). With the Russia-Ukraine crisis which stoked inflatory pressures and created a looming risk of recession, and energy shortfalls. Clean energy becomes a huge opportunity for growth and economic competition (World Energy Outlook, 2019).

2.1 Biofuels

Biofuels are non-toxic, biodegradable and free of sulphur and carcinogenic compounds like benzene (Awogbemi et al., 2021a). Given the enormous wastes generated from the agricultural sector, and their impact on humans, animals, and the environment, its best they are converted into biofuel. The transformation of agricultural wastes to biofuel, therefore, is one of the sustainable waste management strategies to ensure sanitation, resource recovery, and maintaining the carbon balance in the environment (Awogbemi, Kallon & Owoputi, 2022). Biochemical or thermochemical processes remain the two sustainable ways to convert agricultural wastes into biofuels. The biochemical conversion pathway, which is a combination of biological and chemical processes, is commonly used to convert agricultural wastes with high moisture, cellulose, and hemicellulose contents, and a C/N ratio greater than 30% to biodiesel, biomethane, bioethanol, and biobutanol. Conversely, the thermo-chemical conversion pathway is best suited for the conversion of agricultural wastes with high lignin percentage, low moisture content.

The thermochemical route combines the thermal and chemical methods for the synthesis of biosyngas, biooil, biochar and biocoal. From the standpoint of energy utilization and greenhouse gas (GHG) emission, biochemical conversion is preferred to thermochemical conversion (Ibarra-Gonzalez & Rong, 2019; Pattanaik et al., 2019).

Bioethanol is one of the most important and prevalent biofuels in the market and replaces some portion of fossil fuels (IEA, 2022). Bioethanol produced from inexpensive and abundant biomass has been considered as one of the most attractive and promising renewable energy sources (Energy Information Administration, 2023).

2.2 Ethanol

Ethanol, also called ethyl alcohol, is either produced from petroleum or biomass. It is the ethanol produced from biomass that is most often referred to as bioethanol. Ethanol is a chemical that is volatile, colourless and flammable as shown in Table 2.1. It can be produced from petroleum via chemical transformation of ethylene, but it can also be produced by fermentation of glucose, using yeast or other microorganisms. Ethanol burns with a smokeless blue flame that is not always visible in normal light. It is slightly more refractive than water, having a refractive index of 1.36242 (at $\lambda = 589.3$nm and 18.35°C or 65.03°F) (Abdulrahman, Nasirudeen, Timothy, 2022). Ethanol is a 2-carbon alcohol with the molecular formula CH_3CH_2OH. Its empirical formula is C_2H_6O. An alternative notation is $CH_3–CH_2–OH$, which indicates that the carbon of a methyl group ($CH_3–$) is attached to the carbon of a methylene group ($–CH_2–$), which is attached to the oxygen of a hydroxyl group ($–OH$). Ethanol is the systematic name defined by the international union of pure and applied chemistry (IUPAC) nomenclature of organic chemistry for a molecule with two carbon atoms (prefix "eth-"), having a single bond between them (suffix "-ane"), and an attached -OH group (suffix "-ol") (Abdulrahman, 2022).

Table 2.1: Physico-Chemical Characteristics of Ethanol as a Liquid Fuel

Parameter	**Characteristic Properties**
Molecular formula	C_2H_5OH
Molecular mass	46.07 g/mol
Appearance	Colourless liquid
Water solubility	between –117°C and 78°C∞ (miscible)
Density	0.789 kg/l
Boiling temperature	78.5°C (173°F)
Freezing point	–117°C
Flash point	12.8°C (lowest
temperature of ignition)	
Ignition temperature	
Explosion limits	425°C
19% (v/v)	Lower 3.5% (v/v) Upper
Vapour pressure @ 38°C	50 mm Hg
Higher heating value (at 20°C)	29,800 KJ/kg
Lower heating value (at 20°C)	21,090 KJ/kg
Specific heat	Kcal/Kg 60°C
Acidity (pKa)	15.9
Viscosity	1.200 mPa.s (20°C)
Refractive index (nD)	1.36 (25°C)
Octane number	99

Source: Kumari (2019)

Bioethanol has long been considered as a suitable alternative to fossil fuels either as a sole fuel in cars with dedicated engines or as an additive in fuel blends with no engine modification requirement when mixed up to 30%. Today, bioethanol is the most dominant biofuel and its global production showed an upward trend over the last 25 years with a sharp increase from 2000 (Awogbemi et al., 2021b). Ethanol-blended fuel is widely used in Brazil, the United States, and Europe (Abel, Coney, Johnson, Thornton, Zigler & Mccormick, 2023). Most cars on the road today in the U.S. can run on blends of up to 83% ethanol (Alexandre, 2021).

2.3 Grades of Ethanol

2.3.1 Denatured Ethanol

Denatured alcohol also called methylated spirits or denatured rectified spirit is ethanol that has additives (denaturants) to make it poisonous, bad tasting, foul smelling or nauseating to discourage recreational consumption. These include bittering agents such as denatonium benzoate and toxins such as methanol, naphtha, and pyridine. Products of this kind are called denatured alcohol. Denatured alcohol is used as a solvent and as fuel for alcohol burners and camping stoves (Song et al., 2016).

2.3.2 Absolute Alcohol

Absolute or anhydrous alcohol refers to ethanol with low water content. There are various grades with maximum water contents ranging from 1% to a few parts per million (ppm) levels. If azeotropic distillation is used to remove water, it will contain trace amounts of the material separation agent (e.g. benzene) (National Center for Biotechnology Information, 2022). Absolute alcohol is not intended for human consumption. Absolute ethanol is used as a solvent for laboratory and industrial applications, where water will react with other chemicals, and as fuel alcohol. It is hygroscopic (it attracts water). Spectroscopic ethanol is an absolute ethanol with a low absorbance in ultraviolet and visible light, fit for use as a solvent in ultraviolet-visible spectroscopy (NCBI, 2022).

2.3.3 Rectified Spirit

Rectified spirit, an azeotropic composition of 96% ethanol containing 4% water, is used instead of anhydrous ethanol for various purposes. Wine spirits are about 94% ethanol. The impurities are different from those in 95% laboratory ethanol (Bomin, Shinje, Hyunjun, Jinwon, Hankwon & Wangyun, 2021).

2.4 Bioethanol Fuel

Bioethanol is made from biomass which is organic matter (plant or animal material) used for energy production. Ethanol produced from biomass is called bioethanol. It is most often used as a motor fuel, mainly as a biofuel additive for gasoline. The plants used in the production of bioethanol are called energy crops and are grown specifically for energy use. These include corn, maize and wheat crops, waste straw, willow and popular trees, saw dust, reed canary grass, cord grasses, Jerusalem artichoke, myscanthus and sorghum plants. The chemical energy in biomass is released as heat, when it is burned. Biomass can be burned directly or converted to liquid biofuels or biogas that can be burned as fuels (Alexandre, 2021). Examples of biomass and their uses for energy:

1. **Wood and wood processing wastes:** burned to heat buildings, to produce process heat in industry and to generate electricity.
2. **Agricultural crops and waste materials:** burned as a fuel or converted to liquid biofuels.

3. **Food, yard, and wood waste in garbage:** burned to generate electricity in power plants or converted to biogas in landfills.
4. **Animal manure and human sewage:** converted to biogas which can be burned as a fuel.

2.5 Bioethanol, an Eco-friendly Biofuel

Bioethanol is mainly used as an internal combustion engine fuel to blend with gasoline. This approach offers performance and economic benefits to the consumers. The use of bioethanol/gasoline blend in transport engines leads to 90 % CO_2, 60–80 % SO_2, and about 40 % particulate matter emissions (Halder, Azad, Shah, Sarker, 2019; Hoang & Nghiem, 2021). The drastic reduction in these toxic emissions helps to minimize air pollution, ensures environmental security, and reduces the emission of GHGs and other cancer-causing compounds such as ethyl benzene, xylene, toluene, and benzene. Also, because bioethanol is produced mainly from waste biomass as feed stocks, it is generally cost-effective and contributes to waste management and sanitation.

Ethanol production process only uses energy from renewable energy sources; no net carbon dioxide is added to the atmosphere, making ethanol an environmentally beneficial energy source. Ethanol contains 35% oxygen that helps complete combustion of fuel and thus reduces particulate emission that poses health hazard to living beings. The toxic level of the exhaust emissions from ethanol is lower than that of petroleum sources (Abel et al., 2023). Thus, the use of even 10% ethanol blends reduces GHG emissions by 12-19 % compared with conventional fossil fuels. Burning E 85 (85 % ethanol) reduces the nitrogen oxide, particulate and sulfate emissions by 10, 20 and 80 %, respectively, compared to conventional gasoline.

The use of bioethanol could reduce greenhouse gases (GHGs) by as much as 86% (Statista, 2020). A greenhouse gas is a gas that absorbs and emits radiant energy within the thermal infrared range. The primary greenhouse gases in Earth's atmosphere are water vapour, carbondioxide, methane, nitrous oxide and ozone. Greenhouse gases cause the greenhouse effect. Greenhouse effect is the process by which radiation from a planet's atmosphere warms the planet's surface to a temperature that is higher than what it would be without this atmosphere (Statista, 2022c).

According to the United States Energy Information Administration, unlike gasoline, pure bioethanol is non-toxic, biodegradable and quickly breaks down into harmless substances if spilled. However, ethanol has a lower energy density than that of gasoline, meaning more ethanol, in terms of volume and mass, needs to be combusted to produce the same amount of energy. Ethanol fuel has a gasoline gallon equivalency (GGE) value of 1.5. This means that, to replace the energy of 1 volume of gasoline, 1.5 times the volume of ethanol is needed (Alternative Fuels Data Center, 2021). The energy per unit volume of ethanol is 65% lower than that of gasoline, (Alternative Fuels Data Centre, 2021) therefore 1.5 gallons of ethanol contains approximately the same amount of energy as one gallon of gasoline.

Compared to fossil fuel, bioethanol fuel has the following special qualities:

1. It has a high-octane number than gasoline, thereby providing premium blending properties. Minimum octane number requirements for gasoline prevent engine knocking and ensure drivability. Lower octane gasoline is blended with 10% ethanol to attain the standard 87 octane (Alternative Fuels Data Center, 2021).

2. Bioethanol is able to reduce the level of particle emission that endangers health (carbon monoxide, CO).
3. It is similar to gasoline, so its use does not require engine modification.
4. It is regarded as renewable fuel that does not have CO_2 emissions.
5. It has low-cost production.
6. It is an alternative solution in facing globalization since it is eco-friendly.
7. It can be made at home and low-cost production than fossil fuel.
8. The physical characteristics of bioethanol in its pure form are dissolvable in all proportions with water and even with ether, acetone, benzene, and some other organic solvents (Deshmukh, Pande, Choudhari, & Pendse, 2023).
9. Ethanol's characteristic chemical function is dominated by the OH-group, which can readily help reactions in chemical industries such as dehydration, halogenation, ester formation and oxidation (Deshmukh et al., 2023).

2.6 Feedstock used in Bioethanol Production

The raw materials used in the manufacture of bioethanol are classified into three main types: sugars, starches, and cellulose materials.

Sugars like the cane or sweet sorghum juice, molasses, can be used directly for ethanol production through fermentation.

Starches gotten from corn, cassava, potatoes, and root crops, must first be hydrolyzed to fermentable sugars by the action of enzymes which are gotten from malt or molds.

Cellulose from wood, agricultural residues, waste sulfite liquor from pulp, and paper mills, must also be converted into sugars, generally by the action of acids or cellulolytic enzymes (Jitendra, Cheng, Peeta, Anil, Peetu & Chiu-wen, 2022).

Maize, wheat, rice, and sugarcane are the four agricultural crops with maximum production as well as area under cultivation. The use of renewable biomass resources for second-generation biofuel production, has received greater focus in the world. Renewable ‘plant biomass’ refers particularly to cheap and abundant non-food lignocellulose-rich materials that are available from the plants. The global production of major agro wastes and their bioethanol production potential are shown in the Table 2.2 below.

Table 2.2: Worldwide Availability of Major Agricultural Wastes and Their Bioethanol Production Potential

Agricultural wastes	Availability (million tons)	Estimated Bioethanol Potential
Wheat straw	354.34	104
Rice straw	731.3	205
Corn straw	128.2	58.6
Sugarcane bagasse	180.73	51.3

Source: Offor-Emenike, Ibekwe, Akujobi & Braide (2020)

2.7 Agro Wastes in Bioethanol Production

In countries like India, agricultural production of various crops like cotton, mustard, chilli, sugarcane, sorghum, sweet, pulses, oilseeds, etc. results in generation of huge amounts of wastes that do not find any alternative use, they are either left in the fields or are burned. These can serve as good alternative resources to generate biofuels such as bioethanol, in an environmentally friendly manner. The use of agricultural residues helps in decreasing our reliance on food crops, forest woody biomass, hence reduction of deforestation. Crop residues that have short harvest period render them readily available to bioethanol production (Singh, Sevda, Abu Reesh, Vanbroekhoven, Rathore & Pant, 2015; Sharma & Dubey 2020; Pan, Chi, Zhou, Li, Du & Wei, 2020; Shafiei, Alavijeh, Karimi & van den Berg, 2020).

Table 2.3: Composition of Various Agricultural and other Lignocellulosic Residues

Material	Cellulose[a]	Hemicellulose	Lignin	Ash	Extractives
Algae (green)	20–40	20–50	–	–	–
Cotton, flax,	80–95	5–20	–	–	–
Grasses	25–40	25–50	10–30	–	–
Hardwoods	45 ± 2	30 ± 5	20 ± 4	0.6 ± 0.2	5± 3
Hardwood barks	22–40	20–38	30–55	0.8 ± 0.2	6 ± 2
Softwoods	42 ± 2	27 ± 2	28 ± 3	0.5 ± 0.1	3 ± 2
Softwood barks	18–38	15–33	30–60	0.8 ± 0.2	–
Cornstalk	39–47	26–31	3–5	12–16	–
Wheat straw	37–41	27–32	13–15	11–14	–
Newspaper	40–55	25–40	18–30	–	–
Chemical pulp	60-80	20–30	2–10	–	–
Sorghum stalks	27	25	11	–	–
Corn stover	38-40	28	7–21	3.6–7.0	–
Coir	36–43	0.15–0.25	41–45	2.7–10.2	–
Bagasse	32–48	19–24	23–32	1.5–5	–
Rice straw	28–36	23–28	12–14	14–20	–
Wheat straw	33–38	26–32	17–19	6–8	–
Barley straw	31–45	27–38	14–19	2–7	–
Sorghum straw	32	24	13	12	–
Sweet sorghum Bagasse	34–45	18–28	14–22	–	–

Source: Kumar et al. (2019); Offor-emenike et al. (2020)

2.8 Lignocellulosis Biomass

In the last decades, lignocellulosic biomass has attracted many researchers as one of the most promising and sustainable sources for producing bioethanol (Ketsub, Latif, Kent, Doherty, O'Hara, Zhang & Kaparaju, 2021). The Low-cost and sustainable lignocellulosic materials which are feed stocks for bioethanol production are the forestry and agricultural residues (Funseca, Mateo, Roberto, Sanchez, Moya, 2020). Three major components create the structure of lignocellulosic biomass: cellulose, hemicellulose and lignin. Cellulose, hemicellulose, and lignin contents of lignocellulosic materials are different in amount and their entangled structure. These components create a complex matrix where lignin and hemicellulose surround cellulose and protect it from access to the enzyme which causes recalcitrance of enzymatic hydrolysis of cellulose (Naik, Poonia & Chaudhari, 2021; Xue, Jingying, Yan, Dong, Yan, Li, Mei, Jinguang & Fei, 2023; Zhang, Wang, Gou, Zhou, Liu, Xu, Liu, Zhou & Gong, 2022a; Uzoejinwa, He, Wang, El-Fatah Abomohra, Hu & Wang, 2018; Statista, 2022a; Zhang et al., 2020). Various amounts of cellulose, hemicellulose, and lignin are found in different lignocellulosic biomass sources.

Lignin is a random aromatic compound which hinders cellulosic bioethanol production due its strong linkages to cellulose and hemicellulose. The complex nature of lignin polymerization makes a challenge for cellulose separation and further depolymerisation. Before the hydrolysis of cellulose, the lignin content of biomass must be removed in order to provide access for the enzyme to reach the cellulose. Lignin content of softwood is higher than for other biomass which means that cellulose and hemicellulose amount of softwood is lower which makes the softwood pretreatment more severe. In addition to this, amongst the same categories of biomass, hemicellulose content also varies in sub-components as shown in Table 2.3 as Xylan is a major part of hemicellulose in agriculture residues and even hardwoods while the majority of hemicellulose content in softwoods is mannan. These different compositions in major components will cause a different output combination of the pretreated product and highly influence the subsequent ethanol production units, hydrolysis and fermentation (Tse, Wiens & Reaney, 2021; Md-Razali, Ibrahim, Kamal-Bahrin & Abd-Aziz, 2018; John, Yaragarla & Appusamy 2020; Zhang, Liu, Kou, Zhang & Tan, 2019; Kumari & Singh, 2018; Chilakamarry et al., 2022).

2.9 Tigernut Waste

Tiger nut is a perennial tuber or nut commonly found wild and cultivated in Nothern Nigeria. Tiger nut is also known as chufa, earth almond, yellow nut sedge and Zulu nuts (Adedara, Ogunsuyi & Akinnawo, 2020). It is known in Nigeria as "Aya" in Hausa, 'Ohio" in Yoruba and "Akiausa" in Igbo where three varieties (black, brown and yellow) are cultivated The spherical underground tubers are edible and have a sweet nutty flavour and are consumed fresh, dried and in rusted forms (Nata'alal, Farouq, Magashi, & Liman, 2018). Fresh tubers are also fermented to produce a local alcoholic drink, thus releasing secondary waste products which are potentially rich source of fibre and can be converted to value added product of bioethanol.

2.10 Groundnut Shell

Groundnuts (*Arachis hypogeae L.*) are species of legumes. Residues from groundnut pods are called shell and the residue from the roasted nut is called peels. The production of peanuts according to the Food and Agriculture Organization (FAO) statistical yearbook in 2013 was 43,982,066t, produced in 27,660,802 hectares (Ogunsuyi & Adejumobi, 2020). Peanuts are grown mainly in Asia, with a global production rate of 65.3%, followed by Africa with 26.2%, the Americas with 8.4%, and Oceania with 0.1% (Ogunsuyi & Adejumobi, 2020). Only small part of the groundnut shell is used as compost and animal feed. Lately, groundnut shell has been used as a feedstock in oil production. In recent years (2018) the world has been experiencing a tremendous increase in the search for alternative energy sources to replace the conventional fossil fuels due to the finite nature of crude oil and other fossil fuels which must increase by 60% in 2050 (Awogbemi et al., 2021a).

2.11 Beans Husk

Beans (*Phaseolus vulgaris*) is one of the most frequently consumed legume globally, and it is an important legume produced for consumption by humans directly (Awolu & Oyeyemi, 2015; Zhang et al., 2020).

Bean husk is formed or gotten from the removal of beans outer layers, leaving only the seeds; this process is called dehusking. Seeds are removed from the husks and the remaining husks are then used for different purposes such as a supplemental fiber for ruminants, animal feed and can as well be converted into compost or biodisel. After harvesting and processing, the bean husk is discarded and ultimately causes environmental pollution. However, this can be managed to reduce pollution and its effects on humans via converting to bioethanol or composting (Elaigwu & Oluwatosin, 2017).

2.12 Bioconversion of Biomass to Bioethanol

Biomass to ethanol bioconversion process consists of several steps, including pretreatment of biomass, enzymatic hydrolysis, fermentation and product recovery. Proper combination of each step is important for achieving higher bioethanol yield in a cost-effective and sustainable manner.

2.12.1 Processing and Pretreatment

Feedstock pretreatment is the main processing challenge in ethanol production from cellulosic biomass. During pretreatment, the matrix of cellulose and lignin bound by hemicellulose is broken. This reduces the crystallinity of cellulose and increases the fraction of amorphous cellulose, which is the most suitable form for enzymatic attack. The yield of cellulose hydrolysis after pretreatment often exceeds 90% of theoretical as compared to 20% when pretreatment is not carried out (Dahunsi, 2019). For pretreatment of biomass, several physical, physicochemical and biological processes are employed that improve lignocellulose digestibility in very different ways (Antwi, Engler, Nelles, & Schüch, 2019; Dong, Cao, Zhao, Liu, & Ren, 2018; Fonseca, Mateo, Roberto, Sanchez & Moya, 2020).

2.12.2 Pretreatment Methods

2.12.2.1 Physical Pretreatment Methods

Cellulosic biomass can be milled by chipping, grinding, shearing, or milling, which reduces the particle size and increases surface area, facilitating the access of enzymes to the biomass surface and increasing the conversion of cellulose. Primary size reduction should produce particles that can pass through 3- to 5-mm diameter sieve. Other useful physical treatment methods include pyrolysis, irradiation with gamma radiation, microwave, infrared, or sonication (Kasmuri, Kamarudin, Abdullah, Hasan & Som, 2019; Ge, Yek, Cheng, Xia, Wan-Mahari & Liew, 2021).

2.12.2.2 Physico-chemical Methods

Physico-chemical methods combine the physical and chemical methods of pretreatment. Different chemical agents employed during these processes are ozone, acids, alkali, peroxide and organic solvents. Several physicochemical methods are employed for pretreament of biomass before its saccharification, such as ammonia fiber explosion (AFEX) (Sharma & Larroche, 2020; Liu et al., 2021), autohydrolysis (steam explosion) (Ziaei-Rad, et al., 2021; Owuna & Makut, 2018), SO_2 steam explosion (Zhang, Jiang, Zhang, Zhang & Zhang, 2022b), acid treatment (Pattanaik et al., 2019; Stastista, 2022f) and alkali treatment (Zhang et al., 2020).

2.12.3 Biological Treatment

The brown rot, white rot and soft-rot fungi such as *Phaner ochaete chrysosporium, Trametes versicolor, Ceriporiopsis subvermispora, and Pleurotus ostreatus* are employed for biological pretreatment of cellulosic biomass. Besides lignin peroxidases and manganese dependent peroxidases, polyphenol oxidases, laccases and quinosine-reducing enzymes also degrade lignin in lignocelluloses, by producing aromatic radicals. Biological treatment requires low energy and normal environmental conditions but the hydrolysis yield is low and requires long treatment times (Awogbemi et al., 2022).

2.12.4 Enzymatic Hydrolysis

Cellulose hydrolysis, also known as saccharification, is the process in which the cellulose is converted into glucose. Enzymatic hydrolysis is the key to cost-effective ethanol production from cellulosic substrates in the long run, as it is very mild process, gives potentially high yields, and the maintenance costs are low compared to acid or alkaline hydrolysis (Zhang et al., 2020). The process is compatible with many pretreatment methods, but materials poisonous to the enzymes need to be removed or detoxified when chemical pretreatment precedes enzymatic hydrolysis. Factors affecting enzymatic saccharification process involve substrate concentration, enzyme loading, temperature and time of saccharification (Md-Razali et al., 2018).

2.12.5 Microbial Cellulases

In nature there are many microorganisms that produce cellulolytic enzymes (cellulases). The cellulolytic organisms can be sorted into two different subcategories depending on their enzyme organization in the cell:

1. The microorganism with their cellulases organized into multi enzyme complexes called cellulosomes, e.g. *Clostridium thermocellum* and Cellulomonas.
2. The cellulolytic organisms producing non-complexed cellulase that are not attached to one another, and act individually and cooperatively on cellulose, and by doing this gain strong synergistic effects. Examples of fungi from this class are *T. reesei* and *Humicola grisea* and of bacteria, *Streptomyces vividans* and *Cellulomonas fimi* (da Costa Filho, de Araújo Padilha, Matias, Ribeiro, dos Santos & de Santana Souza, 2023). *Trichoderma spp.* (e.g. *T. reesei, T. viride, T. longibrachiatum, T. pseudokoningii* and *T. harzianum*) are ideal cellulolytic model organisms for studying cellulose degradation since these secrete large amounts of cellulases. Today several species of cellulase producing *Penicillium* spp. are known (e.g. *P. citrinum, P. occiantalis, P. italicum,* etc.). Moreover, many species of *Aspergillus* such as *A. nidulans, A. niger* and *A. oryzae* are also known as potential cellulase producers (Zhang et al., 2019).

2.13 Microorganisms of Bioethanol Production

Microorganisms, termed ethanologens, presently convert an adequate portion of the sugars from biomass to bioethanol. There are a number of microorganisms that produce significant quantities of bioethanol (Braide et al., 2016). Yeast is the most commonly used microorganism in fermentation processes. Yeasts are minute, often unicellular, fungi. The yeasts used are typically bakery yeasts. Yeasts capable of fermenting the decaying biomass include, but are not limited to, *Saccharomyces cerevisiae* and *Saccharomyces uvarum*. Non-Saccharomyces yeasts, also known as non-conventional yeasts, are also used to make a number of commercial products. Some examples of non-conventional yeasts include *Kuyberomyces lactis*, *Yarrowiali polytica*, *Hansenula polymorpha* and *Pichia pastoris* (Abdulrahman, 2022).

2.14 Microorganisms of Interest in This Research Work

2.14.1 *Aspergillus niger isolate HEFAPhR*

Aspergillus niger, one of the most common species of the genus *Aspergillus* that causes a disease called black mold on certain fruits and vegetables such as grapes, onions, and peanuts, and is a common contaminant of food. It is ubiquitous in soil and most commonly reported from indoor environments, where its black colonies can be confused with those of Stachybotrys which have also been called black mould (Simpfendorfer, Kyne, Noble, Goldsbury, Basiit. & Jerry, 2016). World Health Organization supports the view that *Aspergillus niger* can be cultured for the industrial production of citric acid and gluconic acid that are safe for human consumption (Abdulrahman, 2022). The organism is also explored for production of enzymes like glucosidase, amylase, cellulase, pectinase and protease. When *Aspergillus niger* is cultured on Sabouraud dextrose agar, Czapek dox agar or potato dextrose agar and incubated at 25^{o}C, they tend to produce spores within 7 days (Gani, Abdulkadir, Usman, Maiturare & Gabriel, 2018).

Macroscopic and morphological identification is based on colony pigmentation and the structure of the conidial head. Microscopic mounts can be done using a cellotape flag or slide culture preparation mounted in lactophenol cotton blue. A drop of alcohol is needed to detach the cellotape flag from the stick, and to act as a wetting agent (Gani et al., 2018). Colonies on potato dextrose agar are wooly initially white, quickly becoming black with conidial production (Simpfendorfer et al., 2016). Hyphae are septate and hyaline. Conidial heads are radiate initially, splitting into columns at maturity. Conidiophores are long, smooth, and hyaline, becoming darker at the apex and terminating in a globose vesicle. Conidia are brown to black, very rough, globose, and measure up to 6 or 7µm diameter (Simpfendorfer et al., 2016). According to the research work of Offor-emenike et al. (2020), the hydrolyses of wheat using *Aspergillus niger*, shows the optimum range of temperature, pH and particle size are 45 – 50^{o}C, 4.5 - 5.0 and 75µm-150µm respectively and that the substrate concentration increases from 1.0gL-1 to 10gL-1, glucose concentration increases from 10mg/dl to about 90mg/ dl after a hydrolysis time of 8 hours, and when cell loading increases, glucose concentration also increases.

2.14.2 *Saccharomyces cerevisiae*

Saccharomyces cerevisiae belong to the order Saccharomycetales under the phylum Ascomycota. It was the first microorganism known to possess the ability to ferment sugars for the production of ethanol and carbon dioxide (Abdulrahman, 2022). *Saccharomyces cerevisiae* has been explored through history for the production of alcoholic drinks and used in the rise of the dough during bread production, hence, the name brewer’s and baker’s yeast. *Saccharomyces cerevisiae* breaks down glucose to ethanol through aerobic and anaerobic fermentations. The aerobic process requires the presence of oxygen while anaerobic process does not require the presence of oxygen (Abdulrahman, 2022).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Sample Collection

Tigernut waste, Beans husk and Groundnut shell were collected from various markets in Owerri, Imo State and used for the experiment. The substrates were sun-dried for 2 weeks and grounded separately using a laboratory blending machine. These were sieved to obtain fine powdered stock and were stored in labelled transparent polyethylene bags, at room temperature.

3.2 Chemical Composition of the Substrates

In the proximate analysis and amino acid profile of substrates, the main compositional components of interest are moisture, fat, protein, ash and available and unavailable carbohydrate.

3.2.1 Amino Acids composition

3.2.1.1 Crude Protein (Nitrogen Determination by Micro Kjeldahl Method)

This was carried out as described by AOAC (2019). The nitrogen component of protein and other compounds was converted to ammonium sulphate, by acid digestion, with boiling sulphuric acid. Five millilitre (5mL) of sample was placed in Kjeldahl flask, and 200mg of catalyst mixture (potassium sulphate, copper sulphate and selenium powder) was added. Then 10 mL of concentrated sulphuric acid was added to the content of the flask. It was gently heated for a few minutes, until frothing ceased. Temperature was increased to digest it for 1 hour. Afterward, it was allowed to cool, and then made up to 100mL with distilled water. Then 5mL of digested solution was pipetted into the distillation chamber of micro-Kjeldhal distillation apparaatus, and 10mL of 40% sodium hydroxide solution was added. It was distilled into 10mL of 4% boric acid, containing mixed indicator (colour from red-green was noted), and titrated with standard 0.01N or 0.02N hydrochloric acid to grey end point.

$$\%\mathrm{N} = \frac{(\mathrm{a} - \mathrm{b}) \times 0.01 \times 14.0057 \times \mathrm{c} \times 100}{\mathrm{d}\sqrt{\mathrm{e}}} \tag{3.1}$$

a = Titre value for the sample

b = Titre value for the blank

c = Volume to which digest is made up with distilled water

d = Aliquot taken for distillation

e = Weight of dried sample (mg)

To percentage crude protein, then multiplied by necessary conversion factor (6.25).

3.2.1.2 Crude Fibre

The method of AOAC (2019) was adopted. Defatted ground sample of substrates was transferred from fat determination into 250ml quick fit flask, 150ml of 1.25% sulphuric acid was added and fitted to reflux condenser. I refluxed for 30 minutes, cool and filter using Buchner funnel fitted with Whatman filter paper, rinsed three times with hot distilled water, dried and transfered the residue into quick fit flask. One hundred and fifty milliliter (150ml) of 1.25% NaOH and reflux was added for 30 minutes, and filtered using Buchner funnel, and rinsed three times with hot distilled water, once with 1.25% sulphuric acid and finally with 95% ethanol. The filter paper containing residue was removed into porcelain crucible and dried in oven for 2 hours at 130^{o}C.

It was cooled in desiccator, ashed at 5500 ± 100^{o}C in muffle furnace, cooled in desiccator and weighed as crude fibre % materials, which provides the estimate of the ash handling requirement.

Crude fibre is the combustible organic residue that is left after other biomolecules like protein have been removed by successive treatments with boiling acid and alkalis, alcohols and ether.

This treatment provides a crude fibre consisting largely of the cellulose content together with a proportion of the lignin and hemicelluloses content of the sample.

3.2.1.3 Ash Content Determination

The method of AOAC (2019) was adopted. Two grams (2g) test portion was weighed into porcelain crucible and placed in muffle furnace. It was then preheated to 6000C, and held at this temperature for 2 hours. Crucible was directly transferred to desiccator, cooled, and weighed immediately; percent ash was reported to two decimal places % (w/w).

The ash content of a material is the residue remaining after ignition at 575 ± 25^{o}C for 3 hours or longer to burn off all the organic matter or carbon. It is a measure of the mineral content in the sample but is not necessarily quantitatively equal to them; there may be loss due to volatilization.

3.2.1.4 Moisture Content Analysis of Waste Samples

The method of AOAC (2019) was adopted with slight modifications. The water content was determined by weighing out 2g into glass Petri dish, which has been previously dried and weighed. The dish, containing the samples, was placed inside hot air oven and allowed for 5 hours at 130±3^{o}C, to dry to constant weight. It was then removed and allowed to cool for ten minutes in a desiccator, before weighing. Moisture content of the samples was computed using Equation 3.2 $\text{Moisture content} = \frac{\text{Weight loss on drying (g)}}{\text{Weight test portion (g)}} \times 100 \qquad (3.2)$

This involves the measurement of the mass loss due to the evaporation of water at or near the boiling point. The loss in mass may also depend on other factors including particle size and mass of sample used, type of dish and temperature variations in the oven from shelf to shelf.

3.2.1.5 Ether Extract (Crude Fat)

The method of AOAC (2019). A soxhlet extraction apparatus and 250ml quick fit flask which has been previously dried in the oven was fitted up. Two grams (2g) of sample was weighed and transferred to a fat free extraction thimble, plugged lightly with cotton wool. The thimble was placed in the extractor and about 150cm^3 of petroleum ether (B.P. 40-60^{o}C) was added into the flask, until it siphons over once. The source of heat (electro thermal heating mantle) was adjusted so that the ether boils gently and left to siphon over for at least 6 hours. The flask (which now contains all the oil) was detached. Extract (oil) was filtered through Whatman filter paper into weighed beaker, washing paper finally with small portion of hot fresh ether. Solvent Evaporated at 100 degree centigrade and dry beaker containing residue in an air oven 1 hour at 100-105 degree centigrade reported as % oil to second decimal place.

The fatty constituents of food/feed samples consist of a number of lipid substances. The fat content (ether extract or crude fat) which may be considered as consisting of the ‘free’ lipid constituents is that which can be extracted by the less polar solvent such a light petroleum fractions, diethyl ether while the ‘bound’ lipid constituents require more polar solvent such as alcohols for extraction.

3.2.1.6 Nitrogen Free Extract

This is used to estimate the quantity of carbohydrate present in samples. The carbohydrate is calculated as weight by difference between 100 and the summation of other proximate parameter as Nitrogen free Extract (NFE) percentage carbohydrate $(NFE) = 100 - (M + P + F1 + A + F2)$ using Equation (3.3)

Where; M = Moisture, P = Protein, F1 = Fat, A = Ash, F2 = Crude fibre

3.2.2 Determination of Amino Acid

Amino acid analysis was determined as described by Benitez (1989):

The 1 g of sample was dried to constant weight, defatted, hydrolyzed, evaporated in a rotary evaporator and loaded into an Applied Biosystems PTH Amino Analyzer.

3.2.2.1 Defatting Sample

The sample was defatted using chloroform/methanol mixture in the ratio of 2:1. About 4g of the sample was put in extraction thimble and extracted for 15 hours in soxhlet extraction apparatus (AOAC, 2019).

3.2.3 Nitrogen Determination

Two hundred milligram (200mg) of ground sample was weighed, wrapped in whatman filter paper (No. 1) and put in the Kjeldhal digestion flask. Concentrated sulphuric acid (10ml) was added. Catalyst mixture (0.5g) containing sodium sulphate (Na_2SO_4), copper sulphate ($CuSO_4$) and selenium oxide (SeO_2) in the ratio of 10:5:1 was added into the flask to facilitate digestion. Four pieces of anti-bumping granules were added.

The flask was put in Kjeldhal digestion apparatus for 3 hours until the liquid turned light green. The digested sample was cooled and diluted with distilled water to 100ml in standard volumetric flask. Aliquot (10ml) of the diluted solution with 10ml of 45% sodium hydroxide was put into the Markham distillation apparatus and distilled into 10ml of 2% boric acid containing 4 drops of bromocresol green/methyl red indicator until about 70ml of distillate was collected.

The distillate was then titrated with standardize 0.01N hydrochloric acid to grey coloured end point.

$$\text{Percentage Nitrogen} = \frac{(a - b) \times 0.01 \times 14 \times V \times 100}{W \times C} \qquad (3.4)$$

Where:

a = Titre value of the digested sample

b = Titre value of blank sample

v = Volume after dilution (100ml)

W = Weight of dried sample (mg)

C = Aliquot of the sample used (10ml)

14 = Nitrogen constant in mg.

3.2.4 Hydrolysis of the sample

A 1g weight of the defatted sample was weighed into glass ampoule. Seven milliliters (7 ml) of 6N HCl was added and oxygen was expelled by passing nitrogen into the ampoule (this is to avoid possible oxidation of some amino acids during hydrolysis e.g methionine and cystine). The glass ampoule was then sealed with Bunsen burner flame and put in an oven preset at 105^{o}C ± 5^{o}C for 22 hours. The ampoule was allowed to cool before being broken open at the tip and the content was filtered to remove the humans. It should be noted that tryptophan is destroyed by 6N HCL during hydrolysis. The filtrate was then evaporated to dryness using rotary evaporator. The residue was dissolved with 5 ml to acetate buffer (pH 2.0); stored in plastic specimen bottles, and stored in a freezer.

3.2.5 Loading of the hydrolysate into analyzer

The amount loaded was 60 microlitres. This was dispensed into the cartridge of the analyzer. The analyzer is designed to separate and analyze free acidic, neutral and basic amino acids of the hydrolysate.

3.2.6 Method of Calculating Amino Acid Values from the Chromatogram Peaks

An integrator attached to the analyzer calculates the peak area proportional to the concentration of each of the amino acids.

3.3 Pre-treatment of Substrates

The substrates consist of components such as protein, sugar, fats and lignin. Pre-treatment helps to break down the substrates so that other processes in the fermentation can be easily carried out. There are physical, biological and chemical methods of pre-treatment. In this study, the physical and biological pretreatment methods were employed.

Size reduction is the first step in bioethanol production using agricultural materials (Jafari, 2016). Substrates include tigernut waste (TNW), groundnut shell (GS) and beans husk (BH). Large quantity of substrates: TNW, BH, and GS, was collected, sun-dried and ground into powdered form using a stainless-steel grinder to increase the surface area for the biological treatment (Braide et al., 2016). The ground substrates were stored in well labeled transparent polyethylene bags at room temperature.

3.3.1 Experimental Design

Design Expert 13 was used to design the experiment. It was employed to check for the interdependence of more than one factor by identifying their overall effect (Olufemi &

Eniodunmo, 2018). Box–Behnken design was adopted for the optimization of biological pretreatment of substrates and production of bioethanol in a 5×3 design, i.e. five factors in three levels. The factors and levels include: Substrate weight (10g, 15g and 20g), pH (6, 7 and 8), Inoculum size (0.5, 0.6 and 0.7), Temperature (25^oC, 30^oC and 35^oC) and Incubation time (48h, 72h and 96h).

Table 3.1: Experimental Design Table (Uncoded)

Std Order	pH	Temp (^{O}C)	Time (h)	Inoculum size (cfu/g)	Substrate (g)
1	7	30	48	5	15
2	7	35	48	6	15
3	7	25	48	6	15
4	6	30	96	6	15
5	7	35	72	7	15
6	8	30	72	6	10
7	7	25	72	7	15
8	7	30	48	6	20
9	7	35	72	5	15
10	7	30	96	7	15
11	7	25	96	6	15
12	8	35	72	6	15
13	7	25	72	6	20
14	6	30	48	6	15
15	7	35	72	6	20
16	7	30	96	5	15
17	8	30	72	5	15
18	7	30	72	6	15
19	6	25	72	6	15
20	6	30	48	6	15
21	7	30	72	5	10
22	7	30	96	6	10
23	7	25	72	6	10
24	7	30	48	7	15
25	8	25	72	6	15
26	7	30	72	6	15
27	6	30	72	7	15
28	7	30	72	6	15
29	8	30	96	6	15
30	7	30	72	6	15
31	6	30	72	6	20
32	7	30	72	7	20
33	6	30	72	6	10
34	6	30	72	5	15
35	7	30	72	6	15
36	8	30	72	6	20
37	7	30	48	6	10
38	7	35	96	6	15
39	8	30	72	7	15
40	6	35	72	6	15
41	7	30	72	6	15
42	7	35	72	6	10
43	7	30	72	5	20
44	7	30	96	6	20
45	7	30	72	7	10
46	7	25	72	5	15

Two stages of pre-treatments were used:

3.3.2 Hydrothermal Treatment

As shown in the parameters in Table 3.1, different weights (10g, 15g and 20g) of the three substrates (TNW, GS and BH) were mixed with 150ml of deionized water in 46 separate Erlenmeyer flasks for each of the substrates. After sealing, the flasks were sterilized in batches in an autoclave at 121^{o}C for 15mins, and reheated in the autoclave at 121^{o}C for 45mins to convert the carbohydrate into sugary liquid. The samples were filtered using a filter bag (Braide, Orji, Adeleye & Korie, 2018).

3.3.3 Enzymatic Hydrolysis

Cellulase enzyme with an activity of 5000u/g was dissolved in buffer solution at pH range of 6.0 and added to the flasks according to the parameters in the table of design. The enzyme breaks down cellulose into dissolved sugars. This was allowed to stand for 24 hours at 37^{o}C (Akujobi & Wesley, 2020). After 48 hours of addition of enzyme, the contents of the flasks were autoclaved to stop the action of the enzyme activties (Zhang et al., 2020).

The heat treatment is to breakdown lignin partially and exposes cellulose for enzymatic hydrolysis.

3.4 Test Microorganisms

3.4.1 *Aspergillus niger isolate HEFAPhR*

Macro culture method was used to identify the organisms (Abdulrahman, 2022). The organisms were isolated from food samples (irish potatoes). Ten grams was immersed in 100 mL of distilled water. A five-fold serial dilution was carried out and 1 ml of the diluents was inoculated on potatoes' dextrose agar (PDA) using a spread-plate technique and incubated for 3 days at 28°C.

Sub-culturing was carried out until pure cultures of *Aspergillus niger isolate HEFAPhR* was obtained. After 10 days of cultivation, a small portion of the mycelia growth was carefully picked with the aid of a sterile wire loop and placed on a drop of lactophenol cotton blue on a microscope slide and covered with a cover slip. The slide was examined under the microscope with (40x) objective lens for morphological examination as described by Cheesbrough (2006); Abdulrahman (2022).

3.4.2 *Saccharomyces cerevisiae*

Saccharomyces cerevisiae was isolated from brewers' yeast. Aliquot of 0.1ml of 10 folds serial dilutions of the brewers yeast were spread on the surface of a solidified potato dextrose agar plate (PDA) and was incubated for 48hours at 28^{o}C. Colonies suspected to be *Saccharomyces cerevisiae* based on their colonial characteristics were sub-cultured on sterile PDA slants. A smear of the isolate was examined microscopically after Gram stained and was examined under the microscope with (40x) objective lens for morphological examination (Simpfendorfer et al., 2016). The isolates were identified by comparing their characteristics with those of known taxa using the scheme as described by Gani et al. (2018).

3.4.3 Identification and Characterization of Isolated Organism

3.4.3.1 Microscopic and Biochemical Test

Microbial isolates were characterized based on cultural (colonial), microscopic and biochemical methods with reference to Cheesbrough (2006). The identities of the isolates were cross-matched for the identification of yeast and molds (Cheesbrough, 2006).

1. Gram Staining Test

The Lactophenol cotton blue (LPCB) gram staining technique was used to identify fungal isolates. A Preparation lactophenol cotton blue staining solution which is made up of distilled water, cotton blue (Aniline blue) 0.25g, phenol crystals 50g, glycerol 100ml, lactic acid 50ml and 70% ethanol, this solution was allowed to stand for over two days before use to allow dissolving and maturation, on a clean microscopic glass slide, add a drop of 70% ethanol, the fungal isolates added using a sterile inoculating loop, the fungal isolates were teased using a needle mounter to ensure proper mixes with alcohol lactophenol cotton blue solution was dropped on the slide and the stain was covered with a sterile cover slip (Senthil, 2018).

2. Sugar Fermentation Test

The test is used to differentiate between microorganisms that oxidise carbohydrate. One millilitre of 10% of some sugars such as; glucose, maltose, lactose, fructose, mannitol and sucrose were separately transferred into duplicate tube containing 9mls of sterile Hugh and Leifson’s medium to obtain a final concentration of 1% of each of the sugars. The tubes were inoculated in duplicates while two non-inoculated tubes served as control. Vaseline was used to cover one set for the duplicate tubes and the control to avoid oxidative utilization of the sugars. The tubes were incubated at 37^oC for 24 hours. After incubation, they were observed for acid production. Yellow colouration in the open tubes indicates acid production while acid production in the sealed tubes suggests a fermentative reaction (Thakur, Kaviti, Mehra & Mer, 2017)

3. Citrate Utilization Test

The principle of this test is based on the ability of an organism to use citrate as its only carbon source. Simmon’s citrate agar was prepared and slants of the media were made in bijou bottles.

From a saline suspension of the test organisms, inoculation was made on the slants. The bottles were incubated at 35^{o}C for 48hours. Bright blue colours in the medium indicate positive test while no colour change indicates of the medium indicates negative test (Cheesbrough, 2006).

3.4.3.2 Molecular Identification of Organism

1. **DNA Extraction**

Extraction was done using a ZR fungal DNA mini prep extraction kit supplied by Inqaba South Africa. A heavy growth of the pure culture of the suspected isolates was suspended in 200 microliters of isotonic buffer into a ZR Bashing Bead Lysis tubes, 750 microliter of lysis solution was added to the tube. The tubes were secured in a bead beater fitted with a 2ml tube holder assembly and processed at maximum speed for 5 minutes. The ZR bashing bead lysis tubes were centrifuged at 10,000xg for 1 minute.

Four hundred (400) microliters of supernatant were transferred to a Zymo-Spin IV spin Filter (orange top) in a collection tube and centrifuged at 7000 xg for 1 minute. One thousand two hundred (1200) microliters of fungal/mold DNA binding buffer were added to the filtrate in the collection tubes bringing the final volume to 1600 microliter, 800 microliters were then transferred to a Zymo-Spin IIC column in a collection tube and centrifuged at 10,000xg for 1 minute, the flow through was discarded from the collection tube. The remaining volume was transferred to the same Zymo-spin and spun. Two hundred (200) microliters of the DNA Pre-Washed buffer was added to the Zymo-spin IIC in a new collection tube and spun at 10,000xg for 1 minute followed by the addition of 500ml microliters of fungal/mold DNA Wash Buffer and centrifuged at 10,000xg for 1 minute.

The Zymo-spin IIC column was transferred to a clean 1.5 microliter centrifuge tube, 100 microliter of DNA elution buffer was added to the column matrix and centrifuged at 10,000xg microliter for 30 seconds to elute the DNA. The ultra-pure DNA was then stored at -20 degree for other downstream reaction (Tibarewala, Bhattachrya, Das & Das, 2020).

2. DNA quantification

The extracted genomic DNA was quantified using the Nanodrop 1000 spectrophotometer. The software of the equipment was lunched by double clicking on the Nanodrop icon. The equipment was initialized with 2 ul of sterile distilled water and blanked using normal saline. Two microlitre of the extracted DNA was loaded onto the lower pedestal; the upper pedestal was brought down to contact the extracted DNA on the lower pedestal. The DNA concentration was measured by clicking on the “measure” button.

3. Internal Transcribed Spacer (ITS) Amplification

The ITS region of the isolates was amplified using the ITS1F: 5'-CTTGGTCATTTAGAGGAAGTAA-3' and ITS4: 5'- TCCTCCGCTTATTGATATGC-3, primers on a ABI 9700 Applied Biosystems thermal cycler at a final volume of 30 microlitres for 35 cycles. The PCR mix included: the X2 Dream Taq Master mix supplied by Inqaba, South Africa (taq polymerase, DNTPs, MgCl), the primers at a concentration of 0.4M and the extracted DNA as template. The PCR conditions were as follows: Initial denaturation, 95ºC for 5 minutes; denaturation, 95ºC for 30 seconds; annealing, 53ºC for 30 seconds; extension, 72ºC for 30 seconds for 35 cycles and final extension, 72ºC for 5 minutes. The product was resolved on a 1% agarose gel at 120V for 15 minutes and visualized on a blue light transilluminator.

4. Sequencing

Sequencing was done using the BigDye Terminator kit on a 3510 ABI sequencer by Inqaba Biotechnological, Pretoria South Africa. The sequencing was done at a final volume of 10ul, the components included 0.25 ul BigDye® terminator v1.1/v3.1, 2.25µl of 5 x BigDye sequencing buffer, 10uM Primer PCR primer, and 2-10ng PCR template per 100bp. The sequencing conditions were as follows: 32 cycles of 96°C for 10s, 55°C for 5s and 60°C for 4min.

5. Phylogenetic Analysis

Obtained sequences were edited using the bioinformatics algorithm Trace edit, similar sequences were downloaded from the National Center for Biotechnology Information (NCBI) data base using BLASTN. These sequences were aligned using ClustalX. The evolutionary history was inferred using the Neighbor-Joining method in MEGA 6.0 (Weiwen, James & Thomas, 2022). The bootstrap consensus tree inferred from 500 replicates (Tibarewala et al., 2020) is taken to represent the evolutionary history of the taxa analyzed. The evolutionary distances were computed using the Jukes-Cantor method (Schewende & Tuan, 2013).

3.4.4 Measurement of Brix and Estimation of Bioethanol Production by Optimizing selected Operating Parameters

Three substrates were used for this study; TNW, BH and GS and they were treated with *Saccharomyces cerevisiae* and *Aspergillus niger isolate HEFAPhR* individually. The operating parameters include substrate weight, pH, inoculum size, temperature and time.

3.4.5 Determination of Reducing Sugar (Brix Level)

Brix is the sugar content of an aqueous solution (Jaywant, Singh & Arif, 2022). After fermentation, the amount of converted sugar can be determined. Brix was measured using the refractometer. A drop of the sample was placed on the refractometer and the brix level was displayed for all the samples.

3.4.6 Fermentation with *Saccharomyces cerevisiae*

According to the parameters in Table 3.1, different weights (10g, 15g and 20g) of the finely ground TNW were dissolved in 150ml of deionized water in 46 separate Erlenmeyer flasks. After sealing, the flasks were sterilized in batches in an autoclave at 121^oC for 45 mins, to breakdown lignin partially and expose cellulose for enzymatic hydrolysis. The samples were filtered using a filter bag.

3.4.7 Fermentation with *Aspergillus niger isolate HEFAPhR*

According to the parameters in Table 3.1, different weights (10g, 15g and 20g) of the finely ground TNW were dissolved in 150ml of deionized water in 46 separate Erlenmeyer flasks. After sealing, the flasks were sterilized in batches in an autoclave at 121^oC for 15mins, break down lignin partially and expose cellulose for enzymatic hydrolysis. The samples were filtered using a filter bag.

3.4.8 Alcoholic Fermentation Process

One-tenth normality (0.1 N) of NaOH and 0.1 N H_2SO_4 were prepared (Henze & Jimenez-Gamero, 2019; Offor-Emenike et al, 2020) and used to adjust the pH of the contents of the flasks to pH 6, 7 and 8 respectively to conform to the design of experiment. Buffer solution was innoculated to the flasks to adjust the respective pH. The contents of all the flasks were made up to a volume of 100ml each, to ensure uniform fermentation volume. According to the design of the experiment, 0.5, 0.6 and 0.7 MacFalands standards of the mold *Aspergillus niger isolate HEFAPhR* were aseptically introduced into the flasks. The content of the 46 flasks was allowed to ferment according to the parameters in the table of design. Fermentation was stopped after 48, 72h, 96h respectively as defined by the design and brix/liquid mixture of the samples in the flasks was measured using the refractometer. The mold, *Aspergillus niger isolate HEFAPhR* was used to treat the other two substrates (BH and GS) following the same process and readings were taken accordingly.

3.5 Determination of the Efficiency of *Saccharomyces cerevisiae* and *Aspergillus niger isolate HEFAPhR* for Bioethanol Production under Stressors

The ability of *Saccharomyces cerevisiae* and *Aspergillus niger isolate HEFAPhR* to withstand some stress conditions like pH, temperature etc. during the fermentation process, were determined. The organisms were subjected to stressors such as ethanol tolerance test, temperature tolerance test and stress exclusion test (Simpfendorfer et al., 2016).

3.5.1 Stress Exclusion Test

The isolates of *Saccharomyces cerevisiae* and *Aspergillus niger isolate HEFAPhR* were plated on YPG medium at 28^{o}C for *Saccharomyces cerevisiae* and 25^{o}C for *Aspergillus niger isolate HEFAPhR* for 72 hours. The grown colonies were plated on YPG supplemented with 80 ml/l ethanol and incubated at 25ºC and 28ºC for 72 hours. The colonies were then plated on YP supplemented with 200g/l glucose and grown at the same conditions. After incubation the colonies were plated in medium YP supplemented with 200g/l sucrose and 80 ml/l ethanol and incubated at same conditions.

3.5.2 Ethanol Tolerance Test

The isolates of *Saccharomyces cerevisiae* and *Aspergillus niger isolate HEFAPhR* were inoculated in 10ml of liquid YPG supplemented with 100, 130 and 150 ml/l ethanol and incubated at 28ºC for the yeast and 25^{o}C for *Aspergillus niger isolate HEFAPhR*. This was allowed to stand for 72 hours.

3.5.3 Temperature Tolerance Test

The isolates that were grown at 28^{o}C for *Saccharomyces cerevisiae* and 25^{o}C for *Aspergillus niger isolate HEFAPhR* were plated in YPG and incubated at 35, 40, 45 and 50ºC for 72 hours and viability was monitored.

3.6 Determination of Ethanol Percentage and Weight

The specific gravity of the distillate was determined after the density was measured. These were done by using a pycnometer. The pycnometer (specific gravity bottle) was weighed with stopper after cleaning, drying and the weight was X1 at 20^{o}C. The pycnometer was filled with distilled water and the weight of the water at 20^{o}C was noted as X3. The pycnometer was emptied, cleaned, dried and then filled with distillate. The weight of the distillates was determined at 20^{o}C and noted as X2. The net weight in grams of the alcoholic liquid in the pycnometer was calculated by subtracting the weight of the empty specific gravity bottle or pycnometer (Mustapha et al., 2019). The percentage ethanol by volume and weight were determined using standard ethanol table as described by Abdulrahman (2022).

The density was calculated using Equation 2.

Mass Density [g/ml] = Density of Distillate Ethanol

$$Sp.Gravity = Density\ of\ Distilled\ Water \tag{3.5}$$

X1 - Weight (g) of Empty Pycnometer

X2 - Weight (g) of Pycnometer + Distillate

X3 - Weight (g) of Pycnometer + Water

3.7 Confirmatory Test for Bio-ethanol Produced

Confirmatory test was carried out on the extracted bio-ethanol sample using potassium dichromate test as indicated by Mustapha, Dahiru, Abdulrahman & Abdullahi (2019). About 5mL of the distillate sample was taken and 2 drops of potassium dichromate was added into the distillate, heated in a water bath for 30 minutes.

3.8 Viability Count

After treating the organisms with the stressors, the viability of the isolates were determined by staining with methylene blue. Methylene blue stains the dead yeast cells which then appear as dark blue cells. Methylene (0.1) blue was dissolved in 100ml of distilled water and flooded on the plates. This was allowed to react for one minute then the cells were counted (Eigenfeld, Kerpes & Becker, 2021).

$$Viability\ (\%) = \frac{Total\ counted\ cells - Total\ counted\ dead\ cells}{Total\ counted\ cells} \times 100 \tag{3.6}$$

3.9 Distillation Process

The ethanol extraction process was carried out in two stages: distillation and dehydration. Distillation was carried out using a rotary evaporator at a temperature, pressure and rotary speed of 60°C, 175 mbar and 100 rpm, respectively. The vaporized fermentation solution was vacuumed by a pump and flowed to the other end to be condensed, resulting in an improved quality of ethanol solution.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Results and Discussion

The results of the proximate analysis of tiger nut residue show that the residue contained a high proportion of carbohydrate as Nitrogen free extract (NFE) (54.75 ± 0.3) (Fig 4.1). This is consistent with the value (58.80 ± 1.6) as reported by Nata'ala, Farouq, Magashi and Liman in Kano (2018). Their sample size might explain the high error margin compared to the present study. From the current study, the high carbohydrate level observed might be attributed to the fact that tigernut is a tuber crop like cassava and potato, which contain a high amount of carbohydrate of about 46.99% (Gambo & Da'u, 2014). The carbohydrate might not be fully extracted during the process of making tigernut milk that gives rise the residue as a by-product. This is agreement with the findings of Wayah and Shehu (2013) who reported a carbohydrate content of 43.0%, which is lower than the reported (54.75%) and might be attributed to the differences in the processes of extraction and making of tiger nut milk (kunun aya).

The crude fat content of the residue was found to be (4.035), which is far lower than 17.50% reported by Nata'ala, Farouq, Magashi and Liman in Kano (2018) and 35.43% reported for dried tiger nut (Oladele & Aina, 2007). This might be attributed to the extreme extraction procedure adopted. Other compositions, Crude protein (10.15), Ash content (2.68), Crude fibre (23), Moisture (5.38) are consistent with the work of Wayah and Shehu, (2013) with moisture content (%) 7.83 ± 0.58, Ash Content (%) 2.33 ± 0.29, Crude fat (%) 17.50 ± 0.50, Crude fiber (%) 10.67 ± 0.29, Crude Protein (%) 2.86 ± 0.13, and Soluble Carbohydrate (%) 58.80 ± 1.6.

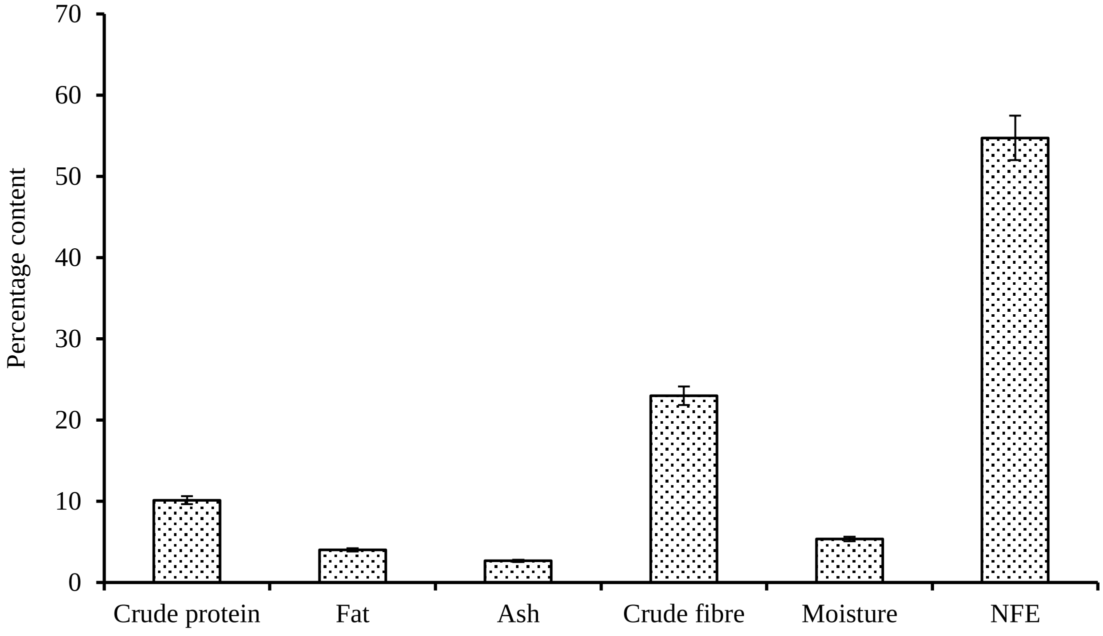


Figure 4.1: Proximate composition of tigernut waste (TNW)

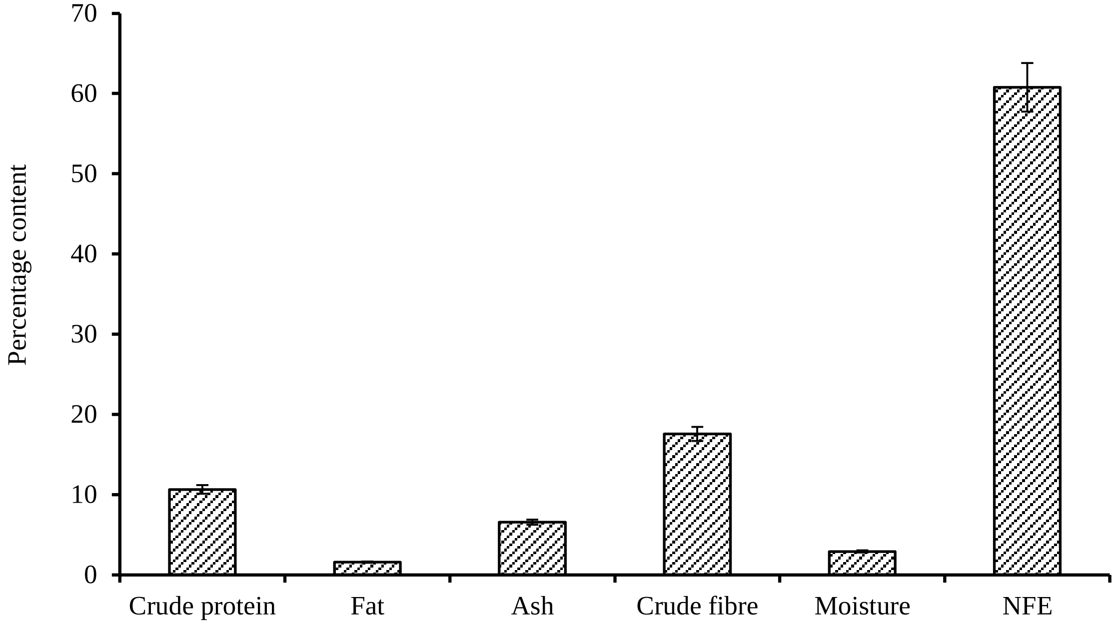


Figure 4.2: Proximate composition of beans husk (BH)

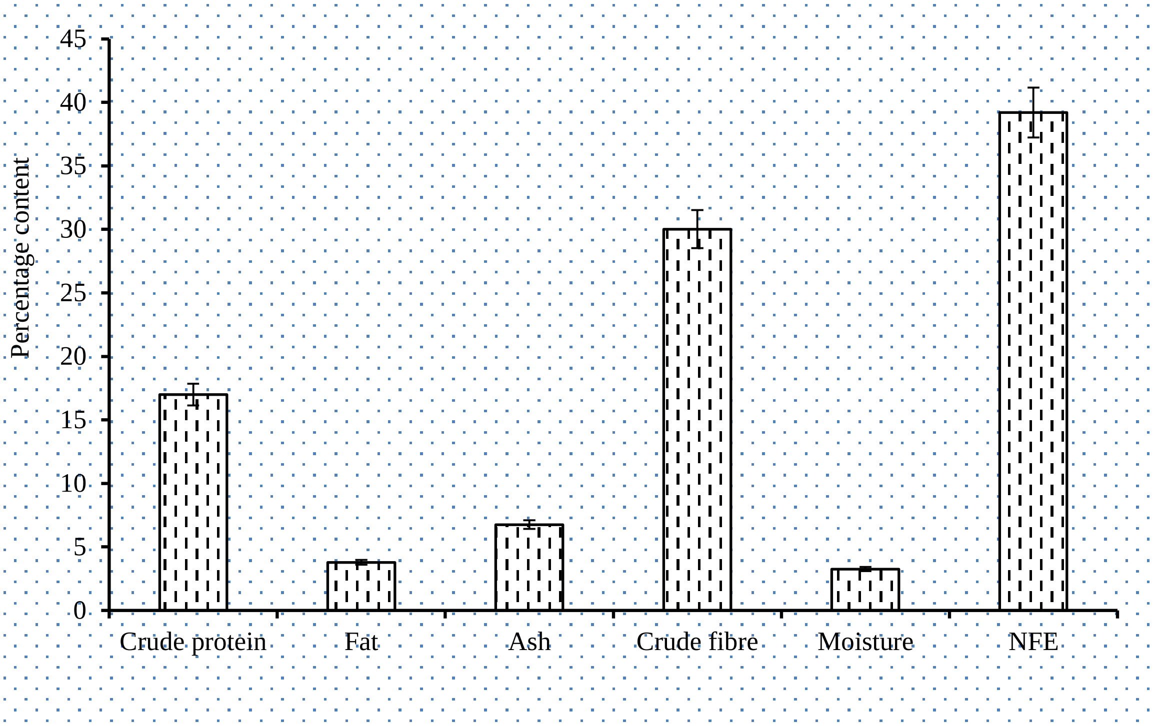


Figure 4.3: Proximate composition of groundnut shell (GS)

Result in Figure 4.2 shows that the highest occuring amino acid in tigernut waste is glutamic acid, while the least occuring is tryptophan. TNW contains roughly the same quantity of lysine, proline, tyrosine, glycine, alanine, and serine; the presence of glutamic acid as the highest occurring amino acid in TNW is noteworthy because glutamic acid can serve as a valuable nutrient source for these microorganisms, potentially enhancing their fermentation efficiency. The low occurrence of tryptophan in TNW is not necessarily negative for bioethanol production, as tryptophan is not typically a critical nutrient for ethanol-producing microorganisms (Arijana et al., 2023).

The fact that TNW contains roughly the same quantity of lysine, proline, tyrosine, glycine, alanine, and serine is important because these amino acids can collectively contribute to the overall nutrient content available to fermentation microorganisms. A balanced supply of these amino acids is advantageous for supporting microbial growth and the fermentation process (Singh, 2018).

In bioethanol production, the efficiency of fermentation is a crucial factor. The nutrient content in the feedstock, including amino acids, can influence the growth and metabolic activity of the ethanol-producing microorganisms. The balanced amino acid composition in TNW suggests that it could be a suitable substrate for fermentation, potentially leading to more efficient and bioethanol production.

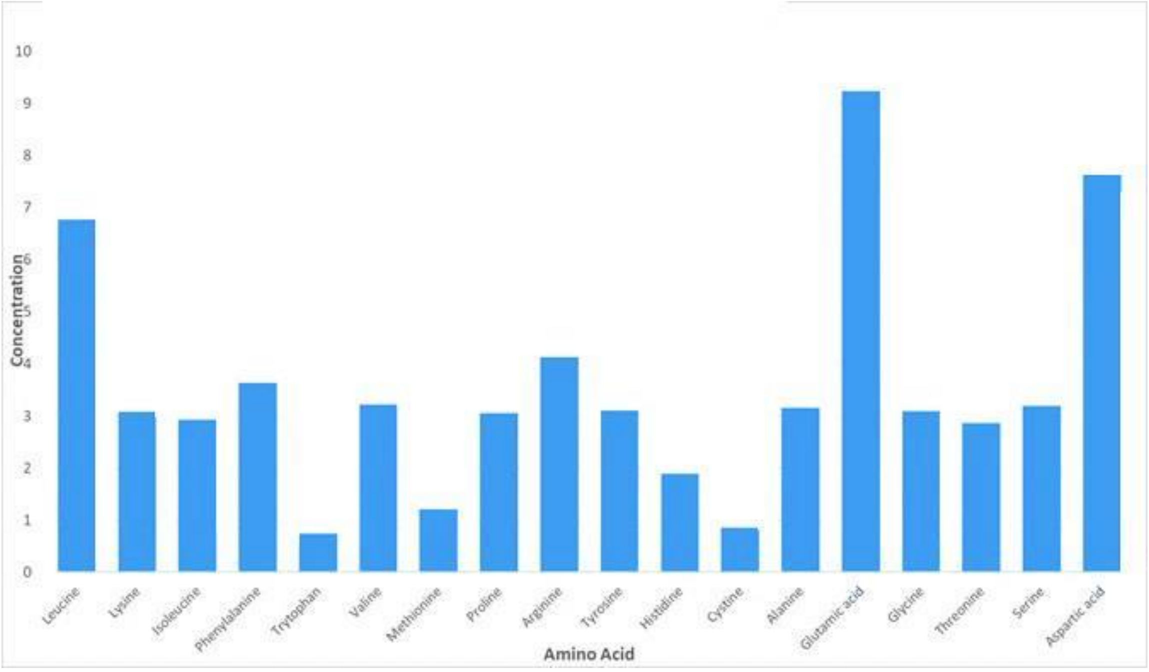


Figure 4.4: Amino acid profile of tigernut waste

The results of the proximate analysis of BH show that the residue contained a high proportion of carbohydrate as nitrogen free extract (NFE) (60.75 ± 0.3) (Figure 4.3). This is different from the NFE content of reported works of other beans waste (cocoa pod) 13.2–70.3 g/100g (Botella-Martínez et al., 2021; Agus, Mohamad & Hussain, 2018). The fibre content (17.545%) falls within the range of that reported for cocoa bean pod - 13.8–65.6 g/100g (Agus, Mohamad & Hussain, 2018). Moisture is lower (2.91%) compared with -4–13.1g/100g as reported by (Botella-Martínez et al., 2021; Rojo-Poveda et al., 2019). Ash content is consistent with 6.0–9.1g/100g (Rojo-Poveda et al., 2019; Grillo et al., 2019); protein lower compared to -18.2–27.4g/100g (Vojvodić, Komes, Vovk, Belščak-Cvitanović & Bušić, 2016; Mancini, Papirio, Lens & Esposito, 2018); crude fat is far higher 17.545%) than 2.3–6.5g/100g reported by (Belščak-Cvitanović, Vojvodić, Bušić, Keppler, Steffen-Heins & Komes, 2018). These differences can be accounted for because of the biochemical constituents and methods of extraction.

Figure 4.4 shows the amino acid composition of beans husk used in the investigation. The highest occurring amino acid the in common beans husk waste is glutamic acid, followed by leucine, while the least occurring is tryptophan. BH contains roughly the same quantity of serine, glycine, alanine, valine, and isoleucine. Lysine and phenylalanine occurred roughly at the same amounts. The presence of glutamic acid as the highest occurring amino acid in BH is noteworthy because glutamic acid can serve as a valuable nutrient source for the fermenting microorganisms, potentially enhancing their fermentation efficiency. The low occurrence of tryptophan in BH is not necessarily negative for bioethanol production, as tryptophan is not typically a critical nutrient for ethanol-producing microorganisms.

Beans husk containining roughly the same quantity serine, glycine, alanine, valine, and isoleucine is important because these amino acids can collectively contribute to the overall nutrient content available to fermentation microorganisms. A balanced supply of these amino acids is advantageous for supporting microbial growth and the fermentation process.

In bioethanol production, the efficiency of fermentation is a crucial factor. The nutrient content in the feedstock, including amino acids, can influence the growth and metabolic activity of the ethanol-producing microorganisms. The balanced amino acid composition in BH suggests that it could be a suitable substrate for fermentation, potentially leading to more efficient and productive bioethanol production.

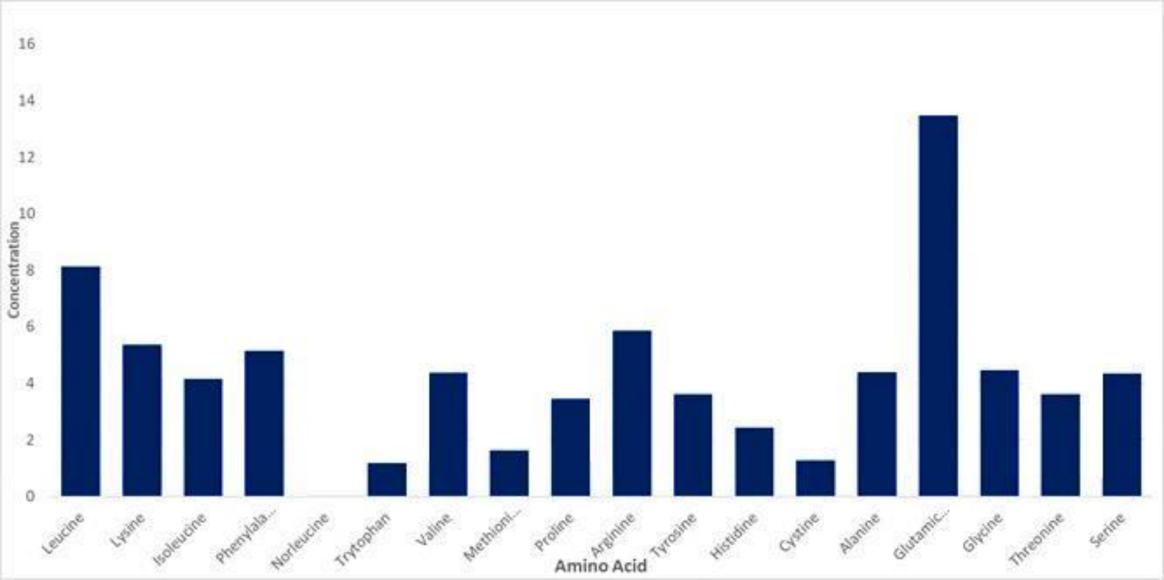


Figure 4.5: Amino acid profile of beans husk

The highest occurring amino acid composition of GS is glutamic acid, while the least occurring is tryptophan as shown in Figure 4.6 revealed that. GS contains roughly the same concentration of alanine, glycine, valine, and isoleucine in agreement with Imran, Humiyion, Arshad, Saeed, Arshad & Al Jbawi (2022). Glutamic acid occurring highest as shown in Figure 4.6 is essential valuable nutrient source for fermenting microorganisms. A balanced supply of the acids as seen in the substrates is beneficial for supporting microbial growth and fermentation activity.

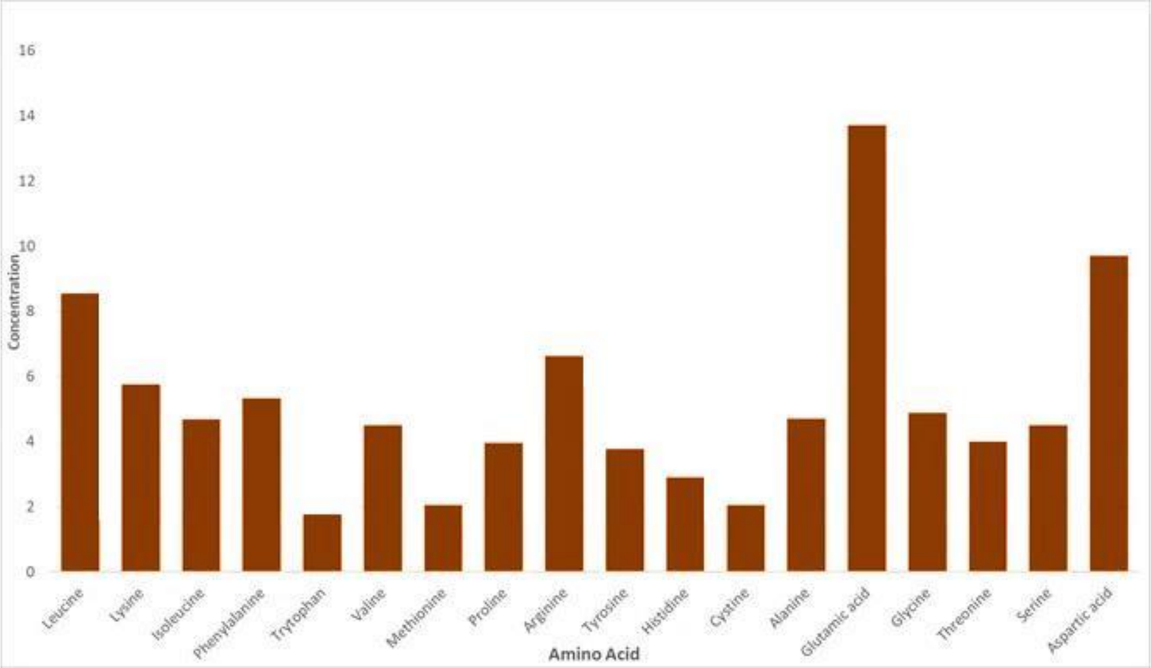


Figure 4.6: Amino acid profile of groundnut shell

Table 4.1: Identification of the Yeast and Mould

Cultural characteristics	Microscopic characteristics	Inferences/organism
Black colony with granular surface and black revere	Septate hyphae, Dark brown large globose conidia heads. Hyaline smooth-wall conidiophores which turn dark towards the vesicles. Conidia heads biseriate	*Aspergillus niger*
White to cream, smooth and glabrous yeast like colony	Large globose to ellipsoidal budding, yeast like cell or blastoconidia.	*Saccharomyces cerervisiae*

4.1.1 Identification of the *Aspergillus niger isolate HEFAPhR*

The organism, identified and grouped by several molecular approaches such as ITS as shown in Figure 4.7 and Figure 4.8. These figures showed bands of agarose gel electrophoresis. Further classification of the organism was carried out molecularly as shown in Figure 4.7.

4.1.2 Molecular Identification of Fungal Isolates

The obtained 18s rRNA sequence from the isolate produced an exact match during the megablast search for highly similar sequences from the NCBI non-redundant nucleotide (nr/nt) database. The 18S rRNA of the isolate W1 showed a percentage similarity to other species at 100%. The evolutionary distances computed using the Jukes-Cantor method were in agreement with the phylogenetic placement of the 16S rRNA of the isolate within the *Aspergillus niger isolate HEFAPhR* and revealed a closely relatedness to *Aspergillus niger isolate HEFAPhR* respectively.

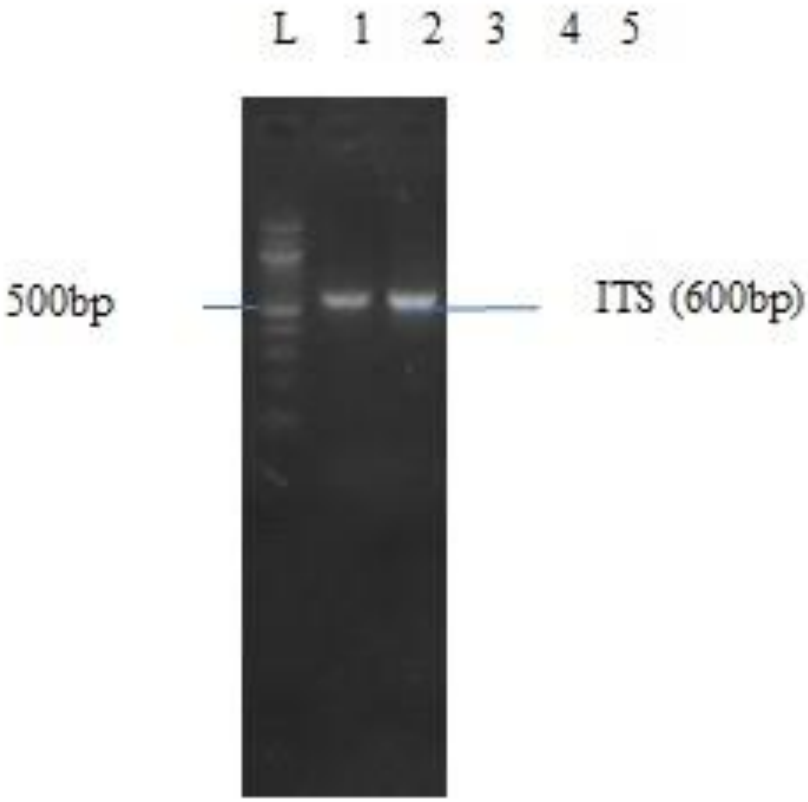


Figure 4.7: Agarose gel electrophoresis showing the amplified ITS (Internal Transcribed Spacers) of the fungal isolates. Lane 1, 2 represent the ITS bands at 600bp while Lane L represents the 500bp molecular ladder

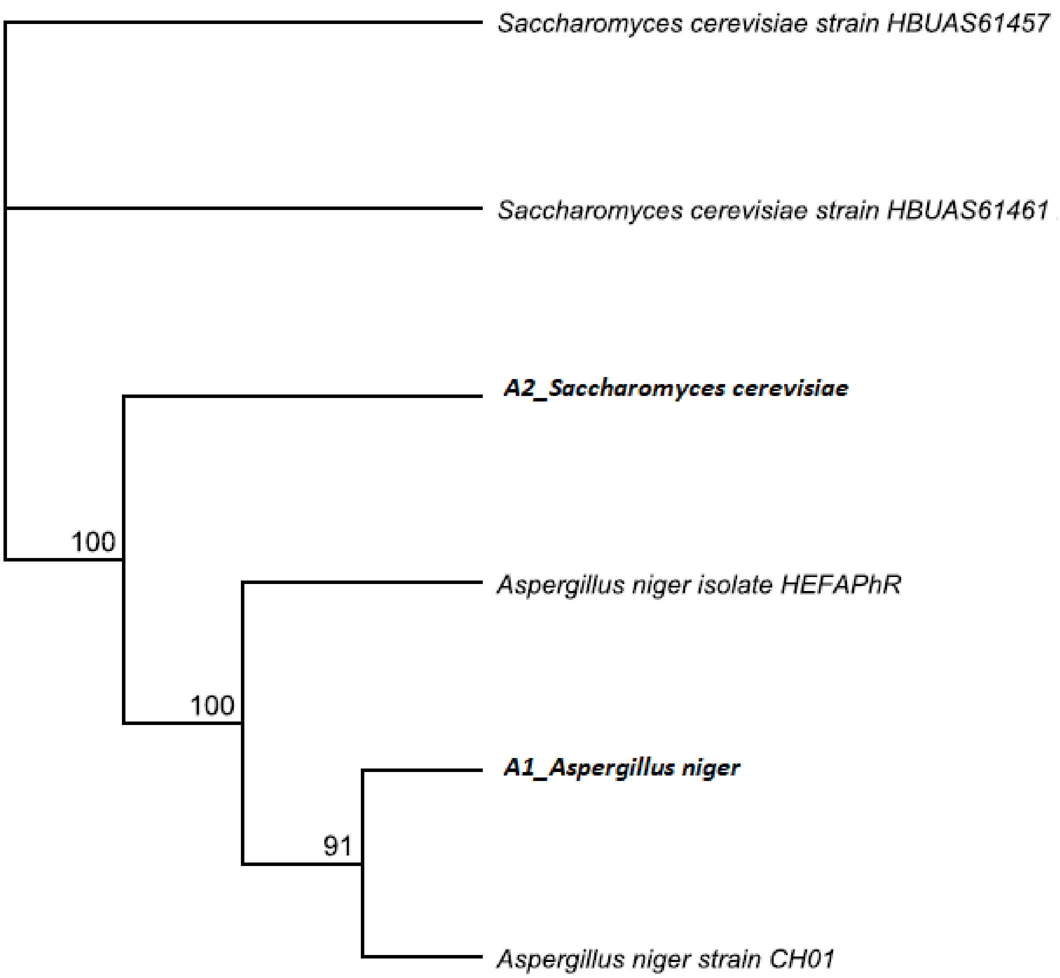


Figure 4.8: The phylogenetic tree showing the evaluation relationship/distance between the fungal isolates

4.1.3 Efficiency of *Saccharomyces cerevisiae* and *Aspergillus niger isolate HEFAPhR* for Bioethanol Production under Stressors

When the cultures of *Saccharomyces cerevisiae* and *Aspergillus niger isolate HEFAPhR* were incubated at different temperatures, the organisms retained 95% viability. The organisms were able to withstand stress and tolerated exposure to ethanol at different concentrations up to 94%.

Results of the observed values of brix and ethanol level of TNW at the experimental stage as shown in Figure 4.9a-b using the refractormeter showed that the optimized flask for TNW with Saccharomyces cerevisiae was Flask 8 indicating that the higher the brix level the higher the ethanol yield.

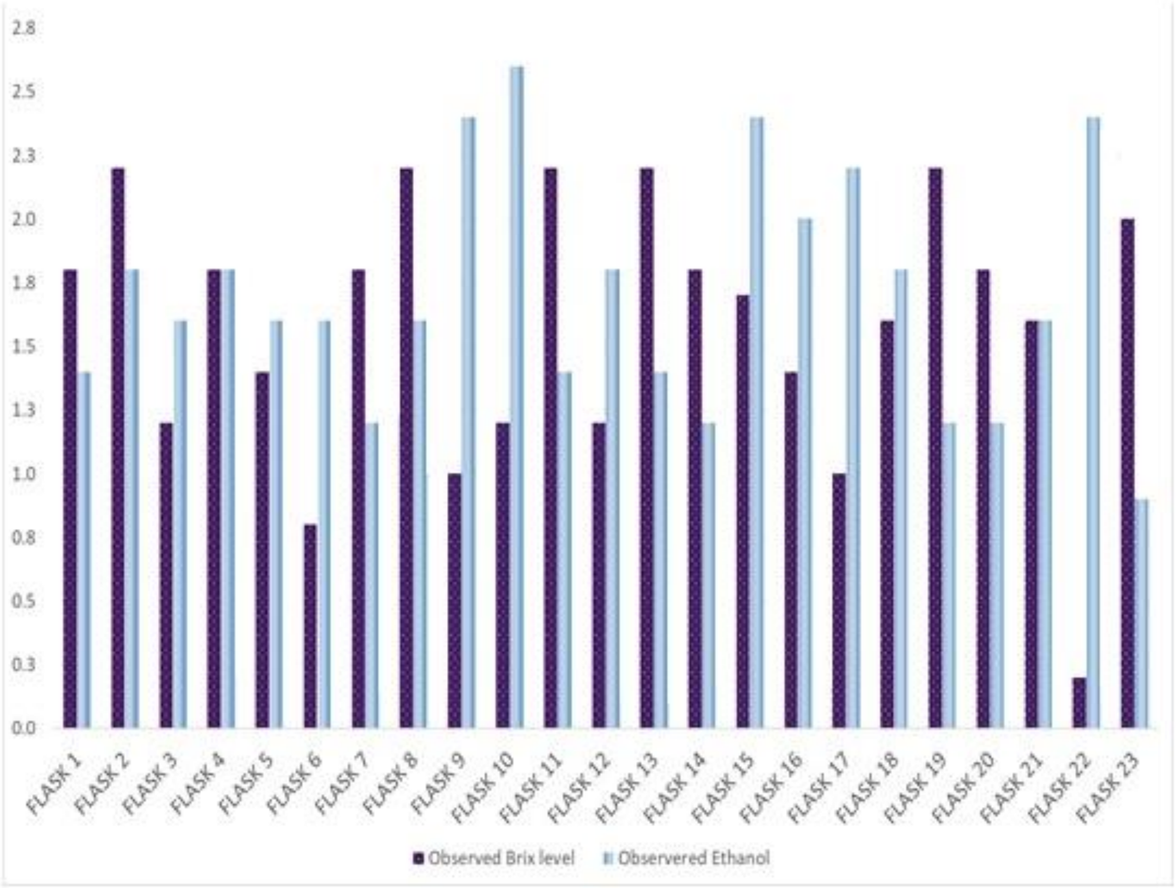


Figure 4.9a: Observed brix level and bioethanol level of tigernut waste with *Saccharomyces cerevisiae*

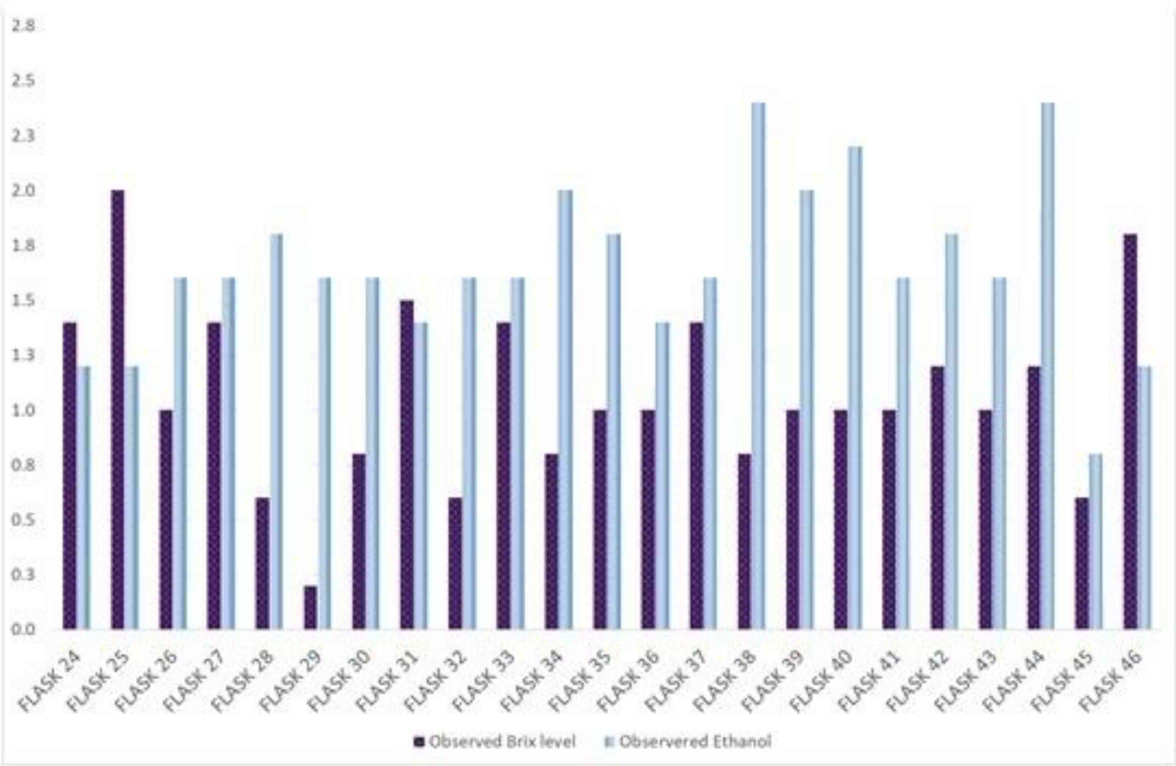


Figure 4.9b: Observed brix level and bioethanol level of tigernut waste with *Saccharomyces cerevisiae*

The main factors (pH, substrate weight, innoculum size, temperature, fermentation time) were selected, as well as their factor levels, coded as –1 (low) and +1 (high), Box–Behnken design was employed and a matrix generated. It generated 46 experimental runs. The selected responses were brix and bioethanol production at optimal conditions.

Result showed that the five factors optimized have great effect on brix and ethanol production from agro waste. The model summary values suggested that a linear, 2FI, Quadratic model best fitted the optimization process. The focus was on the model hat maximized the adjusted R2 predicted R2. The cubic model was aliased and aliases are false signals of any sort present hence the linear model, 2FI, Quadractic model was suggested responses respectively.

The ANOVA in the Responses1-12 (appendix) was used to analyse the result and validate the Responses. The lack of fit test and the adequacy of the regression models were equally performed. A significance level of 5% was used hence P–values greater than 0.05 are considered insignificant while those at 0.05 or less are significant.

The P values check the significance of the factors and equally help to understand the pattern of the mutual interactions between the test variables (Ositadinma et al., 2019).

4.1.4 ANOVA for 2FI Model Response 1 Table A-E (Appendix): Brix Level (Tigernut Waste with *Saccharomyces's cerevisiae*)

P-values <0.0500 indicate model terms are significant indicating that the interaction between substrate weight and innoculum size (Appendix Response 1 Table C) are significant model terms showing that the main effect of substrate weight is statistically significant in affecting the brix level in the treatment of tigernut with of *Saccaromyceses cerevisiae*. Also, for the AC interaction term that is significant at 5% level of significance, it means that using a substrate weight of 10g probability values and inoculum size of 0.5 of MacFaland standard will significantly decrease the brix level as the microorganisms utilizes the sugars. P-Values >0.1000 indicate the model terms are not significant to produce ethanol. The Lack of Fit F-value of 0.42 implies the Lack of Fit is not significant relative to the pure error.

4.1.4.1 Coefficients in Terms of Coded Factors

Only the significant main effect or two factor interaction terms are interpreted. The -0.3063 value of the coefficient associated with substrate weight (A) represents that the brix level is expected to reduce by 0.31 units per unit change in factor levels (10 – 20 g/cm3) when all remaining factors are held constant. The AC interaction term is significant and it means that using a substrate weight of 10g/cm^3 and varying inoculum size from 0.5 of MacFaland standard will significantly decrease the brix level by 0.5 units. The overall average response of all the runs is represented by the intercept term and this value is 1.2 units. This means that on the average, the brix level will decrease by 1.33 units in fermentation of tigernut waste with of *Saccaromyceses cerevisiae* to produce bioethanol.

These plots are obtained by calculating from the model, the values taken by one factor where the second varies with constraint of a given response value. The response surface curves were plotted to understand the interaction of the variables and to determine the optimum levels of each variable for maximum response. The nature of the response surface curves shows the interaction between the variables. The elliptical shape of the curve indicates good interaction of the two variables and circular shape indicates minute interaction between the variables (Ositadinma et al., 2019). There was a relative significant interaction between every variable and there was a maximum predicted efficiency as indicated by the surface confined in the smallest ellipse in the contour diagrams. It was also observed from contour and 3D representation that interaction between the factors inoculum size, fermentation time, temperature, pH, substrate weight, increases the response and maximizes the bioethanol yield.

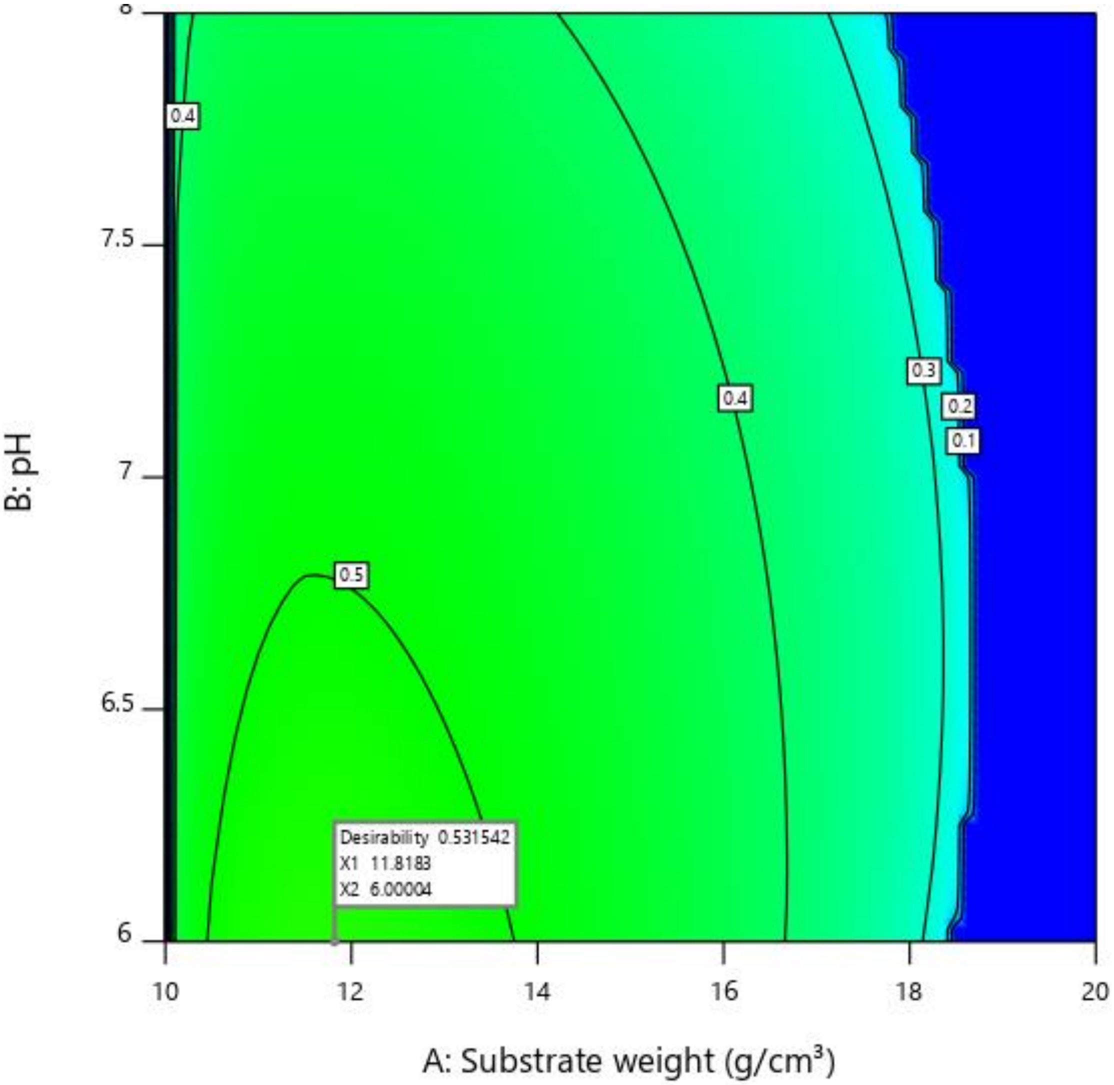


Figure 4.10: Contour plot of tigernut waste's brix level with *Saccharomyces cerevisae*

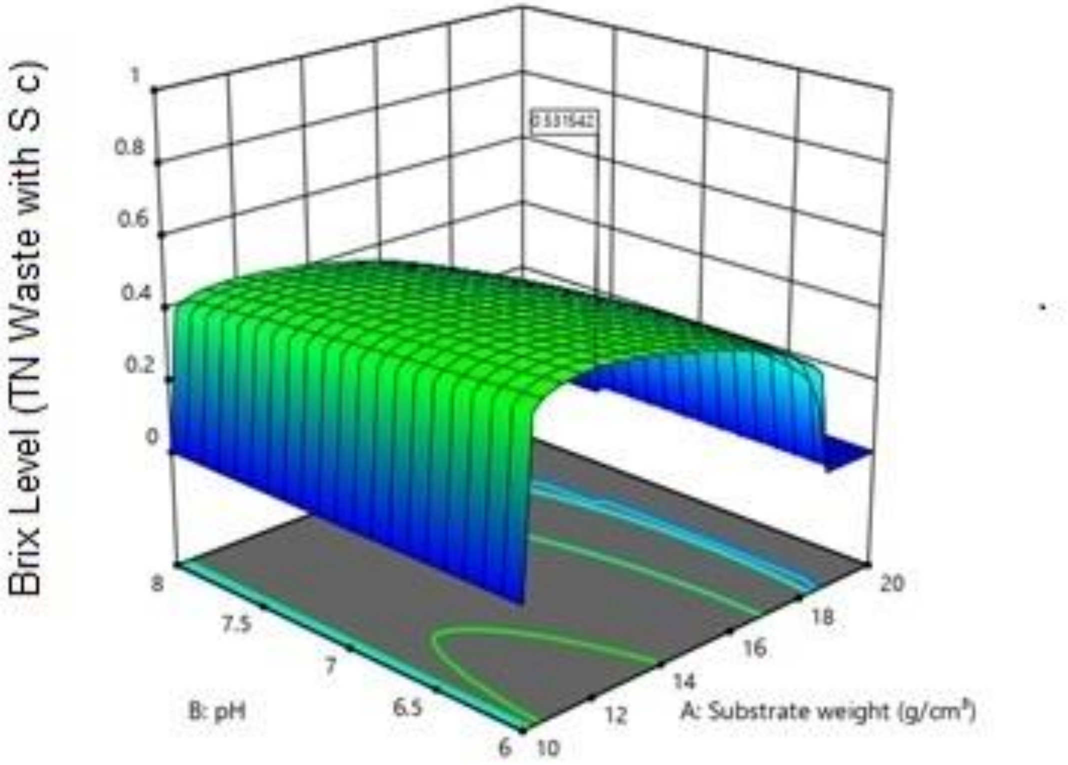


Figure 4.11: 3D surface plot of tigernut waste's brix level with *Saccharomyces cerevisae*

The contour and 3D surface plots shows that the optimum conditions to produce the optimal level of brix 0.5315 (^{o}Bx) are substrate weight 11.81 (g/cm^{3}), pH level 6.00004, Innoculum size 5.63827 (CFU/ml), Temperature 34.9966 (^{o}C), and Time 96 (hours).

4.1.5 ANOVA for Quadratic Model

4.1.5.1 Response 2 Table A-D (Appendix): Bioethanol Level (tigernut waste with *Saccaromyceses cerevisiae*)

The Model F-value of 1.50 implies the model is not significant relative to the variations. There is a 16.58% chance that an F-value this large could occur due to experimental variations/noise.

P-values less than 0.0500 indicate model terms are significant indicating Substrate weight (g/cm³) and Temperature (AD), the square of Innoculum size (CFU/ml) (C^2) is significant model terms meaning that in treatment of TNW with *Saccaromyceses cerevisae*, substrate weight interacts with temperature to significantly increase or decrease the bioethanol level produced. For the quadratic term (C^2), the greater the coefficient, the narrower the parabola, the lesser the quadratic term, the wider the parabola (the curve). P-values greater than 0.1000 indicate the model terms are not significant.

The Lack of Fit F-value of 0.47 Response 2 (appendix) implies the Lack of Fit is not significant relative to the pure error. There is 89.39% chance that a Lack of Fit F-value this large could occur due experimental variation. Non-significant lack of fit is good since we want the model to fit.

4.1.5.2 Coefficients in Terms of Coded Factors

The coefficient estimate represents the expected change in response per unit change in factor value when all remaining factors are held constant indicating that the fermentation of TNW with *Saccaromyceses cerevisiae*, substrate weight interacts with temperature to significantly increase the bioethanol level produced by 0.5 units when all remaining factors are held constant. The C^2

coefficient is negative (-0.3854), this means that that the presence of the different levels of Innoculum size (cfu/ml) causes the curve to point downwards by 0.3854 units. The intercept term has a coefficient of 1.83 which is the overall average response of all the runs. The coefficients are adjustments around that average based on the factor settings..

4.1.5.3 Final Equation in Terms of Actual Factors

The equation in terms of actual factors can be used to make predictions about the response for given levels of each factor. Here, the levels are specified in the original units for each factor.

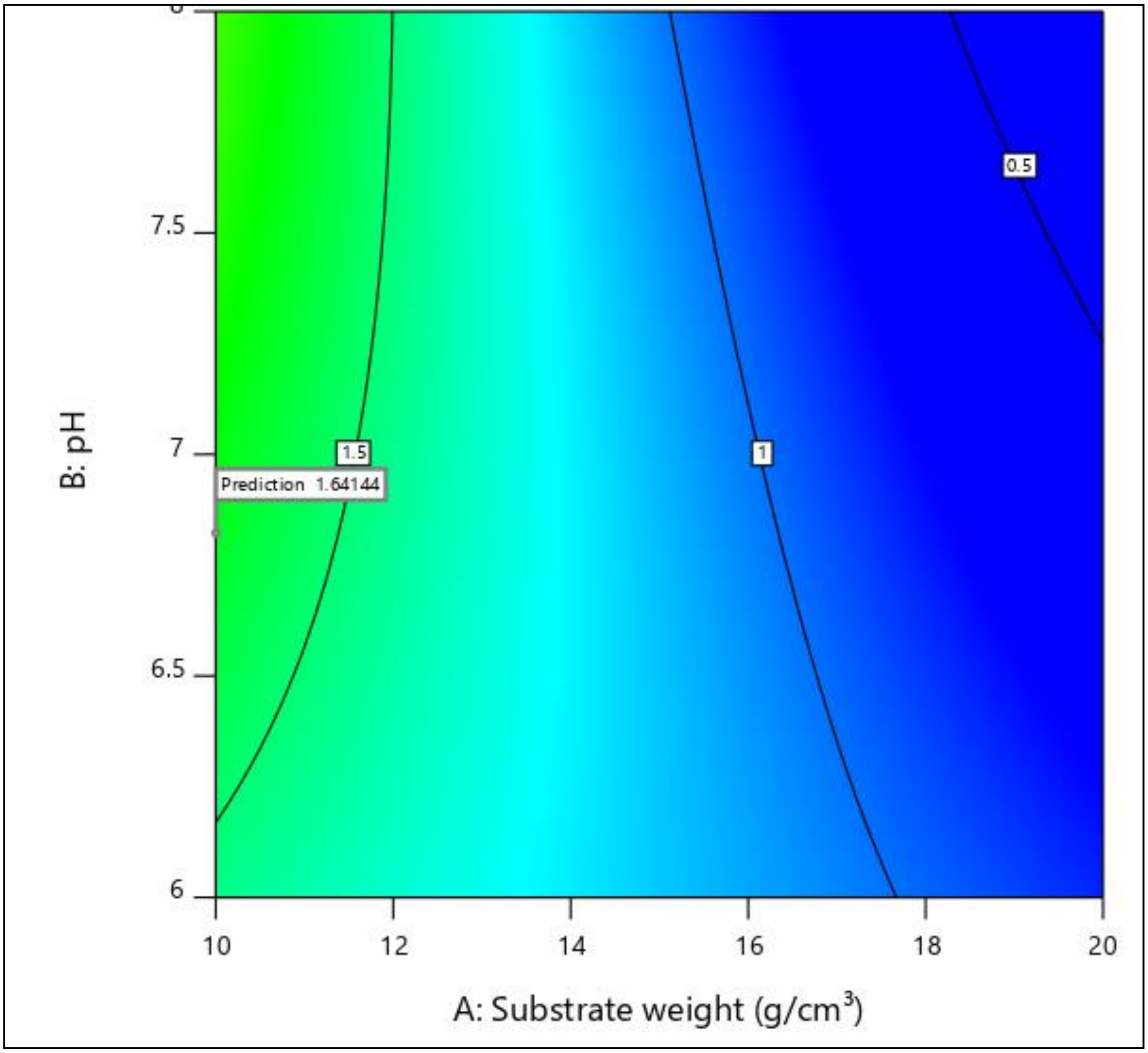


Figure 4.12: Contour plot of tigernut waste's bioethanol level with *Saccharomyces cerevisae*

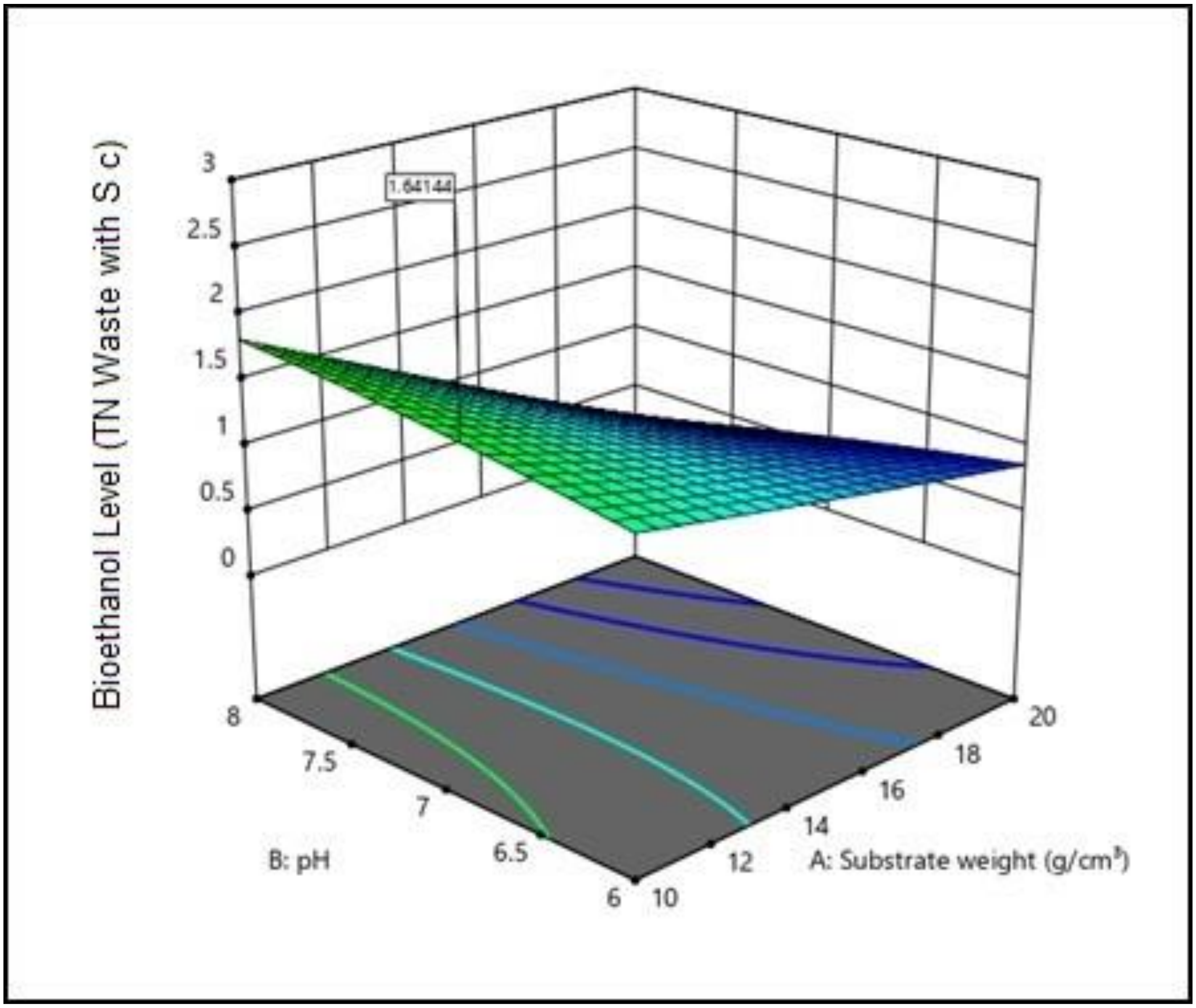


Figure 4.13: 3D surface plot of tigernut waste's bioethanol level with *Saccharomyces cerevisae*

The contour and 3D surface plots show that the optimum condition to produce the highest bioethanol level 1.64 (g/l) are substrate weight 10.003 (g/cm^3), pH level 6.82, Innoculum size 0.5 Macfaland standard (CFU/ml), Temperature 25 (^{o}C), and Time 93.51 (hours).

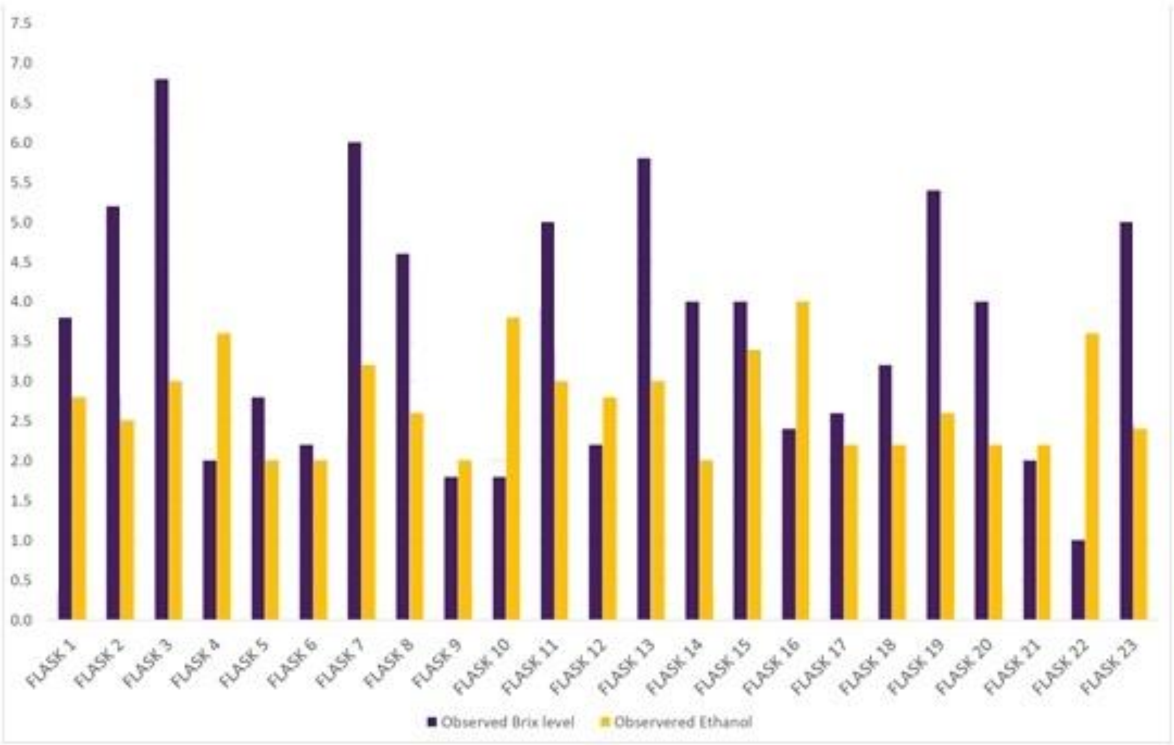


Figure 4.14a: Observed brix level and bioethanol level of beans husk with *Saccharomyces cerevisiae*

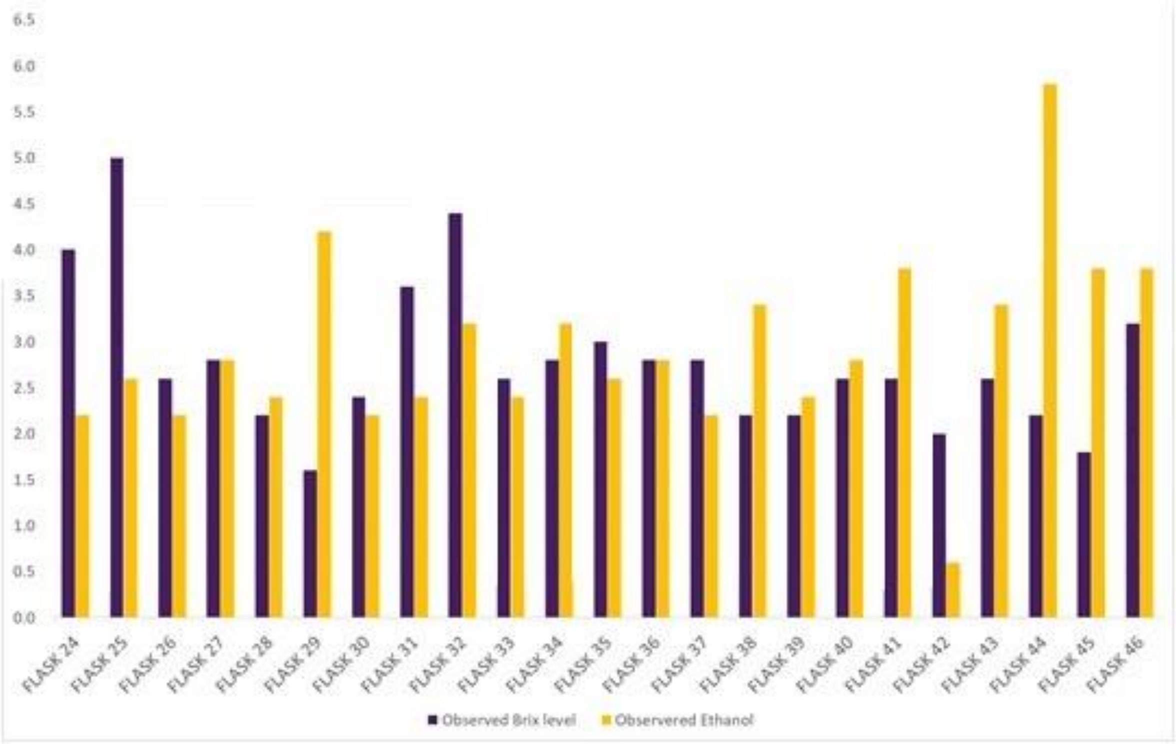


Figure 4.14b: Observed brix level and bioethanol level of beans husk with *Saccharomyces cerevisiae*

4.1.6 ANOVA for Quadratic Model

4.1.6.1 Response 3 Table A-D (Appendix): Brix Level (beans husk with *Aspergillus niger* *isolate HEFAPhR*

P-values less than 0.0500 indicate model terms are significant. In this case there are no significant model terms. Values greater than 0.1000 indicate the model terms are not significant meaning that all factors are not significant in either increasing or decreasing the bioethanol level in the treatment of BH with *Saccharomyces cerevisae*.

The Lack of Fit F-value of 2.97 Response 3 Table B (appendix) implies the Lack of Fit is not significant relative to the experimental variables.

4.1.7 ANOVA for Quadratic Model

4.1.7.1 Response 4 Table A-D (Appendix): Brix Level (beans husk with *Saccharomyces* *cerevisiae*

Transform: Inverse Sqrt

Constant: 0

The model software suggested an inverse-sqrt with zero constant transformation for the response in this model and was used to obtain the model coefficients. P-values less than 0.0500 indicate model terms are significant. In this case the interaction between pH and Innoculum size (CFU/ml) - BC, and the square of Innoculum size (CFU/ml) - C^2 are significant model terms. This means that pH and Innoculum size are significant factors that affect the brix level in the fermentation of BH with *Saccaromyceses cerevisiae*.

There is a 83.63% as shown in Response 4 Table D (appendix) chance that a Lack of Fit F-value this large could occur due to experimental variations. Therefore, non-significant lack of fit is good for model to fit.

The coefficient estimate represents the expected change in response per unit change in factor value when all remaining factors are held constant. The interaction between pH and Innoculum size (CFU/ml) – BC reduces the brix level by 0.1 while the square of Innoculum size (CFU/ml) - C^2 reduces the parabola downwards by 0.08 units in the treatment of BH with coefficients are adjustments around that average based on the factor settings. When the factors are orthogonal the VIFs are 1;

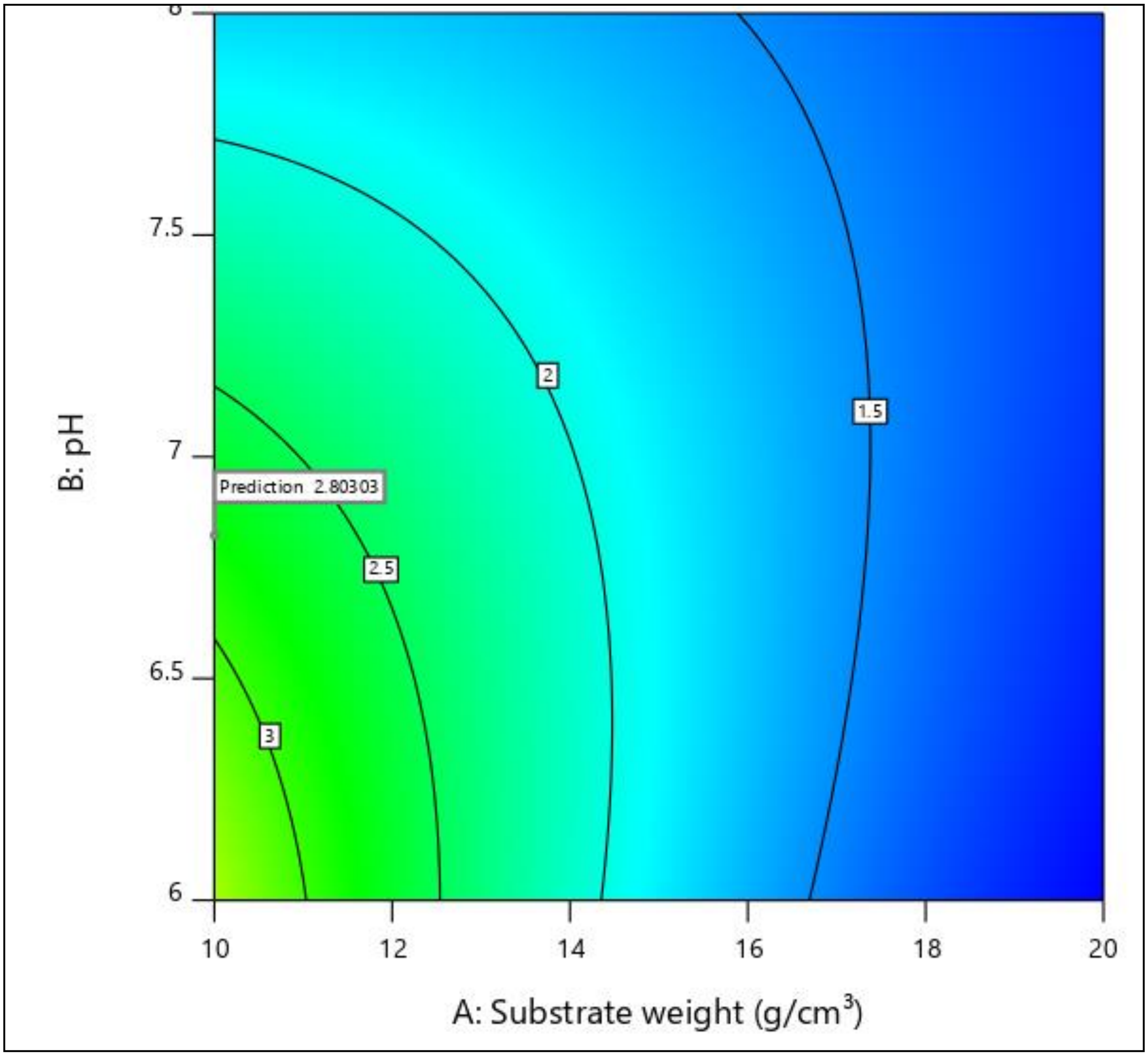


Figure 4.15: Contour plot of beans husk brix level with *Aspergillus niger isolate HEFAPhR*

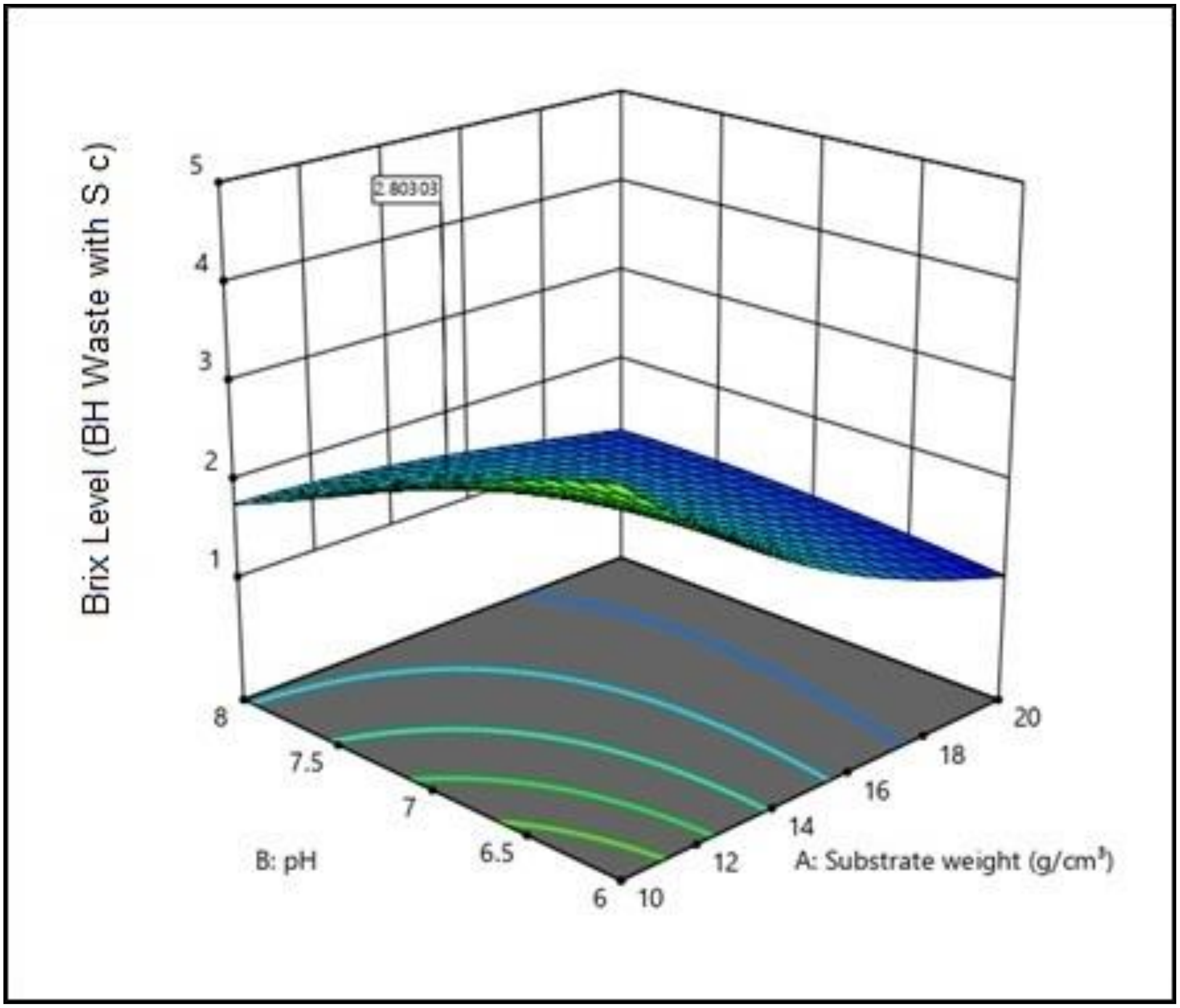


Figure 4.16: 3D surface plot of beans husk brix level with *Aspergillus niger isolate HEFAPhR*

The contour and 3D surface plots show that the optimum condition to produce the highest brix level 2.803 (^{o}Bxl) are substrate weight 10.003 (g/cm^3), pH level 6.82, Innoculum size 0.5(CFU/ml), Temperature 25 (^{o}C), and Time 93.5 (hours).

4.1.8 ANOVA for Linear Model

4.1.8.1 Response 5 Table A-D (Appendix): Bioethanol Level (beans Husk Waste with *Saccaromyceses cerevisiae*)

The Model F-value of 2.53 Response 5 Table B (appendix) implies the model is significant. There is only a 4.42% chance that an F-value this large could occur due to experimental variations.

P-values less than 0.0500 indicate model terms are significant. In this case Substrate weights (g/cm³), Temperature are significant model terms. This means that substrate weight and temperature significantly affected the brix level when using beans husk with *Saccharomyces cerevisiae*.

4.1.8.2 Coefficients in Terms of Coded Factors

The coefficient -0.625 associated with substrate weight as shown in Response 5 Table C (appendix) represents the expected change in response per unit change in the level of substrate weight (10-20 g/cm3) when all remaining factors are held constant. Since this coefficient is negative, it means that substrate weight significantly reduces the brix level. Temperature also reduces the brix level by 0.6875 units when increased from 25 to 35 degrees. The intercept in an orthogonal design is the overall average response of all the runs. The coefficients are adjustments around that average based on the factor settings. where $VIF = Variance\ Inflation\ Factor$.

4.1.8.3 Final Equation in Terms of Actual Factors

The equation in terms of actual factors can be used to make predictions about the response for given levels of each factor. Here, the levels are specified in the original units for each factor.

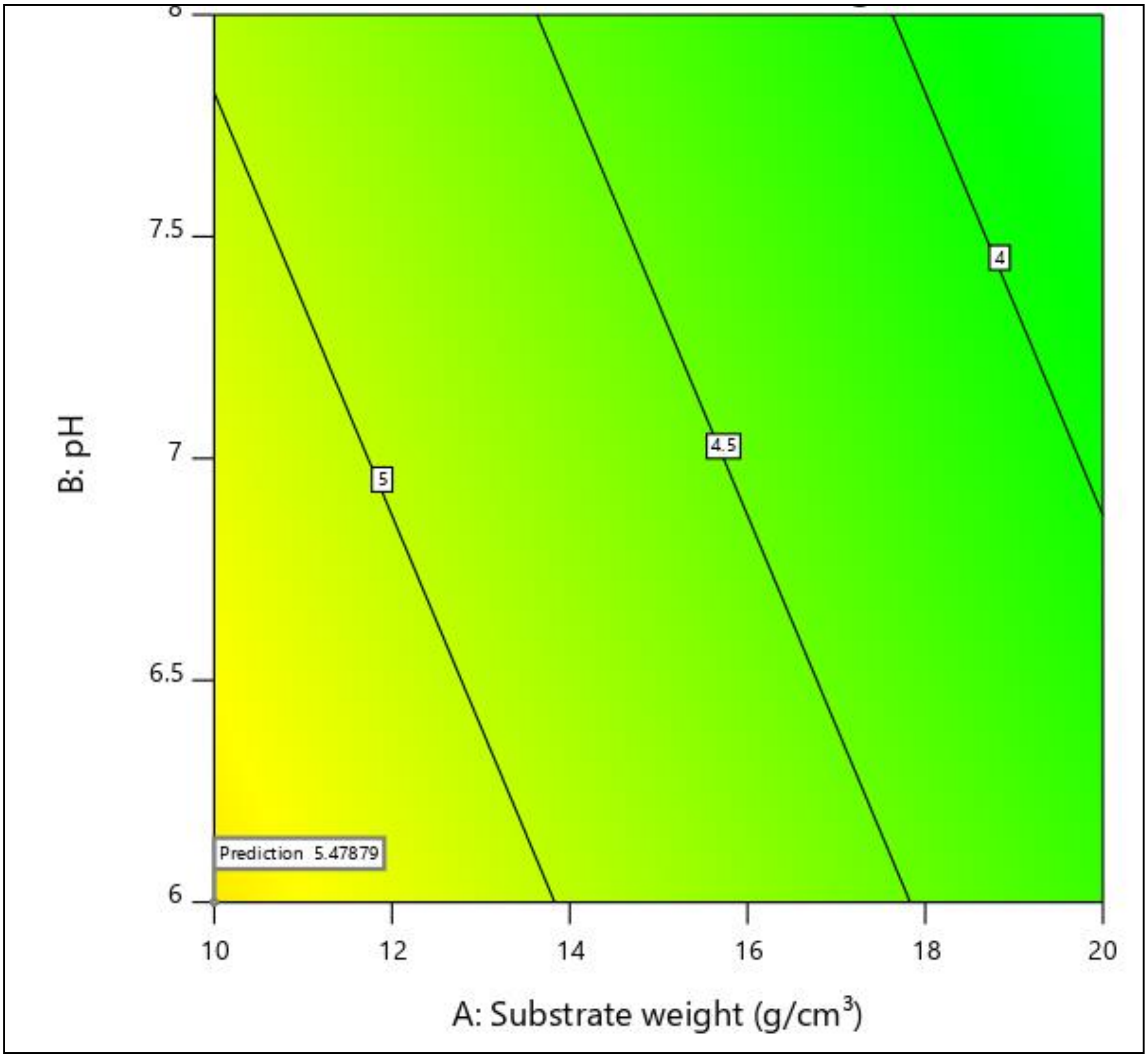


Figure 4.17: Contour plot of beans husk bioethanol level with *Saccharomyces cerevisiae*

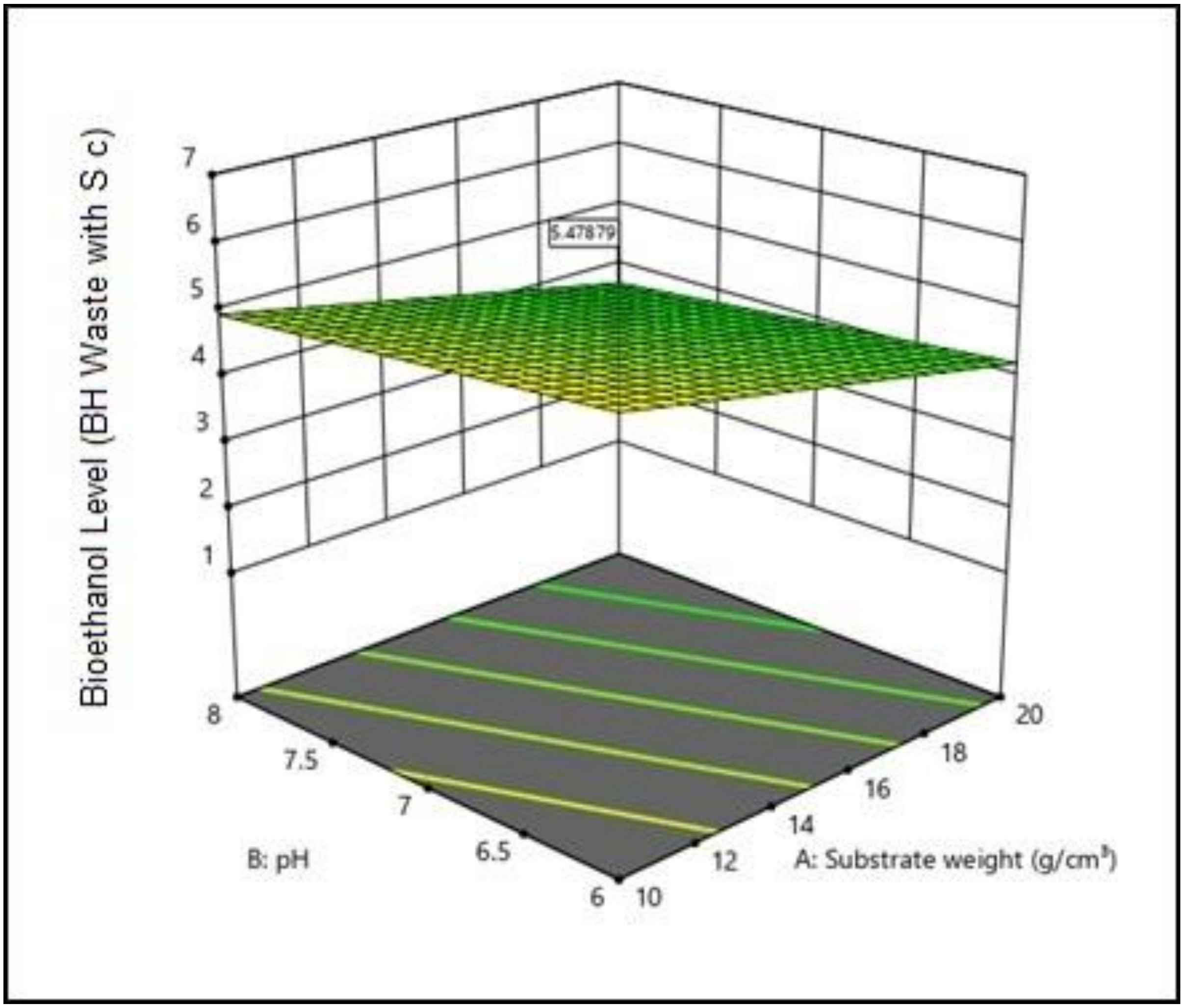


Figure 4.18: 3D surface plot of beans husk bioethanol level with *Saccharomyces cerevisiae*

The contour and 3D surface plots show that the optimum condition to produce the highest bioethanol level 5.478 (g/l) are substrate weight 10.003 (g/cm^3), pH level 6.00, Innoculum size 0.5 Macfland standard (CFU/ml), Temperature 25 (^{o}C), and Time 48 (hours).

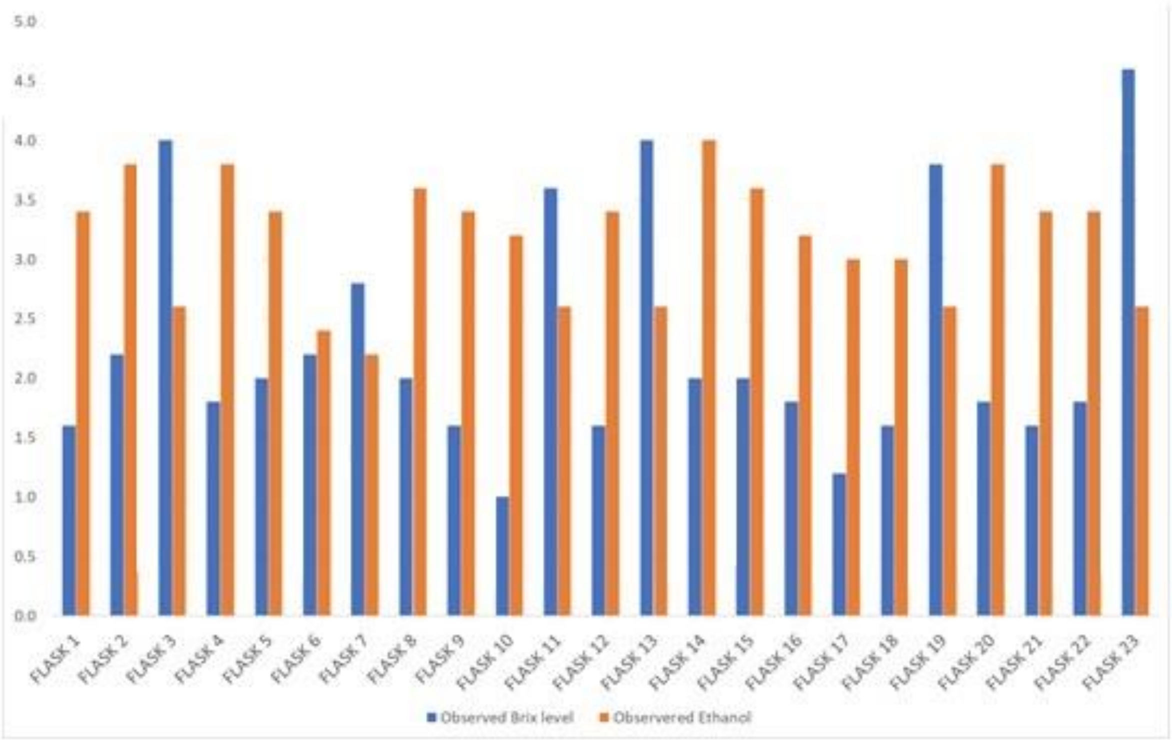


Figure 4.19a: Observed brix level and bioethanol level of groundnut shell with *Saccharomyces cerevisiae*

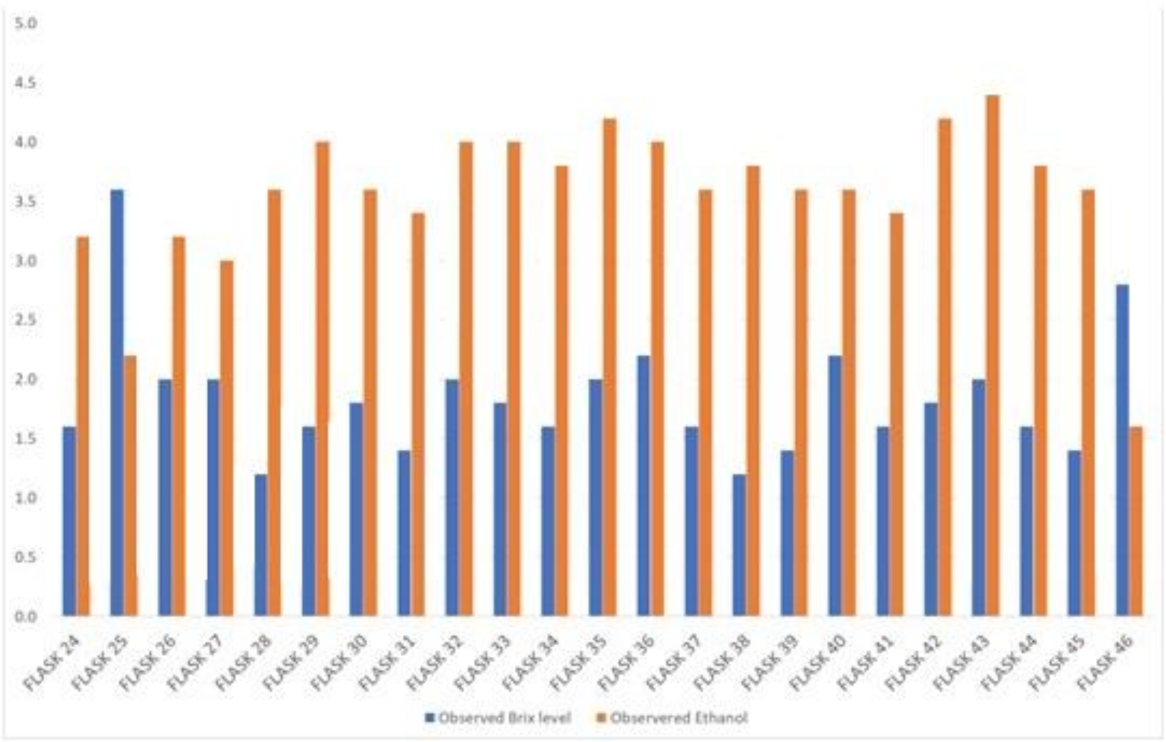


Figure 4.19b: Observed brix level and bioethanol level of groundnut shell with *Saccharomyces cerevisiae*

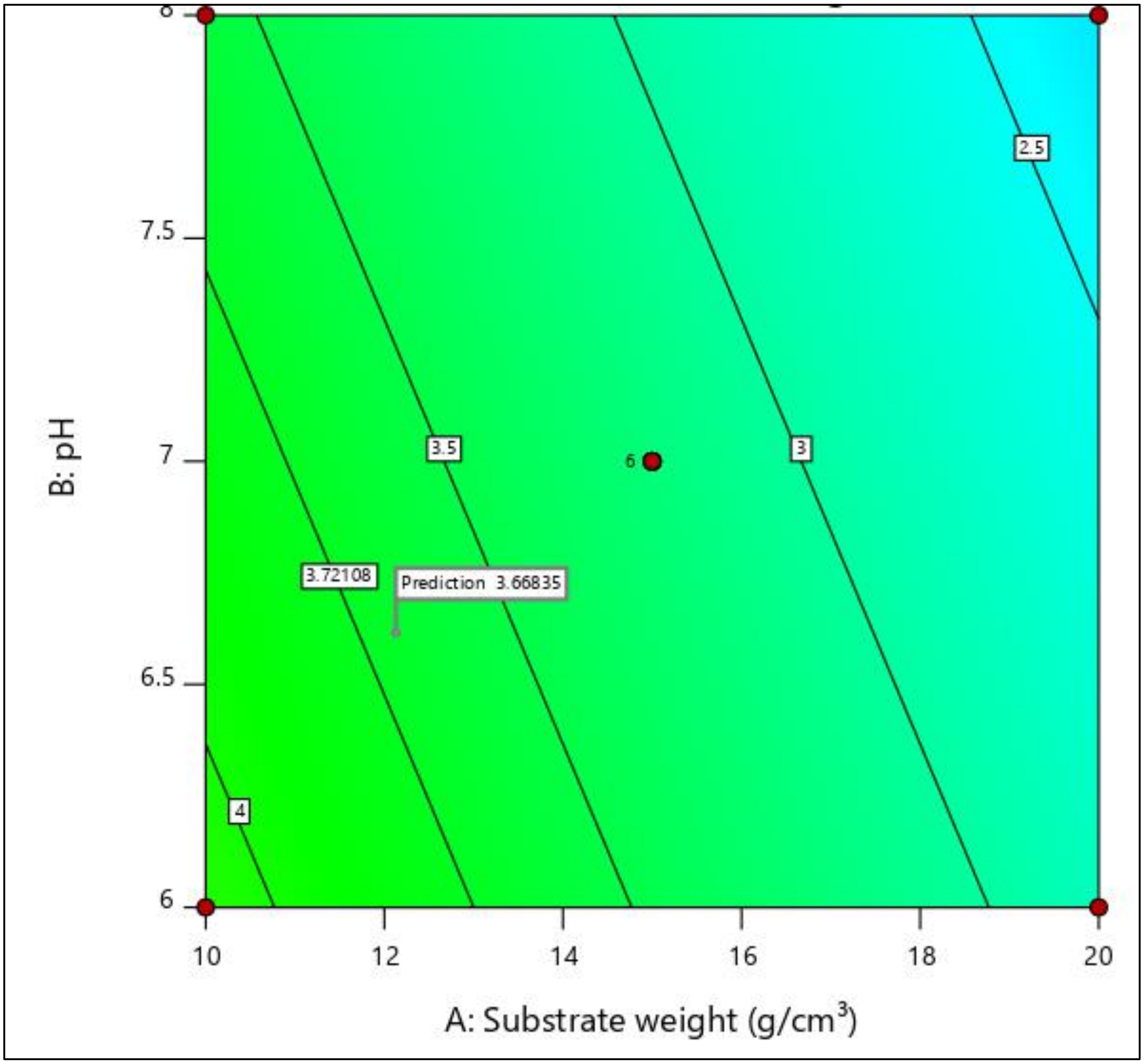


Figure 4.20: Contour plot of groundnut shell brix level with *Aspergillus niger isolate HEFAPhR*

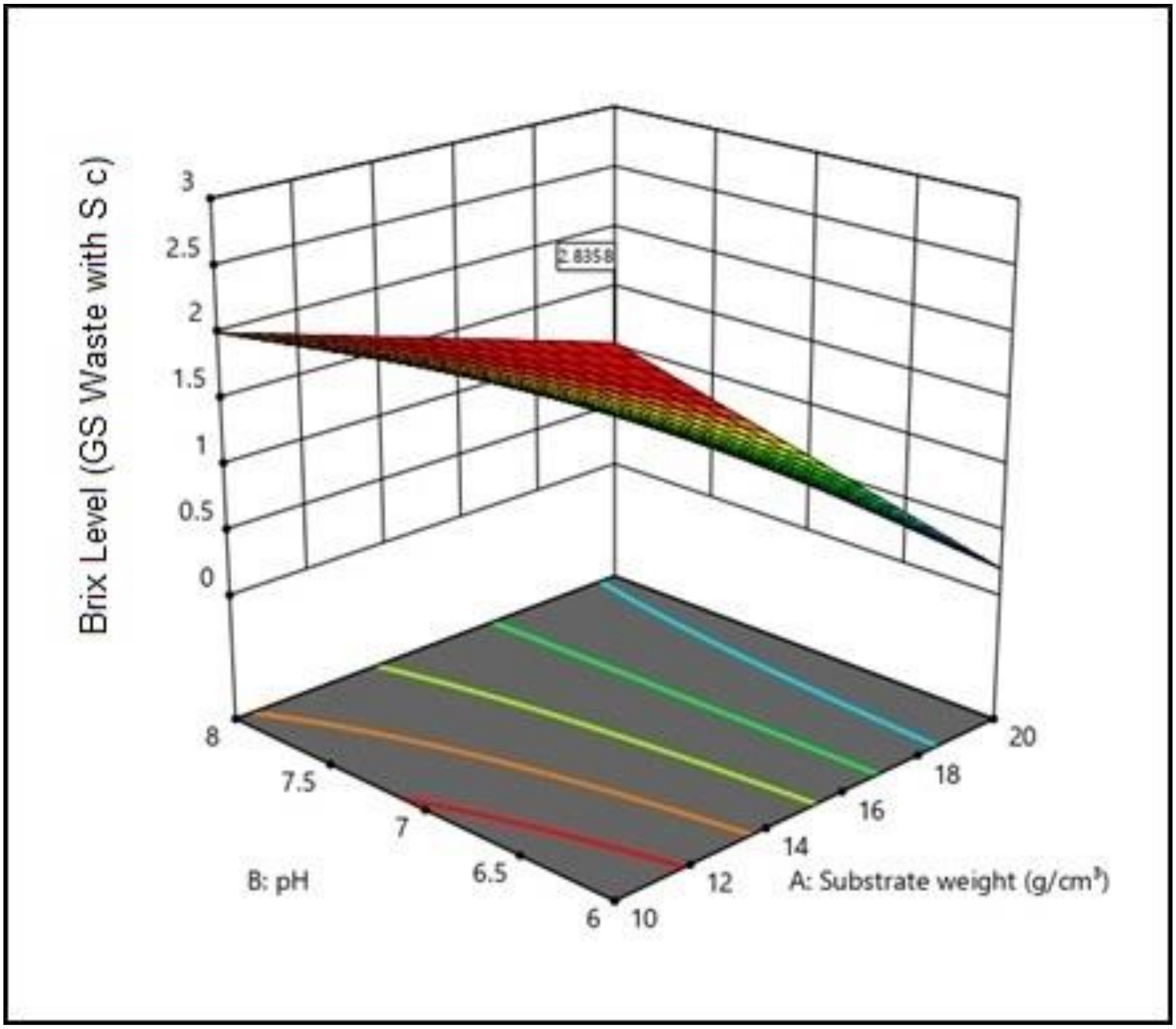


Figure 4.21: 3D surface plot of groundnut shell brix level with *Saccharomyces cerevisiae*

The contour and 3D surface plots show that the optimum condition to produce the highest bioethanol level 2.845 (g/l) are substrate weight 10.0069 (g/cm^3), pH level (6.00001), Innoculum size 0.5 Macfland (CFU/ml), Temperature 34.9999 (^{o}C), and Time 96 (hours).

4.1.9 Bioethanol Level (groundnut shell with *Saccaromyceses cerevisae*)

P-values less than 0.0500 indicate model terms are significant meaning that the interaction between pH and Innoculum size - BC, and the square of Temperature - D^2 are significant model terms for maximizing the production of bioethanol from GS and *Aspergillus niger isolate HEFAPhR*. Values greater than 0.1000 indicate the model terms are not significant.

The Lack of Fit F-value of 2.09 Response 6 (appendix) implies the Lack of Fit is not significant relative to the pure error. There is a 21.27% chance that a Lack of Fit F-value this large could occur due to experimental variations. Non-significant lack of fit is good since we want the model to fit.

The coefficient estimate represents the expected change in response per unit change in factor value when all remaining factors are held constant. In this case the interaction between pH and Innoculum size – BC reduces the bioethanol level by 0.95 units per unit change in the factor level of either of the two factors, while the square of Temperature - D^2 increases the curve upwards (increase in bioethanol yield) per unit increase in temperature for maximizing the production of bioethanol from GS and *Saccharomyces cerevisiae*. The intercept (3.20) units in an orthogonal design are the overall average response of all the runs. The coefficients are adjustments around that average based on the factor settings. When the factors are orthogonal the VIFs are 1; VIFs greater than 1 indicate multi-colinearity, the higher the VIF the more severe the correlation of factors. As a rough rule, VIFs less than 10 are tolerable.

The equations in terms of actual factors are used to make predictions about the response for given levels of each factor. Here, the levels are specified in the original units for each factor.

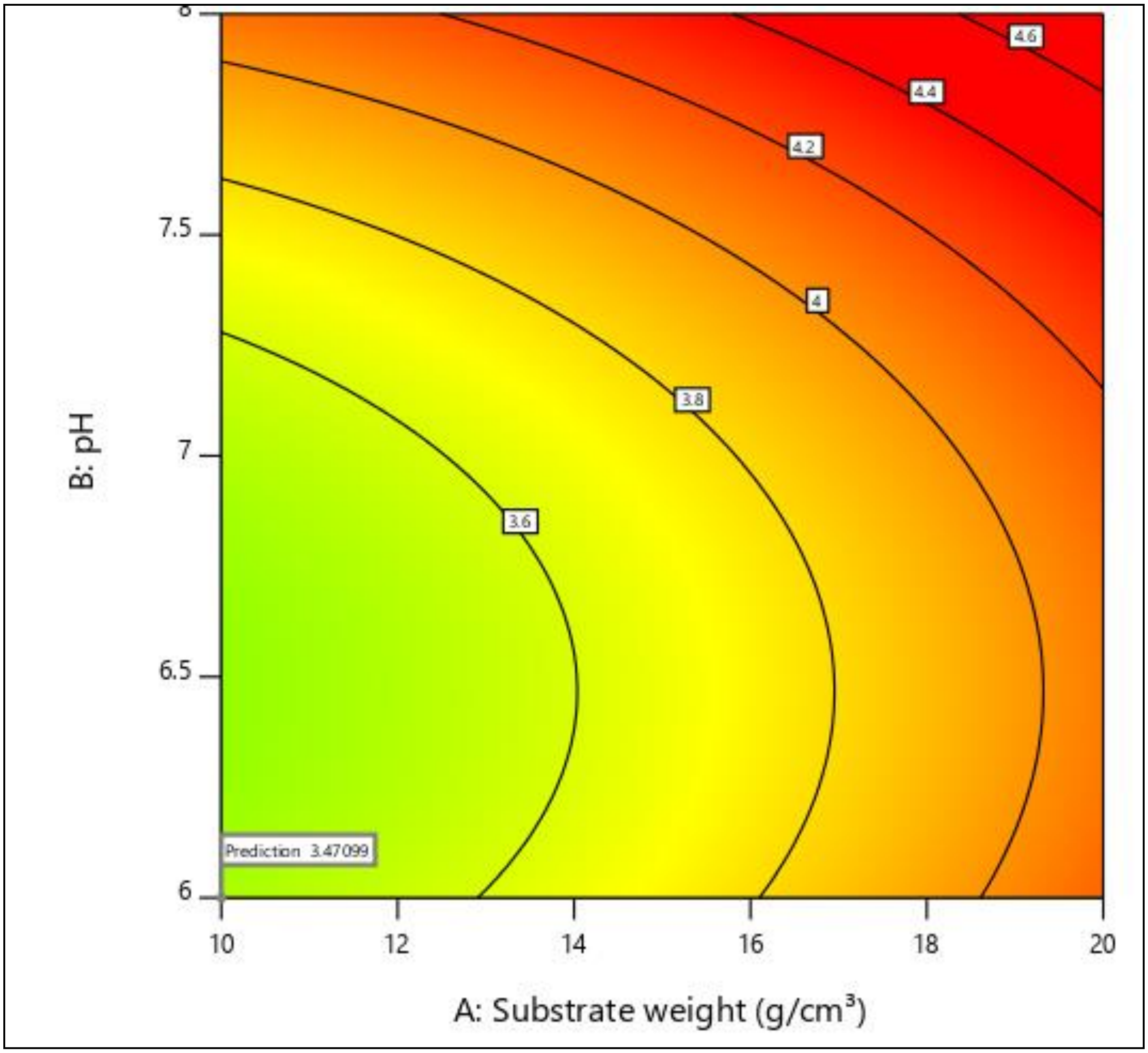


Figure 4.22: Contour plot of groundnut shell bioethanol level with *Saccharomyces cerevisiae*

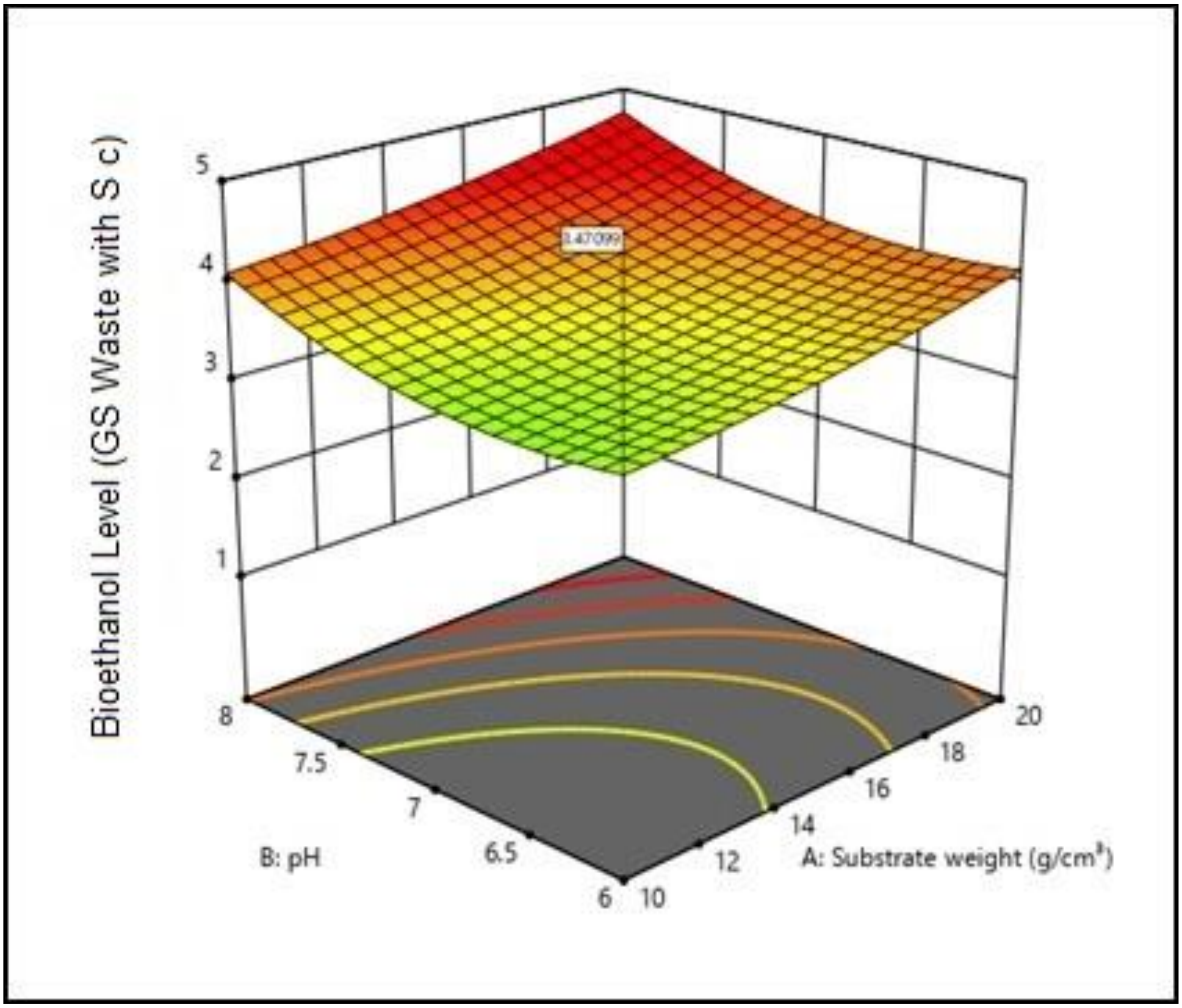


Figure 4.23: 3D surface plot of groundnut shell bioethanol level with *Saccharomyces cerevisiae*

The contour and 3D surface plots show that the optimum condition to produce the highest bioethanol level 3.47 (g/l) are substrate weight 10.003 (g/cm^3), pH level 6.00, Innoculum size 5.512 (CFU/ml), Temperature 35 (^{o}C) and Time 70.13 (hours).

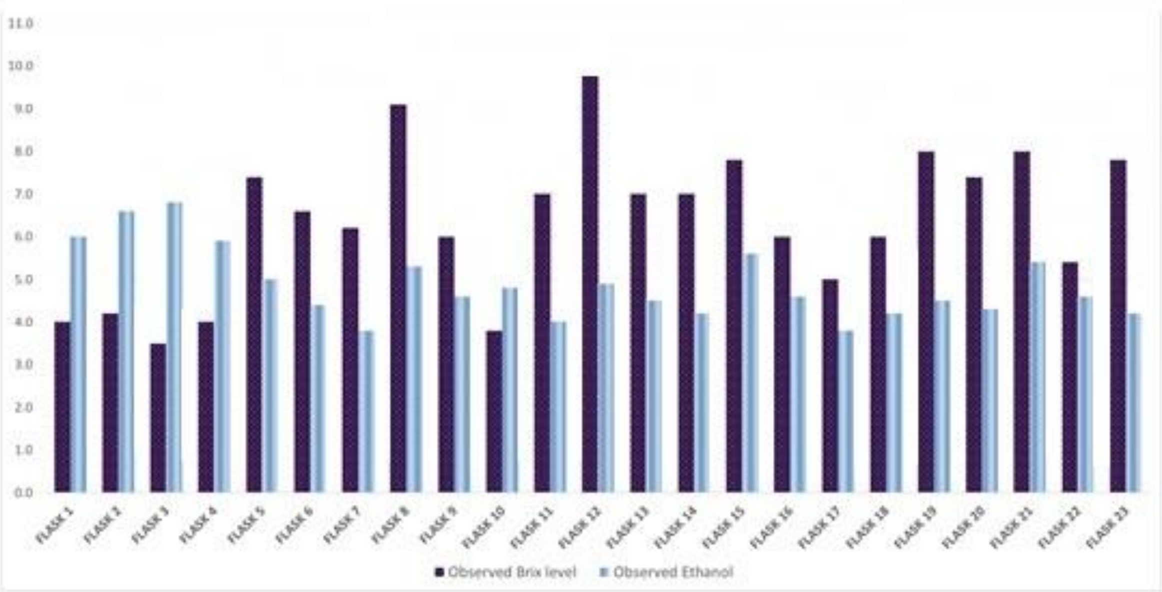


Figure 4.24a: Observed brix level and bioethanol level of tigernut waste with *Aspergillus niger isolate HEFAPhR*

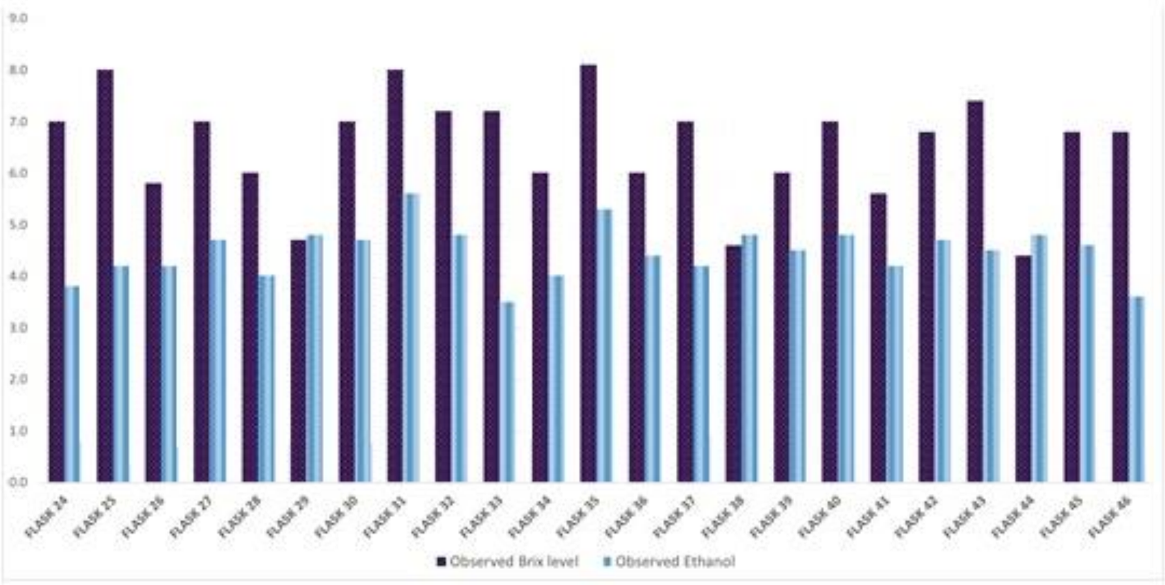


Figure 4.24b: Observed brix level and bioethanol level of tigernut waste with *Aspergillus niger isolate HEFAPhR*

4.1.10 Brix Level (Tiger nut Waste with *Aspergillus niger isolate HEFAPhR*)

ANOVA for 2FI model

4.1.10.1 Response 6 Table A-D: Brix Level (Tiger nut Waste with *Aspergillus niger isolate HEFAPhR*)

P-values less than 0.0500 indicate model terms are significant meaning that the interaction between Temperature and Time (DE interaction effect) is the only significant model term. Values greater than 0.1000 indicate the model terms are not significant.

There is a 22.93% chance that a Lack of Fit F-value this large occurred due to noise. Non-significant lack of fit is good since we want the model to fit.

The coefficient estimate represents the expected change in response per unit change in factor value when all remaining factors are held constant indicating that keeping Temperature constant at 25^{o}C and increasing Time from 48 hours to 96 hours will significantly reduce the brix level by 1.55 units in the fermentation of TNW with *Aspergillus niger*. The intercept (6.47) in an orthogonal design is the overall average response of all the runs. The coefficients are adjustments around that average based on the factor settings. The equation in terms of actual factors are used to make predictions about the response for given levels of each factor.

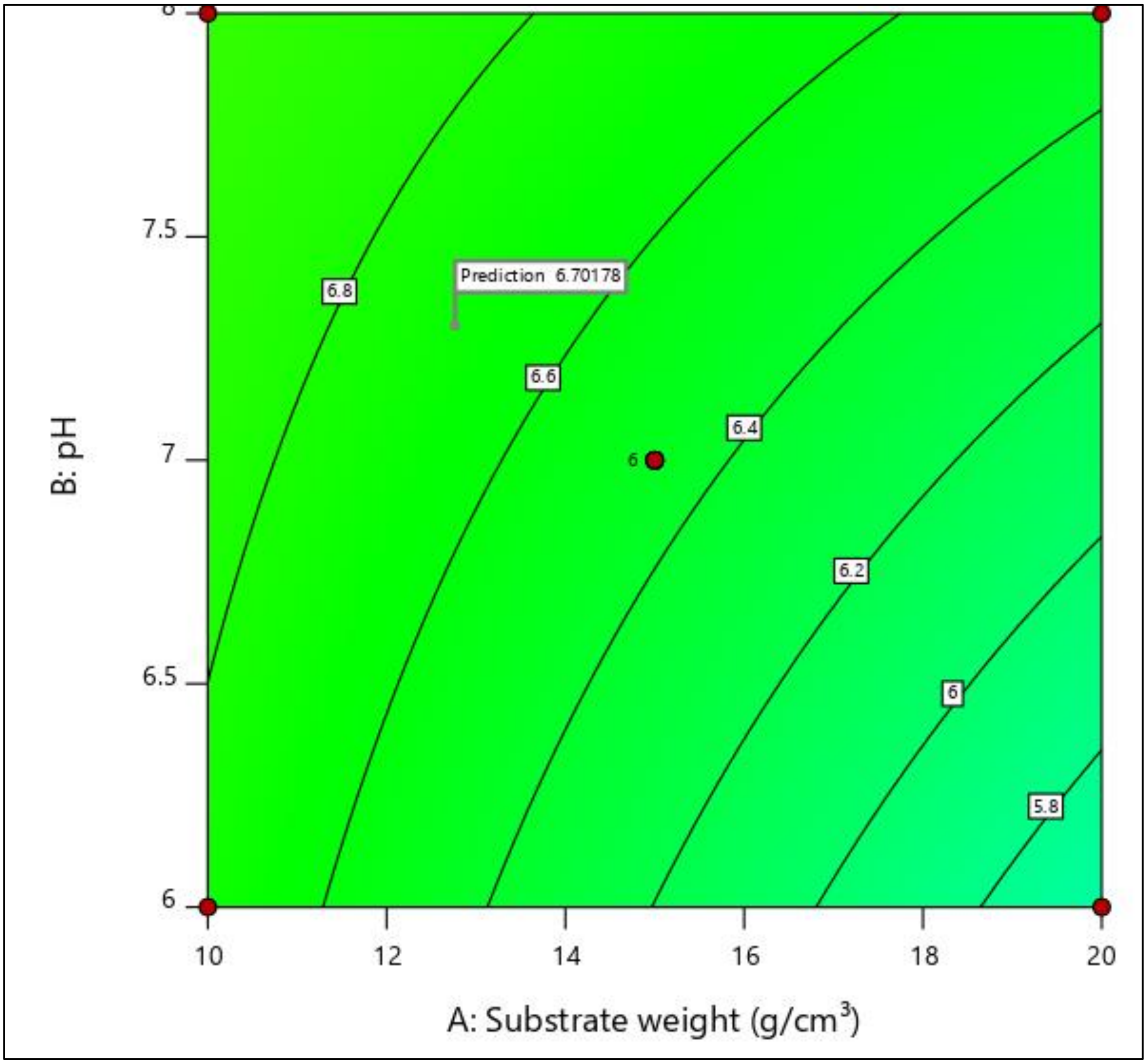


Figure 4.25: Contour plot of tigernut waste's brix level with *Aspergillus niger isolate HEFAPhR*

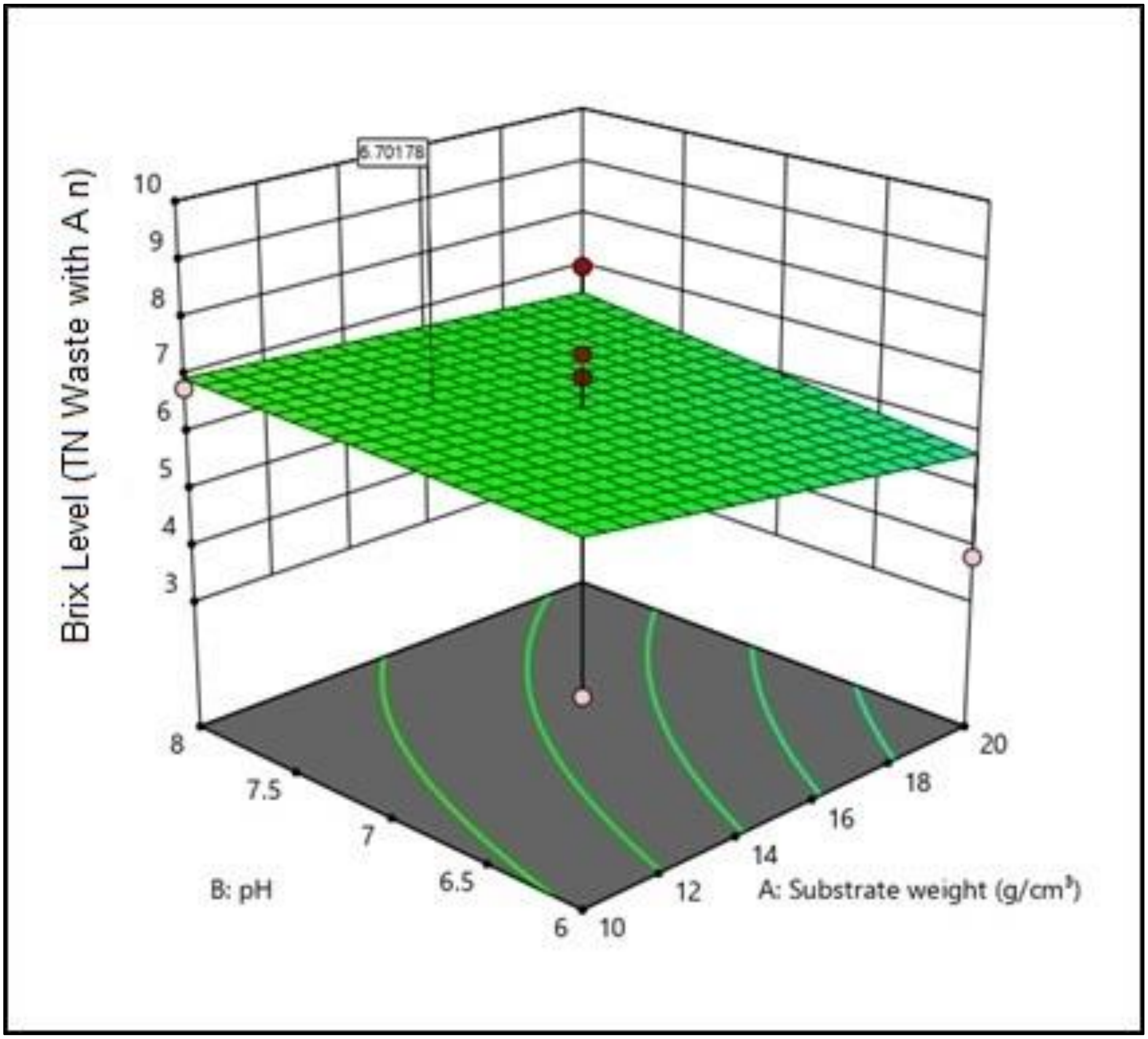


Figure 4.26: 3D surface plot of tiger nut waste's brix level with *Aspergillus niger isolate HEFAPhR*

The contour and 3D surface plots show that the optimum condition to produce the highest bioethanol level 6.70 (^{o}Bx) are substrate weight 12.79 (g/cm^3), pH level 7.300, Innoculum size 6.00 (ml), Temperature 30.00 (^{o}C), and Time 72.00 (hours).

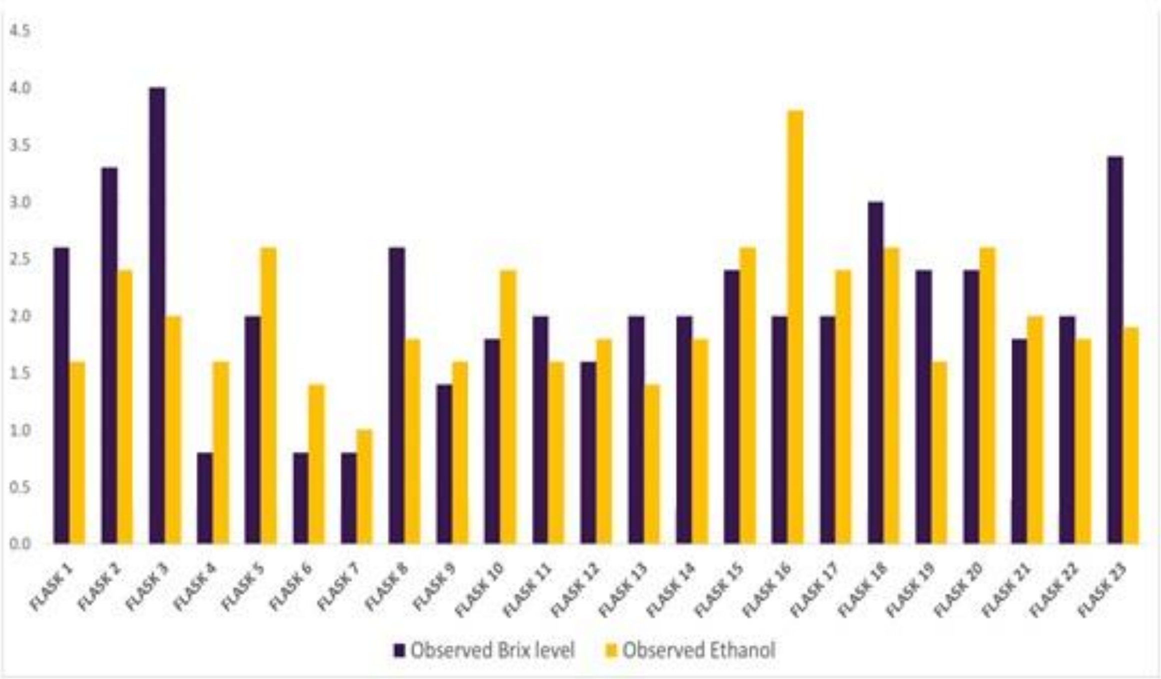


Figure 4.27a: Observed brix level and bioethanol level of beans husk with *Aspergillus niger* isolate HEFAPhR

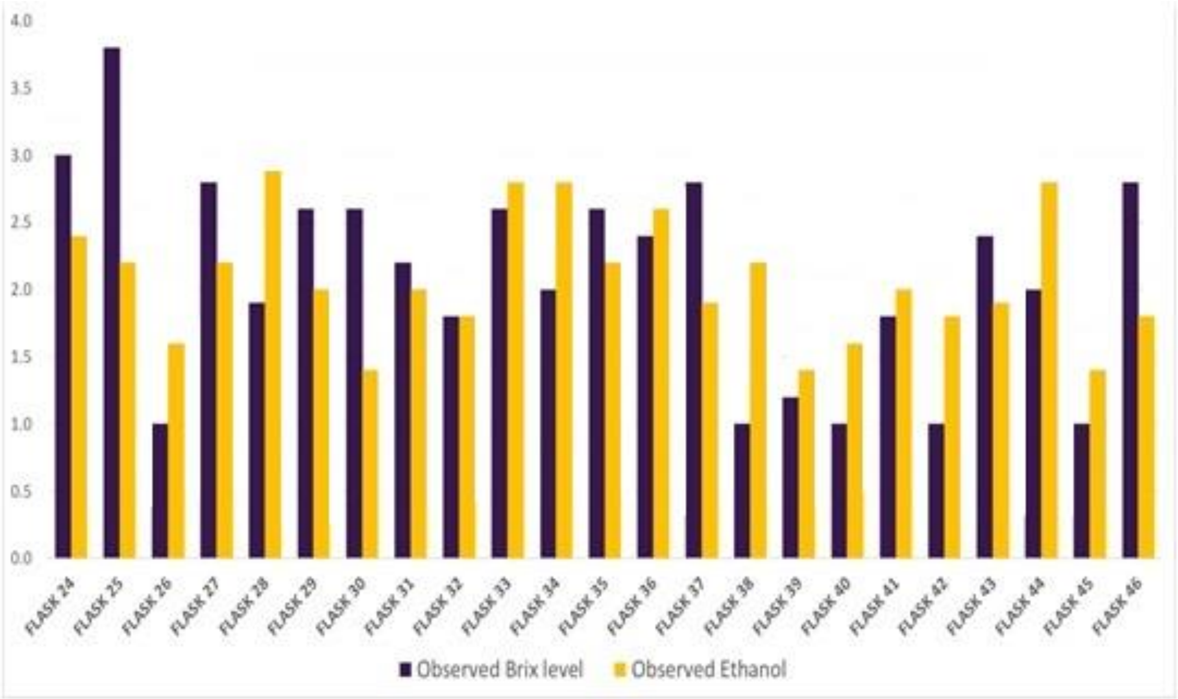


Figure 4.27b: Observed brix level and bioethanol level of beans husk with *Aspergillus niger* *isolate HEFAPhR*

4.1.11 Brix Level (beans husk with *Aspergillus niger isolate HEFAPhR*)

Model Summary Statistics

ANOVA for Quadratic model

4.1.11.1 Response 9 Table A-D: Brix level (beans husk with *Aspergillus niger isolate HEFAPhR*)

The Model F-value of 0.68 implies the model is not significant relative to the noise. There is 80.88% chance that an F-value this large could occur due to noise.

P-values less than 0.0500 indicate model terms are significant indicating that only the main effect due to Time squared (E^2 main effect) is a significant model term. Values greater than 0.1000 indicate the model terms are not significant. The Lack of Fit F-value of 0.55 implies the Lack of Fit is not significant relative to the pure error. There is a 84.20% as shown in Response 9 (appendix) chance that a Lack of Fit F-value this large could occur due to noise. Non-significant lack of fit is good since the modelled to fit.

4.1.11.2 Coefficients in Terms of Coded Factors

The coefficient estimate represents the expected change in response per unit change in factor value when all remaining factors are held constant. The positive value for the E^2 coefficient (1.47) indicates that Time factor increases the parabola of the response curve upwards, thereby increasing the corresponding brix level by 1.47 units in the treatment of BH with *Aspergillus niger isolate HEFAPhR*. The intercept (5.53) in an orthogonal design is the overall average response of all the runs. The coefficients are adjustments around that average based on the factor settings.

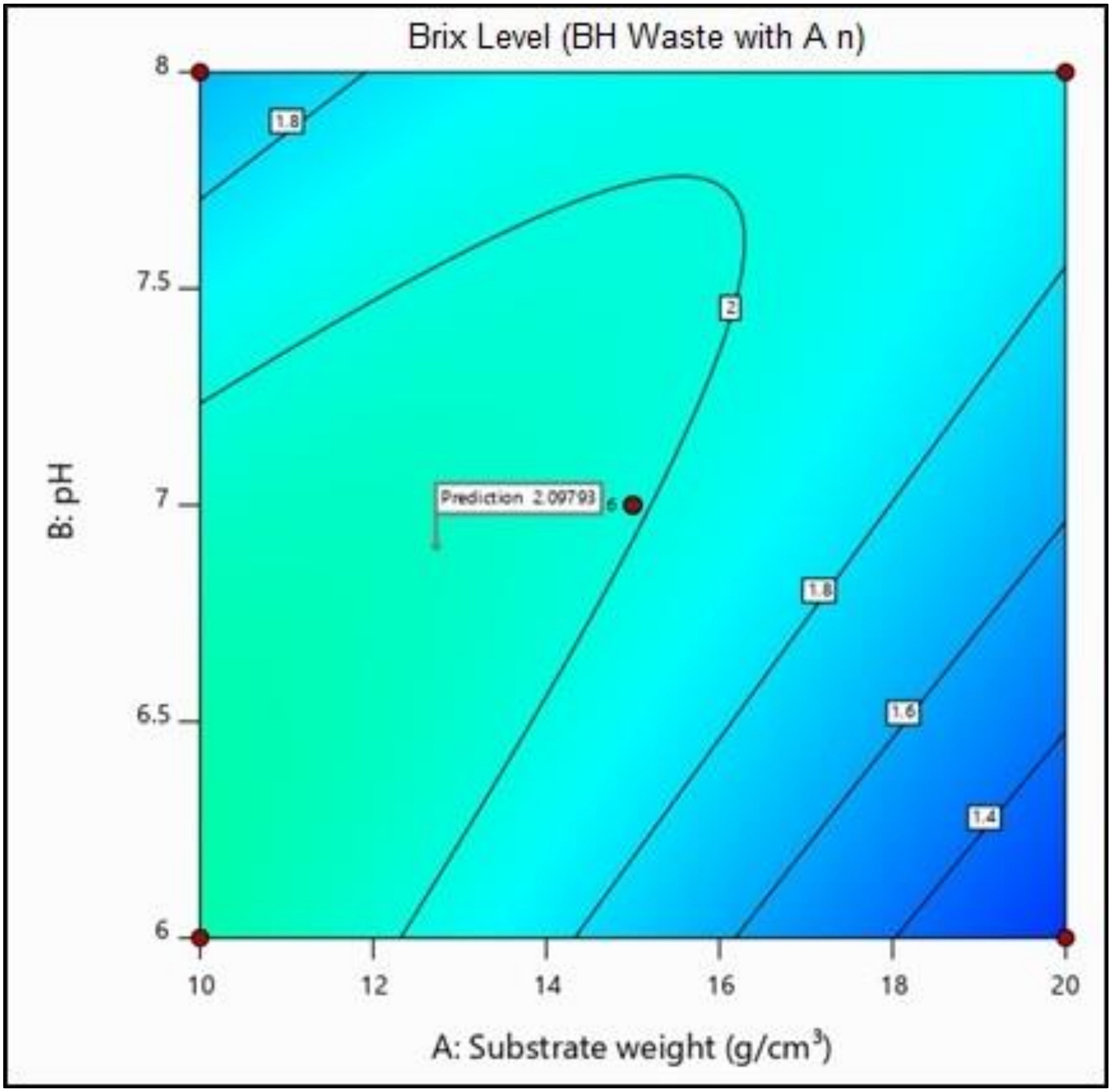


Figure 4.28: Contour plot of beans husk's brix level with *Aspergillus niger isolate HEFAPhR*

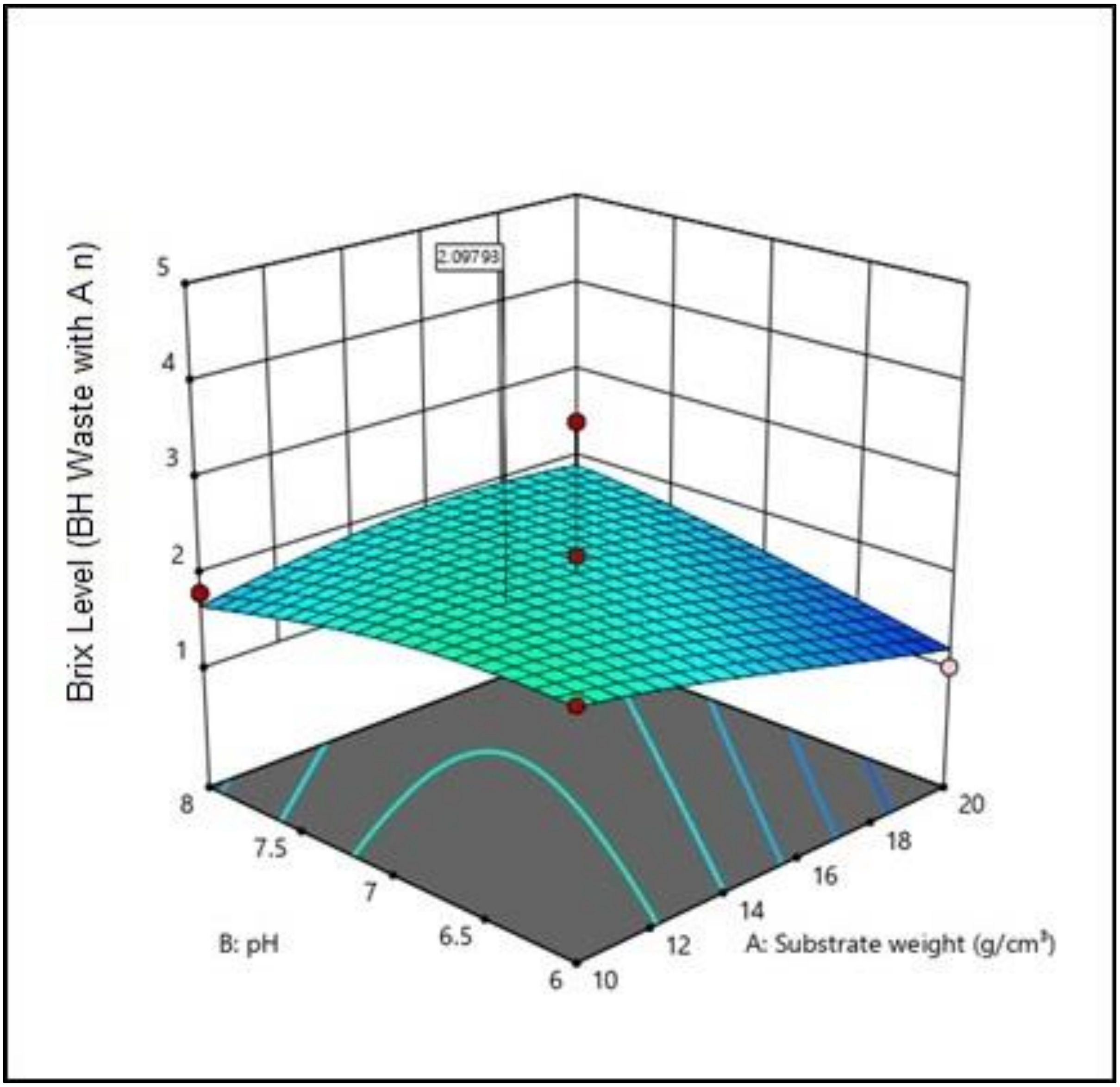


Figure 4.29: 3D surface plot of beans husk's brix level with *Aspergillus niger isolate HEFAPhR*

The contour and 3D surface plots show that the optimum condition to produce the highest brix level 2.09793 (^{o}Bxl) are substrate weight 12.705 (g/cm^3), pH level 6.9097, Innoculum size 5.00 Macfaland (CFU/ml), Temperature 30 (^{o}C), and Time 72 (hours).

4.1.12 Model Summary Statistics

ANOVA for Quadratic model

4.1.12.1 Response 10 Table A-D: Bioethanol Level (beans husk with *Aspergillus niger isolate HEFAPhR*)

The Model F-value of 1.24 implies the model is not significant relative to the noise. There is a 30.21% chance that an F-value this large could occur due to noise.

P-values less than 0.0500 indicate model terms are significant. In this case, it is only the interaction between substrate weight (g/cm^3) and Time (hours) - AE interaction effect that is a significant model term. Values greater than 0.1000 indicate the model terms are not significant. If there are many insignificant model terms (not counting those required to support hierarchy), model reduction may improve your model.

4.1.12.2 Coefficients in Terms of Coded Factors

The coefficient estimate represents the expected change in response per unit change in factor value when all remaining factors are held constant. In this case, keeping substrate weight (g/cm^3) constant at 10g/cm^3) and increase the Time (hours) from 48 hours to 96 hours will significantly reduce the bioethanol level by 10.10 units when using beans husk waste and *Aspergillus niger* to produce bioethanol. The intercept (4.37) in an orthogonal design is the overall average response of all the runs. The coefficients are adjustments around that average based on the factor settings. When the factors are orthogonal the VIFs are 1; VIFs greater than 1 indicate multi-colinearity, the higher the VIF the more severe the correlation of factors. As a rough rule, VIFs less than 10 are tolerable.

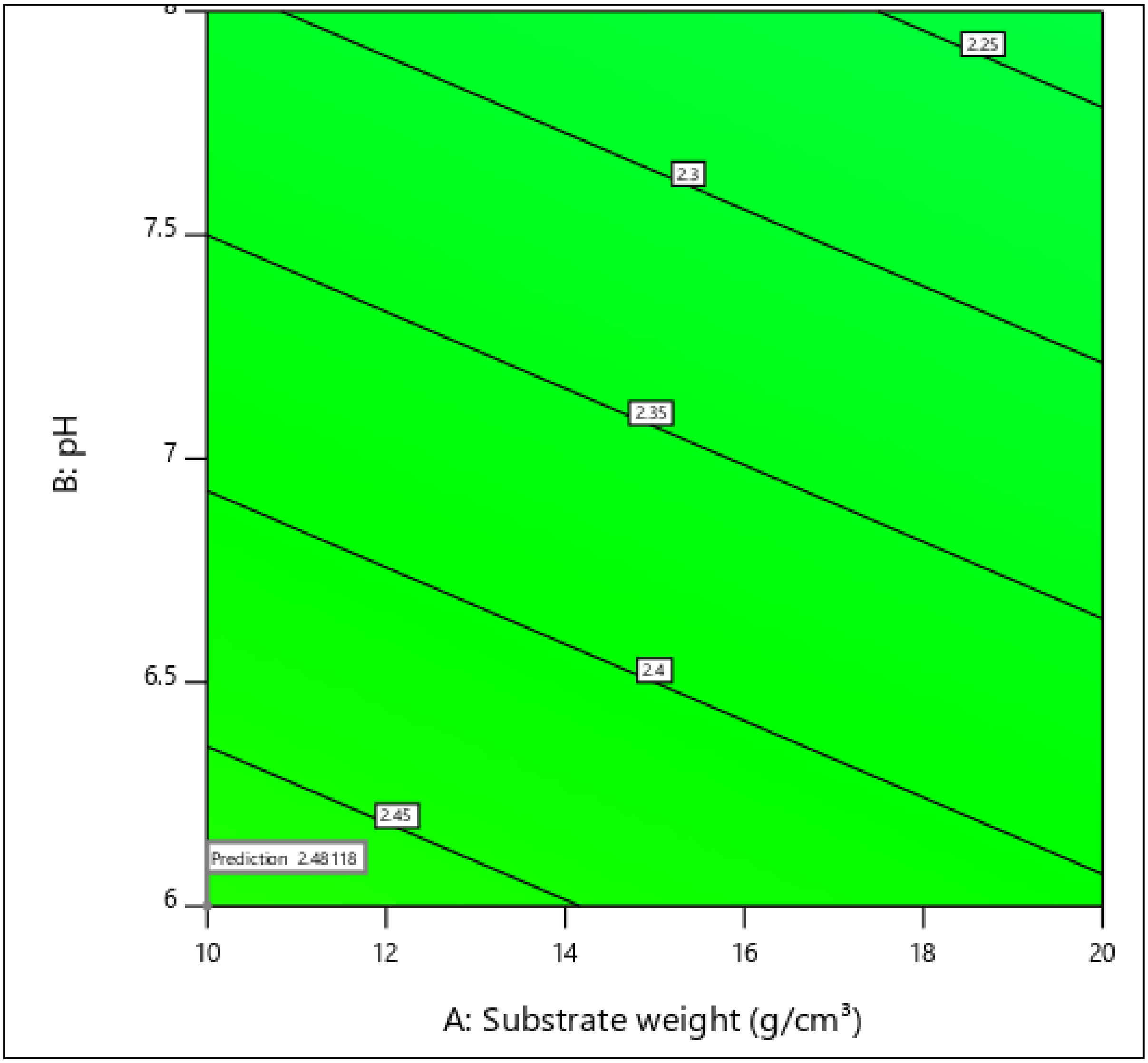


Figure 4.30: Contour plot of beans husk's bioethanol level with *Aspergillus niger isolate HEFAPhR*

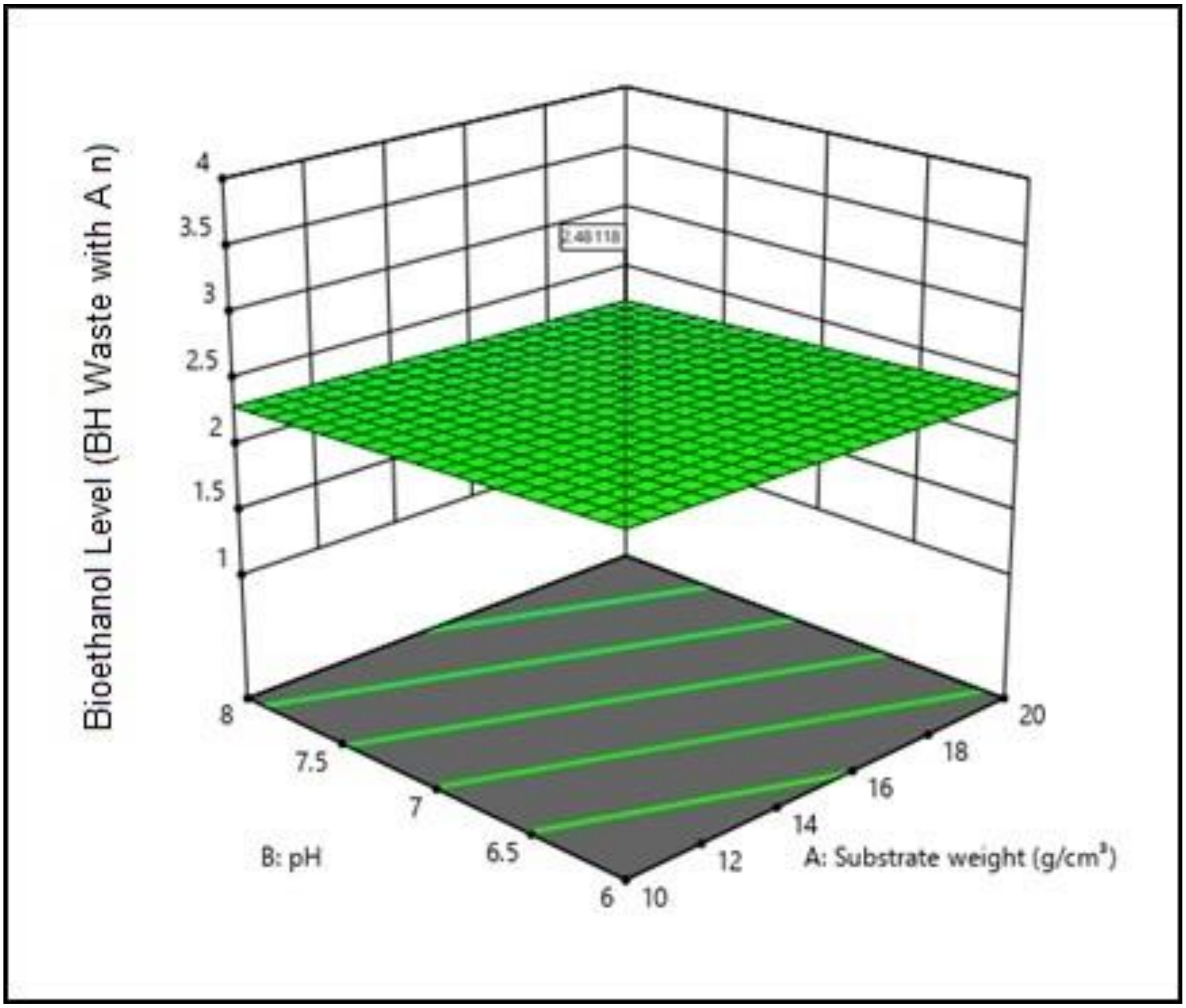


Figure 4.31: 3D surface plot of beans husk's bioethanol level with *Aspergillus niger isolate HEFAPhR*

The contour and 3D surface plots show that the optimum condition to produce the highest bioethanol level 2.48 (g/l) are substrate weight 10 (g/cm^3), pH level 6.0, Innoculum size 5.57 (CFU/ml), Temperature 34.88 (^{o}C) and Time 96 (hours).

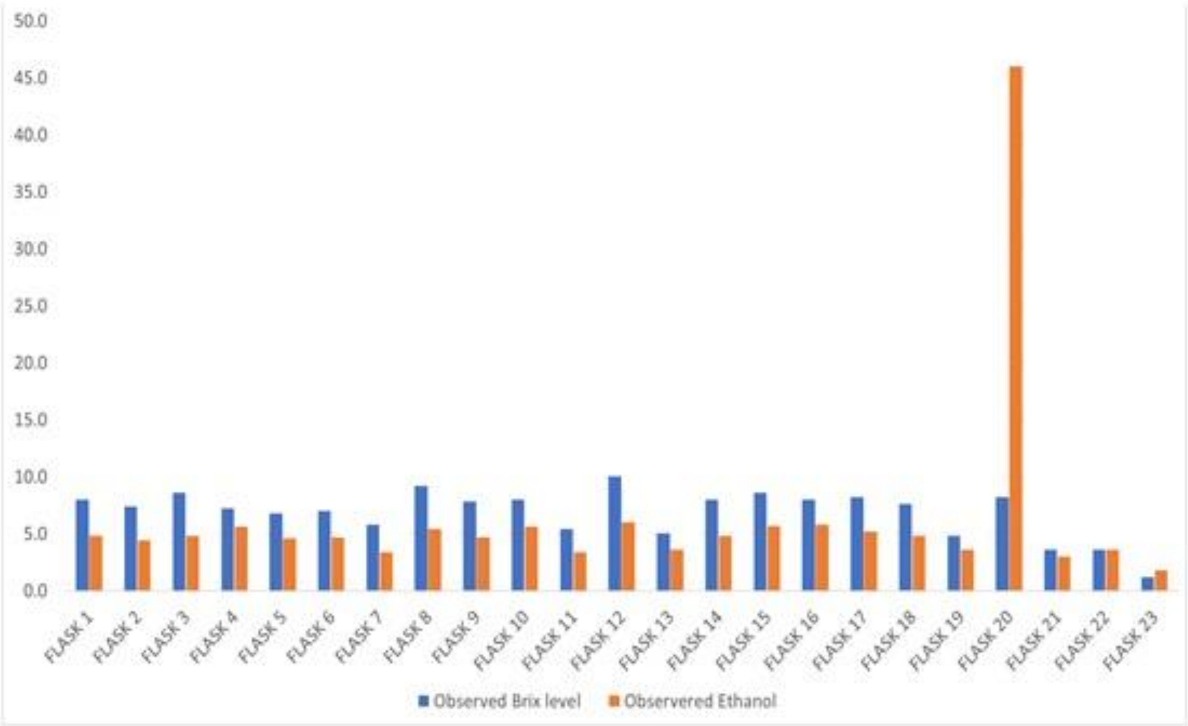


Figure 4.32a: Observed brix level and bioethanol level of groundnut shell with *Aspergillus niger isolate HEFAPhR*

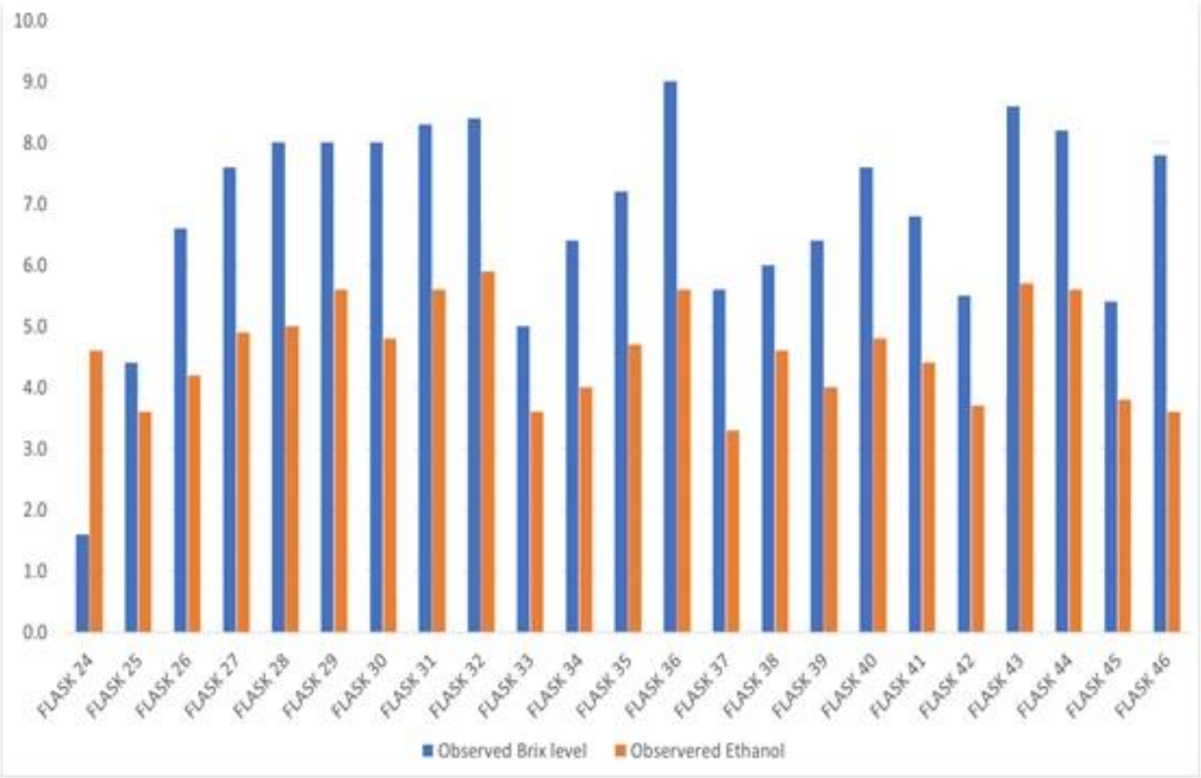


Figure 4.32b: Observed brix level and bioethanol level of groundnut shell with *Aspergillus niger isolate HEFAPhR*

4.1.13 Brix Level (Groundnut Shell Waste with *Aspergillus niger isolate HEFAPhR*)

Model Summary Statistics

ANOVA for Linear model

4.1.13.1 Response 11 Table A-D: Brix level (groundnut shell with *Aspergillus niger isolate HEFAPhR*)

P-values less than 0.0500 indicate model terms are significant. In this case there are no significant model terms. Values greater than 0.1000 indicate the model terms are not significant.

The Lack of Fit F-value of 1.53 implies the Lack of Fit is not significant relative to the pure error. There is a 33.91% chance that a Lack of Fit F-value could occur due to labouratory variations. Non-significant lack of fit is good since we the modelled to fit.

4.1.13.2 Coefficients in Terms of Coded Factors

The coefficient estimate represents the expected change in response per unit change in factor value when all remaining factors are held constant. The intercept in an orthogonal design is the overall average response of all the runs. The coefficients are adjustments around that average based on the factor settings. When the factors are orthogonal the VIFs are 1; VIFs greater than 1 indicate multi-colinearity, the higher the VIF the more severe the correlation of factors. As a rough rule, VIFs less than 10 are tolerable.

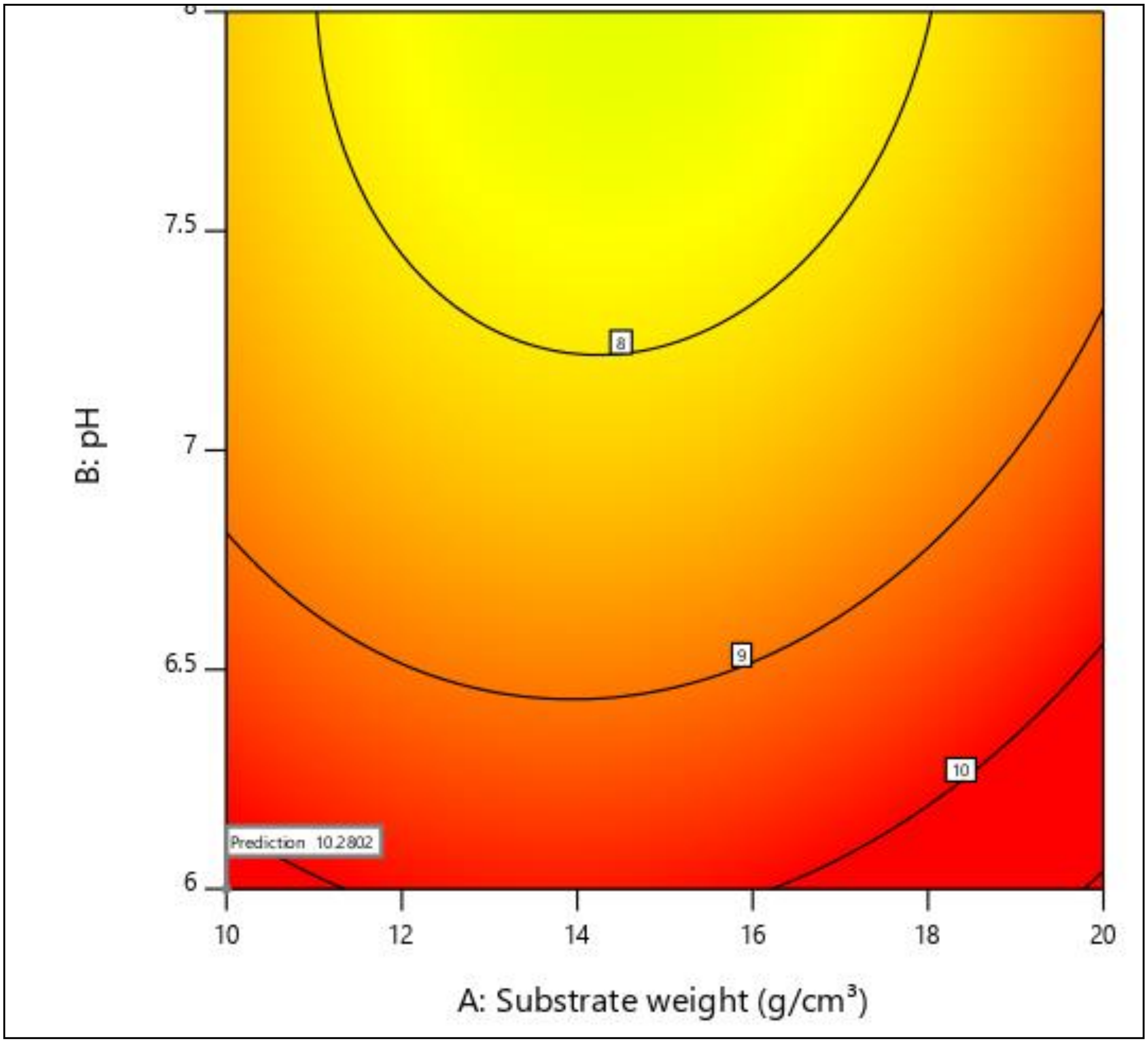


Figure 4.33: Contour plot groundnut shell's brix level with *Aspergillus niger isolate HEFAPhR*

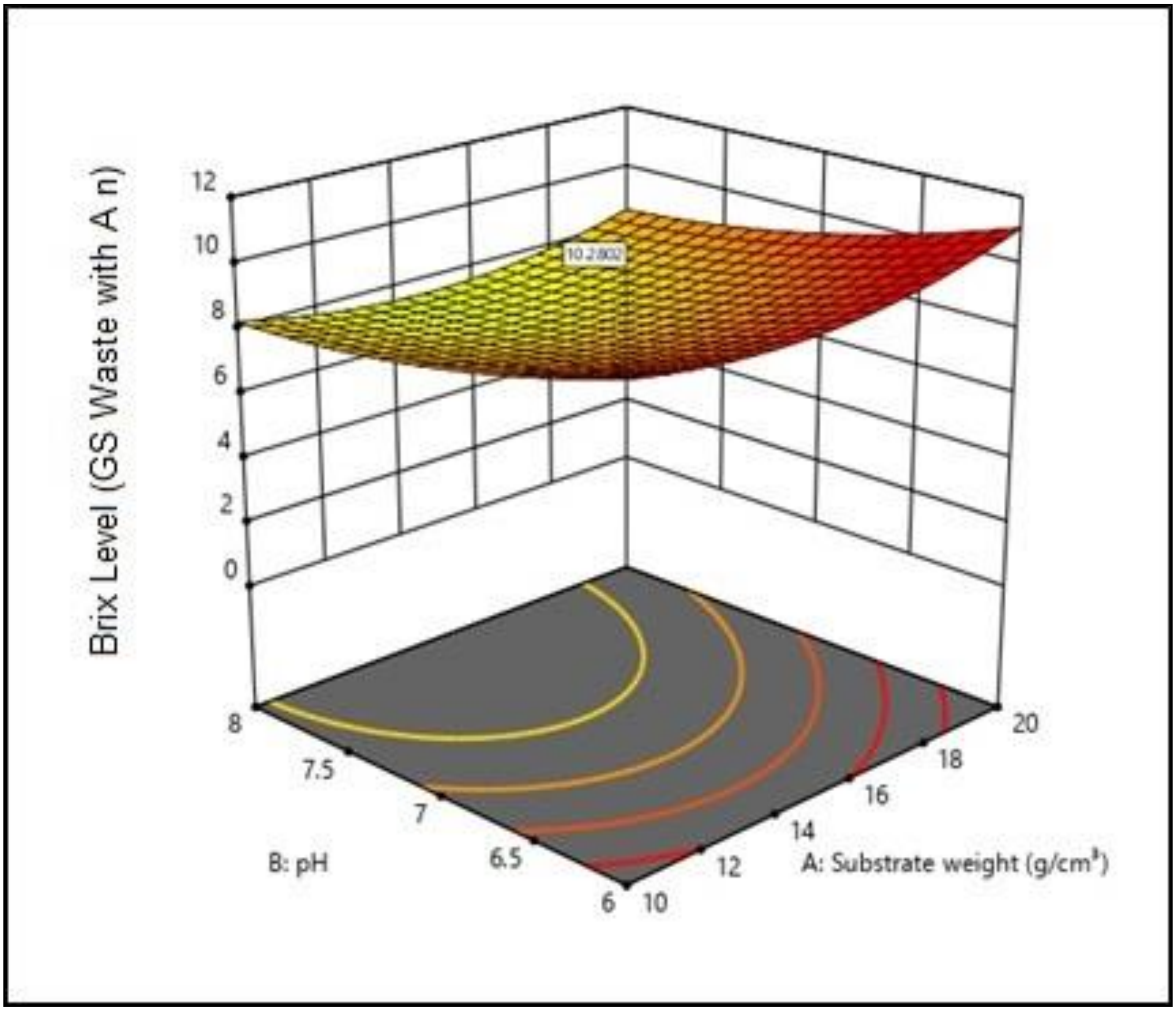


Figure 4.34: 3D Surface plot groundnut shell's brix level with *Aspergillus niger isolate HEFAPhR*

The contour and 3D surface plots show that the optimum condition to produce the highest brix level 10.2 (^{o}Bx) are substrate weight 10 (g/cm^3), pH level 6.0, Innoculum size 5.62 (CFU/ml), Temperature 35.0 (^{o}C) and Time 95.96 (hours).

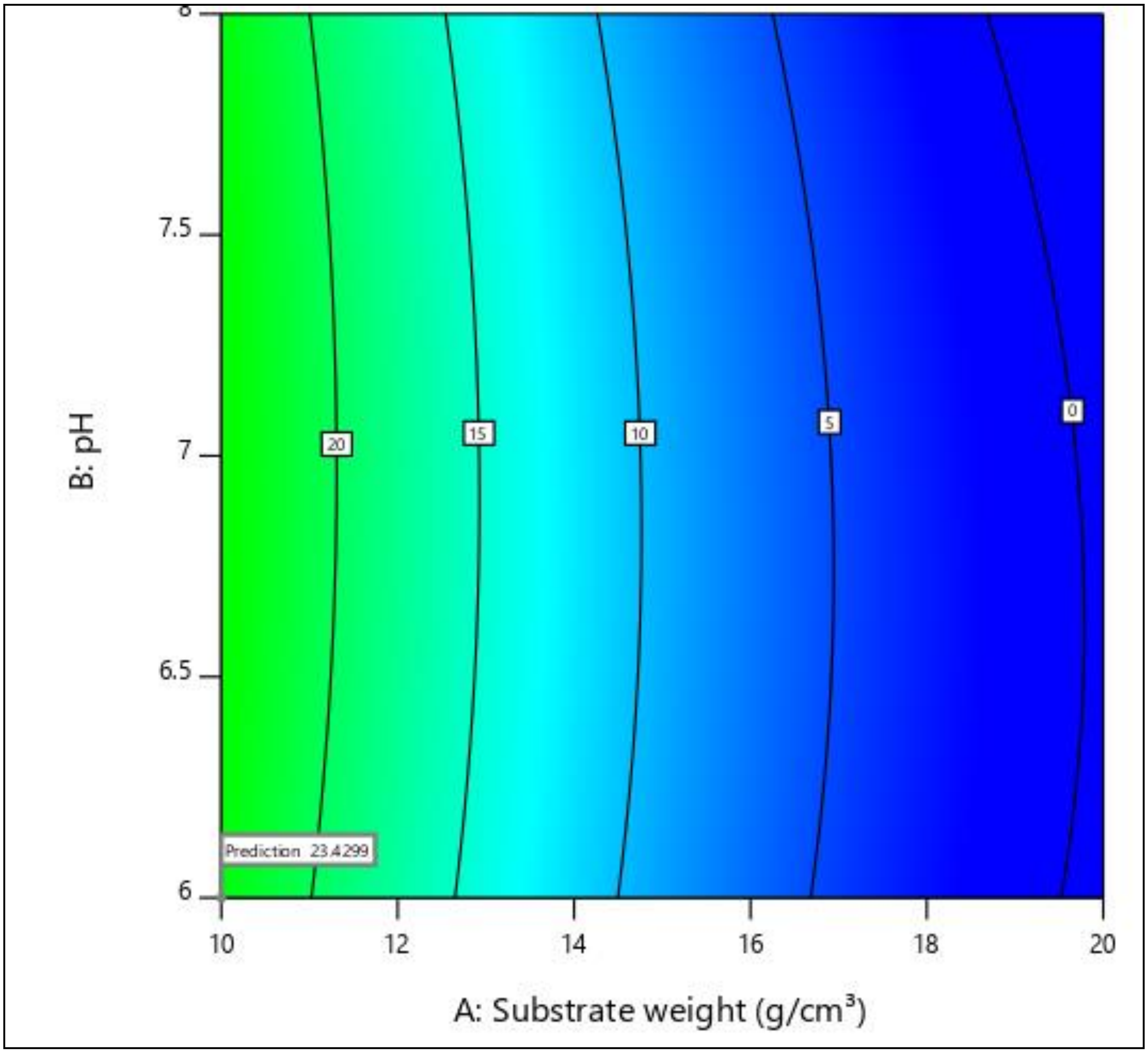


Figure 4.35 Contour plot of groundnut shell's bioethanol level with *Aspergillus niger isolate HEFAPhR*

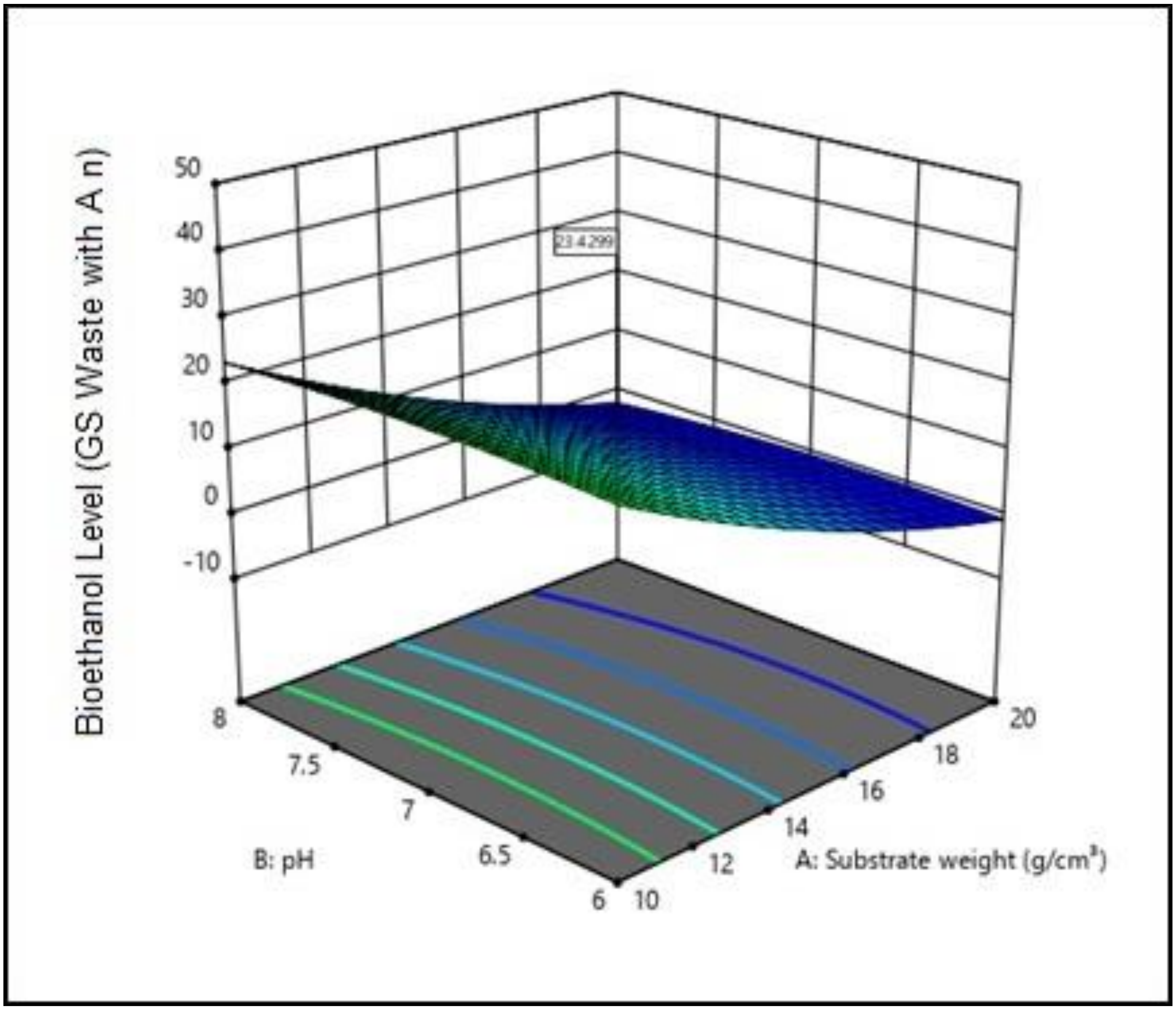


Figure 4.36: 3D Surface plot of groundnut shell's bioethanol level with *Aspergillus niger* *isolate HEFAPhR*

The contour and 3D surface plots show that the optimum condition to produce the highest bioethanol level 23.43 (g/l) are substrate weight 10 (g/cm3), pH level 6.0, Innoculum size 5.5974 (CFU/ml), Temperature 34.99 (^{o}C) and Time 95.98 (hours).

Table 4.2: Bioethanol for tigernut waste

	Predicted Value	
	Saccharomyces cerevisiae	***Aspergillus niger isolate HEFAPhR***
Subtrate Weight (g/cm^3)	10.003	10
Ph Level	6.82	6
Innoculum Size (CFU/ml)	5.0003	5.56043
Temperature (^{o}C)	25	34.9
Time (Hr)	93.51	95.9475
Bioethanol	1.64	5.1
Ethanol volume (ml)	0.246715475	0.77186964
% Yield	0.164476983	0.51457976

Table 4.3: Bioethanol for beans husk

	Predicted Value	
	Saccharomyces cerevisiae	***Aspergillus niger isolate HEFAPhR***
Subtrate Weight (g/cm^3)	10.003	10
Ph Level	6	6
Innoculum Size (CFU/ml)	5.0003	5.57
Temperature (^{o}C)	25	34.88
Time (Hr)	48	96
Bioethanol	5.478	2.48
Ethanol volume (ml)	0.829664782	0.37371911
% Yield	0.553109855	0.24914607

Table 4.4: Bioethanol for groundnut shell

	Predicted Value	
	Saccharomyces cerevisiae	***Aspergillus niger isolate HEFAPhR***
Subtrate Weight (g/cm^3)	10.003	10
Ph Level	6	6
Innoculum Size (CFU/ml)	5.512	5.5974
Temperature (^{o}C)	35	34.99
Time (Hr)	70.13	95.98
Bioethanol	3.47	23.43
Ethanol volume (ml)	0.52379994	3.639705882
% Yield	0.34919996	2.426470588

4.2 Discussion

4.2.1 Tigernut Waste with *Aspergillus niger isolate HEFAPhR*

The plots of the observed brix and ethanol as shown in Figures 4.23a-b depicts that the optimized flask was Flask 32 with brix value 8.0^{o}Bx and 4.8g/l ethanol while the contour plots revealed the interactions between the factors that effected the production of bioethanol.

The optimization plot shows that at pH 6, temperature of 34.9^{o}C, fermentation time of 96h (time), inoculum size of 5 and substrate weight of 10g, maximum value of brix was 6.70^{o}Bx with ethanol yield of 5.10g/l, as shown in the Figures 4.24-4.27 above.

Results from the study showed that optimum temperature for alcohol production was 34.9^{o}C. According to Adejoju, Adedara, Ogunsuiyi & Satyarolu (2020), high temperature above 40^{o}C is not favourable for cells growth and it is a stress factor for microorganisms. Ogunsuiyi (2020) also recorded an ethanol concentration of 78.6g/l at 30^{o}C. Ugheoke (2007) and Zhang et al. (2020) also observed a reduction in ethanol yield as temperature increased beyond 35^{o}C. Ogunsuiyi & Badiru (2016) achieved maximum concentration of ethanol yield from tiger nut waste at 40^{o}C which is in conformity with this work. Although the optimum temperature of *Aspergillus niger isolate HEFAPhR* is 30^{o}C, results show that the maximum ethanol concentration was lower compared to the yield at higher temperature. Optimum temperature supports the hydrolysis of starch into monomers which leads to maximum glucose concentration throughout the experiment. Temperature that is too high kills yeast, and low temperature slows down yeast activity. Higher yield of ethanol was recorded at fermentation time of almost 96h when compared with the yield at 120h and above. According to Offoremenike et al. (2020), when the fermentation time becomes too long, it gives toxic effect on microbial growth, especially in batch mode, due to the high concentration of ethanol in the fermented broth.

Fermentation time has an effect on the growth of microorganisms. Brix conversion works with the fermentation time. The result showed that 10g of the substrate gave the highest yield of brix conversion. The lowest yield was found with substrate weight of 20g. This negates the work of Zabed et al. (2017) who stated that high ethanol productivity and yield in batch fermentation can be obtained by using higher initial sugar concentration; the maximum rate of ethanol production was achieved when using sugars at the concentration of 150g/L. However, it needs longer fermentation time and higher recovery cost. High substrate loading for industrial fermentation is feasible and hence always desired (Nya & Etukudo, 2023). Although inoculum size of MacFalands standard 5 gave a higher yield compared with inoculum size of MacFalands standard 7, increase in the inoculum size did not really have a great effect in the yield of brix converted. This result corroborates with the work of Laopaiboon (2023), which reported that inoculum concentration does not give significant effect on the final ethanol concentration but it affects the consumption rate of sugar and ethanol productivity. From the result, highest yield of ethanol was obtained at an acidic pH of 6. This agrees with the works of Candry, Ituang & Carvajal (2020) who reported that when ethanol is continuously produced from the glucose fermenting culture, other acids like carbonic acid and acetic acid are continuously generated making the system more acidic and low pH could trigger the production of ethanol.

4.2.2 Tigernut waste with *Saccharomyces cerevisiae*

The plots of the observed brix and ethanol as shown in fig 4.7a-b depicts that the optimized flask was Flask 8 with brix value 2.28^{o}Bx and 1.6g/l ethanol, while the predicted response surface plots of Figures 4.8-4.11 showed the interactions between the factors that affected the production of bioethanol. At optimum conditions of pH 6.82, temperature 25°C, fermentation time of 93.5h inoculum size 5.6 of 0.5 MacFaland standard and substrate weight 11g; brix value of 0.53°Bx and ethanol content of 1.64g/l were obtained, while actualized bioethanol volume 0.2467(ml). Results show that highest ethanol yield was seen with the inoculum size increased to MacFalands standard 5.59. According to Mohammed, Nafiz, Muhammad, Irfah, Yue, Shagufta, Lijing, (2022), an increase in inoculum size leads to increased biomass concentration and results in increased bioethanol concentration.

4.2.3 Bean Husk with *Aspergillus niger isolate HEFAPhR*

The plots of the observed brix and ethanol as shown in fig 4.26a-b depicts that the optimized flask was Flask 25 with brix value 3.8°Bx and 2.20g/l ethanol while the contour and response surface plots showed the interactions between the factors that affected the production of bioethanol as shown in Figures 4.27-4.30. Optimization plot shows that at pH 6, temperature of 35°C, fermentation time of 96h, inoculum size of 5.57(ml) and substrate weight of 10g, maximum concentration of brix was 2.09°Bx while the ethanol yield predicted was 2.48g/l as shown in the figure 4.15, while the actualized volume is 0.3737(ml).

Results from the study depict that optimum temperature for alcohol production was at 25°C. This is in line with the works of Elaigwu & Oluwatosin (2017) who achieved maximum concentration of ethanol yield from beans at 25°C. The optimum temperature of *Aspergillus niger isolate HEFAPhR* is 30°C (Xiaoyu, Ming, Yuan, Wang & Xiaohong, 2022), the maximum ethanol concentration was lower compared to the yield with Saccharomyces. High temperature supports the hydrolysis of starch into monomers which led to maximum glucose concentration throughout the experiment. Fermentation time has an effect on the growth of microorganisms. Brix conversion works with the fermentation time. Higher yield of ethanol was seen at fermentation time of 96h. Shorter fermentation time causes inefficient fermentation due to inadequate growth of microorganisms (Wesley et al., 2020). The longer the fermentation time, the toxic the microbial growth becomes and the more the potential inhibitory materials. As a result of high concentration of ethanol in the fermented broth, the system may become unfavourable for the organism (Nya et al., 2023). The result also showed that 20g of the substrate gave the highest yield of brix conversion. The lowest yield was found with substrate weight of 10g. This agrees with the work of Laopaiboon et al. (2021) who stated that high ethanol productivity and yield in batch fermentation can be obtained by using higher initial sugar concentration; the maximum rate of ethanol production was achieved when using sugars at the concentration of 150 g/L. High substrate loading for industrial fermentation is feasible and hence always desired (Nya et al., 2023). Although inoculum size of 5 gave a higher yield compared with inoculum size of 7. Increase in the inoculum size did not really have a great effect in the yield of brix converted. This result corroborates with the work of Laopaiboon et al. (2021) who reported that the final ethanol concentration is not significantly affected by the concentration of inoculum, even though it affects the consumption rate of sugar and production of ethanol. From the result, highest yield of ethanol was obtained at an acidic pH of 6. Kida, Dige, Abba, Muhammed, Ibrahim & Musa (2023) stated that a wide range of optimum pH 4.0-5.0 is required for the activity of *Aspergillus niger isolate HEFAPhR*. This agrees with the work of Ganigue et al. (2016) who reported that when ethanol is continuously produced from the glucose fermenting culture, other acids like carbonic acid and acetic acid are continuously generated making the system more acidic and low pH could trigger the production of ethanol.

This work has shown that the importance of optimization cannot be over emphasized. The maximum ethanol yield equals to 66.58% as shown in Table of result comparism in the appendix.

4.2.4 Beans Husk with *Saccharomyces cerevisiae*

The plots of the observed brix and ethanol as shown in Figures 4.13a-b depicts that the optimized flask was Flask 3 with brix value 6.7^{o}Bx and 3.0g/l ethanol, while the predicted result of interactions between factors brix and ethanol as shown in Figures 4.14-4.17 were 2.80^{o}Bx and 5.47g/l. With optimum condition of pH 6, temperature 30^{o}C, fermentation time 48h, inoculum size of MacFalands standard 5.4 and substrate weight of 12.13g/l. Previous of Awolu & Oyeyemi (2015) and Zhang et al. (2020) study, BH was optimized with other agro wastes which showed higher temperature and pH stability and obtained a yield of 0.7706 and 0.5642 respectively at optimum condition of temperature 80^{o}C, fermentation time of 144h and pH 8. This result improves the yield of bioethanol as compared with what Awolu & Oyeyemi (2015) obtained with the actualized ethanol volume 0.8296(ml) and yield of bioethanol equals to 67%.

4.2.5 Groundnut Shell with *Aspergillus niger isolate HEFAPhR*

The plots of the observed brix and ethanol as shown in fig 4.31a-b depicts that the optimized flask was Flask 12 with brix value 9.9^{o}Bx and 5.20g/l ethanol while the contour and response surface plots response surface plots showed the interactions between the factors that effected the production of bioethanol as shown.in Figures 4.32-4.35 with the predicted brix of 10.0^{o}Bx and 23.43g/l ethanol, and actualized volume of 3.6397(ml) with the optimum temperature for ethanol production as seen at 35^{o}C. This conforms to the work of Adejumobi (2020) who achieved maximum concentration of ethanol yield from groundnut shell at 35^{o}C. High temperature supports the hydrolysiof starch into simple sugars which leads to constant availability of glucose concentration throughout the experiment. Higher yield of ethanol was seen at fermentation time of 96h. Shorter fermentation time causes inefficient fermentation due to inadequate growth of microorganisms (Zabed et al., 2017). The longer the fermentation time, the more the toxic inhibitory materials produced. As a result of high concentration of ethanol in the fermented broth, the system may become unfavourable for the organism (Braide et al., 2018). In another study, Batt & Shilpa (2014) reported that the highest ethanol concentration of 40.11g/l was gotten after 96h and this dropped to 27.34g/l after 48h of fermentation with groundnut shell.

The result also showed that 15g of the substrate gave the highest yield of brix conversion of 3 and alcohol volume of 3.36(ml). The lowest yield was found with substrate weight of 10g. This agrees with the work of Offor-emenike et al. (2020) who stated that high ethanol productivity and yield in batch fermentation can be obtained by using higher initial sugar concentration. High substrate loading for industrial fermentation is feasible and hence always desired (Pattanaik et al., 2019). Although inoculum size of 5 gave a higher yield compared with inoculum size of 7. Increase in the inoculum size did not really have a great effect in the yield of brix converted. This result corroborates with the work of Annika, Tejas, Vineetha & Pratima (2017) who reported that the final ethanol concentration is not significantly affected by the concentration of inoculum, even though it affects the consumption rate of sugar and production of ethanol. From the result, highest yield of ethanol was obtained at pH of 6. Ganigue et al. (2016) reported that when ethanol is continuously produced from the glucose fermenting culture, other acids like carbonic acid and acetic acid are continuously generated making the system more acidic and low pH could trigger the production of ethanol.

The actualized higher ethanol volume equals 0.829(ml) from beans husk with *Saccharomyces cerevisiae* compared to the highest volume of 3.6397(ml) of bioethanol from groundnut shell with *Aspergillus niger isolate HEFAPhR*, followed by tiger nut waste ethanol volume of 0.7718(ml) at optimal conditions of pH 6, temperature of 35^{o}C, inoculum size of MacFalands standard 5, substrate weight of 10g and fermentation time of 96h, maximum brix concentration was 6.70^{o}Bx and the bioethanol yield of 66.7% this result proves that better yield of bioethanol can be produced from these agro wastes especially with the novel strain of *Aspergillus niger isolate HEFAPhR* at optimized conditions.

4.2.6 Groundnut Shell with *Saccharomyces cerevisiae*

The plots of the observed brix and ethanol as shown in Figures 4.18a-b depicts that the optimized flask was flask 23 with brix value 4.6^{o}Bx and 2.6g/l ethanol, while the contour and response surface plots which showed the interactions between the factors that affected the production of bioethanol as shown. In Figures 4.19-4.22, the optimized condition of temperature of 35°C with pH 6 and substrate weight of 12.7g, time 70h of ethanol was realised. From the predicted result, 2.84^{o}Bx and 3.47g/l. With optimum condition of pH 6, temperature of 35^{o}C, substrate weight of 20g, inoculum size of MacFalands standard 6 and fermentation time of 72h; bioethanol yield equals 66.67%.

CHAPTER FIVE

CONCLUSION, CONTRIBUTION AND RECOMMENDATIONS

5.1 Conclusion

The study shows that *Saccharomyces cerevisiae* and *Aspergillus niger isolate HEFAPrh* isolated and characterised to molecular level has the potential for ethanol production at optimal conditions. The amiono acid composition of wastes was found to conserve nutrients and mineral elements that could serve as fertilizer to increase soil fertility. The proximate analysis of agricultural waste (tiger nut, beans husk, groundnut shell) indicated that both are better choice in ethanol production as a result of high carbohydrate, crude fibre and moisture content. Furthermore, the treatment of beans husk with *Saccharomyces cerevisiae* yielded higher brix and ethanol at observed, predicted and actualized levels of 66% while the treatment of groundnut shell waste with the novel strain of *Aspergillus niger isolate HEFAPrh* yielded higher brix and ethanol 66.7%. The findings of this work suggest that more ethanol can be produced from the agrowastes especially with the novel strain of *Aspergillus niger isolate HEFAPrh* and serve as a better substitute than synthetic ethanol produced from petrochemical sources because it is pollution free and eco-friendly.

5.2 Contribution to Knowledge

The agricultural waste tiger nut, beans husk, groundnut shell is proven sustainable alternative feedstock for bioethanol production because of its availability, low cost, higher ethanol yields, and efficiency.

Based on the result bioethanol from groundnut shell followed by tiger nut waste with the novel strain of *Aspergillus niger isolate HEFAPrh* and beans husk waste *Saccharomyces cerevisiae* can easily reduce the fossil fuel crisis and high cost.

The use of agricultural waste (tiger nut, beans husk, groundnut shell) contributes on a large scale to the renewable energy mix. Additionally, it can quickly meet the target of 5% blending for transportation fuel.

Agricultural waste used to produce bioethanol in this research enhances the use of biomass resources due to environmental concerns, future economic concerns, and a desire to have a positive impact on the environment with the lowest CO_2 emissions (880-kg CO_2 equivalent per 1000 kg of ethanol).

The hydrolyzed residues from the agricultural waste (TNW, BH, and GS) are effective soil fertilizers for agricultural activities.

Sustainable waste management strategies to ensure sanitation, resource recovery, and maintaining the carbon balance in the environment.

5.3 Recommendations

1. The use of *Saccharomyces cerevisiae* with beans husk waste and *Aspergillus niger isolate HEFAPrh* with groundnut shell waste and tiger nut waste yielded more ethanol and should be used in large scale production.
2. The use of these agro waste (tiger nut waste, beans husk waste and groundnut) should be encouraged to alleviate the problem of waste disposal and environmental pollution.
3. There is need for further studies on mechanism adopt by fermentation organism used in these research.
4. The organisms used in this work should be employs on other types of agro waste for high ethanol yield.
5. Public and Private cooperation will play a vital role in harnessing the use this agricutural waste as a sustainable energy source.

REFERENCES

Abdulrahman, B., Nasirudeen, M.B., Timothy, M.A. (2022). Process Optimization and Characterization of Bioethanol from Yam (*Dioscorea rotundata*) Peels. *International Journal of Scientific and Research Publications,* Volume 12, Issue 5, [ISSN 2250-3153]

Abdulrahman, M. (2022). Production of Bioethanol from Selected Agro Waste using *Saccharomyces cerevisiae* Strain CBS 1171 and *Lactobacillus delbrueckii* Strain MN945906. http://repository.futminna.edu.ng:8080/xmlui/handle/123456789/15031

Abel, R.C., Coney, K., Johnson, C., Thornton, M.J., Zigler, B.T. & Mccormick, R.L. (2023). Global Ethanol-Blended-Fuel Vehicle Compatibility Study. Available online: https://www.nrel.gov/docs/fy22osti/81252.pdf.

Adedara, A.O., Ogunsuyi, H.O. & Akinnawo, C.A. (2020). Evaluation of Tigernut Waste for Production of Bioproducts. www.researchgate.net

Agus, B. A. P., Mohamad, N. N & Hussain, N. (2018). Composition of Unfermented, Unroasted, Roasted Cocoa Beans and Cocoa Shells from Peninsular Malaysia. *Journal of Food Measurement Characteristics*, 12: 2581–2589.

Ajah, C. P., Akujobi, C. and Wesley, B. (2020); Extraction of Bioethanol from Pretreated Sawdust. *International Journal of Advance Research,* **8:** 23-33, 2320-5407. www.journalijar.com

Alexandre, A. (2021). Electric Vehicles Powered by Ethanol. https://www.revistapesquisa.fapesp.br/en/electric-vehicles-powered-by-ethanol/

Alternative Fuels Data Center (2021). Biobutanol, https://afdc.energy.gov/fuels/emerging_biobutanol.html.

Annika, D.G., Tejas, S., Vineetha, N. & Pratima, P. (2017). Extraction of Cellulose and Biofuel Production from Groundnut Shells and its Application to Increase Crop Yield. *World Journal of Pharmacy and Pharmaceutical Sciences*.

Antwi, E., Engler, N., Nelles, M. and Schüch, A. (2019). Anaerobic digestion and the effect of hydrothermal pretreatment on the biogas yield of cocoa pods residues. *Waste Manage,* (Tucson, Ariz.) 88: 131–140.

Apilak, S., Niphaphat, P., Pattana, L. & Lakkana, L. (2023). Mathematical modeling of bioethanol production from sweet sorghum juice under high gravity fermentation: Applicability of Monod-based, logistic, modified Gompertz and Weibull models. *Electronic Journal of Biotechnology,* 64: 18-26.

Arijana, B., Nenad, M., Semjon, K., Galina, M., Halina, B., Mirela, I. Š., Draženka, K., Srđan, N. and Božidar, Š. (2023). Bioethanol Production from Renewable Raw Materials and Its Separation and Purification: A Review.

Association of Analytical Chemists International, AOAC (2019). Official Methods of Analysis, 21st edition. Gaithersburg, MD U.S.A. Official Methods, 01. https://www.aoac.org

Awogbemi, O., Kallon, D.V.V. & Aigbodion, V.S. (2021b). Trends in the development and utilization of agricultural wastes as heterogeneous catalyst for biodiesel production. *J. Energy Inst.,* 98: 244–258.

Awogbemi, O., Kallon, D.V.V. & Owoputi, A.O. (2022a). Biofuel generation from potato peel waste: current state and prospects. *Recycling,* **7(2):** 23.

Awogbemi, O., Kallon, D.V.V., Aigbodion, V.S. & Panda, S. (2021c). Advances in biotechnological applications of waste cooking oil. Case Stud. *Chem. Environ. Eng.* **4**(100158).

Awogbemi, O., Kallon, D.V.V., Onuh, E.I. & Aigbodion, V.S. (2021a). An overview of the classification, production and utilization of biofuels for internal combustion engine applications. *Energies,* **14(18):** 5687.

Awolu, O.O. & Oyeyemi, S.O. (2015). Optimization of Bioethanol production from Cocoa (Theobroma cacao) Bean Shell. *Int. J. Curr. Microbiol. App. Sci.,* **4(4):** 2319-7706.

Baghbanzadeh, M., Savage, J., Balde, H., Sartaj, M., VanderZaag, A.C., Abdehagh, N. & Strehler, B. (2021). Enhancing hydrolysis and bio-methane generation of extruded lignocellulosic wood waste using microbial pre-treatment. *Renew. Energy,* 170: 438–448.

Bajpai, P. (2021). Global Production of Bioethanol. In Developments in Bioethanol. Green Energy and Technology; Springer: Singapore.

Benitez, L.V. (1989). Amino acid and fatty acid profiles in aquaculture nutrition studies, p. 23-35. in SS De Silva (ed.) Fish Nutrition Research in Asia. Proceedings of the Third Asian Fish Nutrition Network, Meeting Asianfish. Society Special Publication. 4: 166 p.

Bhurat, K.S., Banerjee, T., Pandey, J.K. & Bhurat, S.S. (2021). A lab fermenter level study on anaerobic hydrogen fermentation using potato peel waste: effect of pH, temperature, and substrate pre-treatment. *J. Mater. Cycles Waste Manag.,* **23(4):** 1617–1625.

Bomin, C., Shinje, L., Hyunjun, L., Jinwon, L., Hankwon, L. & Wangyun, W. (2021). Integrated strategy for coproducing bioethanol and adipic acid from lignocellulosic biomass. *Journal of Cleaner Production,* 311: 127849.

Botella-Martínez, C., Lucas-Gonzalez, R., Ballester-Costa, C., Pérez-álvarez, J. Á., Fernández-López, J., Delgado-Ospina, J., Chaves-López, C & Viuda-Martos, M (2021). Ghanaian

Cocoa (Theobroma cacao L.) Bean Shells Coproducts: Effect of Particle Size on Chemical Composition, Bioactive Compound Content and Antioxidant Activity. Agronomy.11:401.

Busic, A., Mardetko, N., Kundas, S., Morzak, G., Belskaya, H., Ivanci, Santek, M., Komes, D., Novak, S. & Santek, B. (2018). Bioethanol production from renewable raw materials and its separation and purification: a review. *Food Technol. Biotechnol.*, **56(3):** 289–311.

Cavelius, P., Engelhart-Straub, S., Mehlmer, N., Lercher, J., Awad, D. & Bruck, T. (2023). The potentials of biofuels from first to fourth generation. *PLOS Biol.*, **21(3):** e3002063.

Cheesbrough, M. (2006). *Medical Laboratory Manual.* Tropical health technology, low priced edition. Doddington, Cambridgeshire, England. Pp18-50.

Chilakamarry, C.R.M., Sakinah, A.M., Zularisam, A.W., Sirohi, R., Khilji, I.A., Ahmad, N. & Pandey, A. (2022). Advances in solid-state fermentation for bioconversion of agricultural wastes to value-added products: opportunities and challenges. *Bioresour. Technol.*, 343: 126065.

da Costa Filho, J. D. B., de Araújo Padilha, C. E., Matias, S. C. B., Ribeiro, V. T., dos Santos, E. S., & de Santana Souza, D. F. (2023). Combining glycerol pretreatment with persulfate oxidation to obtain cellulosic ethanol and dye adsorbent from maize cob. Biomass Conversion and Biorefinery, 1-17.

Dahunsi, S. (2019). Mechanical pretreatment of lignocelluloses for enhanced biogas production: methane yield prediction from biomass structural components. *Bioresour. Technol.*, 343: 126065.

Del Río, P.G., Gull, N.P., Rebelo, F., Romaní, A., Garrote, G., Gull, N.B. (2020). A whole-slurry fermentation approach to high-solid loading for bioethanol production from corn stover. *Agronomy,* **10(11):** 1790. *Bioresour. Technol.*, 280: 18–26.

Deshmukh, M.P., Pande, A., Choudhari, V. & Pendse, D.S. (2023). Investigation of bioethanol production from jatropha deoiled cake and its blending effects for environmental sustainability. *Environ Sci Pollut Res Int.*, **30(47):** 103640-103651.

Dhanya, M. (2022). Perspectives of Agro-Waste Biorefineries for Sustainable Biofuels. In: Waste Biorefinery, Zero, Nandabalan, Y.K., Garg, V.K., Labhsetwar, N.K., Singh, A. (Eds.). Springer, Singapore, pp. 207–232.

Dhyani, V. & Bhaskar, T. (2018). A comprehensive review on the pyrolysis of lignocellulosic biomass. Renew. *Energy,* 129: 695–716.

Dong, L., Cao, G., Zhao, L., Liu, B. & Ren, N. (2018). Alkali/urea pretreatment of rice straw at low temperature for enhanced biological hydrogen production. *Bioresour. Technol.,* 267: 71–76.

Eigenfeld, M., Kerpes, R. & Becker, T. (2021). Understanding the impact of industrial stress conditions on replicative aging in *Saccharomyces cerevisiae*. *Front Fungal Biol.,* **2**(17).

Elaigwu, S.E. & Oluwatosin, S.S. (2017). Composting of dry bean (Phaseolus vulgaris) husk: Physicochemical and spectroscopic characterization. *Centrepoint Journal*, **23(2):** 91-106.

Energy Information Administration (2023). *Annual Energy Outlook 2023 (AEO, 2023),* Washington, DC. www.eia.gov

European biogas (2021). Gas for Climate Market State and Trends Report 2021. Source: https://www.europeanbiogas.eu/wp-content/uploads/2021/12/Gas-for-Climate-Market-State-and-Trends-report-2021.pdf. (Accessed 19 May 2022).

Favaro, L., Jansen, T. & van Zyl, W.H. (2019). Exploring Industrial and Natural *Saccharomyces cerevisiae* strains for the bio-based economy from biomas: The case of bioethanol. *Crit Rev. Biotechnology,* **39(6):** 800 – 816.

Fonseca, B.G., Mateo, S., Roberto, I.C., Sanchez, S. & Moya, A.J. (2020). Bioconversion in batch bioreactor of olive-tree pruning biomass optimizing treatments for ethanol production. *Biochemical Engineering Journal,* 164: 107793.

Food and Agriculture Organization of the United Nation, FAO (2018). The Future of Food and Agriculture-Alternative pathways to 2050. Rome.p 224.CC.BY-NC-SA3.0IGO.

Gambo A. & Da'u, A. (2014). Tigernut (Cyperus esculentus): Composition, products, uses and health benefits – a review. Bayero. *Journal of Pure and Applied Sciences,* **7(1):** 56-61.

Gani, M., Abdulkadir, N., Usman, S.B., Maiturare, H.M. & Gabriel, S. (2018). Production of Bioethanol from Sugarcane Bagasse using *Saccharomyces cerevisiae*. *Biotechnology Journal International,* **22(1):** 1-8.

Ganigue, R., Sanchez-paredes, P., Baneras, I. and Colprim, J. (2016). Low fermentation pH is a trigger to alcohol production but a killer to chain elongation. *Front Microbiology,* **7**: 1-11

Ge, S., Yek, P.N.Y., Cheng, Y.W., Xia, C., Wan Mahari, W.A., Liew, R.K., Peng, W., Yuan, T.-Q., Tabatabaei, M., Aghbashlo, M., Sonne, C. & Lam, S.S. (2021). Progress in microwave pyrolysis conversion of agricultural waste to value-added biofuels: a batch to continuous approach. *Renew. Sust. Energy Rev.*, 135: 110148.

Grillo, A., Salvi, L., Coruzzi, P., Salvi, P., & Parati, G. (2019). Sodium intake and hypertension. Nutrients, **11(9)**, 1970.

Gunam, I., Dewi, I., Antara, N., Anggreni, A. & Setiyo, Y. (2021). Bioethanol production from sugarcane bagasse by Saccharomyces cerevisiae ATCC 9763 immobilized in Na-alginate. *IOP Conf. Ser. Earth Environ. Sci.*, 12054.

Guo, H.-N., Wu, S.-B., Tian, Y.-J., Zhang, J. & Liu, H.-T. (2021). Application of machine learning methods for the prediction of organic solid waste treatment and recycling processes: a review. *Bioresource Technology*, 319: 124114.

Halder, P., Azad, K., Shah, S. & Sarker, E. (2019). Prospects and Technological Advancement of Cellulosic Bioethanol Ecofuel Production. In: Azad, K. (Ed.), Advances in Eco-Fuels for a Sustainable Environment. *Elsevier, Duxford,* 211–236.

Henze, N. & Jimenez-Gamero, M.D. (2019). “A new class of tests for multinorality with I.I.D. and garch data based on the empirical moment generating funtiuon,” - TEST: *An Official Journal of the Spanish Society of Statistics and Operations Research*, 28: 499-521.

Hoang, A.T., Sirohi, R., Pandey, A., Nizeti, S., Lam, S.S., Chen, W.-H., Luque, R., Thomas, S., Arıcı, M. & Pham, V.V. (2022). Biofuel production from microalgae: challenges and chances. *Phytochemistry Rev.,* 1–38.

Hoang, T.D. & Nghiem, N. (2021). Recent developments and current status of commercial production of fuel ethanol. *Fermentation,* **7(4):** 314.

Hope, M.-A. (2021). Unicane Industries expands to export ethanol. https://www.businessday.ng

Ibarra-Gonzalez, P. & Rong, B.G. (2019). A review of the current state of biofuels production from lignocellulosic biomass using thermochemical conversion routes. *Chinese Journal of Chemical Engineering,* **27(7):** 1523–1535.

International Energy Agency, IEA (2021). Global biofuel production 2010-2019. https://www.iea.org/data-and-statisti cs/charts/global-biofuel-production-2010-2019-.

International Energy Agency, IEA (2022). Global biofuel production in 2019 and forecast to 2025. https://www.iea.org/data-and-statistics/charts/global-biofuel-production-in-2019-and-forecast-to-2025.

Imran, A., Humiyion, M., Arshad, M.U., Saeed, F., Arshad, M.S., Afzaal, M., Imran, M., Usman, I., Ikram, A., Naeem, U., Hussain, M. & Al Jbawi, E. (2022). Extraction, amino acid estimation, and characterization of bioactive constituents from peanut shell through eco-innovative techniques for food application, International Journal of Food Properties, 25: 1, 2055-2065.

Jafari Naimi, L. (2016) A study of cellulosic biomasssize reduction (T). University of British Columbia.library.ubc.ca/collections/ubctheses/24/items/1.0224813

Jaywant, S. A., Harshpreet, S., and Khalid, M. A. (2022). "Sensors and Instruments for Brix Measurement: A Review" *Sensors,* 22(6): 2290. https://doi.org/10.3390/s22062290

JenniferHolmgren, Lanza Tech. Annual Report (2021). https://lanzatech.com/wp-content/uploads/2022/08/Lanzatech.

Jitendra, K.S., Cheng, D.D., Peeta, R.S., Anil, K.P., Peetu, S., Chiu-wen (2022). Valorization of pineapple leaves waste for the production of bioethanol. *Bioengineering,* **9(10):** 557. www.mdpi.com

John, I., Yaragarla, P. & Appusamy, A. (2020). Production of Bioethanol from Banana Peel Using Isolated Cellulase from Aspergillus Niger. In: Sivasubramanian, V., Subramanian, S. (Eds.), Global Challenges in Energy and Environment. Springer Nature, Singapore, 9–18.

Karthick, C. & Nanthagopal, K. (2021). A comprehensive review on ecological approaches of waste to wealth strategies for production of sustainable biobutanol and its suitability in automotive applications. *Energy Convers. OR Manag.,* 239: 114219.

Kasmuri, N.H., Kamarudin, S.K., Abdullah, S.R.S., Hasan, H.A. & Som, A.M. (2019). Integrated advanced nonlinear neural network-simulink control system for production of bio-methanol from sugar cane bagasse via pyrolysis. *Energy,* 168: 261–272.

Keskin, T., Nalakath Abubackar, H., Arslan, K. & Azbar, N. (2019). Biohydrogen Production from Solid Wastes. In: Pandey, A., Mohan, S.V., Chang, J.S., Hallenbeck, P.C., Larroche, C. (Eds.), Biohydrogen, second ed. *Elsevier, Netherlands*, 321–346.

Ketsub, N., Latif, A., Kent, G., Doherty, W.O.S., O'Hara, I.M., Zhang, Z. & Kaparaju, P. (2021). A systematic evaluation of biomethane production from sugarcane trash pretreated by different methods. *Bioresour. Technol.,* 319: 124137.

Kumari, D., Singh, R., 2018. Pretreatment of lignocellulosic wastes for biofuel production: a critical review. *Renew. Sust. Energy Rev.,* 90: 877–891.

Kumari, S. & Das, D. (2019). Biohythane production from sugarcane bagasse and water hyacinth: a way towards promising green energy production. *J. Clean. Prod.,* 207: 689–701.

Laopaiboon, L. Suporn, S., Klanrit, P. et al. (2021). Novel effective yeast strains and their performance in high gravity ethanol fermentations from sweet sorghum juice. *Energies,* **14(3):** 557.

Liu, Y., Zheng, X., Tao, S., Hu, L., Zhang, X. & Lin, X. (2021). Process optimization for deep eutectic solvent pretreatment and enzymatic hydrolysis of sugar cane bagasse for cellulosic ethanol fermentation. *Renew. Energy,* 177: 259–267.

Mancini, G., Papirio, S., Lens, P. N., & Esposito, G. (2018). Increased biogas production from wheat straw by chemical pretreatments. *Renewable energy,* 119, 608-614.

Market data forecast (2022). Biodiesel Market. https://www.marketdataforecast.com/mar ket-reports/biodiesel-market.

Md-Razali, N.A.A., Ibrahim, M.F., Kamal-Bahrin, E. & Abd-Aziz, S. (2018). Optimisation of simultaneous saccharification and fermentation (SSF) for biobutanol production using pretreated oil palm empty fruit bunch. *Molecules,* **23(8):** 1944.

Miguel-Angel, P., Francisco, M.A., Quetzalcoatl, H.E. & Alberto-Jesus, P.M (2018). Peanut Shell for Energy: Properties and Its Potential to Respect the Environment. *Sustainability,* 10: 3254.

Miksusanti, Panagan, A. T., Xayasene, T., Fatma, Huu Tho, N., & Hermansyah. (2018). Bioethanol Production from Cassava (*Manihot esculenta*) Peel Using Yeast Isolated from Durian (*Durio zhibetinus*) fruit. *Journal of Advance Biotechnology, 15* (2), 12-66.

Montoya-Rosales, J., Peces, M., Gonzolez-Rodríguez, L., Alatriste-Mondragon, F. & Villa-Gomez, D. (2020). A broad overview comparing a fungal, thermal and acid pre-treatment of bean straw in terms of substrate and anaerobic digestion effect. *Biomass Bioenergy*, 142: 105775.

Muhammad, I., Muhammed, N., Hafiz, A.S., Muhammad, K., Irfan, A., Shagufta, S., Yue, C. & Lijing, C. (2022). Bioethanol Production Optimization from KOH-Pretreated *Bombax ceiba using Saccharomyces cerevisiae* through Response Surface Methodology. www.mdpi.com

Mukhtar, A., Saqib, S., Mubashir, M., Ullah, S., Inayat, A., Mahmood, A., Ibrahim, M. & Show, P.L. (2021). Mitigation of CO_2 emissions by transforming to biofuels: optimization of biofuels production processes. *Renew. Sustain. Energy Rev.*, 150: 111487.

Mustapha, H.M., Dahiru, S., Abdulrahman, B., & Abdullahi, I. (2019). Bio-ethanol production from cassava (*Manihot esculenta*) waste peels using acid hydrolysis and fermentation process. *Africa Journal of Microbiology* **14(2)**, 45-50.

Naik, G.P., Poonia, A.K. & Chaudhari, P.K. (2021). Pretreatment of lignocellulosic agricultural waste for delignification, rapid hydrolysis, and enhanced biogas production: a review. J. Indian Chem. Soc. **98 (10):** 100147.

Nata'alal, M.K., Farouq, A.A., Magashi, A.M. & Liman, M.G. (2018). Assessment of the Potentials of Production of Bioethanol with *Cyperus esculentus L.* (Tiger Nut) Residue by Simultaneous Saccharification and Fermentation. *Asian Journal of Biotechnology and Bioresource Technology,* **3(3):** 1-10.

National Center for Biotechnology Information (2022). PubChem Compound Summary for CID 702, Ethanol. Retrieved October 9, 2023 from https://pubchem.ncbi.nlm.nih.gov/compound/Ethanol

Navarro, R.R., Otsuka, Y., Matsuo, K., Sasaki, K., Sasaki, K., Hori, T., Habe, H., Nakamura, M., Nakashimada, Y., Kimbara, K. & Kato, J. (2020). Combined simultaneous enzymatic saccharification and comminution (SESC) and anaerobic digestion for sustainable biomethane generation from wood lignocellulose and the biochemical characterization of residual sludge solid. *Bioresour. Technol.,* 300: 122622.

Nya, E. (2022). Industrial Potentials of Saccharomyces cerevisiae. *British Journal of Multidisplinary and Advanced Studies,* **4(2):** 23-46.

Offor-Emenike, I., Ibekwe, V., Akujobi, C. & Braide, W. (2020). Brix production from cassava peels and Optimization of Bioethanol synthesis using *Saccharomyces cerevisiae. Food and Environmental Safety Journal (S.I),* **19(4):** 2559 – 6381.

Ogunsuyi, H.O. & Adejumobi, I.B. (2020). Production of Bioethanol de from groundnut (*Arachis hypogeae L.*) Shell and Peels. *International Journal of Engineering Applied Sciences and Technology,* **4(11):** 38-44.

Ojobe, T. O., Temple, V. J., Kapu, M. M., (1990). Chemical analysis of tigernut (*Cyperus esculentis*). *Journal of the science of Food and Agriculture,* 50(2), 261-263.https://doi.org/10.1002/jsfa.2740500214

Oladele, A & Aina, J (2007). Chemical composition and functional properties of flour produced from two varieties of tigernut (Cyperus esculentus). African Journal of Biotechnology, 6(21):2473–2476

Olatunji, K.O., Madyira, D.M., Ahmed, N.A. & Ogunkunle, O. (2022). Biomethane production from Arachis hypogea shells: effect of thermal pretreatment on substrate structure and 8yield. *Biomass Convers. Biorefin.,* 1–14.

Olufemi, B. A. and O. Eniodunmo (2018). Adsorption of Nickel (II) Ions from Aqueous Solution using Banana Peel and Coconut Shell. *International Journal of Technology,* vol. 9, no.3, 434 –445, 2018

Onuh, E. I., Inambao, F. & Awogbemi, O. (2021). Performance and emission evaluation of biodiesel derived from waste restaurant oil and *Moringa oleifera*: a comparative study. *Int. J. Ambient Energy,* **42(8):** 912-919.

Ositadinma, I. C., Tagbor, N. J. and Elijah, O. C. (2019). Optimum Process Parameters for Activated Carbon Production from Rice Husk for Phenol Adsorption. *Current Journal of Applied Science and Technology,* **36(6):** 1–11, 2019

Pan, S., Chi, Y., Zhou, L., Li, Z., Du, L. & Wei, Y. (2020). Evaluation of squeezing pretreatment for improving methane production from fresh banana pseudo-stems. *Waste Manage.*, (Tucson, Ariz.) 102: 900–908.

Pattanaik, L., Pattnaik, F., Saxena, D.K. & Naik, S.N. (2019). Biofuels from Agricultural Wastes. In: Basile, A., Dalena, F. (Eds.), Second and Third Generation of Feedstocks. *Elsevier, Singapore,* 103–142.

Precedence Research (2022). Ethanol Market. https://www.precedenceresearch.com/eth anol-market.

Priyanka, M., Kumari, D., Shankar, U., Yadav, A., Yadav, K. & Priyanka, P. (2019). Agricultural Waste Management for Bioethanol Production. 10.4018/978-1-5225-3540-9.ch001.

Pryshliak, N. & Tokarchuk, D. (2020). Socio-economic and environmental benefits of biofuel production development from agricultural waste in Ukraine. *Environ. Socio-Econ. For. Stud.*, **8(1):** 18–27.

Research and markets (2021). https://www.researchandmarkets.com/reports/4515064/ bio-butanol-market-growth-trends-covid-19.

Rojo-Poveda, O., Barbosa-Pereira, L., Mateus-Reguengo, L., Bertolino, M., Stévigny, C., Zeppa G (2019). Effects of Particle Size and Extraction Methods on Cocoa Bean Shell Functional Beverage. Nutrients, 11:867.

Rose, P.K. (2022). Bioconversion of Agricultural Residue into Biofuel and High-Value Biochemicals: Recent Advancement. In: Nandabalan, Y.K., Garg, V.K., Labhsetwar, N.K., Singh, A. (Eds.), Zero Waste Biorefinery. Springer, Singapore, 233–268.

Schewende, I. & Tuan, D. (2013). Pattern recognition and probabilistic measures in alignment free sequence analysis. *Pubmed.*

Schewende, I., & Tuan, D. Pham, (2013). Pattern recognition and probabilistic measures in alignment free sequence analysis pubmed Doi: 101093/bib/bbt070.

Senthil, P.S. (2018) Identication of fungi www.slideshare.net

Shafiei Alavijeh, R., Karimi, K. & van den Berg, C. (2020). An integrated and optimized process for cleaner production of ethanol and biodiesel from corn stover by Mucor indicus. *J. Clean. Prod.,* 249: 119321.

Shanmugam, S., Hari, A., Ulaganathan, P., Yang, F., Krishnaswamy, S. & Wu, Y.-R. (2018). Potential of biohydrogen generation using the delignified lignocellulosic biomass by a newly identified thermostable laccase from Trichoderma asperellum strain BPLMBT1. *Int. J. Hydrogen Energy,* **43(7):** 3618–3628.

Shanmugam, S., Sun, C., Chen, Z., Wu, Y.-R. (2019). Enhanced bioconversion of hemicellulosic biomass by microbial consortium for biobutanol production with bioaugmentation strategy. *Bioresour. Technol.,* 279: 149–155.

Sharma, B. & Larroche, C. (2020). Comprehensive assessment of 2G bioethanol production. Bioresource Technology, 1-9.

Sharma, H.B. & Dubey, B.K. (2020). Co-hydrothermal carbonization of food waste with yard waste for solid biofuel production: hydrochar characterization and its pelletization. Waste Manage. (Tucson, Ariz.) 118: 521–533.

Simpfendorfer, C.A., Kyne, P.M., Noble, T.H., Goldsbury, J., Basiita, R.K., Lindsay, R., … & Jerry, D.R. (2016). Environmental DNA detects critically endangered largetooth sawfish in the wild. *Endangered Species Research, 30,* 109-116.

Singh, A., Sevda, S., Abu Reesh, I.M., Vanbroekhoven, K., Rathore, D. & Pant, D. (2015). Biohydrogen production from lignocellulosic biomass: technology and sustainability. *Energies,* **8(11):** 13062–13080.

Singh, S. (2018). Food Processing: need and effect on nutrient content. *International Journal of Agricultural Invention,* 3: 185-190. 10.46492/IJAI/2018.3.2.16.

Song, Y., Peng, R., Hensley, D.K., Bonnesen, P.V., Liang, L., Wu, Z., Harry, M., Chi, M., Cheng, S., Bobby, G. & Rondinone, A.J. (2016). High-selectivity Electrochemical Conversion of CO_2 to Ethanol using a Copper Nanoparticle/N-Doped Graphene Electrode. *Chemistry Select,* 1: 6055-6061.

Statista (2020). Annual global CO_2 emissions from 2000 to 2020. https://www.statista.com/statistics/276629/global-co2-emissions/.

Statista (2021a). Global hydrogen market value 2017 and 2023. https://www.statista.com/statistics/933570/global-market-value-hydrogen/.

Statista (2021b). Global hydrogen production outlook by type 2015-2050. https://www.statista.com/statistics/859104/hydrogen-production-outlook-worldwide-by-type/.

Statista (2022a). Global biofuel production 2000-2020. https://www.statista.com/statistics/274163/global-biofuel-production-in-oil-equivalent.

Statista (2022b). Global biofuels market size 2020-2030. https://www.statista.com/statistics/217179/global-biofuels-market-size/#.

Statista (2022c). Global biomethane production 2022 by region and potential. https://www.statista.com/statistics/1296541/global-biomethane-production-and-potential-production/.

Statista (2022d). Global CO_2 emissions 2018-2050. https://www.statista.com/statistics/263980/forecast-of-global-carbon-dioxide-emissions/.

Statista (2022e). Global energy consumption by energy source 1990-2050. https://www.statista.com/statistics/222066/projected-global-energy-consumption-by-source/.

Statista (2022f). Global fuel ethanol production 2016-2021. https://www.statista.com/statistics/274142/global-ethanol-production-since-2000/.

Statista (2022g). Research institute of Sweden. Global n-Butanol market volume 2015-2029. https://www.statista.com/statistics/1245211/n-butanol-market-volume-worldwide/.

Statista (2022h). Global waste management market value 2020-2030. https://www.statista.com/statistics/246178/projected-global-waste-management-market-size/.

Stephen, O. (2020). Explainer: NNPC has aimed ethanol how big is the market? https://www.businessday.ng.

Thakur, A. K., Kaviti, A. K., Mehra, R., & Mer, K. K. S. (2017). Progress in performance analysis of ethanol-gasoline blends on SI engine. *Renewable and Sustainable Energy Reviews*, *69*, 324-340.

Tibarewala, D.N., Bhattacharya, D.K., Das, A. & Das, S. (2020). A new graph-theoretic approach to determine the similarity of genome sequences based on nucleotide triplets Genomics, 112(6).

Tse, T.J., Wiens, D.J. & Reaney, M.J.T. (2021) Production of Bioethanol—A Review of Factors Affecting Ethanol Yield. Fermentation, 7: 268.

Uzoejinwa, B.B., He, X., Wang, S., El-Fatah Abomohra, A., Hu, Y. & Wang, Q. (2018). Co-pyrolysis of biomass and waste plastics as a thermochemical conversion technology for highgrade biofuel production: recent progress and future directions elsewhere worldwide. *Energy Convers. OR Manag.,* 163: 468–492.

Vojvodić, A., Komes, D., Vovk, I., Belščak-Cvitanović, A & Bušić, A (2016). Compositional Evaluation of Selected Agro-Industrial Wastes as Valuable Sources for the Recovery of Complex Carbohydrates. Food Research International, 89:565–573.

Wang, W., Tan, X., Imtiaz, M., Wang, Q., Miao, C., Yuan, Z. & Zhuang, X. (2021). Rice straw pretreatment with KOH/urea for enhancing sugar yield and ethanol production at low temperature. *Ind. Crop. Prod.,* 170: 113776.

Wang, Z., He, X., Yan, L., Wang, J., Hu, X., Sun, Q. & Zhang, H. (2020). Enhancing enzymatic hydrolysis of corn stover by twin-screw extrusion pretreatment. *Ind. Crop. Prod.,* 143: 111960.

Wayah, S. B., & Shehu, S. (2013). Assessment of the nutritional and antinutritional components of tigernut residues. *International Journal of Science and Research*, **4(6):** 342-344.

Weiwen, W., James, B., Thomas, W., Bryan, T., Russ Corbett-Detig, Yatish, T., Robert, L. & Bui, Q.M. (2022). DecentTree: Scalable Neighbour-Joining for the Genomic Era. www.biorxiv.org

Wesley, B., Kanu, I. A., Oranusi, U. S. & Adeleye, S. A. (2016). Production of bioethanol from agricultural waste. *Journal of Fundamental and Applied Science*, **8(2):** 372-386.

Wesley, B., Oji, I. O., Adeleye, S. A. & Korie, M. C. (2018). Comparative study of Bioethanol production from Agricultural wastes by *Zymomonas mobilis* and *Saccharomyces cerevisiae*. *International Journal of Applied Microbiology and Biotechnology*, **6**:50-60.

World Energy Outlook (2019). Analysis – IEA. https://www.iea.org/reports/world-energy-outlook-2019.

Worldometers (2022). World Population Projections. https://www.worldometers.info/wo rld-population/world-population-projections/

Xiaoyu, M., Ming, G., Yuan, L., Qunhui, W. & Xiaohong, S. (2022). Bioethanol through fermentation of spent mushroom substance: Glucose inhibition and elimination approaches. www.sciencedirect.com

Xue, W., Jingying, L., Yan, Z., Dong, T., Yan, L., Li, Z., Mei, H., Jinguang, H. & Fei, S. (2023). Conversion of agricultural and forestry biomass into bioethanol, water-soluble polysaccharides, and lignin nanoparticles by an integrated phosphoric acid plus hydrogen peroxide process. *Industrial Crops and Products*, Volume 191, Part B, 115969, ISSN 0926-6690.

Zabed, H., Sahu, J., Suely, A., Boyce, A. & Faruq, G. (2017). Bioethanol production from renewable sources: current perspective and technological progress. *Renew. Sustain. Energy Rev.*, 71: 475-501.

Zhang, H., Han, L. & Dong, H. (2021). An insight to pretreatment, enzyme adsorption and enzymatic hydrolysis of lignocellulosic biomass: experimental and modeling studies. *Renew. Sustain. Energy Rev.*, 140: 110758.

Zhang, J., Liu, J., Kou, L., Zhang, X. & Tan, T. (2019). Bioethanol production from cellulose obtained from the catalytic hydro-deoxygenation (lignin-first refined to aviation fuel) of apple wood. *Fuel*, 250: 245–253.

Zhang, J., Wang, Y., Gou, Q., Zhou, W., Liu, Y., Xu, J., Liu, Y., Zhou, W. & Gong, Z. (2022a). Consolidated bioprocessing of cassava starch into microbial lipid for biodiesel production by the amylolytic yeast Lipomyces starkeyi. *Ind. Crop. Prod.*, 177: 114534.

Zhang, Y., Yuan, J. & Guo, L. (2020). Enhanced bio-hydrogen production from cornstalk ydrolysate pretreated by alkaline-enzymolysis with orthogonal design method. *Int. J. Hydrogen Energy*, **45(6):** 3750–3759.

Zhang, Z., Jiang, D., Zhang, H., Zhang, H. & Zhang, Q. (2022b). Integrated Technologies for Biohydrogen Production. In: Varjani, S., Pandey, A., Bhaskar, T., Mohan, S.V., Tsang, D.C.W. (Eds.), Biomass, Biofuels, Biochemicals. Elsevier, The Netherlands, 141–159.

Zhou, Q., Le, Q.V., Yang, H., Gu, H., Yang, Y., Sonne, C., Tabatabaei, M., Lam, S.S., Li, C. & Chen, X. (2022). Sustainable conversion of agricultural biomass into renewable energy products: a discussion. *Bioresources,* **17(2):** 1–20.

Ziaei-Rad, Z., Fooladi, J., Pazouki, M. & Gummadi, S.N. (2021). Lignocellulosic biomass pre-treatment using low-cost ionic liquid for bioethanol production: an economically viable method for wheat straw fractionation. *Biomass Bioenergy*, 151: 106140.

APPENDICES

Determination of Chemical Composition of Tiger Nut Waste (TNW), Beans Husk Waste (BHW), Groundnut Shell Waste (GSW)

Sample	Crude Protein	Fat	Ash	Crude Fibre	Moisture	NFE
GSW	16.85 ± 17.13	3.85 ± 3.71	6.8 ± 6.71	30.28 ± 29.76	3.16 ± 3.35	39.19
TNW	10.5 ± 0.11	2.79 ± 1.17	4.64 ± 1.94	20.205 ± 2.605	4.065	54.75
BHW	10.635 ± 0.025	1.585 ± 0.035	6.56 ± 0.02	17.545 ± 0.055	2.91 ± 0.09	60.75

NFE = 100 - (Crude Protein + Fat + Ash + Crude fibre + Moisture) Dr. T. O. Ojobe (Retired) Zoology Dept., UniJos

100% -

Applied Biosystems PTH Amino Acid Analyzer Model 120A

SAMPLE: Tiger Nut Waste

AMINO ACID ANALYSIS

%N (Fat Free) = 1.96

Wt. of sample hydrolysed = 1.020g　　　　Volume loaded: 60 microlitre

Dilution = × 5　　　　C = 0.009880495

Concentration (g/100g protein) = NH × Width @ NH × Sstd × C

Where;

$Sstd = N\ E_{std} \times \text{Mol. Weight} \times \mu\ AA_{std}$

$$C = \frac{Dilution \times 16}{Sample\ Wt\ (g) \times N\% \times Vol. Loaded} \div NH \times W\ (nleu)$$

Amino Acid Analysis of Tigernut Waste

1	2	3	4	5	6
Amino Acid	**Net Height (mm)**	**NH/2 (mm)**	**Width @ NH/2 (mm)**	**Sstd**	**Concentration: g/100g Protein**
Leucine	116	58.00	1.00	5.91	6.77
Lysine	58	29.00	1.00	5.37	3.08
Isoleucine	44.5	22.25	1.00	6.63	2.92
Phenylalanine	20.5	10.25	1.00	17.96	3.64
Norleucine	67.5	33.75	1.00		INTERNAL STANDARD
Trytophan	14	7.00	1.00	5.32	0.74
Valine	55	27.50	1.00	5.92	3.22
Methionine	22.5	11.25	1.00	5.41	1.20
Proline	15	7.50	1.00	20.56	3.05
Arginine	24	12.00	1.00	17.42	4.13
Tyrosine	9	4.50	1.00	34.84	3.10
Histidine	29.5	14.75	1.00	6.47	1.89
Cystine	7	3.50	1.00	12.26	0.85
Alanine	41.5	20.75	1.00	7.68	3.15
Glutamic acid	61	30.50	1.00	15.33	9.24
Glycine	65	32.50	1.00	4.81	3.09
Threonine	51.5	25.75	1.00	5.62	2.86
Serine	59	29.50	1.00	5.47	3.19
Aspartic acid	123.5	61.75	1.00	6.28	7.66

Applied Biosystems PTH Amino Acid Analyzer Model 120A

SAMPLE: Beans Husk

AMINO ACID ANALYSIS

%N (Fat Free) = 3.59

Wt. of sample hydrolysed = 0.557g Volume loaded: 60 microlitre

Dilution = × 5 C = 0.00987837

Concentration (g/100g protein) = NH × Width @ NH × Sstd × C

Where;

Sstd = N E_{std} × Mol. Weight × µ AA_{std}

$$C = \frac{Dilution \times 16}{Sample\ Wt\ (g) \times N\% \times Vol. Loaded} \div NH \times W\ (nleu)$$

Amino Acid Analysis of Beans Husk

1	**2**	**3**	**4**	**5**	**6 (2 × 4 × 5 × C)**
Amino Acid	**Net Height (mm)**	**NH/2 (mm)**	**Width @ NH/2 (mm)**	**Sstd**	**Concentration: g/100g Protein**
Leucine	139.5	69.75	1.00	5.91	8.14
Lysine	101	50.50	1.00	5.37	5.36
Isoleucine	63.5	31.75	1.00	6.63	4.16
Phenylalanine	29	14.50	1.00	17.96	5.15
Norleucine	67.5	33.75	1.00		INTERNAL STANDARD
Trytophan	22.5	11.25	1.00	5.32	1.18
Valine	74.5	37.25	1.00	5.92	4.36
Methionine	30.5	15.25	1.00	5.41	1.63
Proline	17	8.50	1.00	20.56	3.45
Arginine	34	17.00	1.00	17.42	5.85
Tyrosine	120.5	60.25	1.00	34.84	3.61
Histidine	38	19.00	1.00	6.47	2.43
Cystine	10.5	50.25	1.00	12.26	1.27
Alanine	58	29.00	1.00	7.68	4.40
Glutamic acid	89	44.50	1.00	15.33	13.48
Glycine	94	47.00	1.00	4.81	4.47
Threonine	65	32.50	1.00	5.62	3.61
Serine	80.5	40.25	1.00	5.47	4.35
Aspartic acid	149	74.50	1.00	6.28	9.24

Applied Biosystems PTH Amino Acid Analyzer Model 120A

SAMPLE: Groundnut Shell

AMINO ACID ANALYSIS

MODEL: 120A	%N (Fat Free) = 2.74
Wt. of sample hydrolysed = 0.730g	Volume loaded: 60 microlitre
Dilution = × 5	C = 0.009875555

Concentration (g/100g protein) = NH × Width @ NH × Sstd × C

Where;

Sstd = N E_{std} × Mol. Weight × μ AA_{std}

$$C = \frac{Dilution \times 16}{Sample\ Wt\ (g) \times N\% \times Vol.Loaded} \div NH \times W\ (nleu)$$

Amino Acid Analysis of Groundnut Shell

1	2	3	4	5	6 (2 × 4 × 5 × C)
Amino Acid	**Net Height (mm)**	**NH/2 (mm)**	**Width @ NH/2 (mm)**	**Sstd**	**Concentration: g/100g Protein**
Leucine	146.5	73.25	1.00	5.91	8.55
Lysine	108.5	54.25	1.00	5.37	5.75
Isoleucine	71.5	35.75	1.00	6.63	4.68
Phenylalanine	30	15.00	1.00	17.96	5.32
Norleucine	67.5	33.75	1.00		INTERNAL STANDARD
Trytophan	33.5	16.75	1.00	5.32	1.76
Valine	77	38.50	1.00	5.92	4.50
Methionine	38.5	19.25	1.00	5.41	2.06
Proline	1.5	0.75	1.00	20.56	3.96
Arginine	38.5	19.25	1.00	17.42	6.62
Tyrosine	11	5.50	1.00	34.84	3.78
Histidine	45.5	22.75	1.00	6.47	2.91
Cystine	17	8.50	1.00	12.26	2.06
Alanine	62	31.00	1.00	7.68	4.70
Glutamic acid	90.5	45.25	1.00	15.33	13.70
Glycine	103	51.50	1.00	4.81	4.89
Threonine	72	36.00	1.00	5.62	4.00
Serine	83.5	41.75	1.00	5.47	4.51
Aspartic acid	156.5	78.25	1.00	6.28	9.71

Tigernut Waste (TNW) with *Saccharomyces cerevisiae*

R/N S/N	Subs Wt	Temp ^{O}C	Brix Before	Bioethanol Inno. (g/l)	Specific Gravity	Brix After	Bioethanol 48h	Specific Gravity	Brix After	Bioethanol 72h	Specific Gravity	Brix After	Bioethanol 96h	Specific Gravity
1	15	30	3.8	2.0	0.9963	2.8	2.4	0.9956	2.0	3.0	0.9945	1.6	3.4	0.9938
2	15	35	5.0	2.5	0.9954	3.6	3.0	0.9945	2.8	3.4	0.9938	2.2	3.8	0.9932
3	15	25	4.2	2.2	0.9959	4.2	2.2	0.9959	4.2	2.2	0.9959	4.0	2.6	0.9952
4	15	30	4.0	2.0	0.9981	2.8	2.8	0.9948	2.4	3.6	0.9932	1.8	3.8	0.9932
5	15	35	3.7	1.8	0.9966	3.0	2.2	0.9959	2.0	3.0	0.9945	2.0	3.4	0.9938
6	10	30	3.0	1.5	0.9972	2.8	1.8	0.9966	2.6	2.0	0.9963	2.2	2.4	0.9956
7	15	25	3.4	1.7	0.9968	3.4	1.7	0.9968	3.4	1.8	0.9966	2.8	2.2	0.9959
8	20	30	3.8	1.9	0.9964	3.0	2.4	0.9956	2.6	3.0	0.9945	2.0	3.6	0.9934
9	15	35	4.2	2.2	0.9959	3.4	2.8	0.9948	2.2	3.4	0.9938	1.6	3.4	0.9938
10	15	30	3.0	1.5	0.9972	2.2	1.8	0.9932	1.6	2.6	0.9932	1.0	3.2	0.9942
11	15	25	4.0	2.0	0.9963	4.0	2.0	0.9963	3.4	2.2	0.9959	3.6	2.6	0.9952
12	15	35	3.2	1.6	0.9971	2.6	2.0	0.9963	2.2	3.0	0.9945	1.6	3.4	0.9938
13	20	25	4.6	2.3	0.9957	4.6	2.3	0.9957	4.6	2.4	0.9956	4.0	2.6	0.9952
14	15	30	4.2	2.4	0.9956	3.4	2.6	0.9953	2.8	3.2	0.9942	2.0	4.0	0.9928

15	20	35	4.0	2.0	0.9963	3.2	2.4	0.9956	2.4	3.0	0.9945	2.0	3.6	0.9932
16	15	30	3.6	1.8	0.9966	3.0	2.2	0.9959	2.6	2.8	0.9948	1.8	3.2	0.9932
17	15	30	2.8	1.3	0.9976	2.0	1.6	0.9971	1.8	2.4	0.9956	1.2	3.0	0.9945
18	15	30	4.0	2.0	0.9963	3.0	1.8	0.9966	2.2	2.6	0.9953	1.6	3.0	0.9945
19	15	25	4.6	2.3	0.9956	4.6	2.3	0.9957	3.8	2.5	0.9954	3.8	2.6	0.9952
20	15	30	4.4	2.4	0.9956	3.8	2.4	0.9956	2.6	3.4	0.9988	1.8	3.8	0.9932
21	10	30	3.5	1.8	0.9966	2.8	2.6	0.9952	2.2	3.0	0.9945	1.6	3.4	0.9938
22	10	30	4.4	2.2	0.9959	3.2	2.8	0.9948	2.4	3.0	0.9945	1.8	3.4	0.9938
23	10	25	4.8	2.4	0.9956	4.8	2.4	0.9956	4.8	2.4	0.9956	4.6	2.6	0.9952
24	15	30	3.7	1.8	0.9966	2.8	2.2	0.9942	2.2	2.8	0.9948	1.6	3.2	0.9932
25	15	25	4.0	2.0	0.9963	4.0	2.0	0.9963	4.0	2.0	0.9963	3.6	2.2	0.9959
26	15	30	3.8	1.9	0.9964	3.0	2.4	0.9956	2.6	2.8	0.9948	2.0	3.2	0.9942
27	15	30	2.2	1.0	0.9981	2.6	1.8	0.9966	2.8	2.0	0.9963	3.0	2.6	0.9952
28	15	30	3.6	1.8	0.9966	2.8	2.6	0.9952	2.0	3.0	0.9945	1.2	3.6	0.9932
29	15	30	5.0	2.5	0.9954	3.0	3.0	0.9945	2.2	3.6	0.9934	1.6	4.0	0.9928
30	15	30	4.4	2.2	0.9959	3.2	2.8	0.9948	2.4	3.2	0.9942	1.8	3.6	0.9934
31	20	30	3.8	1.9	0.9964	2.8	2.6	0.9952	2.0	3.0	0.9945	1.4	3.4	0.9938
32	20	30	5.0	2.5	0.9957	3.6	3.0	0.9942	2.8	3.6	0.9934	2.0	4.0	0.9928

33	10	30	4.6	2.3	0.9959	3.4	3.2	0.9948	2.6	3.6	0.9938	1.8	4.0	0.9948
34	15	30	4.0	2.2	0.9954	3.0	2.8	0.9945	2.2	3.4	0.9942	1.6	3.8	0.9924
35	15	30	5.0	2.5	0.9956	3.8	3.0	0.9948	3.0	3.2	0.9938	2.0	4.2	0.9928
36	20	30	4.8	2.4	0.9964	4.0	2.8	0.9956	3.2	3.4	0.9928	2.2	4.0	0.9934
37	10	30	3.8	1.9	0.9959	2.8	2.4	0.9948	2.2	2.8	0.9938	1.6	3.6	0.9932
38	15	35	4.2	2.2	0.9966	3.0	2.8	0.9956	2.0	3.4	0.9948	1.2	3.8	0.9934
39	15	30	3.6	1.8	0.9963	2.6	2.4	0.9945	2.0	2.8	0.9942	1.4	3.6	0.9934
40	15	35	4.4	2.0	0.9956	3.6	3.0	0.9934	3.0	3.2	0.9948	2.2	3.6	0.9938
41	15	30	4.8	2.4	0.9954	3.0	2.6	0.9948	2.2	2.8	0.9938	1.6	3.4	0.9924
42	10	35	5.0	2.5	0.9954	3.4	2.8	0.9945	2.6	3.4	0.9934	1.8	4.2	0.9922
43	20	30	5.0	2.5	0.9954	3.6	3.0	0.9945	2.8	3.6	0.9934	2.0	4.4	0.9922
44	20	30	4.4	2.3	0.9957	3.2	2.8	0.9948	2.4	3.2	0.9942	1.6	3.8	0.9932
45	10	30	4.0	2.0	0.9963	3.0	2.6	0.9952	2.0	3.0	0.9945	1.4	3.6	0.9934
46	15	25	3.0	1.5	0.9972	3.0	1.5	0.9972	2.8	1.6	0.9971	2.8	1.6	0.9971

Beans Husk (BH) with *Saccharomyces cerevisiae*

R/N S/N	Subs Wt	Temp ^{O}C	Brix Before	Bioethanol Inno. (g/l)	Specific Gravity	Brix After	Bioethanol 48h	Specific Gravity	Brix After	Bioethanol 72h	Specific Gravity	Brix After	Bioethanol 96h	Specific Gravity
1	15	30	8.1	4.2	0.9924	7.0	4.0	0.9928	6.0	4.3	0.9923	4.0	4.6	0.9918
2	15	35	8.0	4.0	0.9928	7.4	4.2	0.9924	6.6	4.5	0.9919	5.0	4.8	0.9914
3	15	25	7.0	3.5	0.9936	7.0	3.5	0.9936	6.8	3.6	0.9934	6.6	3.8	0.9932
4	15	30	6.3	3.2	0.9941	5.8	3.4	0.9938	5.0	3.6	0.9934	4.0	3.9	0.993
5	15	35	9.2	4.6	0.9918	8.4	4.8	0.9914	7.4	5.0	0.9911	5.4	5.8	0.9898
6	10	30	8.0	4.0	0.9928	7.0	4.2	0.9924	6.6	4.4	0.9922	5.2	4.9	0.9913
7	15	25	6.5	3.7	0.9933	6.5	3.7	0.9933	6.2	3.8	0.9932	6.0	3.8	0.9932
8	20	30	10.1	5.0	0.9911	9.1	5.3	0.9906	7.9	5.6	0.9902	6.4	6.0	0.9895
9	15	35	7.9	4.0	0.9928	7.0	4.2	0.9924	6.0	4.6	0.9918	4.8	5.0	0.9911
10	15	30	6.2	3.6	0.9934	5.8	3.8	0.9932	5.0	4.4	0.9922	3.8	4.8	0.9914
11	15	25	7.6	3.7	0.9933	7.6	3.7	0.9933	7.4	3.8	0.9932	7.0	4.0	0.9928
12	15	35	8.0	4.0	0.9928	7.2	4.4	0.9922	6.4	4.6	0.9918	4.8	5.0	0.9911
13	20	25	10.0	5.1	0.991	10.0	5.1	0.991	9.8	4.9	0.9913	9.6	5.0	0.9911
14	15	30	8.0	4.0	0.9928	7.0	4.2	0.9924	7.0	4.5	0.9919	6.2	5.0	0.9911

15	20	35	9.8	4.9	0.9913	8.6	5.4	0.9905	7.8	5.6	0.9902	6.8	5.8	0.9898
16	15	30	7.2	3.6	0.9934	7.8	3.8	0.9932	6.6	4.2	0.9924	6.0	4.6	0.9918
17	15	30	6.0	3.1	0.9944	5.4	3.4	0.9938	5.0	3.8	0.9932	4.0	4.2	0.9924
18	15	30	7.4	3.8	0.9932	6.6	4.0	0.9928	6.0	4.2	0.9924	5.0	4.6	0.9918
19	15	25	8.5	4.3	0.9923	8.5	4.3	0.9923	8.0	4.5	0.9919	7.8	4.6	0.9918
20	15	30	8.0	4.1	0.9929	7.4	4.3	0.9923	6.8	4.5	0.9919	6.0	4.8	0.9914
21	10	30	9.8	4.9	0.9913	8.9	5.2	0.9908	8.0	5.4	0.9905	6.8	6.0	0.9895
22	10	30	8.0	4.0	0.9928	7.0	3.5	0.9936	6.4	3.8	0.9932	5.4	4.6	0.9918
23	10	25	8.0	4.0	0.9928	8.0	4.0	0.9928	7.8	4.2	0.9924	7.6	4.4	0.9922
24	15	30	7.6	3.6	0.9934	7.0	3.8	0.9932	6.6	4.4	0.9922	5.4	5.0	0.9911
25	15	25	8.4	4.1	0.9929	8.4	4.1	0.9929	8.0	4.2	0.9924	8.0	4.2	0.9924
26	15	30	6.8	3.7	0.9933	6.4	3.9	0.993	5.8	4.2	0.9924	4.9	4.6	0.9918
27	15	30	8.4	4.2	0.9924	8.0	4.5	0.9919	7.0	4.7	0.9916	5.8	5.2	0.9908
28	15	30	7.2	3.6	0.9934	6.8	3.8	0.9932	6.0	4.0	0.9928	5.0	4.4	0.9922
29	15	30	8.0	4.0	0.9928	7.0	4.2	0.9924	5.6	4.4	0.9922	4.7	4.8	0.9914
30	15	30	8.5	4.3	0.9923	7.9	4.5	0.9919	7.0	4.7	0.9916	6.0	5.0	0.9911
31	20	30	10.0	5.1	0.991	9.4	5.4	0.9905	8.0	5.6	0.9902	7.0	6.0	0.9896
32	20	30	8.8	4.4	0.9922	8.2	4.6	0.9918	7.2	4.8	0.9914	6.8	5.0	0.9911

33	10	30	6.8	3.1	0.9944	7.4	3.3	0.994	7.2	3.5	0.9936	6.8	3.8	0.9932
34	15	30	7.5	3.6	0.9934	7.0	3.8	0.9932	6.0	4.0	0.9928	5.4	4.6	0.9918
35	15	30	10.0	5.0	0.9911	9.1	5.2	0.9908	8.1	5.3	0.9906	6.9	5.6	0.9902
36	20	30	8.0	4.0	0.9928	7.2	4.2	0.9924	6.0	4.4	0.9922	5.2	4.8	0.9914
37	10	30	7.8	4.0	0.9928	7.0	4.2	0.9924	6.0	4.6	0.9918	5.0	5.0	0.9911
38	15	35	7.0	3.5	0.9936	6.4	3.8	0.9932	5.6	4.2	0.9924	4.6	4.8	0.9914
39	15	30	7.9	3.9	0.993	6.9	4.3	0.9923	6.0	4.5	0.9919	4.8	5.0	0.9911
40	15	35	8.9	4.4	0.9922	8.0	4.6	0.9918	7.0	4.8	0.9914	5.9	5.2	0.9908
41	15	30	7.0	3.5	0.9936	6.4	3.8	0.9932	5.6	4.2	0.9924	4.8	4.6	0.9918
42	10	35	8.4	4.2	0.9924	8.0	4.4	0.9922	6.8	4.7	0.9916	5.6	5.0	0.9911
43	20	30	10.3	5.0	0.9911	8.9	5.3	0.9906	7.4	5.5	0.9904	6.6	6.0	0.9896
44	20	30	7.4	3.5	0.9936	6.8	3.8	0.9932	5.6	4.2	0.9924	4.4	4.8	0.9914
45	10	30	8.2	4.0	0.9928	7.6	4.2	0.9924	6.8	4.6	0.9918	5.5	5.0	0.9911
46	15	25	7.1	3.6	0.9934	7.0	3.6	0.9934	6.8	3.6	0.9934	6.6	3.8	0.9932

Groundnut Shell (GS) with *Saccharomyces cerevisiae*

R/N S/N	**Subs Wt**	**Temp ^{O}C**	**Brix Before**	**Bioethanol Inno. (g/l)**	**Specific Gravity**	**Brix After**	**Bioethanol 48h**	**Specific Gravity**	**Brix After**	**Bioethanol 72h**	**Specific Gravity**	**Brix After**	**Bioethanol 96h**	**Specific Gravity**
1	15	30	5.8	2.6	0.9952	3.8	2.8	0.9948	2.8	3.0	0.9945	1.4	3.4	0.9938
2	15	35	6.3	3.0	0.9945	5.2	2.5	0.9954	4.6	2.8	0.9955	3.6	3.8	0.9932
3	15	25	6.8	3.0	0.9945	6.8	3.0	0.9945	6.8	3.2	0.9942	6.0	3.4	0.9938
4	15	30	6.2	3.2	0.9942	3.8	2.0	0.9963	3.0	2.8	0.9948	2.0	3.6	0.9934
5	15	35	5.8	2.6	0.9953	3.2	1.8	0.9966	2.8	2.0	0.9963	2.0	2.8	0.9948
6	10	30	4.4	2.2	0.9959	3.2	1.8	0.9966	2.2	2.0	0.9963	1.4	4.0	0.9927
7	15	25	6.0	3.0	0.9945	6.0	3.0	0.9945	6.0	3.2	0.9942	6.0	3.2	0.9942
8	20	30	7.0	3.4	0.9938	4.6	2.6	0.9953	3.8	2.6	0.9952	2.8	3.4	0.9938
9	15	35	4.8	2.2	0.9959	2.0	1.8	0.9966	1.8	2.0	0.9963	1.2	3.2	0.9942
10	15	30	6.2	2.8	0.9948	3.8	2.2	0.9959	3.0	2.8	0.9948	1.8	3.8	0.9932
11	15	25	5.0	2.8	0.9942	5.0	2.8	0.9955	5.0	2.8	0.9948	5.0	3.0	0.9931
12	15	35	6.5	3.2	0.9948	4.0	2.4	0.9956	2.2	2.8	0.9945	2.0	3.8	0.9934
13	20	25	5.8	2.8	0.9945	5.8	2.8	0.9948	5.8	3.0	0.9956	5.8	3.6	0.9934
14	15	30	6.0	3.0	0.9928	4.0	2.0	0.9963	3.2	2.4	0.9938	2.2	3.6	0.9918

15	20	35	8.1	4.0	0.9928	6.0	3.0	0.9945	4.0	3.4	0.9938	2.2	4.6	0.9918
16	15	30	8.3	2.8	0.9948	4.0	2.0	0.9963	3.2	2.0	0.9939	2.4	4.0	0.9928
17	15	30	6.8	3.4	0.9938	4.6	2.0	0.9963	2.6	2.2	0.9939	1.8	3.8	0.9932
18	15	30	6.0	3.0	0.9945	4.0	2.4	0.9956	3.2	2.2	0.9959	1.8	3.4	0.9938
19	15	25	5.4	2.6	0.9953	5.4	2.6	0.9952	5.4	2.6	0.9953	5.4	2.6	0.9953
20	15	30	5.2	3.0	0.9945	4.0	2.2	0.9959	3.4	2.8	0.9948	1.2	4.4	0.9922
21	10	30	4.0	2.0	0.9963	2.2	1.4	0.9974	2.0	2.2	0.9959	1.2	3.4	0.9938
22	10	30	5.0	2.4	0.9956	2.8	2.6	0.9953	2.2	2.8	0.9948	1.0	3.6	0.9934
23	10	25	5.0	2.4	0.9956	5.0	2.4	0.9956	5.0	2.4	0.9956	5.1	2.2	0.9959
24	15	30	6.4	3.2	0.9942	4.0	2.2	0.9959	3.0	2.8	0.9948	2.0	3.6	0.9934
25	15	25	5.0	2.6	0.9953	5.0	2.6	0.9956	5.0	2.6	0.9953	5.0	2.8	0.9948
26	15	30	5.8	2.8	0.9948	3.2	1.6	0.9971	2.6	2.2	0.9959	1.6	3.4	0.9938
27	15	30	6.0	2.6	0.9953	3.8	2.0	0.9963	2.8	2.8	0.9948	1.8	3.8	0.9932
28	15	30	6.0	3.0	0.9945	4.0	2.2	0.9959	2.4	2.8	0.9948	1.4	3.8	0.9932
29	15	30	6.2	2.8	0.9948	4.2	2.4	0.9956	2.8	3.4	0.9938	1.6	4.2	0.9924
30	15	30	5.0	2.6	0.9953	3.0	1.8	0.9966	2.4	2.2	0.9959	1.4	3.8	0.9932
31	20	30	7.0	3.4	0.9938	4.4	2.0	0.9963	3.6	2.4	0.9956	2.2	3.6	0.9934
32	20	30	8.0	4.0	0.9928	5.0	2.8	0.9948	4.4	3.2	0.9942	2.0	4.4	0.9922

33	10	30	4.2	2.0	0.9963	2.8	1.6	0.9971	2.6	2.4	0.9956	1.4	3.8	0.9932
34	15	30	6.2	3.0	0.9945	3.2	2.8	0.9948	2.8	3.2	0.9942	1.6	4.6	0.9918
35	15	30	7.0	3.2	0.9942	4.0	2.2	0.9959	3.0	2.6	0.9953	2.2	3.0	0.9945
36	20	30	5.6	2.8	0.9948	3.6	2.4	0.9956	2.8	2.8	0.9948	1.8	36	0.9934
37	10	30	4.0	2.0	0.9963	2.8	2.2	0.9959	3.4	3.0	0.9945	1.6	3.8	0.9932
38	15	35	6.0	3.0	0.9945	3.8	2.0	0.9963	2.8	2.6	0.9953	2.2	3.4	0.9938
39	15	30	5.0	2.6	0.9953	3.6	1.6	0.9971	2.2	2.4	0.9956	1.6	3.2	0.9942
40	15	35	6.0	3.0	0.9945	3.8	2.0	0.9963	2.8	2.6	0.9953	1.8	3.8	0.9932
41	15	30	6.5	3.2	0.9942	4.0	3.2	0.9963	3.6	3.8	0.9953	2.0	4.6	0.9938
42	10	35	4.0	2.0	0.9963	2.8	1.4	0.9974	2.0	2.6	0.9953	1.6	3.8	0.9942
43	20	30	7.0	3.4	0.9938	3.4	4.0	0.9924	2.6	3.4	0.9938	1.8	3.4	0.9932
44	20	30	8.0	4.0	0.9928	5.0	4.8	0.9914	4.0	5.2	0.9908	2.2	5.8	0.9898
45	10	30	6.2	3.0	0.9945	3.2	3.4	0.9938	1.8	3.8	0.9932	1.6	3.8	0.9932
46	15	25	6.3	3.2	0.9942	4.0	3.6	0.9934	3.2	3.8	0.9932	2.4	4.4	0.9922

Tiger nut waste (TNW) with *Aspergilluls niger isolate H*

R/N S/N	**Subs Wt**	**Temp ^{O}C**	**Brix Before**	**Bioethanol Inno. (g/l)**	**Specific Gravity**	**Brix After**	**Bioethanol 48h**	**Specific Gravity**	**Brix After**	**Bioethanol 72h**	**Specific Gravity**	**Brix After**	**Bioethanol 96h**	**Specific Gravity**
1	15	30	9.0	4.5	0.9919	8.0	4.8	0.9914	6.6	5.0	0.9911	6.8	5.2	0.9908
2	15	35	8.2	4.0	0.9928	7.4	4.4	0.9922	6.6	4.8	0.9914	6.0	5.3	0.9906
3	15	25	9.3	4.6	0.9918	8.6	4.8	0.9914	7.8	5.0	0.9911	6.8	5.4	0.9905
4	15	30	9.8	4.7	0.9916	8.8	5.0	0.9911	8.0	5.2	0.9908	7.2	5.6	0.9902
5	15	35	9.2	4.2	0.9924	8.0	4.4	0.9921	6.8	4.6	0.9918	6.0	5.2	0.9908
6	10	30	8.8	4.2	0.9924	7.8	4.5	0.9919	7.0	4.7	0.9916	6.4	5.0	0.9911
7	15	25	6.4	3.2	0.9942	6.4	3.2	0.9942	5.8	3.4	0.9938	5.6	3.6	0.9934
8	20	30	10.0	5.2	0.9908	9.2	5.4	0.9935	8.6	5.6	0.9902	7.8	5.9	0.9897
9	15	35	8.6	4.3	0.9923	8.0	4.5	0.9919	7.8	4.7	0.9916	7.4	5.1	0.991
10	15	30	9.8	4.8	0.9914	9.0	5.0	0.9911	8.6	5.3	0.9907	8.0	5.6	0.9902
11	15	25	6.2	3.0	0.9945	5.8	3.2	0.9942	5.8	3.4	0.9938	5.4	3.4	0.9938
12	15	35	11.3	5.6	0.9902	10.6	5.8	0.9898	1.0	6.0	0.9896	9.6	6.2	0.9893
13	20	25	6.4	3.0	0.9945	5.8	3.6	0.9934	5.0	3.6	0.9934	4.4	3.8	0.9932
14	15	30	9.0	4.6	0.9918	8.0	4.8	0.9914	7.4	5.0	0.9911	7.0	5.4	0.9905

15	20	35	10.2	5.1	0.991	9.1	5.4	0.9905	8.6	5.7	0.9901	8.0	6.4	0.989
16	15	30	10.0	5.0	0.9911	9.2	5.2	0.9908	8.6	5.6	0.9902	8.0	5.8	0.9898
17	15	30	9.8	4.8	0.9914	9.0	5.0	0.9911	8.2	5.2	0.9908	7.6	5.4	0.9905
18	15	30	8.6	4.3	0.9923	8.0	4.6	0.9918	7.6	4.8	0.9914	7.0	5.2	0.9908
19	15	25	6.0	3.2	0.9941	5.4	3.4	0.9938	4.8	3.6	0.9934	4.0	4.0	0.9928
20	15	30	8.8	4.4	0.9922	8.2	4.6	0.9918	7.8	4.8	0.9918	7.0	5.0	0.9911
21	10	30	3.6	1.6	0.9971	3.0	1..8	0.9966	2.6	2.0	0.9963	2.2	2.4	0.9956
22	10	30	4.4	2.0	0.9963	4.0	2.4	0.9956	3.4	2.4	0.9956	2.6	2.6	0.9953
23	10	25	2.6	1.3	0.9976	2.0	1.6	0.9963	1.2	1.8	0.9966	0.8	2.0	0.9963
24	15	30	8.4	4.4	0.9922	8.8	4.6	0.9918	8.0	4.9	0.9913	7.6	5.2	0.9908
25	15	25	6.0	3.0	0.9948	5.4	3.4	0.9938	4.4	3.6	0.9934	4.0	3.8	0.9932
26	15	30	7.8	3.6	0.9934	7.0	3.8	0.9932	6.6	4.2	0.9924	6.0	4.6	0.9918
27	15	30	8.8	4.5	0.9919	8.0	4.7	0.9916	7.6	4.9	0.9913	7.0	5.2	0.9908
28	15	30	9.4	4.6	0.9918	9.0	4.8	0.9914	8.0	5.0	0.9911	7.0	5.4	0.9905
29	15	30	9.6	4.8	0.9914	9.2	5.0	0.9911	8.6	5.3	0.9907	8.0	5.6	0.9902
30	15	30	9.0	4.5	0.9919	8.4	4.6	0.9918	8.0	4.8	0.9914	7.0	5.0	0.9911
31	20	30	10.3	5.2	0.9908	9.1	5.4	0.9905	8.3	5.6	0.9902	7.8	5.9	0.9897
32	20	30	10.5	5.3	0.9906	9.3	5.6	0.9902	8.4	5.9	0.9897	8.0	6.4	0.989

33	10	30	6.4	3.2	0.9942	5.6	3.4	0.9938	5.0	3.6	0.9934	4.6	3.8	0.9922
34	15	30	7.8	3.6	0.9934	7.0	3.8	0.9932	6.4	4.0	0.9928	6.0	4.2	0.9924
35	15	30	8.6	4.3	0.9923	7.8	4.5	0.9919	7.2	4.7	0.9916	6.6	5.0	0.9919
36	20	30	10.4	5.2	0.9908	9.6	5.4	0.9905	9.0	5.6	0.9902	8.4	6.0	0.9896
37	10	30	6.0	3.1	0.9944	5.6	3.3	0.994	5.0	3.5	0.9936	4.4	3.7	0.9933
38	15	35	8.0	4.0	0.9928	7.2	4.2	0.9924	6.6	4.4	0.9922	6.0	4.6	0.9918
39	15	30	7.6	3.6	0.9934	7.0	3.9	0.993	6.4	4.0	0.9919	5.8	4.2	0.9924
40	15	35	8.8	4.4	0.9922	8.0	4.6	0.9918	7.6	4.8	0.9914	7.0	5.0	0.9911
41	15	30	8.0	4.0	0.9928	7.2	4.2	0.9924	6.8	4.4	0.9921	6.0	4.6	0.9918
42	10	35	6.6	3.3	0.994	6.0	3.5	0.9936	5.5	3.7	0.9933	5.0	4.0	0.9928
43	20	30	10.4	5.2	0.9908	9.2	5.5	0.9904	8.6	5.7	0.9901	8.0	6.0	0.9896
44	20	30	9.8	4.8	0.9914	9.2	5.0	0.9911	8.8	5.2	0.9908	8.2	5.6	0.9902
45	10	30	6.4	3.2	0.9941	6.0	3.5	0.9936	5.4	3.8	0.9932	4.8	4.2	0.9924
46	15	25	7.0	3.5	0.9936	7.9	3.5	0.9936	7.8	3.6	0.9934	7.8	3.6	0.9934

Beans Husk (BH) with *Aspergillus niger isolate H*

R/N S/N	Subs Wt	Temp ^{O}C	Brix Before	Bioethanol Inno. (g/l)	Specific Gravity	Brix After	Bioethanol 48h	Specific Gravity	Brix After	Bioethanol 72h	Specific Gravity	Brix After	Bioethanol 96h	Specific Gravity
1	15	30	2.0	1.0	0.9981	1.8	1.4	0.9974	1.4	1.6	0.9971	1.0	1.8	0.9966
2	15	35	2.6	1.3	0.9976	2.2	1.5	0.9972	1.8	1.9	0.9964	1.4	2.0	0.9963
3	15	25	1.2	0.6	0.9988	1.2	0.6	0.9988	0.8	0.8	0.9985	0.6	1.0	0.9981
4	15	30	2.4	1.2	0.9978	1.8	1.4	0.9974	1.5	1.6	0.9971	1.2	1.8	0.9966
5	15	35	2.5	1.3	0.9976	1.5	1.8	0.9966	1.4	1.4	0.9974	1.0	1.6	0.9971
6	10	30	2.0	0.9	0.9985	1.0	1.4	0.9974	0.8	1.6	0.9971	0.4	1.8	0.9966
7	15	25	2.2	1.1	0.9981	2.2	1.1	0.9981	1.8	1.2	0.9978	1.5	1.4	0.9974
8	20	30	2.8	1.4	0.9974	2.2	1.6	0.9971	1.8	1.8	0.9966	1.4	2.0	0.9963
9	15	35	2.5	1.4	0.9974	1.5	2.0	0.9963	1.0	2.4	0.9956	0.8	2.6	0.9952
10	15	30	2.2	1.2	0.9978	1.8	2.0	0.9963	1.6	2.2	0.9959	1.2	2.6	0.9952
11	15	25	2.4	1.2	0.9978	2.4	1.2	0.9978	2.4	1.2	0.9978	2.2	1.4	0.9974
12	15	35	2.0	1.0	0.9981	1.6	1.6	0.9971	1.2	1.8	0.9966	0.4	2.8	0.9948
13	20	25	2.2	1.4	0.9974	2.2	1.4	0.9974	2.2	1.4	0.9974	2.0	1.6	0.9971
14	15	30	2.0	1.0	0.9981	1.8	1.2	0.9978	1.4	1.8	0.9966	1.2	2.0	0.9963

15	20	35	3.0	1.8	0.9966	2.2	2.2	0.9942	1.7	2.4	0.9956	1.2	2.6	0.9952
16	15	30	2.5	1.3	0.9976	2.0	1.5	0.9972	1.7	1.8	0.9966	1.4	2.0	0.9963
17	15	30	2.2	1.4	0.9974	1.6	1.6	0.9971	1.0	2.2	0.9942	0.8	2.6	0.9953
18	15	30	2.4	1.2	0.9978	2.0	1.6	0.9971	1.6	1.8	0.9966	1.3	2.0	0.9963
19	15	25	2.2	1.2	0.9978	2.2	1.2	0.9978	2.2	1.2	0.9978	2.0	1.4	0.9974
20	15	30	2.0	1.0	0.9981	1.8	1.2	0.9978	1.4	1.6	0.9971	0.9	2.0	0.9963
21	10	30	2.0	1.0	0.9981	1.8	1.4	0.9978	1.6	1.6	0.9971	1.4	1.6	0.9963
22	10	30	2.0	1.0	0.9981	1.5	1.6	0.9974	0.5	2.0	0.9971	0.2	2.4	0.9971
23	10	25	2.0	0.9	0.9981	2.0	0.9	0.9971	2.0	0.9	0.9963	1.8	1.0	0.9956
24	15	30	2.4	1.0	0.9985	1.4	1.2	0.9985	0.8	1.6	0.9985	0.4	2.2	0.9981
25	15	25	2.0	1.2	0.9978	2.0	1.2	0.9978	2.0	1.2	0.9978	1.8	1.6	0.9971
26	15	30	2.2	1.0	0.9981	1.6	1.4	0.9974	1.0	1.6	0.9971	0.8	1.8	0.9966
27	15	30	2.0	0.9	0.9983	1.8	1.2	0.9978	1.4	1.6	0.9971	1.0	2.0	0.9963
28	15	30	2.2	1.0	0.9981	1.2	1.6	0.9971	0.6	1.8	0.9966	0..3	2.0	0.9963
29	15	30	1.4	0.8	0.9985	0.6	1.2	0.9978	0.6	1.4	0.9974	0.2	1.6	0.9971
30	15	30	2.2	1.0	0.9981	1.2	1.4	0.9974	0.8	1.6	0.9971	0.4	1.8	0.9966
31	20	30	2.0	1.0	0.9981	1.8	1.2	0.9978	1.5	1.4	0.9974	1.3	1.6	0.9971
32	20	30	1.0	0.8	0.9985	0.8	1.2	0.9978	0.6	1.6	0.9971	0.2	2.0	0.9963

33	10	30	2.0	1.0	0.9981	1.8	1.2	0.9978	1.4	1.6	0.9971	1.0	1.8	0.9966
34	15	30	2.4	1.2	0.9978	1.5	1.6	0.9971	0.8	2.0	0.9963	0.4	2.6	0.9952
35	15	30	2.2	1.0	0.9981	1.6	1.6	0.9971	1.0	1.8	0.9966	0.8	2.2	0.9959
36	20	30	3.0	1.8	0.9966	1.0	1.2	0.9978	1.0	1.4	0.9974	0.4	1.8	0.9966
37	10	30	2.0	1.2	0.9978	1.4	1.6	0.9971	1.0	1.8	0.9966	0.4	2.0	0.9963
38	15	35	2.2	1.0	0.9981	1.6	1.4	0.9974	1.0	2.2	0.9959	0.8	2.4	0.9956
39	15	30	2.8	1.4	0.9974	1.8	1.8	0.9966	1.0	2.0	0.9963	0.8	2.2	0.9959
40	15	35	2.8	1.5	0.9972	1.6	1.8	0.9966	1.0	2.2	0.9959	0.4	2.4	0.9956
41	15	30	2.0	0.8	0.9985	1.4	1.2	0.9978	1.0	1.6	0.9971	0.8	2.0	0.9963
42	10	35	2.2	1.0	0.9981	1.8	1.6	0.9971	1.2	1.8	0.9966	0.6	2.2	0.9959
43	20	30	2.0	0.9	0.9983	1.6	1.2	0.9978	1.0	1.6	0.9971	0.8	2.0	0.9963
44	20	30	3.0	1.6	0.9971	2.4	1.8	0.9966	1.8	2.0	0.9963	1.2	2.4	0.9956
45	10	30	1.0	0.5	0.999	0.8	0.6	0.9988	0.6	0.8	0.9985	0.2	1.0	0.9981
46	15	25	1.8	0.9	0.9983	1.8	0.9	0.9983	1.8	0.9	0.9983	1.6	1.0	0.9981

Groundnut Shell (GS) with *Aspergilluls niger isolate H*

R/N S/N	Subs Wt	Temp ^{O}C	Brix Before	Bioethanol Inno. (g/l)	Specific Gravity	Brix After	Bioethanol 48h	Specific Gravity	Brix After	Bioethanol 72h	Specific Gravity	Brix After	Bioethanol 96h	Specific Gravity
1	15	30	2.0	1.0	0.9981	2.6	1.6	0.9971	2.0	2.0	0.9963	1.0	2.2	0.9959
2	15	35	3.5	1.7	0.9968	3.3	2.4	0.9956	2.6	2.6	0.9952	2.2	2.8	0.9948
3	15	25	4.0	2.0	0.9963	4.0	2.0	0.9963	3.6	2.4	0.9956	3.4	2.6	0.9952
4	15	30	2.0	1.0	0.9981	1.8	1.2	0.9978	1.4	1.4	0.9974	0.8	1.6	0.9988
5	15	35	2.8	1.4	0.9974	2.5	1.6	0.9972	2.0	2.0	0.9981	1.4	2.6	0.9952
6	10	30	1.4	0.7	0.9987	1.2	1.0	0.9981	0.8	1.4	0.9974	0.6	1.6	0.9971
7	15	25	1.2	0.6	0.9988	1.0	0.6	0.9988	0.8	0.8	0.9985	0.6	1.0	0.9981
8	20	30	3.0	1.5	0.9972	2.6	1.8	0.9966	2.0	2.0	0.9963	1.0	2.2	0.9959
9	15	35	2.0	1.0	0.9981	1.8	1.2	0.9978	1.4	1.6	0.9971	1.0	1.8	0.9966
10	15	30	3.6	1.8	0.9966	3.0	2.0	0.9963	2.6	2.2	0.9959	1.8	2.4	0.9956
11	15	25	2.2	1.4	0.9974	2.2	1.4	0.9974	2.0	1.6	0.9971	2.0	1.6	0.9971
12	15	35	2.8	1.4	0.9974	2.0	1.6	0.9971	1.6	1.8	0.9966	1.2	2.0	0.9963
13	20	25	2.4	1.2	0.9978	2.4	1.2	0.9978	2.0	1.4	0.9974	1.9	1.4	0.9974
14	15	30	2.8	1.4	0.9974	2.0	1.8	0.9966	2.4	2.2	0.9959	1.8	2.6	0.9952

15	20	35	3.7	2.0	0.9963	3.0	2.2	0.9959	2.4	2.4	0.9956	1.6	2.6	0.9952
16	15	30	3.2	1.6	0.9971	3.4	1.8	0.9966	2.8	2.0	0.9981	2.0	3.8	0.9932
17	15	30	3.8	1.7	0.9968	3.0	2.0	0.9963	2.0	2.4	0.9956	1.0	2.5	0.9954
18	15	30	4.3	2.2	0.9959	3.6	2.4	0.9956	3.0	2.6	0.9952	2.0	2.8	0.9948
19	15	25	2.8	1.4	0.9974	2.8	1.4	0.9974	2.4	1.6	0.9971	2.0	2.0	0.9963
20	15	30	4.4	2.2	0.9959	3.4	2.4	0.9956	2.6	2.8	0.9948	2.0	3.0	0.9945
21	10	30	2.7	1.5	0.9972	2.0	1.7	0.9968	1.8	1.9	0.9964	1.4	2.2	0.9942
22	10	30	2.6	1.3	0.9976	2.0	1.4	0.9974	1.8	1.6	0.9971	2.0	1.8	0.9966
23	10	25	3.7	1.6	0.9971	3.6	1.7	0.9968	3.4	1.9	0.9964	2.9	2.0	0.9963
24	15	30	4.2	2.1	0.9961	3.0	2.4	0.9956	2.0	2.6	0.9952	1.4	2.8	0.9948
25	15	25	4.0	2.0	0.9963	4.0	2.1	0.9962	3.8	2.2	0.9959	3.8	2.3	0.9957
26	15	30	2.2	1.0	0.9981	1.6	1.2	0.9978	1.0	1.6	0.9988	0.8	1.8	0.9966
27	15	30	2.6	1.4	0.9974	2.8	1.8	0.9966	3.0	1.9	0.9964	3.2	2.2	0.9959
28	15	30	3.4	1.7	0.9968	3.2	2.0	0.9963	2.8	2.2	0.9959	2.6	2.4	0.9956
29	15	30	4.0	2.0	0.9963	3.4	2.2	0.9959	2.9	2.6	0.9953	1.9	2.9	0.9947
30	15	30	3.4	1.7	0.9968	3.0	1.9	0.9964	2.6	2.0	0.9963	1.4	2.2	0.9959
31	20	30	3.6	1.8	0.9966	3.0	2.0	0.9981	2.6	1.4	0.9974	2.2	2.6	0.9953
32	20	30	3.6	1.6	0.9971	3.0	1.8	0.9966	2.2	2.0	0.9963	1.6	2.4	0.9956

33	10	30	2.8	1.4	0.9974	2.4	1.6	0.9971	1.8	1.8	0.9966	1.6	2.0	0.9963
34	15	30	4.2	2.1	0.9961	3.6	2.3	0.9957	2.6	2.6	0.9952	1.8	2.8	0.9948
35	15	30	3.8	1.6	0.9971	3.0	1.8	0.9966	2.6	2.2	0.9959	2.2	2.4	0.9956
36	20	30	4.0	2.0	0.9963	3.2	2.3	0.9957	2.4	2.6	0.9952	1.6	2.8	0.9948
37	10	30	3.2	1.7	0.9968	2.8	1.9	0.9964	2.0	2.2	0.9959	1.8	2.6	0.9956
38	15	35	2.4	1.2	0.9978	2.0	1.6	0.9971	1.6	1.9	0.9964	1.0	2.2	0.9959
39	15	30	1.8	0.8	0.9985	1.6	1.0	0.9981	1.2	1.4	0.9974	0.6	1.8	0.9966
40	15	35	2.0	1.0	0.9981	1.4	1.4	0.9974	1.0	1.6	0.9971	0.4	2.0	0.9963
41	15	30	3.2	1.6	0.9971	2.6	1.8	0.9966	1.8	2.0	0.9963	1.0	2.0	0.9963
42	10	35	2.2	1.2	0.9978	1.6	1.6	0.9971	1.0	1.8	0.9966	0.6	2.0	0.9963
43	20	30	2.8	1.4	0.9974	2.6	1.6	0.9971	2.4	1.9	0.9964	1.8	2.2	0.9959
44	20	30	4.0	2.0	0.9963	3.4	2.3	0.9957	2.6	2.6	0.9952	2.0	2.8	0.9948
45	10	30	2.0	1.0	0.9981	1.8	1.2	0.9978	1.0	1.4	0.9974	0.4	1.6	0.9971
46	15	25	3.0	1.5	0.9972	3.0	1.5	0.9972	2.8	1.8	0.9966	2.6	2.0	0.9963

Brix Level (Tiger Nut Waste with *Saccharomyces cerevisiae*)

Response 1 Table A: Model Summary Statistics

Source	**Std. Dev.**	**R²**	**Adjusted R²**	**Predicted R²**	**PRESS**	
Linear	0.5055	0.1946	0.0939	-0.0479	13.30	
2FI	**0.4728**	**0.4716**	**0.2074**	**-0.1493**	**14.58**	**Suggested**
Quadratic	0.4758	0.5540	0.1971	-0.3483	17.11	
Cubic	0.5708	0.7432	-0.1556	-4.7850	73.40	Aliased

Among the four models, the computer chose the model that maximized the Adjusted R² and the Predicted R². The best model suggested by the computer was 2FI (two factor interaction model).

Response 1 Table B: Lack of Fit Tests

Source	**Sum of Squares**	**df**	**Mean Square**	**F-value**	**p-value**	
Linear	8.06	35	0.2303	0.5331	0.8763	
2FI	**4.54**	**25**	**0.1818**	**0.4208**	**0.9321**	**Suggested**
Quadratic	3.50	20	0.1750	0.4050	0.9325	
Cubic	1.10	5	0.2197	0.5085	0.7621	Aliased
Pure Error	2.16	5	0.4320			

The selected model (2FI) has insignificant lack-of-fit. This means that the suggested model is a good fit for modeling Brix level data

ANOVA for 2FI Model

Response 1 Table C: Brix level (Tiger Nut Waste with *Saccharomyces cerevisiae*)

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	5.98	15	0.3989	1.78	0.0863	not significant
A-Substrate weight (g/cm³)	1.50	1	1.50	6.71	0.0146	
B-pH	0.3600	1	0.3600	1.61	0.2141	
C-Innoculum size (CFU/ml)	0.4556	1	0.4556	2.04	0.1637	
D-Temperature	0.0900	1	0.0900	0.4027	0.5305	
E-Time (hrs)	0.0625	1	0.0625	0.2796	0.6008	
AB	0.3600	1	0.3600	1.61	0.2141	
AC	1.0000	1	1.0000	4.47	0.0428	
AD	0.4225	1	0.4225	1.89	0.1793	
AE	0.0900	1	0.0900	0.4027	0.5305	
BC	0.8100	1	0.8100	3.62	0.0666	
BD	0.2500	1	0.2500	1.12	0.2987	
BE	1.776E-15	1	1.776E-15	7.948E-15	1.0000	
CD	0.0025	1	0.0025	0.0112	0.9165	
CE	0.4900	1	0.4900	2.19	0.1491	
DE	0.0900	1	0.0900	0.4027	0.5305	
Residual	6.70	30	0.2235			
Lack of Fit	4.54	25	0.1818	0.4208	0.9321	not significant
Pure Error	2.16	5	0.4320			
Cor Total	12.69	45				

Response 1 Table D: Coefficients in Terms of Coded Factors

Factor	Coefficient Estimate	df	Standard Error	95% CI Low	95% CI High	VIF
Intercept	1.33	1	0.0697	1.18	1.47	
A-Substrate weight (g/cm³)	-0.3063	1	0.1182	-0.5476	-0.0649	1.0000
B-pH	-0.1500	1	0.1182	-0.3914	0.0914	1.0000
C-Innoculum size (CFU/ml)	0.1688	1	0.1182	-0.0726	0.4101	1.0000
D-Temperature	-0.0750	1	0.1182	-0.3164	0.1664	1.0000
E-Time (hrs)	-0.0625	1	0.1182	-0.3039	0.1789	1.0000
AB	0.3000	1	0.2364	-0.1827	0.7827	1.0000
AC	0.5000	1	0.2364	0.0173	0.9827	1.0000
AD	-0.3250	1	0.2364	-0.8077	0.1577	1.0000
AE	-0.1500	1	0.2364	-0.6327	0.3327	1.0000
BC	0.4500	1	0.2364	-0.0327	0.9327	1.0000
BD	0.2500	1	0.2364	-0.2327	0.7327	1.0000
BE	0.0000	1	0.2364	-0.4827	0.4827	1.0000
CD	0.0250	1	0.2364	-0.4577	0.5077	1.0000
CE	-0.3500	1	0.2364	-0.8327	0.1327	1.0000
DE	0.1500	1	0.2364	-0.3327	0.6327	1.0000

Response 1 Table E: Final Equation in Terms of Actual Factors

Brix level (TN waste using SC)	=
+37.71984	
-0.601250	Substrate weight (g/cm³)
-5.25000	pH
-3.58125	Innoculum size (CFU/ml)
-0.290000	Temperature
+0.066146	Time (hrs)
+0.060000	Substrate weight (g/cm³) * pH
+0.100000	Substrate weight (g/cm³) * Innoculum size (CFU/ml)
-0.013000	Substrate weight (g/cm³) * Temperature
-0.001250	Substrate weight (g/cm³) * Time (hrs)
+0.450000	pH * Innoculum size (CFU/ml)
+0.050000	pH * Temperature
-1.46060E-16	pH * Time (hrs)
+0.005000	Innoculum size (CFU/ml) * Temperature
-0.014583	Innoculum size (CFU/ml) * Time (hrs)
+0.001250	Temperature * Time (hrs)

Bioethanol Level (Tiger Nut Waste with *Saccharomyces cerevisiae*)

Response 2 Table A: Model Summary Statistics

Source	**Std. Dev.**	**R²**	**Adjusted R²**	**Predicted R²y**	**PRESS**	
Linear	0.4249	0.0819	-0.0329	-0.2033	9.46	
2FI	0.4186	0.3314	-0.0029	-0.5379	12.09	
Quadratic	**0.3778**	**0.5461**	**0.1830**	**-0.4140**	**11.12**	**Suggested**
Cubic	0.4059	0.7904	0.0570	-2.6000	28.31	Aliased

The computer suggested the Quadratic model because it is the only model out of the four models that maximized the Adjusted R² and the Predicted R².

Response 2 Table B: Lack of Fit Tests

Source	**Sum of Squares**	**df**	**Mean Square**	**F-value**	**p-value**	
Linear	5.99	35	0.1710	0.6934	0.7663	
2FI	4.02	25	0.1610	0.6526	0.7843	
Quadratic	**2.34**	**20**	**0.1168**	**0.4735**	**0.8939**	**Suggested**
Cubic	0.4146	5	0.0829	0.3361	0.8717	Aliased
Pure Error	1.23	5	0.2467			

The lack of fit test was conducted to check if the model (Quadratic model) suggested by the computer is a good fit for the data. The model is a good fit since the p –value (0.8939) is greater than the 5% level of significance. Any higher order model example the Cubic model that is aliased is not a good model.

ANOVA for Quadratic Model

Response 2 Table C: Bioethanol Level (Tiger Nut Waste with *Saccharomyces cerevisiae*)

Source	**Sum of Squares**	**df**	**Mean Square**	**F-value**	**p-value**	
Model	4.29	20	0.2147	1.50	0.1658	Not significant
A-Substrate weight (g/cm³)	0.0100	1	0.0100	0.0700	0.7934	
B-pH	0.3306	1	0.3306	2.32	0.1406	
C-Innoculum size (CFU/ml)	0.0306	1	0.0306	0.2145	0.6473	
D-Temperature	0.2500	1	0.2500	1.75	0.1977	
E-Time (hrs)	0.0225	1	0.0225	0.1576	0.6947	
AB	0.2500	1	0.2500	1.75	0.1977	
AC	0.2500	1	0.2500	1.75	0.1977	
AD	1.0000	1	1.0000	7.00	0.0139	
AE	0.1600	1	0.1600	1.12	0.2999	
BC	0.1225	1	0.1225	0.8580	0.3631	
BD	0.0400	1	0.0400	0.2802	0.6013	
BE	0.0000	1	0.0000	0.0000	1.0000	
CD	0.0100	1	0.0100	0.0700	0.7934	
CE	0.0400	1	0.0400	0.2802	0.6013	
DE	0.0900	1	0.0900	0.6304	0.4347	
A^2	0.0003	1	0.0003	0.0024	0.9614	
B^2	0.0109	1	0.0109	0.0767	0.7841	
C^2	1.30	1	1.30	9.08	0.0058	
D^2	0.0464	1	0.0464	0.3250	0.5737	
E^2	0.1064	1	0.1064	0.7453	0.3962	
Residual	3.57	25	0.1428			
Lack of Fit	2.34	20	0.1168	0.4735	0.8939	not significant
Pure Error	1.23	5	0.2467			
Cor Total	7.86	45				

Response 2 Table D: Coefficients in Terms of Coded Factors

Factor	Coefficient Estimate	df	Standard Error	95% CI Low	95% CI High	VIF
Intercept	1.83	1	0.1543	1.52	2.15	
A-Substrate weight (g/cm^3)	0.0250	1	0.0945	-0.1695	0.2195	1.0000
B-pH	-0.1438	1	0.0945	-0.3383	0.0508	1.0000
C-Innoculum size (CFU/ml)	0.0438	1	0.0945	-0.1508	0.2383	1.0000
D-Temperature	0.1250	1	0.0945	-0.0695	0.3195	1.0000
E-Time (hrs)	0.0375	1	0.0945	-0.1570	0.2320	1.0000
AB	-0.2500	1	0.1889	-0.6391	0.1391	1.0000
AC	0.2500	1	0.1889	-0.1391	0.6391	1.0000
AD	0.5000	1	0.1889	0.1109	0.8891	1.0000
AE	0.2000	1	0.1889	-0.1891	0.5891	1.0000
BC	-0.1750	1	0.1889	-0.5641	0.2141	1.0000
BD	0.1000	1	0.1889	-0.2891	0.4891	1.0000
BE	0.0000	1	0.1889	-0.3891	0.3891	1.0000
CD	0.0500	1	0.1889	-0.3391	0.4391	1.0000
CE	0.1000	1	0.1889	-0.2891	0.4891	1.0000
DE	0.1500	1	0.1889	-0.2391	0.5391	1.0000
A^2	0.0063	1	0.1279	-0.2572	0.2697	1.20
B^2	-0.0354	1	0.1279	-0.2988	0.2280	1.20
C^2	-0.3854	1	0.1279	-0.6488	-0.1220	1.20
D^2	0.0729	1	0.1279	-0.1905	0.3363	1.20
E^2	-0.1104	1	0.1279	-0.3738	0.1530	1.20

Response 2 Table E: Final Equation in Terms of Actual Factors

Alcohol level (TN Waste using SC)	=
+0.916667	
-0.672500	Substrate weight (g/cm³)
+1.55208	pH
+4.54375	Innoculum size (CFU/ml)
-0.740000	Temperature
-0.058333	Time (hrs)
-0.050000	Substrate weight (g/cm³) * pH
+0.050000	Substrate weight (g/cm³) * Innoculum size (CFU/ml)
+0.020000	Substrate weight (g/cm³) * Temperature
+0.001667	Substrate weight (g/cm³) * Time (hrs)
-0.175000	pH * Innoculum size (CFU/ml)
+0.020000	pH * Temperature
-9.51640E-17	pH * Time (hrs)
+0.010000	Innoculum size (CFU/ml) * Temperature
+0.004167	Innoculum size (CFU/ml) * Time (hrs)
+0.001250	Temperature * Time (hrs)
+0.000250	Substrate weight (g/cm³)²
-0.035417	pH²
-0.385417	Innoculum size (CFU/ml)²
+0.002917	Temperature²
-0.000192	Time (hrs)²

Brix Level (Beans Husk with *Saccharomyces cerevisiae*)

Response 3 Table A: Model Summary Statistics

Source	**Std. Dev.**	**R²**	**Adjusted R²**	**Predicted R²**	**PRESS**	
Linear	**1.24**	**0.2403**	**0.1453**	**-0.0042**	**80.82**	**Suggested**
2FI	1.16	0.4962	0.2443	-0.1807	95.02	
Quadratic	1.18	0.5657	0.2182	-0.4395	115.85	
Cubic	1.26	0.8016	0.1072	-4.4215	436.31	Aliased

The linear model was suggested because it maximized the Adjusted R² and the Predicted R².

Response 3 Table B: Lack of Fit Tests

Source	**Sum of Squares**	**Df**	**Mean Square**	**F-value**	**p-value**	
Linear	**51.78**	**35**	**1.48**	**0.7903**	**0.6993**	**Suggested**
2FI	31.18	25	1.25	0.6663	0.7748	
Quadratic	25.59	20	1.28	0.6835	0.7542	
Cubic	6.61	5	1.32	0.7058	0.6442	Aliased
Pure Error	9.36	5	1.87			

The selected model should have insignificant lack-of-fit. The p-value (0.6993) associated with the Linear function is not less than 0.05 level of significance, therefore the model is a good fit.

ANOVA for Linear model

Response 3 Table C: Brix level (Beans Husk with *Saccharomyces cerevisiae*)

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	19.34	5	3.87	2.53	0.0442	significant
A-Substrate weight (g/cm³)	6.25	1	6.25	4.09	0.0499	
B-pH	1.10	1	1.10	0.7213	0.4008	
C-Innoculum size (CFU/ml)	0.8100	1	0.8100	0.5299	0.4709	
D-Temperature	7.56	1	7.56	4.95	0.0318	
E-Time (hrs)	3.61	1	3.61	2.36	0.1322	
Residual	61.14	40	1.53			
Lack of Fit	51.78	35	1.48	0.7903	0.6993	not significant
Pure Error	9.36	5	1.87			
Cor Total	80.48	45				

Response 3 Table D: Coefficients in Terms of Coded Factors

Factor	Coefficient Estimate	df	Standard Error	95% CI Low	95% CI High	VIF
Intercept	3.21	1	0.1823	2.84	3.58	
A-Substrate weight (g/cm³)	-0.6250	1	0.3091	-1.25	-0.0003	1.0000
B-pH	-0.2625	1	0.3091	-0.8872	0.3622	1.0000
C-Innoculum size (CFU/ml)	-0.2250	1	0.3091	-0.8497	0.3997	1.0000
D-Temperature	-0.6875	1	0.3091	-1.31	-0.0628	1.0000
E-Time (hrs)	-0.4750	1	0.3091	-1.10	0.1497	1.0000

Response 3 Table E: Final Equation in Terms of Actual Factors

Brix level (BH waste using SC)	=
+13.82120	
-0.125000	Substrate weight (g/cm³)
-0.262500	pH
-0.225000	Innoculum size (CFU/ml)
-0.137500	Temperature
-0.019792	Time (hrs)

Bioethanol Level (Beans Husk with *Saccharomyces cerevisiae*)

Response 4 Table A: Model Summary Statistics

Source	Std. Dev.	R^2	Adjusted R^2	Predicted R^2	PRESS	
Linear	0.8550	0.0456	-0.0736	-0.2882	39.47	
2FI	0.9443	0.1270	-0.3095	-1.2815	69.91	
Quadratic	**0.9825**	**0.2124**	**-0.4178**	**-1.9940**	**91.74**	**Suggested**
Cubic	0.8982	0.7367	-0.1849	-12.0267	399.15	Aliased

The computer suggested Quadratic model as the best model because it maximized the Adjusted R^2 and the Predicted R^2.

Response 4 Table B: Lack of Fit Tests

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Linear	27.37	35	0.7820	2.09	0.2107	
2FI	24.88	25	0.9951	2.66	0.1400	
Quadratic	**22.26**	**20**	**1.11**	**2.97**	**0.1153**	**Suggested**
Cubic	6.19	5	1.24	3.31	0.1077	Aliased
Pure Error	1.87	5	0.3747			

The selected model (Quadratic) should have insignificant lack-of-fit.

ANOVA for Quadratic Model

Response 4 Table C: Bioethanol level (Beans Husk with *Saccharomyces cerevisiae*)

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	6.51	20	0.3253	0.3370	0.9922	Not significant
A-Substrate weight (g/cm³)	0.6806	1	0.6806	0.7050	0.4091	
B-pH	0.1056	1	0.1056	0.1094	0.7436	
C-Innoculum size (CFU/ml)	0.0025	1	0.0025	0.0026	0.9598	
D-Temperature	0.2500	1	0.2500	0.2590	0.6153	
E-Time (hrs)	0.3600	1	0.3600	0.3729	0.5469	
AB	0.0225	1	0.0225	0.0233	0.8799	
AC	0.0900	1	0.0900	0.0932	0.7626	
AD	0.0400	1	0.0400	0.0414	0.8403	
AE	0.0100	1	0.0100	0.0104	0.9197	
BC	3.553E-15	1	3.553E-15	3.680E-15	1.0000	
BD	0.1600	1	0.1600	0.1657	0.6874	
BE	0.0100	1	0.0100	0.0104	0.9197	
CD	0.1600	1	0.1600	0.1657	0.6874	
CE	0.0400	1	0.0400	0.0414	0.8403	
DE	1.96	1	1.96	2.03	0.1666	
A^2	0.0167	1	0.0167	0.0173	0.8964	
B^2	0.0319	1	0.0319	0.0330	0.8573	
C^2	1.06	1	1.06	1.09	0.3055	
D^2	1.16	1	1.16	1.20	0.2834	
E^2	0.0109	1	0.0109	0.0113	0.9160	
Residual	24.13	25	0.9654			
Lack of Fit	22.26	20	1.11	2.97	0.1153	not significant
Pure Error	1.87	5	0.3747			
Cor Total	30.64	45				

Response 4 Table D: Coefficients in Terms of Coded Factors

Factor	Coefficient Estimate	df	Standard Error	95% CI Low	95% CI High	VIF
Intercept	2.63	1	0.4011	1.81	3.46	
A-Substrate weight (g/cm³)	0.2062	1	0.2456	-0.2996	0.7121	1.0000
B-pH	-0.0813	1	0.2456	-0.5871	0.4246	1.0000
C-Innoculum size (CFU/ml)	0.0125	1	0.2456	-0.4934	0.5184	1.0000
D-Temperature	0.1250	1	0.2456	-0.3809	0.6309	1.0000
E-Time (hrs)	0.1500	1	0.2456	-0.3559	0.6559	1.0000
AB	0.0750	1	0.4913	-0.9368	1.09	1.0000
AC	-0.1500	1	0.4913	-1.16	0.8618	1.0000
AD	-0.1000	1	0.4913	-1.11	0.9118	1.0000
AE	0.0500	1	0.4913	-0.9618	1.06	1.0000
BC	0.0000	1	0.4913	-1.01	1.01	1.0000
BD	-0.2000	1	0.4913	-1.21	0.8118	1.0000
BE	0.0500	1	0.4913	-0.9618	1.06	1.0000
CD	0.2000	1	0.4913	-0.8118	1.21	1.0000
CE	-0.1000	1	0.4913	-1.11	0.9118	1.0000
DE	0.7000	1	0.4913	-0.3118	1.71	1.0000
A^2	-0.0438	1	0.3326	-0.7287	0.6412	1.20
B^2	-0.0604	1	0.3326	-0.7454	0.6246	1.20
C^2	0.3479	1	0.3326	-0.3371	1.03	1.20
D^2	0.3646	1	0.3326	-0.3204	1.05	1.20
E^2	-0.0354	1	0.3326	-0.7204	0.6496	1.20

Response 4 Table E: Final Equation in Terms of Actual Factors

Alcohol level (BH waste using SC)	=
+31.46042	
+0.258750	Substrate weight (g/cm³)
+1.58958	pH
-4.61250	Innoculum size (CFU/ml)
-1.17000	Temperature
-0.155729	Time (hrs)
+0.015000	Substrate weight (g/cm³) * pH
-0.030000	Substrate weight (g/cm³) * Innoculum size (CFU/ml)
-0.004000	Substrate weight (g/cm³) * Temperature
+0.000417	Substrate weight (g/cm³) * Time (hrs)
+4.62583E-15	pH * Innoculum size (CFU/ml)
-0.040000	pH * Temperature
+0.002083	pH * Time (hrs)
+0.040000	Innoculum size (CFU/ml) * Temperature
-0.004167	Innoculum size (CFU/ml) * Time (hrs)
+0.005833	Temperature * Time (hrs)
-0.001750	Substrate weight (g/cm³)²
-0.060417	pH²
+0.347917	Innoculum size (CFU/ml)²
+0.014583	Temperature²
-0.000061	Time (hrs)²

Brix Level (Groundnut Shell with *Saccharomyces cerevisiae*)

Response 5 Table A: Model Summary Statistics

Source	Std. Dev.	R^2	Adjusted R^2	Predicted R^2	PRESS	
Linear	0.1180	0.0900	-0.0237	-0.2058	0.7378	
2FI	0.1121	0.3840	0.0760	-0.4641	0.8958	
Quadratic	**0.1033**	**0.5636**	**0.2145**	**-0.4020**	**0.8578**	**Suggested**
Cubic	0.1179	0.7729	-0.0220	-5.1417	3.76	Aliased

Focus on the model maximizing the Adjusted R^2 and the Predicted R^2.

Response 5 Table B: Lack of Fit Tests

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Linear	0.4747	35	0.0136	0.8261	0.6754	
2FI	0.2948	25	0.0118	0.7183	0.7389	
Quadratic	**0.1849**	**20**	**0.0092**	**0.5631**	**0.8363**	**Suggested**
Cubic	0.0569	5	0.0114	0.6928	0.6515	Aliased
Pure Error	0.0821	5	0.0164			

The selected model should have insignificant lack-of-fit.

ANOVA for Quadratic model

Response 5 Table C: Brix level (Groundnut shell with *Saccaromyceses cerevisae*)

Transform: Inverse Sqrt

Constant: 0

Source	**Sum of Squares**	**df**	**Mean Square**	**F-value**	**p-value**	
Model	0.3449	20	0.0172	1.61	0.1278	not significant
A-Substrate weight (g/cm^3)	0.0332	1	0.0332	3.11	0.0900	
B-pH	0.0075	1	0.0075	0.6981	0.4113	
C-Innoculum size (CFU/ml)	0.0002	1	0.0002	0.0219	0.8837	
D-Temperature	0.0028	1	0.0028	0.2585	0.6156	
E-Time (hrs)	0.0114	1	0.0114	1.07	0.3112	
AB	0.0197	1	0.0197	1.84	0.1867	
AC	0.0381	1	0.0381	3.57	0.0706	
AD	0.0093	1	0.0093	0.8716	0.3595	
AE	0.0157	1	0.0157	1.47	0.2360	
BC	0.0470	1	0.0470	4.41	0.0461	
BD	0.0106	1	0.0106	0.9911	0.3290	
BE	0.0001	1	0.0001	0.0064	0.9367	
CD	0.0343	1	0.0343	3.21	0.0852	
CE	0.0009	1	0.0009	0.0841	0.7742	
DE	0.0041	1	0.0041	0.3876	0.5392	
A^2	0.0103	1	0.0103	0.9649	0.3354	
B^2	0.0093	1	0.0093	0.8672	0.3606	
C^2	0.0577	1	0.0577	5.40	0.0285	
D^2	0.0027	1	0.0027	0.2516	0.6203	
E^2	0.0000	1	0.0000	0.0015	0.9699	
Residual	0.2670	25	0.0107			
Lack of Fit	0.1849	20	0.0092	0.5631	0.8363	not significant
Pure Error	0.0821	5	0.0164			
Cor Total	0.6118	45				

Response 5 Table D: Coefficients in Terms of Coded Factors

Factor	Coefficient Estimate	df	Standard Error	95% CI Low	95% CI High	VIF
Intercept	0.7262	1	0.0422	0.6393	0.8131	
A-Substrate weight (g/cm³)	0.0456	1	0.0258	-0.0076	0.0988	1.0000
B-pH	-0.0216	1	0.0258	-0.0748	0.0316	1.0000
C-Innoculum size (CFU/ml)	-0.0038	1	0.0258	-0.0570	0.0494	1.0000
D-Temperature	0.0131	1	0.0258	-0.0401	0.0663	1.0000
E-Time (hrs)	0.0267	1	0.0258	-0.0265	0.0799	1.0000
AB	-0.0701	1	0.0517	-0.1766	0.0363	1.0000
AC	-0.0976	1	0.0517	-0.2040	0.0088	1.0000
AD	0.0482	1	0.0517	-0.0582	0.1547	1.0000
AE	0.0627	1	0.0517	-0.0437	0.1692	1.0000
BC	-0.1085	1	0.0517	-0.2149	-0.0020	1.0000
BD	0.0514	1	0.0517	-0.0550	0.1579	1.0000
BE	-0.0041	1	0.0517	-0.1106	0.1023	1.0000
CD	0.0926	1	0.0517	-0.0138	0.1990	1.0000
CE	-0.0150	1	0.0517	-0.1214	0.0914	1.0000
DE	0.0322	1	0.0517	-0.0743	0.1386	1.0000
A^2	0.0344	1	0.0350	-0.0377	0.1064	1.20
B^2	0.0326	1	0.0350	-0.0395	0.1046	1.20
C^2	-0.0813	1	0.0350	-0.1533	-0.0093	1.20
D^2	0.0175	1	0.0350	-0.0545	0.0896	1.20
E^2	0.0013	1	0.0350	-0.0707	0.0734	1.20

Response 5 Table E: Final Equation in Terms of Actual Factors

1/Sqrt(Brix level (GS waste using SC))	=
-0.408152	
+0.087659	Substrate weight (g/cm³)
+0.087284	pH
+1.51304	Innoculum size (CFU/ml)
-0.270873	Temperature
-0.010150	Time (hrs)
-0.014029	Substrate weight (g/cm³) * pH
-0.019518	Substrate weight (g/cm³) * Innoculum size (CFU/ml)
+0.001930	Substrate weight (g/cm³) * Temperature
+0.000523	Substrate weight (g/cm³) * Time (hrs)
-0.108452	pH * Innoculum size (CFU/ml)
+0.010288	pH * Temperature
-0.000173	pH * Time (hrs)
+0.018521	Innoculum size (CFU/ml) * Temperature
-0.000625	Innoculum size (CFU/ml) * Time (hrs)
+0.000268	Temperature * Time (hrs)
+0.001375	Substrate weight (g/cm³)²
+0.032576	pH²
-0.081297	Innoculum size (CFU/ml)²
+0.000702	Temperature²
+2.31288E-06	Time (hrs)²

Bioethanol Level (Groundnut shell with *Saccharomyces cerevisiae*)

Response 6 Table A: Model Summary Statistics

Source	Std. Dev.	R^2	Adjusted R^2	Predicted R^2	PRESS	
Linear	0.6031	0.1054	-0.0064	-0.2125	19.72	
2FI	0.5654	0.4104	0.1156	-0.5359	24.98	
Quadratic	**0.4890**	**0.6324**	**0.3384**	**-0.3696**	**22.27**	**Suggested**
Cubic	0.5526	0.8123	0.1552	-8.5531	155.37	Aliased

Focus on the model (Quadratic) maximizing the Adjusted R^2 and the Predicted R^2.

Response 6 Table B: Lack of Fit Tests

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Linear	13.91	35	0.3974	3.10	0.1037	
2FI	8.95	25	0.3580	2.80	0.1275	
Quadratic	**5.34**	**20**	**0.2669**	**2.09**	**0.2127**	**Suggested**
Cubic	2.41	5	0.4827	3.77	0.0858	Aliased
Pure Error	0.6400	5	0.1280			

The selected model (Quadratic) have insignificant lack-of-fit.

ANOVA for Quadratic model

Response 6 Table C: Alcohol level (Groundnut shell with *Saccharomyces cerevisiae*)

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	10.29	20	0.5143	2.15	0.0356	significant
A-Substrate weight (g/cm³)	0.2500	1	0.2500	1.05	0.3164	
B-pH	0.0025	1	0.0025	0.0105	0.9194	
C-Innoculum size (CFU/ml)	0.4900	1	0.4900	2.05	0.1647	
D-Temperature	0.7225	1	0.7225	3.02	0.0945	
E-Time (hrs)	0.2500	1	0.2500	1.05	0.3164	
AB	1.776E-15	1	1.776E-15	7.428E-15	1.0000	
AC	0.3600	1	0.3600	1.51	0.2313	
AD	0.3600	1	0.3600	1.51	0.2313	
AE	0.0400	1	0.0400	0.1673	0.6860	
BC	3.61	1	3.61	15.10	0.0007	
BD	0.0900	1	0.0900	0.3764	0.5451	
BE	0.0900	1	0.0900	0.3764	0.5451	
CD	0.0400	1	0.0400	0.1673	0.6860	
CE	0.0100	1	0.0100	0.0418	0.8396	
DE	0.3600	1	0.3600	1.51	0.2313	
A^2	0.0491	1	0.0491	0.2053	0.6544	
B^2	0.7424	1	0.7424	3.10	0.0903	
C^2	0.9218	1	0.9218	3.85	0.0608	
D^2	1.12	1	1.12	4.69	0.0402	
E^2	0.0491	1	0.0491	0.2053	0.6544	
Residual	5.98	25	0.2391			
Lack of Fit	5.34	20	0.2669	2.09	0.2127	not significant
Pure Error	0.6400	5	0.1280			
Cor Total	16.26	45				

Response 6 Table D: Coefficients in Terms of Coded Factors

Factor	Coefficient Estimate	df	Standard Error	95% CI Low	95% CI High	VIF
Intercept	3.20	1	0.1996	2.79	3.61	
A-Substrate weight (g/cm³)	-0.1250	1	0.1223	-0.3768	0.1268	1.0000
B-pH	0.0125	1	0.1223	-0.2393	0.2643	1.0000
C-Innoculum size (CFU/ml)	-0.1750	1	0.1223	-0.4268	0.0768	1.0000
D-Temperature	0.2125	1	0.1223	-0.0393	0.4643	1.0000
E-Time (hrs)	0.1250	1	0.1223	-0.1268	0.3768	1.0000
AB	0.0000	1	0.2445	-0.5036	0.5036	1.0000
AC	-0.3000	1	0.2445	-0.8036	0.2036	1.0000
AD	0.3000	1	0.2445	-0.2036	0.8036	1.0000
AE	-0.1000	1	0.2445	-0.6036	0.4036	1.0000
BC	-0.9500	1	0.2445	-1.45	-0.4464	1.0000
BD	-0.1500	1	0.2445	-0.6536	0.3536	1.0000
BE	0.1500	1	0.2445	-0.3536	0.6536	1.0000
CD	0.1000	1	0.2445	-0.4036	0.6036	1.0000
CE	-0.0500	1	0.2445	-0.5536	0.4536	1.0000
DE	-0.3000	1	0.2445	-0.8036	0.2036	1.0000
A^2	0.0750	1	0.1655	-0.2659	0.4159	1.20
B^2	0.2917	1	0.1655	-0.0493	0.6326	1.20
C^2	-0.3250	1	0.1655	-0.6659	0.0159	1.20
D^2	0.3583	1	0.1655	0.0174	0.6993	1.20
E^2	0.0750	1	0.1655	-0.2659	0.4159	1.20

Response 6 Table E: Final Equation in Terms of Actual Factors

Alcohol level (GS waste using SC)	=
-26.92083	
-0.055000	Substrate weight (g/cm³)
+2.07917	pH
+10.82500	Innoculum size (CFU/ml)
-0.727500	Temperature
+0.042708	Time (hrs)
+9.09793E-15	Substrate weight (g/cm³) * pH
-0.060000	Substrate weight (g/cm³) * Innoculum size (CFU/ml)
+0.012000	Substrate weight (g/cm³) * Temperature
-0.000833	Substrate weight (g/cm³) * Time (hrs)
-0.950000	pH * Innoculum size (CFU/ml)
-0.030000	pH * Temperature
+0.006250	pH * Time (hrs)
+0.020000	Innoculum size (CFU/ml) * Temperature
-0.002083	Innoculum size (CFU/ml) * Time (hrs)
-0.002500	Temperature * Time (hrs)
+0.003000	Substrate weight (g/cm³)²
+0.291667	pH²
-0.325000	Innoculum size (CFU/ml)²
+0.014333	Temperature²
+0.000130	Time (hrs)²

Brix Level (Tiger Nut Waste with *Aspergillus niger isolate H*)

Response 7 Table A: Model Summary Statistics

Source	Std. Dev.	R^2	Adjusted R^2	Predicted R^2	PRESS	
Linear	1.45	0.0720	-0.0440	-0.2564	113.28	
2FI	**1.35**	**0.3922**	**0.0883**	**-0.5754**	**142.05**	**Suggested**
Quadratic	1.46	0.4059	-0.0694	-1.2342	201.44	
Cubic	1.28	0.8188	0.1844	-7.1211	732.23	Aliased

The best model is two factor interactions (2FI) model that maximized the Adjusted R^2 and the Predicted R^2.

Response 7 Table B: Lack of Fit Tests

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Linear	78.66	35	2.25	2.24	0.1869	
2FI	**49.79**	**25**	**1.99**	**1.99**	**0.2293**	**Suggested**
Quadratic	48.56	20	2.43	2.42	0.1659	
Cubic	11.33	5	2.27	2.26	0.1959	Aliased
Pure Error	5.01	5	1.00			

The selected model should have insignificant lack-of-fit.

ANOVA for 2FI model

Response 7 Table C: Brix Level (Tiger Nut Waste with *Aspergillus niger isolate H*)

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	35.36	15	2.36	1.29	0.2673	Not significant
A-Substrate weight (g/cm³)	2.48	1	2.48	1.36	0.2531	
B-pH	1.16	1	1.16	0.6326	0.4326	
C-Innoculum size (CFU/ml)	0.0506	1	0.0506	0.0277	0.8689	
D-Temperature	1.421E-14	1	1.421E-14	7.779E-15	1.0000	
E-Time (hrs)	2.81	1	2.81	1.54	0.2248	
AB	0.0900	1	0.0900	0.0493	0.8258	
AC	0.0900	1	0.0900	0.0493	0.8258	
AD	0.5625	1	0.5625	0.3079	0.5831	
AE	0.3600	1	0.3600	0.1971	0.6603	
BC	2.10	1	2.10	1.15	0.2919	
BD	0.0900	1	0.0900	0.0493	0.8258	
BE	5.76	1	5.76	3.15	0.0859	
CD	4.20	1	4.20	2.30	0.1398	
CE	6.00	1	6.00	3.29	0.0799	
DE	9.61	1	9.61	5.26	0.0290	
Residual	54.80	30	1.83			
Lack of Fit	49.79	25	1.99	1.99	0.2293	not significant
Pure Error	5.01	5	1.00			
Cor Total	90.16	45				

Response 7 Table D: Coefficients in Terms of Coded Factors

Factor	Coefficient Estimate	df	Standard Error	95% CI Low	95% CI High	VIF
Intercept	6.47	1	0.1993	6.06	6.87	
A-Substrate weight (g/cm³)	-0.3938	1	0.3379	-1.08	0.2963	1.0000
B-pH	0.2687	1	0.3379	-0.4213	0.9588	1.0000
C-Innoculum size (CFU/ml)	-0.0563	1	0.3379	-0.7463	0.6338	1.0000
D-Temperature	0.0000	1	0.3379	-0.6901	0.6901	1.0000
E-Time (hrs)	0.4188	1	0.3379	-0.2713	1.11	1.0000
AB	0.1500	1	0.6758	-1.23	1.53	1.0000
AC	0.1500	1	0.6758	-1.23	1.53	1.0000
AD	-0.3750	1	0.6758	-1.76	1.01	1.0000
AE	-0.3000	1	0.6758	-1.68	1.08	1.0000
BC	0.7250	1	0.6758	-0.6551	2.11	1.0000
BD	-0.1500	1	0.6758	-1.53	1.23	1.0000
BE	-1.20	1	0.6758	-2.58	0.1801	1.0000
CD	-1.03	1	0.6758	-2.41	0.3551	1.0000
CE	-1.23	1	0.6758	-2.61	0.1551	1.0000
DE	-1.55	1	0.6758	-2.93	-0.1699	1.0000

Response 7 Table E: Final Equation in Terms of Actual Factors

Brix level (TN waste using AN)	=
-86.65353	
+0.161250	Substrate weight (g/cm³)
-0.031250	pH
+4.24375	Innoculum size (CFU/ml)
+2.59500	Temperature
+1.09870	Time (hrs)
+0.030000	Substrate weight (g/cm³) * pH
+0.030000	Substrate weight (g/cm³) * Innoculum size (CFU/ml)
-0.015000	Substrate weight (g/cm³) * Temperature
-0.002500	Substrate weight (g/cm³) * Time (hrs)
+0.725000	pH * Innoculum size (CFU/ml)
-0.030000	pH * Temperature
-0.050000	pH * Time (hrs)
-0.205000	Innoculum size (CFU/ml) * Temperature
-0.051042	Innoculum size (CFU/ml) * Time (hrs)
-0.012917	Temperature * Time (hrs)

Bioethanol Level (Tiger Nut Waste with *Aspergillus niger isolate H*)

Response 8 Table A: Model Summary Statistics

Source	**Std. Dev.**	**R^2**	**Adjusted R^2**	**Predicted R^2**	**PRESS**	
Linear	0.6869	0.1780	0.0752	-0.0993	25.24	
2FI	**0.6615**	**0.4283**	**0.1424**	**-0.4165**	**32.52**	**Suggested**
Quadratic	0.6710	0.5098	0.1177	-0.8031	41.40	
Cubic	0.7243	0.7715	-0.0282	-9.7722	247.34	Aliased

The best model is two factor interaction (2FI) model because it is the only model that maximized the Adjusted R^2 and the Predicted R^2.

Response 8 Table B: Lack of Fit Tests

Source	**Sum of Squares**	**df**	**Mean Square**	**F-value**	**p-value**	
Linear	17.46	35	0.4989	1.76	0.2751	
2FI	**11.71**	**25**	**0.4686**	**1.66**	**0.3021**	**Suggested**
Quadratic	9.84	20	0.4921	1.74	0.2811	
Cubic	3.83	5	0.7666	2.71	0.1488	Aliased
Pure Error	1.41	5	0.2827			

The selected model should have insignificant lack-of-fit.

ANOVA for 2FI model

Response 8 Table C: Alcohol level (Tiger Nut Waste with *Aspergillus niger isolate H*)

Source	**Sum of Squares**	**Df**	**Mean Square**	**F-value**	**p-value**	
Model	9.83	15	0.6556	1.50	0.1683	Not significant
A-Substrate weight (g/cm³)	1.32	1	1.32	3.02	0.0924	
B-pH	0.1225	1	0.1225	0.2800	0.6006	
C-Innoculum size (CFU/ml)	1.50	1	1.50	3.43	0.0739	
D-Temperature	0.0900	1	0.0900	0.2057	0.6534	
E-Time (hrs)	1.05	1	1.05	2.40	0.1317	
AB	0.4225	1	0.4225	0.9655	0.3337	
AC	0.0225	1	0.0225	0.0514	0.8221	
AD	0.3025	1	0.3025	0.6913	0.4123	
AE	0.0225	1	0.0225	0.0514	0.8221	
BC	0.3600	1	0.3600	0.8227	0.3716	
BD	0.4900	1	0.4900	1.12	0.2984	
BE	0.1225	1	0.1225	0.2800	0.6006	
CD	0.7225	1	0.7225	1.65	0.2086	
CE	1.32	1	1.32	3.02	0.0924	
DE	1.96	1	1.96	4.48	0.0427	
Residual	13.13	30	0.4376			
Lack of Fit	11.71	25	0.4686	1.66	0.3021	not significant
Pure Error	1.41	5	0.2827			
Cor Total	22.96	45				

Response 8 Table D: Coefficients in Terms of Coded Factors

Factor	Coefficient Estimate	df	Standard Error	95% CI Low	95% CI High	VIF
Intercept	4.67	1	0.0975	4.47	4.87	
A-Substrate weight (g/cm³)	-0.2875	1	0.1654	-0.6252	0.0502	1.0000
B-pH	-0.0875	1	0.1654	-0.4252	0.2502	1.0000
C-Innoculum size (CFU/ml)	-0.3062	1	0.1654	-0.6440	0.0315	1.0000
D-Temperature	0.0750	1	0.1654	-0.2627	0.4127	1.0000
E-Time (hrs)	-0.2562	1	0.1654	-0.5940	0.0815	1.0000
AB	0.3250	1	0.3307	-0.3505	1.00	1.0000
AC	0.0750	1	0.3307	-0.6005	0.7505	1.0000
AD	0.2750	1	0.3307	-0.4005	0.9505	1.0000
AE	-0.0750	1	0.3307	-0.7505	0.6005	1.0000
BC	-0.3000	1	0.3307	-0.9755	0.3755	1.0000
BD	0.3500	1	0.3307	-0.3255	1.03	1.0000
BE	-0.1750	1	0.3307	-0.8505	0.5005	1.0000
CD	0.4250	1	0.3307	-0.2505	1.10	1.0000
CE	0.5750	1	0.3307	-0.1005	1.25	1.0000
DE	0.7000	1	0.3307	0.0245	1.38	1.0000

Response 8 Table E: Final Equation in Terms of Actual Factors

Alcohol level (TN waste using AN)	=
+57.42364	
-0.887500	Substrate weight (g/cm³)
-0.837500	pH
-2.70625	Innoculum size (CFU/ml)
-1.57000	Temperature
-0.269010	Time (hrs)
+0.065000	Substrate weight (g/cm³) * pH
+0.015000	Substrate weight (g/cm³) * Innoculum size (CFU/ml)
+0.011000	Substrate weight (g/cm³) * Temperature
-0.000625	Substrate weight (g/cm³) * Time (hrs)
-0.300000	pH * Innoculum size (CFU/ml)
+0.070000	pH * Temperature
-0.007292	pH * Time (hrs)
+0.085000	Innoculum size (CFU/ml) * Temperature
+0.023958	Innoculum size (CFU/ml) * Time (hrs)
+0.005833	Temperature * Time (hrs)

Brix Level (Beans Husk with *Aspergillus niger isolate HEFAPhR*)

Response 9 Table A: Model Summary Statistics

Source	**Std. Dev.**	**R²**	**Adjusted R²**	**Predicted R²**	**PRESS**	
Linear	**0.7653**	**0.1581**	**0.0528**	**-0.1318**	**31.49**	**Suggested**
2FI	0.7508	0.3922	0.0883	-0.5426	42.93	
Quadratic	0.7499	0.4947	0.0905	-0.8371	51.12	
Cubic	0.7245	0.8114	0.1512	-6.5747	210.77	Aliased

The best model suggested by the computer is the linear model because it is the only model that maximized the Adjusted R² and the Predicted R².

Response 9 Table B: Lack of Fit Tests

Source	**Sum of Squares**	**df**	**Mean Square**	**F-value**	**p-value**	
Linear	**21.43**	**35**	**0.6122**	**1.53**	**0.3391**	**Suggested**
2FI	14.91	25	0.5965	1.49	0.3506	
Quadratic	12.06	20	0.6030	1.51	0.3446	
Cubic	3.25	5	0.6497	1.62	0.3038	Aliased
Pure Error	2.00	5	0.4000			

The selected model should have insignificant lack-of-fit

ANOVA for Linear model

Response 9 Table C: Bricks level (Beans Husk with *Aspergillus niger isolate HEFAPhR*)

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	4.40	5	0.8798	1.50	0.2109	Not significant
A-Substrate weight (g/cm³)	2.10	1	2.10	3.59	0.0654	
B-pH	0.1406	1	0.1406	0.2401	0.6268	
C-Innoculum size (CFU/ml)	1.10	1	1.10	1.88	0.1777	
D-Temperature	0.0025	1	0.0025	0.0043	0.9482	
E-Time (hrs)	1.05	1	1.05	1.79	0.1880	
Residual	23.43	40	0.5857			
Lack of Fit	21.43	35	0.6122	1.53	0.3391	Not significant
Pure Error	2.00	5	0.4000			
Cor Total	27.83	45				

Response 9 Table D: Coefficients in Terms of Coded Factors

Factor	Coefficient Estimate	df	Standard Error	95% CI Low	95% CI High	VIF
Intercept	2.12	1	0.1128	1.89	2.35	
A-Substrate weight (g/cm³)	-0.3625	1	0.1913	-0.7492	0.0242	1.0000
B-pH	0.0938	1	0.1913	-0.2929	0.4804	1.0000
C-Innoculum size (CFU/ml)	-0.2625	1	0.1913	-0.6492	0.1242	1.0000
D-Temperature	-0.0125	1	0.1913	-0.3992	0.3742	1.0000
E-Time (hrs)	-0.2563	1	0.1913	-0.6429	0.1304	1.0000

Response 9 Table E: Final Equation in Terms of Actual Factors

Bricks level (BH waste using AN)	=
+4.96739	
-0.072500	Substrate weight (g/cm³)
+0.093750	pH
-0.262500	Innoculum size (CFU/ml)
-0.002500	Temperature
-0.010677	Time (hrs)

Bioethanol Level (Beans Husk with *Aspergillus niger isolate HEFAPhR*)

Response 10 Table A: Model Summary Statistics

Source	Std. Dev.	R²	Adjusted R²	Predicted R²	PRESS	
Linear	**0.5293**	**0.1185**	**0.0083**	**-0.1845**	**15.06**	**Suggested**
2FI	0.5476	0.2923	-0.0615	-0.8084	22.99	
Quadratic	0.5857	0.3255	-0.2142	-1.4941	31.71	
Cubic	0.4941	0.8079	0.1357	-6.3053	92.87	Aliased

Focus on the model maximizing the Adjusted R² and the Predicted R², the linear model is the best and suggested model.

Response 10 Table B: Lack of Fit Tests

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Linear	**10.19**	**35**	**0.2912**	**1.44**	**0.3699**	**Suggested**
2FI	7.98	25	0.3193	1.58	0.3248	
Quadratic	7.56	20	0.3781	1.87	0.2534	
Cubic	1.43	5	0.2857	1.41	0.3578	Aliased
Pure Error	1.01	5	0.2027			

The selected model should have insignificant lack-of-fit.

ANOVA for Linear model

Response 10 Table C: Bioethanol Level (Beans Husk with *Aspergillus niger isolate HEFAPhR*)

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	1.51	5	0.3013	1.08	0.3886	not significant
A-Substrate weight (g/cm³)	0.0225	1	0.0225	0.0803	0.7783	
B-pH	0.1225	1	0.1225	0.4372	0.5123	
C-Innoculum size (CFU/ml)	0.0756	1	0.0756	0.2699	0.6062	
D-Temperature	0.0756	1	0.0756	0.2699	0.6062	
E-Time (hrs)	1.21	1	1.21	4.32	0.0442	
Residual	11.21	40	0.2802			
Lack of Fit	10.19	35	0.2912	1.44	0.3699	not significant
Pure Error	1.01	5	0.2027			
Cor Total	12.71	45				

Response 10 Table D: Coefficients in Terms of Coded Factors

Factor	Coefficient Estimate	df	Standard Error	95% CI Low	95% CI High	VIF
Intercept	2.04	1	0.0780	1.89	2.20	
A-Substrate weight (g/cm³)	-0.0375	1	0.1323	-0.3049	0.2299	1.0000
B-pH	-0.0875	1	0.1323	-0.3549	0.1799	1.0000
C-Innoculum size (CFU/ml)	0.0687	1	0.1323	-0.1987	0.3362	1.0000
D-Temperature	0.0687	1	0.1323	-0.1987	0.3362	1.0000
E-Time (hrs)	0.2750	1	0.1323	0.0076	0.5424	1.0000

Response 10 Table E: Final Equation in Terms of Actual Factors

Alcohol level (BH waste using AN)	=
+1.11848	
-0.007500	Substrate weight (g/cm³)
-0.087500	pH
+0.068750	Innoculum size (CFU/ml)
+0.013750	Temperature
+0.011458	Time (hrs)

Brix Level (Groundnut Shell with *Aspergillus niger isolate HEFAPhR*)

Response 11 Table A: Model Summary Statistics

Source	**Std. Dev.**	**R²**	**Adjusted R²**	**Predicted R²**	**PRESS**	
Linear	1.98	0.0223	-0.0999	-0.2559	201.06	
2FI	2.06	0.2070	-0.1895	-0.7283	276.68	
Quadratic	**2.04**	**0.3524**	**-0.1657**	**-1.0753**	**332.22**	**Suggested**
Cubic	2.03	0.7431	-0.1561	-2.8539	616.95	Aliased

Focus on the model maximizing the Adjusted R² and the Predicted R². The Quadratic term model is suggested.

Response 11 Table B: Lack of Fit Tests

Source	**Sum of Squares**	**df**	**Mean Square**	**F-value**	**p-value**	
Linear	124.30	35	3.55	0.5513	0.8644	
2FI	94.74	25	3.79	0.5882	0.8286	
Quadratic	**71.46**	**20**	**3.57**	**0.5546**	**0.8420**	**Selected**
Cubic	8.92	5	1.78	0.2767	0.9076	Aliased
Pure Error	32.21	5	6.44			

The selected model should have insignificant lack-of-fit.

ANOVA for Quadratic model

Response 11 Table C: Bricks level (Groundnut Shell with *Aspergillus niger isolate HEFAPhR*)

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	56.41	20	2.82	0.6802	0.8088	not significant
A-Substrate weight (g/cm³)	0.6006	1	0.6006	0.1448	0.7067	
B-pH	0.3906	1	0.3906	0.0942	0.7614	
C-Innoculum size (CFU/ml)	0.4556	1	0.4556	0.1099	0.7431	
D-Temperature	0.6806	1	0.6806	0.1641	0.6888	
E-Time (hrs)	1.44	1	1.44	0.3473	0.5610	
AB	0.0625	1	0.0625	0.0151	0.9033	
AC	0.1600	1	0.1600	0.0386	0.8459	
AD	0.4900	1	0.4900	0.1182	0.7339	
AE	0.1600	1	0.1600	0.0386	0.8459	
BC	12.25	1	12.25	2.95	0.0980	
BD	6.76	1	6.76	1.63	0.2134	
BE	1.69	1	1.69	0.4075	0.5290	
CD	3.06	1	3.06	0.7385	0.3983	
CE	3.24	1	3.24	0.7813	0.3852	
DE	1.69	1	1.69	0.4075	0.5290	
A^2	6.06	1	6.06	1.46	0.2380	
B^2	2.04	1	2.04	0.4916	0.4897	
C^2	0.1964	1	0.1964	0.0474	0.8295	
D^2	5.59	1	5.59	1.35	0.2568	
E^2	18.99	1	18.99	4.58	0.0423	
Residual	103.67	25	4.15			
Lack of Fit	71.46	20	3.57	0.5546	0.8420	not significant
Pure Error	32.21	5	6.44			
Cor Total	160.08	45				

Response 11 Table D: Coefficients in Terms of Coded Factors

Factor	Coefficient Estimate	df	Standard Error	95% CI Low	95% CI High	VIF
Intercept	5.53	1	0.8313	3.82	7.25	
A-Substrate weight (g/cm³)	-0.1938	1	0.5091	-1.24	0.8547	1.0000
B-pH	0.1562	1	0.5091	-0.8922	1.20	1.0000
C-Innoculum size (CFU/ml)	0.1687	1	0.5091	-0.8797	1.22	1.0000
D-Temperature	0.2062	1	0.5091	-0.8422	1.25	1.0000
E-Time (hrs)	0.3000	1	0.5091	-0.7485	1.35	1.0000
AB	-0.1250	1	1.02	-2.22	1.97	1.0000
AC	0.2000	1	1.02	-1.90	2.30	1.0000
AD	0.3500	1	1.02	-1.75	2.45	1.0000
AE	0.2000	1	1.02	-1.90	2.30	1.0000
BC	-1.75	1	1.02	-3.85	0.3470	1.0000
BD	-1.30	1	1.02	-3.40	0.7970	1.0000
BE	-0.6500	1	1.02	-2.75	1.45	1.0000
CD	0.8750	1	1.02	-1.22	2.97	1.0000
CE	0.9000	1	1.02	-1.20	3.00	1.0000
DE	0.6500	1	1.02	-1.45	2.75	1.0000
A^2	0.8333	1	0.6893	-0.5863	2.25	1.20
B^2	0.4833	1	0.6893	-0.9363	1.90	1.20
C^2	0.1500	1	0.6893	-1.27	1.57	1.20
D^2	0.8000	1	0.6893	-0.6197	2.22	1.20
E^2	1.47	1	0.6893	0.0553	2.89	1.20

Response 11 Table E: Final Equation in Terms of Actual Factors

Brix level (GSW waste using AN)	=
+7.25417	
-1.64375	Substrate weight (g/cm³)
+14.01458	pH
+2.06875	Innoculum size (CFU/ml)
-1.70875	Temperature
-0.579167	Time (hrs)
-0.025000	Substrate weight (g/cm³) * pH
+0.040000	Substrate weight (g/cm³) * Innoculum size (CFU/ml)
+0.014000	Substrate weight (g/cm³) * Temperature
+0.001667	Substrate weight (g/cm³) * Time (hrs)
-1.75000	pH * Innoculum size (CFU/ml)
-0.260000	pH * Temperature
-0.027083	pH * Time (hrs)
+0.175000	Innoculum size (CFU/ml) * Temperature
+0.037500	Innoculum size (CFU/ml) * Time (hrs)
+0.005417	Temperature * Time (hrs)
+0.033333	Substrate weight (g/cm³)²
+0.483333	pH²
+0.150000	Innoculum size (CFU/ml)²
+0.032000	Temperature²
+0.002561	Time (hrs)²

Bioethanol Level (Groundnut Shell with *Aspergillus niger isolate HEFAPhR*)

Response 12 Table A: Model Summary Statistics

Source	**Std. Dev.**	**R^2**	**Adjusted R^2**	**Predicted R^2**	**PRESS**	
Linear	6.09	0.1376	0.0298	-0.1827	2032.90	
2FI	5.95	0.3821	0.0731	-0.6884	2902.08	
Quadratic	**5.88**	**0.4979**	**0.0963**	**-1.0045**	**3445.25**	**Suggested**
Cubic	4.55	0.8794	0.4575	-6.6237	13103.65	Aliased

The Quadratic model is the suggested and best model because it maximizes the Adjusted R^2 and the Predicted R^2.

Response 12 Table B: Lack of Fit Tests

Source	**Sum of Squares**	**df**	**Mean Square**	**F-value**	**p-value**	
Linear	1479.85	35	42.28	84.11	< 0.0001	
2FI	1059.58	25	42.38	84.32	< 0.0001	
Quadratic	**860.41**	**20**	**43.02**	**85.58**	**< 0.0001**	**Suggested**
Cubic	204.69	5	40.94	81.44	< 0.0001	Aliased
Pure Error	2.51	5	0.5027			

The selected model should have insignificant lack-of-fit.

ANOVA for Quadratic Model

Response 12 Table C: Alcohol level (Groundnut Shell with *Aspergillus niger isolate HEFAPhR*)

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	855.87	20	42.79	1.24	0.3021	Not significant
A-Substrate weight (g/cm³)	111.30	1	111.30	3.22	0.0846	
B-pH	0.0056	1	0.0056	0.0002	0.9899	
C-Innoculum size (CFU/ml)	0.1056	1	0.1056	0.0031	0.9563	
D-Temperature	0.1406	1	0.1406	0.0041	0.9496	
E-Time (hrs)	124.88	1	124.88	3.62	0.0687	
AB	0.6400	1	0.6400	0.0185	0.8928	
AC	0.3600	1	0.3600	0.0104	0.9195	
AD	0.6400	1	0.6400	0.0185	0.8928	
AE	408.04	1	408.04	11.82	0.0021	
BC	6.25	1	6.25	0.1811	0.6741	
BD	1.32	1	1.32	0.0383	0.8464	
BE	0.3600	1	0.3600	0.0104	0.9195	
CD	2.10	1	2.10	0.0609	0.8071	
CE	0.2500	1	0.2500	0.0072	0.9329	
DE	0.3025	1	0.3025	0.0088	0.9262	
A^2	59.85	1	59.85	1.73	0.1999	
B^2	8.26	1	8.26	0.2393	0.6290	
C^2	7.98	1	7.98	0.2312	0.6348	
D^2	2.54	1	2.54	0.0736	0.7884	
E^2	77.35	1	77.35	2.24	0.1469	
Residual	862.92	25	34.52			
Lack of Fit	860.41	20	43.02	85.58	< 0.0001	significant
Pure Error	2.51	5	0.5027			
Cor Total	1718.79	45				

Response 12 Table D: Coefficients in Terms of Coded Factors

Factor	Coefficient Estimate	df	Standard Error	95% CI Low	95% CI High	VIF
Intercept	4.37	1	2.40	-0.5731	9.31	
A-Substrate weight (g/cm³)	-2.64	1	1.47	-5.66	0.3875	1.0000
B-pH	0.0188	1	1.47	-3.01	3.04	1.0000
C-Innoculum size (CFU/ml)	0.0813	1	1.47	-2.94	3.11	1.0000
D-Temperature	0.0938	1	1.47	-2.93	3.12	1.0000
E-Time (hrs)	2.79	1	1.47	-0.2313	5.82	1.0000
AB	-0.4000	1	2.94	-6.45	5.65	1.0000
AC	0.3000	1	2.94	-5.75	6.35	1.0000
AD	0.4000	1	2.94	-5.65	6.45	1.0000
AE	-10.10	1	2.94	-16.15	-4.05	1.0000
BC	-1.25	1	2.94	-7.30	4.80	1.0000
BD	-0.5750	1	2.94	-6.63	5.48	1.0000
BE	-0.3000	1	2.94	-6.35	5.75	1.0000
CD	0.7250	1	2.94	-5.33	6.78	1.0000
CE	0.2500	1	2.94	-5.80	6.30	1.0000
DE	0.2750	1	2.94	-5.78	6.33	1.0000
A^2	2.62	1	1.99	-1.48	6.71	1.20
B^2	-0.9729	1	1.99	-5.07	3.12	1.20
C^2	-0.9563	1	1.99	-5.05	3.14	1.20
D^2	-0.5396	1	1.99	-4.64	3.56	1.20
E^2	2.98	1	1.99	-1.12	7.07	1.20

Response 16 Table E: Final Equation in Terms of Actual Factors

Alcohol level (GSW waste using AN)	=
-182.54375	
+2.11000	Substrate weight (g/cm³)
+26.68958	pH
+14.30625	Innoculum size (CFU/ml)
+0.843750	Temperature
+0.590885	Time (hrs)
-0.080000	Substrate weight (g/cm³) * pH
+0.060000	Substrate weight (g/cm³) * Innoculum size (CFU/ml)
+0.016000	Substrate weight (g/cm³) * Temperature
-0.084167	Substrate weight (g/cm³) * Time (hrs)
-1.25000	pH * Innoculum size (CFU/ml)
-0.115000	pH * Temperature
-0.012500	pH * Time (hrs)
+0.145000	Innoculum size (CFU/ml) * Temperature
+0.010417	Innoculum size (CFU/ml) * Time (hrs)
+0.002292	Temperature * Time (hrs)
+0.104750	Substrate weight (g/cm³)²
-0.972917	pH²
-0.956250	Innoculum size (CFU/ml)²
-0.021583	Temperature²
+0.005169	Time (hrs)²

Observed Plot *Saccharomyces cerevisiae*

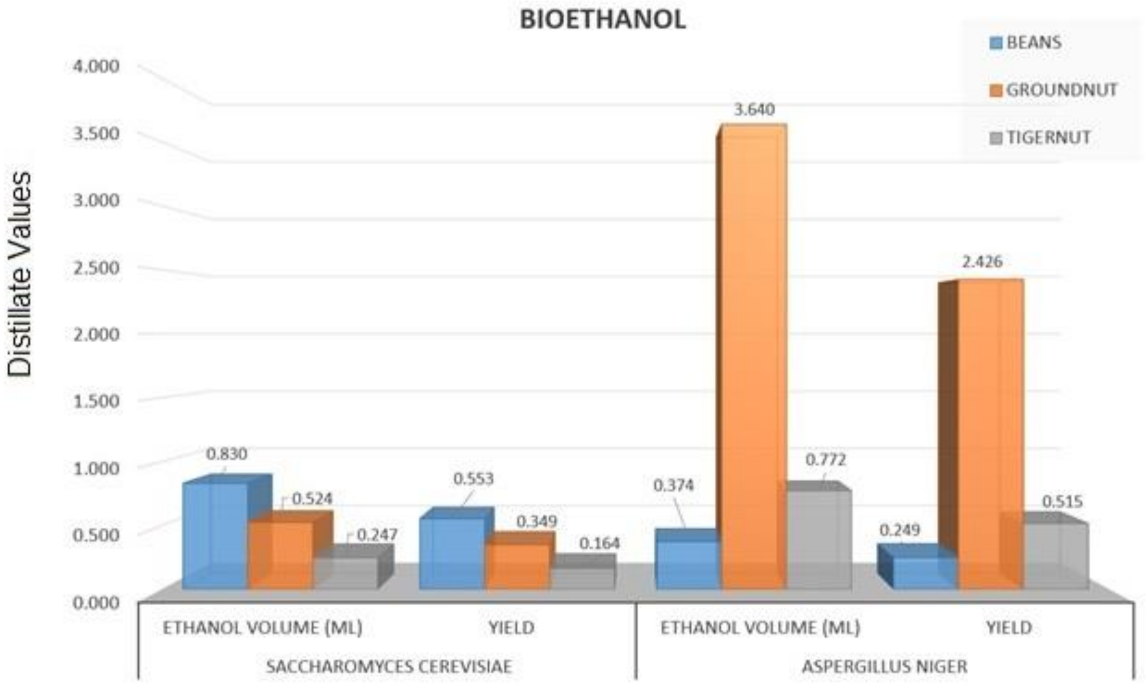
S/N	Time	Tiger nut Waste		Beans Husk		Groundnut Shell	
FLASK 1	48	1.8	1.4	3.8	2.8	1.6	3.4
FLASK 2	48	2.2	1.8	5.2	2.5	2.2	3.8
FLASK 3	48	1.2	1.6	6.8	3.0	4.0	2.6
FLASK 4	96	1.8	1.8	2.0	3.6	1.8	3.8
FLASK 5	72	1.4	1.6	2.8	2.0	2.0	3.4
FLASK 6	72	0.8	1.6	2.2	2.0	2.2	2.4
FLASK 7	72	1.8	1.2	6.0	3.2	2.8	2.2
FLASK 8	48	2.2	1.6	4.6	2.6	2.0	3.6
FLASK 9	72	1.0	2.4	1.8	2.0	1.6	3.4
FLASK 10	96	1.2	2.6	1.8	3.8	1.0	3.2
FLASK 11	96	2.2	1.4	5.0	3.0	3.6	2.6
FLASK 12	72	1.2	1.8	2.2	2.8	1.6	3.4
FLASK 13	72	2.2	1.4	5.8	3.0	4.0	2.6
FLASK 14	48	1.8	1.2	4.0	2.0	2.0	4.0
FLASK 15	72	1.7	2.4	4.0	3.4	2.0	3.6
FLASK 16	96	1.4	2.0	2.4	4.0	1.8	3.2
FLASK 17	72	1.0	2.2	2.6	2.2	1.2	3.0
FLASK 18	72	1.6	1.8	3.2	2.2	1.6	3.0
FLASK 19	72	2.2	1.2	5.4	2.6	3.8	2.6
FLASK 20	48	1.8	1.2	4.0	2.2	1.8	3.8
FLASK 21	72	1.6	1.6	2.0	2.2	1.6	3.4
FLASK 22	96	0.2	2.4	1.0	3.6	1.8	3.4
FLASK 23	72	2.0	0.9	5.0	2.4	4.6	2.6
FLASK 24	48	1.4	1.2	4.0	2.2	1.6	3.2
FLASK 25	72	2.0	1.2	5.0	2.6	3.6	2.2
FLASK 26	72	1.0	1.6	2.6	2.2	2.0	3.2
FLASK 27	72	1.4	1.6	2.8	2.8	2.0	3.0

FLASK 28	72	0.6	1.8	2.2	2.4	1.2	3.6
FLASK 29	96	0.2	1.6	1.6	4.2	1.6	4.0
FLASK 30	72	0.8	1.6	2.4	2.2	1.8	3.6
FLASK 31	72	1.5	1.4	3.6	2.4	1.4	3.4
FLASK 32	72	0.6	1.6	4.4	3.2	2.0	4.0
FLASK 33	72	1.4	1.6	2.6	2.4	1.8	4.0
FLASK 34	72	0.8	2.0	2.8	3.2	1.6	3.8
FLASK 35	72	1.0	1.8	3.0	2.6	2.0	4.2
FLASK 36	72	1.0	1.4	2.8	2.8	2.2	4.0
FLASK 37	48	1.4	1.6	2.8	2.2	1.6	3.6
FLASK 38	96	0.8	2.4	2.2	3.4	1.2	3.8
FLASK 39	72	1.0	2.0	2.2	2.4	1.4	3.6
FLASK 40	72	1.0	2.2	2.6	2.8	2.2	3.6
FLASK 41	72	1.0	1.6	2.6	3.8	1.6	3.4
FLASK 42	72	1.2	1.8	2.0	0.6	1.8	4.2
FLASK 43	72	1.0	1.6	2.6	3.4	2.0	4.4
FLASK 44	96	1.2	2.4	2.2	5.8	1.6	3.8
FLASK 45	72	0.6	0.8	1.8	3.8	1.4	3.6
FLASK 46	72	1.8	1.2	3.2	3.8	2.8	1.6

Observed Plot *Aspergillius niger isolate H*

S/N	Time	Tiger nut Waste		Groundnut Shell Waste		Beans Husk Waste	
FLASK 1	48	4.0	6.0	8.0	4.8	2.6	1.6
FLASK 2	48	4.2	6.6	7.4	4.4	3.3	2.4
FLASK 3	48	3.5	6.8	8.6	4.8	4.0	2.0
FLASK 4	96	4.0	5.9	7.2	5.6	0.8	1.6
FLASK 5	72	7.4	5.0	6.8	4.6	2.0	2.6
FLASK 6	72	6.6	4.4	7.0	4.7	0.8	1.4
FLASK 7	72	6.2	3.8	5.8	3.4	0.8	1.0
FLASK 8	48	9.1	5.3	9.2	5.4	2.6	1.8
FLASK 9	72	6.0	4.6	7.8	4.7	1.4	1.6
FLASK 10	96	3.8	4.8	8.0	5.6	1.8	2.4
FLASK 11	96	7.0	4.0	5.4	3.4	2.0	1.6
FLASK 12	72	9.8	4.9	10.0	6.0	1.6	1.8
FLASK 13	72	7.0	4.5	5.0	3.6	2.0	1.4
FLASK 14	48	7.0	4.2	8.0	4.8	2.0	1.8
FLASK 15	72	7.8	5.6	8.6	5.7	2.4	2.6
FLASK 16	96	6.0	4.6	8.0	5.8	2.0	3.8
FLASK 17	72	5.0	3.8	8.2	5.2	2.0	2.4
FLASK 18	72	6.0	4.2	7.6	4.8	3.0	2.6
FLASK 19	72	8.0	4.5	4.8	3.6	2.4	1.6
FLASK 20	48	7.4	4.3	8.2	46.0	2.4	2.6
FLASK 21	72	8.0	5.4	3.6	3.0	1.8	2.0
FLASK 22	96	5.4	4.6	3.6	3.6	2.0	1.8
FLASK 23	72	7.8	4.2	1.2	1.8	3.4	1.9
FLASK 24	48	7.0	3.8	1.6	4.6	3.0	2.4
FLASK 25	72	8.0	4.2	4.4	3.6	3.8	2.2
FLASK 26	72	5.8	4.2	6.6	4.2	1.0	1.6
FLASK 27	72	7.0	4.7	7.6	4.9	2.8	2.2

FLASK 28	72	6.0	4.0	8.0	5.0	1.9	2.9
FLASK 29	96	4.7	4.8	8.0	5.6	2.6	2.0
FLASK 30	72	7.0	4.7	8.0	4.8	2.6	1.4
FLASK 31	72	8.0	5.6	8.3	5.6	2.2	2.0
FLASK 32	72	7.2	4.8	8.4	5.9	1.8	1.8
FLASK 33	72	7.2	3.5	5.0	3.6	2.6	2.8
FLASK 34	72	6.0	4.0	6.4	4.0	2.0	2.8
FLASK 35	72	8.1	5.3	7.2	4.7	2.6	2.2
FLASK 36	72	6.0	4.4	9.0	5.6	2.4	2.6
FLASK 37	48	7.0	4.2	5.6	3.3	2.8	1.9
FLASK 38	96	4.6	4.8	6.0	4.6	1.0	2.2
FLASK 39	72	6.0	4.5	6.4	4.0	1.2	1.4
FLASK 40	72	7.0	4.8	7.6	4.8	1.0	1.6
FLASK 41	72	5.6	4.2	6.8	4.4	1.8	2.0
FLASK 42	72	6.8	4.7	5.5	3.7	1.0	1.8
FLASK 43	72	7.4	4.5	8.6	5.7	2.4	1.9
FLASK 44	96	4.4	4.8	8.2	5.6	2.0	2.8
FLASK 45	72	6.8	4.6	5.4	3.8	1.0	1.4
FLASK 46	72	6.8	3.6	7.8	3.6	2.8	1.8



Comparison of result yield of bioethanol production from agro waste with *Saccharomyces cerevisiae* and *Aspergillus niger isolate HEFAPhR*