PRODUCTION OF BIO ETHANOL FROM SELECTED AGRO WASTE USING *SACCHAROMYCES CEREVISIAE* STRAIN CBS 1171 AND *LACTOBACILLUS DELBRUECKII* STRAIN MN945906

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A THESIS SUBMITTED TO THE POSTGRADUATE SCHOOL, FEDERAL UNIVERSITYOF TECHNOLOGY, MINNA, NIGERIA IN PARTIAL FULFILMENT OF THE REQUIREMENT FOR THE AWARD OF DEGREE OF MASTERS OF TECHNOLOGY (M.Tech) IN MICROBIOLOGY (ENVIRONMENTAL MICROBIOLOGY)

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ABSTRACT

The production of bioethanol from selected agro waste (banana and cassava) peels was examined using *Saccharomyces cerevisiae* strain CBS 1171and *Lactobacillus delbrueckii* strain MN945906. The results of the proximate analysis shows that carbohydrate content were 67.85±0.23%, nitrogen (0.21%), potassium (64.7%), and phophorus (31.3%). The waste was hydrolyzed with *Aspergillus niger* strain MN945947 and *Zymomonas mobilis* strain MN945907 into 20g, 30g, and 40g, of the substrate for 7 days. The hydrolysed and filtered extracts were fermented with *S. cerevisiae* strain CBS 1171and *L. delbrueckii* strain MN945906 for 5 days. The fermented product was purified by distillation and ethanol collected at 78^{o}C. When combination of *S. cerevisiae*, and *L.delbrueckii* was use for fermentation at 40g, banana+cassava recorded (21.94%), cassava peel (25.99%) and banana (10.32%). When *S. cerevisiae* were used alone for fermentation cassava peel recorded (22.98%), banana (9.36%), and (21.08%) for banana+cassava peel. When *L. delbrueckii* was used alone for fermentation cassava peel recorded (10.61%), banana (8.24%) and banana+cassava peel (15.79%). However, when 30g of substrate were used, banana peel recorded 9.56%, cassava peel had 20.92% and banana+cassava peel recorded 20.86%. At 20g of substrate banana peel recorded 8.76%, cassava peel had 14.14% and banana+cassava peels yielded 13.21%. The average percentage weight and volume for banana+cassava was 13.83% (15.7g/cm^3), cassava peel 12.26% (14.17g/cm^3) and banana peel had 6.8% (18.24g/cm^3).The result revealed that cassava +banana peels a better production of bioethanol using the combination of *S. cerevisiae* and *L. delbruecki.*

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CHAPTER ONE

1.0 INTRODUCTION

1.1 Background to the Study

Renewable energy has become talk of the day worldwide because of concerns on declining supplies of fossil fuels, rising in population and industrialization actuates ever increasing demand of fuels. All over the world, governments have encouraged the use of another source of energy for looming energy crisis. The Federal Government of Nigeria has concluded plans to invest 400 Billion Naira (3.5 Billion US dollars) in Jigawa State for ethanol production programme in other to diversify its sources of revenue (Isah et *al.,* 2019). The US President announced in his state of union speech, an agenda to develop alternative energies such as bioethanol fuel from grains and cellulose in order to terminate Americans dependence on oil (Isah *et al.,* 2019). Bioethanol is the conversion of simple sugar into ethanol and carbon dioxide (CO_2) microbiologically (Oyeleke *et al.,* 2012). These can be used as petrol substitute for motor vehicle. These process is carried out by anaerobic fermentation in accordance with Embden-meyerhoff pathway (EMP) catalysed by enzymes produced by bacteria and fungi. The fermentation process is similar to the one used in alcoholic beverages. It involves the

break down complex sugars into more simple sugars, producing ethanol by using yeast and heat (Oyeleke *et al.,* 2009). The starchy materials are first hydrolysed to reducing sugars and the hydrolysate are subsequently fermented with the required fungi or bacteria to produce ethanol (Nwabanne and Aghadi 2018). So that at end of fermentation process, some part of the sugar is assimilated by the organism while other part is transformed into glycerol, acetaldehydes and lactic acid (Zhan *et al.,* 2010).

Nigeria is the highest producer of cassava in the world, producing higher than Brazil, Thailand and Indonesia (Izah and Ohimain 2015). Cassava and it's by products are renewable source of energy that pose no threat to the environment and has been used by several researchers in the production of bioethanol. The significant use of agricultural wastes to produce bioethanol is motivated by numerous reasons such as: global search for alternative source of energy and transportation fuel to replace the depleting fossil fuel (Virgínio *et al.,* 2018). Ethanol is pollution free, biodegradable, renewable, cause no climate change. It has been estimated that 2.96 million metric tons of cassava peels is generated annually in Nigeria from processing cassava to various food products (Safarian *et al.,* 2019). These enormous wastes that constitute 20 – 35% of the weight of cassava tuber are discarded with consequent implication of environmental pollution. In this regard it has become necessary to convert this waste to useful end products in other not to pose threat to the environment (Gani *et al.,* 2018). Banana peels are not considered very useful and are therefore dried, ground, pelletized, and sold to the feed manufacturers at a low price. Although banana peel is a fruit residue, it accounts for about 30-40% of the total fruit weight and contains carbohydrates, proteins, and fibre in significant amounts. Since banana peels contain lignin in low quantities, it could

serve as a good substrate for production of value added products like ethanol (Chechet, 2016).

1.2 Statement of the Research Problem

The question of sustainable and judicious use of energy resources has become noticeable in the past few years. Most countries relied mainly on fossil fuels, there is the need for diversification into other source of energy because the future of petroleum products is uncertain (Alemayehu, 2015). The Inappropriate municipal solid waste collection and disposal creates a range of environmental problems in this country (Arabia and Wankhade, 2013). A considerable amount of waste ends up in open dumps or drainage system, threatening both surface water and ground water quality and also causes flooding, which provides a breeding ground for diseases-carrying pests. However unless these waste are changed or converted into some useful products like bioethanol.

1.3 Justification for the Study

The production of commercial ethanol is costly, there is the need to use cheap agricultural wastes to produce ethanol so as to maximize profit by the industrialist. The Nigerian economy relied mostly on revenue from fossil fuel which is non-renewable and hence gets depleted on daily basis. Authoritatively, about 2.3 - 2.5 million barrels of crude oil is

produced daily and geologists have cautioned that the Nigerian oil reserves may be totally depleted in 37 years unless alternative sources of fuel are introduced. Meanwhile the developed nations, which are the major consumers of Nigerian crude oil, are making serious efforts to find alternative sources of fuel. Lots of attention is focused on the use of the abundant and renewable waste resources to generate energy in form of bio-fuel (Oyeleke *et al.,* 2012).The aim of this work is to investigate the possibility of using and transforming cassava and banana peel wastes to something valuable, namely ethanol using the fungus *Saccharomyces cerevisiae* as ethanol transforming organism with *Lactobacillus delbrueckii* there by contributing towards alternative energy supply as well as creating an employment opportunity.

1.4 Aim and Objective of Research

The aim of this study is to determine the production of ethanol from selected agro waste (cassava and banana peel) using Saccharomyces *cerevisiae* strain CBS1171and Lactobacillus *delbrueckii* strain MN945906.

The objectives were to:

I. isolation and biochemical characteristics of the isolate from fresh palm wine, cow milk and soil.
II. molecular characteristics of the isolate.
III. screening for enzymatic activity on the isolates.
IV. determine mineral analysis of cassava and banana peels.
V. determine proximate analysis on cassava and banana peels.
VI. determine reducing sugar and pH test on cassava and banana peels.
VII. determine the enzyme hydrolysis of banana peels and cassava peels using *Aspergillus niger* and *Zymomonas mobilis.*
VIII. produce bioethanol from banana peels and cassava peels through fermentation using

Saccharomyces cerevisiae and *Lactobacillus delbrueckii*.

CHAPTER TWO

2. 0 LITERATURE REVIEW

Bioethanol production is usually accomplished by microbial conversion of carbohydrates present in agricultural products to ethanol. Owing to depleting reserves and competing industrial needs of petrochemical feed stocks, there is global emphasis in ethanol production by microbial fermentation process. Increased yield of ethanol production by microbial fermentation depends on the use of ideal microbial strain, suitable fermentation substrate and fitting process (Bekele *et al.,* 2015). Yeasts such as *Saccharomyces cerevisiae* is one of the most important microorganisms in the production of bio-ethanol due to its high ethanol yield, high tolerance to ethanol concentration, high selectivity, low accumulation of by-products, high fermentation rate, good tolerance to substrate concentrations and lower pH value (Bekele *et al.,* 2015).

Several authors Oyeleke *et al.,* (2012) Duhan *et al*., (2013) combined *Saccharomyces cerevisiae* with other group of saccharifying fungi such as *Aspergillus species, Zymomonas mobilis, Kluyveromyces* spp., *Trichoderma* spp., *Gloeophyllum sepiarium* and *Pleurotus*

ostreatus to enhance production of bio-ethanol. The study of Oyeleke, *et al*., 2012 showed that culturing of an efficient sugar fermenter such as *Saccharomyces cerevisiae* with an *Aspergillus* species in a starch medium would prevent accumulation of inhibitory concentrations of reducing sugar and hence enhance the amylolytic activity, the amount of starch metabolizable, and the total ethanol. In such a case *Aspergillus* species hydrolyze starch to glucose, and *S. cerevisiae*, which is non amylolytic ferments glucose to ethanol under anaerobic condition. Yeast is a facultative anaerobe. In an aerobic environment, it converts sugars into carbon dioxide and water. In an anaerobic environment, it converts sugars into carbon dioxide and ethanol. Thus, for an ethanol industry, it is important to exclude significant oxygen from its system.

The commonest ways of producing ethanol is fermentation of feed stocks which are rich in sugar or starch such as sugarcane, sugar beet, sweet sorghum, corn and cassava (Alemayehu, 2015). However, the major disadvantage of this process is that most of these crops are food crops and tend to increase the cost of production. In order to make the fermentation method cost effective and to meet the great demand for ethanol, research studies are now being directed in two areas namely, the production of ethanol from cheaper raw materials and the study of new microorganisms or yeast strains efficient in ethanol production (Alemayehu, 2015). Inexpensive raw materials such as agricultural wastes, municipal and industrial wastes can be used to produce ethanol (Alemayehu, 2015).Cassava are one of the alternative feedstock utilized for ethanol production (Duhan *et al*., 2013).

2.1. Bioethanol

The ethanol obtained from biomass based waste materials or renewable sources is called bioethanol. It can be used as a fuel, chemical feedstock, and solvent in various industries. It has certain advantages over petroleum substitutes, viz., alcohol can be produced from a number of renewable resources, alcohol as fuel burns cleaner than petroleum this aspect is

environmentally more acceptable. It is biodegradable and thus, keeps a check on pollution and it is far less toxic than fossil fuels (Domínguez *et al.,* 2014).

Ethanol is a clear, colourless liquid with a characteristic, agreeable odour. In dilute aqueous solution, it has somewhat sweet flavour, but in more concentrated solutions it has a burning taste. Ethanol, CH_3CH_2OH, is an alcohol, a group of chemical compounds whose molecules contain a hydroxyl group, - OH, bonded to a carbon atom. The word alcohol derives from Arabic al-kuhul, which denotes a fine powder of antimony used as an eye makeup. Alcohol originally referred to any fine powder, but medieval alchemists later applied the term to the refined products of distillation, and this led to the current usage (Wondal, 2012).

Ethanol has been made since ancient times by the fermentation of sugars. All beverage ethanol and more than half of industrial ethanol is still made by this process. Simple sugars are the raw material. Zymase, an enzyme from yeast, changes the simple sugars into ethanol and carbon dioxide. The fermentation reaction, symbolized by the simple equation is actually very complex, impure cultures of yeast produce varying amounts of other substances, including glycerine and various organic acids.

$C_6H_{12}O_6 \longrightarrow 2CH_3CH_2OH + 2CO_2$

(Wondal, 2012).

2.1.1 Physicochemical characteristic of bioethanol

Bioethanol is a transparent, colourless liquid with pleasant odour. The taste of ethanol is varies according to its concentration; when it is diluted has sweet flavour and has a burning taste if it is concentrated. It is the second member alcoholic group which contain hydroxyl group. The melting and boiling points of ethanol are -114.1^oC and 78.5^oC, respectively. The density of ethanol is 0.789 g/mL at 20^oC. It forms homogeneous mixture with both types of solvents i.e. polar as well as non-polar solvents. It is also used as organic solvent and utilized in perfumes, paints, lacquer and explosive industry. Ether is formed after the dehydration of

ethanol. It can be further oxidized to acetaldehyde and then into acetic acid. The detailed physicochemical properties of bioethanol are shown in the Table 1. Alcoholic solutions containing non-volatile substances are called tinctures and solution having volatile substances is called spirit (Kumar *et al.*, 2019).

Table 2.1 Physicochemical characteristics of ethanol

Parameter	Characteristics
Molecular formula	C_2H_5OH
Molecular mass	46.07 g/mol
Appearance	Colorless liquid
Water solubility	Between –117°C and 78°C
Density	0.789 kg/l
Boiling temperature	78.5°C
Freezing point	–117°C
Flash point	12.8°C
Ignition temperature	lowest temperature of ignition
Explosion limits	Lower 3.5% (v/v) Upper 19%(v/v)
Vapour pressure at 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
PKa	15.9
Viscosity	1.200 mPa.s (20°C)
Refractive index ($_nD$)	1.36 (25°C)
Octane number	99

Source: Kumar *et al.*(2019)

2.1.2 Historical background of bioethanol

Bioethanol is a readily available clean fuel for combustion engines made from plant-based feed stocks. It produces considerably lower emissions on combustion and it only releases the same amount of carbon dioxide as plants bound while growing. Ethanol or ethyl alcohol has existed since the beginning of recorded history; the ancient Egyptians produced alcohol by naturally fermenting vegetative materials. Also in ancient times, the Chinese discovered the

art of distillation, which increases the concentration of alcohol in fermented solutions. Ethanol was first prepared synthetically in 1826, through the independent effort of Henry Hennel Ford in Britain. Michael Faraday prepared ethanol by the acid-catalysed hydration of ethylene in 1828, in a process similar to that used for industrial synthesis of ethanol today (Danmaliki *et al.,*2016). In 1893, Henry Ford built a small one-cylinder gasoline combustion engine after which he invented a quadricycle; the first horseless carriage that is propelled by bioethanol powered engine. Ford later turns his interest to automobiles where he designs a delivery wagon and finally he invented the first Ford Motor Company Automobile which was designed to use corn alcohol called ethanol. After the incorporation of this company, the association of license automobile manufacturers threatened to put Ford out since he was not a licensed manufacturer; however, Ford fought the claim and won which open doors for the rapid growth of automobile industries. Ethanol was used as fuel for lamp in United States in 1840, but a tax levied on industrial alcohol during the civil war made this use uneconomical, but this tax was repelled in 1906. In 1907, Henry Ford reintroduced ethanol to the Americans motoring public by producing his first vehicle to run on ethanol (Otulugbu, 2012).

The most common substrate used for nearly 99% of ethanol production in the United States today is starch from agricultural crops, primarily corn (Danmaliki *et al.,* 2016).In 1940s the first fuel ethanol plant was built in the U.S. army built and operated an ethanol plant in Omaha, Nebraska, to produce fuel for the army and for regional fuel blending. Major quantities were not manufactured until the 1970s due to low cost of gasoline between 1940s and 1970s; however, the ethanol industry began to re-emerge when ethanol was used as a fuel extender during gasoline shortages.(Danmaliki *et al.,* 2016)

Brazil kept bioethanol production programme alive by investing heavily on renewable fuels leading to the development of extensive bioethanol industry and also the number of ethanol running cars increased to about 90% of all the new cars sold in Brazil there by making the

country the biggest market for an alternative fuel in the world, in 1988 ethanol began to be added to gasoline in order to reduce CO_2 emission. However, on market conditions, all fuels are required to be blended with 20-25% of ethanol. As the production has increased the effect of biofuel on agricultural markets and the environment has become increasingly important topics, yet much uncertainty still remains. Biofuel has the potential to displace the use of petroleum as a transportation fuel and lower toxic emissions, the evolution of new biofuel production technologies could help alleviate some of the concerns regarding the use of food for fuel by facilitating the use of non-food feedstock's, and could alleviate some of the environmental concerns associated with grain ethanol production. In particular, cellulosic ethanol is believed to hold great promise in this regard, even though there are currently no commercial scale plants in the United State (Oso *et al.,* 2018).

2.2 Uses of Bioethanol

2.2.1 Ethanol as disinfectants

Alcohols are important disinfectants; they evaporate quickly without leaving any residue. They are capable of dissolving lipids which makes them effective against lipid wrapped viral and bacteria cells but are ineffective against spores. They are inexpensive and relatively easy to handle, although their vapours are flammable, it is usually used in concentrations of 70% because higher concentration evaporates too quickly and lower concentration are ineffective (Pooja *et al.,* 2018)

2.2.2 Ethanol as domestic lighting agent

Alcohol burning stoves based on ethanol can be used for cooking or water boiler for households, institutions and industries. Ethanol that are produced from plant biomass has the advantage of not producing air pollution problems of simple biomass burning for cooking purpose as ethanol produces a higher heat flux with no soot or smoke, cooking and hot water

production can take pace faster and pollution free. The equipment required for ethanol burning stoves is similar to existing kerosene stoves. Some ethanol stoves are made from stainless steel in order to minimize corrosion (Pooja *et al.,* 2018).

2.2.3 Ethanol as transportation fuel

Bioethanol is a product of microbial fermentation as opposed to synthetic ethanol produced from petrochemical sources. It is obtained from fermentation of sugars and used as biofuel for internal combustion engines or blend with petroleum. Ethanol has been used as a motor fuel since cars were first manufactured. It is a fuel well-suited for petrol engines; it has a clean combustion process resulting in lower emissions of particulate matter, hydrocarbons and carbon monoxide.

The greatest benefit of using bioethanol is the reduction in CO_2 emissions based on life-cycle processes. Bioethanol is a perfect transportation fuel that has high compression ratios to internal combustion engines because of it high octane ratio compares to petroleum. This low volatility enhances engine output per cycle and prevents automobile engine from getting knock. Although, vehicles running on pure ethanol have high fuel consumption rate of one litre per kilometre that is10-20% less than petrol, the fact that the by-product of its combustion could be used by plants for growth makes it a neutral carbon fuel (Pooja *et al.,* 2018).

Bioethanol has been found to have a potential to decrease greenhouse gases, depending on the production method. Bioethanol represents the best alternative transportation fuel because it uses is projected to increase significantly and remain high. Plant biomass when used as transportation fuel, it helps to keep engines running smoothly without the need for lead or other chemical additives (Graeme *et al.,* 2010).

2.3 Bioethanol Production

The basic steps for large scale production of ethanol are Milling and hydrolysis, Microbial fermentation and Distillation.

2.3.1 Milling and hydrolysis

Milling and hydrolysis involves the breakdown of starch molecules into glucose by enzymatic process. Prior to fermentation, some crops require saccharification or hydrolysis of carbohydrates such as starch or cellulose in to sugars (Chechet, 2016).

2.3.2 Fermentation

Fermentation is the process that converts glucose to ethanol. Ethanol is produced by microbial fermentation of the sugar; microorganism will only work directly with sugars. Two major components of plants are; starch and cellulose, both are made up of sugars and can be converted to sugars for fermentation. Currently, only the sugar (for example, sugarcane) and starch (for instance, corn) portions can be economically converted. However, there is much activity in the area of cellulosic ethanol, where the cellulosic part is broken down to sugars and subsequently converted to ethanol (Chechet, 2016).

2.3.3 Distillation

Further treatment in order to burn in combination with gasoline in gasoline engines, dehydration of the ethanol to be useable as a fuel must be done. Most of the water is removed by distillation, but the purity is limited to 95-96% due to the formation of a low-boiling water ethanol zoetrope. 95.5% v/v ethanol, 3.3% v/v water mixture may be used as a fuel alone, but unlike anhydrous ethanol, is immiscible in gasoline, so the water fraction is typically removed (Kravchenko *et al*., 2014).

2.4 Bio-Ethanol Production in Nigeria

Nigeria being eager to meet up with the demand of bio-fuels has a policy to meet ten percent bio-ethanol content in fuel by 2020 (Halilu, 2008). In pursuance of this policy, the Chief Olusegun Obasanjo's regime marked thousands of hectares of virgin land for cultivation of cassava to supply feedstock for production of bioethanol. The cassava, as it was called, did not however, succeed. In a new development, Global Bio-fuels Limited has embarked on a project which use about 10,000 hectares of virgin land (forest and grasslands). Covering seven states (Osun, Oyo, Kwara, Ondo, Ekiti, Niger and Kogi) in Nigeria, to cultivate sweet sorghum for bioethanol fuel production (Azih, 2007). Global Bio-fuels Limited plans set up seven plants each valued at over 3 billion U.S dollars (345billion naira) in the seven states, to produce about 1 million litres of ethanol per plant on an ethanol fuel production project in Nigeria. Nigerian National Petroleum Cooperation (NNPC) as at 2006 has so far worked out plans to acquire farmlands in Anambra, Benue, and Cross River for large scale cultivation of cassava. It has acquired a large plantation site of over 20,000 hectares at Agufa village for the large scale production of cassava and sugarcane in Jigawa State. In addition, the venture will enable the Jigawa Government to acquire world-class technology and farm management best practices in the large scale production of sugarcane (Azih, 2007).

2.5 Global Bioethanol Production

World production of ethanol was 14.1 billion gallons in 2007 and 65.7 billion litres in 2008 and it is believed that it will soon exceed 100 billion litres with the largest increase in production in United States of America and Brazil. One of the reasons is that attention has been globally concentrated to ethanol production in the quest for biofuel. United States is producing 46% of the world ethanol and Brazil is producing 37% making them the highest producer of ethanol in the world. There is a continuous increase in global demand for bio-ethanol in the market for this essential commodity is expected to reach100 x109 litres. This is

necessitated by the use of ethanol for various industrial purposes such as fuel, industrial solvents, cleansing agents and preservatives (Bekele *et al.*, 2015)

Recently, China became one of the largest ethanol producers as a result of enormous investment in the production of ethanol (Ivanova *et al.*, 2011).Recent analysis of sugarcane and sweet sorghum as bio-ethanol feedstock in Nigeria revealed that sweet sorghum is better suited in terms of its adaptability to harsh climatic conditions and the position Nigeria occupies in the world in terms of cassava production and waste generated annually in cassava processing placed the country in a better position to join the world of bio-ethanol production (Oyeleke *et al.,* 2012).

2.6 Current Ethanol Production

Ethanol is manufactured from microbial conversion of biomass material through fermentation. The production process consists of conversion of biomass to fermentable sugars, fermentation of sugar to ethanol and the separation and purification of ethanol. Fermentation initially produces ethanol containing a substantial amount of water. Then this solution is distilled using distillation column the majority of water to yield up to 95 percent purity ethanol, the balance being water. This mixture is called hydrous ethanol. If the remaining water is removed in further process, the ethanol is called anhydrous ethanol and suitable for blending with gasoline. Ethanol is "denatured "prior to leaving the plant to make it unfit for human consumption by addition of small amount of products such as gasoline (Aloe *et al.,* 2012).

Bioethanol is used in cosmetic, thermometer, used as solvent, as a preservative and most importantly, as a motor fuel (additive for gasoline) (Adiotomre, 2015).

These indispensable uses of bioethanol have led to a high demand for the product but the feedstock (cassava, yam, potatoes, sugar cane, and cereals) used for the first generation biofuels are also used in the food industries. This dichotomy has raised an inevitable tension

between the production of food and bioethanol (Adiotomre, 2015).

2.7 World Market of Ethanol

Today, bio-ethanol is the most dominant bio-fuel and its global production showed an upward trend over the last 25 years with a sharp increase from 2000. As of 2005, worldwide production capacity for bio-ethanol fuel was about 45 billion litres per year, with approximately 15% annual growth between 2000 and 2005. This value increased to 49 billion litres in 2006, when the Americans produced 75% of the total world ethanol output, followed by Asia/Pacific and Europe/Africa with respective values of 15 and 10% (Aloe *et al.,* 2012).

The industrial alcohol market showed a rather modest rate of growth similar to the increase in Gross Domestic Product in many countries. The market for beverage alcohol in most developed countries is stagnating, due to increased health awareness. Fuel ethanol production is predicted to have the strongest increase in the Americans, where the production is expected to rise to around 75 billion litres by 2015, representing about 42 billion litres increase in the projection period. In Asia this value is anticipated to increase to 8 billion litres during the same period, and in Europe, with the policy of increasing the share of bio-fuels in the transportation sector, the production will rise strongly. Therefore, total output in 2015 is forecast to reach over 115 billion litres (Aloe *et al.,* 2012). In 2009, production of fuel ethanol reached an estimated 76 billion litres, an increase of 10 percent over 2008. The United States and Brazil accounted for 88 percent of global ethanol production in 2009. Most of the increased production occurred in the United States (Aloe *et al.*, 2012).

2.8 Chemistry of Bioethanol

Glucose is synthesized in plant by photosynthesis (Shubhra *et al.,* 2014).

$$6CO_2+6H_2O+light\rightarrow C_6H_{12}O_6+6O_2$$

During ethanol fermentation, glucose is decomposed into ethanol and carbon dioxide.

$$C_6H_{12}O_6\rightarrow 2C_2H_5OH+2CO_2+heat$$

During combustion, ethanol reacts with oxygen to produce carbon dioxide, water and heat.

$2C_2H_5OH+3O_2 \rightarrow 2CO_2+3H_2O+heat$

Plant also contains other sugars apart from glucose such as fructose, sucrose, starch and cellulose that can be fermented. Ethanol may also be produced industrially from ethene (ethylene) by the addition of water to the bond in the presence of acid which can catalyse the reaction but it is not consumed (Shubhra *et al.,* 2014).

$CH_2 = CH_2 + H_2O \rightarrow CH_3CH_2OH.$

Microorganisms other than yeast can also be useful in making fermentation products. There are bacteria which are used in ethanol production called *Clostridium ijungdahlii*. Mid to long term technology under development are expected to improve the fermentation efficiency of the organism, producing higher yields in less time, and an organism requiring less detoxification of the hydrolysate (Wondale, 2012). Although *Zymomonas mobilis*, *Kluyveromyces* spp., *Schizo saccharomyces pombe,* and some recombinant bacteria and yeast can ferment sugars to ethanol, *Saccharomyces cerevisiae* is still the standard microorganism in the industry (Braide *et al.,* 2012).

2.9 Cassava

Cassava (*Manihot esculenta*), also known as manioc, tapioca or yucca, is one of the most important food crops in the humid tropics, being particularly suited to conditions of low nutrients availability and is able to survive drought. It is the third largest source of carbohydrates for human consumption in the world. The major harvested organ is the tuber, which is actually swollen root. The nutrient reserve of cassava is made up of starch(Pooja *et al.,* 2018).

2.9.1 Cassava production in Africa

Nigeria became the highest producer of cassava in the world in 2010, producing 37 million tons of the 230 million tons of world cassava production. This production was higher than

that of Brazil and double the amount of cassava produced in Thailand and Indonesia. It was also higher than that of Mozambique, Angola and Ghana (Wobiwo *et al.*, 2019). However, this high cassava production shows the importance and ability of Nigeria to produce sufficient quantity of cassava to satisfy the need for food carbohydrate in the country's population (Pooja*et al.,* 2018).

2.9.2 Cassava peels

Cassava peels is gotten during the processing of the cassava tuber and it is an agricultural waste. It is 1 – 4mm thick and account for 20 – 35% of the weight of the tuber (Olanbiwoninu and Odunfa, 2012). Processing about 300 tons of cassava tubers gives 1.16 tons of the peels with 85% moisture (Euis, *et al.,* 2012). According to Pitcha, *et al.,* (2012), cassava peels is a solid fibrous dry waste that consist of 56 – 60% starch, 15 – 18% hemicellulose, 2 – 3% lignin, 1.5 – 2% protein, 2% pentosan and 0.4 – 5% reducing sugar making it a good source of bioethanol. Cassava peels can easily be degraded by microorganism because it is rich in organic manure and starch content that serve as source of their carbon. This is an advantage over other crop residue like sugarcane bagasse (Pitcha *et al.*, 2012). These peels are usually discarded and allow rotting on the environment rendering the vegetation and soil unproductive. Therefore, there is the need for revalorization of cassava peels waste into useful products. The application of using cassava peels for ethanol production could be of great advantage to a country's economy(Pooja*et al.,* 2018).

2.10 Banana Production

Bananas (*Musa acuminata*) are perennial crops that take the appearance of trees as they mature. Diverse cultivars are grown, *Musa* are believed to have originated in Southeast Asia but their introduction into Africa is unclear. Throughout history *Musa* has provided humans

with food, medicine, clothing, tools, shelter, furniture, paper, and handicrafts. It could be termed as the "first fruit crop" as its cultivation originated during a time when hunting and gatherings were still the principal means of acquiring food (Nwabanne and Aghadi, 2018).

Bananas are important staple foods in many developing countries, especially in Africa of the numerous edible varieties. They provide food security and income for small scale farmers who represent the majority of producers. Banana starch, flour, and chips are processed into banana products whose markets are yet to be fully developed (Happi *et al.*,2011).Black Sigatoka disease is considered the most economically important disease of banana worldwide, causing typical yield losses up to 50%. The fungus grows on the leaves producing dark spots and causes the fruits to ripen prematurely.

Banana *Xanthomonas Wilt* (BXW) attacks almost all varieties of Musa, destroying the fruits and devastating the crop. *Fusarium wilt* has had a huge impact on the world banana trade and is found in every banana producing area, it is spread through the corms used for planting. The major banana pests are the burrowing nematode and the banana weevil. Nematode species attack the plant's roots, resulting in reduced yield. The banana weevil, *Cosmopolites sordidus*, attacks the plant's underground corm, weakening the plant and causing stem breakage (Oyeleke *et al.,* 2012).

2.10.1 Banana peel

Banana peel also known as a banana skin, is the outer covering of the banana fruit. Bananas are popular fruit consumed worldwide with a yearly production of over 145million tones in 2011. Once the peel is removed, the fruit can be eaten raw or cooked and the peel is generally discarded. Because of the removal of the banana peel, there is a significant amount of organic waste being generated (Oyeleke *et al.,* 2012).Banana peels are used as feedstock as they have some nutritional value; the peels are widely used for that purpose on small farms in regions where bananas are grown. Banana peels are used as feedstock for cattle, goats, pigs, poultry,

rabbits, fish and several other species (Oyeleke *et al.,* 2012).

The specific nutrient contained in peels depends on the stage of maturity and the cultivar; for example plantain peels contain less fibre than dessert banana peels, and lignin content increases with ripening (from 7 to 15% dry matter). On average, banana peels contain 6-9% dry matter of protein and 20-30% fibre (measured as NDF). Green plantain peels contain 40% starch that is transformed into sugars after ripening. Green banana peels contain much less starch (about 15%) and ripe banana peels contain up to30% free sugars. Banana peels are also used for ethanol production, cellulase, and as fertilizer and in composting (Kalemelawa *et al.,* 2012).

2.11 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.,* 2012). 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* (Wondale, 2012).

2.12 Microorganisms of Interest in This Research Work

2.12.1 *Aspergillus niger*

*Aspergillus niger*is 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 *et al.,* 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 (Schuster *et al.*,2002). 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 (Verweij and Brandt, 2007).

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 (Verweij and Brandt, 2007). Colonies on potato dextrose agar are wooly initially white, quickly becoming black with conidial production (Larone, 2002). 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 (Larone, 2002). According to the research work of Highina *et al,* (2012), the hydrolyses of wheat using *Aspergillus niger,* shows the optimum range of temperature, pH and particle size are 45 - 50^{0}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.

2.12.2 *Zymomonas mobilis*

Zymomonas mobilis belongs to the family of *Sphingomonadaceae* it moves by means of 1 to

4 flagella that are grouped on one or both end of the cell even though motility is not its essential feature. They are gram negative short rod bacterium that can be found in sugar rich plant saps. It is usually 2 – 6μm long and 1 – 1.4μm wide which some times vary. In high CO_2 or ethanol concentrations slime and granular layers have been seen around the cell. It has been isolated from sugar cane as well as alcoholic beverages such as African palm wine. *Zymomonas mibilis* cause limited spoiling of beer due to it is optimal temperature range of 25 - 30°C. This organism has been of considerable interest in recent years for ethanol production due to high ethanol yield from glucose, osmotic pressure and ethanol tolerance with high specific rates of glucose uptake and ethanol production via Entner-Doudoroff pathway under anaerobic conditions (Isah *et al*., 2019).

It has an overall G+C content of 46.3% and can only metabolize glucose through the Entner-Doudoroff pathway because the genome does not recognize genes for 6-phosphofructokinase, an enzyme essential for the Embden-Meyerhof-Parnas pathway. It also lacks the genes for the enzymes 2-oxoglutarate dehydrogenase complex and malate dehydrogenase both of which are involved in the tricarboxylic acid cycle (Seo *et al.,* 2005). *Zymomonas mobilis* has hopanoids in it is plasma membrane that confer the organism with the ability to survive high ethanol environment. The organism is unable to use other pathways to obtain energy as it appears to lack key enzymes for the Embden-Meyerhof-Parnas pathway and the tricarboxylic acid cycle. The presence of acetic, propionic acids and higher concentrations of oxygen and carbon dioxide can inhibit ethanol production in *Zymomonas mobilis*. Ethanol itself seems to be the biggest inhibitor because it increases permeability of the membrane allowing some cofactors and coenzymes from the Entner-Doudurоff pathway to escape (Lee and Huang, 2000).

2.12.2.1 *The metabolic pathway of Zymomonas mobilis*

Zymomonas mobilis is an obligate fermentative microorganism that ferments glucose, fructose and sucrose via the Entner-Doudoroff (ED) pathway in conjunction with the enzymes, pyruvate decarboxylase and alcohol dehydrogenase, producing ethanol and carbon dioxide (Jungwoo, 2011). The bacterium do not employ Embden-Meyerhof-Parnas (EMP) pathway in its metabolic activity because it lacks the gene for phosphofructokinase and enzymes of the pentose phosphate pathway (Seo *et al.,* 2005). It is also reported that the bacterium do not possess enzymes and genes for α-ketoglutarate dehydrogenase, succinyl thiokinase, succinate dehydrogenase and fumarase and malate dehydrogenase (Seo *et al.,* 2005). The two enzyme activities found in cell free extracts and genes are those of phosphoenol pyruvate (PEP) carboxylase, malic enzyme and citrate lyase, malic and fumarate dehydratase (Oyeleke *et al.,* 2012).

The glycolytic pathway in *Zymomonas mobilis* lacks allosterically regulated pyruvate kinase and phosphofructokinase, typical of Embden-Meyerhof-Parnas glycolysis. Thus, the enzymes take control of the glycolytic flux to some extent. The flux control coefficient of glucose-6-phosphate dehydrogenase for the early stages of batch growth was found to be 0.4, or even higher. Glucokinase and the glucose transporter might also contribute to the flux control and when ethanol is present at high concentrations particularly at late fermentation stages, the flux control is shifted to enolase and phosphoglycerate mutase. The rate at which cells of *Zymomonas mobilis* convert glucose into ethanol and CO_2 is three to five times faster than observed in yeast and 1.2 – 1.5 times faster than in the Gram-positive obligate fermentative *Streptococcus bovis* among bacteria (Jungwoo, 2011).

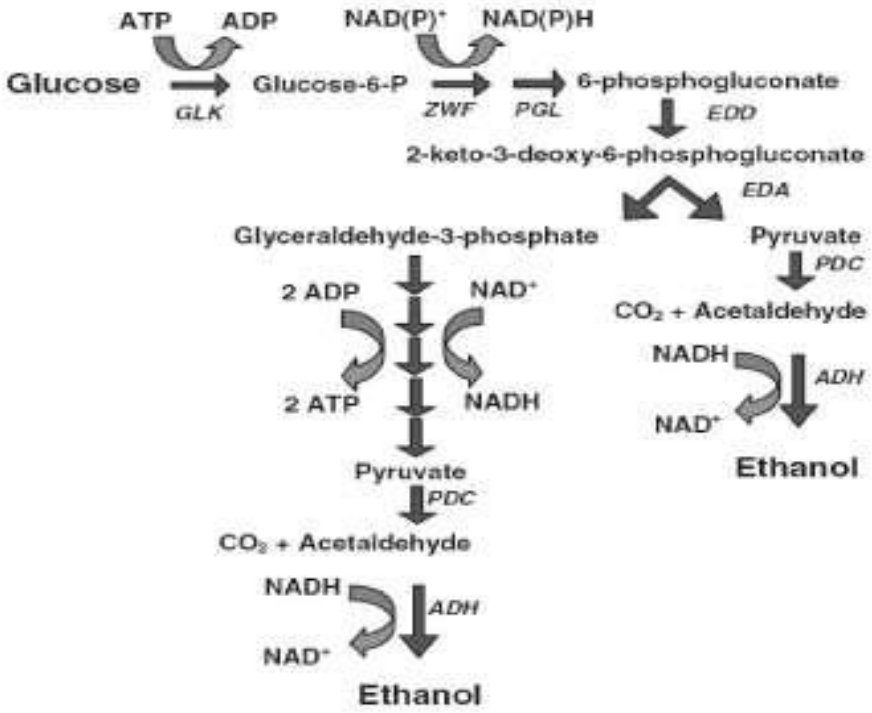

Figure 2.1: The Entner-Doudoroff pathway and ethanolo-genesis.

The branch from glyceraldehyde-3-phosphate to pyruvate is identical to the Embden-Meyerhof-Parnaspathway. The key enzyme of the Entner - Doudoroffpathway is pyruvate decarboxylase, which is only rarely found in bacteria. The Entner - Doudoroff pathway produces only 1 mole of ATP per mole of consumed glucose. Abbreviations: GLK, Glucokinase; ZWF, glucose-6-phosphate dehydrogenase; PGL, phosphogluconolastonase; EDD, 6-phosphogluconate dehydratase; EDA, 2-keto-3-deoxy-gluconate adolase; PDC, pyruvate decarboxylase; ADH, alcohol dehydrogenase (Uldis, 2006).

2.12.3 *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(Endurance, 2018).*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 (Endurance, 2018).

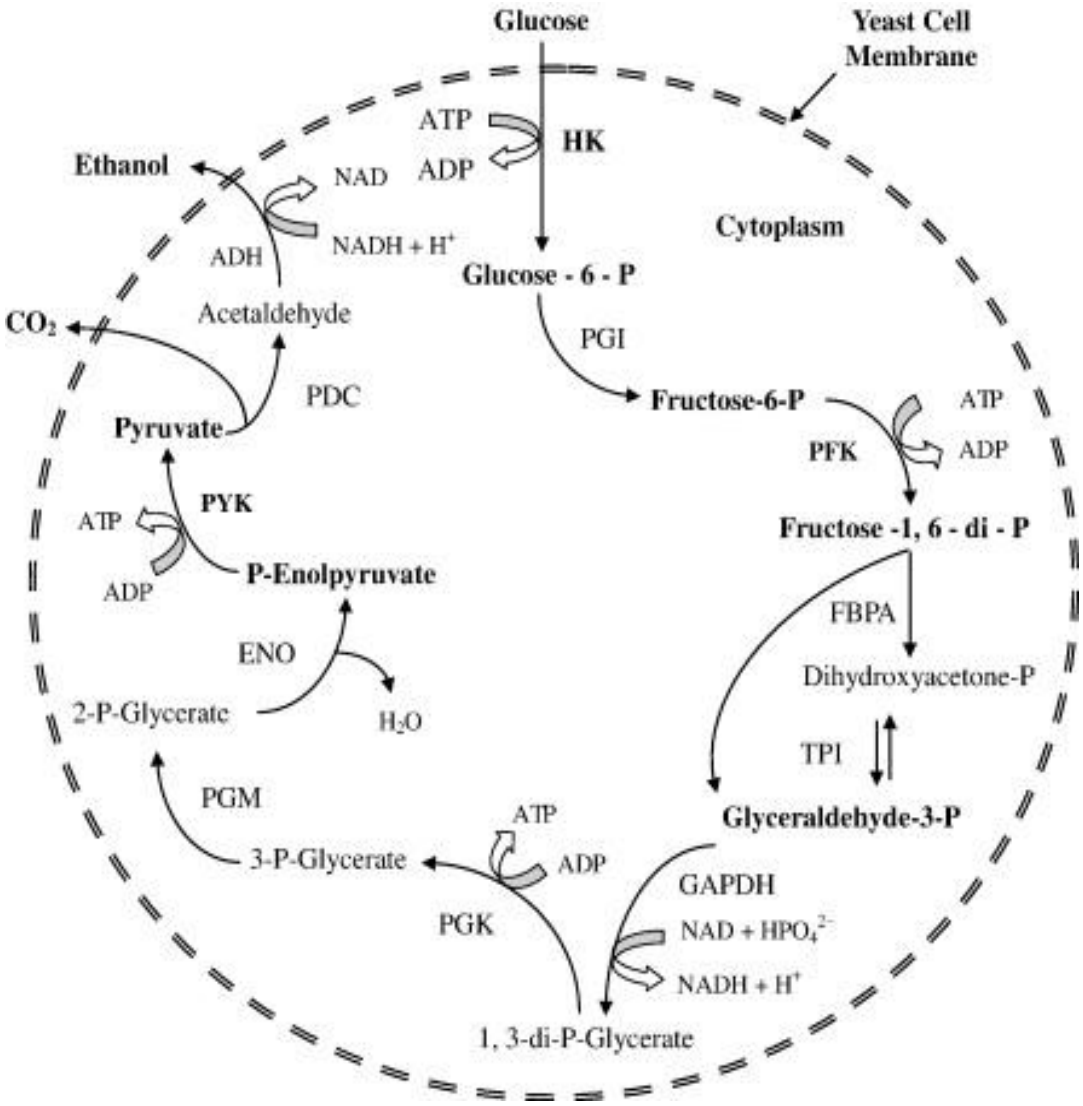

Figure 2.2: Metabolic pathway of ethanol fermentation in *Saccharomyces cerevisiae.*

Source*:* (Bai *et al*, 2008).

KEY: HK: hexokinase, PGI: phosphoglucoisomerase, PFK: phosphofructokinase, FBPA: fructose bisphosphate aldolase, TPI: triose phosphate isomerase, GAPDH: glyceraldehydes-3-phosphate dehydrogenase, PGK: phosphoglycerate kinase, PGM: phosphoglyceromutase, ENO: enolase, PYK: pyruvate kinase, PDC: pyruvate decarboxylase, ADH: alcohol dehydrogenase.

Currently, most ethanol production uses strains of *Saccharomyces cerevisiae* that are highly

adapted to industrial process of converting feed stocks to ethanol. These Yeast strains combine efficient conversion of sugars into ethanol, with other important industrial characteristics such as low nutrient requirement, ethanol resistance, tolerance to pH, and general robustness (Karhumaa *et al.*, 2005).

2.12.3.1 *Mechanism of yeast fermentation*

Yeast can metabolize monosaccharides and disaccharides but preferred glucose and fructose as substrates. Yeast is known to employ glycolytic, tricarboxylic acid and pentose pathways in the metabolism of hexoses and disaccharides and differ only in the initial basic steps of metabolism. The sugar breakdown may occur anaerobically or aerobically and the anaerobic step is called fermentation while the aerobic processes occur in the presence of oxygen. Alcoholic fermentation of glucose is the most common of yeast and the process yields ethanol and CO_2. When yeast sense sugar in its environment, it utilizes the sugar by transporting it across the plasma membrane (Kruckeberg and Dickinson, 2004).

The amount of glucose influence the enzyme levels through several processes like alteration of mRNA translation rates; mRNA stability or protein degradation, and the concentration of intracellular metabolites. This processes lead to the extensive transcriptional regulation of a large number of genes leading to adaptation to fermentative metabolism. These processes is required for the release of genes for the utilization of glucose, such as genes encoding glycolytic pathway enzymes, whereas genes required for the metabolism of alternative substrates, and those encoding proteins in the gluconeogenic and respiratory pathways are repressed by glucose (Gancedo, 2008). *Saccharomyces cerevisiae* consists of more than 20 members for hexose transporters: 18 genes encoding transporters, being the most relevant, with a low affinity for glucose and high transport capacity genes with a high affinity, and low transport capacity and; ii) at least two genes encoding sensors (SNF3, RGT 2), although several points of evidence suggest that GPR1 and HXK 2 also sense and signal glucose levels

(Gancedo, 2008). All these, sensors and transporters are therefore the primary interveners on sugar metabolism.

After glucose uptake, it enters into glycolytic pathway in order to be metabolized to pyruvate, whereby production of energy in form of ATP is coupled to the generation of intermediates and reducing power in form of NADH for biosynthetic pathways (Rodrigues and Leao, 2006). The first step of the glycolytic pathway consists on the phosphorylation of glucose to glucose 6-phosphate by the action of the hexokinases (Hxkp) and the glucokinase (Glkp); which are linked to high-affinity glucose uptake. Then glucose-6-phosphate is isomerized by the phosphoglucose isomerase, encoded by PGI gene, to fructose-6-phosphate. The next step, done by the phosphofructokinase (Pfkp) requires energy, in the form of ATP, to convert fructose-6-phosphate into fructose 1, 6-biphosphate (Fábio, *et al.,* 2013).

2.12.3.2 *Inhibition of ethanol productivity in yeast cells*

During ethanol fermentations, yeast cells are negatively affected by various environmental factors which includes; nutrient deficiency, high temperature and contamination, others are the yeast cell metabolism such as high sugar content tolerance, ethanol tolerance and production (Elena, *et al.,* 2009).These factors causes structural and metabolic changes in an organism acting as expression activator for genes involved in the synthesis of specific compounds that protect cells. This living and non-living factors can trigger the synthesis of compounds that changes the gene expression of the organism thereby inhibiting ethanol productivity. The inhibition is favoured by the presence of other fermentation by-products such as acetaldehyde and acetate, and other stresses such as high temperature (Bai and Moo-Young, 2008).

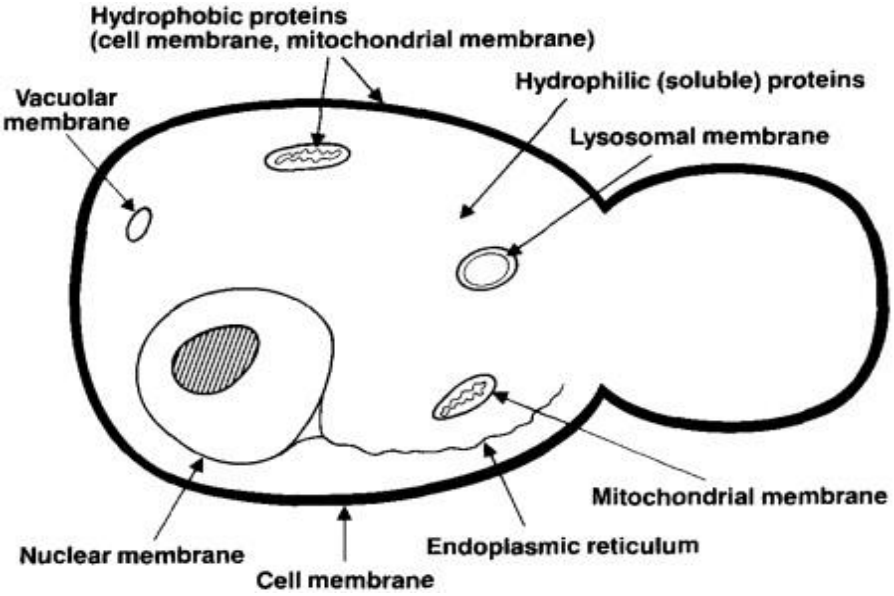

Figure 2.3: Possible target sites for ethanol inhibition in yeast cells.

Source: (Zhao and Bai, 20019).

The research work of Bai *et al*, 2009 critically looked at some key aspects of ethanol fermentation technologies that have been neglected or misunderstood. In comparing the ethanol yield and productivity of *Zymom onas mobilis* with *Saccharomyces cerevisiae*, they found that *Zymomonas mobilis* produces higher ethanol with less biomass when higher metabolic rate of glucose is maintained through its special Entner–Doudoroff pathway. They however, recommend that due to its specific substrate spectrum as well as the undesirability of its biomass to be used as animal feed, this species cannot readily replace *Saccharomyces cerevisiae* in ethanol production. The steady state kinetic models developed for continuous ethanol fermentations show some discrepancies, making them unsuitable for predicting and optimizing the industrial processes. The dynamic behaviour of the continuous ethanol fermentation under high gravity or very high gravity conditions has been neglected, which needs to be addressed in order to further increase the final ethanol concentration and save the energy consumption.

2.12.4 Lactic acid bacteria

Lactic acid bacteria or Lactobacillales are defined as a group of Gram-positive bacteria that ferment sugars such as lactose to produce primarily lactic acid. They are diverse group of bacteria which phylogenetically belongs to the order Lactobacillales. This diverse order includes 6 families, over 30 genera and over 300 species. These microorganisms produce lactate as the main end products from metabolism of glucose, and certain species also produce ethanol, CO_2, and acetate (Nagaoka, 2019). The LAB can be found ubiquitously on plants, in decomposing plant material, in dairy and in animal mucosa. The great variety in niches reflects that the LAB constitutes a group of highly versatile organisms that are able to ferment many different substrates, ranging from simple disaccharides to complex carbohydrates like starch. Because of the huge varieties, culture condition of LAB is varied it requires a rich nutrients for growth, e.g., carbohydrates, amino acids, vitamins, minerals, and sometimes fatty acids and peptides. They usually lack most part of TCA cycle and quinone or ubiquinone biosynthesis systems, meaning that they do not conduct respiration (Fossi*et al.*, 2016).

Oxygen does not usually support their growth, and they prefer anaerobic conditions rather than aerobic conditions for growth. They ferment sugars in a homofermentative (leading to lactic acid) or heterofermentative (leading to a mixture of lactic acid, carbon dioxide, acetic acid and ethanol) way (Sieuwerts, 2009).

2.12.3.1 *Isolation and identification of Lactobacillus*

LAB generally inhabit in nature with other microbes, including molds, yeasts, aerobes, and anaerobes. Several chemicals, including antibiotics, are thus usually used for a selective isolation of LAB from environmental samples. Identification is essential after the isolation of LAB. Identification is usually conducted by phylogenetic analysis based on 16S rRNA gene

sequences, whereas species in certain LAB groups, e.g. *Lactobacillus plantarum* group, *Lactobacillus casei* group, and *Enterococcus faecium* group, are known to share high sequence similarities of 16S rRNA gene within the groups (Fossi *et al.*, 2016). Housekeeping genes are alternative markers for an accurate identification of such LAB groups. In addition, identification based on whole genome sequence similarities, e.g., all nucleotide identity (ANI) and in silico DNA-DNA hybridization (DDH), is becoming common in recent years(Arabia & Wankhade, 2013).

Major culture media for isolation of LAB are de Man, Rogosa, and Sharpe (MRS) medium, *Lactobacillus* selection (LBS) medium, and M17 medium. These media are commercially available in several producers. LAB isolation medium are also used for isolation of specific LAB species. These media usually contain rich nutrients but not antibiotics for selective isolation of LAB. Supplement of 10 mg/L of sodium azide and 10 mg/L of cycloheximide is useful to suppress growth of aerobes and fungi, respectively (Zhai *et al.*, 2015).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study Area

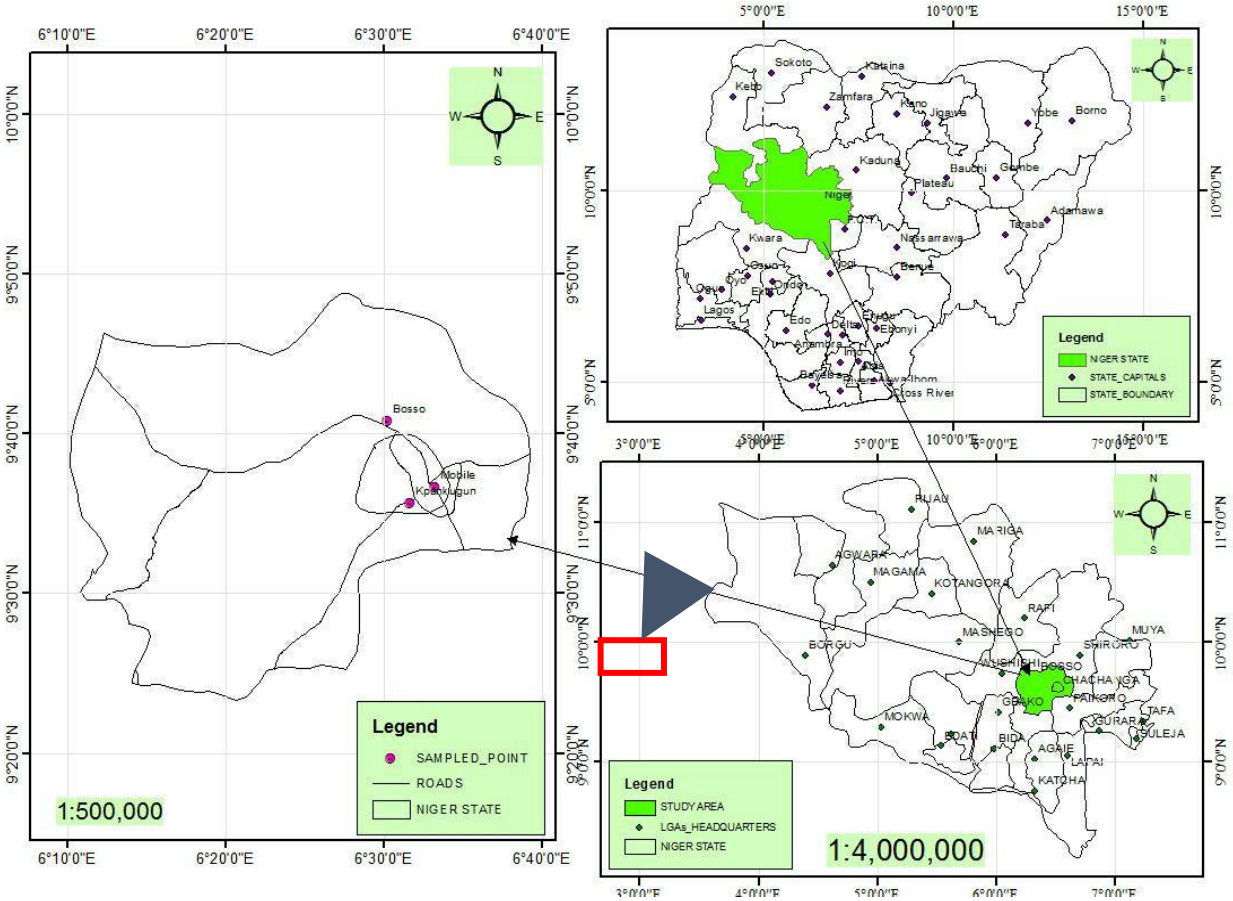

Figure 3.1 Location of Samples Collection North East of Minna, Niger State.
Source: Hui *et al*. (2011).

3.2 Collection and Processing of Agro Waste

Eight thousand (8,000) grams of cassava (*Manihot esculenta*) peels and Banana (*Musa acuminata*) peels were collected in clean polythene bag from Kpakungu, via Bida Road Chanchaga Local Government Area of Niger State. The waste was sun dried for three days after washing with clean water to remove the soil and dirty. It was finally milled into powder using mortar and pistil. The sample was divided into two portions; the first portion was used for analysis while the second was used for hydrolysis fermentation.

3.3 Microorganisms

Lactobacillus bulgaricus, *Saccharomyces cerevisiae*, *Aspergillus niger*and *Zymomonas mobilis*, are the organisms used in this study. *Aspergillus niger* were obtained from the soil surrounding the Department of Microbiology, Federal University of Technology, Minna, *Saccharomyces cerevisiae* and *Zymomonas mobilis* were isolated from fresh sweet palm wine from F.M area of Maitunbi, Minna, Niger state while *Lactobacillus delbrueckii* were isolated from fresh cow milk obtained from Ruga chita Barkin sale Minna Niger state.

3.4 Identification of Microorganisms

3.4.1 *Aspergillus niger*

Macro culture method was used to identify the organisms (Steinbach and Stevens, 2003). The organisms were isolated from soil surrounding of the department of Microbiology. The soil was taken to the laboratory after which 10 g was immersed in 100 mL of distilled water. A fourfold serial dilution was carried out and 1 ml of the diluents was inoculate on sabouraud's dextrose agar (SDA) using a Pour-plate method and incubated for 3 days at 28°C. Sub-culturing was carried out until pure cultures of *Aspergillus niger* was obtained. After 3 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); Oyeleke *et al.*

(2012); Simpfendorfer *et al.* (2016).

3.4.2 *Saccharomyces cerevisiae*

Saccharomyces cerevisiae that was used in the research was isolated from fresh palm wine. Aliquot of 0.1ml of 10^{-5} serial dilution of the palm wine was spread on the surface of a solidified saboraud dextrose agar plate (SDA) and was incubated for 48hours at 30^{o}C. Colonies suspected to be *S. cerevisiae* based on their colonial characteristics were sub-cultured on sterile SDA 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 Isolation of *Zymomonas mobilis* from fresh sweet Palm wine

Zymomonas mobilis was isolated according to the method of Hermann *et al.* (2006). A broth containing 0.3mL Maltina drink, 0.3g Yeast extract, 0.3g glucose, 2g peptone water was prepared and the pH was adjusted to 4.0. It was sterilized at 121^{o}C for 15 minutes and allowed to cool. This was dispensed into three (3) screw cap test tubes and 0.5mL actidione (Cycloheximide) was added to inhibit yeast growth. 5ml each of the fresh palm wine were dispensed into the test tubes after dipping Durham tubes and cultured in an anaerobic jar at room temperature for 72 hours. The growth in the medium was streak on sterile nutrient agar plates and cultured for 24 hours. Biochemical test was carried out and the organism identified using Bergey's manual of determinative bacteriology and also compared with other research work. This was finally sub cultured on agar slant and stored at 4^{o}C for further used.

3.4.4 Isolation of *Lactobacillus bulgaricus*

The media used were deMan Rogosa and Sharpe (MRS) agar for isolation of *Lactobacillus bulgaricus* (LAB).The fresh milk Samples for the isolation of lactic acid bacteria were cultured on MRS Agar using pour plate method. One millilitre (1ml) of the fresh milk sample was pipette into a sterile plates and about 15ml of the prepared molten MRS agar was poured on it. The plates were then gently rotated clockwise and anti-clockwise so as to allow for a homogeneous distribution of the agar and the sample. The agar was allowed to solidify, then inverted in anaerobic jar and incubated at 30°C for 48h.At the end of the incubation period, the MRS plates were observed for colony formation. Colonies observed was subculture on MRS Agar by streaking on plates to obtained pure colony present (Oluyege *et al.*, 2018).

3.5 Characterization and Identification of Bacteria Isolate

3.5.1 Gram staining

This was done as described by Cheesbrough (2000). A little portion of the colony was emulsified in few drops of sterile distilled water on clean glass slide there by making a thin smear. The smear was heat- fixed by rapidly passing the slide, with the smear uppermost, three times through the flame of spirit lamp. The smear was allowed to cool before staining it. The fixed smear was flooded with crystal violet stain for 60 second and was rapidly washed off with clean water. The water was tipped off and was cover with lugol's iodine, allowed for 30 seconds and rinsed with clean water. The smear was decolorize rapidly (30 seconds) with alcohol and was washed immediately with clean water. It was counter stain with safranin, allowed for 60 seconds and was washed with clean water. The back of the slide were wiped with clean cotton wool and placed in a draining rack for the smear to air-dry. The smear was observed microscopically using oil immersion objective (X100). The gram negative appears red/pink and gram positive appear purple/blue.

3.6 Biochemical Test

3.6.1 Catalase test

A drop of hydrogen peroxide (H_2O_2) was placed on a slide and a 24 hours growth culture was emulsified with the drop of H_2O_2 on the slide. Immediately it was observed for the present of bubbles as indication for positive reaction and absence of bubbles indicate negative reaction (Cheesbrough, 2006).

3.6.2 Oxidase test

A drop of freshly prepared oxidase reagent was place on pieces of filter paper placed on clean Petri-dish, and a sterilized wire loop was used to collect the test organism and smear on the filter paper. The appearance of a blue purple colour within ten second was recorded as a positive while absence of blue–purple colour after fifteen second was recorded as negative result (Cheesbrough, 2006).

3.6.3 Citrate test

Heavy inoculums of the test organisms were incubated into a sterile citrate medium with the aid of the sterile wire loop. The inculcated test tubes were incubated at 37^{o}C for 72 hours. A positive test were indicated by turbid and change of colour of the medium from light green to blue (Cheesbrough, 2006).

3.6.4 Urease test

This test applied for bacteria that can decompose urea by enzymatic reaction to produce ammonia. The test organisms were inoculate into urea ager base medium and incubated at 37^{o}C for 48 hours. A positive test is indicated by a change in colour from yellow to pink as result of ammonia production (Oyeleke and Manga 2008).

3.6.5 Sugar Fermentation test

The isolate were tested for fermentation of sugars viz. glucose, fructose, lactose, sucrose, maltose, by adding the specific sugar to the basal carbohydrate media. Acid production was indicated by a change in colour and gas production was detected by observation of gas collection in the inverted Durham tubes (Thakur *et al.,* 20017).

3.7 Molecular Identification of Organisms

3.7.1 Fungi DNA Extraction Protocol

One hundred milligram (100mg) of fungal mycelia was taken into sterile mortal, and 1ml of DNA Extraction Buffer (DEB) containing proteinase K (0.05mg/ml) was added and macerate with sterile pestle. The extract was transferred into 1.5mL eppendorf tube. 50µL of 20% Sodium Dodecyl Sulphate (SDS) was added and incubated in a water bath at 65^{o}C for 30minutes, and the tubes were allowed to cool to a room temperature, 100µL of 7.5M Potassium Acetate were added and briefly mixed. The resultant mixture was centrifuge at 13000rpm for 10minutes. The supernatant were transferred into new fresh autoclaved tubes and added 2/3 volumes of cold Isopropanol / Isopropyl alcohol, inverted the tubes 3-5 times gently and incubated the tubes at -20^{o}C for an hour. These were centrifuged at 13000rpm for 10minutes and discarded the supernatant. 500µL of 70% ethanol were added and centrifuge for 5minutes at 13000rpm. The supernatant were carefully discarded with the DNA pellet intact. The traces of ethanol were removed and the DNA pellets were dried at 37oC for 10-15 minutes. The DNA pellets were re-suspended in 50µL of Tris-EDTA (TE) buffer. The Aliquot of DNA was stored at -20^{o}C for further lab analysis (Mandal and Ghosh, 2016).

3.7.2 PCR Analysis

To use the ITS gene for characterization of fungi, ITS universal primer set which flank the ITS1, 5.8S and ITS2 region were used for PCR. PCR reaction cocktail consisted of 10 µL of 5x GoTaq colourless reaction, 3 µl of MgCl2, 1 µL of 10 mM of dNTPs mix, 1 µL of 10 pmol each ITS 1: 5' TCC GTA GGT GAA CCT GCG G 3'and - ITS 4: 5' TCC TCC GCT TAT TGA TAT GC 3''. primers and 0.3units of Taq DNA polymerase (Promega, USA) made up to 42 µL with sterile distilled water 8µL DNA template. PCR carried out in a GeneAmp 9700 PCR System Thermalcycler (Applied Biosystem Inc., USA); PCR conditions include a cycle of initial denaturation at 940C for 5 min, followed by 35cycles of each cycle comprised of 30secs denaturation at 940C, 30secs annealing of primer at 55^{o}C, 1.5 min extension at 72^{o}C and a final extension for 7min at 72^{o}C (Sieuwerts, 2009).

3.7.3 Integrity

The integrity of the amplified about 1.5Mb gene fragment was checked on a 1% Agarose gel ran to confirm amplification. The buffer (1XTAE buffer) was prepared and subsequently used to prepare 1.5% agarose gel. The suspension was boiled in a microwave for 5 minutes. The molten agarose was allowed to cool to 60°C and stained with 3µl of 0.5 g/mL ethidium bromide (which absorbs invisible UV light and transmits the energy as visible orange light). A comb was inserted into the slots of the casting tray and the molten agarose was poured into the tray. The gel was allowed to solidify for 20 minutes to form the wells. The 1XTAE buffer was poured into the gel tank to barely submerge the gel. Two microliter (2 mL) of 10X blue gel loading dye (which gives colour and density to the samples to make it easy to load into the wells and monitor the progress of the gel) was added to 4µL of each PCR product and loaded into the wells after the 100bp DNA ladder was loaded into well 1. The gel was electrophoresed at 120V for 45 minutes visualized by ultraviolet trans-illumination and photographed. The sizes of the PCR products were estimated by comparison with the

mobility of a 100bp molecular weight ladder that was ran alongside experimental samples in the gel (Sieuwerts, 2009).

3.7.4 Purification of amplified product

After gel integrity, the amplified fragments were ethanol purified in order to remove the PCR reagents. Briefly, 7.6 µL of Na acetate 3M and 240 µL of 95% ethanol were added to each about 40µl PCR amplified product in a new sterile 1.5 µl tube eppendorf, mix thoroughly by vortexing and keep at -20°C for at least 30 min. Centrifugation for 10 min at 13000 g and 4°C followed by removal of supernatant (invert tube on trash once) after which the pellet were washed by adding 150 µL of 70% ethanol and mix then centrifuge for 15 min at 7500 g and 4°C. Again remove all supernatant (invert tube on trash) and invert tube on paper tissue and let it dry in the fume hood at room temperature for 10-15 min. then resuspend with 20 µL of sterile distilled water and kept in -20ºC prior to sequencing. The purified fragment was checked on a 1.5% Agarose gel ran on a voltage of 110V for about 1hr as previous, to confirm the presence of the purified product and quantified using a nanodrop of model 2000 from thermo scientific.(Hamed *et al.*,2016).

3.7.5 Sequencing

The amplified fragments were sequenced using a Genetic Analyser 3130xl sequencer from Applied Biosystems using manufacturers' manual while the sequencing kit used was that of BigDye terminator v3.1 cycle sequencing kit. Bio- Edit software was used for all genetic analysis (Hamed *et al.,* 2016).

3.8 Molecular Identification of Bacteria

3.8.1 Bacteria DNA extraction

DNA was extracted using the protocol stated by (AOAC. 2006). Briefly, Single colonies grown on medium were transferred to 1.5 ml of liquid medium and cultures were grown on a shaker for 48 h at 28 ºC. After this period, cultures were centrifuged at 4600g for 5 min. The resulting pellets were resuspended in 520 µL of TE buffer (10 mMTris-HCl, 1mM EDTA, pH 8.0). Fifteen microliters of 20% SDS and 3 µL of Proteinase K (20 mg/ml) were then added. The mixture was incubated for 1 hour at 37 ºC, then 100 µL of 5 M NaCl and 80 µL of a 10% CTAB solution in 0.7 M NaCl were added and votexed. The suspension was incubated for 10 min at 65 ºC and kept on ice for 15 min. An equal volume of chloroform: isoamyl alcohol (24:1) was added, followed by incubation on ice for 5 min and centrifugation at 7200g for 20 min. The aqueous phase was then transferred to a new tube and isopropanol (1: 0.6) was added and DNA precipitated at –20 ºC for 16 hours. DNA was collected by centrifugation at 13000g for 10 min, washed with 500 µL of 70% ethanol, air-dried at room temperature for approximately three hours and finally dissolved in 50 µL of TE buffer.

3.8.2 Polymerase chain reaction

PCR sequencing preparation cocktail consisted of 10 µl of 5x GoTaq colourless reaction, 3 µl of 25mM MgCl2, 1 µl of 10 mM of dNTPs mix, 1 µl of 10 pmol each 27F 5'- AGA GTT TGA TCM TGG CTC AG-3' and - 1525R, 5′-AAGGAGGTGATCCAGCC-3′ primers and 0.3units of Taq DNA polymerase (Promega, USA) made up to 42 µl with sterile distilled water 8µl DNA template. PCR was carried out in a GeneAmp 9700 PCR System Thermalcycler (Applied Biosystem Inc., USA) with a Pcr profile consisting of an initial denaturation at 94°C for 5 min; followed by a 30 cycles consisting of 94°C for 30 s, 50°C for

60s and 72°C for 1 minute 30 seconds ; and a final termination at 72°C for 10 mins. And chill at 4^{o}C.GEL (Odeyemi *et al.,* 2018).

3.8.3 Integrity

The integrity of the amplified about 1.5Mb gene fragment was checked on a 1% Agarose gel ran to confirm amplification. The buffer (1XTAE buffer) was prepared and subsequently used to prepare 1.5% agarose gel. The suspension was boiled in a microwave for 5 minutes. The molten agarose was allowed to cool to 60°C and stained with 3µl of 0.5 g/mL ethidium bromide (which absorbs invisible UV light and transmits the energy as visible orange light). A comb was inserted into the slots of the casting tray and the molten agarose was poured into the tray. The gel was allowed to solidify for 20 minutes to form the wells. The 1XTAE buffer was poured into the gel tank to barely submerge the gel. Two microliter of 10X blue gel loading dye (which gives colour and density to the samples to make it easy to load into the wells and monitor the progress of the gel) was added to 4µL of each PCR product and loaded into the wells after the 100bp DNA ladder was loaded into well 1. The gel was electrophoresed at 120V for 45 minutes visualized by ultraviolet trans-illumination and photographed. The sizes of the PCR products were estimated by comparison with the mobility of a 100bp molecular weight ladder that was ran alongside experimental samples in the gel (AOAC. 2006)

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were washed by adding 150 µL of 70% ethanol and mix then centrifuge for 15 min at 7500 g and 4°C. Again remove all supernatant (invert tube on trash) and invert tube on paper tissue and let it dry in the fume hood at room temperature for 10-15 min. then resuspend with 20 µL of sterile distilled water and kept in -20ºC prior to sequencing. The purified fragment was checked on a 1.5% Agarose gel ran on a voltage of 110V for about 1hr as previous, to confirm the presence of the purified product and quantified using a nano drop of model 2000 from thermo scientific (AOAC. 2006).

3.8.5 Sequencing

The amplified fragments were sequenced using a Genetic Analyzer 3130xl sequencer from Applied Biosystems using manufacturers' manual while the sequencing kit used was that of BigDye terminator v3.1 cycle sequencing kit. Bio- Edit software and MEGA 6 were used for all genetic analysis (Odeyemi *et al.,* 2018).

3.9 Determination of Enzyme Activity on the Isolate

3.9.1 Screening for cellulase activity

A loop full of grown culture of the isolate were inoculated on sabouraud's dextrose agar (SDA) for fungi and nutrient agar for bacteria, and supplemented with 0.1% Carboxymethyl cellulose (CMC). The SDA plates were incubated for 3days at 25ºC for fungi and 24 hours at 37°C for bacteria isolate and observed for growth. After the incubation period, freshly prepared iodine solution was added to the culture plate to identify the zones around the cultures. The diameter formed after the addition of iodine solution was measured to represent the amylolytic activity (Oyeleke *et al.,* 2012).

3.9.2 Screening for amylase production

The fungal and bacteria isolates were tested for amylase production by starch hydrolysis test in order to check if the isolate have amylase production potential which is essential for the breakdown of complex sugar contained in the substrate. Modified starch agar medium consisting of soluble starch (2g), peptone (2g), yeast extract for fungi (1g) and agar (2g) was used and the isolates were inoculated and incubated for 72h at 28^{0}C. After incubation, the plates were flooded with iodine solution and observed for blue-black colour around colonies to change to brown or milky colour which described for their ability to digest the starch and thus indicates the presence of alpha-amylase (Ekka and Namdeo, 2018).

3.9.3 Screening for pectinase enzyme

One gram (1g) of pectin was added into sabouraud dextrose agar for fungi and nutrient agar for bacteria. This medium was sterilized and distributed aseptically in Petri dishes and the isolate were inoculated unto the plates. The plates were observed for a zone of clearance after 3 to 5 days for fungi and 1 to 2 days for bacteria as described by Oyeleke *et.al.*, (2012)**.**

3.10 Mineral Analysis of Agro Waste

3.10.1 Determination of phosphorus in agro waste

The vanadate colorimetric method (Gregory, 2005) was used. Three (3) grams of the ashed fermentation waste was dissolved in 20mL of distilled water. 2mL of the ashed solution was pipetted into100mL volumetric flask followed by addition of 2.5mL of vanadate molybdate reagent and mixed thoroughly. It was make up to mark with distilled water and allow stand for 10 minutes. A control was prepared containing 2mL distilled water and 2.5mL vanadate reagent in 100mL volumetric flask and make up to mark with distilled water. Absorbance of the test and control were read at 540nm and compared with standard curve of potassium dihydrogen phosphate (KH_2PO_4). Percentage phosphorus = A ×10/W x V. where A =

concentration of dilute ash, W = weight of original food ashed, V = volume of ashed solution to 100mL.

3.10.2 Determination of nitrogen in agro waste

Nitrogen was determined by Micro Kjeldahl method (Gregory, 2005). One point two grams (1.2g) of fermentation waste in a tube was digested with 4ml H_2SO_4 with 2 Klehdahl tablets at 420^0C for 30 minutes until a clear solution is obtained. It was cooled and diluted with water. The tube containing diluted sample was connected to the distillation unit and a conical flask containing 25mL of boric acid was attached to the condenser outlet. 25mL of 40% NaOH was dispensed into the conical flask and distillation carried out for 4 minutes. The ammonium borate solution formed was titrated with 0.1M HCl to a purplish – grey end point. Percentage Nitrogen = 0.14 × A/weight of the cassava in gram. A = volume (ml) of 0.1M HCl used in the titration.

3.10. 3 Determination of potassium in agro waste

Potassium was determined by flame photometry (Gregory, 2005). Five (5) grams of the waste was ashed and digested with 10ml HCl. The ash was transferred into 100mL volumetric flask and make up to mark with deionized water. A standard of varying concentration of absolute KCl was prepared. The absorbance of both sample and standard were read with flame photometer by setting it at potassium wavelength. The graph of KCl concentration was plotted against the absorbance of the sample and percentage potassium calculated

3.11 Proximate Analysis of Agro Waste

The proximate analysis of the samples for moisture, total ash, crude fibre, fat were carried out in triplicate using methods described by Onwuka (2005). The nitrogen was determined by micro Kjeldah method described by Onwuka (2005) and the nitrogen content was converted to protein by multiplying by a factor of 6.25. Total carbohydrate content was estimated by ‘difference’. All the proximate values were reported in percentage (%).

3.11.1 Determination of Moisture

Moisture was determined by oven drying method. Two gram of well-mixed samples was accurately weighed in clean, dried crucible (W_1). The crucible was allowed in an oven at 100-105 C for 6-12 h until a constant weight was obtained. Then the crucible was placed in the desiccators for 30min to cool. After cooling, it was weighed again (W_2). The percentage moisture was calculated using Equation 1.

$$\text{Moisture} = \frac{W_1 - W_2 \times 100}{\text{Weight of sample}} \qquad (1)$$

Where

W = Initial weight of crucible + Sample 1

W = Final weight of crucible + Sample 2

3.11.2 Determination of Ash

The ash content was determine by obtaining a clean empty crucible that was placed in a muffle furnace at 550^0C for an hour, cooled in desiccator and then weight of empty crucible was noted (W_1). Two gram of each of sample was taken in crucible (W_2) and was purchased over a burner, until it was charred. Then the crucible was placed in muffle furnace for ashing at 550^0C for 2-4 h. the appearance for gray white ash indicate complete oxidation of all organic matter in the sample. After ashing the crucible was cooled and weighed (W_3). Percentage ash was calculated using Equation 2.

$$\% \text{ Ash} = \frac{\text{Difference in Weight of Ash} \times 100}{\text{Weight of Sample}} \qquad (2)$$

Difference in weight of ash = $W_3 - W_1$

3.11.3 Determination of Crude Protein

Protein in the sample was determined by kjeldahl method 0.25g of dried samples was taken in digestion flask, with 6ml of concentrated H_2SO_4 and a speck of kjeldah catalyst (mixture of 10g Na_2SO_4+5g $CuSO_4$+ 0.05g selenium). The flask was swirled in order to mix the contents

thoroughly then digested on the digestion block till the mixtures become clear (colourless or greenish in colour). The digest was cooled and transferred to 100mL volumetric flask and volume was made up to mark by the addition of distilled water. Distillation of the digest was performed in Markham Distillation Apparatus.

Ten milliliters of digest was introduced in the distillation tube then 10 ml of 40% NaOH was gradually added through the same way. Distillation was continued for at least 10 min and NH_3 produced was collected as NH_4OH in conical flask containing 5ml of 4% boric acid solution with few drops of methyl red indicator. During distillation yellowish colour appears due to NH_4OH. The distillate was then titrated against standard 0.1 N HCI solutions till the appearance of pink color. A blank was also run through all steps as above. Percentage crude protein content of the sample was calculated using Equation 3.

% Crude Protein = 6.25* x %N (*. Correction factor)

$$\%N = \frac{(S-B) \times N \times 0.014 \times D \times 100}{\text{Weight of the sample} \times V} \quad (3)$$

Where

S = Sample titration reading
B = Blank titration reading
N = Normality of HCI
D = Dilution of sample after digestion
V = Volume taken for distillation
0.014 – Milli equivalent weight of Nitrogen

3.11.4 Determination of Crude Fat:

Crude fat was determined by ether extract method using Soxhlet apparatus. Approximately 2g of moisture free sample was wrapped in filter paper, placed in fat free thimble and then introduced in the extraction tube. A weighed, cleaned and dried receiving flask was filled with petroleum ether and fitted into the apparatus. The soxhlet apparatus was assembled and allow refluxing for 6hrs; extract was transferred into clean glass dish with either washing

which was evaporated on water bath. Then the dish placed in an oven at 105^{o}C-110^{o}C for 1hr and cooled it in a desiccator. The percentage crude fat was determined using Equation 4.

$$\% \text{ Crude Fat} = \frac{\text{Weight of either} \times 100}{\text{Weight of sample}} \quad (4)$$

3.11.5 Determination of crude Fibre

Two gram (2g) of sample was defatted with per ether; boiled under reflux for 30min with 200ml a solution containing 1.25g of H_2SO_4 per 100mL of solution. The solution was filtered through linen or several layers of sieve cloth on fluted funnel, washed with boiling water until the washings are no longer acidic then the residue was transferred into a beaker and boiled for 30min with 200ml of solution containing 1.25g of carbonate free NaOH per 100mL, the final residue was filtered through a thin but close pad of washed and ignited asbestos in a Gooch crucible, then dried in an electric oven and weighed after which it was incinerated, cooled and reweighed. The loss in weight after incineration x 100 is the percentage crude fibre.

3.11.6 Carbohydrate Content Determination:

The nitrogen free method described by A.O.A.C (1990) was used. 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+F+A+F_2) using Equation 5.

Where; M=moisture, P=protein,F_1=Fat , A=ash, F_2=crude fibre (5)

3.12 pH Test

The pH meter was calibrated and inserted separately into each of the substrate (raw and hydrolysed agro waste) after the pH meter was standardized with buffer of pH 7.0 and 4.0. The readings were taken as described by Mustapha *et al.* (2019).

3.13 Determination of Reducing Sugar

The reducing sugar content of the hydrolyzed agro wastes was determined using the dinitros alicylic acid colorimetric method of Miller (2007) with glucose as standard. It was assayed by adding 3mL of DNS reagents to 3 ml of the sample. The mixture was heated in boiling water for 10 min to develop the red-brown colour. Then 1 mL of 40% potassium sodium tartarate solution was added to stabilize the colour and cooled to room temperature under running tap water. The absorbance of the samples was measured at 491 nm using ultraviolet (UV-VIS) spectrophotometer. The reducing sugar content was subsequently determined by making reference to a standard curve of known glucose concentrations.

3.14 Production of Bioethanol

The methods used for bioethanol production includes; Inoculums preparation, enzyme hydrolysis, fermentation and distillation process.

3.14.1 Inoculums preparation

The fungi used for inoculation was grown in 10 ml test tube of YPD medium containing 1% yeast extract, 2% peptone, 2% glucose. The bacteria were grown on peptone. After incubating in a room temperature for 72h for fungi, and 24h for bacteria at 37^{o}C the cell culture was aseptically transferred into the substrate, 5ml each for hydrolysis (Miksusanti *et al.,* 2018).

3.14.2 Enzyme hydrolysis of cassava and banana peels

Different quantities of the substrates were weighed inside separate 500cm3 conical flasks, carried out in quadruple (20grams each in four different conical flaks, 30grams each in another sets of four conical flasks and 40grams in another four different conical flasks for cassava, banana peel and mixture of both peel). Sterile distilled water was added to make up to the mark and the flasks were plunged with sterile cotton wool wrapped in aluminium foil to avoid contamination. The mixtures were sterilized in an autoclave at 121^0C for 15minutes, allowed to cool and sterile distilled water was aseptically added to make up to mark again. 5ml of freshly harvested cells of *Zymomonas mobilis* was inoculated into a set of 20grams, 30grams and 40grams of each substrates mixture under aseptic condition. *Aspergillus niger* was also added aseptically to another set of each of the substrate mixtures (20g, 30g and 40g). *Aspergillus niger* and *Zymomonas mobilis* was added into another set of the flasks containing the mixtures while the other set serves as control for the two substrates. The flasks were covered and were then incubated at room temperature (28^0C) and 37^0C for seven days. The flasks were shaken at interval to produce a homogenous solution and even distribution of the organisms in the substrates mixture. The mixtures were separately filtered after seven days using No 1Whatman filter paper (Oyeleke *et al.,* 2012).

3.13.3 Fermentation of hydrolysed cassava and banana peels

Supernatant from the above hydrolysis process were transferred into another sets of conical flasks correctly labelled, covered, autoclaved at 121^oC for15 minutes and allowed to cool. 5ml of freshly harvested cells of *Lactobacillus delbrueckii* was aseptically added into a set of flasks containing the hydrolysed supernatants (20g, 30g and 40g supernatants) and *Saccharomyces cerevisiae* was also added into another set of hydrolysed supernatant. The two organisms were combined into the third set of the hydrolysed supernatants while the

control set still served as control. The flasks were corked using cotton wool, shake and incubated 37^0C and at room temperature 28^0C for five days. The flasks were shaken at interval to produce a homogenous solution and even distribution of the organisms in the substrates mixture(Oyeleke *et al.*, 2012).

3.14.4 Distillation of fermented cassava and banana peels

This was carried out at using distillation apparatus (set up). The fermented liquid was transferred into round bottom flask and placed on a heating mantle fixed to a distillation column enclosed in running tap water. Another flask was fixed to the other end of distillation column to collect the distillate at 78^{o}C. This was done for each of the fermented broth according to the method described by Izah and Ohimain,(2015).

3.15 Determination of Quantity of Ethanol Produced

The distillate collected was measured using a measuring cylinder and expressed as quantity of ethanol produced in g/l by multiplying the volume of the distillate by the density of ethanol (0.8033g/cm3) (Oyeleke *et al.*, 2012).

3.16 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 weigh 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 note as X3.The pycnometer was empty, clean, dry and then filled with distillate. the weight of the distillate weredetermine at 20^{o}C and note 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 Suomalainen *et al.*(2012).

The density was calculated using Equation 6.

$$\text{Density [g/ml]} = \frac{\text{Mass}}{\text{Volume}}$$

$$\text{Sp. gravity} = \frac{\text{Density of distillate ethanol}}{\text{Density of distilled water}} = \qquad (6)$$

Where:

X1- weight (g) of empty pycnometer,

X2- weight (g) of pycnometer + distillate,

X3 - weight (g) of pycnometer + water

3.18 Confirmatory Test for Bio-ethanol Produced

Confirmatory test was carried out on the extracted bio-ethanol sample using potassium dichromate test as indicated by Mustafa *et al.* (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.

3.19 Data Analysis

Data obtained were statistically analysed by one-way analysis of variance. Comparison of means were made by the New Duncan's multiple range test (P = 0.05).

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

4.1 Result

4.1.1 Isolation and Characterization of fungi and bacteria isolates

4.1.1.1 Cultural and Microscopic Observation of *S. cerevisia*e and *Aspergillus niger*

The result of cultural and microscopic identification of fungi isolated from soil and fresh palm wine are shown in Table 4.1, the isolates identified were *Aspergillus niger* having a black colony with granular surface and black reveres while *Saccharomyces cerervisiae* has white to cream, smooth and glabrous yeast like colony with large globose to ellipsoidal budding.

Table 4.1 Cultural and Microscopic Characteristic of fungi isolated from soil and fresh palm wine

Cultural characteristics	Microscopic characteristics	Inferences/organism
Black colony with granular surface and black reveres	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*

4.1.1.2 Morphological and biochemical characteristic of *Z.mobilis* and *L. delbrueckii*

The result of morphological and biochemical characteristics of organisms isolated from fresh palm wine grown in an anaerobic jar are shown in Table 4.2. *Z. mobilis* appears Gram negative, catalase positve, motility positive, glucose positive, fructose positive, sucrose positive, maltose negative and lactose negative while *Lactobacillus delbrueckii* is gram positive, catalase negative, motility negative, glucose positive, fructose positive, sucrose positive, maltose positive and lactose positive.

Table 4.2 Morphological and biochemical characterization of isolated organisms from fresh palm wine and fresh cow milk

Gram reaction	Catalase	Motility	Glucose	Fructose	Sucrose	Maltose	Lactose	urease	Citrate	Suspected Organism
–	+	+	+	+	+	–	–	–	–	*Zymomonas mobilis*
+	–	–	+	+	+	+	+	–	–	*Lactobacillus delbrueckii*

Key: +: fermentation/positive; –: no fermentation/negative

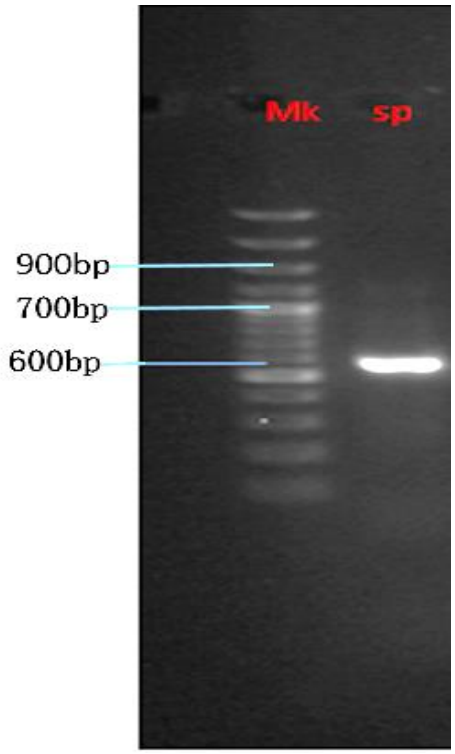

Plate I **Gelelectrophoresis micrograph of amplified product of *Saccharomyces cerevisiae* strain CBS 1171)**

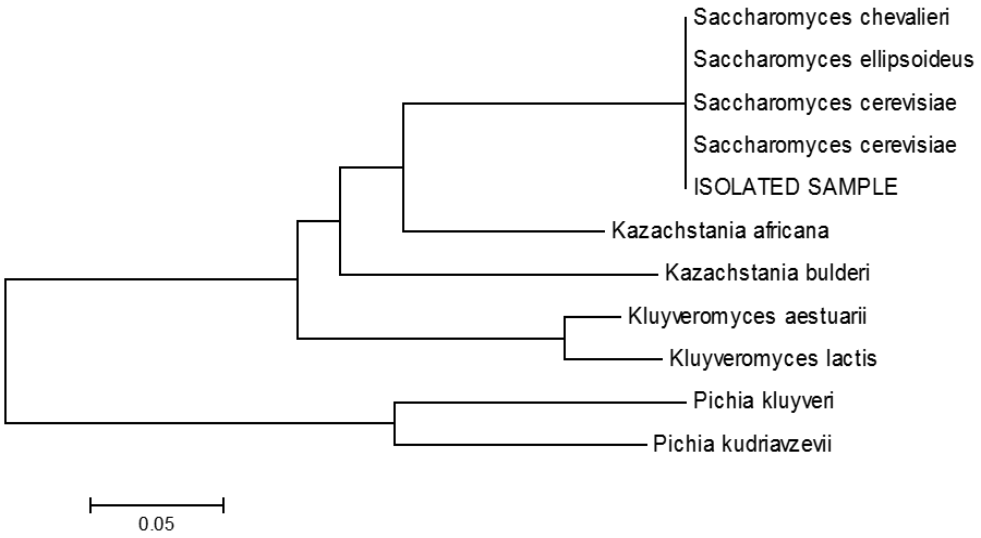

Figure 4.1 Phylogeny tree of *Saccharomyces cerevisiae* strain CBS 1171)

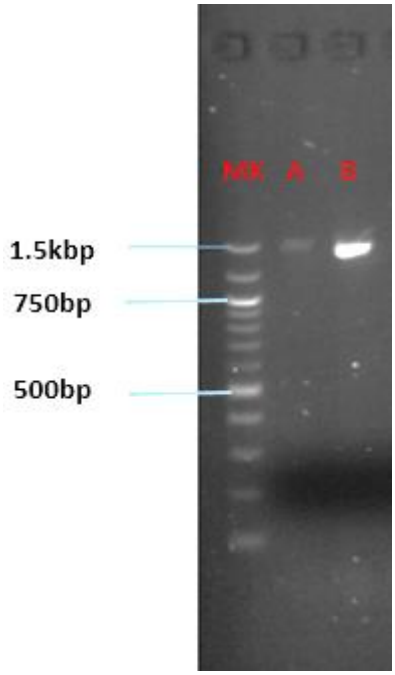

Plate II **Gel electrophoresis micrograph of amplified product of sample A; *Lactobacillus delbrueckii* strain MN945906 and sample B; *Zymomonas mobilis* strain MN945907**

Agarose gel confirming the positive amplification of bacteria 16s rRNA gene region using the 16s prokaryotic universal primers. A 1.5kbp amplification indicates a positive amplification. Loading arrangement molecular marker (MK) sample A (A) and sample B (B).

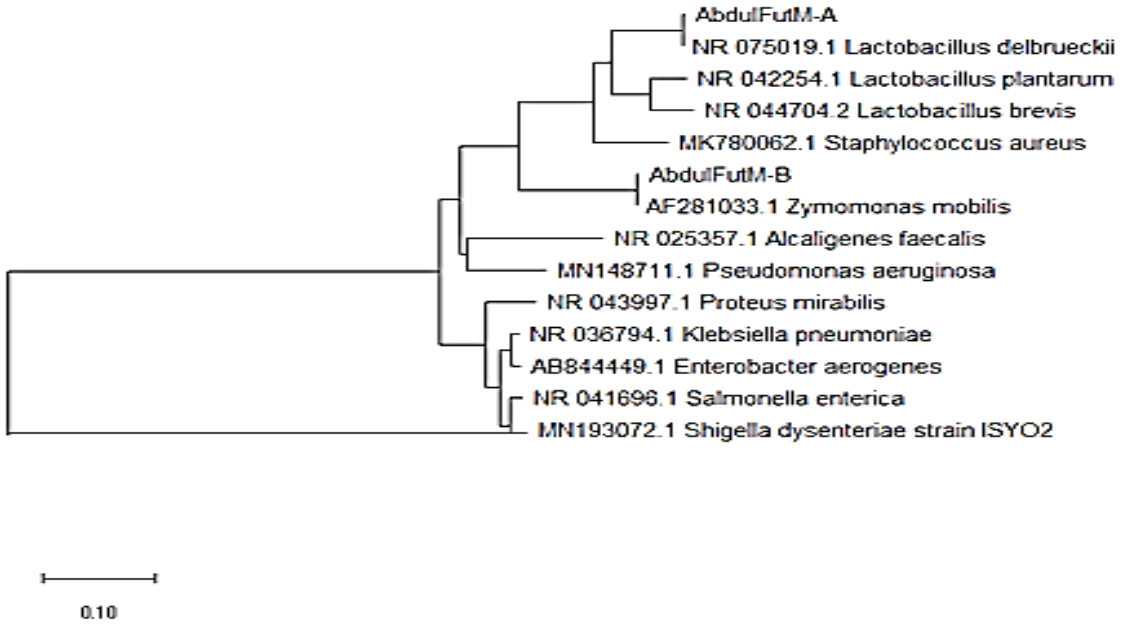

Figure 4.2 Phylogeny tree of *Lactobacillus delbrueckii* strain MN945906

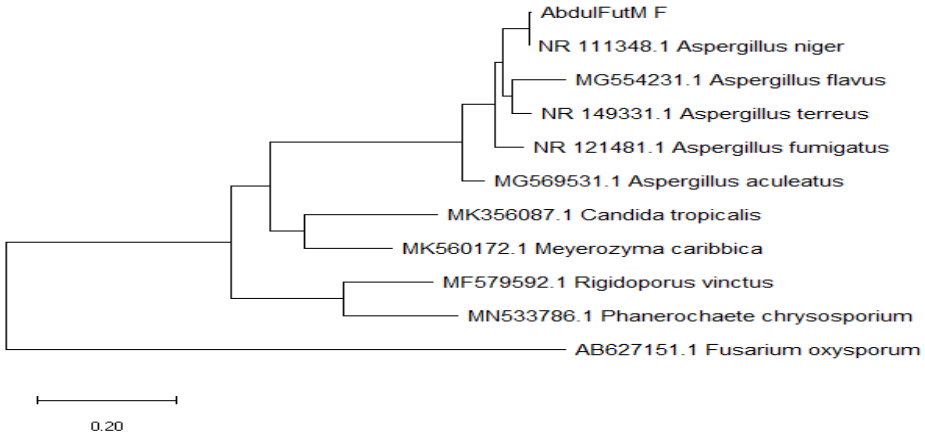

Figure 4.3 Phylogeny tree of *Zymomonas mobilis* strain MN945907

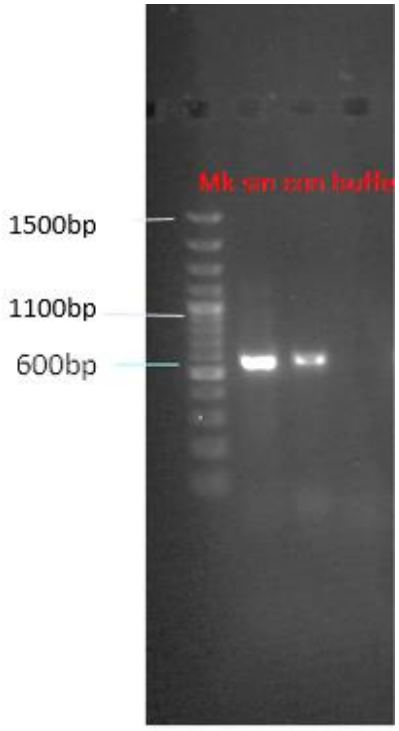

Plate III **Gelelectrophoresis micrograph of amplified product of *Aspergillus niger* strain MN945947. Agarose gel confirming the positive amplification of fungi ITS gene region using the ITS universal primers. A 600pb amplification indicates a positive amplification. Loading arrangement molecular marker (MK) fungi isolate (Sm) fungi positive control (con) and buffer control.**

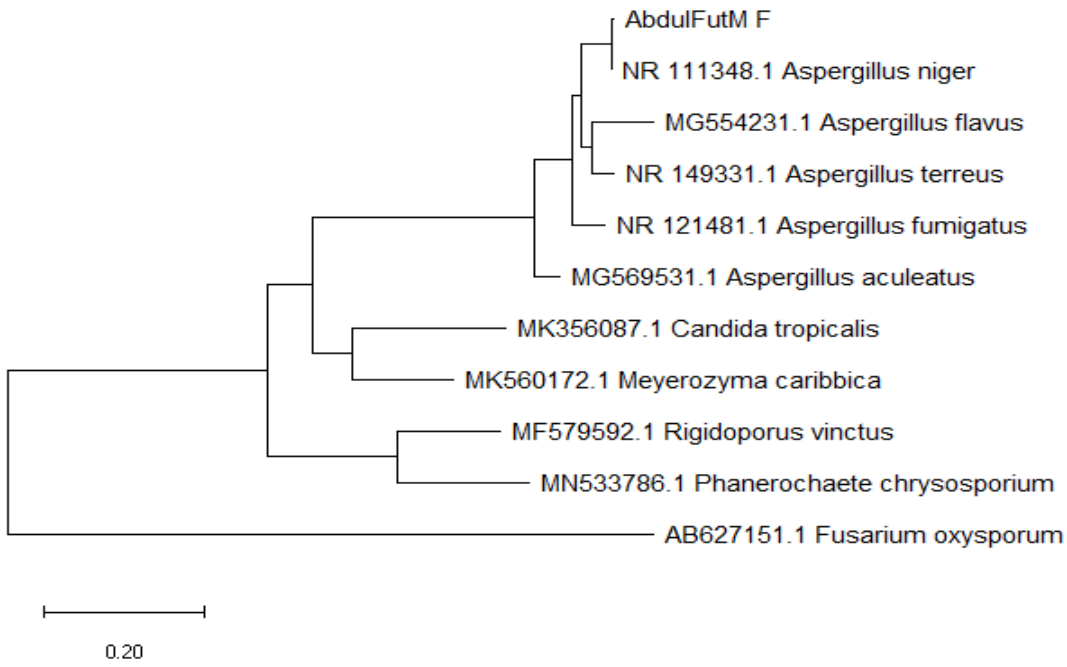

Figure 4.4 Phylogeny tree of *Aspergillus niger* strain MN945947.

4.1.2 The enzymatic activity of isolated organism and their zone of clearance

The result of enzymatic activity on the isolated organism as presented on Figure 4.5 shows the presence of zone of clearance on amylase, cellulose, and pectinase respectively.

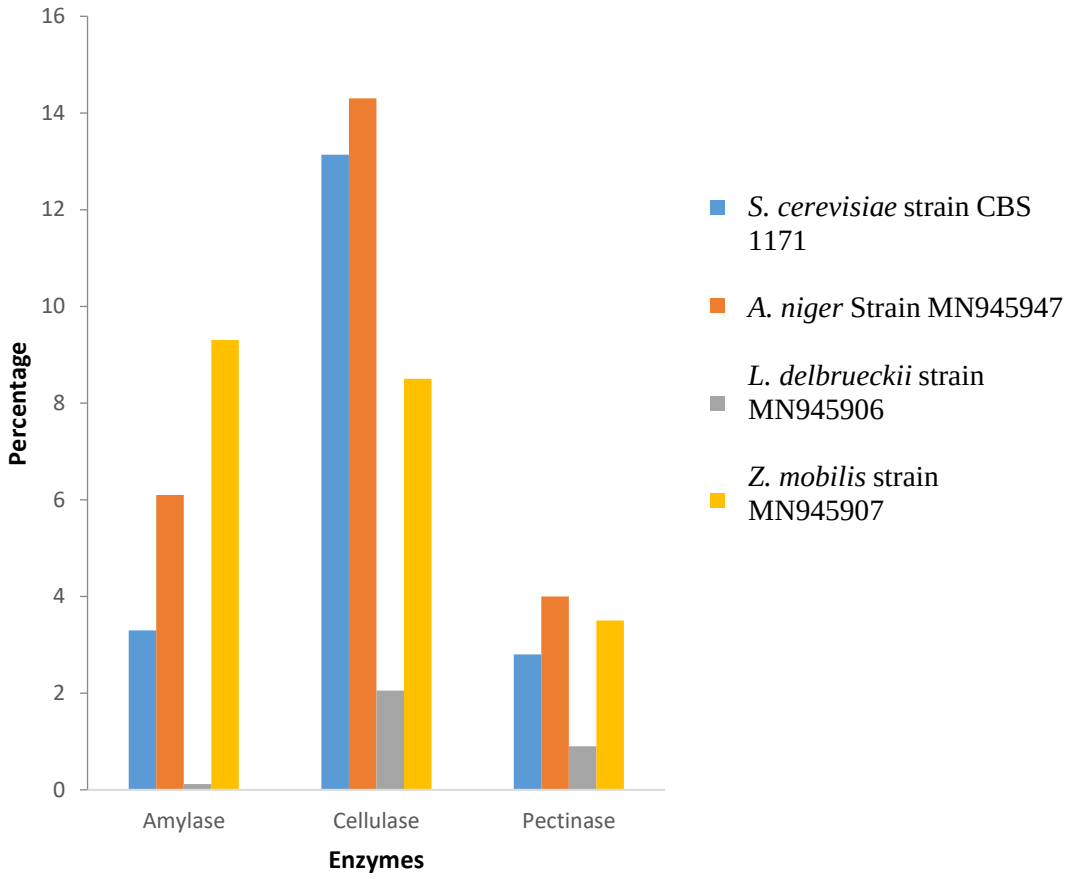

Figure 4.5: The enzymatic activity of isolated organism and their zone of clearance

4.1.3 Mineral Composition of Cassava and Banana peel

The result of mineral composition of agro-waste used in ethanol production are presented in Figure 4.6.The values recorded ranges from 64.7% to 0.21% for Cassava peel with potassium having (64.7%), phosphorus 31.3% and nitrogen 0.21%. The potassium content of banana peel recorded 43.5%, phosphorus 40.8% while nitrogen had 0.29%.

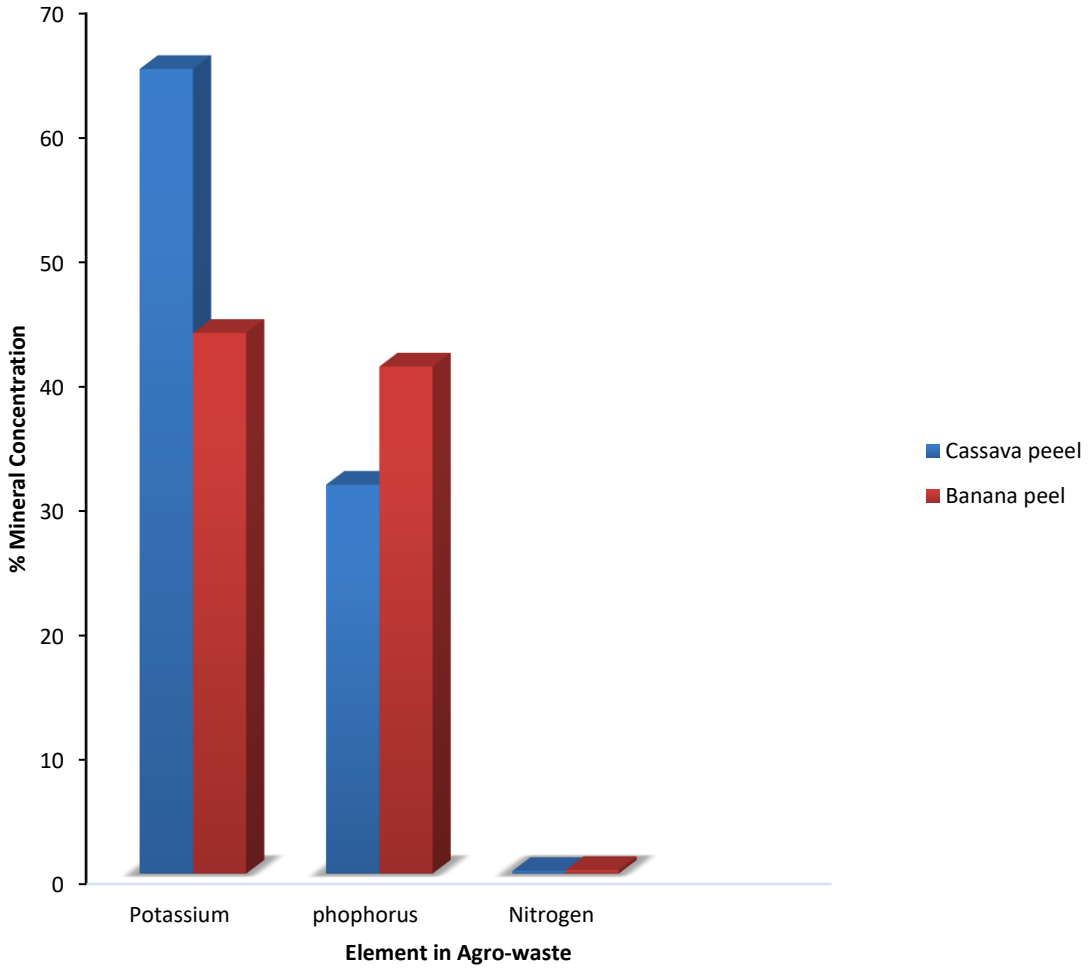

Figure 4.6: Mineral Composition of agro waste (Cassava and Banana peel)

4.1.4 Proximate Composition of Raw and Hydrolysed Agro-waste

The result of proximate composition of raw and hydrolysed agro-waste are presented in Table 4.3. The moisture value ranges from 5.41±0.00 % to 7.44±0.49 % (for raw agro waste) and 55.12±0.23 % to 63.16±0.85 % (for hydrolysed agro waste). The ash content ranges from 9.28±0.07 % to 10.50±0.39 % (for raw banana+ cassava peel). The carbohydrate content was 67.85±0.23 for Cassava peel, 63.47±0.28% banana + cassava peel and 60.13±0.64% for banana peel. The values of fibre, protein and Fat for banana peel are 14.21±0.59 %, 5.31±0.68 % and 3.62±0.00 %. The cassava peel recorded 10.49±0.43 %, 3.52±0.26 % and 2.21±0.07% while banana+ cassava peel recorded 12.22±0.59 %, 3.48±0.33 % and 3.80±0.18%. The result of hydrolysed agro waste for five days shows that banana peel has the highest moisture content of 63.16±0.85 %, banana+ cassava peel 60.61±0.30 % while cassava peel had the lowest content of 55.12±0.23 %. The ash content recorded 4.81±0.00 % for hydrolysed Cassava peel, 5.19±0.05 % for hydrolysed banana peel and 5.98±0.15% banana+ cassava peel. The average value recorded for fibre, fat, protein and carbohydrates were 12.64±0.28 %, 1.52±0.00 %, 3.98±0.30 % and 11.49±1.49 % for hydrolysed banana peel. Hydrolyzed cassava peel recorded 10.69±0.29 %, 1.35±0.05 %, 3.41±0.40 %, and 22.56±0.84 %. Hydrolysed banana+ cassava peel recorded 11.49±0.64 %, 1.30±0.05 4 %. 60±0.51% and 18±2.08 %.

Table 4.3: Proximate composition (%) of raw and hydrolysed Agro-waste

Sample	Moisture	Ash	Fibre	Fat	Protein	Carbohydrate
Raw B.P	7.44±0.49	9.28±0.07	14.21±0.59	3.62±0.00	5.31±0.68	60.13±0.64
Raw C.P	5.41±0.00	10.50±0.39	10.49±0.43	2.21±0.07	3.52±0.26	67.85±0.23
Raw B.P+C.P	6.81±0.00	10.21±0.21	12.22±0.59	3.80±0.18	3.48±0.33	63.47±0.28
Hydrolyzed B.P	63.16±0.85	5.19±0.05	12.64±0.28	1.52±0.00	3.98±0.30	11.49±1.49
Hydrolyzed C.P	55.12±0.23	4.81±0.00	10.69±0.29	1.35±0.05	3.41±0.40	22.56±0.84
Hydrolyzed B.P+C.P	60.61±0.30	5.98±0.15	11.49±0.64	1.30±0.05	4.60±0.51	18±2.08

Key: B.P: Banana peel, C.P: Cassava peel.

4.1.5 Percentage of reducing sugar in raw and hydrolysed Agro waste

The results presented in Table 4.4 Shows the percentage reducing sugar of raw and hydrolyzed agro waste. The highest yield of 37.82±0.68 % was recorded for raw banana peel while the lowest reducing sugar of 14.57±1.03 % was recorded for raw cassava peel. The reducing sugar values for hydrolyzed agro waste were 15.51±0.73 % for banana peel. Hydrolysed cassava peel had recorded 10.72±0.00 % and 7.98±0.66 % for mixture of peels.

Table: 4.4: Percentage of reducing sugar in raw and hydrolysed Agro waste

Sample	Reducing sugar (%)
Raw B.P	37.82±0.68
Raw C.P	14.57±1.03
Raw B.P+C.P	35.34±1.18
Hydrolyzed B.P	15.51±0.73
Hydrolyzed C.P	10.72±0.00
Hydrolyzed B.P+C.P	7.98±0.66

Key: B.P: Banana peel, C.P: Cassava peel.

4.1.6 Percentage of ethanol yield from the agro waste

The percentage ethanol yield from agro waste through microbial fermentation with *Saccharomyces cerevisiae*, *Lactobacillus delbrueckii* and *S. cerevisiae+ L. delbrueckii* are shown in figure 4.7. Cassava peel ethanol yield ranges from 8% to 25.99 %. The value of ethanol yield from banana+ cassava peel ranges from 9.54% to 21.94 % While Banana peel ranges from 6.25 % to 10.32 % of ethanol.

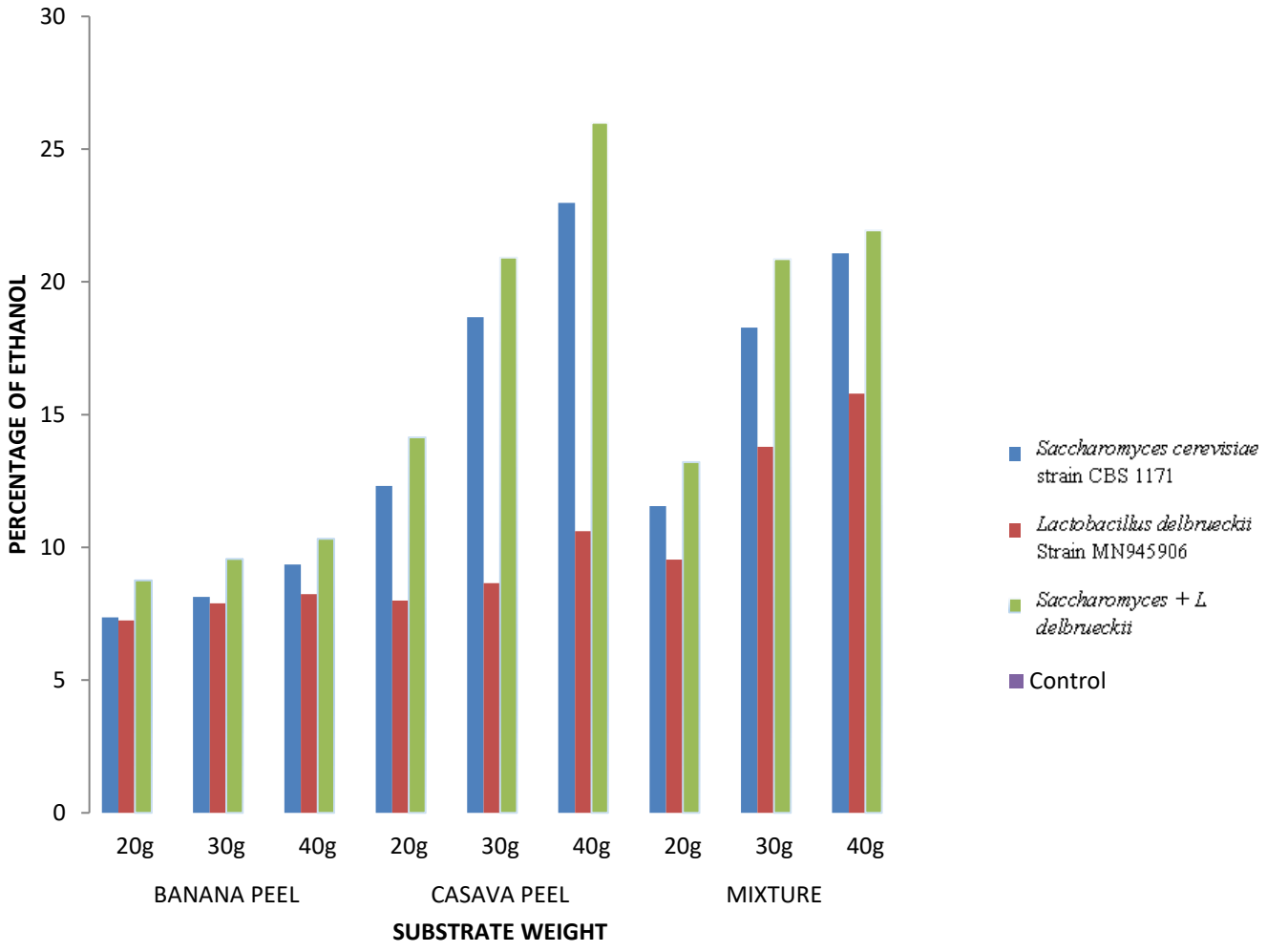

Figure 4.7: percentage of ethanol yield from the agro waste.

4.1.7 Average percentage of ethanol yield from the agro waste

The average percentage yield of ethanol from Banana, cassava peel and banana+ cassava peel are presented in figure 4.8. Mixture of banana+ cassava peel recorded the 16.22 %, cassava peel recorded 15.08 while banana peel recorded 8.54 % as the lowest yield. The average yield between the mixture of banana+ cassava peel and cassava peel are not significant.

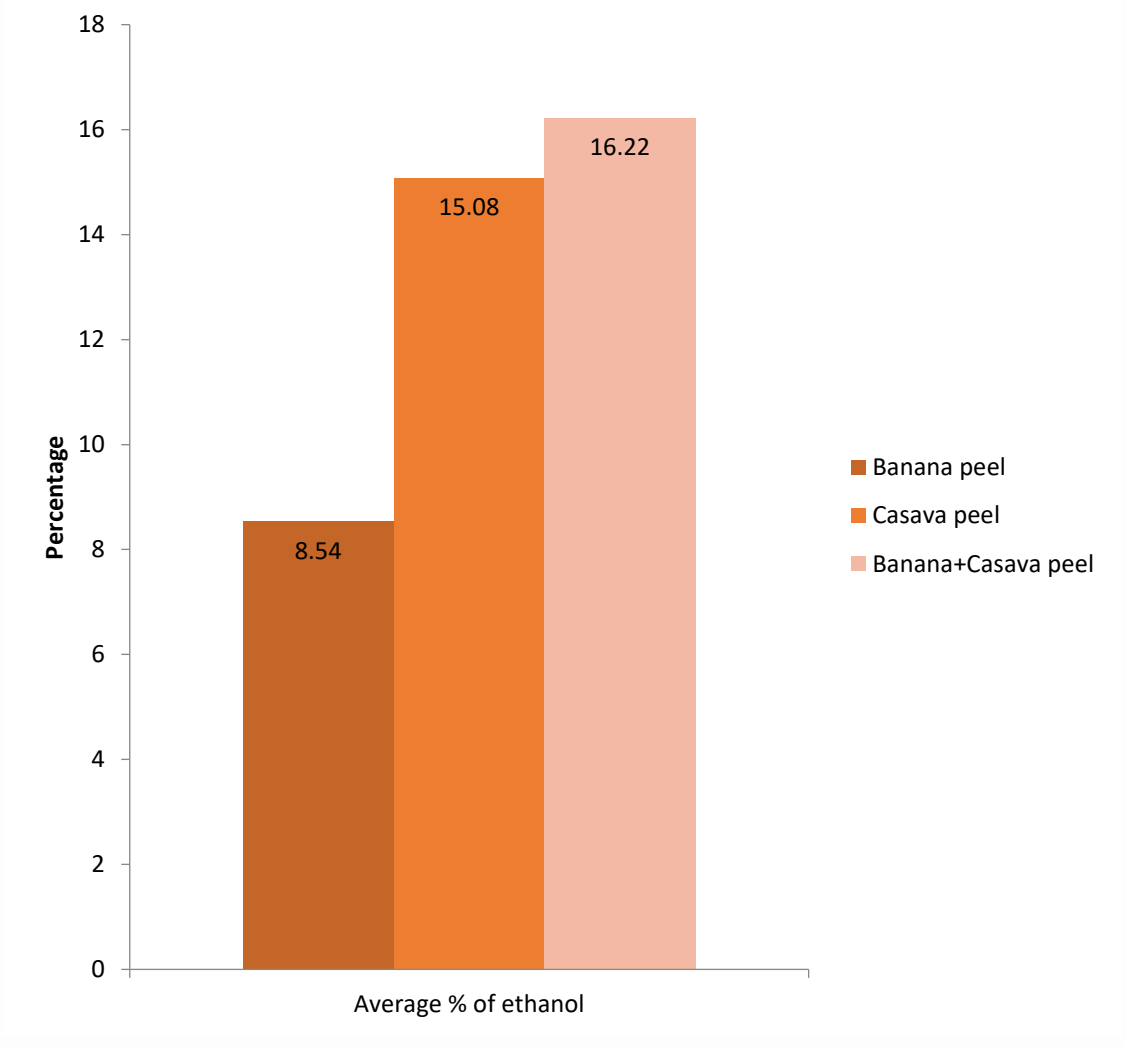

Figure 4.8: Average percentage of ethanol yield from the agro waste

4.1.8 Average percentage of ethanol yield from fermentation organism

The average percentages of ethanol yield from fermentation organism are show in Figure 4.9. *Saccharomyces cerevisiae* + *L. delbrueckii* recorded ethanol yield of 16.18 %. *S.cerevisiae* recorded 14.14 % while *L. delbrueckii* recorded 9.97 % as the lowest ethanol percentage yield.

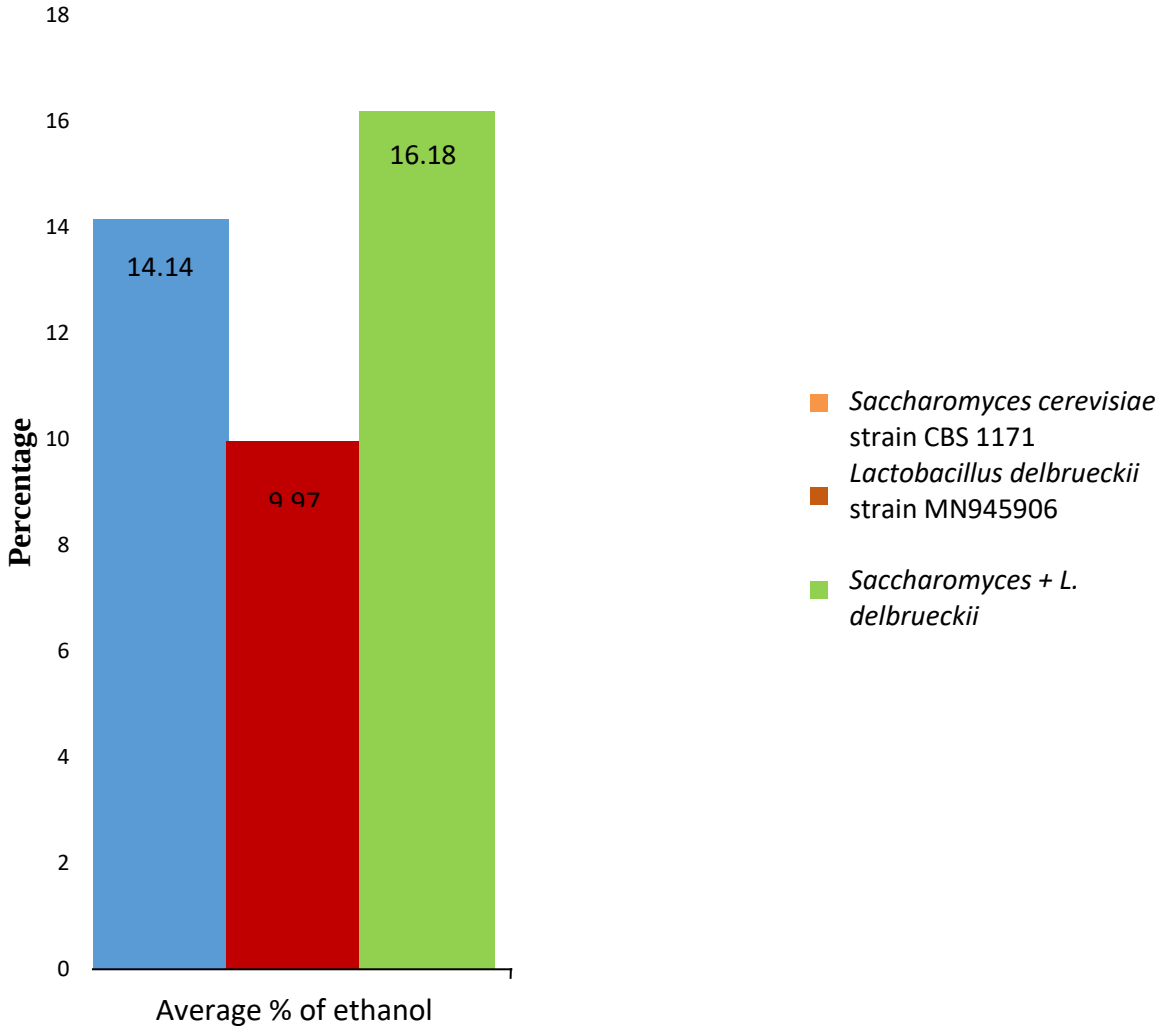

Figure 4.9: Average percentage of ethanol yield from fermentation organism

4.1.9 Average percentage weight and volume of ethanol from fermentation organism

The average percentage weight and volume of ethanol from fermentation organism are represented in Figure 4.10. *S. cerevisiae* + *L. delbrueckii* recorded 13.29 % (17.23 g/cm^3). *S. cerevisiae* recorded 11.39 % (15.05 g/cm^3) while *L. delbrueckii* recorded 8.22 % (15.05 g/cm^3) as the lowest.

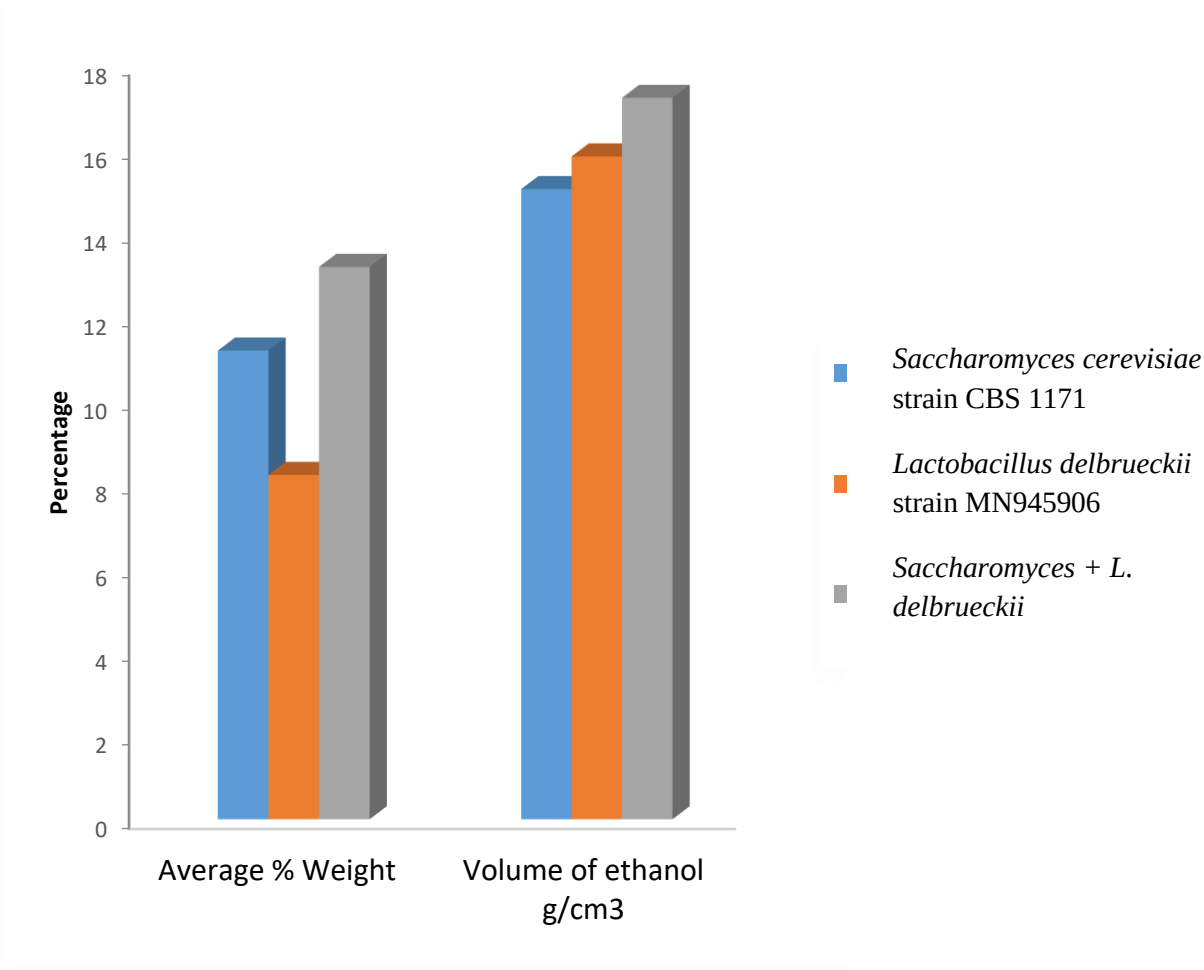

Figure 4.10: Average percentage weight and volume of ethanol from fermentation organism

4.1.10 Average percentage weight and volume of ethanol yield from agro waste

The average percentage weight and volume of ethanol yield from banana, cassava peel and mixture (banana+ cassava peel) are presented in Figure 4.11. The mixture recorded 13.83 % (15.7 g/cm^3). Cassava peel recorded 12.26 % (14.17 g/cm^3) while banana peel had 6.8 % (18.24 g/cm3).

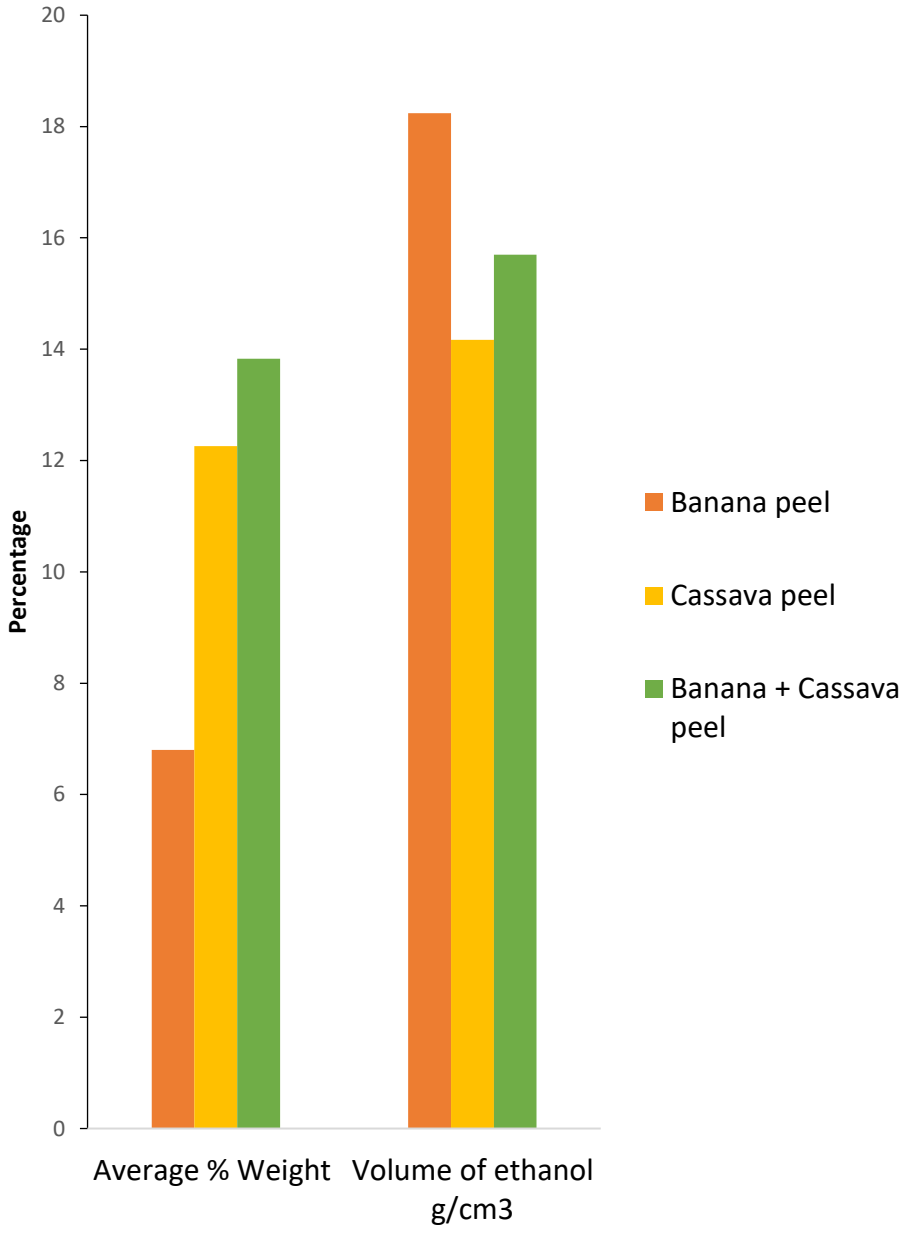

Figure 4.11: Average percentage weight and volume of ethanol yield from agro waste

4.1.11 pH result of raw and hydrolysed Agro waste

The results presented in Table 4.5 shows the pH of values of raw and hydrolysed agro waste. The pH values of 5.23±0.02, 6.185±0.01 and 6.14±0.01 were recorded for raw cassava, banana peel and mixture. Hydrolyzed banana, cassava peel and Mixture recorded 6.11±0.01, 5.24±0.02 and 5.81±0.0283 respectively.

Table 4.5: pH result of raw and hydrolysed Agro waste

Sample	pH
Raw B.P	5.23±0.02
Raw C.P	6.18±0.01
Raw B.P+C.P	6.14±0.01
Hydrolyzed B.P	6.11±0.01
Hydrolyzed C.P	5.24±0.02
Hydrolyzed B.P+C.P	5.81±0.02

Key: B.P: Banana peel, C.P: Cassava peel,

4.1.12 Confirmatory Test for Bio-ethanol Produced

Table 4.6 shows the confirmatory test on bioethanol produced. The distillate from Cassava, Banana peel and Mixture produced a green colour as an indication of ethanol presence.

Table 4.6: Confirmatory Test for Bio-ethanol Produced

Produced bio-ethanol	**Result in colour changes**
Banana	Green
Cassava	Green
Mixture	Green
Control	Negative

4.2 Discussion

The morphological, microscopic identification of fungi and biochemical characteristics of bacteria isolated from soil, fresh palm wine and cow milk are presented in Table 4.1and 4.2 respectively. The result shows that the suspected isolates were *Aspergillus niger*, *Saccharomyces cerevisiae*, *Lactobacillus delbrueckii* and *Zymomonas mobilis*. These findings were compared with Ellis *et al.* (2007) and Bergey's manual of bacteriology (2006) who reported that the conidial heads of *Aspergillus niger* are dark brown to black while *Saccharomyces cerevisiae* are white to cream, smooth, globrous yeast-like colonies, with a large globose to ellipsoidal budding, *Zymomonas mobilis* are anaerobic gram negative rods with single polar flagellum that ferments fructose, sucrose and glucose while *Lactobacillus bulgaricus* are rod-shaped, gram-positive, non-spore-forming bacteria. The result also conform to the work of Rabah *et al.* (2011) who isolated *Zymomonas mobilis* from rotten sweet orange and quantified that the organism can thrive in sweet mediums. In another findings by Fossi*et al.* (2016) also isolated *Lactobacillus delbrueckii* from corn-beer and palm-wine. The isolate were subjected to molecular identification and were identified as *Lactobacillus delbrueckii* strain MN945906, *Zymomonas mobilis* strain MN945907, *Aspergillus niger* strain MN945947 and *Saccharomyces cerevisiae* strain CBS 1171. This isolates shows clear zone of inhibition in their enzymatic activities as presented in Figure 4.1.

The mineral analysis of the agro-waste revealed that cassava peel recorded 64.0% of potassium, phosphorus 31.3% and nitrogen 0.21% as presented in Figure 4.6. The potassium content of banana peel recorded 43.5%, phosphorus 40.8% while nitrogen had 0.29%. However, the 0.21% nitrogen obtained in this work is in line with the work reported by Wantanee (2004) who also obtained 0.20±0.016% of nitrogen on yam peel. While the

potassium (64.0%) obtained was higher than 1.84±0.15 reported by Gani *et al.* (2018) for fermented cassava. This increase may be as result of raw nature of the substrates.

The proximate composition of raw and hydrolysed agro-waste is presented in Table.4.3. In raw agro waste the mean value of protein and fat have the lower percentage, than the fibre, ash and carbohydrate content. These differences may be as a result of starch content present in the substrate amounting to less protein and fat in the peels. However, the high carbohydrate value recorded in raw agro waste was 67.85±0.23 %, 63.47±0.28 % and 60.13±0.64 % respectively. This may be as a result of preliminary milling of the waste which potency has increased the surface area present in the substrate to take out more carbohydrate from the peels. 67.85±0.23 % carbohydrate yield in raw cassava peel is in agreement with the work of Oso *et al.* (2018) and Isah *et al.* (2019) produce of bioethanol from cassava peels and sugarcane bagas. Though hydrolysed agro waste recorded low percentage of fat, protein and ash content while moisture and carbohydrate content were high in the substrate. These values differ significantly as a result of heat used in the hydrolysis of the substrate which reduced the starch intake resulting in high increase in moisture content. This is in agreement with the work of Nwabanne and Aghadi (2018) who work on statistical modelling of enzymatic hydrolysis of banana peels and not in agreement with the work of Oyeleke *et al.* (2012) this may be as a result of differences in the species of peels used.

The result of reducing sugar and pH of agro -waste are presented in Table 4.4 and 4.5. There was no significant difference in the mean pH values of raw and hydrolysed waste. The pH values obtained are neutral and slightly alkaline. This signifies that at pH values 5.23±0.02 to 6.18±0.01 the micro-organism can thrive well in fermentation medium this correlate to the work of Aloe *et al.*(2012) who recorded 5.0 -6.0 pH values.

The percentage ethanol yield from banana peels, cassava peels and its mixture is presented in figure 4.8.The result revealed that the combination of *S. cerevisiea* and *L. delbrueckii* have a maximum yield of 25.99%, 10.14%, and 21.94% from cassava peels, banana peels and mixture respectively. *S.cerevisiea* has a maximum yield of 22.92%, 9.36% 21.94% while *L. delbrueckii* has a maximum ethanol yield of 10.61, 8.24 and 15.79 % for cassava peels, banana peels and mixture respectively. This result revealed a high production by *S. cerevisiae* than *L. delbrueckii*. This may be due to complex mechanism which enables them to breakdown sugar in either anaerobic or aerobic condition Karhumaa *et al.* (2005). As alcoholic fermentation yeasts, it utilizes sucrose, glucose, fructose, maltose and maltotriose as carbon sources to produce alcohol under anaerobic conditions as reported by Hemalatha *et al*. (2015). The yeast undergoes several physiological changes during the fermentation process. These resulted in a build-up of unsaturated fatty acids and sterols at the start of fermentation, which are vital nutrients for the yeast. The yeast consumes these nutrients and depletes the amount of sugar as the fermentation progresses. Fermentation was considered complete when the supply of sugar was completely converted to ethanol Braide *et al.* (2016).

When the organisms are combined together, the maximum ethanol yield of 25.99 %, for cassava peel, 10.14 %, for banana peel and 21.94 % for mixture were recorded. The highest yield from the mixtures could be because that the two organisms have syntrophic relationship which makes them to yield more when in combination than individually. These almost collaborate with the work of Oyeleke *et al.* (2012) produced 26 % from cassava and sweat potato peel using *S. cerevisiae* and *Z. mobilis* as fermentation organism. But not in agreement with the work of Hemalatha *et al.* (2015) who reports ethanol yield of 7% and 5% from fruit waste.

The average ethanol weight and volume from agro-waste is shown in Figure 4.10 and 4.11. The average ethanol weight and volume from mixture of *S.cerevisiae* and *L.delbrueckii* is

16.22 % (17.23 g/cm^3), that of *S.cerevisiae* is 14.14 % (15.05 g/cm^3) while average ethanol weight and volume of *L.delbrueckii* is 9.97 % (15.05 g/cm^3). This is in agreement with the work of Agulejika *et al.* (2005) who reported an average ethanol of 16 % from spoilt mangoes and Oyeleke *et al.* (2012) who reports an average ethanol yield of 17.6 % from cassava peel. The result of this research established that ethanol can be produced from agro waste (cassava and banana peels and its mixture). More ethanol is produced from mixture and cassava peels than from banana peels, and makes it a better alternative to banana peels. These has make agro waste as useful product which when properly harness will solve the problem of environmental pollution because it is pollution free, biodegradable, renewable and cause no climate change.

CHAPTER FIVE

5.0 CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

The study shows that *S. cerevisiae* strain CBS 1171 and *L. delbrueckii* strain MN945906 isolated and characterised to molecular level has the potential for ethanol production. The hydrolysed wastes were found to conserve nutrients and mineral elements that could serve as fertilizer to increase soil fertility. The proximate analysis of cassava and banana peels indicated that both are better choice than banana peel and cassava peels in ethanol production as a result of high carbohydrate, crude fibre and moisture content. Furthermore, the combination of *S. cerevisiae* strain CBS 1171 and *L. delbrueckii* strain MN945906 yielded more ethanol at 25% than when not in combined form. The findings of this work suggest that more ethanol can be produced from the combination of the organisms and serve as a better choice than synthetic ethanol produced from petrochemical sources because its pollution free and eco-friendly.

5.2 Recommendations

1. The combination of *S. cerevisiae* strain CBS 1171 and *L. delbrueckii* strain MN945906 yielded more ethanol and should be used in large scale production.
2. The use of these waste cassava and banana peels 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 use in these research.
4. The organism used in these work should be employ on other types of agro waste for high ethanol yield.

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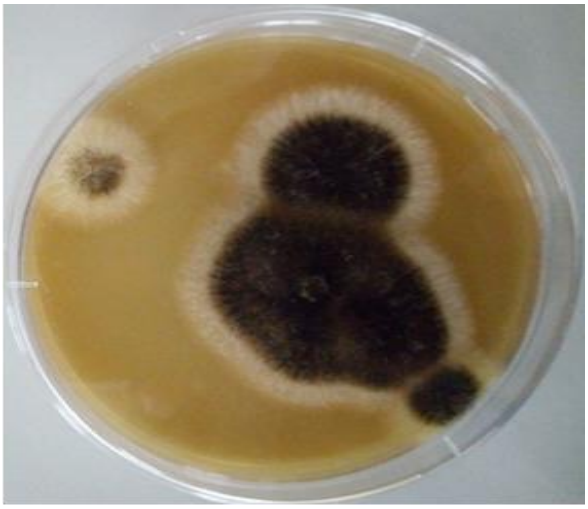
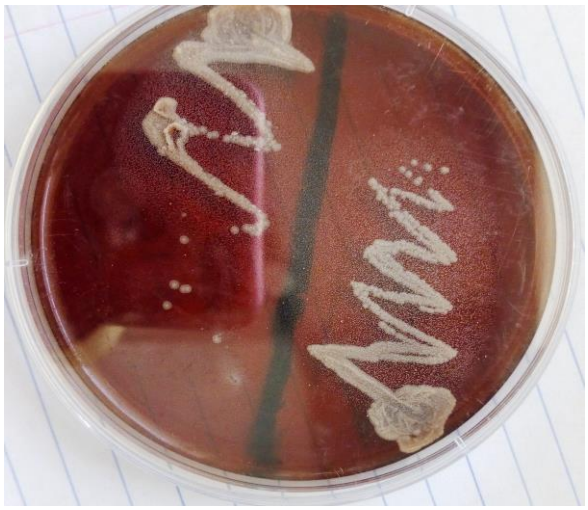
Appendix A

MACROSCOPIC VIEW OF BACTERIA AND FUNGI USED FOR THE PRODUCTION OF BIOETHANOL

Appendix A1: *A.niger* Strain MN945947 **Appendix A2:** *L. delbrueckii* Strain MN945906

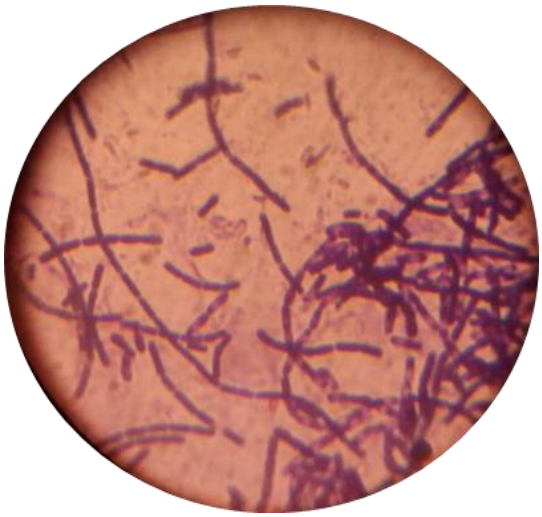




Appendix A3: *S. cerevisiae* Strain CBS117 **APpendix A4:** *Z. mobilis* Strain MN945907

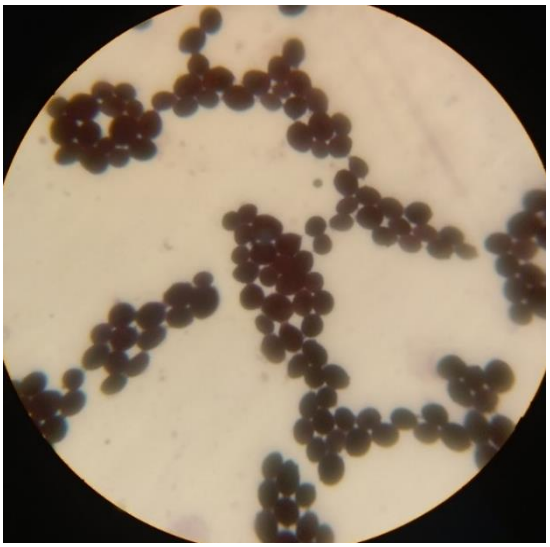
MICROSCOPIC VIEW OF BACTERIA AND FUNGI USED FOR THE PRODUCTION OF BIOETHANOL

Appendix A5: *L. delbrueckii* Strain MN945906

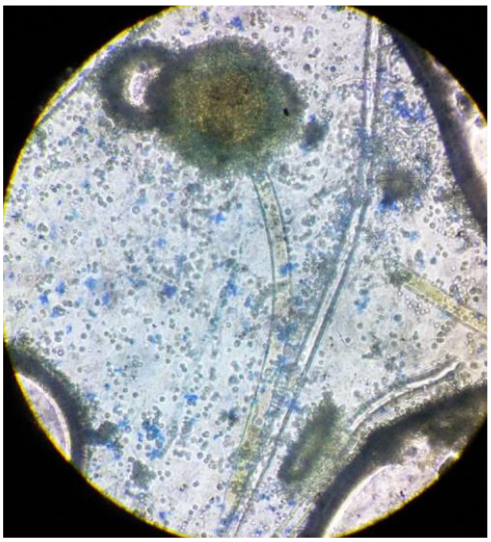


Appedix A6: *Z. mobilis* Strain MN945907

Appedix A7: *S. cerevisiae* Strain CBS117



Appedix A8: *A.niger* Strain MN945947



Appendix A9: Dried cassava peel

Appedix A10: Dried banana peel

Appendix A11: Cassava dump site

Appendix A12: Banana dump site

Appendix A13: Distillation apparatus used in extraction bioethanol

Appendix B

SEQUENCING RESULTS OF IDENTIFIED FUNGI AND BACTERIA ISOLATES

Sequencing results shows that the sample is 99% identical to *Saccharomyces cerevisiae* strain CBS 1171)

AAAAAAATTTAAAAATTTTGAAAATGGATTTTTTTTTTTTGGGCAAGAGCATGAGAGCTT
TTACTGGGCAAGAAGACAAGAGATGGAGAGTCCAGCCGGGCCTGCGCTTAAGTGCGCGG
TCTTGCTAGGCTTGTAAGTTTCTTTCTTGCTATTCCAAACGGTGAGAGATTTCTGTGCTTT
TGTTATAGGACAATTAAAACCGTTTCAATACAACACACTGTGGAGTTTTCATATCTTTGC
AACTTTTTCTTTGGGCATTCGAGCAATCGGGGCCCAGAGGTAACAAACACAAACAATTTT
ATCTATTCATTAAATTTTTGTCAAAAACAAGAATTTTCGTAACTGGAAATTTTAAAAATA
TTAAAAACTTTCAACAACGGATCTCTTGGTTCTCGCATCGATGAAGAACGCAGCGAAATG
CGATACGTAATGTGAATTGCAGAATTCCGTGAATCATCGAATCTTTGAACGCACATTGCC
CCCTTGGTATTCCAGGGGGCATGCCTGTTTGAGCGTCATTTCCTTCTCAAACATTCTTTTT
GAGTGAGTGATACTCTTTGGAGTTAACTTGAAATTGCTGGCCTTTTCATTGGATGTTTTTT
TTCCAAAGGAGGTTTCTCTGCGTGCTTGAGGTATAATGCAAGTACGGTCGTTTTAGGTTTT
ACCAACTGCGGCTAATCTTTTTTATACTGAGCGTATTGGAACGTTATCGATAAGAAGAGA
GCGTCTAGGCAACAATGTTCT

Sequence of sample A; 99% identical to *Lactobacillus delbrueckii* strain MN945906

GCTCAGGACGAACGCTGGCGGCGTGCCTAATACATGCAAGTCGAGCGAGCTGAA
TTCAAAGATTCCTTCGGGATGATTTGTTGGACGCTAGCGGCGGATGGGTGAGTAA
CACGTGGGCAATCTGCCCTAAAGACTGGGATACCACTTGGAAACAGGTGCTAAT
ACCGGATAACAACATGAATCGCATGATTCAAGTTTGAAAGGCGGCGTAAGCTGT
CACTTTAGGATGAGCCCGCGGCGCATTAGCTAGTTGGTGGGGTAAAGGCCTACC
AAGGCAATGATGCGTAGCCGAGTTGAGAGACTGATCGGCCACATTGGGACTGAG
ACACGGCCCAAACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCACAATGGACG
CAAGTCTGATGGAGCAACGCCGCGTGAGTGAAGAAGGTTTTCGGATCGTAAAGC
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CAACCAGAAAGTCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTG
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CTGATGTGAAAGCCCACGGCTCAACCGTGGAACTGCATCGGAAACTGTCATTCTT
GAGTGCAGAAGAGGAGAGTGGAATTCCATGTGTAGCGGTGTAATGCGTAGATAT
ATGGAAGAACACCAGTGGCGAAGGCGGCTCTCTGGTCTGCAACTGACGCTGAGG
CTCGAAAGCATGGGTAGCGAACAGGATTAGATACCCTGGTAGTCCATGCCGTAA
ACGATGAGCGCTAGGTGTTGGGGACTTTCCGGTCCTCAGTGCCGCAGCAAACGC
ATTAAGCGCTCCGCCTGGGGAGTACGACCGCAGGTTGAAACTCAAAGGAATTGA
CGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAAGCGAAGAA
CCTTACCAGGTCTTGACATCCTGTGCTACACCTAGAGATAGGTGGTTCCCTTCGG

GGACGCAGAGACAGGTGGTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTG
GGTTAAGTCCCGCAACGAGCGCAACCCTTGTCTTTAGTTGCCATCATTAAGTTGG
GCACTCTAAAGAGACTGCCGGTGACACCGGAGGAAGGTGGGGATGACGTCAAGT
CATCATGCCCCTTATGACCTGGGCTACACACGTGCTACATGGGCAGTACAACGAG
AAGCGAACCCGCGAGGGTAAGCGGATCTCTTAAAGCTGTTCTCAGTTCGGACTCA
GGCTGAACTCGCCTGCACGAAGCTGGAATCGCTAGTAATCGCGGATCAGCACGC
CGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCATGGAAGTC
TGCAATGCCCAAAGTCGGTGGGATAACCTTTATAGAGTCAGCCGCCTAAGGCAG
GGCAGATGACTGGGGTGAAGTCGTAACAAGGTAGCCGTAGGGGCAT

Sequence of sample B; 99% identical to *Zymomonas mobilis*strain MN945907

TGGCCCAGAACGAACGCGGGGGGCATCCTTAACACATGCAAGTCGAACGAAGGC
TTCGGCCTTAGTGGCGCACGGGTGCGTAACGCGTGGGAATCTGCCTTCAGGTACG
GAATAACTAGGGGAAACTCGAGCTAATACCGTATGACATCGAGAGATCAAAGAT
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CCAAGGCGACGATCCATAGCTGGTCTGAGAGGATGATCAGCCACACTGGACTGA
GACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGGACAATGGGG
GAAACCCTGATCCAGCAATGCCGCGTGAGTGAAGAAGGCCTTAGGGTTGTAAAG
CTCTTTTACCCGGGATGATAATGACAGTACCGGGAGAATAAGCTCCGGCTAACTC
CGTGCCAGCAGCCGCGGTAATACGGAGGGAGCTAGCGTTGTTCGGAATTACTGG
GCGTAAAGCGTACGTAGGCGGTTTAATAAGTCAGGGGTGAAAGCCCAGAGCTCA
ACTCTGGAACTGCCTTTGAGACTGTTAGACTAGAACATAGAAGAGGTAAGTGGA
ATTCCGAGTGTAGAGGTGAAATTCGTAGATATTCGGAAGAACACCAGTGGCGAA
GGCGACTTACTGGTCTATAGTTGACGCTGAGGTACGAAAGCGTGGGTAGCAAAC
AGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGATAACTAGCTGTCGGGT
ACATGGTATCTGGGTGGCGGAGCTAACGCATTAAGTTATCCGCCTGGGGAGTAC
GGTCGCAAGATTAAAACTCAAAGAAATTGACGGGGGCCTGCACAAGCGGTGGAG
CATGTGGTTTAATTCGAAGCAACGCGCAGAACCTTACCAGCGTTTGACATCCTGA
TCGCGGAAAGTGGAGACACATTCTTTCAGTTCGGCTGGATCAGAGACAGGTGCT
GCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGC
GCAACCCTCACCTCTAGTTGCCATCATTAAGTTGGGCACTTTAGAGGAACTGCCG
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TGGGCTACACACGTGCTACAATGGCGGTGACAGAGGGCCGCAAGCCTGCAAAGG
TTAGCTAATCTCAAAAAGCCGTCTCAGTTCGGATTGTTCTCTGCAACTCGAGAGC
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CCAGGCCTTGTACACACCGCCCGTCACACCATGGGAGTTGGATTCACCCGAAGG
CGCTGCGCTAACCCGCAAGGGAGGCAGGCGACCACGGTGGGTTTAGCGACTGGG
GTGAAGTC

99% identical to *Aspergillus niger*strain MN945947

TAAACCTGCGGAAGGATCATTACCGTGCGGGTCCTTTGGGCCCAACCTCCCATCC
GTGTCTATTGTACCCTGTTGCTTCGGCGGGCCCGCCGCTTGTCGGCCGCCGGGGG
GGCGCCTCTGCCCCCCGGGCCCGTGCCCGCCGGAGACCCCAACACGAACACTGT
CTGAAAGCGTGCAGTCTGAGTTGATTGAATGCAATCAGTTAAAACTTTCAACAAT
GGATCTCTTGGTTCCGGCATCGATGAAGAACGCAGCGAAATGCGATAACTAATG
TGAATTGCAGAATTCAGTGAATCATCGAGTCTTTGAACGCACATTGCGCCCCCTG
GTATTCCGGGGGGCATGCCTGTCCGAGCGTCATTGCTGCCCTCAAGCCCGGCTTG
TGTGTTGGGTCGCCGTCCCCCTCTCCGGGGGGACGGGCCCGAAAGGCAGCGGCG
GCACCGCGTCCGATCCTCGAGCGTATGGGGCTTTGTCACATGCTCTGTAGGATTG
GCCGGCGCCTGCCGACGTTTTCCAACCATTCTTTCCAGGTTGACCTCGGATCAGG
TAGGGATACCCGCTGAACAAA

APPENDIX C

Appendix C1: Determination of the Alcohol Content of Beverages

Specific gravity (20/20)	*Per cent* by weight	by volume	*G in* 100ml	*Specific gravity* (20/20)	*Per cent* by weight	by volume	*G in* 100ml
0·9700	20·27	24·86	19·62	0·9650	23·88	29·14	23·00
0·9699	20·34	24·95	19·69	0·9649	23·95	29·22	23·07
0·9698	20·41	25·04	19·76	0·9648	24·02	29·31	23·13
0·9697	20·49	25·12	19·83	0·9647	24·09	29·39	23·20
0·9696	20·56	25·21	19·90	0·9646	24·16	29·47	23·26
0·9695	20·64	25·30	19·97	0·9645	24·23	29·55	23·33
0·9694	20·71	25·39	20·04	0·9644	24·30	29·64	23·39
0·9693	20·78	25·48	20·11	0·9643	24·37	29·72	23·46
0·9692	20·86	25·56	20·18	0·9642	24·44	29·80	23·52
0·9691	20·93	25·65	20·25	0·9641	24·51	29·88	23·59
0·9690	21·00	25·74	20·32	0·9640	24·58	29·96	23·65
0·9689	21·08	25·83	20·39	0·9639	24·65	30·04	23·71
0·9688	21·15	25·91	20·45	0·9638	24·71	30·12	23·78
0·9687	21·22	26·00	20·52	0·9637	24·78	30·20	23·84
0·9686	21·30	26·09	20·59	0·9636	24·85	30·29	23·91
0·9685	21·37	26·17	20·66	0·9635	24·92	30·37	23·97
0·9684	21·44	26·26	20·73	0·9634	24·99	30·45	24·03
0·9683	21·52	26·35	20·80	0·9633	25·06	30·53	24·10
0·9682	21·59	26·43	20·86	0·9632	25·13	30·61	24·16
0·9681	21·66	26·52	20·93	0·9631	25·19	30·69	24·22
0·9680	21·73	26·61	21·00	0·9630	25·26	30·77	24·28
0·9679	21·81	26·69	21·07	0·9629	25·33	30·85	24·35
0·9678	21·88	26·78	21·14	0·9628	25·40	30·92	24·41
0·9677	21·95	26·86	21·20	0·9627	25·47	31·00	24·47
0·9676	22·02	26·95	21·27	0·9626	25·53	31·08	24·54
0·9675	22·10	27·04	21·34	0·9625	25·60	31·16	24·60
0·9674	22·17	27·12	21·41	0·9624	25·67	31·24	24·66
0·9673	22·24	27·21	21·47	0·9623	25·74	31·32	24·72
0·9672	22·31	27·29	21·54	0·9622	25·80	31·40	24·78
0·9671	22·38	27·38	21·61	0·9621	25·87	31·47	24·84
0·9670	22·46	27·46	21·68	0·9620	25·94	31·55	24·91
0·9669	22·53	27·55	21·74	0·9619	26·00	31·63	24·97
0·9668	22·60	27·63	21·81	0·9618	26·07	31·71	25·03
0·9667	22·67	27·72	21·88	0·9617	26·13	31·78	25·09
0·9666	22·74	27·80	21·94	0·9616	26·20	31·86	25·15
0·9665	22·81	27·89	22·01	0·9615	26·27	31·94	25·21
0·9664	22·89	27·97	22·08	0·9614	26·33	32·01	25·27
0·9663	22·96	28·05	22·14	0·9613	26·40	32·09	25·33
0·9662	23·03	28·14	22·21	0·9612	26·46	32·17	25·39
0·9661	23·10	28·22	22·28	0·9611	26·53	32·24	25·45
0·9660	23·17	28·31	22·34	0·9610	26·59	32·32	25·51
0·9659	23·24	28·39	22·41	0·9609	26·66	32·39	25·57
0·9658	23·31	28·47	22·48	0·9608	26·72	32·47	25·63
0·9657	23·38	28·56	22·54	0·9607	26·79	32·54	25·69
0·9656	23·46	28·64	22·61	0·9606	26·85	32·62	25·75
0·9655	23·53	28·73	22·67	0·9605	26·92	32·69	25·81
0·9654	23·60	28·81	22·74	0·9604	26·98	32·77	25·87
0·9653	23·67	28·89	22·81	0·9603	27·05	32·84	25·93
0·9652	23·74	28·98	22·87	0·9602	27·11	32·92	25·98
0·9651	23·81	29·06	22·94	0·9601	27·17	32·99	26·04

Appendix C2: The percentage of ethanol yield from Cassava, Banana peel and (Banana +Cassava) peel through the activities of fermentation organisms

SUBTRATE	BANANA PEEL			CASAVA PEEL			BANANA+CASAVA PEEL		
Fermentation organisms	**20g**	**30g**	**40g**	**20g**	**30g**	**40g**	**20g**	**30g**	**40g**
S. cerevisia	$7.36^{b}\pm 0.10$	$8.13^{c}\pm 0.05$	$9.36^{c}\pm 0.05$	$12.31^{c} \pm 0.30$	$18.67^{c} \pm 1.18$	$22.98^{c} \pm 0.09$	$11.56^{c} \pm 0.02$	$18.28^{c} \pm 0.12$	$21.08^{c} \pm 0.15$
L.bulgaricus	$7.25^{b}\pm 0.02$	$7.89^{b}\pm 0.02$	$8.24^{b}\pm 0.13$	$8.00^{b}\pm 0.05$	$8.65^{b}\pm 0.21$	$10.61^{b} \pm 0.36$	$9.54^{b}\pm 0.02$	$13.79^{b} \pm 0.03$	$15.79^{b} \pm 0.06$
Sacch+lac	$8.76^{c}\pm 0.08$	$9.56^{d}\pm 0.11$	$10.14^{d} \pm 0.02$	$14.14^{d} \pm 0.41$	$20.92^{d} \pm 0.31$	$25.99^{d} \pm 0.17$	$13.21^{d} \pm 0.15$	$20.86^{d} \pm 0.09$	$21.94^{d} \pm 0.39$
Control	$0^{a}\pm 0.00$	$0^{a}\pm 0.00$	$0^{a}\pm 0.00$	$0^{a}\pm 0.00$	$0^{a}\pm 0.00$	$0^{a}\pm 0.00$	$0^{a}\pm 0.00$	$0^{a}\pm 0.00$	$0^{a}\pm 0.00$

Means value with the letter in the same column do not differ significantly at P<0.005

Appendix C3: Percentage of organism identity and there Accession number

Sample code	**Strain**	**Accession number**	**Organism**	**% identity to most identical organism**
Abdulfut A	MN945906	FT9516	*Lactobacillus delbrueckii*	99.41%
Abdulfut B	MN945907	FT9517	*Zymomonas mobilis*	99.44%
Abdulfut C	MN945947	FT5927	*Aspergillus niger*	99.63%
Isolate D	CBS1171	FT9524	*Saccharomyces cerevisiae*	99.65%

APPENDIX D

Case Processing Summary

	Cases					
	Included		Excluded		Total	
	N	Percent	N	Percent	N	Percent
20g * Fermentation_organisms	12	100.0%	0	0.0%	12	100.0%
30g * Fermentation_organisms	12	100.0%	0	0.0%	12	100.0%
40g * Fermentation_organisms	12	100.0%	0	0.0%	12	100.0%

Report

Fermentation_organisms		20g	30g	40g
Saccharomyces	Mean	7.3633	8.1367	9.3667
	Std. Error of Mean	.10667	.05333	.05333
Lactobacillus	Mean	7.2567	7.8967	8.2467
	Std. Error of Mean	.02667	.02667	.13667
Sacch+Lacto	Mean	8.7600	9.5600	10.1433
	Std. Error of Mean	.08000	.11000	.02667
Control	Mean	.0000	.0000	.0000
	Std. Error of Mean	.00000	.00000	.00000
Total	Mean	5.8450	6.3983	6.9392
	Std. Error of Mean	1.03349	1.13050	1.22535

ANOVA

		Sum of Squares	Df	Mean Square	F	Sig.
20g	Between Groups	140.878	3	46.959	3386.494	.000
	Within Groups	.111	8	.014		
	Total	140.989	11			
30g	Between Groups	168.605	3	56.202	4786.511	.000
	Within Groups	.094	8	.012		
	Total	168.699	11			
40g	Between Groups	198.063	3	66.021	3959.282	.000
	Within Groups	.133	8	.017		
	Total	198.196	11			

Post Hoc Tests

Homogeneous Subsets

20g

Duncan[a]

Fermentation_organisms	N	Subset for alpha = 0.05		
		1	2	3
Control	3	.0000		
Lactobacillus	3		7.2567	
Saccharomyces	3		7.3633	
Sacch+Lacto	3			8.7600
Sig.		1.000	.299	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

30g

Duncan[a]

Fermentation_organisms	N	Subset for alpha = 0.05			
		1	2	3	4
Control	3	.0000			
L.bulgaricus	3		7.8967		
S.cerevisiae	3			8.1367	
Sacch+Lacto	3				9.5600
Sig.		1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

40g

Duncan[a]

Fermentation_organisms	N	Subset for alpha = 0.05			
		1	2	3	4
Control	3	.0000			
L.bulgricus	3		8.2467		
S.cerevisiae	3			9.3667	
Sacch+Lacto	3				10.1433
Sig.		1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Case Processing Summary

	Cases					
	Included		Excluded		Total	
	N	Percent	N	Percent	N	Percent
20g * Fermentation_organisms	12	100.0%	0	0.0%	12	100.0%
30g * Fermentation_organisms	12	100.0%	0	0.0%	12	100.0%
40g * Fermentation_organisms	12	100.0%	0	0.0%	12	100.0%

Report

Fermentation_organisms		20g	30g	40g
S.cerevisia	Mean	12.3133	18.6700	22.9800
	Std. Error of Mean	.30667	1.18568	.09000
L.bulgaricus	Mean	8.0033	8.6567	10.6133
	Std. Error of Mean	.05333	.21667	.36333
Sacch+Lacto	Mean	14.1467	20.9267	25.9967
	Std. Error of Mean	.41333	.31333	.17333
Control	Mean	.0000	.0000	.0000
	Std. Error of Mean	.00000	.00000	.00000
Total	Mean	8.6158	12.0633	14.8975
	Std. Error of Mean	1.64735	2.53358	3.12304

ANOVA

		Sum of Squares	Df	Mean Square	F	Sig.
20g	Between Groups	356.608	3	118.869	591.979	.000
	Within Groups	1.606	8	.201		
	Total	358.214	11			
30g	Between Groups	838.008	3	279.336	240.141	.000
	Groups	9.306	8	1.163		
	Total	847.314	11			
40g	Between Groups	1286.424	3	428.808	3360.125	.000
	Within Groups	1.021	8	.128		
	Total	1287.445	11			

Post Hoc Tests

Homogeneous Subsets

20g

Duncan[a]

Fermentation_organisms	N	Subset for alpha = 0.05			
		1	2	3	4
Control	3	.0000			
L.bulgaricus	3		8.0033		
S.cerevisiae	3			12.3133	
Sacch+Lacto	3				14.1467
Sig.		1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

30g

Duncan[a]

Fermentation_organisms	N	Subset for alpha = 0.05			
		1	2	3	4
Control	3	.0000			
L.bulgaricus	3		8.6567		
S.cerevisiae	3			18.6700	
Sacch+Lacto	3				20.9267
Sig.		1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

40g

Duncan[a]

Fermentation_organisms	N	Subset for alpha = 0.05			
		1	2	3	4
Control	3	.0000			
L.bulgaricus	3		10.6133		
S.cerevisiae	3			22.9800	
Sacch+Lacto	3				25.9967
Sig.		1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Case Processing Summary

	Cases					
	Included		Excluded		Total	
	N	Percent	N	Percent	N	Percent
20g * Fermentation_organisms	12	100.0%	0	0.0%	12	100.0%
30g * Fermentation_organisms	12	100.0%	0	0.0%	12	100.0%
40g * Fermentation_organisms	12	100.0%	0	0.0%	12	100.0%

Report

Fermentation_organisms		20g	30g	40g
S.cerevisiae	Mean	11.5633	18.2833	21.0833
	Std. Error of Mean	.02667	.12667	.15667
L.bulgaricus	Mean	9.5433	13.7900	15.7900
	Std. Error of Mean	.02333	.03000	.06000
Sacch+Lacto	Mean	13.2100	20.8667	21.9433
	Std. Error of Mean	.15000	.09333	.39667
Control	Mean	.0000	.0000	.0000
	Std. Error of Mean	.00000	.00000	.00000
Total	Mean	8.5792	13.2350	14.7042
	Std. Error of Mean	1.54426	2.42737	2.65801

ANOVA

		Sum of Squares	Df	Mean Square	F	Sig.
20g	Between Groups	314.645	3	104.882	5886.712	.000
	Within Groups	.143	8	.018		
	Total	314.787	11			
30g	Between Groups	777.604	3	259.201	13470.832	.000
	Within Groups	.154	8	.019		
	Total	777.758	11			
40g	Between Groups	931.473	3	310.491	2231.874	.000
	Within Groups	1.113	8	.139		
	Total	932.585	11			

Post Hoc Tests

Homogeneous Subsets

20g

Duncan[a]

Fermentation_organisms	N	Subset for alpha = 0.05			
		1	2	3	4
Control	3	.0000			
L.bulgaricus	3		9.5433		
S.cerevisiae	3			11.5633	
Sacch+Lacto	3				13.2100
Sig.		1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

30g

+Duncan[a]

Fermentation_organisms	N	Subset for alpha = 0.05			
		1	2	3	4
Control	3	.0000			
L.bulgaricus	3		13.7900		
S.cerevisiae	3			18.2833	
Sacch+Lacto	3				20.8667
Sig.		1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Duncan[a]**40g**

Fermentation_organisms	N	Subset for alpha = 0.05			
		1	2	3	4
Control	3	.0000			
L,bulgaricus	3		15.7900		
S.cerevisia	3			21.0833	
Sacch+Lacto	3				21.9433
Sig.		1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Case Processing Summary

	Cases					
	Included		Excluded		Total	
	N	Percent	N	Percent	N	Percent
Moisture * SAMPLE	12	100.0%	0	0.0%	12	100.0%
Ash * SAMPLE	12	100.0%	0	0.0%	12	100.0%
Fibre * SAMPLE	12	100.0%	0	0.0%	12	100.0%
Fat * SAMPLE	12	100.0%	0	0.0%	12	100.0%
Protein * SAMPLE	12	100.0%	0	0.0%	12	100.0%
Carbohydrate * SAMPLE	12	100.0%	0	0.0%	12	100.0%
pH * SAMPLE	12	100.0%	0	0.0%	12	100.0%
Reducing_sugar * SAMPLE	12	100.0%	0	0.0%	12	100.0%

Report

SAMPLE		Moisture	Ash	Fibre	Fat	Protein	Carbohydrate	pH	Reducing sugar
Raw B.P	Mean	7.4400	9.2800	14.2150	3.6200	5.3100	60.1350	5.2300	37.8200
	Std. Error of Mean	.49000	.07000	.59500	.00000	.68000	.64500	.02000	.68000
RAW C.P	Mean	5.4100	10.5050	10.4900	2.2150	3.5250	67.8500	6.1850	14.5750
	Std. Error of Mean	.00000	.39500	.43000	.07500	.26500	.23000	.01500	1.03500
RAW MIXTURE	Mean	6.8100	10.2100	12.2200	3.8050	3.4800	63.4750	6.1400	35.3400
	Std. Error of Mean	.00000	.21000	.59000	.18500	.33000	.28500	.01000	1.18000
HYDROLYZED B.P	Mean	63.1650	5.1950	12.6400	1.5200	3.9850	11.4950	6.1150	15.5150
	Std. Error of Mean	.85500	.05500	.28000	.00000	.30500	1.49500	.01500	.73500
HYDROLYZED C.P	Mean	55.1250	4.8100	10.6900	1.3500	3.4100	22.5650	5.2400	10.7200
	Std. Error of Mean	.23500	.00000	.29000	.05000	.40000	.84500	.02000	.00000
HYDROLYZED MIXTURE	Mean	60.6150	5.9850	11.4900	1.3050	4.6050	18.0000	5.8100	7.9800
	Std. Error of Mean	.30500	.15500	.64000	.05500	.51500	2.08000	.02000	.66000
Total	Mean	33.0942	7.6642	11.9575	2.3025	4.0525	40.5867	5.7867	20.3250
	Std. Error of Mean	8.03735	.72255	.40952	.31529	.24832	7.11208	.12324	3.55962