BIOETHANOL (BIO-FUEL) PRODUCTION FROM AGRO-WASTE USING YEAST ISOLATES FROM NIGERIA

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ERTIFICATION

This work titled “Bioethanol (Bio-fuel) production from agro-waste using yeast isolates from Nigeria” submitted to the school of Postgraduate studies, University of Lagos, Lagos, Nigeria for the award of Doctor of Philosophy in Botany is an original research carried out by EBABHI, Abosede Margaret in the Department of Botany, University of Lagos, under the supervision of Professor A. A. Adekunle, Dr. A. A. Osuntoki and Dr. W. O. Okunowo.

This work has not been submitted previously, in whole or part, to qualify for any other academic award.

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DEDICATION

This study is dedicated to the glory of GOD for his mercy and compassion that endureth forever.

To Engr. Larry C. Ebabhi

To Daniel and Marvellous

To Bukola Ikumapayi.

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ABSTRACT

Bio-fuels are fuels derived from biological materials or their by-products such as agricultural waste. A study was carried out to assess the production of bio-fuel (bioethanol) from some hydrolyzed agricultural wastes using yeast species isolated from *Cola acuminata, Ipomoea batatas, Manihot esculenta, Pennisetum glaucum, Sorghum bicolor, Solanum tuberosum, Zea mays* and palmwine (from *Elaeis guineensis*). Eight yeast species including *Candida krusei, Candida tropicalis* strain A, *Candida tropicalis* strain B, *Kluyveromyces marxianus, Pichia caribbica, Saccharomyces cerevisiae* strain A, *Saccharomyces cerevisiae* strain B and *Schizosaccharomyces pombe* were used. The agro-wastes and starchy substrates were pretreated through milling, saccharification with mineral acid (H_2SO_4/HCl) and *Anacardium occidentale* (cashew) Nut Shell Extract (CNSE). Substrates were distilled after 72 h of fermentation. The quantity of bioethanol produced varied with substrates and organisms used. The analysis of the CNSE revealed the presence of hydrolytic enzymes such as endoglucanase, exoglucanase and xylanase in varying concentrations. Biochemical analysis and DNA sequencing revealed that some of the fungal species are probably new strains. Using 100 g hydrolyzed substrates, the highest amount (33.34 ± 2.81 g/L) of bioethanol was obtained from sweet potato tuber hydrolyzed with 50 ml of 50% HCl and fermented with *S. pombe*. Plantain peel hydrolyzed with CNSE and fermented with *S. pombe* produced 28.12±1.61 g/L of bioethanol. The quantity of reducing sugar in the agro-waste hydrolyzed with CNSE was maximum of 491 mg/g in sugarcane chaff and minimum of 46 mg/g in rice husk. Sugar fermentation test of the yeasts showed that they can ferment sugars such as glucose, lactose, maltose, fructose and xylose. Lactose and xylose were fermented

atypically by *S. cerevisiae* strain B. All strains tested were resistant to 30 µg/l of chloramphenicol and they were able to grow at 37°C except *Candida krusei.* In the urease hydrolysis test, *S. pombe, P. caribbica* and *S. cerevisiae* strain B were positive. Growth study of the yeast strains on eight broth media showed that potato dextrose broth, malt peptone broth and millet dextrose broth are the best media for growth and reproduction. This is probably the first report of isolation and characterization of fermenting yeast from maize, kolanut and sweet potato in Nigeria. Gas chromatographic (GC) assay of the CNSE hydrolysates showed the presence of simple sugars in varying concentrations with cassava peel possessing the highest concentration of glucose (38.19 mg/L) while plantain peel yielded the lowest concentratiom of 5.44 mg/L. The GC analysis of some of the agro-waste distillates showed considerable concentrations of bioethanol. Highest concentration of 2.41 x 10^4 mg/L was obtained from sweet potato peel fermented with *S. cerevisiae* strain A whereas the lowest concentration of $2.75x10^3$ was obtained from plantain peel fermented with *S. pombe.* The cashew nutshell extract was able to biodegrade the agro-wastes due to the synergy of the cellulases that are present in it. This study showed that agro-waste which are diverse and commonly pose significant disposal problems can be used for the production of bio-fuel and other organic compounds. Fossil fuel causes environmental pollution and replacing it with bio-fuel like bioethanol from agro-waste will be more environmental friendly and will also reduce pressure on crop plants.

CHAPTER ONE

1.0 INTRODUCTION

1.1. BACKGROUND STUDY

Plants are very important to life because they support the existence of all living things directly or indirectly. The ability of plant to capture solar energy sustains almost all life forms on earth. Plant based organic matter is consumed by herbivores and forms the basis of every food chain (Marinelli, 2004). Plant materials serve other diverse purposes such as food for man and animal (Etejere and Bhat, 1985; Brand-miller and Holt, 1998; McGee, 2004; Abdulrahaman and Kolawole**,** 2006); medicine (Ainslie, 1937; Hutchinson and Dalziel, 1958; Oliver, 1960; Sofowora, 1982; Burkill, 1995, 2000; Adekunle, 2001; Adekunle and Ikumapayi, 2006); and raw materials in industries (Liggett and Koffler, 1948; Burkill, 1998). Today, plants are used in biotechnology for bio-monitoring (Martin, 1998; Mertens *et al.,* 2005) and phytoremediation (Meagher, 2000; Mukred *et al*., 2008). The stored solar energy in plant is also used as fuel in wood, alcohol; methane and these are referred to as bio-fuels (Aiba *et al.,* 1973, Ellington *et al.,* 1993; Giampietro *et al.,* 1997; Hammerschlag, 2006**;** Bowyer *et al.,* 2007; Demirbas, 2009; Mathiyazhagan *et al.,* 2011).

Bio-fuel can be defined as any solid, liquid or gaseous fuel derived from biological materials (biomass) or their by-products (Giampietro *et al.,* 1997; Yunqiao *et al.,* 2007). Theoretically, it can be produced from any (biological) carbon source (Righelato, 1980); although, the most common sources are photosynthetic plants. Bio-fuel is also called non-conventional fuel (Birol and Davie, 2001) whereas fossil fuel,

petroleum, coal, propane, natural gases and nuclear materials like uranium are called conventional fuel. Today, the use of bio-fuel has expanded throughout the world. Some of the major producers and consumers are Brazil, China, Germany and United States of America (Goldemberg, 2007; Luciano *et al.,* 2007). There are currently lots of researches on the use of biomass for bio-fuel production and many crop plants are specifically grown for this purpose. Examples of such crop plants and countries where they are used are found in Table 1 below:

TABLE 1: SOME CROPS USED IN BIOFUEL PRODUCTION

AGRICULTURAL PRODUCE	**COUNTRY**
Saccharum officinarum(Sugarcane)	Brazil, Mozambique, Tanzania
Manihot esculenta (Cassava) and	
Sorghum bicolor (Sorghum)	China
Beta vulgaris (Sugar beet)	Germany
Jatropha curcas (Barbados nut)	India
Triticum aestivum (Wheat) and	
Hurdeum vulgare (Barley)	Spain
Cereals	Sweden
Zea mays (Corn), *Panicum virgatum*	
(switch grass) and *Glycine max* (Soybean)	United States of America

Source**:** Kolachov and Nicholson, 1951**;** Borgelt *et al.,* 1994; Luciano *et al.,* 2007

Bio-fuel is used for purposes such as heating homes, alcohol, chemicals, for cooking stoves but the main use is in the transportation sector (Hill *et al.,* 2006; Azih, 2007; Goldemberg, 2007; Luciano *et al*., 2007; Demirbas, 2009). They include fuels like biodiesel, bioethanol, non-fossil methane, biobutanol, oilgae (diesel from algae) and other biomass sources (Kurtzman, 1983; Shay 1993; Hill *et al*., 2006; Azih, 2007; Mathiyazhagan *et al*., 2011).

1.2. STATEMENT OF PROBLEM

Bioethanol is the commonest and the most widely used liquid bio-fuel in the world (Righelato, 1980; Farrell *et al*., 2006). It is used particularly as an alternative fuel to petrol (gasoline). In Brazil, the ethanol-based bio-fuel sector has had a successful development since the 1980's and has become the envy of other countries that depend on importation of petroleum (Goldemberg *et al.,* 1985). In the United States of America, China, Australia, Japan, Europe and parts of Africa, it is becoming the major source of fuel (Hill *et al.,* 2006; Yunqiao *et al*., 2007; Klass, 2008). Nigeria has also followed suit (Azih, 2007). The country (Nigeria) is presently looking at the prospect of using *Manihot esculenta* (cassava), *Saccharum officinarum* **(**sugarcane) and Z*ea mays* (maize) as sources of bio-fuel (Azih, 2007; Agba *et al*., 2010; Agboola *et al.,* 2011). Bioethanol is the same as alcohol found in beverages. It is considered renewable because it is the main result of the conversion of the sun's energy into usable energy through photosynthesis, provided that all minerals required for growth are returned to land.

Currently, interest in bio-fuel mainly lies in bioethanol production from starch or sugar substrate found in a wide variety of crops such as bagasse, sugar beet, sorghum, switch grass, barley, potatoes, sweet potatoes, corn, wheat, cassava, palm sap (Abouzied and Reddy, 1986; Shay, 1993; Mosier *et al.,* 2005). There are controversies by farmers, politicians and environmentalists all over the world, over the production of bio-fuel from crop plants despite the production success that accompanies it. The effect of bioethanol production on other food crop prices is indirect. The use of maize for bioethanol production for example increases the

demand, and therefore increases price of maize. This in turn results in farm acreage being diverted from other food crops to maize production. Supply of the other food crops is reduced while the price of maize is increased.

Clearly, substantial food price increase would occur if ethanol derived from crop plants replaces even a small percentage of petrol. The Food and Agriculture Organization's World Food Program (2008) reported that it foresees an urgent problem with world hunger resulting from rising food prices. In Nigeria, the land degradation problem in the Niger Delta, climate change and the pointer to the fact that oil reserves will be exhausted in the nearest future, 2050 (Agba *et al.,* 2010) coupled with the need for economic development would necessitate a shift towards bio-fuel production.

The basic steps for large scale production of bioethanol include hydrolysis, microbial (yeast) fermentation of sugars, distillation, dehydration and denaturing (Kolachov and Nicholson, 1951; Abouzied and Reddy, 1986; Buzas *et al.,* 1989; Tyiagi *et al*., 1992; De Figueroa *et al*., 2000; Zafar and Owais, 2006). Prior to fermentation, some biomass type require saccharification or hydrolysis of the polysaccharides into simple sugar because of microbial fermentation (Rhee *et al*., 1984; Odunfa and Shasore, 1987; Ofuya and Nwajuiba, 1990; Amutha and Gunasekaran, 2001; Ueda *et al*., 2004). Two major components of plants, starch and cellulose, are both made up of sugars and can in principle be converted to sugar for fermentation. Currently, only the starch portions can be economically converted and this account for the bulk of energy constituents of crop plants while the cellulose linked with lignin (lignocelluloses) which make up the stalk, peels, husk, chaff of crop plants are difficult to break due to the complexity involved. Lignocellulose is the woody structural material of plants

which gives them strength and rigidity. It is usually composed of three primary constituents including cellulose, hemicelluloses and lignin **(**Hayes, 1977; Reshamwala *et al*., 1995; Cheung and Anderson, 1997). These feedstocks are abundant, diverse, and in some cases pose a significant disposal problem.

There is a lot of energy stored in lignocellulose (Mosier *et al.*, 2005) and so, it is important to find ways of making it easier to get at this energy and extract it in the form of sugars that can be fermented to produce bioethanol and other products. This is because, the sugars suitable for fermentation processes and alcohol yield are only found in the cellulose and hemicellulose constituents. The cellulose is the most abundant organic biomolecule on earth and the major constituent of all plant materials (Murai *et al.*, 1998; Narasimha *et al*., 2006). It is a linear biopolymer that contains hexose sugars (mainly glucose) and linked by a β1- 4 glycosidic bond (Gielkens *et al*., 1999). The hemicellulose which is the second most abundant component of lignocellulosic biomass contains pentose sugars (mainly xylose) and sugar acids (Zheng *et al.*, 2009). The xylose in wood and straw represents about a third of the sugars that could potentially be used to make bioethanol, but it is not readily available. Releasing the energy from lignocellulose is an important challenge to tackle as it will allow the production of fuels from plants in a sustainable way that does not affect the food chain. In order to obtain the sugars needed for fermentation pre-treatments such as chemical pre-treatment or biological pre-treatment of lignocellulosic biomass is necessary (Mosier *et al.*, 2005).

The most widely used pre-treatment method is mineral acid hydrolysis (Zheng *et al.*, 2009). Concentrated acid hydrolysis, which is one of the acid hydrolysis methods involves the use of concentrated acid (sulfuric acid - H_2SO_4 or hydrochloric acid -

HCl) under high temperature (190 - 215°C) to split the cellulose and hemicellulose into simple sugars (Zheng *et al.*, 2009). However, the products of mineral acid hydrolysis (acid hydrolysate) become very acidic and may contain a variety of inhibitory compounds such as furfural or furfuraldehyde 5-hydroxymethyl-furfural (5-HMF), acetate and other phenolic compounds (Palmqvist and Hahn-Hagerdal 2000; Luo *et al*., 2002; Hayes *et al*., 2005). The use of these mineral acids in breaking down the lignocellulose makes the bioethanol produced corrosive on pipes and other containers. A lot of developing nations will have to depend on importation of the mineral acids and a lot of hazards are encountered in the transportation from place to place. Also there are problems of emission of poisonous gases in the disposal of the mineral acid containers. Finding alternative means of hydrolyzing cellulolytic waste will reduce the corrosive nature of ethanol produced and make the production of bioethanol more economical.

It has become essential to produce bioethanol from residues of biomass which are materials derived from recently living organisms. These include plant and animal by-products, such as garden waste, crop residue, animal dung and waste plant materials. Also, biodegradable outputs from industries, agriculture, forestry and households can be used for bioethanol production. These cellulosic ethanol production using non-food crops or inedible waste products, does not divert food away from the animal or human food chain. Using waste feedstock to produce fuel can therefore contribute to waste management, fuel security, reduced pollution and reduction in global warming. However, there is little or no scientific investigation into the genetic constituents and biochemical properties of the yeast strains isolated in Nigeria and there is dearth of information on plant-based acids for the purpose of hydrolysis.

1.3. AIM AND OBJECTIVES OF STUDY

The aim of this work is therefore to isolate and characterize yeasts from various substrates. It is also aimed at using the isolated yeast to produce bio-ethanol from agro-waste hydrolyzed with cashew nut shell extract.

The objectives are to:

1. isolate and characterize yeasts from tropical plant sources: cassava, sorghum, kola nut, sweet potato, millet, Irish potato, maize and palm wine.
2. identify the yeasts phenotypically based on standard conventional techniques and genotypically using molecular analysis.
3. Extract constituents from cashew nut shell (*Anacardium occidentale*) and examine its efficacy in hydrolyzing agro-waste.
4. inoculate pure culture of the yeast (individually) on the hydrolyzed agro-waste and determine the best yeast isolate for production of bioethanol from agro-waste.
5. produce bioethanol from the agro-waste hydrolyzed with the cashew nut shell extract and compare with bioethanol produced from mineral acid hydrolyzed waste.

1.4. SIGNIFICANCE OF THE STUDY

This study shall provide information on yeast strains from tropical plants which can be useful in microbial fermentation in industries, plant base extract that can be used for hydrolysis and agro-waste that can be useful in the production of bio-fuel in Nigeria.

1.5. RESEARCH QUESTIONS

1. Can we use yeast isolates from substrates in Nigeria to produce bioethanol?
2. Which Nigerian plant can produce extract for hydrolysis of agro-waste?
3. Are there agro-waste that can be used for the production of bioethanol?
4. Which part of Lagos produces the various kinds of waste?

1.6 OPERATIONAL DEFINITION OF TERMS

Arthrospores: Arthrospores are conidia that are produced very simply by the last cell on a hypha breaking off and dispersing as a propagule.

Ascocarp: Fruiting body in the sub division ascomycotina.

Batch fermentation: Batch fermentation process refers to the process that starts with the inoculation and end with the retrieval of the product. This happens inside a single fermenter with no intermediate steps.

Biodegradation: The transformation of a substance into new compounds through biochemical reactions or by the actions of microorganisms such as fungi.

Biodeterioration: The breakdown of biomass by microorganisms.

Bioethanol: A colourless, volatile, flammable organic compounds that contain the hydroxyl group (OH) and that form esters with acids. With the chemical formula C_2H_5OH, synthesized or obtained by fermentation of sugars and starches. It is also called alcohol, ethyl alcohol, grain alcohol.

Biofuel: Bio-fuel can be defined as any solid, liquid or gaseous fuel derived from relatively dead biological materials or their by-products.

Biomass: The term biomass is actually an abbreviation of the term biological mass. It describes the total quantity or mass of organic material produced by living organisms in a particular area, at a given time.

Bio-waste: Biodegradable waste is a type of waste that originates typically from plant and animal sources which can be broken down by other living organism.

Cellulose: This is a polysaccharide that is made up of glucose units that constitute the main part of the cell wall of plants.

Continuous fermentation: The process by which cells are maintained in culture in the exponential growth phase by the continuous addition of fresh medium that is exactly balanced by the removal of cell suspension from the bioreactor.

Distillation: This is the process of separating mixtures based on differences in their volatilities in a boiling liquid mixture. It is a physical separation process.

Feedstock: This refers to the raw material that is required for some industrial process.

Fermentation: The process by which micro-organisms break down complex organic substances generally in the absence of oxygen to produce alcohol and carbon dioxide.

Fossil fuels: A general term for buried combustible geologic deposits of organic materials, formed from decayed plants and animals that have been converted to crude oil, coal, natural gas, or heavy oils by exposure to heat and pressure in the Earth's crust over hundreds of millions of years.

Hemicelluloses: Hemicelluloses are plant cell wall polysaccharides that are not solubilized by water but are solubilized by aqueous alkali or mineral acid.

Hydrolysate: This is the product of hydrolysis.

Hydrolysis: The chemical process by which carbohydrates or starches are simplified into organic soluble, usually by facultative anaerobes.

Lignocellulose: This is a matrix of cross-linked polysaccharide networks, glycosylated proteins, and lignin which consist of three main components: cellulose (38–50%), hemicellulose (17–32%), and lignin (15–30%).

Non-conventional fuel: Also known as alternative fuels or advanced fuels, are any materials or substances that can be used as fuels, other than conventional fuels.

Pseudohyphae: A chain of easily disrupted fungal cells that is intermediate between a chain of budding cells and a true hypha, marked by constrictions rather than septa at the junctions.

Saccharification: Is the process of breaking a complex carbohydrate (starch or cellulose) into its monosaccharide components.

Starch: Complex carbohydrate used by plants as a way to store glucose (sugar). It is found in grains, roots, tubers and other foods.

Yeast: This is a microscopic, unicellular fungus (usually in sub-division Ascomycotina) consisting of single oval cells that reproduce vegetatively by budding or fission and are capable of converting sugar into ethanol and carbondioxide.

CHAPTER TWO

2.0. LITERATURE REVIEW

2.1. GENERAL DESCRIPTION OF BIO-FUEL

Bio-fuels are fuels produced directly or indirectly from organic materials (biomass) including plant materials and animal waste. As stated by Warabi *et al.* (2004), the term covers solid biomass, liquid fuels and various biogases. They include a wide range of fuels which are in some way derived from biomass **(**Demirbas, 2009). Bio-fuels may be derived from agricultural crops, including conventional food plants such as corn, soybean, cassava or from special energy crops like switch grass (Birol and Davie, 2001; Yunqiao *et al.,* 2007) also from oil plants such as canola, Barbados nut and sunflower. Using raw potato starch for example, Abouzied and Reddy (1986) were able to produce a type of bio-fuel. Bio-fuel may also be derived from forestry, agricultural or fishery products or municipal wastes, as well as from agro-industry, food industry, food service by-products and wastes (Subhadra and George, 2011).

There are basically two common strategies of producing bio-fuels. One is to grow crops high in either sugar (sugar cane, sugar beet, and sweet sorghum) or starch (Borgelt *et al.,* 1994; Hammerschlag, 2006; Luciano *et al.,* 2007) and then use yeast fermentation to produce ethyl alcohol (ethanol). The second is to grow plants that contain high amounts of vegetable oil, such as oil palm, rape seed, cottonseed, soybean, algae (Subhadra and George, 2011), or Barbados nut (Agarwal and Agarwal, 2007). When these oils are heated, their viscosity is reduced, and they can be burned directly in a diesel engine, or the oils can be chemically processed to produce fuels such as biodiesel (Demirbas, 2008). Wood and its byproducts can also be converted into bio-fuels as reported by Bowyer *et al.* (2007),

with common examples like wood gas and methanol. It is also possible to make cellulosic ethanol from non-edible plant parts, but this can be difficult to accomplish economically. Bio-fuels are gaining increased public and scientific attention, driven by factors such as oil price, the need for increased energy security, economic stability and concern over greenhouse gas emissions from fossil fuels (Righelato, 1980).

There are two forms of biofuel namely:

- Primary bio-fuel
- Secondary bio-fuel

Primary bio-fuels are traditional unprocessed biomass such as fire wood, charcoal, wood chips and pellets, organic materials and animal dung (Bowyer *et al.,* 2007; Laird, 2008). They represent the main source of energy for a large number of people in developing countries who use it mainly for cooking, heating or electricity production. **Secondary bio-fuels** result from processing of biomass and include liquid bio-fuels such as bioethanol and biodiesel that can be used in vehicles and industrial processes. Hill *et al*., (2006) reported the development of bioethanol based stoves in Malawi to reduce dependence on charcoal, firewood and paraffin. All bio-fuels are the results of varying production processes which can be categorized into four generations: first, second, third and fourth generation bio-fuels.

2.2 CLASSIFICATION OF BIO-FUEL

2.2.1. **First generation bio-fuels**

First generation bio-fuels are sourced from natural compounds such as sugars, starches, vegetable oils and fats, which are then, processed using conventional technologies. As such, they encompass the fuel types of biodiesel, bioalcohols, syngas and vegetable oil.

However, while these sources of bio-fuels offer a great alternative to the traditional fossil fuels, they do present some economic difficulties of their own. One of the greatest controversies facing the first generation bio-fuels is that they require the use of major food crops in their production. Thus, with first generation bio-fuels, there is an incredible stress on the agricultural sector, resulting in food shortages, or the expansion of farmlands. This in itself has huge environmental implications such as deforestation, soil erosion, water shortages and on the larger scale, climate change. Many countries are expanding or contemplating on the expansion of their first generation bioethanol production with Brazil and the United States of America having by far the largest plants (Goldemberg *et al*., 1985; Luciano *et al*., 2007). Interest in first generation bio-fuel is also growing in South East Asia, Malaysia, Indonesia and Thailand where majority of the world's palm oil for food is grown (Schott, 2009). Looking at the researches of Borgelt *et al.* 1994**,** Hill *et al*. 2006, Goldemberg 2007, Luciano *et al*. 2007 and Demirbas 2009, with all these detrimental impacts, the excessive use of first generation bio-fuels may defeat the purpose of bio-fuels in the first place, which is to provide a clean and renewable source of energy.

2.2.2. Second generation bio-fuels

Second generation bio-fuels comprise the answer to the issues presented by their first generation counterparts as they are manufactured from inedible plant matter or non-food crops as well as the waste biomass produced by the agricultural sector. This includes the left over stalks, stems and leaves from the processing of maize, cassava (Adesanya *et al.*, 2009), sugar cane, wheat, millet (Oyeleke and Jibrin, 2009), soybeans and other food crops. Because of the vast and diverse array of inedible biomass types and sources, second generation bio-fuels largely surpass the limitations of the first generation as they do not threaten our food reserves, food production or biodiversity. They are also a far more

sustainable resource, environmentally friendly and completely cost-effective because we are making fuel from what would previously be thought of as useless waste materials. Second generation bio-fuel can further be defined based on the mode being used for converting the bio-waste into fuel which can be either biochemical or thermochemical means. Some second generation bio-fuel like bioethanol and biobutanol can be made through biochemical processes while most others like biodiesel can be processed thermochemically (Spath and Dayton, 2003). The biochemical process includes pre-treatment, saccharification, fermentation and distillation (Coelho, 2006). According to Coelho (2006), the second generation ethanol is often referred to as cellulosic ethanol because it is sourced from lignocelluloses bio-waste.

2.2.3. Third generation bio-fuels

The bio-fuel obtained from algae also referred to as oilgae in the bio-fuel industry, is the third generation bio-fuel. Microalgae are photosynthetic microorganisms that can produce lipids, proteins and carbohydrates in large amounts over short periods of time. These products can be processed into both biofuels and useful chemicals (Demirbas, 2011). Recent research into the use of algae as a source of fuel has shown that it can produce as much as 30 times more energy per unit growing area than land crops (corn, soybeans, wheat, etc.), although this is yet to be commercially implemented. The advantage of third generation bio-fuels is that it is 100% environmentally friendly, biodegradable and easy to grow, although the oil extraction process is a bit complex (Mathiyazhagan *et al*., 2011). Algae also has the benefits of naturally producing ethanol as a byproduct, which can easily be extracted without disturbing the plants, as well as absorbing carbon dioxide in the process of photosynthesis. This sink of carbon dioxide is fundamental in an environment which is suffering under the strain of excessive greenhouse gas emissions.

2.2.4. Fourth generation bio-fuels

This is an idea of capturing and storing carbon, the fourth generation technology looks at the possibility of combining genetically modified feedstocks which can capture enormous amount of carbon. In this, the production of bio-fuels is aimed at not only producing sustainable energy but also a way of capturing and storing CO_2. Biomass materials, which have absorbed CO_2 while growing, are converted into fuel using the same processes as second generation biofuels. This process however, differs from second and third generation production as at all stages of production the carbon dioxide is captured using processes such as oxy-fuel combustion (Dmitri and Ross, 2011). The carbon dioxide can then be geosequestered by storing it in old oil and gas fields or saline aquifers. This carbon capture makes fourth generation biofuel production carbon negative rather than simply carbon neutral, as it locks away more carbon than it produces. This system not only captures and stores carbon dioxide from the atmosphere but it also reduces CO_2 emissions by replacing fossil fuels. Although, it is concluded that fourth generation biofuel production has introduced the cell factory concept and shifted the research paradigm. There are still several technical bottlenecks in this biofuel research and development, which can only be solved by the use of post-genome tools on these photosynthetic organisms (Jing *et al.*, 2011).

Bio-fuels can also be referred to as alternative fuels which can be called non-conventional or advanced fuels. Bio-fuels are also considered as renewable source, some form of renewable energy is used to create alternative fuels. Renewable energy is used mostly to generate electricity.

2.3. HISTORY OF BIO-FUELS

Ancient people have used biomass fuels in the form of solid bio-fuels (wood) for heating and cooking since the discovery of fire. With the discovery of electricity, man discovered another way of utilizing the bio-fuel. This form of fuel was discovered even before the discovery of the fossil fuels, but with the exploration of the fossil fuel like gas, coal and oil the production and use of bio-fuel suffered a severe impact. With the advantages placed by the fossil fuels, they gained a lot of popularity especially in the developed countries. Interestingly, Dorado *et al.* (2004) stated the use of liquid bio-fuel in the automobile industry since its inception which dates back to 1853 when scientists E. Duffy and J. Patrick conducted the first trans-esterification of a vegetable oil many years before the first diesel engine became functional. This concept was later used by Rudolf Diesel the German inventor of the diesel engine (Diesel, 1913). He designed his diesel engine (a single 10 feet iron cylinder with a flywheel at its base) to run on peanut oil and later Henry Ford designed the Model T car which was produced from 1903 to 1926. This car was completely designed to use hemp derived bio-fuel as fuel. Diesel believed that the utilization of biomass fuel is the future of his engine, as he stated in his 1912 speech saying, "the use of vegetable oils for engine fuels may seem insignificant today, but such oils may become in the course of time, as important as petroleum and the coal-tar products of the present time" (Diesel, 1913). However, with the exploration of huge supplies of crude oil in some parts of the United States, petroleum became very cheap and thus led to the reduction of the use of bio-fuels. The manufacturers of diesel engines then decided to alter their engines to utilize the lower viscous fossil fuel best known as petro-diesel rather than the biomass vegetable oil fuel. Most of vehicles like trucks and cars began using this form of fuel which was much cheaper and efficient ignoring the fact that decades later it would bring high pollution costs.

In the period of World War II bio-fuel again became valuable as a strategic alternative for imported fuel. In this period, Germany was one of the countries that underwent a serious shortage of fuel. It was during this period that various other inventions took place like the use of gasoline along with alcohol that was derived from potatoes. Britain was the second country to come up with the concept of grain alcohol mixed with petrol (Sheehan *et al.*, 2004). The war frames were the periods when the various major technological changes took place but, during the period of peace, cheap oil from the gulf countries as well as the Middle East again eased off the pressure. With the increased supply the geopolitical and economic interest in bio-fuel faded away. According to Sheehan *et al.* (2004), a serious fuel crisis again hit various countries during the period of 1973 and 1979, because of geopolitical conflict. Thus, the Organization of Petroleum Exporting Countries (OPEC), made a heavy cut in exports especially to the non-OPEC nations. The constant shortage of fuel attracted the attention of the various academics and governments to the issues of energy crisis and the use of bio-fuels. The twentieth century came with the attention of the people towards the use of bio-fuels. Some of the main reasons for the people shifting their interest to bio-fuels were the rising prices of oil, emission of the greenhouse gases and interest like rural development (Bender, 1999).

2.4. TYPES OF BIO-FUELS

There are different categories of bio-fuel depending on their chemical make-up or composition, but what they all have in common is that they are completely organic in origin.

2.4.1. Bioethanol

The most commonly used and widely researched of the bioalcohols is bioethanol. Bio-ethanol is a type of alcohol that can be produced primarily through the fermentation of any

feedstock containing significant amounts of sugar or by the hydration of ethylene from petroleum and other sources (Myers and Myers, 2007). Ethanol can be blended with petrol or burned in nearly pure form in slightly modified spark-ignition engines. A litre of ethanol contains approximately two thirds of the energy provided by a litre of petrol. However, when mixed with petrol, it improves the combustion performance and lowers the emissions of carbon monoxide and sulphur oxide. Ethanol has widespread use as a solvent of substances intended for human contact or consumption (Chastain, 2006), including scents, flavourings, colourings, and medicines. In chemistry, it is both an essential solvent and a feedstock for the synthesis of other products. It has a long history as a fuel for heat and light, and more recently as a fuel for internal combustion engines (Reshamwala *et al.*, 1995).

Ethanol is the systematic name defined by the IUPAC nomenclature of organic chemistry for a molecule with two carbon atoms (prefix "eth-"), having a single bond between them (suffix "-ane"), and an attached -OH group (suffix "-ol") (Lide, 2000).

2.4.1.1 Physical properties

Ethanol is a volatile, colourless liquid that has a slight odour (Anonymous, 2008). It burns with a smokeless blue flame that is not always visible in normal light. Ethanol is a versatile solvent, miscible with water and with many organic solvents, including acetic acid, acetone, benzene, carbon tetrachloride, chloroform, diethyl ether, ethylene glycol, glycerol, nitromethane, pyridine, and toluene (Windholz, 1976**;** Lide, 2000). It is also miscible with light aliphatic hydrocarbons, such as pentane and hexane, and with aliphatic chlorides such as trichloroethane and tetrachloroethylene (Windholz, 1976**).** According to Morrison and Boyd (1972), ethanol's miscibility with water decreases sharply as the number of carbons increases. The miscibility of ethanol with alkanes is limited to alkanes

up to undecane, mixtures with dodecane and higher alkanes show a miscibility gap below a certain temperature (about 13°C for dodecane) (Dahlmann and Schneider, 1989). The miscibility gap tends to get wider with higher alkanes and the temperature for complete miscibility increases.

Ethanol-water mixtures have less volume than the sum of their individual components at the given fractions. The Mixture of equal volumes of ethanol and water results in only 1.92 volumes of mixture (Lide, 2000). Mixing ethanol and water is exothermic. At 298 K, up to 777 J/mol are set free (Costigan *et al*., 1980). Mixtures of ethanol and water form an azeotrope at about 96 volume percent ethanol and 4% water at normal pressure and temperature of 351 K. This azeotropic composition is strongly temperature and pressure dependent and vanishes at temperatures below 303 K (Pemberton and Mash, 1978). The addition of even a few percent of ethanol to water sharply reduces the surface tension of water. This property partially explains the "tears of wine" phenomenon. When wine is swirled in a glass, ethanol evaporates quickly from the thin film of wine on the wall of the glass. As the wine's ethanol content decreases, its surface tension increases and the thin film "beads up" and runs down the glass in channels rather than as a smooth sheet. Mixtures of ethanol and water that contain more than about 50% ethanol are flammable and easily ignited. Ethanol-water solutions that contain less than 50% ethanol may also be flammable if the solution is first heated. Ethanol is slightly more refractive than water, having a refractive index of 1.36242 (Lide, 2000) (at λ=589.3 nm and 18.35°C).

The physical properties of ethanol stem primarily from the presence of its hydroxyl group and the shortness of its carbon chain. Ethanol's hydroxyl group is able to participate in hydrogen bonding, rendering it more viscous and less volatile than less polar organic compounds of similar molecular weight. Hydrogen bonding causes pure ethanol to be

hygroscopic to the extent that it readily absorbs water from the air. The polar nature of the hydroxyl group causes ethanol to dissolve many ionic compounds, notably sodium and potassium hydroxides, magnesium chloride, calcium chloride, ammonium chloride, ammonium bromide, and sodium bromide while sodium and potassium chlorides are slightly soluble in ethanol (Windholz, 1976). Thus, ethanol cannot be transported in metallic pipes or vessels and because the ethanol molecule also has a non-polar end, it will also dissolve non-polar substances, including most essential oils and numerous flavouring, colouring and medicinal agents.

2.4.1.2. Steps in bioethanol production

According to Kolachov and Nicholson (1951), after harvesting the feedstock such as sugarcane, corn, there are seven steps to making ethanol suitable to use as a petrol additive. These are:

1. **Milling:** The feedstock is ground into a fine powder called meal.

2. Liquefaction: The meal is mixed with water and alpha-amylase, passing through cookers where the starch is heated to 120-150°C and liquefied and then cooked further at 95°C.

3. Saccharification: The mash from the cookers is cooled and enzymes are added to convert the liquefied starch to fermentable sugars.

4. Fermentation: Yeast is added to ferment the sugars to ethanol and carbon dioxide. Using a continuous process, the fermenting mash is allowed to flow, or cascade, through several fermenters until the mash is fully fermented and then leaves for the final tank.

5. Distillation: The fermented mash, now called "beer," contains about 10% alcohol, as well as all the non-fermentable solids from the meal and the yeast cells. The mash is pumped to the distillation system where the alcohol will be removed from the solids and

the water. The alcohol leaves the final column at about 96% strength, and the residue mash, called stillage, is transferred from the base of the column to the co-product processing area.

6. Dehydration: The alcohol then passes through a dehydration system where the remaining water will be removed. Most ethanol plants use a molecular sieve to capture the last bit of water in the ethanol. The alcohol product at this stage is called anhydrous (pure, without water) ethanol and is approximately 200 proof.

7. Denaturing: Ethanol that will be used for fuel is then denatured with a small amount (2-5%) of some product, like gasoline, to make it unfit for human consumption.

However, the simplified process for the production of bioethanol can be given as follows: Through the process of photosynthesis, plant produces sugar in the form of glucose:

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

During fermentation performed primarily by yeast, glucose is decomposed into ethanol and carbondioxide: $C_6H_{12}O_6 \rightarrow 2C_2H_6O + 2CO_2 + \text{heat}$

During combustion, ethanol is heated with oxygen to produce carbondioxide and water

$$2C_2H_6O + 3O_2 \rightarrow 2CO_2 + H_2O + \text{heat}$$

The net reaction for the overall production and consumption of ethanol is simply light plus heat (Uno *et al.,* 2001; Agarwal and Agarwal, 2007). Ethanol and methanol fuel are typically primary sources of energy; they are convenient fuels for storing and transporting energy. These alcohols can be used in "internal combustion engines as alternative fuels". Methanol can be produced from a wide variety of sources including fossil fuels,

agricultural products, municipal waste, wood and varied biomass. More importantly, it can also be made from chemical recycling of carbondioxide (such as from the CO_2 rich fuel gases of fossil fuel, burning power plant or exhaust cement and other factories). Recent developments with cellulosic ethanol production and commercialization may allay some of the concerns on use of large arable farm land for growing energy crops.

2.4.1.3. Source of raw materials

The raw materials used in the manufacture of ethanol through fermentation vary and are conveniently classified under three types of agricultural raw materials: sugar, starch and cellulose materials. Sugars (from sugar cane, sugar beet, molasses and fruits) can be converted to ethanol directly. Starch (from grains, potatoes, root crops) must first be hydrolyzed to fermentable sugars using mineral acids or by the actions of enzymes from malt or molds (Righelato, 1980). Cellulose (from wood, agricultural residues, waste sulfite, liquor from pulp and paper mills) must also be converted to sugars generally by the actions of mineral acids. Once simple sugars are formed, enzymes from yeast can readily ferment to ethanol.

All alcoholic beverages: wine and brandy from natural sugars present in fruits; beer and whiskey from grain starches and vodka, rum from cane sugar are produced through the process of fermentation (Cook, 1958; Aiba, 1973; Kurtzman, 1983). In all cases, the fermentation must take place in a vessel that allows carbon dioxide to escape, but prevents outside air from coming in, as exposure to oxygen would prevent the formation of ethanol. Similarly, yeast fermentation of various carbohydrate products is used to produce much of the ethanol used for fuel (Righelato, 1980; Rhee *et al.*, 1984).

2.4.2. Biobutanol

Butanol is a 4-carbon alcohol (butyl alcohol). Biobutanol is butanol produced from biomass feedstocks (Atsumi *et al.,* 2008) such as sugar beets, sugar cane, corn grain, wheat and cassava; non-food energy crops such as switch grass, as well as agricultural by-products such as straw and corn stalks. The difference from ethanol production is primarily in the fermentation of the feedstock and minor changes in distillation. Dürre (2007) reported the use of *Clostridium acetobutylicum* specifically for the fermentation process. These are introduced to the sugars produced from the biomass; the sugars are broken down into various alcohols, which include ethanol and butanol. Unfortunately, a rise in alcohol concentration causes the butanol to be toxic to the microorganisms, killing them off after a period of time. This makes the fermentation process expensive and unrealistic when compared to the petroleum costs of the late 50's. However, new technological advances and the discovery of new microbes have improved the efficiency and cost of the fermentation process tremendously. Through genetic engineering, researchers have been able to modify the most efficient microbes to be able to withstand high alcohol concentrations (Huang *et al.,* 2010). New modifications are constantly being researched, including the modification to enzymes and genes involved in butanol formation from biomass fermentation.

2.4.3 Biodiesel

Biodiesel refers to a non-petroleum-based diesel fuel consisting of short chain alky1 (methyl or ethyl) esters, made by trans-esterification of vegetable oil or animal fat (tallow), which can be used (alone, or blended with conventional petrol or diesel) in unmodified diesel-engine vehicles (Demirbas, 2008). Trans-esterification is the process of using an alcohol (ethanol or methanol) in the presence of a catalyst like sodium hydroxide (NaOH)

or potassium hydroxide (KOH) to chemically breakdown the molecule of raw renewable oil into methyl or ethyl esters of the renewable oil with glycerol as a by-product (Dorado *et* al., 2004). Biodiesel is distinguished from the straight vegetable oil (SVO) (sometimes referred to as waste vegetable oil, used vegetable oil, pure plant oil) used (alone or blended) as fuels in some converted diesel vehicles. It is standardized as mono-alky1 ester and other kinds of diesel-grade fuels of biological origin are not included. Biodiesel can be derived from a wide range of oils, including rapeseed, soybean, palm, coconut or *Jatropha curcas* oils (Agarwal and Agarwal, 2007) and therefore the resulting fuels can display a greater variety of physical properties than ethanol.

Bio-oil or triglyceride that is, completely organically sourced oil obtained from plants such as *Jatropha*, canola flowers, soy-bean, rapeseed, sunflowers, palms (Shay, 1993), algae and used vegetable oil are all considered to be great sources of biodiesel (Dorado *et al.*, 2004). The bio-oil is usually heated to reduce its viscosity, after which it can be used as an additive in diesel fuel. Alternatively, it can be further treated to produce biodiesel, which can be used to power absolutely any technology that subsists off of diesel fuel. It can be blended with traditional diesel fuel or burned in its pure form in compression ignition engines. Its energy content is somewhat less than that of diesel (88 to 95%). Diesel engines can also run on vegetable oils and animal fats**,** for instance used cooking oils from restaurants and fat from meat processing industries. The production processes for both bioethanol and biodiesel yield additional by-products such as animal feed.

2.4.4. Biogas

Biogas is the result of anaerobic transformation of organic materials (such as animal dung) with the help of anaerobic organisms to produce a mixture of gases (containing up to 60 percent methane and CO_2). Through specially designed collectors and closed systems, the

gas can be captured off landfills or waste deposit sites and used for a number of applications such as the source of electricity and to heat buildings and water. As a fuel, its primary use is in engine with internal combustion. It is about 20% lighter than air and has an ignition temperature in the range of 650 to 750°C. It is an odourless and colourless gas that burns with a clear blue flame similar to liquefied petroleum gas (LPG). The solid by-product of bacterial digestion can also be used as a potent fertilizer, ideal for agricultural use.

2.4.5. Syngas

Syngas is essentially a mixture of carbon monoxide (a poisonous and potent greenhouse gas) and hydrogen (a harmless and naturally found atmospheric gas). This is produced through the partial combustion of biomass in an oxygen-starved environment that yields a more potent and efficient form of fuel, which can be used to drive vehicle engines as well as the generation of electricity by turbines. It production begins with gasification or pyrolysis (Devil *et al.,* 2003). The gasification is based on the process by which various biofuel can be produced including Fisher Tropsch Liquid (FTL), dimethyl ether (DME) and various alcohols. During gasification, bio-waste is heated for easy conversion. The mixture of combustible and non-combustible gases contaminant in the gas is removed. This is followed by adjusting the composition of the gas to prepare it for further downstream process. The major components of non-clean and concentrated syngas are carbon monoxide (CO) and hydrogen (H_2) with a little amount of methane (CH_4). The carbon monoxide and hydrogen react when passed over a catalyst to produce liquid fuel. The design of the catalyst determines what fuel is produced. In most plant designs, not all the syngas passed over the catalyst is converted to liquid fuel.

2.5. FERMENTATION

Fermentation is the process of deriving energy from the oxidation of organic compounds, such as carbohydrates without the use of oxygen as an electron acceptor (Klein *et al.*, 2004). It is also a process by which complex organic compounds, such as glucose, are broken down by the action of enzymes into simpler compounds without the use of oxygen. Fermentation results in the production of energy in the form of two ATP molecules, and produces less energy than the aerobic process of cellular respiration. The other end products of fermentation differ depending on the organism. In many bacteria, fungi, protists, and animals cells (notably muscle cells in the body), fermentation produces lactic acid and lactate, carbon dioxide, and water. In yeast and most plant cells, fermentation produces ethyl alcohol, carbon dioxide, and water.

During fermentation, pyruvate is metabolized to various different compounds. In contrast, respiration is where electrons are donated to an exogenous electron acceptor, such as oxygen, through an electron transport chain.

Sugars are the most common substrate of fermentation and typical examples of fermentation products are ethanol, lactic acid, and hydrogen. However, other compounds such as butyric acid and acetone can be produced by the process of fermentation. Typically, fermentation in food processing industries is the conversion of carbohydrates to alcohols and carbon dioxide or organic acids using yeasts, bacteria, or a combination thereof, under anaerobic conditions. Yeast carries out fermentation in the production of ethanol in beers, wines and other alcoholic drinks, along with the production of large quantities of carbondioxide. Voet and Voet (1995), had also stated the process of fermentation in mammalian muscle during periods of intense exercise where oxygen supply becomes limited, resulting in the production of lactic acid.

2.5.1. Process of fermentation

Fermentation is a process of energy yielding metabolism that involves a sequence of oxidation-reduction reaction in which both the substrate (electron donor) and the terminal electron acceptor(s) are organic compounds. In aerobic respiration, molecular oxygen is the final electron acceptor while in anaerobic respiration or fermentation molecular energy is not required. Before fermentation takes place, one glucose molecule is broken down into two pyruvate molecules. This is known as glycolysis (Klein *et al.*, 2004). Glycolysis is summarized by the chemical equation:

$$C_6H_{12}O_6 + 2ADP + 2P_i + 2NAD^+ \rightarrow 2CH_3COCOO^- + 2ATP + 2NADH + 2H_2O + 2H^+$$

The chemical formula of pyruvate is CH_3COCOO^-. P_i stands for the inorganic phosphate. As shown by the reaction equation, glycolysis causes the reduction of two molecules of NAD^+ to NADH. Two ADP molecules are also converted to two ATP and two water molecules via substrate-level phosphorylation. The chemical equation below summarizes the fermentation of glucose, whose chemical formula is $C_6H_{12}O_6$ (Purves *et al*., 2004). One glucose molecule is converted into two ethanol molecules and two carbon dioxide molecules:

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

In some organisms, the pyruvate goes through ethanol fermentation also referred to as alcoholic fermentation. This is a biological process in which sugars such as glucose, fructose, and sucrose are converted into cellular energy and thereby produce ethanol and carbon dioxide as metabolic waste products. Because yeasts perform this conversion in the absence of oxygen, ethanol fermentation is classified as anaerobic**.** Usually only one of the products is desired; in bread-making, the alcohol is baked out and in alcohol production,

the carbon dioxide is released into the atmosphere or used for carbonating the beverage. When the ferment (enzyme) has a high concentration of pectin, minute quantities of methanol can be produced.

In animals and some bacteria, the pyruvate can go through lactic acid fermentation. Lactic acid fermentation is the simplest type of fermentation. Essentially, it is a redox reaction. In anaerobic conditions, the cell's primary mechanism of ATP production is glycolysis. Glycolysis reduces (transfers) electrons to NAD^+, forming NADH. For glycolysis to continue, NADH must be oxidized (have electrons taken away) to regenerate the NAD^+. This is usually done through an electron transport chain in a process called oxidative phosphorylation; however, this mechanism is not available without oxygen (Uno *et al.,* 2001). Instead, the NADH donates its extra electrons to the pyruvate molecules formed during glycolysis. Since the NADH has lost electrons, NAD^+ regenerates and is again available for glycolysis. Lactic acid, for which this process is named, is formed by the reduction of pyruvate (Uno *et al.,* 2001). Lactic acid fermentation can be heterolactic or homolactic.

In aerobic respiration, the pyruvate produced by glycolysis is further oxidized completely, generating additional ATP and NADH in the citric acid cycle and by oxidative phosphorylation. However, this can only occur in the presence of oxygen. Oxygen is toxic to organisms which are obligate anaerobes and are not required by facultative anaerobic organisms. If oxygen is present, some species of yeast (*Kluyveromyces lactis*, *Kluyveromyces lipolytica*) oxidize pyruvate completely to carbon dioxide and water (respiration). Thus these yeasts produce ethanol only in an anaerobic environment. However, many types of yeast such as the commonly used baker's yeast *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe* prefer fermentation to respiration. These yeasts will produce ethanol even under aerobic conditions given the right sources of

nutrition. In the absence of oxygen, one of the fermentation pathways occurs in order to regenerate NAD^+; lactic acid fermentation is one of these pathways. Hydrogen gas can also be produced in many types of fermentation (mixed acid fermentation, butyric acid fermentation, caproate fermentation, butanol fermentation, glyoxylate fermentation), as a way to regenerate NAD^+ from NADH. Electrons are transferred to ferredoxin, which in turn is oxidized by hydrogenase, producing H_2.

2.5.2. History of fermentation

Ancient man considered fermentation as some mystery not knowing that he was dealing with the activities of microorganisms. Man had knowledge of fermentation a long time ago when he observed that meat left to stand for a few days was tastier than meat eaten after killing and that intoxicating drinks could be made from grains and fruits (Aiba *et al.,* 1973). This concept has been known to man for many years and humans have been controlling the fermentation process. The earliest evidence of winemaking dates from eight thousand years ago, in Georgia, in the Caucasus area (Cavalieri *et al.,* 2003). There is strong evidence that people were fermenting beverages in Babylon around 5000 BC (FAO, 2007), ancient Egypt around 3150 BC (Cavalieri *et al.,* 2003), pre-Hispanic Mexico around 2000 BC and Sudan around1500 BC. There is also evidence of leavened bread in ancient Egypt around 1500 BC (Dirar, 1993; FAO, 2007) and of milk fermentation in Babylon around 3000 BC.

According to Dubos (1951), the French chemist, Louis Pasteur was the first known scientist to connect yeast to fermentation in 1856. Pasteur originally defined fermentation as "respiration without air". He later proved that alcoholic fermentation was brought about by yeast, when studying the fermentation of sugar to alcohol by yeast. He concluded that the fermentation was catalyzed by a vital force (ferments) within the yeast cells. The ferments were thought to function only within living organisms. He concluded that,

alcoholic fermentation is an act correlated with the life and organization of the yeast cells and not with the death or putrefaction of the cells (Dubos, 1951).

Nevertheless, it was known that yeast extracts ferment sugar even in the absence of living yeast cells. Many scientists, including Pasteur, had attempted unsuccessfully to extract the fermentation enzyme from yeast (Lagerkvist, 2005). Success came in 1897 when the German chemist Eduard Buechner ground up yeast, extracted a juice from them, then found to his amazement that this "dead" liquid would ferment a sugar solution, forming carbon dioxide and alcohol much like living yeasts. The "unorganized ferments" behaved just like the organized ones. From that time on the term “enzyme” came to be applied to all ferments. It was then understood that fermentation is caused by enzymes which are produced by microorganisms (Lagerkvist, 2005). He termed the ferment secretion zymase (Harden and Young, 1906).

It is on record that it took scientists/researchers one hundred years to uncover the enzymatic process of degradation of carbohydrate by yeast into alcohol and carbondioxide (Cook, 1958; Aiba *et al.,* 1973). Fermentation usually implies that the action of microorganisms is desirable and the process is used to produce alcoholic beverages such as wine, beer, and cider. It is also employed in the leavening of bread and for preservation techniques to create lactic acid in sour foods such as yogurt or vinegar (acetic acid) for use in pickling foods.

2.5.3. Types of fermentation

There are basically two types of fermentation namely:

1. Batch fermentation process:

This is a process whereby a fermenter tank is filled with the prepared mash of raw materials to be fermented. The temperature and pH for microbial fermentation is properly

adjusted and occasionally nutritive supplements are added to the prepared mash. The mash is steam sterilized in a pure culture process. The inoculum of a pure culture is added to the fermenter, from a separate pure culture vessel. Then the fermentation proceeds and after the proper time the contents of the fermenter are taken out for further processing. The fermenter is cleaned and the process is repeated. Thus, each fermentation process is a discontinuous process divided into batches.

2. Continuous fermentation process:

In continuous fermentation, an open system is set up. Sterile nutrient solution is added to the bioreactor continuously and an equivalent amount of converted nutrient solution with microorganisms is simultaneously removed from the system (Maxon, 1960). Two basic types of continuous fermentations can be distinguished:

Homogeneously Mixed Bioreactor: This is run as either a chemostat or a turbidostat. In the chemostat in the steady state, cell growth is controlled by adjusting the concentration of one substrate. Any required substrate (carbohydrates, nitrogen compounds, salts, O_2) can be used as a limiting factor. In the turbidostat, cell growth is kept constant by using turbidity to monitor the biomass concentration and the rate of feed of nutrient solution is appropriately adjusted.

Plug Flow Reactor: In this type of continuous fermentation, the culture solution flows through a tubular reactor without back mixing. The composition of the nutrient solution, the number of cells, mass transfer, and productivity vary at different locations within the system. At the entrance to the reactor, cells must be continuously added along with the nutrient solution

2.6. YEAST

Yeasts are chemoorganotrophs as they use organic compounds as a source of energy and do not require sunlight to grow. The main source of carbon is obtained by hexose sugars such as glucose and fructose, or disaccharides such as sucrose and maltose (Kurtzman, 2006). Some species can metabolize pentose sugars, alcohols, and organic acids (Barnett, 1975). Yeast species either require oxygen for aerobic cellular respiration (obligate aerobes), or are anaerobic but also have aerobic methods of energy production (facultative anaerobes). Unlike bacteria, there are no known yeast species that grow only anaerobically (obligate anaerobes). According to Alexopoulos *et al.* (1996) yeasts are referred to as ascomycetes which possess a predominately unicellular thallus which reproduce asexually by budding or transverse division or both and produce ascospores in naked ascus. While Kurtzman and Fell, (2006) described yeasts as a growth form of eukaryotic microorganisms classified in the kingdom Fungi, with about 1,500 species described. It is estimated that only 1% of all yeast species have been described. Some organisms which are not known to produce ascospores but which possess all other characteristics above, and are not related to other group of fungi are listed as yeast because many have lost the ability to form ascospores or form ascospores under stringent conditions. The shape and size of the individual cell of some species vary slightly but in other species the cell morphology is extremely heterogenous. Cook (1958) gave the shape of yeast cells to be spherical, globose, ellipsoidal, elongate to cylindrical with rounded ends, more or less rectangular. This was also correlated by Alexopoulos *et al.* (1996), who added pear shaped, apiculate or lemon shaped, ogival or pointed at one end or tetrahedral to the shapes of yeast cells. The sizes of yeast vary in unicellular forms between 2 µm-10 µm in length (Cook, 1958). The length of cylindrical cells is often 20-30 µm and in some cases even greater.

2.6.1. Occurrence of yeast

Yeast is ubiquitous in nature, existing on plants, animals, in water, sediments, soil, in terrestrial, aquatic and marine habitats (Cook, 1958; Alexopoulos *et al.,* 1996; Sláviková and Vadkertiová, 2003); in the guts (Odiete and Akpata, 1981) and in decomposing organic matter (Alexander, 1977). Many species have high specific habitats whereas others are found on a variety of substrates in nature. Yeasts occur in an environment with temperature range of 25ºC – 30ºC and relative humidity of 80-90ºC. Optimum temperature for cultivation varies from species but those isolated from soil, air or water usually grow best at 28ºC (Arthur and Watson, 1976; Alexander, 1977). Yeast can grow both in aerobic and anaerobic conditions. All these factors are conducive for the cultivation and storage of yeast. Common media used for the cultivation of yeasts include; potato dextrose agar (PDA) or potato dextrose broth, Wallerstien Laboratories Nutrient agar (WLN), yeast peptone dextrose agar (YPD), and yeast mould agar or broth (YM). The antibiotic cycloheximide is sometimes added to yeast growth media to inhibit the growth of *Saccharomyces* yeasts and select for wild/indigenous yeast species (Seeliger, 1956). Methylene Blue is used to test for the presence of live yeast cells (Lee *et al.*, 1981).

2.6.2. Reproduction in yeast

Yeast basically undergoes asexual and sexual reproduction. Vegetative (asexual) reproduction is characterized by budding or fission. In the asexual reproduction where multiplication of yeast occur by budding process, the protoplasm pushes out of the cell wall in the form of a bud and forms a daughter cell that enlarges until it is separated from the mother cell by constriction at the base (Alexopoulos *et al.,* 1996). Yeast buds are sometimes called blastospores or blastoconidia. When yeast reproduces by fission, the parent cell first elongates, the nucleus divides and a transverse wall is then formed to

separate the mother cell into two uninucleate daughter cells. The product of this mechanism is termed arthrospores or arthroconidia (Henrici, 1941).

Sexual reproduction in yeast is differentiated from that of other fungi by sexual states that are not enclosed in a fruiting body. Yeasts are categorized into two groups based on their methods of sexual reproduction; the ascomycetous and basidiomycetous yeast. The sexual spores of the ascomycetous yeast are termed ascospores which are formed in simple structure often a vegetative cell. Such asci are called naked asci because of the absence of an ascocarp, which is a more complex fruiting body found in the higher ascomycotina. Two ascospores may assume the function of copulating gametangia which unite to form a zygote cell. Subsequently, an ascus is formed. This contains ascospores, the number of which depends on the proceeding development of the nuclei. Four to eight ascospores per ascus are usually formed but other numbers may also occur (Alexopoulos *et al.*, 1996). If the vegetative cells are diploid, a cell may transform directly into an ascus after the 2N nucleus undergoes reduction or meiotic division. Some yeasts are heterothallic, that is, sporulation occurs when strains of opposite mating type are mixed on sporulation media. However, some strains may be homothallic (self-fertile) with reduction division and karyogamy (fussion of two haploid nuclei) taking place during the formation of sexual spores (Henrici, 1941).

2.6.3. Life cycle of yeast

Yeast exhibits any of the three forms of life cycles below:

a. The first form is a process when the diploid stage is short and confined to the zygote cell which undergoes meiosis immediately after karyogamy and develops ascospores. Example is found in *Schizosacharomyces octosporus*

b. The second form possesses a long diploid phase and very short haploid phase. This is the ascospores that sporulate in the yeast which undergoes the method. Example is found in *Saccharomyces ludwigii*

c. In the third type of life cycle, both the haploid and diploid phases are perpetuated by budding so that both phases are of equal importance which may constitute a form of alternation of generation. Copulation takes place between haploid cells to form diploid cell by the process of plasmogamy and karyogamy (Henrici, 1941: Alexopoulos *et al.*, 1996). Thus mature yeast cells are diploid containing double (2N) number of chromosomes.

Basidiospores and teliospores are the sexual spores that are produced in the three classes of basidiomycetous yeast. Sexual reproduction and life cycle in these yeasts are typical in that it include both unifactorial (bipolar) and bifactorial (tetra polar) mating systems.

The useful physiological properties of yeast have led to their use in the field of biotechnology. Fermentation of sugars by yeast is the oldest and largest application of this technology. Some yeasts have the ability to carry out an alcoholic fermentation while others lack this property. Fermentative yeasts have a fermentative type of metabolism whereas non-fermentative yeasts have only a respiratory or oxidative metabolism (Henrici, 1941)**.** Both reactions produce energy, the energy from respiration is used for synthetic reactions such as assimilation and growth while part is lost as heat. When a fermenting (anaerobic) yeast culture is aerated, fermentation is suppressed and respiration increases. This is the Pasteur's phenomenon (Barnett, 2003).

Many types of yeasts are used for making many foods: Baker's yeast in bread production, brewer's yeast in beer fermentation, yeast in wine fermentation and for xylitol (Prior et al., 1989)**.** Instances are bound where yeasts like *Kluyveromyces marxianus* teleomorph of

Candida kefyr is used commercially to produce lactase enzyme similar to the use of other fungi such as those in the genus *Aspergillus* (Seyis and Aksoz, 2004). *Saccharomyces cerevisiae* and *K. marxianus* were yeasts used by Tyiagi *et al.* (1992) and Zafar and Owais, (2006) respectively in the production of ethanol. Some are able to grow in foods with a low pH, (5.0 or lower) and in the presence of sugars, organic acids and other easily metabolized carbon sources (Kurtzman, 2006). During their growth, yeasts metabolize some food components and produce metabolic end products. This causes the physical, chemical, and sensory properties of a food to change and the food is spoiled. Yeast also play other vital roles in industrial fermentation processes such as the production of industrial enzymes, chemicals, food products, malt, beverages and wine; genetic engineering and medical mycology (Boundy-Mills, 2012).

2.7. BIOETHANOL PRODUCTION IN NIGERIA

In Nigeria the production of ethanol (alcohol) via local methods is as old as the people. Researches are currently on-going for large scale production of ethanol as bio-fuel. Some of the plants that have been used in other countries which are presently being advocated for use in Nigeria include corn (*Zea mays*), sugarcane (*Saccharum officinarum*) and cassava (*Manihot esculenta*) (Agba *et al.,* 2010). The need for alternative fuel has become an important issue in Nigeria as a result of the periodic fuel crisis, especially with the way and manner the price is hiked by each successive government. Oil spillages, oil well blow-out, oil blast discharges (Owolabi and Okwechime, 2007) and improper disposal of drilling mud from petroleum drilling and refinery companies (Staney, 1990) have resulted also into a multitude of unresolved problems such as damage to wildlife, modification of the ecosystem through fauna and flora elimination, loss of aesthetic values of natural beaches

as well as decrease in fishery resources (Staney, 1990). The nation is also battling with global environmental changes like climate change and global warming which is due to the increase in the concentration of atmospheric CO_2, which has presently risen to about 380 parts per million (PPM) from 280 ppm in the 1800s (Adeyinka *et al*., 2005). While agriculture has been the mainstay of Nigeria's economy in the pre-independence days, crude oil has been her major source of foreign exchange from the post-independence period especially in the last 3-4 decades. With the current cover clamour for resource control, it is therefore time for the nation to start looking into alternatives available for the production of fuel for the transport sector (Bugaje and Mohammed, 2007). The transportation sector consumes fuel in two main ways; as petrol for most small capacity vehicles and as diesel for most heavy duty vehicles (Agba *et al.,* 2010). It is a well establish fact that agriculture can provide Nigeria bio-fuel that can be used as substitute for both petrol and diesel. The technology has been developed in many countries, including developing countries such as Brazil, Ethiopia, India and South Africa (Bugaje and Mohammed, 2007). Producing bio-fuel in Nigeria has enormous potential for the people, with changes in land tenure, the people will have new security in their work and more small scale farmers will have more income from production of crops. The aim for sourcing bio-fuel is to develop better and cleaner methods for extracting and burning traditional fuels like wood, coal and oil as well as to develop new sources of energy such as liquid fuel from biomass. According to Azih, (2007), Nigeria will be $150 million annually richer when she adopts the development and application of bio-fuel as an alternative fuel to crude oil.

The federal government of Nigeria national policy on bio-fuels in 2007 seeks to establish a thriving bioethanol industry by utilizing agricultural products as means of improving the quality of automotive fossil-fuel based in the country (Azih, 2007). It hopes to prepare

regulations for sale and use, and guarantee the take-off under contractual terms. It aims to create jobs, rural and agricultural development, technology acquisition and transfer, attract foreign investment and streamline the roles of federal, state and local governments in bio-fuels development. Implementation of these plans include initial market seeding (E10), bio-fuel production programmes (BPP) to achieve 100% domestic production by 2020, complete bio-fuel uptake arrangement, joint-venture distillers. This is anchored on agricultural productivity and competitiveness. Already $4 billion have been committed to sugarcane sourced ethanol project in the northern states of Jigawa and Benue while cassava-sourced ethanol projects are earmarked for the southern Anambra and Ondo states (Azih, 2007).

2.8. SELECTED PLANTS USED FOR ISOLATION OF YEAST AND PRODUCTION OF BIO-FUEL

2.8.1. *Manihot esculenta* Crantz.

Manihot esculenta belongs to the family Euphorbiaceae. It is a tropical Africa shrub and it is identified in the different West African countries by their native names:

Tapioca, cassava, cassada (English); manioc, mandioc, cassave (French); mandioca (Portugese). Nigeria: Igai (Edo); Akparamka (Efik); Rogo (Hausa); Akphunkoro, abacha, akpu, awaranoku (Igbo); Ege, gbaguda, ogege, elugbe (Yoruba) and Doya (Kanuri).

Manihot esculenta grows to about 4 m high with a markedly articulated stem and large fleshy to woody tubers, native of Brazil and now dispersed throughout the tropical world (Hutchinson and Dalziel, 1958). The plant had assumed such importance in the world long before the voyages of discovery from Europe of the fifteenth and sixteenth centuries that it

had been dispersed in the American tropics from 25^{o} south of the equator to 25^{o} north. The origin of the plant was also cited as Brazil by Olsen and Schaal (1999). Economically, the tuber is used as food to prepare delicacies from the tropics to the temperate regions of the world.

The plant is extremely variable especially in the roots. The main distinction of races lies between 'bitter and sweet'. In the bitter races a cyanogenetic glycoside, linamarin, occurs throughout the plant tissues and this by enzymatic action is reduced to glucose, acetone and hydrocyanic acid, in the roots. The latter may amount to upwards of 0.01% concentration. It has also been reported that the peels of cassava contain toxic cyanogenic glucosides (Oke, 1968). In the sweet races the glycoside occurs only in the roots in much lower amounts and then principally in the root-skin, facilitating easy removal by pairing in preparation for eating. Concentration of hydrocyanic acid varies also according to soil type, moisture, temperature and age, unfavourable conditions leading to higher amounts. High potash availability is desirable for improved starch-production and lower hydrocyanic acid level. Aregheore and Agunbiade (1991) showed that the toxic compound cyanogenic glucosides in cassava can cause acute cyanide poisoning and death of man and animal when consumed.

The tuber is the highest source of carbohydrate except for sugarcane and it is deficient in protein, fat, some minerals and vitamins (Olsen and Schaal, 1999). Its nutritional value is less than that of cereals, legumes and some other roots like yam. The leaves contain more protein but lack essential amino acids and methionine. However, they are useful for human and animal consumption. Solid media fermentation of cassava products have been reported in Canada where *Aspergillus fumigatus* was used, in Burundi and Nigeria where *Rhizopus oryzae* and *Aspergillus niger* were respectively used in enriching cassava products (Akindahunsi *et al.,* 1999; Oboh *et al.,* 2002). The liquid contains a lot of microorganisms,

lactic acids, lysine from *Lactobacillus oryneformis*, amylase from *Saccharomyces* sp and tinamarase from *Lactobacillus delbruckii* capable of hydrolyzing the glucosides (Raimbault, 1998; Akindahunsi *et al.,* 1999). The amylase present in the fermented water was characterized by Oboh (2005).

2.8.1.1. Biofuel from cassava

Nigeria and Ghana are already establishing cassava-to-ethanol plants. Cassava is a major staple food in Nigeria which also serves as raw materials for the ethanol, pharmaceutical, confectionery, textile, beverages and packing industries (Henk *et al.,* 2007). Prior to the take-off of the presidential initiative on cassava production and export, about 90% of cassava produced was consumed as food (Ololade, 2007). Production of ethanol from cassava is currently economically feasible when crude oil prices are above US$120 per barrel. New varieties of cassava are being developed, so the future situation remains uncertain. Currently, cassava can yield between 25-40 tonnes per hectare (with irrigation and fertilizer), and from a tonne of fresh cassava roots about 150 litres of ethanol is produced (Kuiper *et al*., 2007). The overall energy efficiency of cassava-root to ethanol conversion is approximately 32%. The yeast currently used for processing cassava is *Endomycopsis fibuligera*, sometimes used together with bacterium *Zymomonas mobilis* (Gonzalez *et al.,* 1998; Amutha and Gunasekaran, 2001).

2.8.2. *Elaeis guineensis* Jacq.

E. guineensis, also known as the African oil palm, is a very large feather-palm and one of the two species of the family Arecaceae. It is native to West Africa, occurring between Angola and Gambia, while the American Oil Palm *Elaeis oleifera* is native to tropical

Central America and South America. The generic name is derived from the Greek word for oil, '*elaion'* while the species name refers to its country of origin (Burkill, 1997)

Matured trees are single-stemmed and grow to 20 m tall. The leaves are pinnate and reach between 3-5 m long. A young tree produces about 30 leaves a year. Established trees over 10 years produce about 20 leaves a year. The flowers are produced in dense clusters; each individual flower is small, with three sepals and three petals. Trunk being clad in the persisting leaf bases from shed leaves for many years. The palm is monoecious with male and female inflorescences borne separately in the leaf axils (Burkill, 1997). It is easily recognizable by its arching dark-green leaves and straight trunk-clothed when young with petiole bases. The palm oil extracted from both the pulp of the fruit and the kernel is used in foods and for manufacturing soaps. The increasing use in the commercial food industry in many parts of the world is buoyed by its cheaper pricing (United States Department of Agriculture, 2006), the high oxidative stability of the refined product (Che Man *et al.,* 1999; Matthäus, 2007) and high levels of natural antioxidants (Sundram *et al.,* 2003). Since palm oil contains more saturated fats than oils made from canola, corn, linseed, soybeans and sunflowers, it can withstand extreme deep-frying heat and resists oxidation (De Marco *et al.,* 2007).

Palm sap is extracted from the inflorescence by means of opposite cutting in a mature trunk. Cunningham and Wehmeyer (1988) reported that palm wine is a rich source of vitamins A and C while the oil is a good source of edible oil and wine. Many workers have researched into the isolation and characterization of yeast species from palm wine (Nwachukwu *et al*., 2006; Bechem *et al*., 2007)

2.8.3. *Zea mays* Linn.

This is an annual of the grass family (Poaceae). It is single-stemmed which occasionally tillering with stout culm, sometimes stilt root at the basal nodes (Burkill, 1995). It is known by various names such as: Maize (English speaking nations); Corn (America); Sweet corn (India); mais (French); milho, milhogrande, milhogrosso, milhomaees (Portuguese).

Vernacular names in some African countries include: ekontibaba, ekuntibaba, mala (Senegal); manyo, tubah-nyo (Gambia); nkangntol, nkuskus (Sierra Leone); kroju, eburo, aburow (Ghana); Nigeria: masaraa (Hausa); ikpapka (Igbo): agbado, yangan, oka (Yoruba).

Zea mays are monoecious plants with the male inflorescence (tassel) at the terminal end and the female flowers on a short lateral auxiliary branch borne down the stem. It grows to a height of about 6 m and 3-4 cm in diameter (Welch and Graham, 1999). The stems superficially resemble bamboo canes and the internodes can reach 44.5 cm. The plant has a distinct growth form; the lower leaves being like broad flags, generally 50–100 cm long and 5–10 cm wide; the stems are erect, conventionally 2–3 m in height, with many nodes, casting off flag-leaves at every node. Under these leaves and close to the stem grow the ears (female inflorescence). They grow to about 3 mm a day (Garrison, 2004). Maize is predominantly a cross-pollinating species, a feature that has contributed to its broad morphological variability and geographical adaptability. Depending on the latitude and the climate in which it is grown, maize is classified into three distinct types, tropical, temperate and subtropical.

Maize was introduced into Nigeria probably in the 16th century by the Portuguese (Ihelarouye and Ngoddy, 1965). It is the most important cereal in the world after wheat

and rice with regard to cultivation areas and total production (Purseglove, 1972) The global production of maize is estimated to about 300 million tonnes per year. 145 million (or about 50 per cent) are produced in USA alone (Ihelarouye and Ngoddy, 1965; Purseglove, 1972). In Nigeria, its production is quite common in all parts of the country, from the north to the south, with an annual production of about 5.6 million tones (Central Bank of Nigeria, 1992). Maize is an important staple food for more than 1.2 billion people in Sub Sahara Africa and Latin America. All parts of the crop can be used for food and non-food products. In industrialized countries, maize is largely used as livestock feed and as a raw material for industrial products. Maize accounts for 30−50% of low-income household expenditures in Eastern and Southern Africa. A heavy reliance on maize in the diet, however, can lead to malnutrition and vitamin deficiency diseases such as night blindness and kwashiorkor (Gopalan, 1968; Alastair, 1999). In addition to being an economically important crop, maize is also a classical genetic model for plant research. As a result of its shallow roots, maize is susceptible to droughts, intolerant of nutrient-deficient soils, and prone to be uprooted by severe winds. The corn steep liquor, a plentiful watery byproduct of maize wet milling process, is widely used in the biochemical industry and research as a culture medium to grow many kinds of microorganisms (Liggett and Koffler, 1948; USDA, 2010).

2.8.3.1. Biofuel from maize

Maize is increasingly used as a feedstock for the production of ethanol fuel. Feed maize is being used increasingly for heating; specialized corn stoves are being made with either feed maize or wood pellets to generate heat. Maize cobs are also used as a biomass fuel source. Maize is relatively cheap and home-heating furnaces have been developed in many temperate regions which use maize kernels as a fuel. They feature a large hopper that feeds the uniformly sized maize kernels (or wood pellets or cherry pits) into the fire.

Ethanol from maize is mixed with petrol in order to decrease the amount of pollutants emitted when used to fuel motor vehicles. According to Jacob (2007), the main feedstock for the production of ethanol in the United States of America is currently corn with approximately 2.8 gallons of ethanol being produced from one bushel of corn (0.42 liter per kg). While much of the corn turns into ethanol, some of the corn also yields by-products such as DDGS (distillers dried grains with soluble) that can be used as feed for livestock (Lumpkins *et al.,* 2004). A bushel of corn produces about 18 pounds of DDGS (320 kg of DDGS per metric ton of maize). Corn-based gasohol (a combination of unleaded gasoline and ethanol made from corn) reduces fossil energy used by 50 to 60% and pollution by 35 to 46%t. More than 11% of all automotive fuels sold in the United States of America are ethanol-blended, and that percentage may increase in the future (Jacob, 2007)**.**

2.8.4. *Cola acuminata* Schott and Endl.

Cola belongs to the family Sterculiaceae. It is a large genus of about 125 species restricted to the forest region of Africa particularly West Africa (Cheek, 2002), with various names such as:

Kola, true or commercial kola (English); kola (French); coleira (Portuguese); we-eh, gole, toolu (Sierra Leone); bisi, bese (Ghana); Nigeria: gooro (Hausa), oji (Igbo), oobi-abata (Yoruba).

Cola is a generic name coined by Schott and Endlicher in 1832 (Bouquet and Debray, 1974) with West Africa as the generic Centre. It is the first fruit taken into cultivation by Africans in West Africa. It is the largest genus of the family Sterculiaceae. Its habitat ranges from slender-stemmed shrubs to large trees.

C. acuminata is a tree of about 20 m high with natural forest distribution to the east of *C. nitida*. As reported by Abaka (2000), it is one of the five species of edible kola nut grouped into the Subgenus Eucola by Chevalier and Perrot in 1911**.** Others are *Cola nitida*, *Cola ballayi*, *Cola verticillata* and *Cola sphaerocarpa*. The latter three species are not known to be cultivated. Both *C. acuminata* and *C. nitida* are commonly planted around villages with *C. nitida* having higher caffeine content. *C. acuminata* is differentiated from *nitida* by having 3-6 cotyledons while *nitida* has 2. The fruit is composed of up to five carpels, up to 20 cm long by 6 cm broad with as many as fourteen seeds covered with white skin (Burkill, 2000). The seeds are generally reddish or pink but sometimes whitish. The sapwood is whitish to slightly pink when fresh while the heart wood is dull yellowish-brown or reddish. The stock of trees is maintained by transplanting of naturally produced seedlings.

The fruits are eaten raw and the seeds are chewed for their stimulating properties. Chewing kolanut can ease hunger pangs. Kolanuts are used mainly for their stimulant and euphoriant qualities. They have effects similar to other xanthine containing herbs like cocoa, tea etc. However, the effects are distinctively different, producing a stronger state of euphoria and wellbeing (Newall *et al.*, 1996). A recent study by Shonibare *et al*., (2009) confirmed the phytochemical and antimicrobial properties of *C. acuminata.* The nut contains 1-2.5% caffeine, 0.023% theobromine, 1.618% tannin, 1.28-3.0% fixed oil, 9% protein, 75% carbohydrates, kolatin and kolatein (Bouquet and Debray, 1974). It also contains the glucoside, kolanin that is used as a heart stimulant. Industrially, the bark is mixed with *Khaya* to produce brown dye.

2.8.5. *Solanum tuberosum* Linn.

This belongs to Solanaceae, a family of shrubs or small trees with fragrant white pendant flowers. It is a hybrid known only to be cultivated to Andean Cordillera of Southern Columbia but now present in tropical and subtropical countries worldwide. Known with varying names worldwide:

Potato, Irish potato (English); pomme de terre (apple of the earth) (French); batata-the tuber, batateira-the plant (Portuguese); airish-petete (Sieera Leone); baa (Ivory Coast); Nigeria: kalin tuuraawaa (Hausa);

S. tuberosum is a tuberous, herbaceous annual or perennial if the tuber is considered to be evergreen. It is a native of temperate areas of the Southern America Andes and grows between 500-2500 m in altitude. The tuber is a modified thickening of the underground stems. It produces the most important starch crop of the temperate countries and ranks fourth amongst leading staple food of the world after rice, wheat and maize (Mcneill, 1999).

Plant growth and tuber formation require a temperate climate, soil temperature not above 20ºC, evenly distributed rainfall, preferably a free draining loamy soil, day length 12-13 h but variable. Some varieties need 15-16 h, crop length 3-10 months according to varieties (Burkill, 2000). The leaves contain tannin, alkaloid, solanine, and solanidine (Hutchinson and Dalziel, 1958; Chadha, 1976)**.** The leaves can be boiled and eaten as spinach. The leaves and green parts are diuretic and biologically active against microorganisms. Potato pulp is a by-product of starch manufacture and is an animal feed. Butane and acetone are by-products of potato processing (Zaag and Horton, 1983)**.** Cattle, pigs and horses fed with large amount of uncooked tubers suffer eruption even fatality.

2.8.6. *Musa paradisiaca* Linn.

This is a gigantic herb with pseudostems of concentric leaf sheath from a few centimeters to over 30 cm; it can be up to 5 m high. Known by various names such as:

Plantain (English); banana-the fruit, bananier-the plant (French); bananeira (Portuguese); forondo, bongui (Guinea); gbola (Sierra Leone); Nigeria: ayabar (Hausa), abirjka (Igbo), ogede (Yoruba).

Musa paradisiaca belongs to family Musaceae with about 50 species. Its origin dates back to 600-500 B.C. in Indo-Malesian. Minimum rainfall of about 100 mm per month or adequate irrigation and temperature not under 16ºC is necessary for optimal cultivation. The principal centers of cultivation in West Africa are in the humid parts of the Ivory Coast and Ghana (Burkill, 1998). It serves as staple in over 120 countries in the world (Debabandya *et al.,* 2009). It is grown on plantations in pure stands or as mixed crops. It serves as a shade plant for coffee and cacao in Gabon (Burkill, 1998). It is an important component in the general cropping system providing shade and mulch to the ground and a continuity of food (Kobayashi *et al.,* 2001).

After harvesting the fruit, the plantain shoot can be cut and the layers peeled (like an onion) to get a cylinder-shaped soft shoot. This is made into a sort of salad or a dry curry or as a wet curry in some parts of India (Oke *et al*., 1998). The fruit pulp is eaten in many ways such as cooked, fried, baked, mashed or added to other types of food. The fruit pulp can also be sundried, smoked, and mealed into flour for storage (Busson, 1965; Okiy, 1960). Plantain shoot is considered rich in fibers and is considered as a very good remedy for avoiding constipation. The stems reduce to ash makes a powder dressing for ulcer and strips of the stem serve as tourniquets and as bandages (Oliver, 1960; Abiw, 1990). The dried stem peels are also slit into fine threads and are used for weaving mats, stringing

garlands and packaging wrapper. Tryptamine is present in the leaf and fruit peel (Burkill, 1998). Plantain ripening metabolism is halted and requires heat in cooking for the carbohydrate to be broken into sugar.

2.8.7. *Oryza* Linn.

Oryza is an annual and perennial grass with about 1,925 species in Africa, Asia, Australia and Southern America (Burkill, 1995). Commonly known as rice (English); riz (French); orroz (Portuguese); diuna (Senegal); pendekio (Guinea); iraa (Ghana); Nigeria: shinkaafaa (Hausa); oskapa, obara (Igbo); ireesi (Yoruba).

O. glaberrima Steud. (African rice) is one of the six species indigenous to Africa. It arises by selection from *O. barthii* and has been domesticated. *O. glaberrima* is similar to *O. sativa* with minor differences in the pubescence of the glume and size of the lignule (Carney, 2001). It originated in the middle and upper Niger River valley over 3000 years ago. There is no known date of its domestication (Burkill, 1995). It is an annual grass with stout culm of 1-1.5 m high, very variable and the grain panicles are black to white. The species shows immerse diversity and over 1500 varietal cultivars are known with the greatest quantity found west of longitude 4º West in Ivory Coast. Various cultivars are adopted to swamp, hill, flood and floating conditions. Some grow in water up to 2.5 m deep and can withstand immersion for 3-4 days to produce culm of 4-5 m long. The grains shatter thus requiring reaping of the panicle before final maturity.

Brown rice commonly known as ‘ofada rice’ is a variety of *Oryza glaberrima* commonly grown in Ifo area of Ogun state. Rice is not a complete diet, milled rice have about 86-90% carbohydrate, 7-8% protein (Carney, 2001). The grain is commonly fermented to make wine, spirit, beer and industrial alcohol. The husk is of no value as food but burns well as fuel in rice mill engine (Chand *et al.,* 1987). The straw has about 32-33%

carbohydrate, 1.4% fat, low digestibility but can be mixed with other fodders for cattle. As recorded by Burkill (1995) it is also use in Nigeria for producing boards, for building huts, making of basket and mat.

2.8.8. *Ipomoea batatas* (L.) Lam.

Ipomoea batatas is a dicotyledonous plant that belongs to the family Convolvulaceae. This is the only crop plant of major importance among the approximately 50 genera and more than 1,000 species of Convolvulaceae, The genus *Ipomoea*, also includes several garden flowers called morning glories, though that term is not usually extended to *I. batatas*. Some cultivars of *I. batatas* are grown as ornamental plants. Some others are used locally, but many are actually poisonous.

Common names of *I. batatas* include: Sweet potato, Spanish potato (English); batate, patate (French); batata (Portuguese); g-bami, gbam-de, n-joule (Sierra Leone); anago, atomo, asikuma, kampi (Ghana); Nigeria: dankalii (Hausa); otoli, ji-oyibo, ji-bekee (Igbo); odunkun (Yoruba).

The sweet potato as it is commonly called is only distantly related to the potato (*Solanum tuberosum*) with about 400 varieties; the white, purple and the orange skinned varieties are the commonest (Austin, 1988). It is botanically very distinct from the other vegetable called a yam, which is native to Africa and Asia and belongs to the monocot family Dioscoreaceae. The plant is a herbaceous perennial vine, bearing alternate heart-shaped or palmately lobed leaves and medium-sized sympetalous flowers. The edible tuberous root is long and tapered, with a smooth skin whose colour ranges between red, purple, brown and white. Its flesh ranges from white through yellow, orange, and purple.

The plant does not tolerate frost; it grows best at an average temperature of 24°C (75 °F), abundant sunshine and warm nights. Annual rainfalls of 750–1,000 mm (30–39 in) are considered most suitable, with a minimum of 500 mm (20 in) in the growing season. The crop is sensitive to drought at the tuber initiation stage 50–60 days after planting and it is not tolerant to water-logging, as it may cause tuber rots and reduce growth of storage roots if aeration is poor (Abidin, 2004). Depending on the cultivar and conditions, tuberous roots mature in two to nine months. They can be grown in poor soils with little fertilizer. However, sweet potatoes are very sensitive to aluminum toxicity and will die about 6 weeks after planting if lime is not applied at planting in this type of soil. Raw sweet potato contains about 20.1% carbohydrate, 1.6% protein, 0.6% oil. Besides simple starches, sweet potatoes are rich in complex carbohydrates, dietary fiber, beta carotene, vitamin C, and vitamin B_6. Pink, yellow and green varieties are high in carotene, the precursor of vitamin A and a lot of antioxidants (Tumuhimbise *et al*., 2009; Zhang *et al.,* 2009; Mei *et al.,* 2010). The peptic substance (0.78% total, 0.43% soluble) present in fresh tubers contains uronic acid (60%) and methoxyl (4 to 5%). Other constituents include phytin (1.05%), two monoamino phosphatides (probably lecithin and cephalin), organic acids (oxalic acid), phytosterolin, phytosterol, resins, tannins, and colouring matter (Noda and Horiuchi, 2008; Mei *et al*., 2010).

According to FAO (1990), sweet potato leaves and shoots are a good source of vitamins A, C, and B_2 (Riboflavin). Low *et al.* (2007) also reported that the dark orange flesh varieties have more beta carotene than those with light coloured flesh and their increased cultivation is encouraged in Africa, where vitamin A deficiency is a serious health problem. This was correlated by the work of Failla *et al*. (2009) and despite the name "sweet", it may be a beneficial food for diabetics, as preliminary studies on animals have revealed that it helps to stabilize blood sugar levels and to lower insulin resistance (Han *et*

al., 2007; Zhang *et al.*,2009). All parts of the plant are used for animal fodder. Researchers at North Carolina State University are breeding sweet potato varieties that would be grown primarily for bio-fuel production.

2.8.9. *Saccharum officinarum* Linn.

This is a perennial clump grass that is very polymorphic with canes of varying distinct dimensions 1.5 to 6.0 cm in diameter; 5 to 25 cm node length and 2.5 to 6.0 m high (Burkill, 1998). It is a domesticated cultigen grown throughout the world as the commercial sugarcane. It is known by various vernacular names in different regions.

Sugarcane (English); canne a sucre (French); cana-de-acucar; canasacarina (Portuguese); sukara, banta, sukar (Senegal); ahrana (Ivory Coast); Nigeria: arakke (Hausa), acharammako (Igbo), reke (Kanuri), ireke (Yoruba).

Sugarcane refers to any of 6 to 37 species (depending on which taxonomic system is used) in the genus *Saccharum* (family Poaceae, tribe Andropogoneae). Sugarcane is native to warm temperate to tropical regions of the world. They have stout, jointed, fibrous stalks that are rich in sugar. All sugar cane species interbreed, and the major commercial cultivars are complex hybrids. This is because the original species which probably came from South West of the Pacific had been subjected to selection from numerous ranges of forms derived from bud mutations and man had picked those capable of yielding sweeter sap, then raw molasses and finally crystalline sugar. The resultant thick canes have become known as noble canes to distinguish them from the thin canes of the other species (Purseglove, 1972; Burkill, 1998).

Today, sugarcane is grown in over 110 countries. In 2009, an estimated 1,683 million metric tons were produced worldwide which amounts to 22.4% of the total world

agricultural production by weight. About 50% of production occurs in Brazil and India (FAO, 2010). Sugarcane cultivation requires a tropical or temperate climate, with a minimum of 60 cm (24 in) of annual moisture. It is one of the most efficient photosynthesizers in the plant kingdom. It is a C_4 plant, able to convert up to 1 percent of incident solar energy into biomass. Although sugarcanes produce seeds, modern stem cutting has become the most common reproduction method.

The cane is chewed from its sweet sap, a use still prevalent in many parts of West Africa (Irvine, 1948). Chewing the cane promotes salivation with a cleansing action synergistic with friction of the cane as a chew-stick. The sap goes into cooking for making sweetmeat, sweet drinks and sometimes alcoholic drinks. Ainslie (1937) reported the use of the sap as sweetening agent in medicine for cancer and other illnesses. Ethanol is generally available as a byproduct of sugar production. It can be used as a bio-fuel alternative to gasoline, and is widely used in cars in Brazil (Goldemberg, 2007). It is an alternative to gasoline, and may become the primary product of sugarcane processing, rather than sugar.

2.8.10. *Pennisetum glaucum* (Linn.) Moench

The millets are a group of small-seeded species of cereal crops or grains, widely grown around the world for food and fodder. The millets include species in several genera, mostly in the subfamily Panicoideae, of the grass family Poaceae (Jere *et al*., 1977). The exceptions, finger millet and teff are in the subfamily Chloridoideae. They are grown in difficult production environments such as those at risk of drought. They have been in cultivation in East Asia for the last 10,000 years (Lu *et al.*, 2009).

According to the FAO (1996), the most widely cultivated species in order of worldwide production are:

Pearl millet (*Pennisetum glaucum*), Foxtail millet (*Setaria italica*), Proso millet (*Panicum miliaceum*), Finger millet (*Eleusine coracana*), Indian barnyard millet (*Echinochloa frumentacea*), Japanese barnyard millet (*Echinochloa esculenta*), Kodo millet (*Paspalum scrobiculatum*), Little millet (*Panicum sumatrense*), Guinea millet (*Urochloa deflexa*) and Browntop millet (*Panicum ramosum*)

Millets are major food sources in arid and semi-arid regions of the world and feature in the traditional cuisine of many others. Nutritionally, millets are rich in B vitamins, especially niacin, B6 and folic acid, calcium, iron, potassium, magnesium, and zinc. Khetarpaul and Chauhan (1989) reported the presence of other phytochemicals like phytic acid which is believed to lower cholesterol and phytate which is associated with reduction in cancer risk. Millets contain no gluten, so they are not suitable for raised bread. When combined with wheat, (or xanthan gum for those who have celiac disease), they can be used for raised bread. Alone, they are suited for flatbread. As none of the millets are closely related to wheat, they are appropriate foods for those with celiac disease or other forms of allergies/intolerance of wheat. However, millets are also a mild thyroid peroxidase inhibitor and probably should not be consumed in great quantities by those with thyroid disease. The protein content in millet is very close to that of wheat; both provide about 11% protein by weight. It is also used as bird and animal feed. Millets are traditionally important grains used in brewing millet beer in some cultures, for instance by the Tao people of Orchid Island and, along with sorghum, by various peoples in East Africa. There is no report of isolation and characterization of yeast from *P. glaucum.*

2.8.11. *Sorghum bicolor* (Linn.) Moench

This plant is known as grain sorghum, great millet (English); mil, gros mil, sorgo, guernotte (French); sorgo, migliozaburro (Portuguese); da-gave, be-ndab, gi-ndab, basit-

bantaku (Senegal); basso, kinti, kous (Gambia); koko, atoko, bammatica, kulia (Ghana); Nigeria: yara- syinang (Hausa); okaokiri (Igbo); oka-baba (Yoruba).

Sorghum bicolor is an annual grass with stout culms of about 4 m high. A matured plant is about 3-5 cm in diameter, with a panicle of loose or dense grains. It is erect and sometimes goose-neck, generally very variable. It is a very important dry area cereal with numerous strains (31 species, 157 cultivars and 571 forms) (Burkill, 2000). It is a major staple food in West Africa. A cyanogenic glycoside (dhurrin) is present in the young foliage, probably as a result of plant metabolism. This is also promoted by drought. Tiller shoots after harvesting are very poisonous (Hutchinson and Dalziel, 1958; Burkill, 2000).

The stem culms are used for fencing, hut-building, and mat making. The stem contains pentosan (27.8%) for the production of furfural. The plant is grown specifically for its grains which rank fourth in the world after wheat, rice and maize (Food and Agriculture Organization, 2009).The polished grain is deficient in protein, Vitamins A and B. There is no report of isolation and characterization of yeast from *Sorghum bicolor.*

2.8.12. *Anacardium occidentale* Linn.

Cashew is a tree of about 10 m high with a crooked trunk and sprawling crown in the family Ancardiaceae. The family contains 73 genera and about 600 species. *Anacardium* contains 8 species, native to tropical America (Mexico, Peru and Brazil) of which the cashew is by far the most important economically. It was dispersed in the 17th century to Africa, India and the Far East by Portuguese adventurers. The Brazilian name ‘caju’ is adopted under various forms in many European languages and other parts of the world (Burkill, 1985).

Some of the known names include: Cashew (English); acajou, cajou, pommed'acajou (French); cajueiro (the tree), caju, castanha de caju (the nut) (Portuguese); abemen, yalagere, kadu, bukayu, finza (Senegal); kasuu, kasuwa (Gambia); kashu, kundi (Sierra Leone); Nigeria: blighia, kanjuu (Hausa); kaju, kantonoyo (Yoruba).

The plant grows well in waste sandy places and has been satisfactorily used for reclamation of sand-dune near the sea (Hutchinson and Dalziel, 1958). It is also drought resistant. It grows well at soil pH 4.5-6.5. The fruit is the swollen pear-shaped pedicel, likened to an apple in the French name pommed'acajou. The nut is borne at the distal end. The unripe fruit is high in astringent and the ripe fruit is rich in sugar and vitamins. In Ghana, the fruit is used in making intoxicating drinks (Irvine, 1961). The pulp is a potential source of alcohol and has been used to make vinegar. The nut is contained in a tough leathery shell of two layers enclosed in a soft honeycombed mesocarp filled with a caustic oil known as cashew nut shell oil that amount to 15-20% of the fresh shell in the West Africa material. The three main cashew products traded in the international market are: raw cashew nuts, cashew kernels and cashew nut shell liquid (Azam-Alli and Judge, 2001). Cashew kernel contains vital minerals which are seldom found in daily diets (Holland *et al.*, 1991). It is widely consumed as roasted, fried, salted or sugared snacks, as material for confectionery, bakery products and as a food ingredient (Azam-Alli and Judge, 2001). The roughness of the shell is much that it cannot be cracked without heat treatment to make it brittle although heating sends off the shell oil (Ogunsina and Bamgboye, 2007). If the shell is roasted in a confined space to obtain the kernels, the oil vapour can cause swelling, rubefaciation, vesication and dermatitis. The shell oil has local uses in tattooing, to remove warts, to put in a carious tooth; it is used for refractive leprosy and ulcer. The oil also has industrial applications in manufacture of brake lining, industrial belting, clutching, for reinforcing synthetic rubber. The oil is dark coloured, viscous,

poisonous and strongly vesicant due to the presence of two phenolic substances cardol which is about 10% and anacardic acid 90%. The kernel extracted from the husk is edible and contains 40-57% oil, 20% protein. Recently, Trox *et al.* (2010) found appreciable levels of certain bioactive compounds such as beta-carotene, lutein, zeaxanthin, alpha-tocopherol, gamma-tocopherol, thiamin, stearic acid, oleic acid and linoleic acid in raw cashew nut kernels. Several compounds including esters, terpenes, hydrocarbons, carboxylic acids, aldehydes, alcohols, ketones, lactones and norisoprenoids have been isolated from *A. occidentale,* characterized and quantified by gas chromatography-mass spectrometry analyses (Bicalho and Rezende, 2001).

The roots are boiled and used as a foot bath for yaws on the soles (Irvine, 1961). The wood of the tree is reddish-brown, moderately hard and termite resistant. It is used in boat-building, for box, chest, mortar, house making and as firewood (Hutchinson and Dalziel, 1958; Irvine, 1961).

2.9. WHAT IS WASTE?

Waste has variously been defined globally by different people, organization and government under varying concepts. It is basically unwanted or useless materials. In biology, waste is any of the many unwanted substances or toxins that are expelled from living organisms. Okebukola (1996) sees waste as a material that is no longer needed and therefore, discarded. Pongrácz and Pohjola (2004) also proposed some definitions one of which defined waste as "Things with well-defined purpose, and acceptable performance, but their users failed to use them for the intended purpose". Simply defined, Lawal (2010) define waste as any substance, solid, liquid or gaseous that remains as residue or an incidental by-product of the processing of a substance or for which no use can be found by

the organism or system that produces it. Waste is directly linked to human development, both technologically and socially. The compositions of different wastes have varied over time and location, with industrial development and innovation being directly linked to waste materials. Examples of this include plastics and nuclear technology. Some components of waste have economic value and can be recycled once correctly recovered.

There are many waste types defined by modern systems of waste management, notably including:

- Municipal Waste includes household waste, commercial waste, demolition waste
- Hazardous Waste includes Industrial waste
- Bio-medical Waste includes clinical waste
- Special Hazardous waste includes radioactive waste, Explosives waste, E-waste
- Agricultural Waste includes crop residue.

Every human activity generates waste but it is the accumulation of wastes that constitute environmental health hazards. Osinowo (2001) observes that waste generation occurs through domestic, commercial, industrial, agricultural and other social activities. Lawal (2010) sited human activities such as environmental sanitation, food preparation and consumption of packaged foods, laundry, washing of utensils, discharge of unwanted household items or unserviceable household equipment and old furnishing as domestic contributors of huge volume of waste. Activities like retailing and distributive trade, small, medium and large scale industrial operations were also cited as activities that bring about the generation of both solid and liquid waste. On-farm operation and in-farm gate activities are usually characterized by waste generation. Typical examples include timber and wood-processing industry which generate large quantities of waste in form of sawdust and shavings. Waste could be photodegradable, biodegradable or non-degradable.

Nigeria as a developing nation generates a lot of waste annually. Ali (1995) gave the composition of the various wastes that are generated in Nigeria as follows:

Household waste (85%); commercial waste (8%); sewage sludge (3.5%); industrial waste (1.6%); agricultural waste (1.1%); mini waste (0.5%); hazardous waste (0.3%) and radioactive waste (0.02%).

2.9.1. WASTE GENERATION IN LAGOS STATE

Lagos as a rapid urbanizing city shares majority of the waste generated in the nation. The high population growth rate and rapid urbanization make the generation of some particular kinds of waste in large volumes highly unavoidable. For instance, in 1994 alone, the tonnage of refuse collected by Lagos State Waste Management Authority (LAWMA) was as much as 185,417 tonnes of waste. This was reported by Lawal (2010) quoting Alo (2001). The summary of the most common ones are presented in the table below:

TABLE 2: TYPES OF WASTE GENERATED IN LAGOS STATE

	Types of Waste	Description
1.	Garbage	Waste from the preparation and cooking of food and market refuse as well as those from the handling, storage and sale of meat e.g. blood, bones, etc.
2.	Rubbish	Combustibles (primary organic) – paper and paper plates, cardboard, wood, boxes, etc. plastic containers, nylon, polythene bags, rubber, yam trimmings. Non-combustibles (primarily inorganic) - metal, broken bottles, cans and tins.
3.	Bulk Waste	Discarded large motor parts, tyres, refrigerators, furniture, abandoned vehicles, large crates and other large appliance.
4.	Street Refuse	Content of liter receptacles, biscuits, sausage and sweet wrappers, "pure water" sachet and street sweeping dirt.
5.	Human and Animal Waste	Faeces in different heaps and sizes, dead small and large animals, abandoned human corpse on major highways, poultry waste and dung especially around abattoir.
6.	Construction	Roofing scraps, broken concrete wire insulation scraps rubbles and

	And Demolition Waste	plaster.
7.	Industrial Refuse	Solid, liquid and gaseous waste resulting from production processes especially from breweries, rubber and plastics, paints and chemical well as detergent industries.
8.	Sewage Treatment Residue	Dewatered sludge, septic tank sludge and coarse screening grit.
9.	Special Waste	Hazardous waste such as pathological waste usually from surgical operations in the hospitals, explosives from bullet cartridges and t materials

Source: Lawal, 2010

2.9.2. WASTE MANAGEMENT

Waste Management is the appropriate method of disposing waste safely in such a way that makes it less harmful. It involves appropriate disposal of sewage and household refuse. It could come in form of waste treatment or energy conservation. The latter requires the recycling of the nutrients and useful materials contained in domestic or industrial waste. On the other hand, waste treatment can come in different forms. That is, as primary treatment which allows screens to be used to filter out large debris through a sedimentation tank for the suspended solids to settle as sludge, or as secondary treatment, that uses a biological process to break down waste? There is also the tertiary treatment that requires series of specialized chemical and physical processes to reduce the quantity of one or more of the pollutants remaining after primary and secondary treatments through precipitation, absorption and electro-dialysis or reverse osmosis (Lawal, 2010). According to Wilson *et al.* (2006), the location of waste treatment and disposal facilities often has an impact on property values due to noise, dust, pollution, unsightliness, and negative stigma. The informal waste sector consists mostly of waste pickers who scavenge for metals, glass, plastic, textiles, and other materials and then trade them for a profit. This sector can

significantly alter or reduce waste in a particular system, but other negative economic effects come with the disease, poverty, exploitation, and abuse of its workers.

2.9.3. TREATMENT OF PROCESSING WASTES

Until recently, wastes seldom attracted the interest of the well-educated or the investment dollars of the well-heeled. This is now changing, partly because of a growing recognition that solid waste is a potential source of abundant high-grade fuel, close to energy markets (Hayes, 1977). The waste streams easiest to tap for fuels are probably those flowing from food production. Bagasse, the residue from sugarcane, has long been used as an energy source in most cane-growing regions. Various agricultural and industrial processing wastes are quite high in organic material, and frequently these pollutants may be removed through the growth of appropriate microorganisms. Many of the treatment devices and chemicals employed depend upon the character of the waste and consequently modification of a method must be adopted for individual industries within a group. For example, paper pulping wastes are a serious threat to lakes and streams in many areas of the world, partly because of the presence of D-xylose and other low molecular weight organic compounds. The ability of *Candida utilis* (Henneberg) to assimilate this pentose, coupled with its tolerance to lignin degradation products, makes it ideal for pulping waste treatment (Kurtzman, 1983). Cheese whey represents another waste that may be fermented. This had frequently been disposed through municipal sewage systems or by spraying onto fields. The high lactose content of whey has made it attractive for the production of ethanol in dairying areas. The species most commonly used for fermentation is the yeast *Kluyveromyces fragilis* (Jorgensen) van der Walt (Kurtzman, 1983). Hayes (1977) while quoting Poole and Williams (1976) stated that collectible crop residues and feedlot wastes in the United States contain about 5 quadrillion kilojoules of energy more energy than is used by the nation's farmers. The generation of methane from such residues is often

economical (Jewell, 1977). However, the development of a total energy self-sufficient farm probably requires a broader goal than simple short-term maximization of food output.

Consequently, money can often be saved in waste management with more efficiently designed collection routes, modifying vehicles, and with public education (Carlsson, 2005). Environmental policies such as pay as you throw can reduce the cost of management and reduce waste quantities. Waste recovery (that is, recycling, reuse) can curve economic costs because it avoids extracting raw materials and often cuts transportation costs.

CHAPTER THREE

3.0. MATERIALS AND METHODS

3.1. SAMPLE COLLECTION AND PROCESSING OF SUBSTRATES

3.1.1. *Manihot esculenta* (Cassava)

The cassava tubers (*M. esculenta 92/0057*) used in this investigation were collected from the International Institute of Tropical Agriculture (IITA) Ibadan, Oyo State. The tubers were peeled in the laboratory and washed clean using distilled water. The tubers and the peels were separately cut into bits and sun dried for two weeks. They were then milled into flour and stored in an air tight container.

3.1.2. *Elaeis guineensis* sap (Palm wine)

Fresh palm wine sample tapped from *Elaeis guineensis* trees in the University of Lagos, Akoka was obtained from the tapper in a sterile container. 100 ml was transferred into a 250 ml conical flask and corked to ferment for 72 h. The wine remaining was stored for 360 h in opaque plastic container plugged with wool wrapped in aluminium foil at 28-30°C to encourage longer fermentation. This was distilled to obtain the ethanol that was used for extraction in section 3.9. This was done using a modified method of Nwachukwu *et al*. (2006).

3.1.3. *Zea mays* (Maize)

Maize seeds were bought from the market (Oyinbgo market, Lagos) in the month of May, 2009. Dirts were removed by hand picking. The maize was ground into powder form using Warring blender (Binatone) while maize cobs were collected from refuse dump around Idioro market in Mushin Local Government area of Lagos state. These were washed under running tap to remove sand and other dirt particles. The samples

were cut into bits and sundried for a period of three weeks. These were then milled into powder and stored for further use (Oyeleke and Jibrin, 2009).

3.1.4. *Solanum tuberosum* (Irish potato)

Irish potatoes were bought from the market (Mile 12 market, Kosofe Local Government Area, Lagos). The tubers were peeled in the laboratory, washed clean using distilled water. The tubers were then separately cut into bits and sun dried for two weeks. They were then milled into flour and stored in separate air tight containers.

3.1.5. *Cola acuminata* (Kolanut)

Kola nuts were bought from the market (Imota market, Ikorodu Local Government Area of Lagos). The nuts were washed clean in the laboratory using distilled water and allowed to dry. The nuts were then grated and ground into powder using the warring blender (Binatone). This was stored in polythene bag for further use.

3.1.6. *Musa paradiaca* (Ripe plantain)

Plantain was bought from Idioro market in Mushin Local Government Area of Lagos state. These were peeled and sliced into bits before sun drying for two weeks. Some peels were collected from the refuse sites around major plantain markets in Lagos and from homes. These were washed with the peels from the laboratory, cut into pieces and sun dried for three weeks. The dried plantain and the dried peels were ground separately and stored separately in polythene bags for further use.

3.1.7. *Oryza glaberrima* (Ofada rice)

Rice husk was collected from milling site in Ifo Local Government Area of Ogun state. These were sorted to remove stones and other dirt particles. The sample was milled into powder and stored in air tight container for further use.

3.1.8. *Ipomoea batatas* (Sweet potato)

Sweet potatoes were bought from the market (Mile 12 market, Kosofe Local Government Area, Lagos). The tubers were peeled in the laboratory and washed clean using distilled water. The tubers and the peels (some collected from food stalls) were then separately cut into bits and sun dried for three weeks. They were then milled into flour and stored in separate air tight containers.

3.1.9. *Saccharum officinarum* (Sugarcane)

Sugarcane was bought from Agege market in Lagos while sugarcane chaff and peels were picked from dumping sites around Lagos metropolis. The sugarcane was squeezed to extract the juice; this was stored in a sterile container and refrigerated. The chaff and those from refuse dumps were washed under running tap and sun dried for about three weeks. They were then milled into flour and stored in separate air tight containers.

3.1.10 *Pennisetum glaucum* (Pearl Millet)

Millet seeds were bought from the market (Oyingbo market, Lagos), dirt were removed by hand picking. The seeds were milled into powder and stored in air tight container for further use.

3.1.11 *Sorghum bicolor* (Guinea corn)

Sorghum seeds were bought from the market (Oyingbo market, Lagos), dirt were removed by hand picking. The seeds were milled into powder and stored in air tight container for further use.

3.2. ISOLATION OF THE ORGANISMS

Twenty grams of each sample material (cassava, maize, guinea corn, Irish potato, sweet potato, Sorghum, kolanut, millet) was soaked with distilled water in 250 ml conical flask for 72 h. The steep water was stirred with sterile glass rod to uniformly distribute the organisms. Ten millilitre of each solution was taken using 10 ml sterile pipette to prepare a serial dilution in sterile McCartney bottles. From the stock, 1 ml of the solution was obtained and transferred to another 9.0 ml of distilled water in sterile McCartney bottle to give the 10^{-1} dilution. This procedure continued until the dilution reached 10^{-4}**.** Aliquot (0.1ml) of this was pipetted onto sterile potato dextrose agar (PDA) plate. The inocula were spread using sterile hockey stick (spreader). The plates were prepared in duplicates and incubated at room temperature (28 ± 2°C) for a period of 48 h. A pure culture of each plate was obtained by sub-culturing using streaking technique and stored on PDA slope in McCartney bottles (bottles were stored in refrigerator at 4°C). The above procedure was also applied for the palm wine which had been allowed to ferment for 72 h. Drops of lactic acid were added to each plate before solidification to inhibit the growth of other organisms especially bacteria.

3.3. IDENTIFICATION OF ISOLATES

The isolates that emerged from the pure culture plates were aseptically picked with inoculating needle onto slides and stained with a drop of methylene blue solution and observed under the light microscope. The identities of the organisms were certified based on the morphological and cultural features as well as comparing them with confirmed representatives of different species in relevant texts like Rhode and Hartmann (1980); Alexpoulous *et al*. (1996) and Ellis *et al*. (2007). The authentication was also done by a mycologist in the Department of Botany, University of Lagos. Photomicrographs of the isolates were taken with motic camera 2.0. Some isolates were genetically identified by growing them on malt extract agar slants in 5.0 ml McCartney bottles and sent to the Centre for Agriculture and Bioscience International (CABI) identification service, Royal Botanical Garden Kew, England. These isolates were sequenced according to CABI standard protocols while the DNA extracts of the other isolates were sent in microtubes to Macrogen Incorporated, Maryland, USA for sequencing.

3.4. GRAM STAINING TECHNIQUE

The pure isolates were stained according to the method described by Beveridge (2001**).** A thin smear of an isolate was made on a clean glass slide and heat fixed by flaming with a blue flame. Two drops of crystal violet were added to the smear for 1-2 min. The crystal violet was washed with distilled water and stained with Gram's iodine solution for a min. The iodine solution was carefully poured off. The stain was decolourized by flooding the slide with acetone until no more light colouration was observed. The slide was thoroughly washed with water to remove the acetone. Two

drops of safranin reagent was added to counter stain for two min and was rinsed again with tap water. Excess water was blotted out and the slide was dried in hand over Bunsen flame. Observation was made using oil immersion objective of the light microscope. Gram positive organisms were characterized by purple colouration while Gram negative cells were pinkish in colour. This staining technique also shows the different shapes of the organisms and the arrangement of the cells.

3.5. BIOCHEMICAL ANALYSIS

The biochemical analyses of the isolates were carried out as follows:

3.5.1. GERM TUBE TEST

Fresh cultures of the isolated organisms were introduced into different test-tubes containing blood plasma. These were incubated overnight. The organisms were then placed on slides and observed under the light power microscope. This was done according to a modified method of Tierno and Milstoc (1977).

3.5.2. SUGAR FERMENTATION

During the course of the test, eight samples of sugar were used. These were glucose, maltose, lactose, sucrose, fructose, dextrose, galactose, starch and xylose. The modified method of Olutiola *et al.* (2000) was used.

One percent of each sugar was dissolved in 100 ml of distilled water in a sterilized conical flask. Each flask was cinderlized by placing in a water bath to boot for three consecutive times at 30 min intervals. One percent of peptone was also dissolved into 100 ml of distilled water in a sterile conical flask, a few drops of Bromcresol purple

indicator was added to it. This was then autoclaved for 20 min. Eight millilitres of the prepared peptone solution was pipetted into a sterile test tube containing inverted Durham tube for each of the sugars.

One millilitre of each sugar was aseptically introduced into the peptone solution in the test tubes. Each test tube was replicated. Then pure seed isolates of the yeast from one source was aseptically introduced into one set of test tubes while the second pair of test tubes were left unseeded. The tubes were sealed with non-absorbent cotton wool wrapped in aluminium foil and then incubated at room temperature for four to seven days. This was repeated for the other yeast isolates. Tubes in which Bromocresol purple changed yellow and bubbles observed in the Durham tube indicated growth and acid production respectively. Tubes with no colour change indicated negative results. Uninoculated tubes served as controls.

3.5.3. UREA HYDROLYSIS TEST

The cultures were grown on Sabouraud agar and were transferred to fresh slants before the urea test was done. Fresh cultures from the slants were transferred with a loop to the surface of Christensen urea medium consisting of the following: 0.1 g peptone; 0.1 g glucose; 0.5 g NaCl; 0.2 g KH_2PO_4; 1.5 g agar and 0.012 g phenol red per 1000 ml of distiiled water. The ingredients were mixed and melted in a water bath. After adjusting the pH to 6.8 the medium was dispensed into test tubes in 4.5 ml amounts and autoclaved for 10 min at 121°C. The tubes where allowed to cool to 50 °C. To every tube of the autoclaved medium 0.5 ml of a 20 percent Seitz-filtered solution of urea was added aseptically. After mixing with the base the contents of the tubes were allowed to solidify with a long slant and a deep butt. After inoculation, the tubes were incubated at the optimal temperature (28-30°C) of the organism for 72 h.

Urea hydrolysis was indicated by a distinct colour change of the indicator from a deep pinkish red to an orange-yellow colour starting at the slanted part of the medium and progressing rapidly to the deep part of the butt (Seeliger, 1956).

3.5.4. SENSITIVITY TO CHLORAMPHENICOL

The organisms were tested for sensitivity to chloramphenicol by growing isolates in malt extract agar (MEA) plates in the presence of 30 µg/ml chloramphenicol discs. The plates were incubated at room temperature for 3-5 days and they were observed daily for growth, Kirby *et al*. (1966).

3.6. DETERMINATION OF SPORE COUNT OF YEAST ISOLATES

The total number of yeast cells and viable cells was calculated by the methylene blue method of Lee *et al.* (1981) using a haemocytometer. A drop of spore suspension was placed on the ruled area of a clean Neubauer counting chamber. A cover slip was placed first and the spore suspension was permitted to run underneath by capillary action from Pasteur pipette tip. The counting cell was allowed to stand for 10 min to permit the spores to settle into the same focal plane as much as possible. Using a light microscope, the spores were counted with 10x ocular and 5 mm objective and this was done for the total grid in triplicate. The cell count was calculated thus:

Yeast cells/ml = Number of cells in total grid x dilution factor x 10^{-4}.

3.7. GROWTH STUDY OF THE ISOLATES AT 37°C

Fresh inocula were aseptically transferred from the slant of each isolate onto MEA plates and incubated for 24 h. A drop of the slur of the 24 h old culture of each isolate was placed at the centre of each plate; a cross was drawn with a permanent marker at

the bottom of each plate to indicate the centre as the origin. The plates were incubated at 37°C for 48 h. They were observed daily at 24 h intervals. The growth diameter were measured and recorded. This is as described by Booth (1971).

3.8. GROWTH STUDY OF THE ISOLATES ON DIFFERENT MEDIA

Growth study was carried out according to the modified methods of Ibrahim *et al*. (2002) to determine the best media for the cultivation of the isolates. The following media were used for the purpose of this studies: potato dextrose agar broth (PDAB), malt extract peptone broth (MEPB), carrot dextrose broth (CB), cassava dextrose broth (CDB), crayfish dextrose broth (CrayDB), maize dextrose broth (MDB), millet dextrose broth (MiDB) and sorghum dextrose broth (SDB).

Preparation: Three grams of PDA was measured into 250 ml conical flask while 3 g of malt extract and 2 g peptone were measured into another conical flask. To each flask, 250 ml of distilled water was added and autoclaved at 121°C for 20 min.

Fifty grams each of carrot, cassava, crayfish, maize, millet and sorghum were boiled in 100 ml distilled water respectively in a water bath for one hour. Each was strained with cheese cloth into another 250 ml conical flask and 10 g of dextrose was weigh into each of the flask. The volume made up to 250 ml by adding distilled water and these were autoclaved. Each was aseptically poured into nine test-tubes in duplicates. Each of the eight yeast isolate from pure culture plates was aseptically transferred into each tube with the ninth tube serving as the control. The tubes were incubated at room temperature (28 ± 2°C) for 7 days. They were observed daily at 24 h intervals. The turbidity of the content and optical density were measured in a spectrophotometer and recorded at wavelength 530 nm for each day.

3.9. DNA EXTRACTION FROM YEAST ISOLATES AND ANALYSES

The DNA extraction and analysis was carried out at the Biotechnology Centre of the Federal University of Agriculture, Abeokuta (FUNAAB), Ogun State. Total genomic DNA was extracted with Cetyl Trimethyl Ammonium Bromide (CTAB) buffer as described by Graham *et al.* (1994). This procedure was repeated for each of the samples for 2 days.

3.9.1. Procedure

Some of the cell pellets of each isolate were aseptically picked into Eppendorf tubes from the incubated stock in the McCartney bottles. 500 µl of Cetyl Trimethyl Ammonium Bromide (CTAB) buffer was added. It was later mixed for homogenisation. Afterwards the mixture was incubated at 65°C for 15 min and equilibrated to room temperature. To this, 10 µl of Protenase K solution (an equal volume of a mixture of phenol: chloroform: isoamyl alcohol (25:24:1, v/v) was then added and incubated at 37°C for 30 min. This was done in order to remove the RNA molecules in the samples. On cooling, 500 µl of chloroform was added again to the mixture and mixed for 5 min. The resultant mixture was then centrifuged at 10,000 rpm for 10 min in order to separate each component in the homogenous mixture. The supernatant was collected into a fresh Eppendorf tube without taking the white interphase.

Cold isopropanol (500 µl) was added to the supernatant. The mixture was kept at the temperature of -20°C inside the refrigerator for 1 h. It was later brought out for centrifugation at 10,000 rpm for 10 min in order to separate the mixture. Afterwards

the supernatant was decanted carefully. 500 µl of 70% ethanol was added to the decanted supernatant which was mixed very well to achieve a homogenous solution. This was centrifuged at 10,000 rpm for 10 min and the supernatant was discarded. The pellet left was air dried for 1 h and then re-suspended in 200 µl sterile water.

This procedure was repeated for each of the samples for 2 days. The samples were kept in sterilized microtubes for subsequent DNA sequencing analysis.

3.9.2. Preparation of 1% Agarose Gel

One percent (1%) of Agarose gel was prepared by mixing 1.5 g agarose with 150 ml 1X TBE Buffer. The mixture was boiled in a microwave until all agarose has dissolved. This was allowed to cool down under running cold tap water. 6 µl of Ethidium Bromide was added (this was done in the fumehood) and swirled to mix. The gel was then poured into the tray and allowed to stand for at least 30 min before removing the combs. The gel was then placed in an electrophoresis tank. Five microlitres of loading dye was spotted for 10 µl of each DNA sample on a parafilm paper. Using a pipette, 15 µl of each sample was loaded into each "well" of the prepared 1 % agarose gel; also one-half microgram of 1 kb ladder (Lambda DNA HindIII) was loaded into the "marker" well. It was run for 45 min at 110 Milli Amps. Genomic DNA was visualized in a Gel Documentation System LG 2020 (Hangzhou Langqi, Inco., China) under Ultra violet light source **(Plate 9).**

3.9.3. DNA Sequencing

Isolated yeast samples coded EAM 2, EAM 3, EAM 4 and EAM 5 were sent on MEA slant to CABI (Centre for Agriculture and Bioscience International) Royal Botanical Garden, Kew, England for DNA sequencing of the Internal Transcribed Spacer (ITS) region of the ribosomal DNA segment. The DNA sequencing of the remaining yeasts

(EAM 1, EAM 6, EAM 7 and EAM 8) was done by sending the DNA extracts in microtubes to Macrogen Incorporated Maryland, USA. The sequenced ITS 1 and ITS 4 generated were aligned using clusteral and blasted against Genbank database using the BLAST (Basic Local Alignment Search Tool) program as described by Altschul *et al.* (1990). This was done to find the similarity between sequences. Also for comparison of the yeast genomic DNA sequences with the library database for phylogenetic relationship and identification.

3.10. EXTRACTION FROM ANACARDIUM OCCIDENTALE SHELL

This was done according to the method described by Sofowora (1982). Cashew nuts (*Anacardium occidentale* L.) were bought at the fruit stall of Ketu market in Kosofe Local Government Area of Lagos State. The nuts were cracked using a nut cracker to separate the kernel from the shells. The solvent used was the ethanol distilled from 360 h old palm wine in the Botany Research Laboratory of the University of Lagos (as earlier described in section 3.1.2.). The cashew shells were pounded into bits using a wooden pestle and mortal. Five hundred grams of it was soaked in 600 ml of ethanol in a sterile 1 L conical flask for 72 h. The mixture was filtered through No. 1 Whatman filter paper. The filtrate was concentrated through the rotary evaporator under reduced pressure and controlled temperature of 50°C and the pH was obtained as 3.2 using a pH meter. The acid was stored in opaque glassware for further use.

3.11. STUDY ON ENZYME ACTIVITY FROM PLANT EXTRACT

The study was done according to the modified method of Zaldivar *et al*. (2001). Cellulolytic enzymes production by the plant extract was determined using three carbon sources: carboxymethylcellulose (substitution degree 0.7, Sigma), microcrystalline cellulose and xylan.

3.11.1. β 1, 4-ENDOGLUCANASE ACTIVITY

The β -1,4-endoglucanase activity was determined using carboxymethylcellulose as substrate and the formation of reducing sugars was measured by reaction with 3,5-Dinitrosalicylic acid (DNS). The reaction mixtures containing 10 mg CMC (carboxymethyl cellulose) in 1 ml of 0.05 M sodium acetate buffer (pH 5.0) and 1 ml cashew nut extract were incubated at 50 °C for 30 min. tubes were removed to an ice-bath for sediment to settle. The reducing sugar formed was measured with DNS. Three milliliters (3 ml) of DNS reagent was added to 1ml of the test sample. The colour was developed by boiling the mixture in water bath for 5 min. Absorbance was read at 540 nm using spectrophotometer (UNICO 2100, Germany). Reducing sugar concentration was obtained from a standard glucose concentration curve. This was done in triplicate.

3.11.2. β -1,4-EXOGLUCANASE ACTIVITY

The β-1,4-exoglucanase activity was assayed as above using microcrystalline cellulose (Avicel) as substrate and the formation of reducing sugars was measured by reaction with DNS. The reaction mixtures containing 10 mg of microcrystalline cellulose in 1 ml of 0.05 M sodium acetate buffer (pH 5.0) and 1 ml cashew nut extract were incubated at 50°C for 30 min. The reducing sugar formed was measured with DNS as previously described above.

3.11.3. XYLANASE ACTIVITY

Xylanase activity was determined by measuring the release of reducing sugars from a solution of water soluble birch wood xylan (Fluka BioChemika, 95588) using the DNS method. The reaction mixtures containing 10 mg Xylan (Fluka BioChemika,

95588) in 1 ml of 0.05 M sodium acetate buffer (pH 5.0) and 1 ml cashew nut extract were incubated at 50°C for 30 min. The xylose formed was measured with dinitrosalicyclic acid (DNS) as described above.

3.12. HYDROLYSIS OF SAMPLES

The hydrolysis of substrates with mineral (synthetic) acid was carried out according to a modified method described by Kolachov and Nicholson (1951).

3.12.1. HYDROLYSIS OF SAMPLES WITH H_2SO_4

One hundred grams of cassava flour was weighed into a 2 L sterilized container. Distilled water (1 L) was added to the container to make a slurry. This was done in duplicate. The mixture was cooked until gelatinized and allowed to cool. Fifty millilitres of 50% H_2SO_4 was added into the first container and 50 ml of 40% H_2SO_4 was added to the second container for saccharification of the gelatinized mixtures. These were transferred into seperate 1 L conical flask for each mixture. The flasks were corked with sterile cotton wool wrapped in aluminium foil and then autoclaved at 121°C for 20 min. The flasks were allowed to cool, filtered through muslin cloth and the pH of each was adjusted to between 3.5 and 4.0 with 0.4 M NaOH after returning to the initial containers. The same procedures were repeated for plantain, sweet potato, Irish potato, maize and kola nut. For the sugarcane chaff, cassava peel, plantain peel, sweet potato peel, maize cob and rice husk, 100 ml of 50% H_2SO_4 and 40% H_2SO_4 were used for the hydrolysis. Control containers were set up for each experiment without the addition of mineral acid. All experiments were done in duplicates. The above procedures were repeated for all the substrates using 50% HCl and 40% HCl.

3.12.2. HYDROLYSIS OF SAMPLES WITH CASHEW NUT SHELL EXTRACT (CNSE)

One hundred grams of cassava peel flour was weighed into a 2 L sterilized container. Distilled water (1 L) was added to the flask and the mixtures cooked until gelatinized. This was allowed to cool to about 50°C and 20ml of the CNSE was added to the flask. The flask was allowed to stand for an hour for proper enzymatic reactions. The flask was corked with sterile cotton wool wrapped in aluminium foil and then autoclaved at 121°C for 20 min. The same procedures were repeated for plantain peel, sweet potato peel, sugarcane chaff, maize cob and rice husk. Control flasks were set up for each experiment without the plant acid and each experiment was carried out in duplicate.

3.13. PREPARATION OF BROTH

A modified method of Booth (1971) was used in preparing the broth. Three grams of MEA was dissolved in 250 ml of distilled water in a sterilized conical flask and 2 g of peptone was added to serve as source of nitrogen. The mouth of the flask was plugged with non-absorbent cotton wool wrapped in aluminium foil and tapped round. The flask was gently shaken to avoid air bubbles and then heated in water bath to homogenize the medium. The medium was autoclaved at 121°C for 20 min and then allowed to cool before use. At 55°C, drops of Lactic acid were added to acidify the medium against bacterial growth. Inocula from the PDA plates were carefully transferred into the flask and incubated for 48 h. Several flasks were prepared for each isolate source.

3.14. FERMENTATION

The fermentation was carried out according to a modified method of Oyeleke and Jibrin (2009) by aseptically inoculating isolates from the broth into each flask as described below:

To each of the hydrolysates (mineral acid and plant extract), 20 ml of each broth was added respectively. The palmwine and sugarcane juice were not hydrolyzed but were autoclaved at 121°C for 20 min. Broth was also inoculated into flasks containing 1 L of each substrate. All flasks were also replicated for the palmwine and sugarcane juice. All flasks were incubated at 28-30°C for 72-96 h. The fermentation flasks were shaken periodically with the use of manual agitator.

3.15. FRACTIONAL DISTILLATION

Distillation was done using a heating mantle to heat the flask (round-bottom) containing the fermented broth. The distillate collected over a slow heat at 78°C was measured with the aid of a measuring cylinder, and expressed as the quantity of ethanol produced in g/L by multiplying the volume of distillate collected by the density of ethanol (0.8033 g/ml).

3.16. DETERMINATION OF THE CONCENTRATION ETHANOL PRODUCED

This was done for the distilled fermented hydrolysates from the samples using the procedure of Templeton (1994). Gas Chromatography (HP 6890 powered with HP ChemStation Rev. A) was used. The carrier gas used was Nitrogen. Standard solutions of ethanol, methanol and furfural were prepared. Two to three micro litres of the standards were injected through an injection port with micro syringe. The injector temperature was 250°Cand detector temperature was 370°C. The GC was

programmed at an initial temperature of 60°C which was held for 5 min, then ramped at 10°C/min for 12 min and finally, ramped at 20°C/min for 12°C. Column type and dimension were HP 5 and 30 m by 0.25 mm by 0.25 µm respectively.

3.17. PROXIMATE ANALYSIS AND DETERMINATION OF CELLULOSE CONTENT IN THE AGRO-WASTE

Proximate analyses of the agro-wastes were carried out in accordance with standard methods of FAO (1986). The modified method of Updegraff (1969) was used for cellulose content. The agro-wastes used were cassava peel, maize cob, plantain peel, sweet potato peel, sugarcane chaff and rice husk. Two milligram of each ground sample was weighed into tubes and 100 µl of inositol solution was added. The tubes were capped tightly and incubated for 90 min at 121°C. The tubes were allowed to cool and then centrifuged at 10 000 rpm for 10 min. The supernatant was transferred to glass screwed cap vials by ensuring that the pellet was not disturbed. One millilitre of Updegraff reagent (acetic acid, nitric acid, water, 8:1:2 v/v) was added to the pellets, the tubes were capped, vortexed and heated in a water bath at 100°C for 30 min. The samples were cooled on ice at room temperature and centrifuged at 10 000 rpm for 15 min, the supernatant was discarded. Water (1.5 ml) was added to the pellets, mixed thoroughly, centrifuged and the supernatant discarded. This was repeated using 1.5 ml acetone instead of water. The pellets were carefully air dried. Then 175 µl of 75% H_2SO_4 was added and incubated at room temperature for 30 min. The content was vortexed and incubated for another 15 min. Then 825 µl of water was added, vortexed and centrifuged at 10 000 rpm for 5 min. To 10 µl of the mixture 90 ml of water was added and the glucose content of the supernatant was assayed using colorimetric anthrone assay by adding 200 µl of freshly prepared anthrone reagent.

The mixture was heated on a microtiter plate for 30 min at 80°C in an oven. The plates were cooled to room temperature and shaken thoroughly. The absorbance of the pellets was read at 625 nm using a microtiter plate reader. The glucose was calculated based on absorbance compared to the standard curve prepared with varying concentrations of glucose.

3.18. DETERMINATION OF REDUCING SUGAR IN THE HYDROLYSATES USING 3-5-DINITROSALICYCLIC ACID (DNS) PRINCIPLE

This was carried out according to the method described by Miller (1959). A standard solution was prepared by adding 1.0 ml of DNS reagent to 3.0 ml of sugar solution of known concentration in a test-tube. A blank was prepared by adding 1.0 ml of DNS to 3.0 ml of distilled water in a test-tube and the experiment was prepared by adding 1.0 ml of the DNS reagent to 3.0 ml of each of the supernatant substrates in a test-tube. These were covered with sterile cotton wool wrapped in aluminum foil and placed in a boiling water-bath for 15 min and allowed to cool to room temperature. The absorbance of each was read at 540 nm in a spectrophotometer against the blank. The concentration of each substrate was calculated from the standard curve of the known sugar.

3.19. IDENTIFICATION OF SPECIFIC SIMPLE SUGARS IN THE PLANT EXTRACT HYDROLYSATES

The hydrolysates were analyzed on an HP 6890 Series GC powered with an HP ChemStation Rev. A 09.01 (1206) and a flame ionization detector (FID). Sample (2-3 µl) was injected from slit injector. The carrier gas was hydrogen at the flow rate of 1.0 ml/min. the fractionation was carried out in an isothermal temperature of 210°C. the

injector and detector temperature were 250°C and 325°C respectively. Typical coefficient of correlation for standard curve was 0.95-0.99. Pecks were identified by comparison of retention times with those of standard glucose, xylose, arabinose, maltose, rhamnose, lactose, sucrose, ribose and fructose.

3.20. STATISTICAL ANALYSES

Results were presented in tables as mean ± standard error of mean (MEAN ± SE). The comparisons between the mean values were tested using Duncan Multiple Range Test and the ANOVA was performed to find the level of significance at $P< 0.05$. Tables, figures and line graphs were used for graphical representations according to Parker (1979).

CHAPTER FOUR

4.0 RESULTS

4.1. IDENTIFICATION OF YEAST ISOLATES

The results showed that some yeasts species were associated with 72 h fermentation process of the substrates used in the course of this study (cassava tuber, palm wine, maize seed, kola nut, guinea corn, millet grains, Irish potato and sweet potato tubers). Eight yeast species belonging to five genera which include *Candida*, *Kluyveromyces*, *Pichia*, *Schizosaccharomyces* and *Saccharonyces* were identified as yeasts associated with natural fermentation of crop plants. The isolate from each substrate had different characteristics; most were ovoid-like, almost translucent with smooth demarcated colonies. Some showed bud formation and pseudohyphae emergence while the characteristic yeast odour was evident in all isolate plates. A summary of the cultural characteristics of the isolates and their origin are represented in Table 3. They all exhibited the characteristic creamy-white colouration of yeast-like organisms with slimy growth forms. The sizes of the yeasts were read using a microscope ruler and the length ranged from 1.2 - 15.6 µm while the breadth range is from 1.2 to 6.0 µm. Table 4 depicts a summary of the yeast while the cellular morphology are displayed in Plates 1-8.

TABLE 3: CULTURAL CHARACTERISTICS OF YEAST ISOLATES.

STRAIN CODE	SOURCE	NAME OF ORGANISM	PIGMENTATION	COLONY MORPHOLOGY	CELL LENGTH (µm)	CELL BREADTH (µm)	CELL/ML
EAM 1	Palm wine	*Schizosaccharo -myces pombe*	White	Flat, entire smooth	2.0 –15.6	2.0 – 5.0	$7.1x10^7$
EAM 2	Cassava tuber	*Kluyveromyces marxianus*	Creamy; white	Raised, smooth clustered	5.0 – 10.5	2.0 - 5.0	$3.7x10^7$
EAM 3	Maize	*Pichia caribbica*	Creamy; white	Flat, ovoid	1.2 – 10.8	1.5 – 3.5	$1.3x10^8$
EAM 4	Irish potato	*Candida tropicalis* A	Creamy	Raised, scattered	4.0 – 9.0	3.0 – 4.5	$3.2x10^7$
EAM 5	Kolanut	*Candida tropicalis* B	Creamy	Flat, smooth	1.5 – 2.0	5.0 – 6.0	$1.1x10^8$
EAM 6	Guinea corn	*Saccharomyces cerevisiae* A	White; creamy	Oblong/ Eclipse	2. 0 – 8.0	0.5 – 3.0	$6.1x10^7$
EAM 7	Millet	*Saccharomyces cerevisiae* B	White; creamy	Ovoid	1.2 – 2.0	4.5 – 5.7	$7.6x10^7$
EAM 8	Sweet potato	*Candida krusei*	Creamy	Rough contour	2.0 –12.0	1.5 – 5.0	$5.2x10^7$

TABLE 4: MORPHOLOGICAL CHARACTERISTIC OF THE YEAST ISOLATES ON POTATO DEXTROSE AGAR (PDA)

S/NO	YEAST	CHARACTERISTICS
1.	***Candida krusei***	Colony growth was rather slow forming ramiform pattern. The entire 9 cm Petri dish was not covered. Each branch ending in petal forms at 72 h of growth at 28-30°C. The cells were rather ovoid in shape measuring 2.0 – 5.0 µm in diameter with no pseudohypha.
2.	***Candida tropicalis*** **strain A**	Colonies grew moderate to rapid forming single colonies and covered 9 cm Petri dish in 48 h of growth at 28 - 30°C. The cells form single, creamy, scattered colonies. The cells were elongated to ellipsoidal having compact pseudohyphae and smooth-wall measuring 4.0 -9.0 µm.
3.	***Candida tropicalis*** **strain B**	Colonies grew moderate to rapid forming single colonies and covered 9 cm Petri dish in 48 h of growth at 28 - 30°C. The cells form raised, clustered, creamy, colonies. The cells were ovate to ellipsoidal having compact pseudohyphae with smooth-walled measuring 1.5 -2.0 µm. Under the microscope the

		cells appear to be moving in bunches forming long chains. The colony showed pinkish colouration after a period of two weeks.
4.	***Kluyveromyces marxianus***	Colonies grew moderately slow in culture, without covering a 9 cm Petri dish after 72 h of growth at 28-30°C. It covered part of the surface of the media with cloudy white foams. The colonies mature to form creamy-white clusters. The cells were short ovoid to elongate with some at the point of constriction measuring 5.0 - 10.5 µm in diameter. The cells were produced singly.
5.	***Pichia caribbica***	Colonies grew fast but the 9 cm Petri dish was not covered after 72 h growth. Colonial growth was flat with cloudy white, entire, smooth surface. Cells were ellipsoidal in cluster, measuring 1.2 – 10.8 µm in diameter.
6.	***Saccharomyces cerevisiae* A**	Colonies grew moderately covering a 9 cm Petri dish in about 48 h at 28 - 30°C. They formed whitish colonies which became creamy and slimy as the culture matured. At maturity, the cells showed bipolar budding with bunches of spores in different clusters.

7.	***Saccharomyces cerevisiae* B**	Colonies grew fast and covered 9 cm Petri dish within 48 h at 28-30°C. At first the growth form was translucent white which turned cloudy white in 72 h. The cells are predominantly small without pseudohypha. Spores were somewhat oval at maturity with smooth wall formation and ascosporic cell formation developing.
8.	***Schizosaccharomyces pombe***	In culture, colonies grew rapidly covering a 9 cm Petri dish in about 36 h at 28°C. They formed at first watery almost translucent flat entirely smooth surface. The growth form later became raised with distinct colonies development in a somewhat chain pattern. The colonies appeared whitish. At maturity (in 72 h), the spores were producing constriction showing point of fission. A typical yeast odour was emitted as culture aged. The spores were about 2.0-15.6 x 2.0-5.0 µm in diameter. The cells were oval to round in shape.

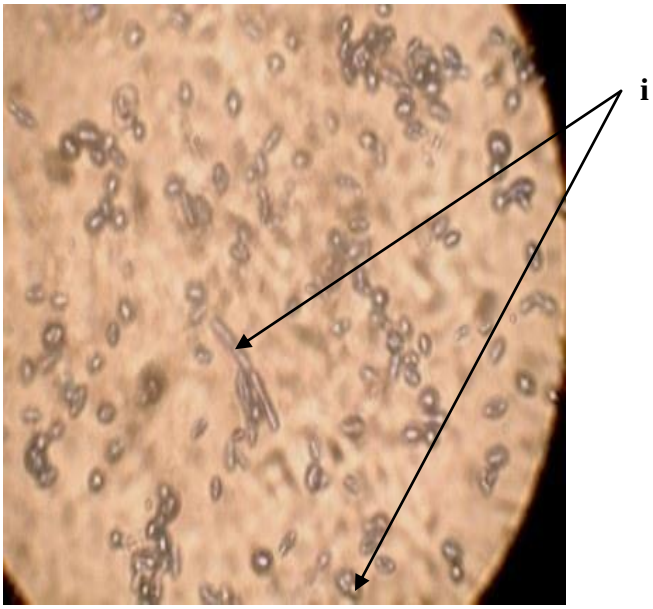


PLATE 1 = Photomicrograph of *Schizosaccharomyces pombe* showing (i) cells at the point of division. (X40)

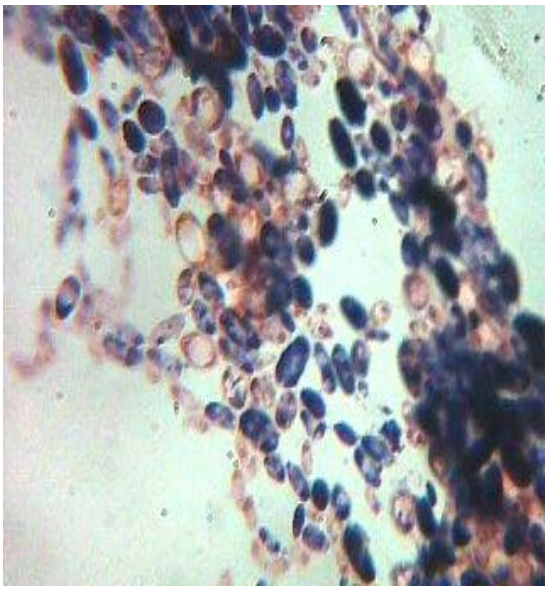


PLATE 2 = Photomicrograph of *Kluyveromyces marxianus* (x40)

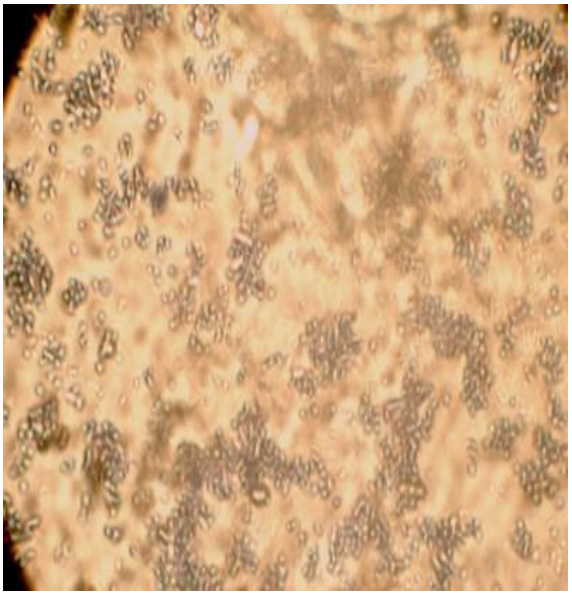


PLATE 3 = Photomicrograph of *Pichia caribbica* (X40)

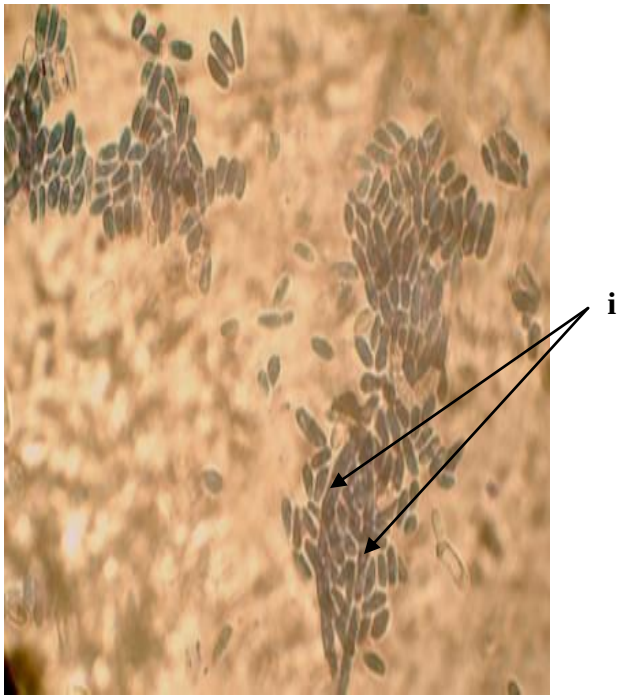


PLATE 4 = Photomicrograph of *Candida tropicalis* strain A showing (i) pseudohyphae (x40)

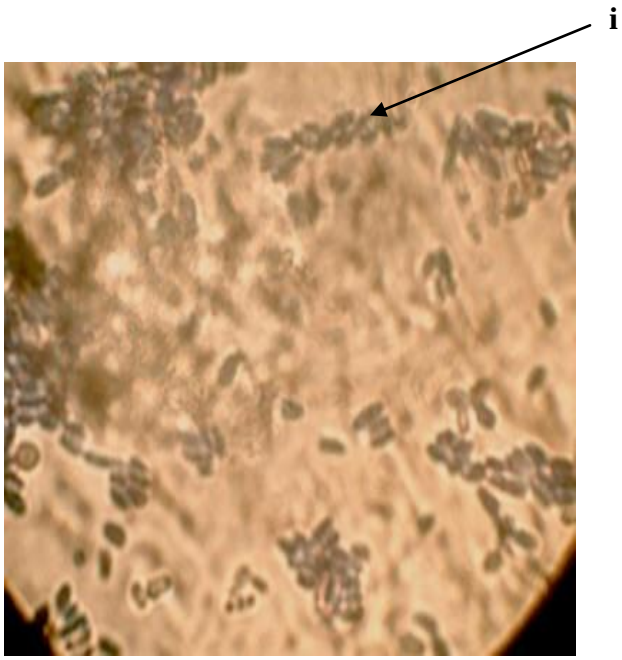


PLATE 5= Photomicrograph of *Candida tropicalis* strain B (i) spores in bunches (X40)

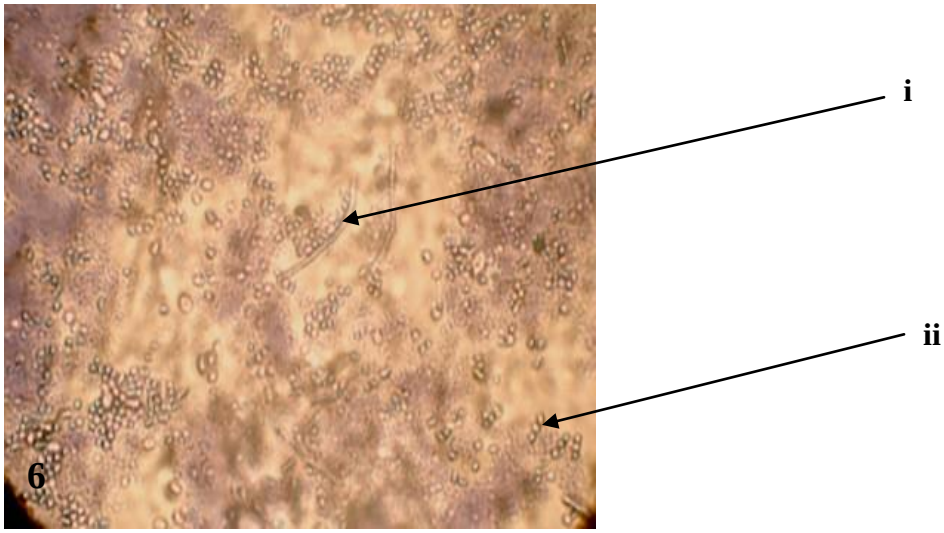


PLATE 6= Photomicrograph of *Saccharomyces cerevisiae* strain A showing (i) spores in chain, (ii) pseudohyphae (x40)

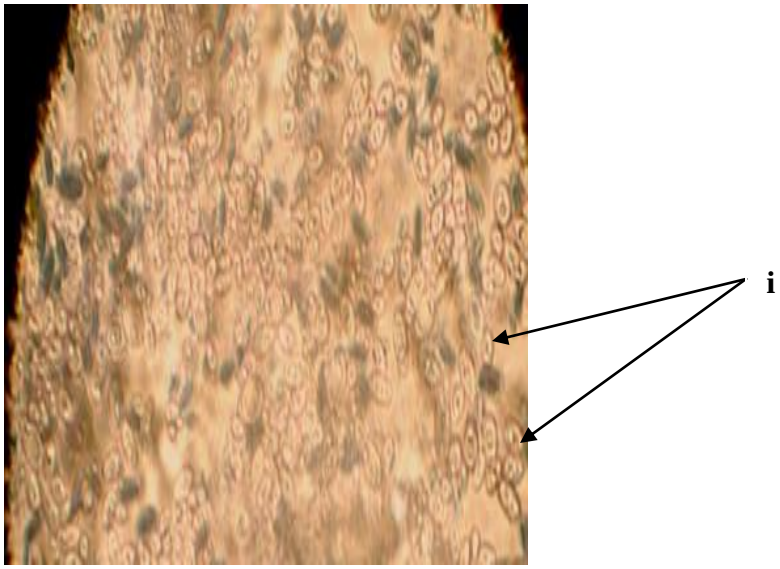


PLATE 7= Photomicrograph of *Saccharomyces cerevisiae* strain B showing (i) ascosporic development (X40)

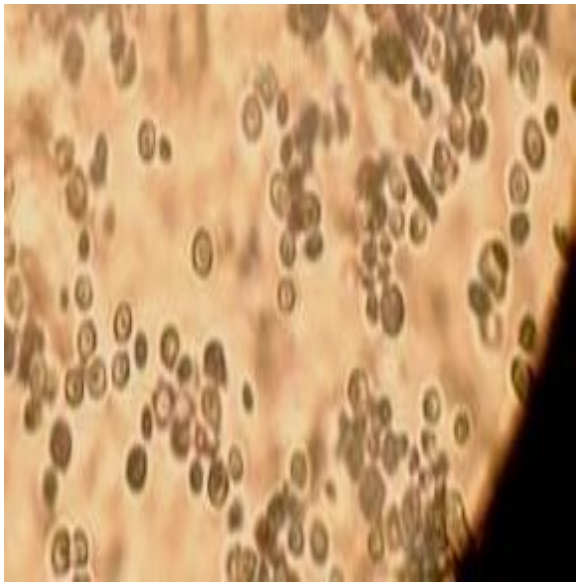


PLATE 8= Photomicrograph of *Candida krusei (*x40)

4.2. BIOCHEMICAL TEST

The isolates were examined based on the type of growth, shape, size, pigmentation and consistency using the Gram staining technique.

4.2.1. GRAM STAIN AND SUGAR FERMENTATION

The yeast isolates fermented the glucose but none fermented the starch. All isolates were able to ferment galactose except the *Candida krusei* (isolate from sweet potato) while the *Schizosaccharomyces pombe* and *Candida krusei* could not ferment lactose. The *Saccharomyces* species (Strains A and B) were only able to assimilate the sucrose sugar but could not ferment it. A colour change from purple to yellow and production of gas in the Durham tube is an indication of fermentation of the substrate which is a positive result while a colour change without 1production of gas is an indication of assimilation which is not a positive result. The control experiment had neither colour change nor gas production. The result of the fermentation test is as shown in Table 5.

4.2.2. EFFECT OF CHLORAMPHENICOL ON YEAST ISOLATES

All isolated organisms showed resistance to chloramphenicol which was indicated by the growth in the presence of the discs. The antibiotic is usually added to fungi growth media to inhibit the growth of Gram positive and Gram negative bacteria. Result is as shown in Table 5.

4.2.3. GROWTH STUDY AT SPECIFIC TEMPERATURE

Result of growth study at 37°C showed that only *Pichia caribbica* and *Saccharomyces cerevisiae* strain B were able to grow successfully after 24 h of incubation. All others except the *Candida krusei* showed significant growth after 36 h. *Candida krusei* did not grow at 37°C after 48 h of

study. The growth of the organisms were observed by drawing a cross at the back of the Petri dishes and the spread of the colonies were measured daily at 24 h intervals for 3 days. The standard error of the mean was obtained. The highest mean growth of 5.53 ± 0.60 cm was by *P. caribbica. Candida tropicalis* strain B (EAM 5) had the lowest mean growth after 48 h of study.

4.2.4. GERM TUBE TEST

The Germ tube test gave negative result for all the isolates as viewed under a high power microscope. After growing the organisms in blood plasma in an aseptic environment for a period of 24 h, spores were aseptically transferred onto a slide and viewed for any shape change. A change in shape from oval to sickle indicates a positive result.

4.2.5. UREA TEST

All the isolates were unable to break the urea at 72 h of incubation except the *Candida krusei* isolate which was indicated by a colour change from pinkish-red to orange-yellow colour. The set up experiment was observed for 4 days. After 72 h of incubation, *S. pombe, P. caribbica* and *Saccharomyces cerevisiae* (strain B) were able to breakdown the urea which was indicated by the colour change. The lack of colour change is an indication of a negative result which means that the yeast strain cannot synthesize urease, an enzyme necessary for the breakdown of urea.

TABLE 5: PHENOTYPIC CHARACTERIZATION OF THE ISOLATES

Biochemical characteristic	ISOLATE							
	EAM 8	EAM 4	EAM 5	EAM 2	EAM 3	EAM 6	EAM 7	EAM 1
	Candida krusei	*C. tropicalis* strain A	*C. tropicalis* strain B	*K. marxianus*	*Pichia caribbica*	*S. cerevisiae* A	*S. cerevisiae* B	*Schizosaccharomyces pombe*
Cell morphology	Ovoid	Ovoid	Ovoid	Elongated	Ellipsoids	Ovoid	Ellipsoids	Round to oval
Gram reaction	+	+	+	+	+	+	+	+
Sucrose	-	**V**	-	+	+	+	**V**	+
Glucose	+	+	+	+	+	+	+	+
Starch	-	-	-	-	-	-	-	-
Lactose	-	+	+	**V**	+	+	+	-
Galactose	-	+	+	+	-	+	+	+
Maltose	-	+	+	--	+	-	-	+
Xylose	-	+	-	-	+	-	+	+
Fructose	-	-	-	+	+	+	+	+
Dextrose	-	+	-	+	-	-	-	-
Urease	-	-	-	-	+	-	+	+
Growth at 37°C	-	+	+	+	+	+	+	+
Resistance to chloramhenicol	+	+	+	+	+	+	+	+

+ = PRESENT - = ABSENT V = Variable

4.3. GENOTYPIC CHARACTERIZATION OF THE YEAST ISOLATES

Cultural and biochemical procedures were utilized to phenotypically characterize the isolates. Genotypic identification was accomplished through the DNA extraction and sequencing the PCR amplified ITS rDNA gene fragment. Genomic DNA of the samples visualized in a Gel Documentation System LG 2020 (Hangzhou Langqi, Inco., China) under Ultra violet light source showed that the extraction protocols were effective (Plate 9).

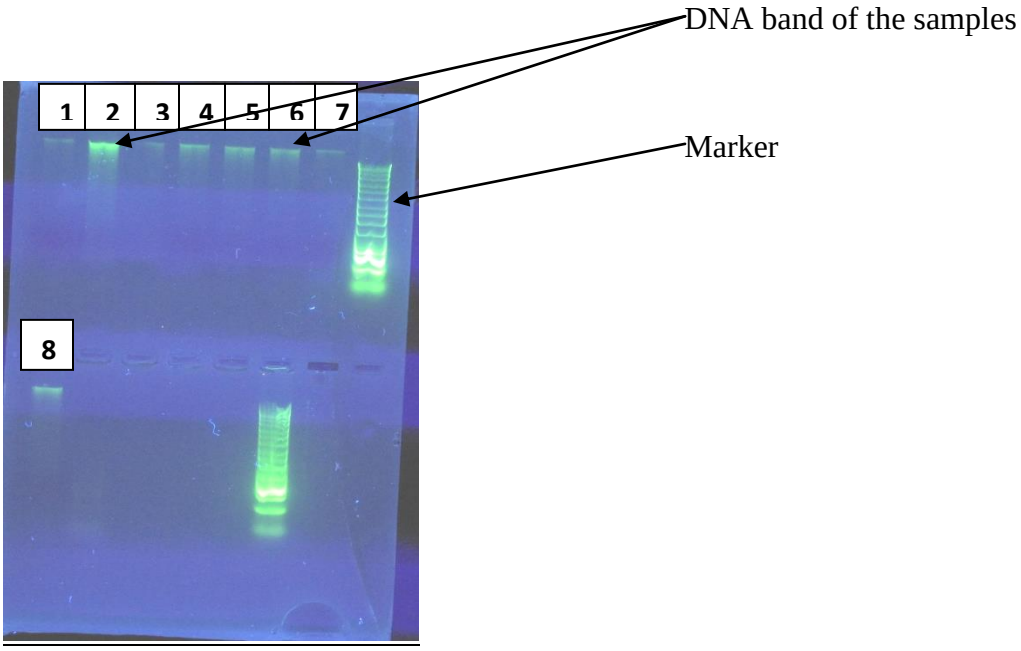


Plate 9: Agarose gel electrophoresis of PCR products with 8 identifying yeast DNA bands viewed under the UV light. Lane 1: EAM1; 2: EAM 2; 3: EAM 3; 4: EAM 4; 5: EAM 5; 6: EAM 6; 7: EAM 7; 8: EAM 8.

The DNA sequence data obtained from some of the isolates (EAM 2, EAM 3, EAM 4 and EAM 5) have been deposited in the database library of the Royal Botanical Garden Kew. The sequence data of the isolate from palm wine (EAM 1) revealed that the isolate belongs to the fission yeast *Schizosaccharomyces.* It is closest to the species *S. pombe* (CU 329671.1) which was isolated from a natural source and deposited in the Genbank database library. It shows 87% homology, which indicates the maximum identification alignment of the isolate with what has been deposited in the Genbank (Table 6).

The blast sequence query showed that *Kluyveromyces marxianus* (IMI 398399) and *Pichia caribbica* (IMI 398400) had the maximum identity (of 100%) with the genomic DNA sequence of EAM 2 and EAM 3 respectively at both ITS.

Isolates EAM 4 and EAM 5 were 100% homologous to *Candida tropicalis* 18S rRNA gene sequence. The isolates were similar in their cultural and cellular characteristics but have slight variation in the biochemical characteristics.

The blast sequence query showed that *Saccharomyces cerevisiae* (GU 931323.1) has the maximum identity (of 88%) with the genomic DNA sequence of EAM 6 and 7 at ITS 1 and ITS 4 sequence with that in the Genbank library database. Biochemical characterization showed some differences between the two isolates. Isolate code EAM 7 fermented xylose and utilized urea while isolate code EAM 6 did not.

The blast sequence query showed that at ITS 1 and ITS 4 no similarity of the EAM 8 genomic DNA sequence was discovered with that in the Genbank library database (htpp://www.ncbi.nlm.nih.gov).

Therefore of all the BLAST query of the eight yeast isolates genomic DNA sequences carried out, it was discovered that there were no similarity with the exact sequence of EAM 8 that was queried with the GenBank library database. This may be attributed to the virtual absence of the DNA sequences in their library database.

TABLE 6: MOLECULAR IDENTITY OF YEAST SPECIES ISOLATED FROM CROP PLANTS

YEAST CODE	Organisms	Origin	% Identification	IMI(KewUK) identification number/accession number
EAM 1	*Schizosaccharomyces pombe*	Palm wine	**87**	**CU 329671.1**
EAM 2	*Kluyveromyces marxianus*	Cassava	**100**	**IMI 398399**
EAM 3	*Pichia caribbica*	Maize	**100**	**IMI 398400**
EAM 4	*Candida tropicalis* strain A	Irish potato	**100**	**IMI 398401**
EAM 5	*Candida tropicalis* strain B	Kolanut	**100**	**IMI 398401**
EAM 6	*Saccharomyces cerevisiae* strain A	Guinea corn	**88**	**GU931323.1**
EAM 7	*Saccharomyces cerevisiae* strain B	Millet	**88**	**GU931323.1**
EAM 8	*Candida krusei*	Sweet potato		_

4.3.1 FASTA SEQUENCES OF THE STUDIED YEASTS GENOMIC DNA

CU329671.1 ITS1 1141bp
TGCGTGCGCGTGCGAATACACCTGCACTAAGTGGATAGGCCCCCTAGTTACT
AGGGCAAATCTACGAGAAAACAAATTAGCCTGTGGCTTGGTGAATTGCGATC
TGTATCTATGAAAAGGTCATTTATGTTTATTATATGAAAGTGATTGTGTTGCA
TCGTTCTATCAGTCGTATCCGGAGTAATATAAACAGAGCATTGTGCCGCAAAT
GAGAGGTTCGAGACTTCTGCGGAGGTCTTTCATGAATCCATTAAAGTTATGG
ACTTGCAAGCTTCACCCCCCTGAACAGGAGGATGCTAGCTGCGAACACTTTT
GTTCTGGGCTTCTAGGGTGCCCAACTCAAGTGTTGCGAGACAACAAATGACT
TGTCCTCAGATTGAGACGATAAATGACGAATAATTCGTTTAACAGTTCTTATC
TAAATAGATTATAATATATGTGATTGTCTATAATTGCTTTTTTCACTTGCTCAC
GCAGAGCTCCTAAATCTGGATGGCGGCTTTAAATCATTTCGATAATAAAAAA
AGAGAGTACTTTCACTTGCAATTGACGAAGAGGCGAAACAGCTTGAATATGC
TTCATCTTGGGAAGAACAAATAATGAAATATTTACTATAATTTCGACGCTGTC
AAAGCTGTCCACAGACAGCGATGCCAAGATATTAAATAAAATAACCTCCTAG
TCCATGTAGTAATTCCGGCAATCCCAAAAATAACACTATATAATAAAAACGC
TGATAATTAAAAATTAAAATAAAATAAAATAAAATAAAGCCTTAAACCGGAG
CCATTATATAACAAAGCGAGACATATTAATCCNACNATATGTAGAATAAGTT
ATAAATAATAAACTAGTATTTCTCTCCTCCTGCGTTAACAAGCGACGCTCAAG
GAAGCAAACAAATGTAAGGAAACACATAAATGATAGGGGGGTTCCGTAACG
ACCTACTGAAAAAAAATCACTTTCCAGGCATTAAAAAAAGTAATCTTTTCAA
TTCAGNGTAACGNTTTCCAAGGGCCCTTTCTCTTGCCGGCTTCTATAAAAGAT
TGTCAAAAAAAACCTTCG

Figure 1: FASTA Sequence data of *Schizosaccharomyces pombe* (87% homology**), CU 329671.1**

IMI398399 ITS 628bp
TGGGGGAATCGTCTGAACAAGGCCTGCGCTTAATTGCGCGGCCAGTTCTTGA
TTCTCTGCTATCAGTTTTCTATTTCTCATCCTAAACACAATGGAGTTTTTTCTC
TATGAACTACTTCCCTGGAGAGCTCGTCTCTCCAGTGGACATAAACACAAAC
AATATTTTGTATTATGAAAAACTATTATACTATAAAATTTAATATTCAAAACT
TTCAACAACGGATCTCTTGGTTCTCGCATCGATGAAGAACGCAGCGAATTGC
GATATGTATTGTGAATTGCAGATTTTCGTGAATCATCAAATCTTTGAACGCAC
ATTGCGCCCTCTGGTATTCCAGGGGGCATGCCTGTTTGAGCGTCATTTCTCTC
TCAAACCTTTGGGTTTGGTAGTGAGTGATACTCGTCTCGGGTTAACTTGAAAG
TGGCTAGCCGTTGCCATCTGCGTGAGCAGGGCTGCGTGTCAAGTCTATGGACT
CGACTCTTGCACATCTACGTCTTAGGTTTGCGCCAATTCGTGGTAAGCTTGGG
TCATAGAGACTCATAGGTGTTATAAAGACTCGCTGGTGTTTGTCTCCTTGAGG
CATACGGCTTTAACCAAAACTCTCAAAGTTTGACCTCAAATCAGGTA

Figure 2: FASTA Sequence data of *Kluyveromyces marxianus* (100% homology), IMI 39899

IMI 398400 ITS 1 535bp

GCGCTTAACTGCGCGGCGAAAAACCTTACACACAGTGTCTTTTTGATACAGA
ACTCTTGCTTTGGTTTGGCCTAGAGATAGGTTGGGCCAGAGGTTTAACAAAAC
ACAATTTAATTATTTTTATTGATAGTCAAATTTTGAATTAATCTTCAAAACTTT
CAACAACGGATCTCTTGGTTCTCGCATCGATGAAGAACGCAGCGAAATGCGA
TAAGTAATATGAATTGCAGATTTTCGTGAATCATCGAATCTTTGAACGCACAT
TGCGCCCTCTGGTATTCCAGAGGGCATGCCTGTTTGAGCGTCATTTCTCTCTC
AAACCCCCGGGTTTGGTATTGAGTGATACTCTTAGTCGAACTAGGCGTTTGCT
TGAAAAGTATTGGCATGGGTAGTACTGGATAGTGCTGTCGACCTCTCAATGT
ATTAGGTTTATCCAACTCGTTGAATGGTGTGGCGGGGTATTTCTGGTATTGTT
GGCCCGGCCTTACAACAACCAAACAAGTTTGACCTCAAATCAGGTAGGAATA
CCCGCTGA

Figure 3: FASTA sequence data of *Pichia caribbica* (100%) IMI 398400

IMI 398401 ITS 469bp

TGATTTGCTTAATTGCACCACATGTGTTTTTTATTGAACAAATTTCTTTGGTGG
CGGGAGCAATCCTACCGCCAGAGGTTATAACTAAACCAAACTTTTTATTTACA
GTCAAACTTGATTTATTATTACAATAGTCAAAACTTTCAACAACGGATCTCTT
GGTTCTCGCATCGATGAAGAACGCAGCGAAATGCGATACGTAATATGAATTG
CAGATATTCGTGAATCATCGAATCTTTGAACGCACATTGCGCCCTTTGGTATT
CCAAAGGGCATGCCTGTTTGAGCGTCATTTCTCCCTCAAACCCCCGGGTTTGG
TGTTGAGCAATACGCTAGGTTTGTTTGAAAGAATTTAACGTGGAAACTTATTT
TAAGCGACTTAGGTTTATCCAAAAACGCTTATTTTGCTAGTGGCCACCACAAT
TTATTTCATAACTTTGACCTCAAATCAGGTAGGACTACCCGCTGA

Figure 4: FASTA sequence of *Candida tropicalis* strain A (100%), IMI 398401

IMI 398401 ITS4 485bp

CACGATTTGAGGTCGAGCTTTTTGTTGTCTCGCAACACTCGCTCTCGGCCGCC
AAGCAGTCCCTGAAAAAAAGTCTAGTTCGCTCGGCCAGCTTCGCTCCCTTTCA
GGCGAGTCGCAGCTCCGACGCTCTTTACACGTCGTCCGCTCCGCTCCCCCAAC
TCTGCGCACGCGCAAGATGGAAACGACGCTCAAACAGGCATGCCCCCCGGA
ATGCCGAGGGGCGCAATGTGCGTTCAAGAACTCGATGATTCACGATGGCTGC
AATTCACACTAGGTATCGCATTTCGCTGCGCTCTTCATCGATGCGAGAACCAA
GAGATCCGTTGTTGAAAGTTTTGTTTGTTTTTCCTAAAATTTTTCTGGCCAACA
ATTGGCTATTTCCCCATTTTAAGGGGTGGTGGTTTCCTTCCCCTTCCCCCATGG
TATAATAAAATCACATAATTAATCCTCCCCAAGGTCCCTTCCGAAACTCAAAA
TTCTGTAATGATCCTTCCGCAAGGTTCACTACGGAA

Figure 5: FASTA sequence data of *Candida tropicalis* strain B (100%), IMI 398401

GU931323.1 ITS1 485bp

GCCTGTTTGAGGTCGAGCTTTTTGTTGTCTCGCAACACTCGCTCTCGGCCGCC
AAGCGTCCCTGAAAAAAAGTCTAGTTCGCTCGGCCAGCTTCGCTCCCTTTCAG
GCGAGTCGCAGCTCCGACGCTCTTTACACGTCGTCCGCTCCGCTCCCCCAACT
CTGCGCACGCGCAAGATGGAAACGACGCTCAAACAGGCATGCCCCCCGGAA
TGCCGAGGGGCGCAATGTGCGTTCAAGAACTCGATGATTCACGATGGCTGCA
ATTCACACTAGGTATCGCATTTCGCTGCGCTCTTCATCGATGCGAGAACCAAG
AGATCCGTTGTTGAAAGTTTTGTTTGTTTTTTCGTAGATTTCTCTTGTCGACTA
TATGCTATATTCCACATTTTAGGTGTTGTTGTTTTCGTTCCGCTCACGCAGTGT
AGTAGTAAATCACAGTAATGATCCTTCCGCAAGGCCCCCCTAAACGAAAG

Figure 6: FASTA sequence data of *S. cerevisiae* strain A (88%), GU931323.1

GU931323.1 ITS1 487bp

CAGATTTGAGGTCGAGCTTTTTGTTGTCTCGCAACACTCGCTCTCGGCCGCCA
AGCGTCCCTGAAAAAAAGTCTAGTTCGCTCGGCCAGCTTCGCTCCCTTTCAGG
CGAGTCGCAGCTCCGACGCTCTTTACACGTCGTCCGCTCCGCTCCCCCAACTC
TGCGCACGCGCAAGATGGAAACGACGCTCAAACAGGCATGCCCCCCGGAAT
GCCGAGGGGCGCAATGTGCGTTCAAGAACTCGATGATTCACGATGGCTGCAA
TTCACACTAGGTATCGCATTTCGCTGCGCTCTTCATCGATGCGAGAACCAAGA
GATCCGTTGTTGAAAGTTTTGTTTGTTTTTTCGTAGATTTCTCTTGTCGACTAT
ATGCTATATTCCACATTTTAGGTGTTGTTGTTTTCGTTCCGCTCACGCAGTGTA
GTAGTAAATCACAGTAATGATCCTTCCGCAACCACCTACGAA

Figure 7: FASTA sequence data of *S. cerevisiae* strain B (88%), GU931323.1

The above sequence data were used to generate a phylogenetic tree using the neighbour-joining method (Figure 8). Three major clusters were revealed in the UPGMA (Unweighted Pair Group Method with Arithmetic) dendrogram in agreement with BLAST analysis. The species in the first cluster belong to the genus *Candida* while the species in the second cluster belong to the genus *Saccharomyces.* The species in the third cluster belongs to the genus *Schizosaccharomyces* (CU 329671.1). The first cluster contains a sub-cluster formed by EAM 2 (IMI 398399) and EAM 3 (IMI 398400) indicating that these species are closely related with a logical variation as they form different telomorphic state of the genus *Candida* and they are somewhat different from species EAM 4 and 5 (IMI 398401). Species EAM 1 (CU329671.1) is distantly related to the others while EAM 6 and EAM 7 are closely related species.

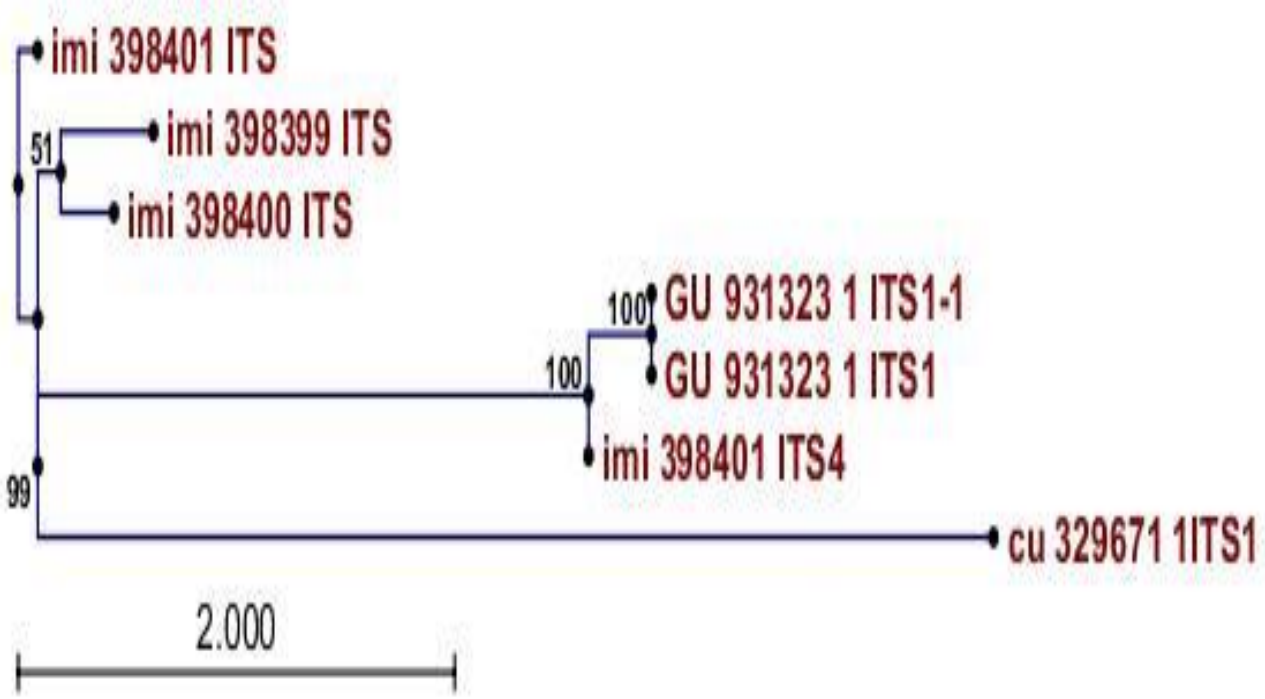


FIGURE 8: Phylogenetic tree of yeast isolates ITS sequences. Sequences were aligned and masked manually in CLCbio 2.1. Trees representing evolutionary relatedness were determined by the Neighbour-Joining/Unweighted Pair Group Method with Arithmetic (UPGMA) version 6.5. The scale bar represents 2.0% substitution per nucleotide position.

4.4. CHEMICAL COMPOSITION OF THE AGRO-WASTE USED

4.4.1. PROXIMATE ANALYSIS

The data representing the chemical composition of the agro-waste are displayed in Table 7. These agro-wastes were selected based on the abundance and availability as their produce are the major common staple tropical food crops in Nigeria.

4.4.2. CELLULOSE CONTENT OF THE AGRO-WASTE

The result from the quant1ification of the cellulose present in the agro-waste showed that the various substance have varying amount of cellulose as summaried in Table 8. Rice husk had the highest amount of cellulose (808 mg/g) while the plantain peel was observed to contain the lowest concentration of cellulose (416 mg/g) amounting to 41.6% (Table 7).

TABLE 7: PROXIMATE COMPOSITION OF AGRO-WASTE

SAMPLES	MOISTURE (%)	ASH (%)	PROTEIN (%)	LIPID (%)	FIBRE (%)	CELLULOSE (**mg/g**)
Cassava peel	14.16	2.25	5.23	7.20	5.10	498
Maize cob	7.71	2.35	3.99	1.20	26.87	734
Plantain peel	11.09	4.57	7.70	4.66	1.81	575
Rice husk	18.33	2.77	4.50	3.19	10.54	416
Sugarcane chaff	11.44	2.42	2.36	1.42	25.73	808
Sweet potato peel	13.04	1.12	7.70	4.66	1.81	542

4.5. REDUCING SUGAR CONCENTRATION IN THE HYDROSYLATES

Test of reducing sugar in the CNSE hydrolysates showed that sugarcane chaff contained the highest amount of reducing sugar of 491 mg/g while rice chaff had the lowest of 46 mg/g. This showed that the lignocellulosic and hemicellulosic substance in the substrates had been broken down into simple sugars which the fermenting organisms can utilize. Result is shown in Figure 9.

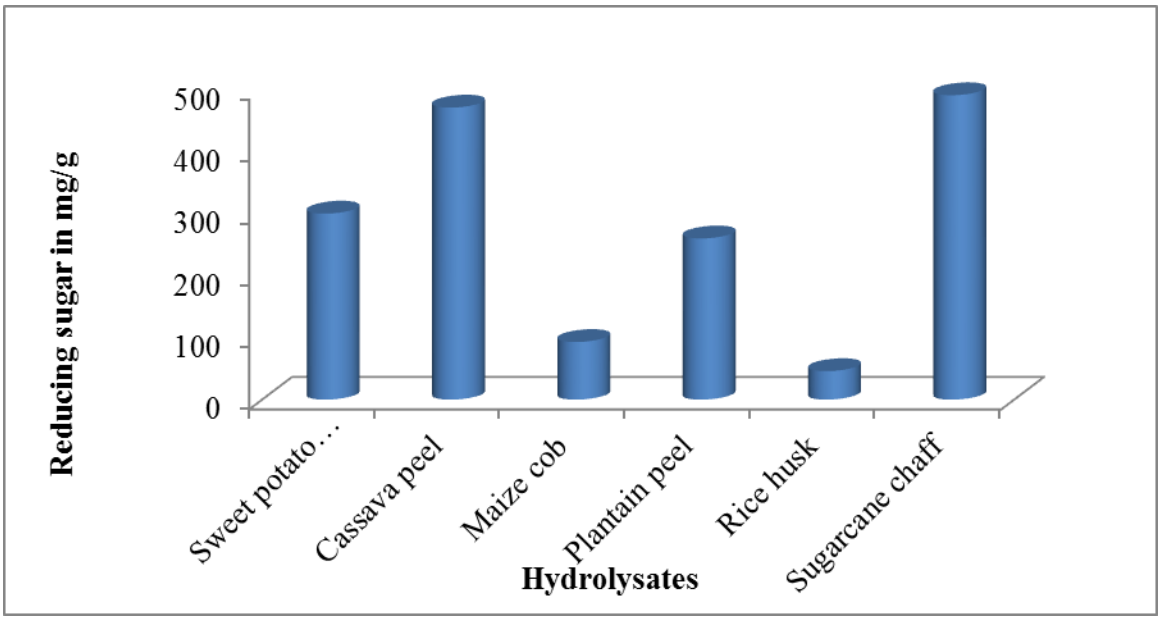


FIGURE 9: AMOUNT OF REDUCING SUGAR IN HYDROLYSED AGRICULTURAL WASTE

4.6. TYPES AND CONCENTRATION OF SUGARS PRESENT IN CASHEW NUT SHELL EXTRACT (CNSE) HYDROLYZED AGRO-WASTE

Plant extract hydrolysates were subjected to Gas Chromatographic analysis in order to obtain the type and concentration of sugars present. The standards used include glucose, fructose, sucrose, arabinose, xylose among others. The correlation co-efficient of each the selected standards is 0.99 g/L. The results showed the presence of the test sugars in varying concentrations. The highest concentration of glucose was obtained in cassava peel while the lowest was in the plantain peel. Sweet potato peel had the highest concentration of sucrose and the highest concentration of fructose as shown in Table 8. Appendix VIA shows the GC profile of the various sugars in the hydrolyzed agro-waste while appendix VIB shows the chromatogram of the mixture of the standard and the calibration curve along with the correlation co-efficient as attached.

TABLE 8: CONCENTRATION OF DIFFERENT SUGAR COMPONENTS OF THE AGRICULTURAL WASTE (PLANT EXTRACT HYDROLYSATES)

Substrate	Ribose	Xylose	Arabinose	Rhamnose	Fructose	Glucose	Maltose	Lactose	Sucrose
	mg/g								
Cassava peel	1.10×10^{-4}	3.93	5.38×10^{-5}	4.87	1.21×10^{-5}	38.19	5.68×10^{-5}	5.13×10^{-5}	1.10×10^{-2}
Maize cob	2.06×10^{-4}	3.20	3.80	3.55×10^{-5}	11.42	7.27	8.05	9.96	4.25×10^{-5}
Plantain peel	2.06×10^{-4}	1.28×10^{-4}	2.06×10^{-4}	3.55×10^{-5}	7.09	5.44	2.07×10^{-5}	1.67×10^{-4}	14.02
Rice husk	5.19×10^{-1}	2.57×10^{-1}	1.17	3.55×10^{-5}	4.95	11.60	5.68×10^{-5}	5.17×10^{-5}	6.67×10^{-1}
Sweet potato peel	2.06×10^{-4}	1.28×10^{-4}	2.07×10^{-4}	5.55×10^{-5}	12.68	15.70	2.07×10^{-5}	1.67×10^{-4}	23.76

4.7. BIOETHANOL PRODUCTION

The results obtained from the Analysis of variance (Anova test) and the Duncan Multiple Range Test at $P< 0.05$ showed that there were considerable differences in the amount of bioethanol produced.

4.7.1. AMOUNT OF BIOETHANOL FROM HCl HYDROLYSATES

From the results on Tables 9-14, the mean amount of bioethanol obtained ranged from 01.81±1.81 g/L to 36.56 ± 3.62 g/L. The H_2SO_4 and HCl hydrolysates yielded bioethanol with all the yeast strains tested. All control experiment did not produced bioethanol after fermentation and distillation. From the hydrolyzed substrates, sweet potato produced the highest amount of ethanol while rice husk produced the least. Bioethanol production varied with all the substrates and yeast strains used.

Tables 9 and 10 show the amount of bioethanol produced from HCl hydrolysates. The 40% HCl could not hydrolyze agro-waste such as sweet potato peels and cassava peels. It also produced low amounts of ethanol from all substrates compared with the 50% HCl hydrolysates; and was unable to hydrolyze Irish potato and maize cob. The highest amount of bioethanol from the crop plant in the HCl hydrolysate was obtained from sweet potato (33.34 ± 2.81 g/L) while the highest amount from the agricultural wastes was from plantain peel (26.31± 1.41 g/L). Significant difference ($P<0.05$) was observed in the sweet potato tuber fermented with *S. pombe* and in sugarcane chaff fermented with *P. caribbica* compared with all other substrates fermented with the same organism in the 50% HCl hydrolysates respectively (Table 9). The sweet potato and maize fermented with *S. pombe* and *K. marxianus* respectively together with the plantain peel fermented

with *Candida tropicalis* (strain A) showed significant differences in the 40% HCl hydrolysates at 5% confidence limit.

TABLE 9: AMOUNT OF BIOETHANOL FROM SUBSTRATE HYDROLYSED WITH 50% HCl

	S. pombe	***K. marxianus***	***P. caribbica***	***C. tropicalis* strain A**	***C. tropicalis* strain B**	***Saccharomyces cerevisiae* strain A**	***S. cerevisiae* strain B**	***Candida krusei***
Substrate	g/L							
Plantain	19.48±1.00^{D}	11.85±1.00^{BC}	25.31±2.82^{EF}	14.06±1.21^{CD}	30.53±3.62^{A}	15.67±1.21^{E}	18.48±3.22^{C}	19.68±7.23^{E}
Sweet potato	33.34±2.81^{E}	12.25±5.42^{BC}	18.08±2.82^{CDE}	11.65±2.01^{CD}	28.11±3.21^{A}	9.84±0.20^{C}	12.25±2.61^{B}	6.03±1.21^{AB}
Maize	20.69±2.21^{D}	21.89±7.03^{D}	24.50±3.21^{EF}	15.47±1.41^{D}	28.71±3.82^{A}	14.46±1.61^{DE}	28.12±2.41^{D}	7.43±1.81^{ABCD}
Irish potato	00.00±0.00^{A}	00.00±0.00^{A}	00.00±0.00^{A}	00.00±0.00^{A}	00.00±0.00^{A}	00.00±0.00^{A}	00.00±0.00^{A}	00.00±0.00^{A}
Cassava	08.84±4.01^{B}	15.27±1.61^{CD}	18.88±2.41^{CDEF}	11.85±3.82^{CD}	7.63±1.61^{AB}	19.08±1.81^{F}	10.44±0.40^{B}	11.65±3.21^{BCDE}
Kola nut	13.26±0.40^{B}	9.04±1.80^{ABC}	14.06±1.61^{CD}	6.63±1.41^{ABC}	6.63±0.61^{AB}	4.42±0.40^{B}	8.64±0.21^{B}	6.83±1.65^{ABC}
Cassava peel	00.61±0.61^{A}	7.83±0.20^{ABC}	4.62±1.00^{AB}	00.00±0.00^{A}	00.00±0.00^{A}	5.63±0.81^{B}	1.81±1.81^{A}	6.63±0.65^{ABC}
Sugarcane chaff	15.06±2.21^{CD}	3.81±1.41^{AB}	26.31±1.41^{F}	17.07±4.62^{D}	9.84±3.01^{B}	9.84±1.00^{C}	19.07±1.80^{C}	7.84±3.41^{BCD}
Sweet potato peel	00.00±0.00^{A}	00.00±0.00^{A}	11.25±4.55^{BC}	7.64±3.21^{ABC}	10.45±0.81^{B}	4.02±0.81^{B}	23.10±1.40^{CD}	7.43±1.41^{BCD}
Rice husk	00.00±0.00^{A}	00.00±0.00^{A}	5.83±1.41^{AB}	4.02±1.61^{AB}	.82±1.61^{AB}	11.45±0.60^{CD}	00.00±0.00^{A}	16.47±2.02^{CDE}
Plantain peel	19.28±1.61^{D}	11.25±0.81^{BC}	20.69±4.22^{DEF}	11.25±1.61^{BCD}	13.26±4.02^{B}	15.27±1.61^{E}	23.50±1.00^{CD}	17.07±4.22^{DE}
Maize cob	00.00±0.00^{A}	00.00±0.00^{A}	00.00±0.00^{A}	00.00±0.00^{A}	00.00±0.00^{A}	00.00±0.00^{A}	00.00±0.00^{A}	00.00±0.00^{A}

Values are expressed as mean ± SE. Mean with the same letter within a column are not significantly different at P<0.05

TABLE 10: AMOUNT OF BIO ETHANOL FROM SUBSTRATE HYDROLYSED WITH 40% HCl

Yeast	***S. pombe***	***K. marxianus***	***Pichia caribbica***	***C. tropicalis strain A***	***C. tropicalis* strain B**	***Saccharomyces cerevisiae* A**	***Saccharomyces cerevisiae*** B	***Candida krusei***
Substrate				**g/L**				
Plantain	16.47±2.01^{DEF}	12.46±1.21^{C}	17.48±1.01^{DE}	6.83±1.61^{CD}	19.88±2.61^{C}	18.68±2.21^{C}	9.44±0.60^{B}	18.88±2.41^{C}
Sweet potato	21.49±341^{F}	10.64±2.21^{BC}	23.30±2.41^{E}	6.83±1.21^{CD}	8.44±1.21^{ABC}	5.63±1.61^{B}	9.24±0.81^{B}	9.24±0.00^{BC}
Maize	11.85±0.60^{BCD}	25.90±3.42^{D}	16.87±0.80^{DE}	9.44±0.60^{DE}	15.07±13.46^{BC}	6.03±0.81^{B}	18.08±4.41^{C}	16.67±0.20^{C}
Irish potato	0.00±0.00^{A}	0.00±0.00^{A}	0.00±0.00^{A}	0.00±0.00^{A}	0.00±0.00^{A}	0.00±0.00^{A}	0.00±0.00^{A}	0.00±0.00^{A}
Cassava	7.43±1.00^{B}	15.87±0.61^{C}	6.43±06.43^{AB}	12.65±0.60^{F}	9.24±0.00^{ABC}	18.48±1.21 ^{C}	6.83±0.40^{B}	16.07±6.43^{C}
Kola nut	20.88±4.42^{EF}	10.84±0.00^{BC}	15.27±1.61^{CD}	6.23±1.41^{C}	4.22±1.01^{AB}	7.43±0.60^{B}	18.48±0.41^{C}	14.87±1.20^{B}
Cassava peel	0.00±0.00^{A}	0.00±0.00^{A}	0.00±0.00^{A}	0.00±0.00^{A}	0.00±0.00^{A}	0.00±0.00^{A}	0.00±0.00^{A}	0.00±0.00^{A}
Sugarcane chaff	15.27±0.80^{CDE}	13.46±3.02^{C}	10.44±2.41^{BCD}	12.05±0.80^{EF}	4.62±1.41 ^{AB}	4.62±0.60^{B}	9.84±1.41^{B}	8.64±1.41^{B}
Sweet potato peel	0.00±0.00^{A}	0.00±0.00^{A}	0.00±0.00^{A}	0.00±0.00^{A}	0.00±0.00^{A}	0.00±0.00^{A}	0.00±0.00^{A}	0.00±0.00^{A}
Rice husk	11.85±0.60^{BCD}	6.23±1.01^{B}	9.24±0.40^{BC}	3.22±0.81^{B}	4.62±1.00^{AB}	15.87±0.21^{C}	17.68±0.81^{C}	9.04±1.01^{B}
Plantain peel	9.84±1.41 ^{BC}	6.83±1.61 ^{B}	13.86±1.41^{CD}	13.26±1.21^{F}	11.05±1.40^{ABC}	17.27±0.40 ^{C}	19.28±0.80^{C}	18.49±0.82^{C}
Maize cob	0.00±0.00^{A}	0.00±0.00^{A}	0.00±0.00^{A}	0.00±0.00^{A}	0.00±0.00^{A}	0.00±0.00^{A}	0.00±0.00^{A}	0.00±0.00^{A}

Values are expressed as mean ± SE. mean with the same letter within a column are not significantly different at P<0.05

4.7.2. AMOUNT OF BIOETHANOL FROM H_2SO_4 HYDROLYSATES

The H_2SO_4 hydrolysates were able to yield bioethanol from all substrates used with the exception of kola nut. The highest amount of 28.12±1.61g/l bioethanol was from sweet potato and the lowest amount of 02.01±0.40 g/l was obtained from rice husk. It was observed that the 50% H_2SO_4 showed greater yield than the 40% H_2SO_4 (Tables 11 and 12). Sweet potato fermented with *S. pombe* and the cassava tuber fermented with *C. tropicalis* (strain A) had significant differences ($P<0.05$) above all others in the 50% H_2SO_4 as well as the plantain fermented with *C. tropicalis* (strain B) and *Candida krusei.* From Table 12, cassava peels fermented with *S. pombe* and *Saccharomyces cerevisiae* (strain A) gave significant difference ($P<0.05$) above other substrates with the exception of plantain peel fermented with *C. tropicalis* (strain B) while the cassava tuber fermented with *Kluyveromyces marxianus* had significance difference on other substrates in the 40% H_2SO_4.

TABLE 11: AMOUNT OF BIOETHANOL FROM SUBSTRATE HYDROLYSED WITH 50% H_2SO_4

	S. pombe	*K. marxianus*	*Pichia caribbica*	*Candida tropicalis* **A**	*Candida tropicalis* **B**	*Saccharomyces cerevisiae* **A**	*Saccharomyces cerevisiae* **B**	*Candida krusei*
Substrate				g/L				
Plantain	21.69±5.22^{E}	13.06±0.61^{EF}	23.70±0.80^{G}	15.47±1.41^{DE}	20.89±3.62^{F}	14.46±1.61^{BC}	11.25±0.81^{BC}	19.69±1.21^{G}
Sweet potato	28.12±1.61^{F}	16.07±0.81^{FG}	21.49±1.01^{G}	7.03±1.01^{BC}	6.83±1.61^{BC}	12.05±0.80^{B}	13.46±3.41^{C}	7.63±0.40^{CD}
Maize	16.27±0.20^{CDE}	11.64±0.81^{CDE}	12.85±0.40^{D}	20.29±5.43^{EF}	9.64±1.21^{CD}	17.88±0.60^{C}	22.09±0.40^{D}	12.65±3.01^{F}
Irish potato	13.06±0.61^{BCD}	17.47±3.82^{G}	9.04±0.61^{B}	17.68±0.81^{DEF}	12.86±0.81^{DE}	2.41±2.41^{A}	11.25±0.81^{BC}	12.65±3.01^{DEF}
Cassava	10.04±0.40^{B}	12.66±1.01^{DEF}	15.87±0.61^{EF}	22.69±2.61^{F}	9.44±1.81^{CD}	23.70±1.21^{D}	23.51±4.20^{D}	10.85±1.21^{CD}
Kola nut	0.00±0.00^{A}	0.00±0.00^{A}	0.00±0.00^{A}	0.00±0.00^{A}	0.00±0.00^{A}	0.00±0.00^{A}	0.00±0.00^{A}	0.00±0.00^{A}
Cassava peel	9.64±08 ^{B}	8.44±1.21^{BCD}	11.85±1.00^{CD}	12.45±2.01^{CD}	2.81±2.81^{AB}	13.66±2.82^{BC}	15.87±1.41^{C}	8.64±0.61^{DE}
Sugarcane chaff	17.27±0.80 ^{DE}	4.42±1.21^{B}	13.26±0.81^{DE}	16.27±0.20^{DEF}	7.03±1.01^{BC}	13.26±2.01^{BC}	10.25±1.01^{BC}	8.44±0.41^{DE}
Sweet potato peel	12.66±1.01^{BCD}	10.44±0.40^{CDE}	16.67±0.60^{F}	11.45±1.01^{CD}	6.23±1.01^{BC}	12.05±0.80^{B}	11.45±1.81^{BC}	4.42±0.40^{BC}
Rice husk	0.00±0.00^{A}	0.00±0.00^{A}	0.00±0.00^{A}	4.02±0.61^{AB}	0.00 ±0.00^{A}	0.00 ±0.00^{A}	7.03±1.41^{B}	2.01±0.40^{AB}
Plantain peel	10.65±1.41 ^{BC}	9.24±0.81^{CDE}	9.84±1.41^{BC}	15.67±1.21^{DE}	16.27±0.60^{EF}	14.66±1.00^{BC}	15.66±1.21^{C}	12.05±0.80^{EF}
Maize cob	10.05±1.21^{B}	7.83±0.20^{BC}	8.44±1.61^{B}	7.43±1.00^{BC}	11.45±1.01^{CDE}	13.46±1.01^{BC}	6.43± 0.00 ^{B}	9.44±1.01^{DEF}

Values are expressed as Mean ± SE. mean with the same letter within a column are not significantly different at P<0.05

TABLE 12: BIOETHANOL YIELD FROM SUBSTRATE HYDROLYSED WITH 40% H_2SO_4

	S. pombe	***K. marxianus***	***Pichia caribbica***	***Candida tropicalis* A**	***Candida tropicalis* B**	***Saccharomyces cerevisiae* A**	***Saccharomyces cerevisiae* B**	***Candida krusei***
Substrate				g/L				
Plantain	14.46 ± 1.61^{E}	5.22 ± 0.40^{B}	16.07 ± 0.81^{F}	9.44 ± 1.01^{FGH}	12.86 ± 0.81^{E}	15.67 ± 0.81^{E}	11.85 ± 0.60^{ABCD}	14.66 ± 1.81^{F}
Sweet potato	13.05 ± 2.61^{DE}	11.05 ± 1.01^{DE}	11.85 ± 0.60^{DE}	3.82 ± 0.61^{BCD}	4.22 ± 1.01^{BC}	9.44 ± 0.20^{CD}	2.41 ± 2.41^{AB}	8.83 ± 0.41^{DE}
Maize	12.05 ± 0.80^{CDE}	11.25 ± 0.81^{DE}	17.68 ± 0.81^{F}	16.87 ± 0.80^{I}	12.46 ± 1.21^{E}	16.67 ± 0.20^{EF}	11.65 ± 1.21^{ABCD}	11.65 ± 0.40^{EF}
Irish potato	6.23 ± 1.01^{B}	9.64 ± 0.40^{CD}	18.49 ± 0.82^{F}	15.27 ± 1.21^{I}	8.24 ± 1.01^{D}	7.83 ± 0.60^{BCD}	7.03 ± 1.41^{ABC}	0.00 ± 0.00^{A}
Cassava	8.03 ± 2.81^{BC}	22.10 ± 1.21^{F}	12.45 ± 0.80^{E}	10.45 ± 0.81^{GH}	5.23 ± 1.21^{BCD}	17.89 ± 1.41^{EF}	21.29 ± 3.62^{D}	14.46 ± 2.01^{F}
Kola nut	0.00 ± 0.00^{A}	0.00 ± 0.00^{A}	0.00 ± 0.00^{A}	0.00 ± 0.00^{A}	0.00 ± 0.00^{A}	0.00 ± 0.00^{A}	0.00 ± 0.00^{A}	0.00 ± 0.00^{A}
Cassava peel	20.90 ± 1.60^{F}	4.62 ± 0.60^{B}	11.65 ± 1.21^{DE}	6.63 ± 1.81^{DEF}	2.41 ± 2.41^{AB}	18.09 ± 1.21^{F}	15.67 ± 0.81^{CD}	6.23 ± 1.01^{CD}
Sugarcane chaff	6.84 ± 1.21^{B}	8.64 ± 1.41^{CD}	7.83 ± 0.60^{BC}	11.85 ± 0.60^{H}	8.44 ± 1.21^{D}	7.23 ± 0.80^{BC}	11.65 ± 11.65^{ABCD}	0.00 ± 0.00^{A}
Sweet potato peel	9.04 ± 0.61^{BCD}	6.83 ± 1.61^{BC}	18.28 ± 0.21^{F}	2.21 ± 1.00^{AB}	4.42 ± 0.80^{BC}	9.84 ± 0.60^{D}	6.63 ± 0.61^{ABC}	5.43 ± 1.01^{BC}
Rice husk	8.84 ± 0.41^{BCD}	0.00 ± 0.00^{A}	12.05 ± 1.61^{DE}	5.63 ± 0.81^{CDE}	5.83 ± 0.61^{BCD}	0.00 ± 0.00^{A}	5.22 ± 0.40^{ABC}	3.02 ± 0.60^{AB}
Plantain peel	15.87 ± 1.01^{E}	13.66 ± 0.41^{E}	6.03 ± 0.41^{B}	3.02 ± 1.41^{ABC}	17.88 ± 0.60^{F}	7.63 ± 0.80^{BCD}	14.26 ± 0.20^{BCD}	10.24 ± 0.60^{E}
Maize cob	07.43 ± 1.00^{BC}	09.04 ± 0.61^{CD}	9.64 ± 0.40^{CD}	7.43 ± 1.00^{EFG}	7.03 ± 1.01^{CD}	6.03 ± 0.41^{B}	8.64 ± 0.61^{ABC}	2.62 ± 1.01^{AB}

Values are expressed as Mean ± SE. mean with the same letter within a column are not significantly different at P<0.05

4.7.3. AMOUNT OF BIOETHANOL FROM CASHEW NUT SHELL EXTRACT (CNSE) HYDROLYSATES

The CNSE hydrolysates showed that plantain peel fermented with *S. pombe* produced the highest amount of bioethanol while maize cob fermented with *K. marxianus* produced the lowest amount of bioethanol (Table 13). The plantain peel fermented with *S. pombe and C. tropicalis* (strain A) as well as sweet potato peel fermented with *C. tropicalis* (strain A) and maize cob fermented with *Pichia caribbica* showed significant difference ($P<0.05$) against all other substrates.

Besides cassava peel and rice husk having low mean of 1.81±1.81 g/L and 2.01±0.40 g/L respectively, all other substrates tested had mean yield above 2.5 g/L. From the unhydrolyzed substrates which already contain fermentable sugars (palm wine and sugarcane juice), the highest amount of bioethanol was produced from sugarcane juice fermented with *S. pombe* compared with the palm wine (Table14)

Generally, the crop plant substrates produced higher amount of bioethanol than the agricultural waste which had the highest yield of 28.12±1.61 g/L from plantain peel. All substrates were fermented at pH 3.5–4.0 and distillation was done at 78-80°C. The distillation equipment and some of the agricultural waste used in the course of this study are as shown in the Appendix.

The results also showed that the yeast strains have varied fermentation activities. The *Candida tropicalis* strain A, *S. pombe* and *Pichia caribbica* had the highest fermenting activities while the *Candida krusei* had the least fermentation rate on all substrates.

TABLE 13: AMOUNT OF BIOETHANOL FROM SUBSTRATE HYDROLYSED WITH CNSE

	S. pombe	***K. marxianus***	***Pichia caribbica***	***C. tropicalis* Strain A**	***C. tropicalis* strain B**	***Saccharomyces cerevisiae* A**	***Saccharomyces cerevisiae* B**	***Candida krusei***
Substrate				g/L				
Cassava peel	15.67±1.21^{C}	00.00± 0.00^{A}	16.27±1.01$^{B\,C}$	17.47±0.60^{BC}	06.62±0.60^{C}	17.07±0.20^{D}	12.46±1.21^{B}	00.00±0.00^{A}
Sugarcane chaff	00.00±0.00^{A}	11.65±1.21^{B}	20.70±1.40^{C}	20.10±0.80^{C}	18.48±0.41^{D}	00.00 ± 0.00^{A}	00.00± 0.00^{A}	00.00±0.00^{B}
Sweet potato peel	18.08±0.41^{C}	16.47±1.21^{B}	09.04±0.61^{A}	23.90±0.60^{D}	00.00 ± 0.00^{A}	14.86±2.01^{D}	12.05±0.80^{B}	00.00±0.00^{C}
Rice husk	07.43±0.60^{B}	03.22±3.22^{A}	11.25±0.81$^{A\,B}$	10.65±1.01^{A}	00.00 ± 0.00^{A}	10.65±1.01^{C}	00.00 ± 0.00^{A}	00.00±0.00^{D}
Plantain peel	28.12±1.61^{D}	16.27±1.01^{B}	10.85±1.21$^{A\,B}$	23.90±1.01^{D}	00.00 ± 0.00^{A}	07.83±0.60^{BC}	11.05±0.21^{B}	00.00± 0.00^{E}
Maize cob	05.02±0.60^{B}	02.62±2.61^{A}	26.35±3.80^{D}	16.47±0.40^{B}	05.22±0.40^{B}	05.42±0.60^{B}	12.85±0.00^{B}	00.00± 0.00^{F}

Values are expressed as Mean ± SE. mean with the same letter within a column are not significantly different at P<0.05

SE = STANDARD ERROR OF MEAN

TABLE 14: AMOUNT OF BIOETHANOL OBTAINED FROM UNHYDROLYSED SAMPLES (Mean ± SEM) g/L

Substrate	Sugarcane	Palm wine
Candida krusei	06.22 ± 1.01	20.10 ±0.80
Candida tropicalis A	27.32 ± 1.21	08.64 ± 0.61
Candida tropicalis B	08.84 ± 0.61	24.30 ± 2.61
K. marxianus	20.69 ± 1.01	26.91 ± 1.21
Pichia caribbica	23.90 ± 0.60	26.11 ± 3.22
Saccharomyces cerevisiae Strain A	21.09 ± 0.20	17.89 ± 1.41
Saccharomyces cerevisiae Strain B	10.25 ± 1.01	24.10 ± 2.41
Schizosaccharomyces pombe	36.56 ± 3.62	29.32 ± 2.81

4.8. ENYZME ASSAY OF CASHEW NUT SHELL EXTRACT

The substance extracted from empty cashew nut shell used in the course of this study had a pH of 3.2. The experiment for enzyme assay was performed in triplicates and the values are presented as Mean ± SEM of the triplicate results. The endoglucanase (CMCase) is expressed in terms of units. One unit is the amount of enzyme releasing 1 µmole of reducing sugar from carboxymethyl cellulose per minute. Exoglucanase is also expressed in terms of units. One unit is the amount of enzyme releasing one µmole of reducing sugar from microcrystalline cellulose per minute and one unit of xylanase activity is the amount of enzyme liberating one µmole of xylose from xylan per minute.

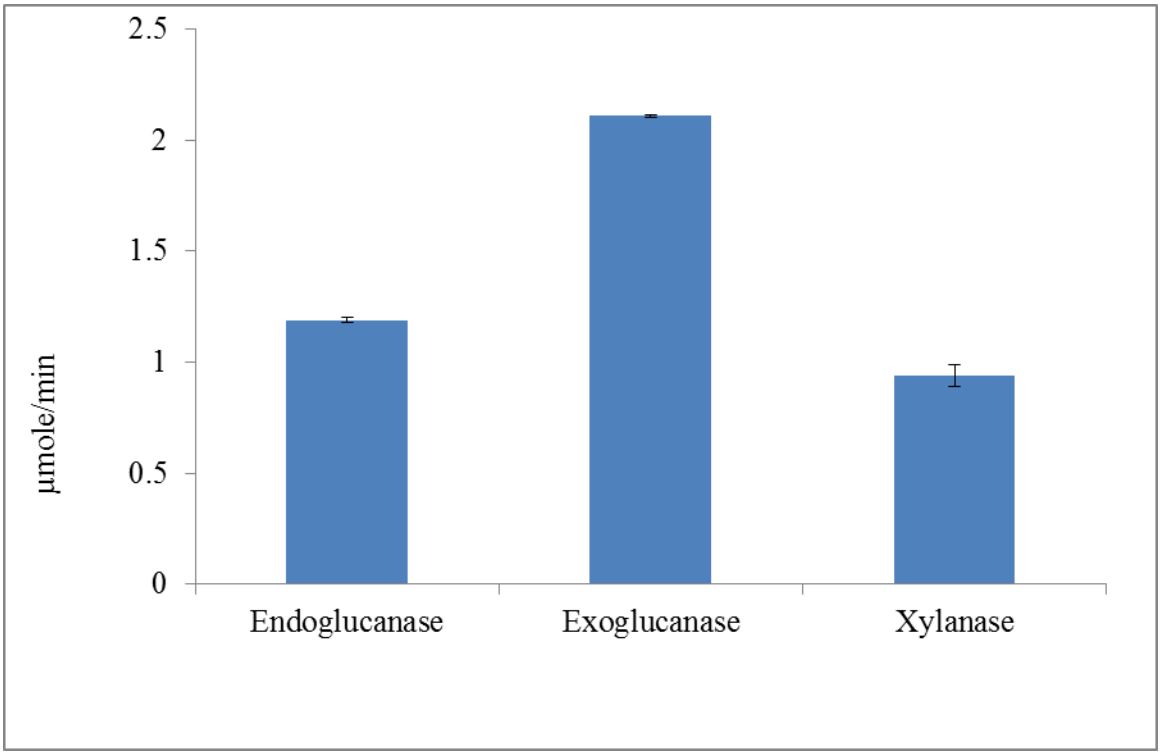


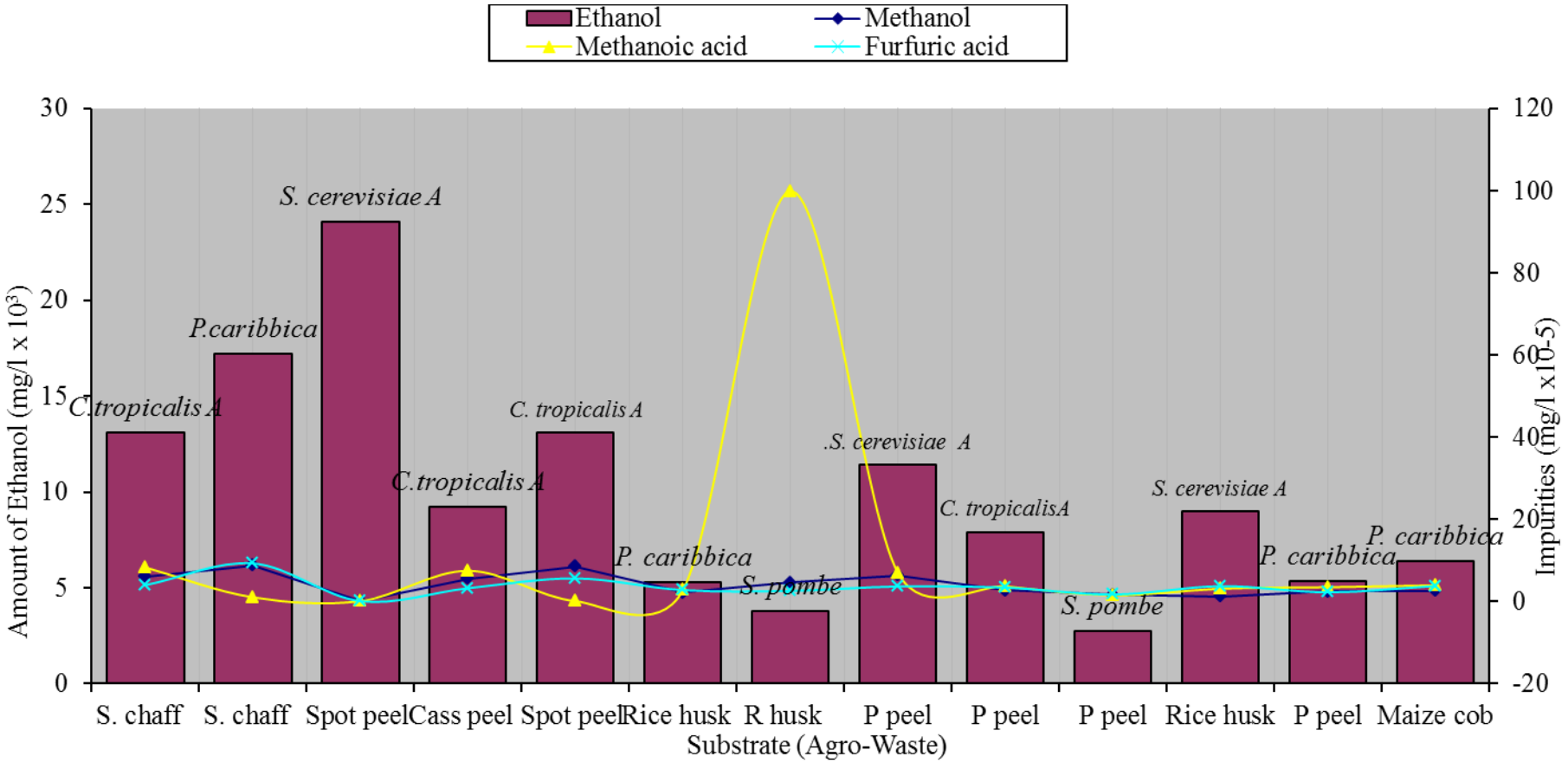
FIGURE 10: AMOUNT OF ENZYME ACTIVITY OF CASHEW NUT SHELL EXTRACT

4.9. IDENTIFICATION AND CONCENTRATION OF ALCOHOL PRODUCED FROM SOME AGRO-WASTE HYDROLYSED WITH CNSE

Ethanol, methanolic acid, furfural and methanol were used as standards during the analysis. Figure 11 gives a graphical representation of the result. The correlation coefficient on the selected calibration curve standard is 0.999 mg/l. The distilled products were found to consist mainly of ethanol while traces of methanol and furfural were found in the hydrolysates. Methanol is poisonous and therefore an undesirable in fermented product and furfural is an inhibitor of the fermentation process. The highest concentration of ethanol was observed in sweet potato peel hydrolysate. This observation showed that the fermentation was efficiently done and the products are safe for use as bioethanol.

4.10. CONCENTRATION OF ALCOHOLS PRODUCED FROM SOME SUBSTRATES HYDROLYSED WITH MINERAL ACID.

As summaried in Table 15, the distilled products were found to consist mainly of ethanol. The highest concentration of ethanol was observed in the cassava peel hydrolysate. This observation showed that the fermentation was efficiently done and the products are safe for use as bioethanol.



S. chaff= sugarcane chaff; Spot peel= sweet potato peel; Cass peel= cassava peel; R husk= Rice husk; Ppeel= Plantain peel

FIGURE 11: CONCENTRATION OF BIOETHANOL IN SOME AGRICULTURAL WASTE

TABLE 15: CONCENTRATION OF BIOETHANOL IN SOME SELECTED CROP PLANT (mg/l)

Substrate	Mineral acid used	Fermenting organism	Ethanol	Methanol
Maize	50% H_2SO_4	*C. tropicalis* Strain A	33.53	-
Maize	40% H_2SO_4	*Saccharomyces cerevisiae* strain /B	30.01	-
Cassava tubers	50% HCl	*Kluyveromyces marxianus*	27.91	-
Sweet potato	50% HCl	*C. tropicalis* strain A	25.61	-
Palm wine	-	*Kluyveromyces marxianus*	18.58	-
Irish potato	50% H_2SO_4	*Pichia caribbica*	16.51	-
Plantain	50% H_2SO_4	*Candida krusei*	13.62	-
Plantain	50% HCl	*C. tropicalis* strain A	9.04	-
Kola nut	40% HCl	*Kluyveromyces marxianus*	6.18	-

4.11. GROWTH STUDIES OF THE YEAST EXTRACTS IN EIGHT MEDIA

Result of the growth studies of the yeast isolates using potato dextrose agar broth (PDAB), malt peptone broth (MPB), carrot dextrose broth (CB), cassava dextrose broth (CDB), crayfish dextrose broth (CrayDB), maize dextrose broth (MDB), millet dextrose broth (MiDB) and sorghum dextrose broth (SDB) with spectrophotometer (Cecil CE 2041, Germany) at wavelength 530 nm is shown in Figures 12 to 19. The isolates grew in the media at pH 2.5 – 4.0 and temperature 28°C - 30°C. The growth studies in the media showed that *Schizosaccharomyces pombe, Kluyveromyces marxianus* and *Pichia caribbica* had a sigmoid growth curve in potato dextrose broth and malt peptone broth which is the normal growth pattern for living organisms. There was a general increase in growth of the organisms in all the media with increase in the number of days from day 2 to day 7 except in the cassava and crayfish broths. The organisms did not grow well in the maize dextrose broth where the growth rate of *P. caribbica, Candida tropicalis* strain A (Irish potato isolate), *Saccharomyces cerevisiae* strain A and *Saccharomyces cerevisiae* strain B fell sharply at day 2 of growth and remained low through the period of study. The highest optical density of 2.88 was obtained by the organisms in millet dextrose broth at day 6. In the lag phase (day 1-2) which can be regarded as adaptation phase, the isolate cells reproduced even though there was no appreciable availability of nutrients. In the crayfish broth, all the organisms had the peak growth between day 2 and 3 after which there were decrease in growth as observed by the low optical density. A gradual decline occurred from day 6 to 7 in carrot broth, malt peptone broth and maize dextrose broth. In millet dextrose broth the optical density showed no decline even on day 7 in all the studied organisms.

LEGENDS FOR FIGURES 12 - 19

MPB	=	Malt Peptone Broth
PDAB	=	Potato Dextrose Agar Broth
CB	=	Carrot Broth
MDB	=	Maize Dextrose Broth
CrayDB	=	Crayfish Dextrose Broth
MiDB	=	Millet Dextrose Broth
SDB	=	Sorghum Dextrose Broth
CDB	=	Cassava Dextrose Broth

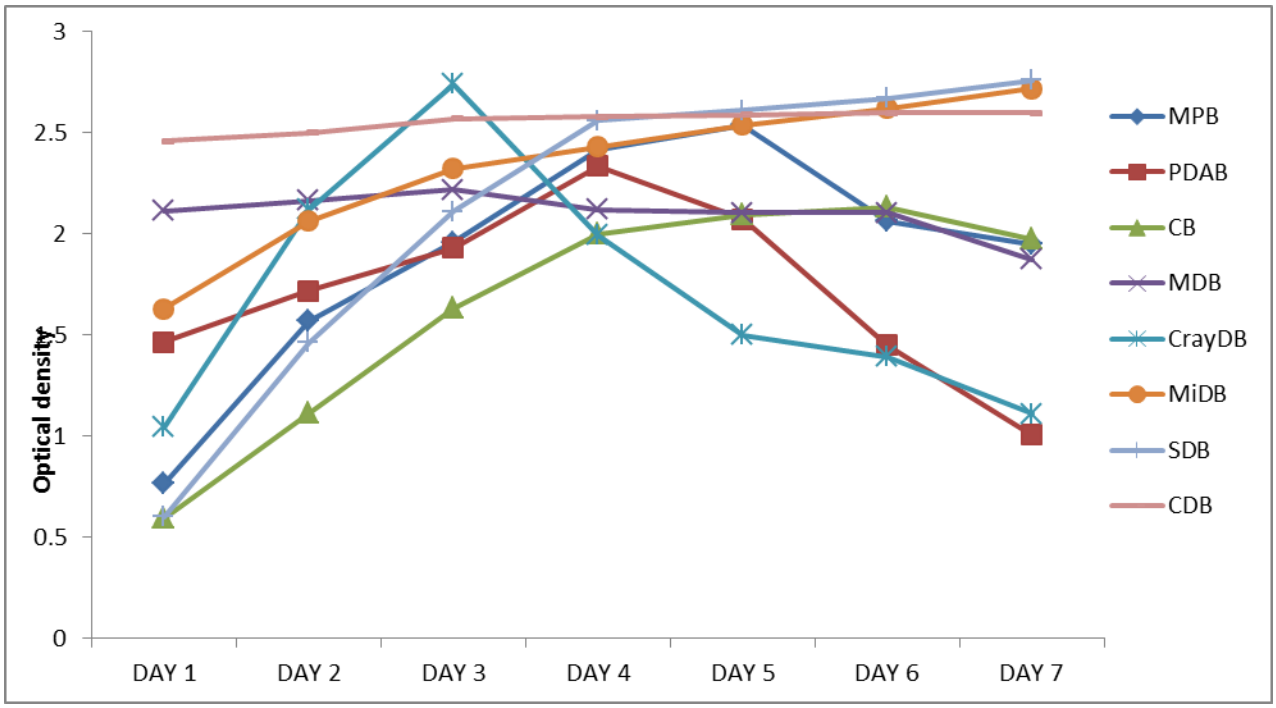


FIGURE 12: Growth curve of *S. pombe* in eight broth media at 530nm

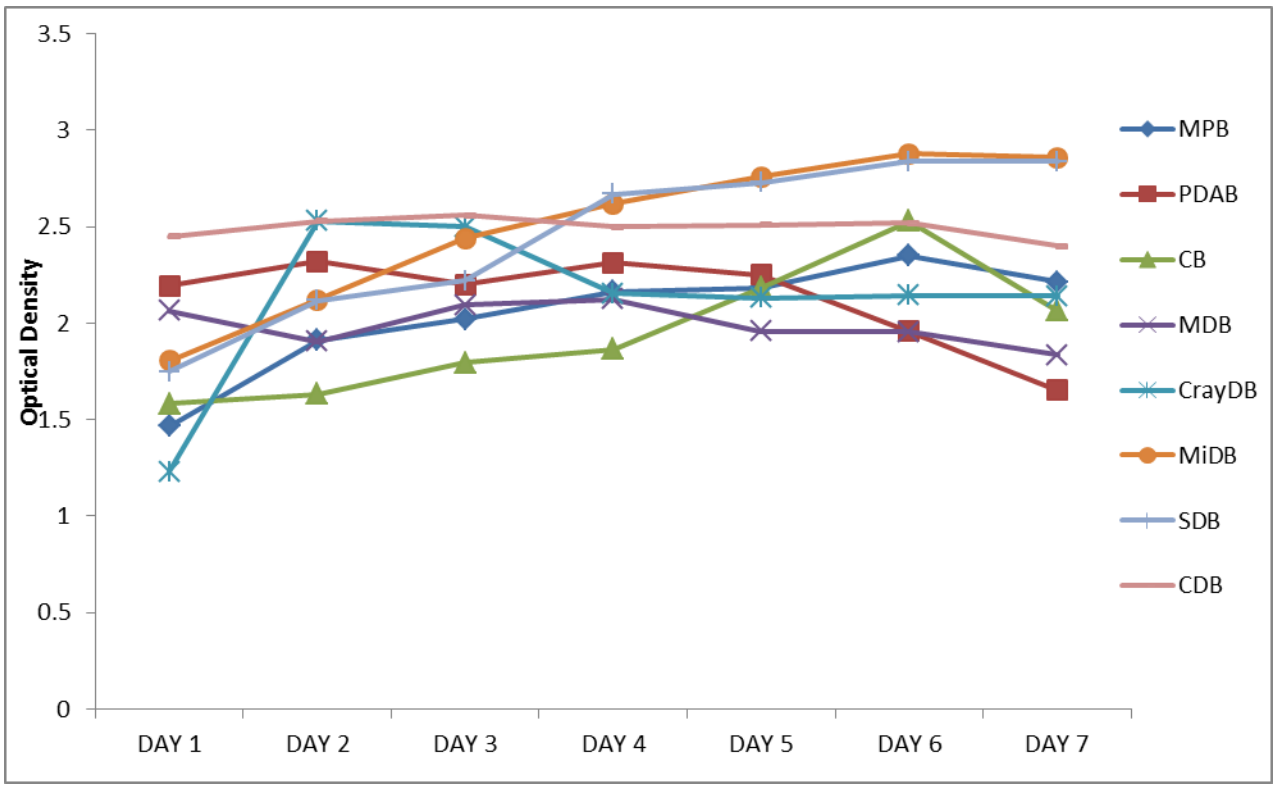


FIGURE 13: Growth curve of *K. marxianus* in eight broth media at 530nm

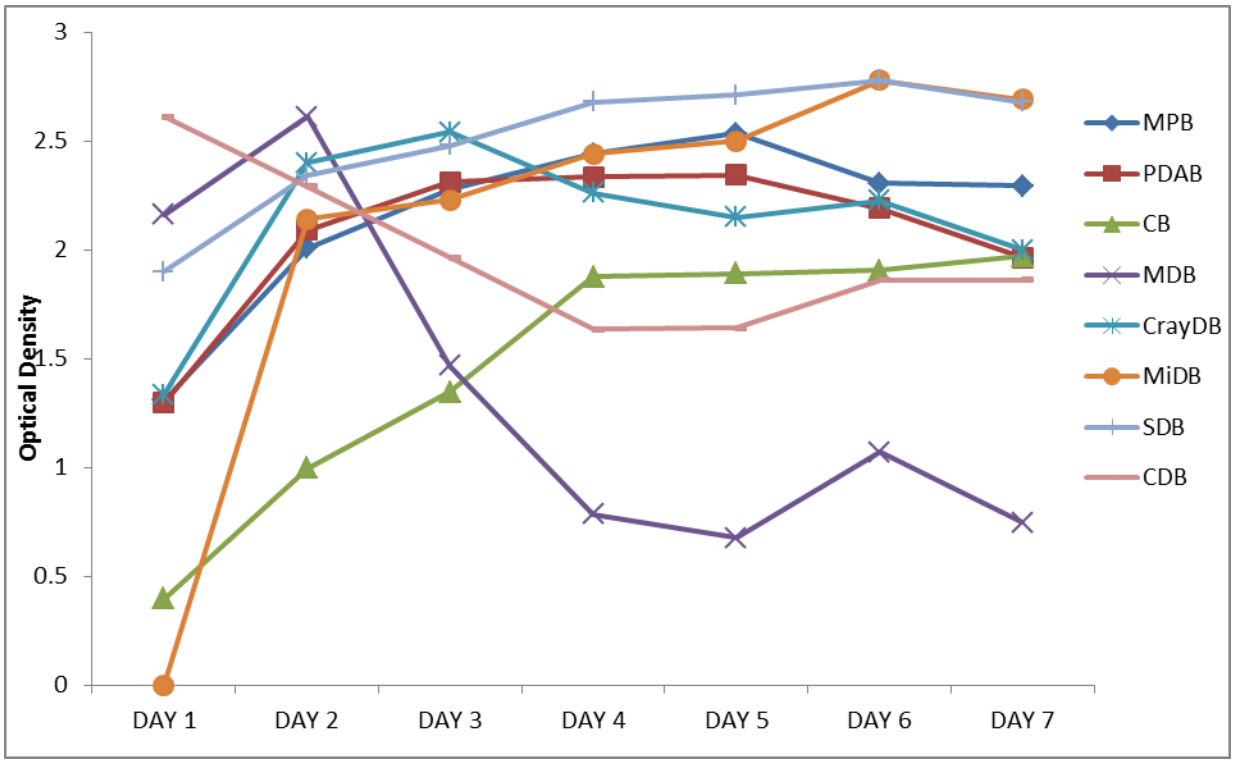


FIGURE 14: Growth curve of *Pichia caribbica* in eight broth media at 530nm

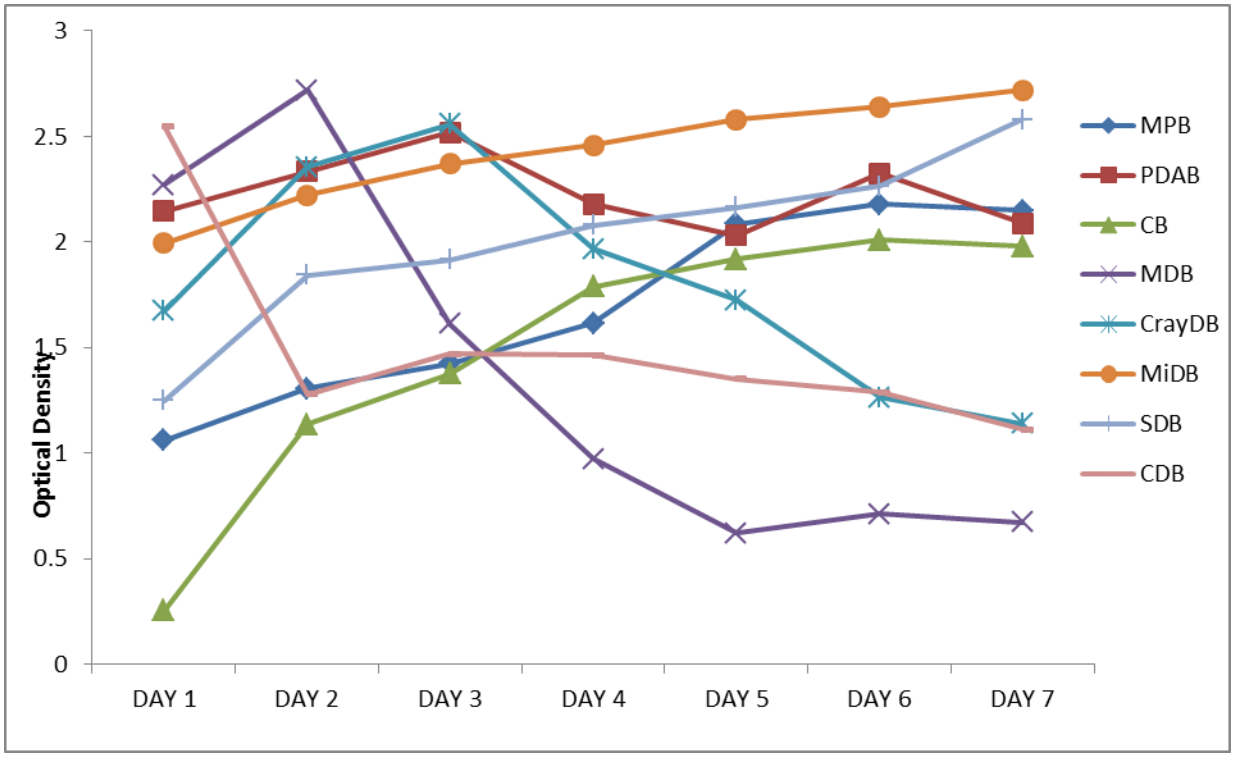


FIGURE 15: Growth curve of *C. tropicalis* strain A in eight broth media at 530nm.

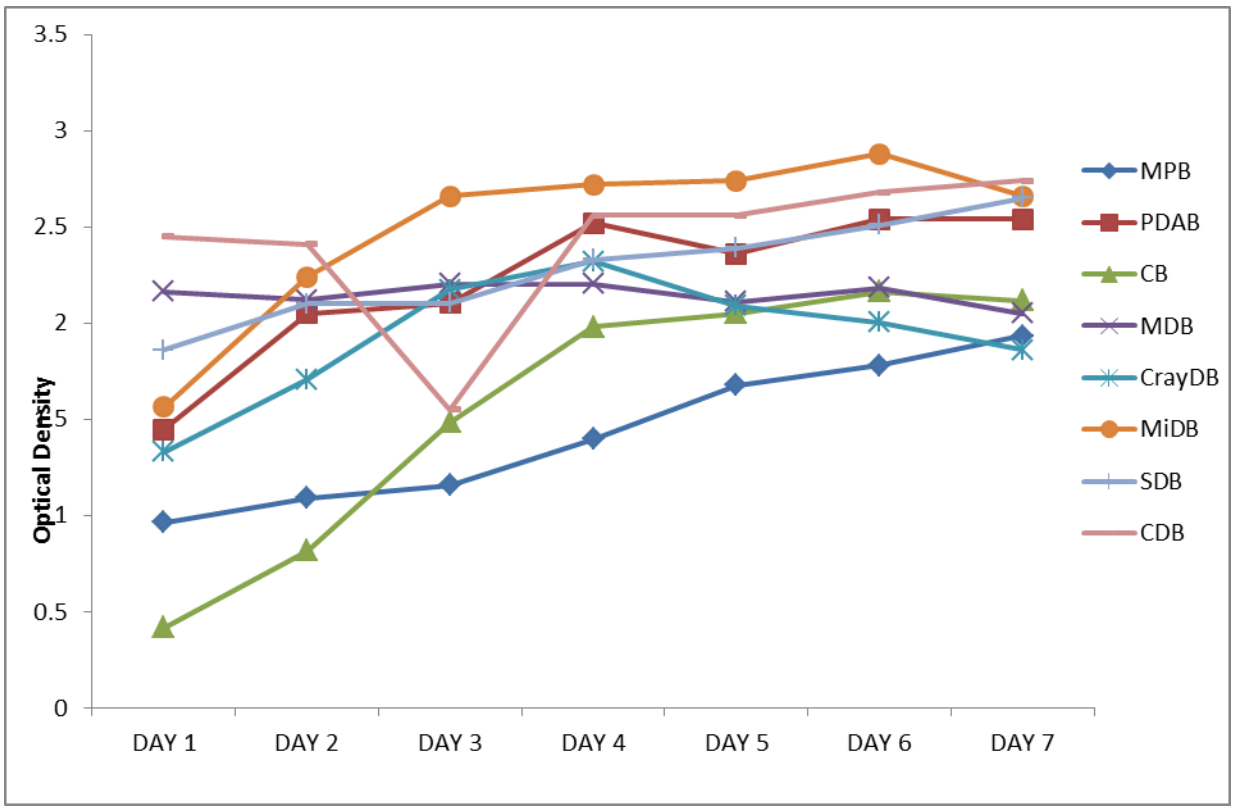


FIGURE 16: Growth curve of *Candida tropicalis* strain B in eight broth media.

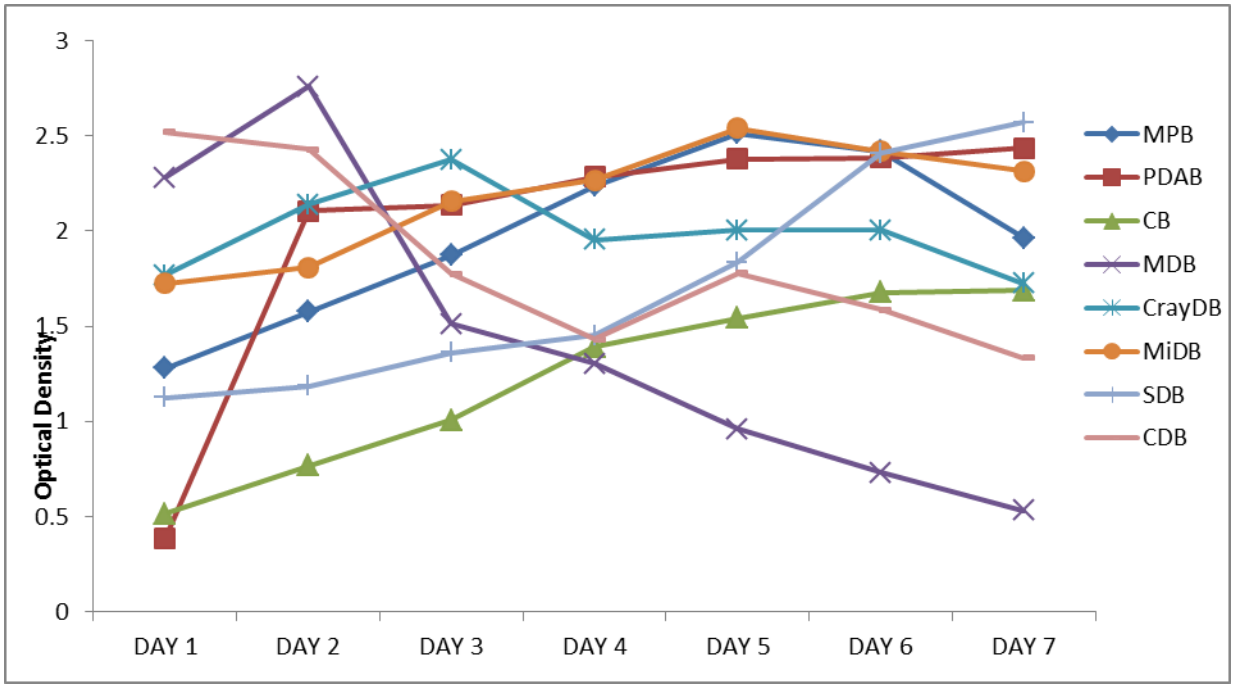


FIGURE 17: Growth curve of *Saccharomyces cerevisiae* Strain A in eight broth media at 530nm.

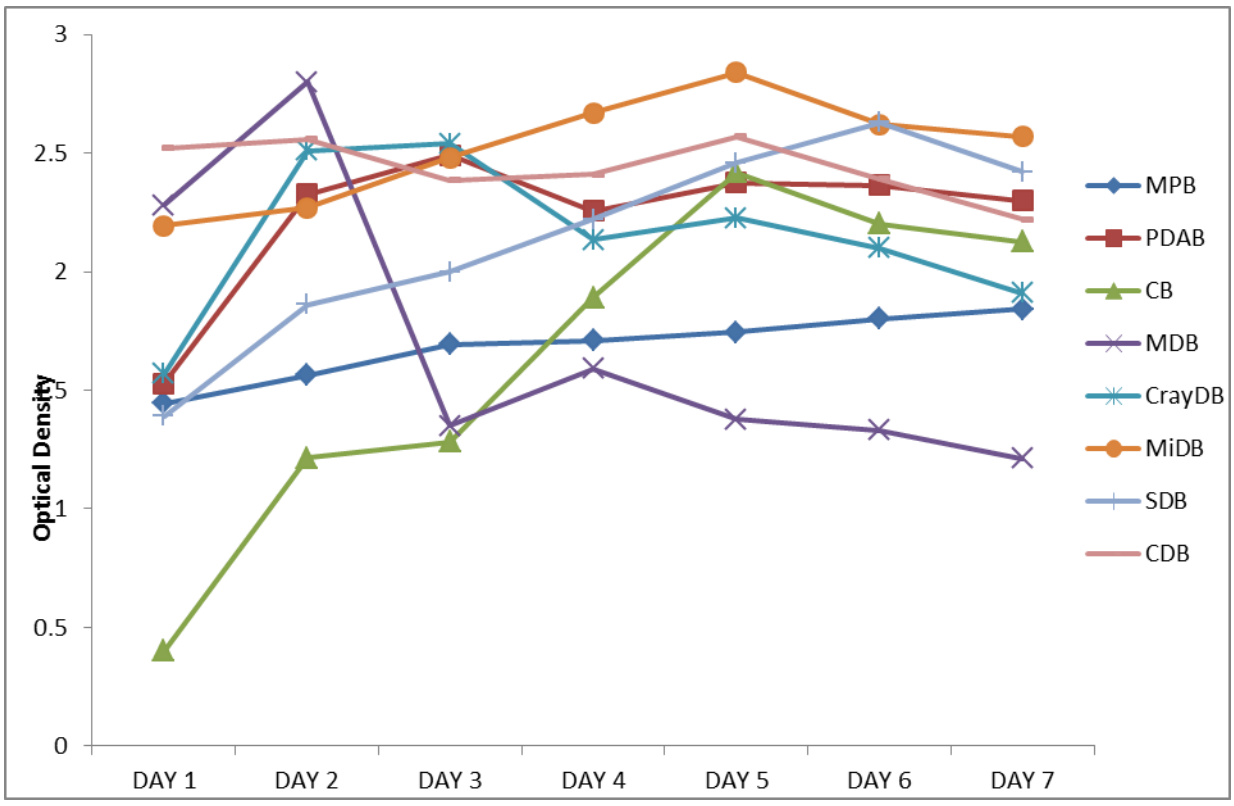


FIGURE 18: Growth curve of *Saccharomyces cerevisiae* Strain B in eight broth media at 530nm.

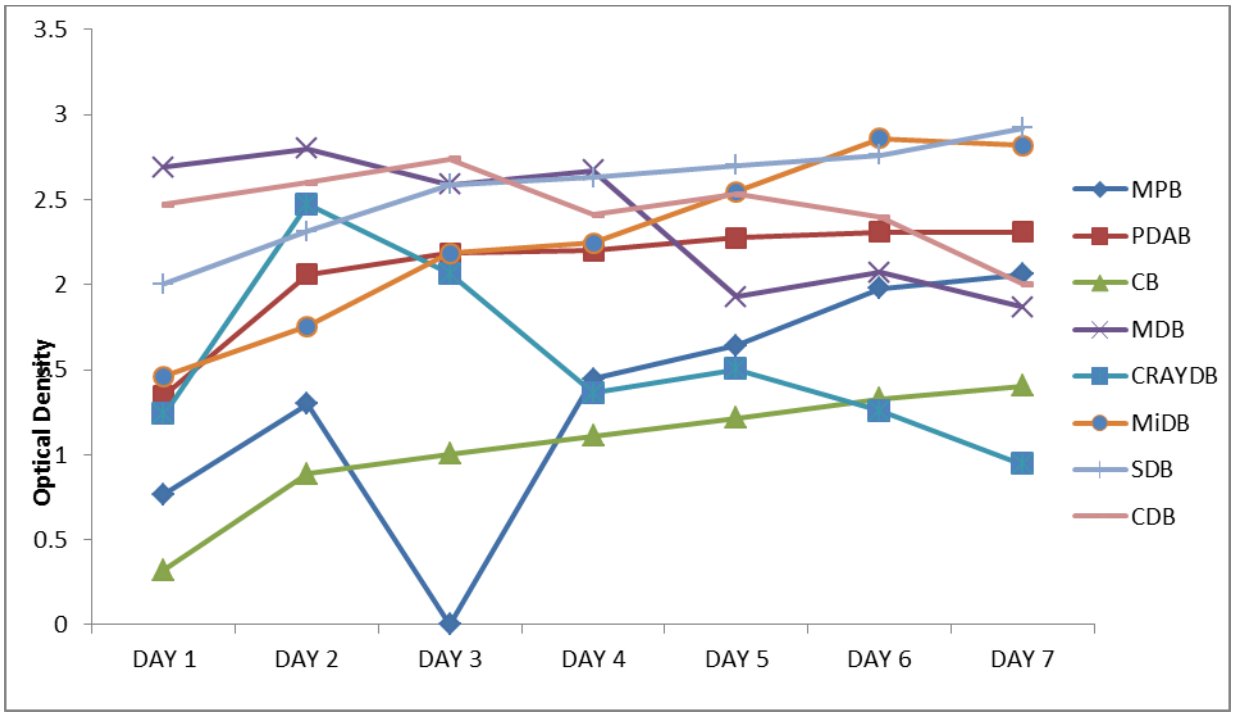


FIGURE 19: Growth curve of *Candida krusei* in eight broth media at 530nm.

CHAPTER FIVE

5.0. DISCUSSION

In this study, eight yeast species from five genera were isolated from palm wine (*Elaeis guineensis* sap), *Cola acuminata, Ipomoea batatas, Manihot esculenta, Pennisetum glaucum, Sorghum bicolor, Solanum tuberosum* and *Zea mays* bought from market places around Lagos State. The yeast species include *Candida krusei, Candida tropicalis* strain A, *Candida tropicalis* strain B, *Kluyveromyces marxianus, Pichia caribbica, Saccharomyces cerevisiae* strain A, *Saccharomyces cerevisiae* strain B and *Schizosaccharomyces pombe.* The yeasts were found to be fermentative in the breakdown of hexose and pentose sugars for the production of ethanol and carbondioxide. Hitherto, several workers such as Schneider *et al*. (1981), Oyeleke and Jibrin (2009), Mohd *et al*. (2011) have reported the activities of some of the yeast strains in bioethanol production. The experimental set-ups in this study were allowed to ferment for about 72-96 h and then distillation was carried out under controlled temperature of 78-80°C.

Alcohol acts as a solvent for an immense range of industrial products, including paints, lacquers, dyes and oils. In addition, some are used as raw material in chemical synthesis and in the form of fuel (Morrison and Boyd, 1972; Goldemberg *et al.,* 1985). Ethanol ranks second only to water as the most widely used solvent in chemical industry and as these industries have expanded, the demand for industrial alcohol has increased. Bioethanol yield in this study varied among substrates. The highest yield was from sugarcane juice fermented with *Schizosaccharomyces pombe.* This is due to the presence of saccharine (sugar containing) materials in the juice. The carbohydrate in the juice is already present in the form of simple, directly fermentable carbon six sugar molecules that is, glucose and fructose which are

monomers of sucrose (Ocloo and Ayernor, 2010). This runs contrary to the starchy and cellulosic substrates that need to be hydrolyzed through the use of enzymes or mineral acids at different concentrations before the introduction of yeast for fermentation. These were observed in the researches of Rhee *et al*. (1984), Atthasampunna *et al.* (1987), De Figueroa *et al*. (2000), Adesanya *et al.* (2008) and Oyeleke and Jibrin, (2009) who had to hydrolyze the various substrates used in their experiments. The bulky substrates had to be grated into smaller pieces of between 0.2 – 2.0 cm when it was discovered during the course of the study that the initial procedure of using sliced tubers and larger chops in the range of 5.0-8.0 cm did not yield any ethanol after a period of 72 h. This was probably due to the large surface area which inhibited the metabolic activities of the yeast strains. Substrates used in the works of De Figueroa *et al*. (2000), Oyeleke and Jibrin (2009) and Ocloo and Ayernor (2010) had to be ground into powdery forms before processing.

Fermentation occurred best at pH 3.5-4.0 while substrates at pH > 4.0 produced little or no ethanol. This could be due to the fact that at pH > 4.0 the yeasts are less tolerant to the environment and thus less efficient in substrate utilization. Some yeast strains like *Candida tropicalis* strain A and B did not produce ethanol at pH above 4.0, these organisms have been reported to be more acid tolerant. The initial pH of the CNSE hydrolysates was higher compared to that of the mineral acid hydrolysates. This could explain the low yield of ethanol as most yeast metabolism ceases at higher pH (Roostita and Fleet, 1996; Mariam *et al*., 2009). It has also been reported that increasing pH value in molasses hydrolysate, reduces alcohol dehydrogenase (enzyme required for the conversion of pyruvic acid to ethanol) activity in *Saccharomyces cerevisiae* cell and leads to the formation of acetic acid instead of ethanol in a

fermentation process (Munene *et al*., 2002). In like manner, ethanol production by *C. krusei* from the natural acid hydrolysates was negligible because the initial pH range of 5.3 and 5.7 was inhibitory to the growth of the organism. Interestingly, workers like Haltrich *et al*. (1996); Roostita and Fleet (1996) have reported that most fungal cultures generally require slightly acidic pH for their growth and metabolism. Also, Chaudhary and Qari (2006) and Manikandan *et al*. (2008) stated that the initial pH has a significant effect on fermentation particularly on the growth and metabolism of yeast, rate of fermentation and on the by-products produced. This depicts the fact that microorganisms have varied tolerance for acidic conditions (Roostita and Fleet, 1996).

Lignocellulosic ethanol (a type of bioethanol) is considered one of the most potential next generation automobile fuels for the future (Kumar *et al*., 2009). Lignocellulosic ethanol can be produced from various feedstocks such as lignocellulosic biomass, starchy materials, sucrose containing feedstock and microalgae (Mathiyazhagan *et al*., 2011; Nigam and Singh, 2011; Templer and Murphy, 2012). Consequently, it was observed that the highest amount of bioethanol from the agro-waste using the CNSE was 28.12±1.61 g/L at 72 h of incubation. Previously, Oyeleke and Jibrin (2009), Kathiresan and Saravanakumar (2011), had respectively obtained 26.31± 1.41 g/L of bioethanol from guinea corn husk and 12.3 g/L of bioethanol from sawdust at 120 h of fermentation. Mohd *et al*. (2011) as well gave a corresponding yield of bioethanol from bio-waste where they obtained a yield between 9.55-10.32 g/L of ethanol from empty fruit bunches of palm oil fermented with *S. cerevisiae.* The highest amount of bioethanol produced from the crop plant was 33.34 ± 2.81 g/L obtained from sweet potato tubers hydrolysed with 50% HCl while the highest from the lignocellulosic waste as stated above was from plantain peels. This further indicates that bioethanol production from agro-

waste hydrolysates is significantly comparable with bioethanol production from other feedstock like crop plants that include sugarcane, cassava and maize. These crop plants can therefore be put into more effective use for man and animal while the waste generated from their processing can be used for biofuel (bioethanol) production. This correlates with the works of Giampietro *et al.* (1997); Mohd *et al*. (2011); Templer and Murphy (2012) that bioethanol in high quantity can be derived from cellulosic biomass through acid or enzymatic hydrolysis followed by fermentation.

Generally, lignocellulosic biomass should undergo numerous processes consisting of pre-treatment, hydrolysis, enzymatic saccharification and fermentation of the sugar to produce bioethanol (Kolachov and Nicholson, 1951; Sun and Cheng, 2002). It is shown from the results that a combination of dilute acid hydrolysis (CNSE) and enzymatic saccharification could be a promising approach for extracting fermentable sugars from agro-waste as feedstock for ethanol production. As in most ethanol production using various lignocellulosic biomasses, the main process of pre-treatment involves alkaline or acid hydrolysis and enzyme saccharification to produce glucose and xylose followed by fermentation with yeast (Demirbas, 2005).

Cashew nut shell extract was successfully used in this study because the mineral acids (H_2SO_4 and HCl) being used in various studies produced substantial amounts of impurities like furfural, acetate and other phenolic compounds (Palmqvist and Hahn-Hagerdal, 2000). It is also economical to produce plant based extract for the purpose of hydrolyzing agro-waste rather than relying on importation of mineral acids with scarce foreign exchange. Though negligible amount of methanol was found in the bioethanol produced, this cannot be compared to the quantity of bioethanol produced. Methanol is a toxic alcohol which is formed during fermentation of naturally occurring pectin in wort (Tomoyuki *et al*., 2000). Okunowo and

Osuntoki (2007) suggested the addition of pectinolytic enzymes before fermentation process to degrade the pectin in order to reduce the production of methanol. It has also been reported by Naidu and Panda (2004) that the pectinolytic enzyme degrade the pectin thus, reducing the viscosity of the solution to be fermented for easy metabolic activities of the fermenting organisms since more fermentable sugars are made available. *Anacardium occidentale* (cashew) nut shell is known for its production of acidic substances and consisting of various amounts of bioactive compounds. Trox *et al.* (2010) found appreciable levels of certain bioactive compounds such as beta-carotene, oleic acid and linoleic acid in raw cashew nut kernels. Likewise, Bicalho and Rezende (2001) have isolated and characterized several compounds including esters, terpenes, hydrocarbons, carboxylic acids, lactones and norisoprenoids from *A. occidentale.* The enzyme assay of the extract from the cashew nut shell showed that it contains β-endoglucanase, β-exoglucanase and xylanase which could break the β-1,4, linkage of the cellulosic and hemicellulosic materials in the substance used in this study. Therefore, confirming the presence of cellulase involved in cellulose hydrolysis in the cashew nut shell extract. Cellulases are complex cellulolytic enzymes whose synergetic activities probably led to the breakdown of the substrates into their monomeric sugars. Najafpour *et al*. (2007), Taherzadeh and Karimi (2007) had earlier reported that proton in acid could catalyze and break the β-1,4, linkages of glucose and xylose monomer, acetyl groups and other products in cellulose and hemicelluloses biomass. Interestingly, the cashew nut shell extract could hydrolyze maize cob, cassava peel and sweet potato peel which were not hydrolyzed by HCl. This proves that extract from cashew nut shell can be used for the hydrolysis of lignocellulosic agro-waste where high cellulose and hemicelluloses can be potentially converted to ethanol. Ohmiya *et al*. (1995) earlier extracted cellulase from poplar cell. According to Coughlan and

Ljungdahl (1988), at least three major groups of cellulases are involved in the hydrolysis process; these are a) endoglucanase which attacks regions of crystalinity in the cellulose, creating free chain-ends, b) cellobiohydrolase (exoglucanase) which degrades the molecule further by removing cellobiose units from free chain-ends and c) β-glucosidase which hydrolyzes cellobiose to glucose. This further shows that some plants contain hydrolytic compounds that can be useful in the biodegradation of agro-wastes to economically produce valuable organic compounds. Hammond and Ayernor (2000) had earlier used malts extracted from various cereals in the hydrolysis of starch and they observed that rice malt gave the highest yield of sugars. The pH of the CNSE used was 3.2, thus showing that the extract is acidic. This as well could aid in the hydrolysis of the agro-waste into monomeric units. Muhammad *et al*. (2010) reported that cellulase from *Trichoderma viride* showed optimum activity at pH 5.5. Workers like Ahmad *et al*. (2009) have earlier stated that the initial medium pH in the range of 4.5-5.5 is the optimum for carboxymethyl cellulase (endoglucanase) production.

Fermentation profile shows that the reducing sugars can produce considerable amount of bioethanol within 72 h. The highest amount of reducing sugar was from sugarcane chaff hydrolysate (491 mg/g). Arumugam and Manikandan (2011), had reported that an initial pretreatment of fibrous peel residues breakdown its structure to make it more susceptible to enzymatic reactions. This can be the reason for the valuable amount of reducing sugars librated from the hydrolysates. Likewise, based on the enzymatic saccharification study, it was observed that the sugar concentration varied in the different agro-waste. Glucose was the dominant monomer sugar obtained from the process. Sun and Cheng (2002) reported that generally, during enzymatic hydrolysis, cellulose is degraded by cellulases to reducing sugars

that can be fermented by yeast to ethanol. Also, Jeffries (2006) had stated that yeast are more advantageous than other microbes for commercial fermentation due to their larger sizes, thicker cell wall, better growth at low pH, less stringent nutritional requirements, and greater resistance to contaminations. Substantial concentration of glucose was also obtained by Mohd *et al*. (2011) from empty fruit bunches of oil palm. This indicates that glucose extracted from agro-waste like cassava peel, rice husk and sweet potato peel can be converted into bioethanol by yeast like species of *Schizosaccharomyces, Pichia* and *Candida*. In like manners, Patle and Lal (2007) observed reducing sugar yield of 48-84 g/L and ethanol production of 23-32 g/L in acid pretreated fruits and vegetable residues. Notably, the generally higher ethanol yield from sweet potato tubers and sweet potato peels can be due to the high concentration of fructose and glucose sugars in the substrates. Michael and Roselind (2000) had stated that high fructose and glucose concentrations in fresh fruits releases higher amount of ethanol. The result of this study revealed that the ethanol production varied significantly between the species ($P<0.05$). This variation may be due to the various sugar utilization mechanisms and to the presence of the gene encoding for the specific enzymes that can degrade the sugars.

Yeast are potential tools used in fermentation and can be used for ethanol production. A typical example is *Saccharomyces cerevisiae* which can efficiently ferment glucose and mannose but has been reported by Ligthelm *et al.* (1988) and Dickinson (1999) to be unable to ferment xylose and lactose into bioethanol. Thus it is not a suitable tool for ethanol production. As reported by Hahn-Hagerdal *et* al. (2007), the commercial production of bioethanol from lignocellulosic hydrolysates by yeast requires the strains that can ferment both hexose and pentose with a high ethanol yield and specific ethanol productivity. Therefore, the ability of

the yeast strains used in this study to utilize xylose and lactose sugars present in the hydrolysates is an important potential for increasing the commercial values of cellulosic ethanol. This is significant because in order to maximize the lignocelluloses derived polymers of carbohydrates and xylan whose main constituents are glucose and xylose, the fermenting organisms must be able to utilize both sugars. Thus, using a larger amount of the raw materials will produce greater yields of ethanol than if only glucose substrate is consumed. Instead of recent developments and testing of genetically modified species as observed in the studies of Govindaswamy and Vane (2007); Bera *et al.* (2010); Wei and Anli (2012), approaches should be geared towards isolating indigenous strains as opposed to genetically modified organisms.

There were differences in the level of ethanol produced by the species of *Saccharomyces cerevisiae* (from guinea corn and millet) and *Candida tropicalis* (from Irish potato and kola nut) and this could have been due to the differences in their sources of origin. Okunowo and Osuntoki (2005) reported a similar finding where *S. cerevisiae* isolated from sugarcane molasses and yam showed different levels of alcohol production when used in the fermentation of orange juice.

Schizosaccharomyces pombe was isolated from fermented palm wine and purified on malt extract agar plate. This is in contrast with most documented reports such as Okafor (1972), Okague (1988), Ejiofor *et al.* (1994), Nwachukwu *et al.* (2006) and Bechem *et al.* (2007) who had reported that palmwine predominantly consist of budding yeast. Species of yeast like *Candida* have not been extensively reported as fermentative yeast for bioethanol production nor in the production of other useful organic compounds except as causal agents of human diseases. Ellis *et* al. (2007) reported *Candida tropicalis* as the causal agent of candidiasis in

man; they are opportunistic fungi which live in most human organs. However, recent reports by Kathiresan and Saravanakumar (2011) and Senthilraja *et al*. (2011) have shown that species of *Candida* are not just pathogens but can be useful tools for bioethanol production, as they were able to use *C. tropicalis and C. albicans* isolated from marine environment to produce bioethanol. There had also been reports of their isolation from dairy products such as yoghurt (Rohm *et al*., 1992) and milk (Gadaga *et al*., 2000). Nevertheless, most species of yeast have been reported to suffer environmental stress such as nutrient deficiency, temperature, pH and by-products of fermentation during ethanol fermentation (Grave *et al.,* 2006 and Yah *et al*., 2010). This could have led to low amount of bioethanol production by some of the species used in the present study. Attfield, (1997) had earlier reported that for the application of yeast in biotechnologies such as baking, brewing, alcohol fermentation and wine making there is need for physiological and genetic improvement of their environmental stress properties. *Pichia caribbica*, *Candida tropicalis* strain B and *Candida krusei* were isolated from *Zea mays*, *Cola acuminata* and *Ipomoea batatas* respectively. This is probably the first report of isolation and characterization of yeasts from these substrates that can be used in fermentation for the production of bioethanol in Nigeria. Most workers had reported the use of *Saccharomyces cerevisiae* for fermentation in the production of bioethanol (Abouzied and Reddy, 1986; Bechem *et al.,* 2007; Adesanya *et al.,* 2008; Oyeleke and Jibrin, 2009). This report therefore gives an array of prospective fermentative species of yeast from locally available substrates.

The Gram stain studies which showed that the organisms were Gram positive (Hill *et al.,* 1967) and germ-tube test which gave negative results further confirmed that the organisms are actually yeast. From urea test, *S. pombe*, *P. caribbica* and *S. cerevisiae* (from millet) were able to hydrolyze urea which was indicated by the colour change from pinkish-red to yellow-

orange. This is in contrast with the work of Seeliger (1956) where the test was carried out to distinguish *Saccharomyces* and *Candida* species from other yeast as members of these genera were reported to lack urease.

The organisms were able to degrade the sugars because they contain the enzymes necessary for the conversion of sugars to ethanol with the release of carbondioxide. *Kluyveromyces marxianus* for instance could breakdown the D-xylose because it contains xylanase. This is in correlation with the findings of Lark *et al.* (1997) who fermented D-xylose from recycled paper sludge to produce ethanol using *K. marxianus.* Roostita and Fleet (1996) earlier reported the isolation of *K. maxianus* from dairy products as it has the ability to ferment lactose, hydrolyze milk fat and proteins. This organism was also able to ferment galactose which is an indication of the presence of *β*-galactosidase. Rajoka *et al.* (2003) had extracted the enzyme from *K. marxianus.* Previously, Kolachov and Nicholson (1951) had stated that yeasts utilize the sugar for growth and reproduction. In the study on resistance to chloramphenicol, all yeast isolates showed resistance to chloramphenicol since they could grow in the presence of the discs. This goes to confirm the reason for the addition of chloramphenicol at low concentrations to the growth medium during the primary isolation of yeast from a mixed culture (Linné and Ringsrud, 1992). The chloramphenicol inhibits the growth of most bacteria while allowing the yeast to grow. Parallel to this, Bechem *et al.* (2007) studied the resistance of yeast isolates to chloramphenicol.

Contrary to literature reports by Kurtzman (1983) that species of *Saccharomyces* cannot ferment lactose as they lack the enzyme lactase, the *Saccharomyces* strains (from guinea corn and millet) isolated in the course of this study were able to ferment the lactose used in the fermentation test. This strain could be used for the purpose of fermentation to produce alcohol

and other derivatives. Although, the rice husk was observed to contain the highest amount of cellulose (808mg/g) it yielded the lowest amount of reducing sugar. This can be as a result of partial hydrolysis of the feedstock. Most workers use base in place of acid for the hydrolysis of rice husk probably as a result of the type of carbohydrate present in it. Bases are generally more expensive than acid and so not an alternative solvent for hydrolysis.

In the classification of yeast (fungi), conventional methods such as physiological and morphological analysis are not enough to adequately identify yeast especially with the emergence of new strains. Molecular identification is known to provide a more objective separation of genera and species than phenotypic analysis. DNA sequence analyses was achieved through DNA extraction using CTAB procedure, amplification of regions of rDNA/ Internal Transcribed Spacer sequence (ITS), purification of the PCR products. The amplified region was done using ITS1 and ITS4 which are recommended universal primers for fungi identifications (Trost *et al*., 2004). Then comparison of the data obtained with known sequence in the database of the Genbank and CABI, Royal Botanical Garden, Kew. The comparison of rDNA gene sequence is an important instrument in determining the phylogenetic and evolutionary relationship of many organisms. Ellepola *et al*. (2003) used this method to separate a species of *Candida* which had earlier been wrongly identified as *Candida albicans* into the correct species of *C. dubliniensis.* Phylogenetics, the study of the evolutionary relationship between different species and population of organisms based on gene analysis enabled the yeast to be grouped under three major clusters. This presented the isolates in the form of a schematic tree where divisions into branches were made to indicate how different the species diversified in the course of evolution. *Schizosacharomyces pombe* is distantly related to the others while *P. caribbica* and *K. marxianus* fell into the same cluster. The isolates from

Irish potato and kolanut are of the same species but maybe of different strains as they do not show consistent activities in the biochemical processes.

This research shows the genome rDNA/ITS sequences of the yeast samples to be more accurate and reliable in phylogenetic typing and identification than the conventional means. The ability to accurately and reproducibly identify fungi has been greatly enhanced through comparative DNA sequencing (Hall, 2007). Fungal taxonomists have used DNA sequences for many years as a basis for reclassification of all fungal taxa and have more recently moved to ITS sequencing as the "gold standard". Highly conserved genes such as ribosomal RNA (rRNA) genes provide information on the general properties of the organism based on the properties of their known relatives. In addition analysis of the rDNA sequences has the advantage that it not only enables species identification but also permits phylogenetic analysis. However, physiological, biochemical and gene sequencing are all necessary to adequately identify an organism as yeast.

Growth study of the isolates in potato dextrose agar broth (PDAB), malt peptone broth (MPB), carrot dextrose broth (CB), cassava dextrose broth (CDB), crayfish dextrose broth (CrayDB), maize dextrose broth (MDB), millet dextrose broth (MiDB) and sorghum dextrose broth (SDB) showed that the organisms grew in the eight broth media in varying proportion. The potential to grow on all the media indicates that the yeast strains investigated in the study are able to breakdown the major components of the substrates. The ability of the organisms to grow at pH 3.0- 4.0 showed that they can tolerate acidic conditions. Kok *et al.* (1992) reported a similar finding. The high OD of MiDB in comparison with others indicates that the organisms degraded the components at a faster rate than in others since millet is a rich source of carbohydrate. Ibrahim *et al.* (2002) reported that growth medium rich in carbon and nitrogen

provides optimal growth for microorganism. Interestingly, Srinubabu *et al.* (2007) previously reported in their study on screening nutritional parameters for production of protease by *Aspergillus oryzae* that glucose in PDA is the best source of carbon for fungal growth and thus may be the reason for more growth of the organisms in the media.

Bioethanol from lignocelluloses agro-waste is another way of developing clean energy that is environment friendly. Its carbon dioxide emission is equal to it absorptions during crop production. The industry is endowed with great opportunities and would assist in rural development in Nigeria. There will be sufficient crop supply for human and industrial use. It would also create jobs, wealth, develop rural infrastructure, expand rural market, and reduce the scourge of poverty. Although there are challenges that may face the development of the industry in Nigeria, these problems can be overcome through careful planning and implementation strategies.

SUMMARY OF FINDINGS

- Eight yeast strains from five genera with fermentative properties were isolated from the following substrates: palm wine, cassava tuber, maize, Irish potato tubers, guinea corn and millet grains as well as kola nut and sweet potato tubers. The identities of the isolates were confirmed through photomicrography and molecular characterization (DNA sequencing).
- Fission yeast (*Schizosaccharomyces pombe*) was isolated from palm wine as against most literature reports of palm wine consisting of only budding yeasts.
- The best media for the cultivation of the organisms were potato dextrose agar, malt extract agar and millet dextrose agar while *Pichia caribbica, Schizosaccharomyces pombe* and *Candida tropicalis* strain have more potential as fermenting organisms than the others.
- Cashew nut shells consist of acidic substances which has hydrolytic enzymes of β-endo and β-exoglucanase which is useful in the hydrolysis of substrates for biofuel production. This is important for hydrolyzing starch and cellulosic substrates into simple sugars for microbial (yeast) fermentation to occur.
- Hydrolyzed agricultural wastes contain varying concentrations of fermentable sugars like glucose, fructose, sucrose and xylose.
- Hydrolysis of agricultural wastes was achieved using extract from *Anacardium occidentale* (cashew nut shell).
- Substantial amount of bioethanol was produced from agricultural wastes which is significant in comparison with what was produced from crop plants.

CONTRIBUTIONS TO KNOWLEDGE

1. This is the first report of isolation and characterization of yeast strains from kola nut, Irish potato, millet, sorghum and sweet potato that can be used for the production of bioethanol in Nigeria.
2. The DNA sequence of the yeast isolates and submission of sequence data to a Gene bank (biodiversity) which is a major contribution to Mycology was done.
3. This study identified crude extract from *Anacardium occidentale* (cashew nut shell) that can be used for hydrolysis of agro-waste (bio-waste). The extract hydrolysates produced simple sugars of varying concentrations from the agro-waste.
4. *Candida tropicalis* strain A, *Pichia caribbica and Schizosaccharomyces pombe* were identified as the best fermenting yeasts in this study.
5. Production of bioethanol from agro-wastes (cassava peels, sugarcane chaff, maize cob, plantain peels, rice chaff and sweet potato peels) using Nigerian yeast isolates was done. This is biotransformation of some agricultural wastes into useable products, thus helping to manage waste more efficiently.

CHAPTER SIX

6.0. CONCLUSION

This study has dealt with the isolation and characterization of yeast from crop plants such as cassava tuber, *E. guineensis* sap, maize seed, kolanut, guinea corn, millet grains, Irish potato and sweet potato tubers in Nigeria. Biochemical test (physiological analysis) and DNA sequence (genotypic analysis) have shown that some of the isolates might be new strains. The sequence data of some of the isolates have hitherto been deposited at the database of CABI Royal Botanical Garden, Kew, England. Fermentation process of the isolates showed that the isolates can be used in the production of bioethanol from agro-wastes. *Pichia caribbica*, *Candida tropicalis* strain A and *Schizosaccharomyces pombe* are prospective better fermenting organisms than the other isolates used in this study. The growth study of the isolates using some of the sources of origin as growth media showed that millet dextrose broth along with potato dextrose broth and malt peptone broth are the best media for the cultivation of the organisms. The millet dextrose broth can further be concentrated and other vital nutrients added to produce indigenous growth medium. Developing the industrial potentiality of the yeasts isolated from these locally available materials will help in reducing dependency on importation and encourage local production of industrial strains.

In cellulosic ethanol production pre-treatment like hydrolysis is a necessary procedure for the liberation of the cellulose and hemicelluloses constituents from lignocelluloses biomass. Investigation on the effect of an alternative agent of hydrolysis using the extract from empty nut shell of cashew revealed that this can be used for the hydrolysis of agro-waste as it was successfully assayed for cellulases like endoglucanase and xylanase.

It was shown that agro-waste (plantain peel, sugarcane chaff, maize cob, cassava peel, rice husk and sweet potato peel) when properly treated can be renewable sources of monomer sugars such as glucose, fructose, xylose, sucrose and arabinose which can be metabolized by yeasts thus producing bioethanol and carbondioxide in the process. The use of these feed stocks in biofuel production in Nigeria can be encouraged since it is reasonable to utilize them due to the low cost, diversity and availability of the materials. It is also another channel of waste management and economic empowerment of the populace.

Bioethanol produced from the CNSE hydrolyzed agro-waste used in this study revealed that the waste can be used in the place of crop plants in the production of biofuel in Nigeria. According to Agboola *et al.* (2011), the bio-ethanol policy in Nigeria has adopted the use of cassava, maize and sugarcane for the production of bio-ethanol knowing that these staple crops are the major food crops in Nigeria. The government road map for achieving the bioethanol target of 5.14 billion litres/year distances itself from how this will not lead to food shortage in Nigeria. Therefore, the establishment of bioethanol plants and diversion of large hectares of lands for growing crops for the production of bioethanol will further impoverish the general populace. Agricultural wastes are cost effective, renewable and abundant. Bioethanol from agricultural waste could be a promising technology though the process has several challenges and limitations such as biomass transport and handling, and efficient pretreatment methods for total delignification of lignocelluloses.

SUGGESTION FOR FURTHER WORK

The crude extract of cashew nut shell was used in this work for hydrolytic purpose. This could be studied further to determine the active hydrolytic content.

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APPENDIX I

ONEWAY ANALYSIS OF VARIENCE FOR:

APPENDIX IA

AMOUNT OF ETHANOL PRODUCED BY SUBSTRATE HYDROLYSED WITH 50% HCl

Descriptives

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
Schizosaccharomyces pombe	1.00	2	19.4800	1.4142	1.0000	6.7738	32.1862	18.48	20.48
	2.00	2	33.3400	3.9739	2.8100	-2.3644	69.0444	30.53	36.15
	3.00	2	20.6850	3.1183	2.2050	-7.3322	48.7022	18.48	22.89
	4.00	2	.0000	.0000	.0000	.0000	.0000	.00	.00
	5.00	2	8.8350	5.6781	4.0150	-42.1804	59.8504	4.82	12.85
	6.00	2	13.2550	.5728	.4050	8.1090	18.4010	12.85	13.66
	7.00	2	.6050	.8556	.6050	-7.0823	8.2923	.00	1.21
	8.00	2	15.0600	3.1254	2.2100	-13.0207	43.1407	12.85	17.27
	9.00	2	.0000	.0000	.0000	.0000	.0000	.00	.00
	10.00	2	.0000	.0000	.0000	.0000	.0000	.00	.00
	11.00	2	19.2800	2.2769	1.6100	-1.1770	39.7370	17.67	20.89
	12.00	2	.0000	.0000	.0000	.0000	.0000	.00	.00
	Total	24	10.8783	10.9915	2.2436	6.2370	15.5197	.00	36.15
Kluyveromyces marxianus	1.00	2	11.8450	1.4213	1.0050	-.9247	24.6147	10.84	12.85
	2.00	2	12.2500	7.6650	5.4200	-56.6176	81.1176	6.83	17.67
	3.00	2	21.8900	9.9419	7.0300	-67.4346	111.2146	14.86	28.92
	4.00	2	.0000	.0000	.0000	.0000	.0000	.00	.00
	5.00	2	15.2650	2.2698	1.6050	-5.1285	35.6585	13.66	16.87
	6.00	2	9.0350	2.5527	1.8050	-13.8997	31.9697	7.23	10.84
	7.00	2	7.8300	.2828	.2000	5.2888	10.3712	7.63	8.03
	8.00	2	3.8100	1.9940	1.4100	-14.1057	21.7257	2.40	5.22
	9.00	2	.0000	.0000	.0000	.0000	.0000	.00	.00
	10.00	2	.0000	.0000	.0000	.0000	.0000	.00	.00
	11.00	2	11.2450	1.1384	.8050	1.0165	21.4735	10.44	12.05
	12.00	2	.0000	.0000	.0000	.0000	.0000	.00	.00
	Total	24	7.7642	7.5262	1.5363	4.5861	10.9422	.00	28.92
Pichia caribbica	1.00	2	25.3050	3.9810	2.8150	-10.4630	61.0730	22.49	28.12
	2.00	2	18.0750	3.9810	2.8150	-17.6930	53.8430	15.26	20.89
	3.00	2	24.5000	4.5396	3.2100	-16.2869	65.2869	21.29	27.71
	4.00	2	.0000	.0000	.0000	.0000	.0000	.00	.00
	5.00	2	18.8800	3.4083	2.4100	-11.7420	49.5020	16.47	21.29
	6.00	2	14.0550	2.2698	1.6050	-6.3385	34.4485	12.45	15.66
	7.00	2	4.6200	1.4142	1.0000	-8.0862	17.3262	3.62	5.62
	8.00	2	26.3050	1.9870	1.4050	8.4528	44.1572	24.90	27.71
	9.00	2	11.2450	4.5467	3.2150	-29.6054	52.0954	8.03	14.46
	10.00	2	5.8250	1.9870	1.4050	-12.0272	23.6772	4.42	7.23
	11.00	2	20.6850	5.9609	4.2150	-32.8717	74.2417	16.47	24.90
	12.00	2	.0000	.0000	.0000	.0000	.0000	.00	.00
	Total	24	14.1246	9.7641	1.9931	10.0016	18.2476	.00	28.12
Candida tropicalis strain A	1.00	2	14.0550	1.7041	1.2050	-1.2560	29.3660	12.85	15.26
	2.00	2	11.6500	2.8426	2.0100	-13.8895	37.1895	9.64	13.66
	3.00	2	15.4650	1.9870	1.4050	-2.3872	33.3172	14.06	16.87
	4.00	2	.0000	.0000	.0000	.0000	.0000	.00	.00
	5.00	2	11.8450	5.3952	3.8150	-36.6292	60.3192	8.03	15.66
	6.00	2	6.6250	1.9870	1.4050	-11.2272	24.4772	5.22	8.03
	7.00	2	.0000	.0000	.0000	.0000	.0000	.00	.00
	8.00	2	17.0700	6.5337	4.6200	-41.6327	75.7727	12.45	21.69
	9.00	2	7.6350	4.5326	3.2050	-33.0884	48.3584	4.43	10.84
	10.00	2	4.0150	2.2698	1.6050	-16.3785	24.4085	2.41	5.62
	11.00	2	11.2450	2.2698	1.6050	-9.1485	31.6385	9.64	12.85
	12.00	2	.0000	.0000	.0000	.0000	.0000	.00	.00
	Total	24	8.3004	6.4799	1.3227	5.5642	11.0366	.00	21.69

Appendix I cont'd

Descriptives

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
Candida tropicalis strain B	1.00	2	30.5250	5.1124	3.6150	-15.4079	76.4579	26.91	34.14
	2.00	2	28.1100	4.5396	3.2100	-12.6769	68.8969	24.90	31.32
	3.00	2	28.7150	5.3952	3.8150	-19.7592	77.1892	24.90	32.53
	4.00	2	.0000	.0000	.0000	.0000	.0000	.00	.00
	5.00	2	7.6300	2.2769	1.6100	-12.8270	28.0870	6.02	9.24
	6.00	2	6.6250	.8556	.6050	-1.0623	14.3123	6.02	7.23
	7.00	2	.0000	.0000	.0000	.0000	.0000	.00	.00
	8.00	2	9.8400	4.2568	3.0100	-28.4057	48.0857	6.83	12.85
	9.00	2	10.4450	1.1384	.8050	.2165	20.6735	9.64	11.25
	10.00	2	6.8250	2.2698	1.6050	-13.5685	27.2185	5.22	8.43
	11.00	2	13.2550	5.6781	4.0150	-37.7604	64.2704	9.24	17.27
	12.00	2	.0000	.0000	.0000	.0000	.0000	.00	.00
	Total	24	11.8308	11.2924	2.3051	7.0625	16.5992	.00	34.14
Saccharomyce cerevisiae A	1.00	2	15.6650	1.7041	1.2050	.3540	30.9760	14.46	16.87
	2.00	2	9.8400	.2828	.2000	7.2988	12.3812	9.64	10.04
	3.00	2	14.4600	2.2769	1.6100	-5.9970	34.9170	12.85	16.07
	4.00	2	.0000	.0000	.0000	.0000	.0000	.00	.00
	5.00	2	19.0800	2.5597	1.8100	-3.9182	42.0782	17.27	20.89
	6.00	2	4.4200	.5657	.4000	-.6625	9.5025	4.02	4.82
	7.00	2	5.6250	1.1384	.8050	-4.6035	15.8535	4.82	6.43
	8.00	2	9.8400	1.4142	1.0000	-2.8662	22.5462	8.84	10.84
	9.00	2	4.0150	1.1384	.8050	-6.2135	14.2435	3.21	4.82
	10.00	2	11.4450	.8556	.6050	3.7577	19.1323	10.84	12.05
	11.00	2	15.2650	2.2698	1.6050	-5.1285	35.6585	13.66	16.87
	12.00	2	.0000	.0000	.0000	.0000	.0000	.00	.00
	Total	24	9.1379	6.2847	1.2829	6.4841	11.7917	.00	20.89
Saccharomyce cerevisiae B	1.00	2	18.4750	4.5467	3.2150	-22.3754	59.3254	15.26	21.69
	2.00	2	12.2500	3.6911	2.6100	-20.9132	45.4132	9.64	14.86
	3.00	2	28.1200	3.4083	2.4100	-2.5020	58.7420	25.71	30.53
	4.00	2	.0000	.0000	.0000	.0000	.0000	.00	.00
	5.00	2	10.4400	.5657	.4000	5.3575	15.5225	10.04	10.84
	6.00	2	8.6350	.2899	.2050	6.0302	11.2398	8.43	8.84
	7.00	2	1.8050	2.5527	1.8050	-21.1297	24.7397	.00	3.61
	8.00	2	19.0650	2.5385	1.7950	-3.7426	41.8726	17.27	20.86
	9.00	2	23.0950	1.9870	1.4050	5.2428	40.9472	21.69	24.50
	10.00	2	.0000	.0000	.0000	.0000	.0000	.00	.00
	11.00	2	23.4950	1.4213	1.0050	10.7253	36.2647	22.49	24.50
	12.00	2	.0000	.0000	.0000	.0000	.0000	.00	.00
	Total	24	12.1150	10.1688	2.0757	7.8211	16.4089	.00	30.53
Candida krusei	1.00	2	19.6800	10.2248	7.2300	-72.1859	111.5459	12.45	26.91
	2.00	2	6.0250	1.7041	1.2050	-9.2860	21.3360	4.82	7.23
	3.00	2	7.4300	2.5597	1.8100	-15.5682	30.4282	5.62	9.24
	4.00	2	.0000	.0000	.0000	.0000	.0000	.00	.00
	5.00	2	11.6450	4.5467	3.2150	-29.2054	52.4954	8.43	14.86
	6.00	2	6.8250	2.2698	1.6050	-13.5685	27.2185	5.22	8.43
	7.00	2	6.6250	.8556	.6050	-1.0623	14.3123	6.02	7.23
	8.00	2	7.8400	4.8225	3.4100	-35.4882	51.1682	4.43	11.25
	9.00	2	7.4300	1.9940	1.4100	-10.4857	25.3457	6.02	8.84
	10.00	2	16.4650	2.8496	2.0150	-9.1380	42.0680	14.45	18.48
	11.00	2	17.0700	5.9680	4.2200	-36.5502	70.6902	12.85	21.29
	12.00	2	.0000	.0000	.0000	.0000	.0000	.00	.00
	Total	24	8.9196	6.8213	1.3924	6.0392	11.8000	.00	26.91

Appendix IA cont'd

Homogeneous Subsets

Schizosaccharomyces pombe

Duncan[a]

		Subset for alpha = .05				
VAR00001	N	1	2	3	4	5
4.00	2	.0000				
9.00	2	.0000				
10.00	2	.0000				
12.00	2	.0000				
7.00	2	.6050				
5.00	2		8.8350			
6.00	2		13.2550	13.2550		
8.00	2			15.0600	15.0600	
11.00	2				19.2800	
1.00	2				19.4800	
3.00	2				20.6850	
2.00	2					33.3400
Sig.		.829	.104	.486	.059	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2.000.

Kluyveromyces marxianus

Duncan[a]

		Subset for alpha = .05			
VAR00001	N	1	2	3	4
4.00	2	.0000			
9.00	2	.0000			
10.00	2	.0000			
12.00	2	.0000			
8.00	2	3.8100	3.8100		
7.00	2	7.8300	7.8300	7.8300	
6.00	2	9.0350	9.0350	9.0350	
11.00	2		11.2450	11.2450	
1.00	2		11.8450	11.8450	
2.00	2		12.2500	12.2500	
5.00	2			15.2650	15.2650
3.00	2				21.8900
Sig.		.056	.070	.105	.110

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2.000.

Pichia caribbica

Duncan[a]

VAR00001	N	Subset for alpha = .05					
		1	2	3	4	5	6
4.00	2	.0000					
12.00	2	.0000					
7.00	2	4.6200	4.6200				
10.00	2	5.8250	5.8250				
9.00	2		11.2450	11.2450			
6.00	2			14.0550	14.0550		
2.00	2			18.0750	18.0750	18.0750	
5.00	2			18.8800	18.8800	18.8800	18.8800
11.00	2				20.6850	20.6850	20.6850
3.00	2					24.5000	24.5000
1.00	2					25.3050	25.3050
8.00	2						26.3050
Sig.		.133	.084	.056	.091	.073	.066

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2.000.

Candida tropicalis strain A

Duncan[a]

VAR00001	N	Subset for alpha = .05			
		1	2	3	4
4.00	2	.0000			
7.00	2	.0000			
12.00	2	.0000			
10.00	2	4.0150	4.0150		
6.00	2	6.6250	6.6250	6.6250	
9.00	2	7.6350	7.6350	7.6350	
11.00	2		11.2450	11.2450	11.2450
2.00	2			11.6500	11.6500
5.00	2			11.8450	11.8450
1.00	2			14.0550	14.0550
3.00	2				15.4650
8.00	2				17.0700
Sig.		.051	.056	.057	.124

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2.000.

Candida tropicalis strain B

Duncan[a]

		Subset for alpha = .05		
VAR00001	N	1	2	3
4.00	2	.0000		
7.00	2	.0000		
12.00	2	.0000		
6.00	2	6.6250	6.6250	
10.00	2	6.8250	6.8250	
5.00	2	7.6300	7.6300	
8.00	2		9.8400	
9.00	2		10.4450	
11.00	2		13.2550	
2.00	2			28.1100
3.00	2			28.7150
1.00	2			30.5250
Sig.		.065	.103	.512

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2.000.

Saccharomyce cerevisiae A

Duncan[a]

		Subset for alpha = .05					
VAR00001	N	1	2	3	4	5	6
4.00	2	.0000					
12.00	2	.0000					
9.00	2		4.0150				
6.00	2		4.4200				
7.00	2		5.6250				
2.00	2			9.8400			
8.00	2			9.8400			
10.00	2			11.4450	11.4450		
3.00	2				14.4600	14.4600	
11.00	2					15.2650	
1.00	2					15.6650	
5.00	2						19.0800
Sig.		1.000	.314	.316	.061	.447	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2.000.

Saccharomyce cerevisiae B

Duncan[a]

VAR00001	N	Subset for alpha = .05			
		1	2	3	4
4.00	2	.0000			
10.00	2	.0000			
12.00	2	.0000			
7.00	2	1.8050			
6.00	2		8.6350		
5.00	2		10.4400		
2.00	2		12.2500		
1.00	2			18.4750	
8.00	2			19.0650	
9.00	2			23.0950	23.0950
11.00	2			23.4950	23.4950
3.00	2				28.1200
Sig.		.487	.165	.069	.062

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2.000.

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Schizosaccharomyces pombe	Between Groups	2702.956	11	245.723	38.917	.000
	Within Groups	75.769	12	6.314		
	Total	2778.725	23			
Kluyveromyces marxianus	Between Groups	1126.155	11	102.378	6.955	.001
	Within Groups	176.635	12	14.720		
	Total	1302.790	23			
Pichia caribbica	Between Groups	2057.594	11	187.054	16.606	.000
	Within Groups	135.174	12	11.265		
	Total	2192.769	23			
Candida tropicalis strain A	Between Groups	844.224	11	76.748	7.578	.001
	Within Groups	121.526	12	10.127		
	Total	965.749	23			

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Candida tropicalis strain B	Between Groups	2794.365	11	254.033	21.998	.000
	Within Groups	138.578	12	11.548		
	Total	2932.943	23			
Saccharomyce cerevisiae A	Between Groups	882.937	11	80.267	37.748	.000
	Within Groups	25.517	12	2.126		
	Total	908.454	23			
Saccharomyce cerevisiae B	Between Groups	2313.054	11	210.278	38.675	.000
	Within Groups	65.245	12	5.437		
	Total	2378.299	23			
Candida krusei	Between Groups	858.658	11	78.060	4.428	.008
	Within Groups	211.528	12	17.627		
	Total	1070.187	23			

Post Hoc Tests

APPENDIX IB

AMOUNT OF ETHANOL PRODUCED BY SUBSTRATE HYDROLYSED WITH 40% HCl

Descriptives

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
Schizosaccharomyces pombe	1.00	2	16.4700	2.8426	2.0100	-9.0695	42.0095	14.46	18.48
	2.00	2	21.4850	4.8295	3.4150	-21.9067	64.8767	18.07	24.90
	3.00	2	11.8500	.8485	.6000	4.2263	19.4737	11.25	12.45
	4.00	2	.0000	.0000	.0000	.0000	.0000	.00	.00
	5.00	2	7.4300	1.4142	1.0000	-5.2762	20.1362	6.43	8.43
	6.00	2	20.8850	6.2438	4.4150	-35.2129	76.9829	16.47	25.30
	7.00	2	.0000	.0000	.0000	.0000	.0000	.00	.00
	8.00	2	15.2650	1.1384	.8050	5.0365	25.4935	14.46	16.07
	9.00	2	.0000	.0000	.0000	.0000	.0000	.00	.00
	10.00	2	11.8500	.8485	.6000	4.2263	19.4737	11.25	12.45
	11.00	2	9.8400	1.9940	1.4100	-8.0757	27.7557	8.43	11.25
	12.00	2	.0000	.0000	.0000	.0000	.0000	.00	.00
	Total	24	9.5896	8.1879	1.6713	6.1321	13.0470	.00	25.30
Kluyveromyces marxianus	1.00	2	12.4550	1.7041	1.2050	-2.8560	27.7660	11.25	13.66
	2.00	2	10.6400	3.1254	2.2100	-17.4407	38.7207	8.43	12.85
	3.00	2	25.9050	4.8295	3.4150	-17.4867	69.2967	22.49	29.32
	4.00	2	.0000	.0000	.0000	.0000	.0000	.00	.00
	5.00	2	15.8650	.8556	.6050	8.1777	23.5523	15.26	16.47
	6.00	2	10.8400	.0000	.0000	10.8400	10.8400	10.84	10.84
	7.00	2	.0000	.0000	.0000	.0000	.0000	.00	.00
	8.00	2	13.4550	4.2639	3.0150	-24.8542	51.7642	10.44	16.47
	9.00	2	.0000	.0000	.0000	.0000	.0000	.00	.00
	10.00	2	6.2250	1.4213	1.0050	-6.5447	18.9947	5.22	7.23
	11.00	2	6.8250	2.2698	1.6050	-13.5685	27.2185	5.22	8.43
	12.00	2	.0000	.0000	.0000	.0000	.0000	.00	.00
	Total	24	8.5175	7.9970	1.6324	5.1407	11.8943	.00	29.32
Pichia caribbica	1.00	2	17.4750	1.4213	1.0050	4.7053	30.2447	16.47	18.48
	2.00	2	23.3000	3.4083	2.4100	-7.3220	53.9220	20.89	25.71
	3.00	2	16.8700	1.1314	.8000	6.7050	27.0350	16.07	17.67
	4.00	2	.0000	.0000	.0000	.0000	.0000	.00	.00
	5.00	2	6.4250	9.0863	6.4250	-75.2124	88.0624	.00	12.85
	6.00	2	15.2650	2.2698	1.6050	-5.1285	35.6585	13.66	16.87
	7.00	2	.0000	.0000	.0000	.0000	.0000	.00	.00
	8.00	2	10.4400	3.4083	2.4100	-20.1820	41.0620	8.03	12.85
	9.00	2	.0000	.0000	.0000	.0000	.0000	.00	.00
	10.00	2	9.2400	.5657	.4000	4.1575	14.3225	8.84	9.64
	11.00	2	13.8550	1.9870	1.4050	-3.9972	31.7072	12.45	15.26
	12.00	2	.0000	.0000	.0000	.0000	.0000	.00	.00
	Total	24	9.4058	8.2941	1.6930	5.9035	12.9081	.00	25.71
Candida tropicalis strain A	1.00	2	6.8250	2.2698	1.6050	-13.5685	27.2185	5.22	8.43
	2.00	2	6.8250	1.7041	1.2050	-8.4860	22.1360	5.62	8.03
	3.00	2	9.4400	.8485	.6000	1.8163	17.0637	8.84	10.04
	4.00	2	.0000	.0000	.0000	.0000	.0000	.00	.00
	5.00	2	12.6500	.8485	.6000	5.0263	20.2737	12.05	13.25
	6.00	2	6.2250	1.9870	1.4050	-11.6272	24.0772	4.82	7.63
	7.00	2	.0000	.0000	.0000	.0000	.0000	.00	.00
	8.00	2	12.0500	1.1314	.8000	1.8850	22.2150	11.25	12.85
	9.00	2	.0000	.0000	.0000	.0000	.0000	.00	.00
	10.00	2	3.2150	1.1384	.8050	-7.0135	13.4435	2.41	4.02
	11.00	2	13.2550	1.7041	1.2050	-2.0560	28.5660	12.05	14.46
	12.00	2	.0000	.0000	.0000	.0000	.0000	.00	.00
	Total	24	5.8738	5.1729	1.0559	3.6894	8.0581	.00	14.46

Descriptives

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
Candida tropicalis strain B	1.00	2	19.8800	3.6911	2.6100	-13.2832	53.0432	17.27	22.49
	2.00	2	8.4350	1.7041	1.2050	-6.8760	23.7460	7.23	9.64
	3.00	2	15.0650	19.0282	13.4550	-155.8970	186.0270	1.61	28.52
	4.00	2	.0000	.0000	.0000	.0000	.0000	.00	.00
	5.00	2	9.2400	.0000	.0000	9.2400	9.2400	9.24	9.24
	6.00	2	4.2150	1.4213	1.0050	-8.5547	16.9847	3.21	5.22
	7.00	2	.0000	.0000	.0000	.0000	.0000	.00	.00
	8.00	2	4.6150	1.9870	1.4050	-13.2372	22.4672	3.21	6.02
	9.00	2	.0000	.0000	.0000	.0000	.0000	.00	.00
	10.00	2	4.6200	1.4142	1.0000	-8.0862	17.3262	3.62	5.62
	11.00	2	11.0450	1.9870	1.4050	-6.8072	28.8972	9.64	12.45
	12.00	2	.0000	.0000	.0000	.0000	.0000	.00	.00
	Total	24	6.4262	7.5803	1.5473	3.2254	9.6271	.00	28.52
Saccharomyce cerevisiae A	1.00	2	18.6800	3.1254	2.2100	-9.4007	46.7607	16.47	20.89
	2.00	2	5.6250	2.2698	1.6050	-14.7685	26.0185	4.02	7.23
	3.00	2	6.0250	1.1384	.8050	-4.2035	16.2535	5.22	6.83
	4.00	2	.0000	.0000	.0000	.0000	.0000	.00	.00
	5.00	2	18.4750	1.7041	1.2050	3.1640	33.7860	17.27	19.68
	6.00	2	7.4300	.8485	.6000	-.1937	15.0537	6.83	8.03
	7.00	2	.0000	.0000	.0000	.0000	.0000	.00	.00
	8.00	2	4.6200	.8485	.6000	-3.0037	12.2437	4.02	5.22
	9.00	2	.0000	.0000	.0000	.0000	.0000	.00	.00
	10.00	2	15.8650	.2899	.2050	13.2602	18.4698	15.66	16.07
	11.00	2	17.2700	.5657	.4000	12.1875	22.3525	16.87	17.67
	12.00	2	.0000	.0000	.0000	.0000	.0000	.00	.00
	Total	24	7.8325	7.5701	1.5452	4.6359	11.0291	.00	20.89
Saccharomyce cerevisiae B	1.00	2	9.4400	.8485	.6000	1.8163	17.0637	8.84	10.04
	2.00	2	9.2350	1.1384	.8050	-.9935	19.4635	8.43	10.04
	3.00	2	18.0750	6.2438	4.4150	-38.0229	74.1729	13.66	22.49
	4.00	2	.0000	.0000	.0000	.0000	.0000	.00	.00
	5.00	2	6.8300	.5657	.4000	1.7475	11.9125	6.43	7.23
	6.00	2	18.4750	.5728	.4050	13.3290	23.6210	18.07	18.88
	7.00	2	.0000	.0000	.0000	.0000	.0000	.00	.00
	8.00	2	9.8400	1.9940	1.4100	-8.0757	27.7557	8.43	11.25
	9.00	2	.0000	.0000	.0000	.0000	.0000	.00	.00
	10.00	2	17.6750	1.1384	.8050	7.4465	27.9035	16.87	18.48
	11.00	2	19.2800	1.1314	.8000	9.1150	29.4450	18.48	20.08
	12.00	2	.0000	.0000	.0000	.0000	.0000	.00	.00
	Total	24	9.0708	7.8395	1.6002	5.7605	12.3812	.00	22.49
Candida krusei	1.00	2	18.8800	3.4083	2.4100	-11.7420	49.5020	16.47	21.29
	2.00	2	9.2400	.0000	.0000	9.2400	9.2400	9.24	9.24
	3.00	2	16.6700	.2828	.2000	14.1288	19.2112	16.47	16.87
	4.00	2	.0000	.0000	.0000	.0000	.0000	.00	.00
	5.00	2	16.0650	9.0863	6.4250	-65.5724	97.7024	9.64	22.49
	6.00	2	14.8650	1.7041	1.2050	-.4460	30.1760	13.66	16.07
	7.00	2	.0000	.0000	.0000	.0000	.0000	.00	.00
	8.00	2	8.6350	1.9870	1.4050	-9.2172	26.4872	7.23	10.04
	9.00	2	.0000	.0000	.0000	.0000	.0000	.00	.00
	10.00	2	9.0350	1.4213	1.0050	-3.7347	21.8047	8.03	10.04
	11.00	2	18.4850	1.1526	.8150	8.1294	28.8406	17.67	19.30
	12.00	2	.0000	.0000	.0000	.0000	.0000	.00	.00
	Total	24	9.3229	7.8347	1.5992	6.0146	12.6312	.00	22.49

Appendix IB cont'd

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Schizosaccharomyces pombe	Between Groups	1462.848	11	132.986	20.175	.000
	Within Groups	79.101	12	6.592		
	Total	1541.949	23			
Kluyveromyces marxianus	Between Groups	1408.808	11	128.073	24.756	.000
	Within Groups	62.081	12	5.173		
	Total	1470.889	23			
Pichia caribbica	Between Groups	1463.718	11	133.065	13.473	.000
	Within Groups	118.514	12	9.876		
	Total	1582.232	23			
Candida tropicalis strain A	Between Groups	596.541	11	54.231	34.388	.000
	Within Groups	18.924	12	1.577		
	Total	615.465	23			

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Candida tropicalis strain B	Between Groups	931.072	11	84.643	2.601	.058
	Within Groups	390.518	12	32.543		
	Total	1321.591	23			
Saccharomyce cerevisiae A	Between Groups	1297.066	11	117.915	67.494	.000
	Within Groups	20.964	12	1.747		
	Total	1318.030	23			
Saccharomyce cerevisiae B	Between Groups	1365.337	11	124.122	30.901	.000
	Within Groups	48.201	12	4.017		
	Total	1413.538	23			
Candida krusei	Between Groups	1307.323	11	118.848	13.653	.000
	Within Groups	104.458	12	8.705		
	Total	1411.781	23			

Post Hoc Tests

Homogeneous Subsets

Schizosaccharomyces pombe

Duncan[a]

VAR00001	N	Subset for alpha = .05					
		1	2	3	4	5	6
4.00	2	.0000					
7.00	2	.0000					
9.00	2	.0000					
12.00	2	.0000					
5.00	2		7.4300				
11.00	2		9.8400	9.8400			
3.00	2		11.8500	11.8500	11.8500		
10.00	2		11.8500	11.8500	11.8500		
8.00	2			15.2650	15.2650	15.2650	
1.00	2				16.4700	16.4700	16.4700
6.00	2					20.8850	20.8850
2.00	2						21.4850
Sig.		1.000	.136	.073	.121	.059	.087

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2.000.

Kluyveromyces marxianus

Duncan[a]

VAR00001	N	Subset for alpha = .05			
		1	2	3	4
4.00	2	.0000			
7.00	2	.0000			
9.00	2	.0000			
12.00	2	.0000			
10.00	2		6.2250		
11.00	2		6.8250		
2.00	2		10.6400	10.6400	
6.00	2		10.8400	10.8400	
1.00	2			12.4550	
8.00	2			13.4550	
5.00	2			15.8650	
3.00	2				25.9050
Sig.		1.000	.084	.058	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2.000.

Pichia caribbica

Duncan[a]

		Subset for alpha = .05				
VAR00001	N	1	2	3	4	5
4.00	2	.0000				
7.00	2	.0000				
9.00	2	.0000				
12.00	2	.0000				
5.00	2	6.4250	6.4250			
10.00	2		9.2400	9.2400		
8.00	2		10.4400	10.4400	10.4400	
11.00	2			13.8550	13.8550	
6.00	2			15.2650	15.2650	
3.00	2				16.8700	16.8700
1.00	2				17.4750	17.4750
2.00	2					23.3000
Sig.		.086	.247	.100	.063	.075

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2.000.

Candida tropicalis strain A

Duncan[a]

		Subset for alpha = .05					
VAR00001	N	1	2	3	4	5	6
4.00	2	.0000					
7.00	2	.0000					
9.00	2	.0000					
12.00	2	.0000					
10.00	2		3.2150				
6.00	2			6.2250			
1.00	2			6.8250	6.8250		
2.00	2			6.8250	6.8250		
3.00	2				9.4400	9.4400	
8.00	2					12.0500	12.0500
5.00	2						12.6500
11.00	2						13.2550
Sig.		1.000	1.000	.658	.070	.060	.379

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2.000.

Candida tropicalis strain B

Duncan[a]

VAR00001	N	Subset for alpha = .05		
		1	2	3
4.00	2	.0000		
7.00	2	.0000		
9.00	2	.0000		
12.00	2	.0000		
6.00	2	4.2150	4.2150	
8.00	2	4.6150	4.6150	
10.00	2	4.6200	4.6200	
2.00	2	8.4350	8.4350	8.4350
5.00	2	9.2400	9.2400	9.2400
11.00	2	11.0450	11.0450	11.0450
3.00	2		15.0650	15.0650
1.00	2			19.8800
Sig.		.110	.113	.092

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2.000.

Saccharomyce cerevisiae A

Duncan[a]

VAR00001	N	Subset for alpha = .05		
		1	2	3
4.00	2	.0000		
7.00	2	.0000		
9.00	2	.0000		
12.00	2	.0000		
8.00	2		4.6200	
2.00	2		5.6250	
3.00	2		6.0250	
6.00	2		7.4300	
10.00	2			15.8650
11.00	2			17.2700
5.00	2			18.4750
1.00	2			18.6800
Sig.		1.000	.072	.071

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2.000.

Candida krusei

Duncan[a]

VAR00001	N	Subset for alpha = .05		
		1	2	3
4.00	2	.0000		
7.00	2	.0000		
9.00	2	.0000		
12.00	2	.0000		
8.00	2		8.6350	
10.00	2		9.0350	
2.00	2		9.2400	
6.00	2		14.8650	14.8650
5.00	2			16.0650
3.00	2			16.6700
11.00	2			18.4850
1.00	2			18.8800
Sig.		1.000	.073	.237

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2.000.

APPENDIX IC

AMOUNT OF ETHANOL PRODUCED BY SUBSTRATE HYDROLYSED WITH 50% H_2SO_4

Descriptives

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
Schizosaccharomyces pombe	1.00	2	21.6900	7.3822	5.2200	-44.6364	88.0164	16.47	26.91
	2.00	2	28.1150	2.2698	1.6050	7.7215	48.5085	26.51	29.72
	3.00	2	16.2700	.2828	.2000	13.7288	18.8112	16.07	16.47
	4.00	2	13.0550	.8556	.6050	5.3677	20.7423	12.45	13.66
	5.00	2	10.0400	.5657	.4000	4.9575	15.1225	9.64	10.44
	6.00	2	.0000	.0000	.0000	.0000	.0000	.00	.00
	7.00	2	9.6400	1.1314	.8000	-.5250	19.8050	8.84	10.44
	8.00	2	17.2700	1.1314	.8000	7.1050	27.4350	16.47	18.07
	9.00	2	12.6550	1.4213	1.0050	-.1147	25.4247	11.65	13.66
	10.00	2	.0000	.0000	.0000	.0000	.0000	.00	.00
	11.00	2	10.6450	1.9870	1.4050	-7.2072	28.4972	9.24	12.05
	12.00	2	10.0450	1.7041	1.2050	-5.2660	25.3560	8.84	11.25
	Total	24	12.4521	8.0101	1.6351	9.0697	15.8345	.00	29.72
Kluyveromyces marxianus	1.00	2	13.0550	.8556	.6050	5.3677	20.7423	12.45	13.66
	2.00	2	16.0650	1.1384	.8050	5.8365	26.2935	15.26	16.87
	3.00	2	11.6450	1.1384	.8050	1.4165	21.8735	10.84	12.45
	4.00	2	17.4750	5.3952	3.8150	-30.9992	65.9492	13.66	21.29
	5.00	2	12.6550	1.4213	1.0050	-.1147	25.4247	11.65	13.66
	6.00	2	.0000	.0000	.0000	.0000	.0000	.00	.00
	7.00	2	8.4350	1.7041	1.2050	-6.8760	23.7460	7.23	9.64
	8.00	2	4.4150	1.7041	1.2050	-10.8960	19.7260	3.21	5.62
	9.00	2	10.4400	.5657	.4000	5.3575	15.5225	10.04	10.84
	10.00	2	.0000	.0000	.0000	.0000	.0000	.00	.00
	11.00	2	9.2350	1.1384	.8050	-.9935	19.4635	8.43	10.04
	12.00	2	7.8300	.2828	.2000	5.2888	10.3712	7.63	8.03
	Total	24	9.2708	5.6374	1.1507	6.8904	11.6513	.00	21.29
Pichia caribbica	1.00	2	23.6950	1.1384	.8050	13.4665	33.9235	22.89	24.50
	2.00	2	21.4850	1.4213	1.0050	8.7153	34.2547	20.48	22.49
	3.00	2	12.8500	.5657	.4000	7.7675	17.9325	12.45	13.25
	4.00	2	9.0350	.8556	.6050	1.3477	16.7223	8.43	9.64
	5.00	2	15.8650	.8556	.6050	8.1777	23.5523	15.26	16.47
	6.00	2	.0000	.0000	.0000	.0000	.0000	.00	.00
	7.00	2	11.8450	1.4213	1.0050	-.9247	24.6147	10.84	12.85
	8.00	2	13.2550	1.1384	.8050	3.0265	23.4835	12.45	14.06
	9.00	2	16.6700	.8485	.6000	9.0463	24.2937	16.07	17.27
	10.00	2	.0000	.0000	.0000	.0000	.0000	.00	.00
	11.00	2	9.8400	1.9940	1.4100	-8.0757	27.7557	8.43	11.25
	12.00	2	8.4350	2.2698	1.6050	-11.9585	28.8285	6.83	10.04
	Total	24	11.9146	7.1567	1.4609	8.8926	14.9366	.00	24.50
Candida tropicalis strain A	1.00	2	15.4650	1.9870	1.4050	-2.3872	33.3172	14.06	16.87
	2.00	2	7.0250	1.4213	1.0050	-5.7447	19.7947	6.02	8.03
	3.00	2	20.2850	7.6721	5.4250	-48.6462	89.2162	14.86	25.71
	4.00	2	17.6750	1.1384	.8050	7.4465	27.9035	16.87	18.48
	5.00	2	22.6900	3.6911	2.6100	-10.4732	55.8532	20.08	25.30
	6.00	2	.0000	.0000	.0000	.0000	.0000	.00	.00
	7.00	2	12.4500	2.8426	2.0100	-13.0895	37.9895	10.44	14.46
	8.00	2	16.2700	.2828	.2000	13.7288	18.8112	16.07	16.47
	9.00	2	11.4450	1.4213	1.0050	-1.3247	24.2147	10.44	12.45
	10.00	2	4.0150	2.2698	1.6050	-16.3785	24.4085	2.41	5.62
	11.00	2	15.6650	1.7041	1.2050	.3540	30.9760	14.46	16.87
	12.00	2	7.4300	1.4142	1.0000	-5.2762	20.1362	6.43	8.43
	Total	24	12.5346	6.9736	1.4235	9.5899	15.4793	.00	25.71

Descriptives

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
Candida tropicalis strain B	1.00	2	20.8850	5.1124	3.6150	-25.0479	66.8179	17.27	24.50
	2.00	2	6.8250	2.2698	1.6050	-13.5685	27.2185	5.22	8.43
	3.00	2	9.6350	1.7041	1.2050	-5.6760	24.9460	8.43	10.84
	4.00	2	12.8550	1.1384	.8050	2.6265	23.0835	12.05	13.66
	5.00	2	9.4400	2.5597	1.8100	-13.5582	32.4382	7.63	11.25
	6.00	2	.0000	.0000	.0000	.0000	.0000	.00	.00
	7.00	2	2.8100	3.9739	2.8100	-32.8944	38.5144	.00	5.62
	8.00	2	7.0250	1.4213	1.0050	-5.7447	19.7947	6.02	8.03
	9.00	2	6.2250	1.4213	1.0050	-6.5447	18.9947	5.22	7.23
	10.00	2	.0000	.0000	.0000	.0000	.0000	.00	.00
	11.00	2	16.2650	.8556	.6050	8.5777	23.9523	15.66	16.87
	12.00	2	11.4450	1.4213	1.0050	-1.3247	24.2147	10.44	12.45
	Total	24	8.6175	6.3429	1.2947	5.9391	11.2959	.00	24.50
Saccharomyce cerevisiae A	1.00	2	14.4600	2.2769	1.6100	-5.9970	34.9170	12.85	16.07
	2.00	2	12.0500	1.1314	.8000	1.8850	22.2150	11.25	12.85
	3.00	2	17.8750	.8556	.6050	10.1877	25.5623	17.27	18.48
	4.00	2	2.4100	3.4083	2.4100	-28.2120	33.0320	.00	4.82
	5.00	2	23.6950	1.7041	1.2050	8.3840	39.0060	22.49	24.90
	6.00	2	.0000	.0000	.0000	.0000	.0000	.00	.00
	7.00	2	13.6550	3.9810	2.8150	-22.1130	49.4230	10.84	16.47
	8.00	2	13.2550	2.8355	2.0050	-12.2209	38.7309	11.25	15.26
	9.00	2	12.0500	1.1314	.8000	1.8850	22.2150	11.25	12.85
	10.00	2	.0000	.0000	.0000	.0000	.0000	.00	.00
	11.00	2	14.6600	1.4142	1.0000	1.9538	27.3662	13.66	15.66
	12.00	2	13.4550	1.4213	1.0050	.6853	26.2247	12.45	14.46
	Total	24	11.4637	7.1798	1.4656	8.4320	14.4955	.00	24.90
Saccharomyce cerevisiae B	1.00	2	11.2450	1.1384	.8050	1.0165	21.4735	10.44	12.05
	2.00	2	13.4550	4.8295	3.4150	-29.9367	56.8467	10.04	16.87
	3.00	2	22.0900	.5657	.4000	17.0075	27.1725	21.69	22.49
	4.00	2	11.2450	1.1384	.8050	1.0165	21.4735	10.44	12.05
	5.00	2	23.5050	5.9468	4.2050	-29.9246	76.9346	19.30	27.71
	6.00	2	.0000	.0000	.0000	.0000	.0000	.00	.00
	7.00	2	15.8650	1.9870	1.4050	-1.9872	33.7172	14.46	17.27
	8.00	2	10.2450	1.4213	1.0050	-2.5247	23.0147	9.24	11.25
	9.00	2	11.4450	2.5527	1.8050	-11.4897	34.3797	9.64	13.25
	10.00	2	7.0250	1.9870	1.4050	-10.8272	24.8772	5.62	8.43
	11.00	2	15.6650	1.7041	1.2050	.3540	30.9760	14.46	16.87
	12.00	2	6.4300	.0000	.0000	6.4300	6.4300	6.43	6.43
	Total	24	12.3513	6.6460	1.3566	9.5449	15.1576	.00	27.71
Candida krusei	1.00	2	19.6850	1.7041	1.2050	4.3740	34.9960	18.48	20.89
	2.00	2	7.6300	.5657	.4000	2.5475	12.7125	7.23	8.03
	3.00	2	12.6500	4.2568	3.0100	-25.5957	50.8957	9.64	15.66
	4.00	2	10.8450	1.7041	1.2050	-4.4660	26.1560	9.64	12.05
	5.00	2	7.6350	1.7041	1.2050	-7.6760	22.9460	6.43	8.84
	6.00	2	.0000	.0000	.0000	.0000	.0000	.00	.00
	7.00	2	8.6350	.8556	.6050	.9477	16.3223	8.03	9.24
	8.00	2	8.4350	.5728	.4050	3.2890	13.5810	8.03	8.84
	9.00	2	4.4200	.5657	.4000	-.6625	9.5025	4.02	4.82
	10.00	2	2.0100	.5657	.4000	-3.0725	7.0925	1.61	2.41
	11.00	2	12.0500	1.1314	.8000	1.8850	22.2150	11.25	12.85
	12.00	2	9.4350	1.4213	1.0050	-3.3347	22.2047	8.43	10.44
	Total	24	8.6192	5.1899	1.0594	6.4277	10.8107	.00	20.89

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Schizosaccharomyces pombe	Between Groups	1403.508	11	127.592	21.203	.000
	Within Groups	72.213	12	6.018		
	Total	1475.721	23			
Kluyveromyces marxianus	Between Groups	688.994	11	62.636	17.914	.000
	Within Groups	41.957	12	3.496		
	Total	730.951	23			
Pichia caribbica	Between Groups	1159.771	11	105.434	69.271	.000
	Within Groups	18.265	12	1.522		
	Total	1178.035	23			
Candida tropicalis strain A	Between Groups	1018.542	11	92.595	11.113	.000
	Within Groups	99.986	12	8.332		
	Total	1118.528	23			

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Candida tropicalis strain B	Between Groups	860.723	11	78.248	14.529	.000
	Within Groups	64.625	12	5.385		
	Total	925.348	23			
Saccharomyce cerevisiae A	Between Groups	1134.739	11	103.158	24.318	.000
	Within Groups	50.905	12	4.242		
	Total	1185.644	23			
Saccharomyce cerevisiae B	Between Groups	934.967	11	84.997	12.602	.000
	Within Groups	80.937	12	6.745		
	Total	1015.904	23			
Candida krusei	Between Groups	587.352	11	53.396	19.928	.000
	Within Groups	32.152	12	2.679		
	Total	619.505	23			

Post Hoc Tests

Homogeneous Subsets

Schizosaccharomyces pombe

Duncan[a]

		Subset for alpha = .05					
VAR00001	N	1	2	3	4	5	6
6.00	2	.0000					
10.00	2	.0000					
7.00	2		9.6400				
5.00	2		10.0400				
12.00	2		10.0450				
11.00	2		10.6450	10.6450			
9.00	2		12.6550	12.6550	12.6550		
4.00	2		13.0550	13.0550	13.0550		
3.00	2			16.2700	16.2700	16.2700	
8.00	2				17.2700	17.2700	
1.00	2					21.6900	
2.00	2						28.1150
Sig.		1.000	.231	.054	.106	.057	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2.000.

Kluyveromyces marxianus

Duncan[a]

		Subset for alpha = .05						
VAR00001	N	1	2	3	4	5	6	7
6.00	2	.0000						
10.00	2	.0000						
8.00	2		4.4150					
12.00	2		7.8300	7.8300				
7.00	2		8.4350	8.4350	8.4350			
11.00	2			9.2350	9.2350	9.2350		
9.00	2			10.4400	10.4400	10.4400		
3.00	2			11.6450	11.6450	11.6450		
5.00	2				12.6550	12.6550	12.6550	
1.00	2					13.0550	13.0550	
2.00	2						16.0650	16.0650
4.00	2							17.4750
Sig.		1.000	.063	.087	.061	.087	.108	.465

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2.000.

Pichia caribbica

Duncan[a]

		Subset for alpha = .05						
VAR00001	N	1	2	3	4	5	6	7
6.00	2	.0000						
10.00	2	.0000						
12.00	2		8.4350					
4.00	2		9.0350	9.0350				
11.00	2		9.8400	9.8400				
7.00	2			11.8450	11.8450			
3.00	2				12.8500			
8.00	2				13.2550	13.2550		
5.00	2					15.8650	15.8650	
9.00	2						16.6700	
2.00	2							21.4850
1.00	2							23.6950
Sig.		1.000	.300	.050	.298	.056	.526	.098

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2.000.

Candida tropicalis strain A

Duncan[a]

VAR00001	N	Subset for alpha = .05					
		1	2	3	4	5	6
6.00	2	.0000					
10.00	2	4.0150	4.0150				
2.00	2		7.0250	7.0250			
12.00	2		7.4300	7.4300			
9.00	2			11.4450	11.4450		
7.00	2			12.4500	12.4500		
1.00	2				15.4650	15.4650	
11.00	2				15.6650	15.6650	
8.00	2				16.2700	16.2700	16.2700
4.00	2				17.6750	17.6750	17.6750
3.00	2					20.2850	20.2850
5.00	2						22.6900
Sig.		.189	.282	.106	.075	.153	.061

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2.000.

Candida tropicalis strain B

Duncan[a]

VAR00001	N	Subset for alpha = .05					
		1	2	3	4	5	6
6.00	2	.0000					
10.00	2	.0000					
7.00	2	2.8100	2.8100				
9.00	2		6.2250	6.2250			
2.00	2		6.8250	6.8250			
8.00	2		7.0250	7.0250			
5.00	2			9.4400	9.4400		
3.00	2			9.6350	9.6350		
12.00	2			11.4450	11.4450	11.4450	
4.00	2				12.8550	12.8550	
11.00	2					16.2650	16.2650
1.00	2						20.8850
Sig.		.272	.118	.065	.197	.071	.070

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2.000.

Saccharomyce cerevisiae A

Duncan[a]

VAR00001	N	Subset for alpha = .05			
		1	2	3	4
6.00	2	.0000			
10.00	2	.0000			
4.00	2	2.4100			
2.00	2		12.0500		
9.00	2		12.0500		
8.00	2		13.2550	13.2550	
12.00	2		13.4550	13.4550	
7.00	2		13.6550	13.6550	
1.00	2		14.4600	14.4600	
11.00	2		14.6600	14.6600	
3.00	2			17.8750	
5.00	2				23.6950
Sig.		.287	.274	.066	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2.000.

Saccharomyce cerevisiae B

Duncan[a]

VAR00001	N	Subset for alpha = .05			
		1	2	3	4
6.00	2	.0000			
12.00	2		6.4300		
10.00	2		7.0250		
8.00	2		10.2450	10.2450	
1.00	2		11.2450	11.2450	
4.00	2		11.2450	11.2450	
9.00	2		11.4450	11.4450	
2.00	2			13.4550	
11.00	2			15.6650	
7.00	2			15.8650	
3.00	2				22.0900
5.00	2				23.5050
Sig.		1.000	.106	.076	.596

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2.000.

Candida krusei

Duncan[a]

VAR00001	N	Subset for alpha = .05						
		1	2	3	4	5	6	7
6.00	2	.0000						
10.00	2	2.0100	2.0100					
9.00	2		4.4200	4.4200				
2.00	2			7.6300	7.6300			
5.00	2			7.6350	7.6350			
8.00	2				8.4350	8.4350		
7.00	2				8.6350	8.6350		
12.00	2				9.4350	9.4350	9.4350	
4.00	2				10.8450	10.8450	10.8450	
11.00	2					12.0500	12.0500	
3.00	2						12.6500	
1.00	2							19.6850
Sig.		.243	.167	.085	.101	.066	.093	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2.000.

APPENDIX ID

AMOUNT OF ETHANOL PRODUCED BY SUBSTRATE HYDROLYSED WITH 40% H_2SO_4

Descriptives

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
Schizosaccharomyces pombe	1.00	2	14.4600	2.2769	1.6100	-5.9970	34.9170	12.85	16.07
	2.00	2	13.0500	3.6911	2.6100	-20.1132	46.2132	10.44	15.66
	3.00	2	12.0500	1.1314	.8000	1.8850	22.2150	11.25	12.85
	4.00	2	6.2250	1.4213	1.0050	-6.5447	18.9947	5.22	7.23
	5.00	2	8.0300	3.9739	2.8100	-27.6744	43.7344	5.22	10.84
	6.00	2	.0000	.0000	.0000	.0000	.0000	.00	.00
	7.00	2	20.8950	2.2557	1.5950	.6286	41.1614	19.30	22.49
	8.00	2	6.8250	1.7041	1.2050	-8.4860	22.1360	5.62	8.03
	9.00	2	9.0350	.8556	.6050	1.3477	16.7223	8.43	9.64
	10.00	2	8.8350	.5728	.4050	3.6890	13.9810	8.43	9.24
	11.00	2	15.8650	1.4213	1.0050	3.0953	28.6347	14.86	16.87
	12.00	2	7.4300	1.4142	1.0000	-5.2762	20.1362	6.43	8.43
	Total	24	10.2250	5.5048	1.1237	7.9005	12.5495	.00	22.49
Kluyveromyces marxianus	1.00	2	5.2200	.5657	.4000	.1375	10.3025	4.82	5.62
	2.00	2	11.0450	1.4213	1.0050	-1.7247	23.8147	10.04	12.05
	3.00	2	11.2450	1.1384	.8050	1.0165	21.4735	10.44	12.05
	4.00	2	9.6400	.5657	.4000	4.5575	14.7225	9.24	10.04
	5.00	2	22.0950	1.7041	1.2050	6.7840	37.4060	20.89	23.30
	6.00	2	.0000	.0000	.0000	.0000	.0000	.00	.00
	7.00	2	4.6200	.8485	.6000	-3.0037	12.2437	4.02	5.22
	8.00	2	8.6350	1.9870	1.4050	-9.2172	26.4872	7.23	10.04
	9.00	2	6.8250	2.2698	1.6050	-13.5685	27.2185	5.22	8.43
	10.00	2	.0000	.0000	.0000	.0000	.0000	.00	.00
	11.00	2	13.6550	.5728	.4050	8.5090	18.8010	13.25	14.06
	12.00	2	9.0350	.8556	.6050	1.3477	16.7223	8.43	9.64
	Total	24	8.5013	5.9637	1.2173	5.9830	11.0195	.00	23.30
Pichia caribbica	1.00	2	16.0650	1.1384	.8050	5.8365	26.2935	15.26	16.87
	2.00	2	11.8500	.8485	.6000	4.2263	19.4737	11.25	12.45
	3.00	2	17.6750	1.1384	.8050	7.4465	27.9035	16.87	18.48
	4.00	2	18.4850	1.1526	.8150	8.1294	28.8406	17.67	19.30
	5.00	2	12.4500	1.1314	.8000	2.2850	22.6150	11.65	13.25
	6.00	2	.0000	.0000	.0000	.0000	.0000	.00	.00
	7.00	2	11.6450	1.7041	1.2050	-3.6660	26.9560	10.44	12.85
	8.00	2	7.8300	.8485	.6000	.2063	15.4537	7.23	8.43
	9.00	2	18.2750	.2899	.2050	15.6702	20.8798	18.07	18.48
	10.00	2	12.0500	2.2769	1.6100	-8.4070	32.5070	10.44	13.66
	11.00	2	6.0250	.5728	.4050	.8790	11.1710	5.62	6.43
	12.00	2	9.6400	.5657	.4000	4.5575	14.7225	9.24	10.04
	Total	24	11.8325	5.4419	1.1108	9.5346	14.1304	.00	19.30
Candida tropicalis strain A	1.00	2	9.4350	1.4213	1.0050	-3.3347	22.2047	8.43	10.44
	2.00	2	3.8150	.8556	.6050	-3.8723	11.5023	3.21	4.42
	3.00	2	16.8700	1.1314	.8000	6.7050	27.0350	16.07	17.67
	4.00	2	15.2650	1.7041	1.2050	-4.5977E-02	30.5760	14.06	16.47
	5.00	2	10.4450	1.1384	.8050	.2165	20.6735	9.64	11.25
	6.00	2	.0000	.0000	.0000	.0000	.0000	.00	.00
	7.00	2	6.6250	2.5527	1.8050	-16.3097	29.5597	4.82	8.43
	8.00	2	11.8500	.8485	.6000	4.2263	19.4737	11.25	12.45
	9.00	2	2.2100	1.4142	1.0000	-10.4962	14.9162	1.21	3.21
	10.00	2	5.6250	1.1384	.8050	-4.6035	15.8535	4.82	6.43
	11.00	2	3.0150	1.9870	1.4050	-14.8372	20.8672	1.61	4.42
	12.00	2	7.4300	1.4142	1.0000	-5.2762	20.1362	6.43	8.43
	Total	24	7.7154	5.2207	1.0657	5.5109	9.9199	.00	17.67

Descriptives

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
Candida tropicalis strain B	1.00	2	12.8550	1.1384	.8050	2.6265	23.0835	12.05	13.66
	2.00	2	4.2150	1.4213	1.0050	-8.5547	16.9847	3.21	5.22
	3.00	2	12.4550	1.7041	1.2050	-2.8560	27.7660	11.25	13.66
	4.00	2	8.2350	1.4213	1.0050	-4.5347	21.0047	7.23	9.24
	5.00	2	5.2250	1.7041	1.2050	-10.0860	20.5360	4.02	6.43
	6.00	2	.0000	.0000	.0000	.0000	.0000	.00	.00
	7.00	2	2.4100	3.4083	2.4100	-28.2120	33.0320	.00	4.82
	8.00	2	8.4350	1.7041	1.2050	-6.8760	23.7460	7.23	9.64
	9.00	2	4.4200	1.1314	.8000	-5.7450	14.5850	3.62	5.22
	10.00	2	5.8250	.8556	.6050	-1.8623	13.5123	5.22	6.43
	11.00	2	17.8750	.8556	.6050	10.1877	25.5623	17.27	18.48
	12.00	2	7.0250	1.4213	1.0050	-5.7447	19.7947	6.02	8.03
	Total	24	7.4146	5.0048	1.0216	5.3012	9.5279	.00	18.48
Saccharomyce cerevisiae A	1.00	2	15.6650	1.1384	.8050	5.4365	25.8935	14.86	16.47
	2.00	2	9.4400	.2828	.2000	6.8988	11.9812	9.24	9.64
	3.00	2	16.6700	.2828	.2000	14.1288	19.2112	16.47	16.87
	4.00	2	7.8300	.8485	.6000	.2063	15.4537	7.23	8.43
	5.00	2	17.8850	2.0011	1.4150	-9.4280E-02	35.8643	16.47	19.30
	6.00	2	.0000	.0000	.0000	.0000	.0000	.00	.00
	7.00	2	18.0850	1.7183	1.2150	2.6470	33.5230	16.87	19.30
	8.00	2	7.2300	1.1314	.8000	-2.9350	17.3950	6.43	8.03
	9.00	2	9.8400	.8485	.6000	2.2163	17.4637	9.24	10.44
	10.00	2	.0000	.0000	.0000	.0000	.0000	.00	.00
	11.00	2	7.6300	1.1314	.8000	-2.5350	17.7950	6.83	8.43
	12.00	2	6.0250	.5728	.4050	.8790	11.1710	5.62	6.43
	Total	24	9.6917	6.2100	1.2676	7.0694	12.3139	.00	19.30
Saccharomyce cerevisiae B	1.00	2	11.8500	.8485	.6000	4.2263	19.4737	11.25	12.45
	2.00	2	2.4100	3.4083	2.4100	-28.2120	33.0320	.00	4.82
	3.00	2	11.6450	1.7041	1.2050	-3.6660	26.9560	10.44	12.85
	4.00	2	7.0250	1.9870	1.4050	-10.8272	24.8772	5.62	8.43
	5.00	2	21.2850	5.1124	3.6150	-24.6479	67.2179	17.67	24.90
	6.00	2	.0000	.0000	.0000	.0000	.0000	.00	.00
	7.00	2	15.6650	1.1384	.8050	5.4365	25.8935	14.86	16.47
	8.00	2	11.6500	16.4756	11.6500	-136.3773	159.6773	.00	23.30
	9.00	2	6.6250	.8556	.6050	-1.0623	14.3123	6.02	7.23
	10.00	2	5.2200	.5657	.4000	.1375	10.3025	4.82	5.62
	11.00	2	14.2600	.2828	.2000	11.7188	16.8012	14.06	14.46
	12.00	2	8.6350	.8556	.6050	.9477	16.3223	8.03	9.24
	Total	24	9.6892	6.9018	1.4088	6.7748	12.6036	.00	24.90
Candida krusei	1.00	2	14.6600	2.5597	1.8100	-8.3382	37.6582	12.85	16.47
	2.00	2	8.8350	.5728	.4050	3.6890	13.9810	8.43	9.24
	3.00	2	11.6500	.5657	.4000	6.5675	16.7325	11.25	12.05
	4.00	2	.0000	.0000	.0000	.0000	.0000	.00	.00
	5.00	2	14.4600	2.8426	2.0100	-11.0795	39.9995	12.45	16.47
	6.00	2	.0000	.0000	.0000	.0000	.0000	.00	.00
	7.00	2	6.2250	1.4213	1.0050	-6.5447	18.9947	5.22	7.23
	8.00	2	.0000	.0000	.0000	.0000	.0000	.00	.00
	9.00	2	5.4250	1.4213	1.0050	-7.3447	18.1947	4.42	6.43
	10.00	2	3.0150	.8556	.6050	-4.6723	10.7023	2.41	3.62
	11.00	2	10.2400	.8485	.6000	2.6163	17.8637	9.64	10.84
	12.00	2	2.6150	1.4213	1.0050	-10.1547	15.3847	1.61	3.62
	Total	24	6.4271	5.4570	1.1139	4.1228	8.7314	.00	16.47

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Schizosaccharomyces pombe	Between Groups	645.988	11	58.726	13.825	.000
	Within Groups	50.973	12	4.248		
	Total	696.961	23			
Kluyveromyces marxianus	Between Groups	800.265	11	72.751	49.211	.000
	Within Groups	17.740	12	1.478		
	Total	818.005	23			
Pichia caribbica	Between Groups	665.673	11	60.516	46.969	.000
	Within Groups	15.461	12	1.288		
	Total	681.134	23			
Candida tropicalis strain A	Between Groups	602.179	11	54.744	26.583	.000
	Within Groups	24.712	12	2.059		
	Total	626.892	23			

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Candida tropicalis strain B	Between Groups	545.676	11	49.607	19.563	.000
	Within Groups	30.429	12	2.536		
	Total	576.105	23			
Saccharomyce cerevisiae A	Between Groups	874.239	11	79.476	74.854	.000
	Within Groups	12.741	12	1.062		
	Total	886.980	23			
Saccharomyce cerevisiae B	Between Groups	775.687	11	70.517	2.645	.055
	Within Groups	319.930	12	26.661		
	Total	1095.617	23			
Candida krusei	Between Groups	662.131	11	60.194	31.691	.000
	Within Groups	22.793	12	1.899		
	Total	684.923	23			

Post Hoc Tests

Homogeneous Subsets

Schizosaccharomyces pombe

Duncan[a]

		Subset for alpha = .05					
VAR00001	N	1	2	3	4	5	6
6.00	2	.0000					
4.00	2		6.2250				
8.00	2		6.8250				
12.00	2		7.4300	7.4300			
5.00	2		8.0300	8.0300			
10.00	2		8.8350	8.8350	8.8350		
9.00	2		9.0350	9.0350	9.0350		
3.00	2			12.0500	12.0500	12.0500	
2.00	2				13.0500	13.0500	
1.00	2					14.4600	
11.00	2					15.8650	
7.00	2						20.8950
Sig.		1.000	.240	.063	.082	.111	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2.000.

Kluyveromyces marxianus

Duncan[a]

VAR00001	N	Subset for alpha = .05					
		1	2	3	4	5	6
6.00	2	.0000					
10.00	2	.0000					
7.00	2		4.6200				
1.00	2		5.2200				
9.00	2		6.8250	6.8250			
8.00	2			8.6350	8.6350		
12.00	2			9.0350	9.0350		
4.00	2			9.6400	9.6400		
2.00	2				11.0450	11.0450	
3.00	2				11.2450	11.2450	
11.00	2					13.6550	
5.00	2						22.0950
Sig.		1.000	.109	.052	.073	.063	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2.000.

Pichia caribbica

Duncan[a]

VAR00001	N	Subset for alpha = .05					
		1	2	3	4	5	6
6.00	2	.0000					
11.00	2		6.0250				
8.00	2		7.8300	7.8300			
12.00	2			9.6400	9.6400		
7.00	2				11.6450	11.6450	
2.00	2				11.8500	11.8500	
10.00	2				12.0500	12.0500	
5.00	2					12.4500	
1.00	2						16.0650
3.00	2						17.6750
9.00	2						18.2750
4.00	2						18.4850
Sig.		1.000	.138	.137	.072	.523	.071

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2.000.

Candida tropicalis strain A

Duncan[a]

VAR00001	N	Subset for alpha = .05								
		1	2	3	4	5	6	7	8	9
6.00	2	.0000								
9.00	2	2.2100	2.2100							
11.00	2	3.0150	3.0150	3.0150						
2.00	2		3.8150	3.8150	3.8150					
10.00	2			5.6250	5.6250	5.6250				
7.00	2				6.6250	6.6250	6.6250			
12.00	2					7.4300	7.4300	7.4300		
1.00	2						9.4350	9.4350	9.4350	
5.00	2							10.4450	10.4450	
8.00	2								11.8500	
4.00	2									15.2650
3.00	2									16.8700
Sig.		.068	.308	.108	.086	.254	.086	.068	.135	.285

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2.000.

Candida tropicalis strain B

Duncan[a]

VAR00001	N	Subset for alpha = .05					
		1	2	3	4	5	6
6.00	2	.0000					
7.00	2	2.4100	2.4100				
2.00	2		4.2150	4.2150			
9.00	2		4.4200	4.4200			
5.00	2		5.2250	5.2250	5.2250		
10.00	2		5.8250	5.8250	5.8250		
12.00	2			7.0250	7.0250		
4.00	2				8.2350		
8.00	2				8.4350		
3.00	2					12.4550	
1.00	2					12.8550	
11.00	2						17.8750
Sig.		.156	.074	.133	.090	.806	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2.000.

Saccharomyce cerevisiae A

Duncan[a]

VAR00001	N	Subset for alpha = .05					
		1	2	3	4	5	6
6.00	2	.0000					
10.00	2	.0000					
12.00	2		6.0250				
8.00	2		7.2300	7.2300			
11.00	2		7.6300	7.6300	7.6300		
4.00	2		7.8300	7.8300	7.8300		
2.00	2			9.4400	9.4400		
9.00	2				9.8400		
1.00	2					15.6650	
3.00	2					16.6700	16.6700
5.00	2					17.8850	17.8850
7.00	2						18.0850
Sig.		1.000	.130	.069	.069	.062	.216

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2.000.

Saccharomyce cerevisiae B

Duncan[a]

VAR00001	N	Subset for alpha = .05			
		1	2	3	4
6.00	2	.0000			
2.00	2	2.4100	2.4100		
10.00	2	5.2200	5.2200	5.2200	
9.00	2	6.6250	6.6250	6.6250	
4.00	2	7.0250	7.0250	7.0250	
12.00	2	8.6350	8.6350	8.6350	
3.00	2	11.6450	11.6450	11.6450	11.6450
8.00	2	11.6500	11.6500	11.6500	11.6500
1.00	2	11.8500	11.8500	11.8500	11.8500
11.00	2		14.2600	14.2600	14.2600
7.00	2			15.6650	15.6650
5.00	2				21.2850
Sig.		.064	.064	.097	.117

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2.000.

Candida krusei

Duncan[a]

VAR00001	N	Subset for alpha = .05					
		1	2	3	4	5	6
4.00	2	.0000					
6.00	2	.0000					
8.00	2	.0000					
12.00	2	2.6150	2.6150				
10.00	2	3.0150	3.0150				
9.00	2		5.4250	5.4250			
7.00	2			6.2250	6.2250		
2.00	2				8.8350	8.8350	
11.00	2					10.2400	
3.00	2					11.6500	11.6500
5.00	2						14.4600
1.00	2						14.6600
Sig.		.069	.075	.572	.083	.075	.059

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2.000.

APPENDIX IE

BIOETHANOL PRODUCED FROM PLANT ACID HYDROLYSATES

Descriptives

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
Schizosaccharomyces pombe	1.00	2	15.6650	1.7041	1.2050	.3540	30.9760	14.46	16.87
	2.00	2	.0000	.0000	.0000	.0000	.0000	.00	.00
	3.00	2	18.0750	.5728	.4050	12.9290	23.2210	17.67	18.48
	4.00	2	7.4300	.8485	.6000	-.1937	15.0537	6.83	8.03
	5.00	2	28.1150	2.2698	1.6050	7.7215	48.5085	26.51	29.72
	6.00	2	5.0200	.8485	.6000	-2.6037	12.6437	4.42	5.62
	Total	12	12.3842	9.7948	2.8275	6.1608	18.6075	.00	29.72
Kluyveromyces marxianus	1.00	2	.0000	.0000	.0000	.0000	.0000	.00	.00
	2.00	2	11.6450	1.7041	1.2050	-3.6660	26.9560	10.44	12.85
	3.00	2	16.4650	1.7041	1.2050	1.1540	31.7760	15.26	17.67
	4.00	2	3.2150	4.5467	3.2150	-37.6354	44.0654	.00	6.43
	5.00	2	16.2650	1.4213	1.0050	3.4953	29.0347	15.26	17.27
	6.00	2	2.6100	3.6911	2.6100	-30.5532	35.7732	.00	5.22
	Total	12	8.3667	7.2545	2.0942	3.7573	12.9760	.00	17.67
Pichia caribbica	1.00	2	16.2650	1.4213	1.0050	3.4953	29.0347	15.26	17.27
	2.00	2	20.6950	1.9728	1.3950	2.9698	38.4202	19.30	22.09
	3.00	2	9.0350	.8556	.6050	1.3477	16.7223	8.43	9.64
	4.00	2	11.2450	1.1384	.8050	1.0165	21.4735	10.44	12.05
	5.00	2	10.8450	1.7041	1.2050	-4.4660	26.1560	9.64	12.05
	6.00	2	31.5050	5.3669	3.7950	-16.7150	79.7250	27.71	35.30
	Total	12	16.5983	8.2890	2.3928	11.3318	21.8649	8.43	35.30
Candida tropicalis strain A	1.00	2	17.4700	.8485	.6000	9.8463	25.0937	16.87	18.07
	2.00	2	20.0950	1.1243	.7950	9.9936	30.1964	19.30	20.89
	3.00	2	23.9000	.8485	.6000	16.2763	31.5237	23.30	24.50
	4.00	2	10.6450	1.4213	1.0050	-2.1247	23.4147	9.64	11.65
	5.00	2	23.8950	1.4213	1.0050	11.1253	36.6647	22.89	24.90
	6.00	2	16.4700	.5657	.4000	11.3875	21.5525	16.07	16.87
	Total	12	18.7458	4.8778	1.4081	15.6466	21.8451	9.64	24.90

Descriptives

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
Candida tropicalis strain B	1.00	2	6.6250	.8556	.6050	-1.0623	14.3123	6.02	7.23
	2.00	2	18.4750	.5728	.4050	13.3290	23.6210	18.07	18.88
	3.00	2	.0000	.0000	.0000	.0000	.0000	.00	.00
	4.00	2	.0000	.0000	.0000	.0000	.0000	.00	.00
	5.00	2	.0000	.0000	.0000	.0000	.0000	.00	.00
	6.00	2	5.2200	.5657	.4000	.1375	10.3025	4.82	5.62
	Total	12	5.0533	6.8747	1.9846	.6854	9.4213	.00	18.88
Saccharomyce cerevisiae A	1.00	2	17.0700	.2828	.2000	14.5288	19.6112	16.87	17.27
	2.00	2	.0000	.0000	.0000	.0000	.0000	.00	.00
	3.00	2	14.8600	2.8426	2.0100	-10.6795	40.3995	12.85	16.87
	4.00	2	10.6450	1.4213	1.0050	-2.1247	23.4147	9.64	11.65
	5.00	2	7.8300	.8485	.6000	.2063	15.4537	7.23	8.43
	6.00	2	5.4200	.8485	.6000	-2.2037	13.0437	4.82	6.02
	Total	12	9.3042	6.0695	1.7521	5.4478	13.1605	.00	17.27
Saccharomyce cerevisiae B	1.00	2	12.4550	1.7041	1.2050	-2.8560	27.7660	11.25	13.66
	2.00	2	.0000	.0000	.0000	.0000	.0000	.00	.00
	3.00	2	12.0500	1.1314	.8000	1.8850	22.2150	11.25	12.85
	4.00	2	.0000	.0000	.0000	.0000	.0000	.00	.00
	5.00	2	11.0450	.2899	.2050	8.4402	13.6498	10.84	11.25
	6.00	2	12.8500	.0000	.0000	12.8500	12.8500	12.85	12.85
	Total	12	8.0667	6.0174	1.7371	4.2434	11.8900	.00	13.66
Candida krusei	1.00	2	.0000	.0000	.0000	.0000	.0000	.00	.00
	2.00	2	.0000	.0000	.0000	.0000	.0000	.00	.00
	3.00	2	.0000	.0000	.0000	.0000	.0000	.00	.00
	4.00	2	.0000	.0000	.0000	.0000	.0000	.00	.00
	5.00	2	.0000	.0000	.0000	.0000	.0000	.00	.00
	6.00	2	.0000	.0000	.0000	.0000	.0000	.00	.00
	Total	12	.0000	.0000	.0000	.0000	.0000	.00	.00

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Schizosaccharomyces pombe	Between Groups	1045.502	5	209.100	127.706	.000
	Within Groups	9.824	6	1.637		
	Total	1055.326	11			
Kluyveromyces marxianus	Between Groups	536.788	5	107.358	15.291	.002
	Within Groups	42.125	6	7.021		
	Total	578.913	11			
Pichia caribbica	Between Groups	716.131	5	143.226	21.675	.001
	Within Groups	39.648	6	6.608		
	Total	755.779	11			
Candida tropicalis strain A	Between Groups	254.661	5	50.932	43.260	.000
	Within Groups	7.064	6	1.177		
	Total	261.725	11			

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Candida tropicalis strain B	Between Groups	518.495	5	103.699	450.833	.000
	Within Groups	1.380	6	.230		
	Total	519.875	11			
Saccharomyce cerevisiae A	Between Groups	393.601	5	78.720	40.646	.000
	Within Groups	11.620	6	1.937		
	Total	405.222	11			
Saccharomyce cerevisiae B	Between Groups	394.035	5	78.807	110.785	.000
	Within Groups	4.268	6	.711		
	Total	398.303	11			
Candida krusei	Between Groups	.000	5	.000	.	.
	Within Groups	.000	6	.000		
	Total	.000	11			

Post Hoc Tests

Homogeneous Subsets

Schizosaccharomyces pombe

Duncan[a]

		Subset for alpha = .05			
VAR00001	N	1	2	3	4
2.00	2	.0000			
6.00	2		5.0200		
4.00	2		7.4300		
1.00	2			15.6650	
3.00	2			18.0750	
5.00	2				28.1150
Sig.		1.000	.109	.109	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2.000.

Kluyveromyces marxianus

Duncan[a]

		Subset for alpha = .05	
VAR00001	N	1	2
1.00	2	.0000	
6.00	2	2.6100	
4.00	2	3.2150	
2.00	2		11.6450
5.00	2		16.2650
3.00	2		16.4650
Sig.		.285	.129

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2.000.

Pichia caribbica

Duncan[a]

VAR00001	N	Subset for alpha = .05			
		1	2	3	4
3.00	2	9.0350			
5.00	2	10.8450	10.8450		
4.00	2	11.2450	11.2450		
1.00	2		16.2650	16.2650	
2.00	2			20.6950	
6.00	2				31.5050
Sig.		.437	.088	.136	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2.000.

Candida tropicalis strain A

Duncan[a]

VAR00001	N	Subset for alpha = .05			
		1	2	3	4
4.00	2	10.6450			
6.00	2		16.4700		
1.00	2		17.4700	17.4700	
2.00	2			20.0950	
5.00	2				23.8950
3.00	2				23.9000
Sig.		1.000	.392	.052	.996

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2.000.

Candida tropicalis strain B

Duncan[a]

VAR00001	N	Subset for alpha = .05			
		1	2	3	4
3.00	2	.0000			
4.00	2	.0000			
5.00	2	.0000			
6.00	2		5.2200		
1.00	2			6.6250	
2.00	2				18.4750
Sig.		1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2.000.

Saccharomyce cerevisiae A

Duncan[a]

VAR00001	N	Subset for alpha = .05			
		1	2	3	4
2.00	2	.0000			
6.00	2		5.4200		
5.00	2		7.8300	7.8300	
4.00	2			10.6450	
3.00	2				14.8600
1.00	2				17.0700
Sig.		1.000	.134	.090	.163

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2.000.

Saccharomyce cerevisiae B

Duncan[a]

VAR00001	N	Subset for alpha = .05	
		1	2
2.00	2	.0000	
4.00	2	.0000	
5.00	2		11.0450
3.00	2		12.0500
1.00	2		12.4550
6.00	2		12.8500
Sig.		1.000	.088

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2.000.

Candida krusei

Duncan[a]

VAR00001	N	Subset for alpha = .05					
		1	2	3	4	5	6
1.00	2	.0000					
2.00	2		.0000				
3.00	2			.0000			
4.00	2				.0000		
5.00	2					.0000	
6.00	2						.0000
Sig.		1.000	1.000	1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2.000.

APPENDIX 1F

AMOUNT OF BIOETHANOL PRODUCED FROM UNHYDROLYZED SUBSTRATE

T-Test

Group Statistics

	VAR00001	N	Mean	Std. Deviation	Std. Error Mean
Schizosaccharomyces pombe	1.00	2	36.5550	5.1124	3.6150
	2.00	2	29.3200	3.9739	2.8100
Kluyveromyces marxianus	1.00	2	20.6850	1.4213	1.0050
	2.00	2	26.9150	1.7041	1.2050
Pichia caribbica	1.00	2	23.9000	.8485	.6000
	2.00	2	26.1050	4.5467	3.2150
Candida tropicalis strain A	1.00	2	27.3150	1.7041	1.2050
	2.00	2	8.6350	.8556	.6050
Candida tropicalis strain B	1.00	2	8.8350	.5728	.4050
	2.00	2	24.3000	3.6911	2.6100
Saccharomyce cerevisiae A	1.00	2	21.0900	.2828	.2000
	2.00	2	17.8850	2.0011	1.4150
Saccharomyce cerevisiae B	1.00	2	10.2450	1.4213	1.0050
	2.00	2	24.1000	3.4083	2.4100
Candida krusei	1.00	2	6.2250	1.4213	1.0050
	2.00	2	20.0950	1.1243	.7950

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
Schizosaccharomyces pombe	Equal variances assumed	1.5E+15	.000	1.580	2	.255	7.2350	4.5787	-12.4655	26.9355
	Equal variances not assumed			1.580	1.885	.262	7.2350	4.5787	-13.6604	28.1304
Kluyveromyces marxianus	Equal variances assumed	.	.	-3.970	2	.058	-6.2300	1.5691	-12.9813	.5213
	Equal variances not assumed			-3.970	1.938	.061	-6.2300	1.5691	-13.1941	.7341
Pichia caribbica	Equal variances assumed	2.8E+17	.000	-.674	2	.570	-2.2050	3.2705	-16.2769	11.8669
	Equal variances not assumed			-.674	1.070	.616	-2.2050	3.2705	-37.8870	33.4770
Candida tropicalis strain A	Equal variances assumed	.	.	13.854	2	.005	18.6800	1.3484	12.8785	24.4815
	Equal variances not assumed			13.854	1.474	.015	18.6800	1.3484	10.3642	26.9958
Candida tropicalis strain B	Equal variances assumed	1.4E+16	.000	-5.855	2	.028	-15.4650	2.6412	-26.8293	-4.1007
	Equal variances not assumed			-5.855	1.048	.100	-15.4650	2.6412	-45.5938	14.6638
Saccharomyce cerevisiae A	Equal variances assumed	7.6E+15	.000	2.243	2	.154	3.2050	1.4291	-2.9438	9.3538
	Equal variances not assumed			2.243	1.040	.259	3.2050	1.4291	-13.3852	19.7952
Saccharomyce cerevisiae B	Equal variances assumed	.	.	-5.306	2	.034	-13.8550	2.6112	-25.0899	-2.6201
	Equal variances not assumed			-5.306	1.338	.074	-13.8550	2.6112	-32.5360	4.8260
Candida krusei	Equal variances assumed	.	.	-10.824	2	.008	-13.8700	1.2814	-19.3835	-8.3565
	Equal variances not assumed			-10.824	1.899	.010	-13.8700	1.2814	-19.6732	-8.0668

APPENDIX II

SEQUENCE DATA OF THE ISOLATES

DB:ID	Srce	Length	Identity%	Similar%	Overlap	E()
EM_GSS:AL422755	T3 end of clone AZ0AA004B07	1028	100.0	100.0	628	4.3e-168
EM_GSS:AL423984	T3 end of clone AZ0AA011F11	1026	100.0	100.0	628	4.3e-168
EM_GSS:AL422682	T7 end of clone AZ0AA003G01	1005	100.0	100.0	628	4.3e-168
EM_FUN:AB365315	Kluyveromyces marxianus gene	671	100.0	100.0	628	4.6e-168
EM_FUN:EF568057	Kluyveromyces marxianus stra	664	100.0	100.0	628	4.6e-168
EM_FUN:AY939806	Kluyveromyces marxianus isol	721	99.8	99.8	628	1.5e-167
EM_FUN:DQ249191	Kluyveromyces marxianus stra	676	99.8	99.8	628	1.5e-167
EM_GSS:AL424146	T7 end of clone AZ0AA012F04	927	99.4	100.0	628	2.1e-167
EM_GSS:AL423937	T3 end of clone AZ0AA011D07	861	99.7	99.7	628	3.1e-167
EM_GSS:AL423331	T7 end of clone AZ0AA007G11	932	99.5	99.7	628	4.5e-167
EM_FUN:AF543841	Kluyveromyces marxianus 26S	8798	99.8	99.8	628	6.2e-167
EM_GSS:AL424387	T7 end of clone AZ0AA014B04	945	99.5	99.8	629	9.8e-167
EM_FUN:DQ249190	Kluyveromyces marxianus stra	673	99.5	99.5	628	1e-166
EM_FUN:GU256755	Kluyveromyces marxianus stra	652	100.0	100.0	623	1.2e-166

EM_GSS:AL423632	T7 end of clone AZ0AA009E12	966	99.4	99.5	628	1.4e-166
EM_GSS:AL423587	T7 end of clone AZ0AA009C12	934	99.4	99.5	628	1.5e-166
EM_FUN:AB480229	Kluyveromyces marxianus gene	910	99.7	99.7	629	1.5e-166
EM_FUN:EU019227	Kluyveromyces marxianus isol	721	99.5	99.5	628	1.5e-166
EM_GSS:AL423922	T7 end of clone AZ0AA011C11	983	99.7	99.8	627	2.4e-166
EM_GSS:AL424075	clone AZ0AA012C02 of library	1057	99.4	99.7	628	5.9e-166
EM_GSS:AL424080	T3 end of clone AZ0AA012C05	1008	99.4	99.7	628	6e-166
EM_GSS:AL424695	T7 end of clone XAZ0AA001H05	917	98.6	99.7	628	6.9e-166
EM_FUN:EU794731	Kluyveromyces marxianus stra	673	99.4	99.4	629	1.6e-165
EM_GSS:AL423939	T3 end of clone AZ0AA011D08	874	98.6	99.4	628	3.3e-165
EM_FUN:EU019224	Kluyveromyces marxianus isol	724	99.0	99.0	630	8.4e-165
EM_FUN:AJ401698	Kluyveromyces marxianus 5.8S	633	100.0	100.0	610	5.5e-163
EM_FUN:AJ401694	Kluyveromyces marxianus 5.8S	633	100.0	100.0	610	5.5e-163
EM_FUN:AY046214	Kluyveromyces marxianus inte	633	100.0	100.0	610	5.5e-163
EM_FUN:AJ401699	Kluyveromyces marxianus 5.8S	633	100.0	100.0	610	5.5e-163
EM_FUN:AJ401692	Kluyveromyces marxianus 5.8S	633	100.0	100.0	610	5.5e-163
EM_FUN:EU266570	Kluyveromyces marxianus ATCC	683	99.2	99.2	627	7e-163

EM_GSS:AL422554	T3 end of clone AZ0AA003A05	949	99.7	99.7	618	1.1e-162
EM_FUN:AJ401701	Kluyveromyces marxianus 5.8S	633	99.8	99.8	610	1.8e-162
EM_FUN:AJ401693	Kluyveromyces marxianus 5.8S	633	99.8	99.8	610	1.8e-162
EM_FUN:AJ401700	Kluyveromyces marxianus 5.8S	633	99.8	99.8	610	1.8e-162
EM_FUN:AJ401697	Kluyveromyces marxianus 5.8S	633	99.8	99.8	610	1.8e-162
EM_FUN:AJ401696	Kluyveromyces marxianus 5.8S	633	99.8	99.8	610	1.8e-162
EM_FUN:AJ401695	Kluyveromyces marxianus 5.8S	633	99.8	99.8	610	1.8e-162
EM_FUN:AB011519	Kluyveromyces marxianus gene	632	99.7	99.7	609	5e-162
EM_FUN:L47107	Kluyveromyces marxianus 18S ribo	720	98.1	98.1	629	7.2e-162
EM_FUN:AB011520	Kluyveromyces marxianus gene	632	99.5	99.5	609	1.6e-161
EM_FUN:AB011518	Kluyveromyces marxianus gene	632	99.5	99.5	609	1.6e-161
EM_GSS:AL423406	T3 end of clone AZ0AA008C06	986	99.3	99.7	608	1.8e-160
EM_FUN:U09325	Kluyveromyces marxianus strain H	635	99.0	99.0	611	1.9e-159
EM_FUN:FJ838775	Kluyveromyces marxianus stra	634	98.5	98.5	611	6.4e-158
EM_GSS:AL423357	T7 end of clone AZ0AA008A01	846	98.2	99.5	597	1.6e-156
EM_GSS:AL424018	T7 end of clone AZ0AA011H07	851	99.5	99.7	582	6.2e-154
EM_FUN:GU133329	Kluyveromyces sp. ZMS1 18S r	621	98.2	98.2	597	3.6e-152

EM_GSS:AL424860	T7 end of clone XAZ0AA002H02	1027	99.7	99.7	577	4.3e-152
EM_FUN:CR382124	Kluyveromyces lactis strain	1715506	95.9	95.9	630	7.6e-152
EM_GSS:AL427558	clone BA0AB020A07 of library	976	95.7	96.0	630	1.4e-151
EM_FUN:GQ376078	Kluyveromyces lactis isolate	743	95.9	95.9	630	1.7e-151
EM_FUN:GQ376079	Kluyveromyces lactis isolate	743	95.9	95.9	630	1.7e-151
EM_FUN:GQ376077	Kluyveromyces lactis isolate	743	95.9	95.9	630	1.7e-151
EM_GSS:AL428678	clone BA0AB027B10 of library	882	95.9	95.9	628	6e-151
EM_FUN:AJ401720	Kluyveromyces dobzhanskii 5.	632	96.9	96.9	612	5.7e-150
EM_FUN:AJ401722	Kluyveromyces dobzhanskii 5.	632	96.9	96.9	612	5.7e-150
EM_FUN:AJ401721	Kluyveromyces dobzhanskii 5.	632	96.9	96.9	612	5.7e-150
EM_FUN:AY046215	Kluyveromyces dobzhanskii in	632	96.7	96.7	612	1.8e-149
EM_FUN:AJ401719	Kluyveromyces dobzhanskii 5.	632	96.7	96.7	612	1.8e-149
EM_FUN:FJ838773	Kluyveromyces marxianus stra	628	96.7	96.7	608	4e-149
EM_FUN:AY626023	Kluyveromyces lactis strain	631	96.1	96.1	612	2e-147
EM_FUN:AY623809	Kluyveromyces lactis strain	631	96.1	96.1	612	2e-147
EM_FUN:AJ401709	Kluyveromyces lactis 5.8S rR	631	96.1	96.1	612	2e-147

EM_FUN:AY623808	Kluyveromyces lactis strain	631	96.1	96.1	612	2e-147
EM_FUN:AY626022	Kluyveromyces lactis strain	631	96.1	96.1	612	2e-147
EM_FUN:AJ401716	Kluyveromyces lactis 5.8S rR	631	95.9	95.9	612	6.3e-147
EM_FUN:AJ401714	Kluyveromyces lactis 5.8S rR	631	95.9	95.9	612	6.3e-147
EM_FUN:AJ401712	Kluyveromyces lactis 5.8S rR	631	95.9	95.9	612	6.3e-147
EM_FUN:AJ401713	Kluyveromyces lactis 5.8S rR	631	95.9	95.9	612	6.3e-147
EM_FUN:AJ401710	Kluyveromyces lactis 5.8S rR	631	95.9	95.9	612	6.3e-147
EM_FUN:AY338968	Kluyveromyces lactis strain	631	96.1	96.1	612	1.2e-146
EM_FUN:AY338969	Kluyveromyces lactis strain	631	96.1	96.1	612	1.2e-146
EM_FUN:AB011514	Kluyveromyces dobzhanskii ge	630	95.9	95.9	610	1.6e-146
EM_FUN:AJ401706	Kluyveromyces lactis 5.8S rR	631	95.8	95.8	612	2e-146
EM_FUN:AJ401717	Kluyveromyces lactis 5.8S rR	631	95.8	95.8	612	2e-146
EM_FUN:AY046213	Kluyveromyces lactis interna	631	95.8	95.8	612	2e-146
EM_FUN:AJ401704	Kluyveromyces lactis 5.8S rR	631	95.8	95.8	612	2e-146
EM_FUN:AJ401718	Kluyveromyces lactis 5.8S rR	631	95.8	95.8	612	2e-146
EM_FUN:AJ401715	Kluyveromyces lactis 5.8S rR	631	95.8	95.8	612	2e-146
EM_FUN:AJ401703	Kluyveromyces lactis 5.8S rR	632	95.9	95.9	610	2.3e-146

EM_FUN:AY628331	Kluyveromyces lactis strain	630	95.9	95.9	612	3.9e-146
EM_FUN:AB011517	Kluyveromyces lactis gene fo	630	95.6	95.6	611	5.7e-146
EM_GSS:AL422973	T3 end of clone AZ0AA005D06	1070	88.4	95.2	629	6e-146
EM_FUN:AB011515	Kluyveromyces lactis gene fo	630	95.4	95.4	611	1.8e-145
EM_FUN:AJ229068	Kluyveromyces dobzhanskii 5.	600	96.2	96.3	601	3.5e-145
EM_FUN:AB011516	Kluyveromyces lactis gene fo	630	95.3	95.3	611	4e-145
EM_FUN:AJ401705	Kluyveromyces lactis 5.8S rR	630	95.6	95.6	612	4e-145
EM_FUN:AJ401707	Kluyveromyces lactis 5.8S rR	630	95.6	95.6	612	4e-145
EM_FUN:AY338967	Kluyveromyces lactis strain	631	95.3	95.3	612	6.7e-145
EM_FUN:AY628330	Kluyveromyces lactis strain	631	95.3	95.3	612	6.7e-145
EM_FUN:AJ401711	Kluyveromyces lactis 5.8S rR	634	95.3	95.3	614	1.1e-144
EM_FUN:AJ401702	Kluyveromyces lactis 5.8S rR	631	95.4	95.4	613	4.1e-144
EM_FUN:AJ401708	Kluyveromyces lactis 5.8S rR	629	95.3	95.3	612	2.5e-143
EM_GSS:AL426475	clone BA0AB013B08 of library	806	96.1	96.1	590	1.5e-142
EM_FUN:AY046212	Kluyveromyces wickerhamii in	635	94.1	94.1	615	7.9e-140

APPENDIX II cont'd

Pichia caribbica IMI 398400 ITS sequence

UBMISSION PARAMETERS			
Title	Sequence	**Database**	em_rel_fun
Sequence length	535	**Sequence type**	N
Program	Fasta	**Version**	35.04 Jan. 24, 2010
Expectation upper value	10.0	**Sequence range**	1-
Number of scores	50	**Number of alignments**	100
Word size	6	**Open gap penalty**	-14
Gap extension penalty	-4	**Histogram**	False

DB:ID	Source	Length	Identity %	Similar %	Overlap
EM_FUN:EU177575	Pichia caribbica strain WB 1	607	100.0	100.0	535
EM_FUN:EU568995	Pichia caribbica isolate CNR	607	99.8	100.0	535
EM_FUN:EU568997	Pichia caribbica isolate CNR	607	99.8	100.0	535
EM_FUN:GU248264	Pichia caribbica strain XTWJ	631	99.8	99.8	535
EM_FUN:EU569001	Pichia caribbica isolate CNR	607	99.8	99.8	535
EM_FUN:EU569029	Pichia caribbica isolate CNR	607	99.8	99.8	535
EM_FUN:EU569027	Pichia caribbica isolate CNR	607	99.8	99.8	535

EM_FUN:EU569023	Pichia caribbica isolate CNR	607	99.8	99.8	535		2.6e-
EM_FUN:EU569013	Pichia caribbica isolate CNR	607	99.8	99.8	535		2.6e-
EM_FUN:EU569003	Pichia caribbica isolate CNR	607	99.8	99.8	535		2.6e-
EM_FUN:EU568913	Pichia caribbica 18S ribosom	607	99.8	99.8	535		2.6e-
EM_FUN:EU569007	Pichia caribbica isolate CNR	607	99.8	99.8	535		2.6e-
EM_FUN:EU569009	Pichia caribbica isolate CNR	607	99.8	99.8	535		2.6e-
EM_FUN:EU569005	Pichia caribbica isolate CNR	607	99.8	99.8	535		2.6e-
EM_FUN:EU569011	Pichia caribbica isolate CNR	607	99.8	99.8	535		2.6e-
EM_FUN:EU569019	Pichia caribbica isolate CNR	607	99.8	99.8	535		2.6e-
EM_FUN:EU569015	Pichia caribbica isolate CNR	607	99.8	99.8	535		2.6e-
EM_FUN:EU569017	Pichia caribbica isolate CNR	607	99.8	99.8	535		2.6e-
EM_FUN:EU569021	Pichia caribbica isolate CNR	607	99.8	99.8	535		2.6e-
EM_FUN:EU569025	Pichia caribbica isolate CNR	607	99.8	99.8	535		2.6e-
EM_FUN:FM199963	Pichia caribbica 18S rRNA ge	606	99.8	99.8	535		2.6e-
EM_FUN:EU400273	Candida sp. GLH-2008 18S rib	568	99.8	99.8	535		2.6e-
EM_FUN:AM158923	Candida fukuyamaensis partia	885	99.6	99.6	535		7.8e-
EM_FUN:EF197951	Pichia guilliermondii strain	639	99.6	99.6	535		8.2e-
EM_FUN:EF197807	Candida carpophila strain HK	626	99.6	99.6	535		8.2e-

EM_FUN:EF198016	Pichia guilliermondii strain	624	99.6	99.6	535		8.2e-
EM_FUN:EF197810	Candida carpophila strain HK	608	99.6	99.6	535		8.3e-
EM_FUN:EU568999	Pichia caribbica isolate CNR	607	99.6	99.6	535		8.3e-
EM_FUN:AM160625	Pichia guilliermondii ITS1,	1182	99.1	99.1	535		2.3e-
EM_FUN:EF222224	Pichia guilliermondii strain	636	99.1	99.1	535		2.6e-
EM_FUN:EF197814	Pichia guilliermondii strain	635	99.1	99.1	535		2.6e-
EM_FUN:EF197809	Pichia guilliermondii strain	624	99.1	99.1	535		2.6e-
EM_FUN:EF197816	Pichia guilliermondii strain	624	99.1	99.1	535		2.6e-
EM_FUN:FJ662408	Pichia guilliermondii isolat	616	99.1	99.1	535		2.6e-
EM_FUN:EF222226	Pichia guilliermondii strain	616	99.1	99.1	535		2.6e-
EM_FUN:EU568983	Pichia guilliermondii isolat	607	99.1	99.1	535		2.6e-
EM_FUN:EU568979	Pichia guilliermondii isolat	607	99.1	99.1	535		2.6e-
EM_FUN:EU568973	Pichia guilliermondii isolat	607	99.1	99.1	535		2.6e-
EM_FUN:EU568977	Pichia guilliermondii isolat	607	99.1	99.1	535		2.6e-
EM_FUN:EU568971	Pichia guilliermondii isolat	607	99.1	99.1	535		2.6e-
EM_FUN:EU568985	Pichia guilliermondii isolat	607	99.1	99.1	535		2.6e-
EM_FUN:EU568989	Pichia guilliermondii isolat	607	99.1	99.1	535		2.6e-
EM_FUN:EU568981	Pichia guilliermondii isolat	607	99.1	99.1	535		2.6e-
EM_FUN:EU568975	Pichia guilliermondii isolat	607	99.1	99.1	535		2.6e-
EM_FUN:EU568991	Pichia guilliermondii isolat	607	99.1	99.1	535		2.6e-

EM_FUN:EU568993	Pichia guilliermondii isolat	607	99.1	99.1	535		2.6e-
EM_FUN:EU568987	Pichia guilliermondii isolat	607	99.1	99.1	535		2.6e-
EM_FUN:FJ969194	Pichia guilliermondii strain	607	99.1	99.1	535		2.6e-
EM_FUN:EF568003	Pichia guilliermondii strain	565	99.1	99.1	535		2.6e-
EM_FUN:EU520225	Mycena pura isolate NW473 18	612	98.9	99.1	535		3.8e-
EM_FUN:AB305098	Pichia guilliermondii genes	833	98.9	98.9	535		7.8e-
EM_FUN:DQ249193	Pichia guilliermondii strain	657	98.9	98.9	535		8.1e-
EM_FUN:EF190233	Pichia guilliermondii strain	642	98.9	98.9	535		8.2e-
EM_FUN:EF532299	Pichia guilliermondii strain	641	98.9	98.9	535		8.2e-
EM_FUN:EF643576	Pichia guilliermondii strain	639	98.9	98.9	535		8.2e-
EM_FUN:DQ680842	Pichia guilliermondii strain	636	98.9	98.9	535		8.2e-
EM_FUN:DQ683005	Pichia guilliermondii strain	635	98.9	98.9	535		8.2e-
EM_FUN:AB369917	Pichia guilliermondii genes	631	98.9	98.9	535		8.2e-
EM_FUN:EF191048	Pichia guilliermondii strain	631	98.9	98.9	535		8.2e-
EM_FUN:EF375704	Pichia guilliermondii strain	628	98.9	98.9	535		8.2e-
EM_FUN:EF193071	Pichia guilliermondii vouche	627	98.9	98.9	535		8.2e-
EM_FUN:EF192233	Pichia guilliermondii strain	624	98.9	98.9	535		8.2e-
EM_FUN:EF190227	Pichia guilliermondii strain	624	98.9	98.9	535		8.2e-
EM_FUN:DQ668346	Pichia guilliermondii isolat	623	98.9	98.9	535		8.2e-
EM_FUN:DQ668353	Pichia guilliermondii isolat	622	98.9	98.9	535		8.2e-

EM_FUN:EU236703	Pichia guilliermondii 18S ri	620	98.9	98.9	535		8.2e-
EM_FUN:DQ663478	Pichia guilliermondii isolat	617	98.9	98.9	535		8.2e-
EM_FUN:DQ663479	Pichia guilliermondii isolat	611	98.9	98.9	535		8.2e-
EM_FUN:DQ534408	Debaryomyces hansenii strain	611	98.9	98.9	535		8.2e-
EM_FUN:DQ663480	Pichia guilliermondii isolat	610	98.9	98.9	535		8.2e-
EM_FUN:GQ149494	Pichia guilliermondii strain	608	98.9	98.9	535		8.2e-
EM_FUN:GQ149493	Pichia guilliermondii strain	607	98.9	98.9	535		8.2e-
EM_FUN:DQ663477	Pichia guilliermondii isolat	607	98.9	98.9	535		8.2e-
EM_FUN:AY939795	Pichia guilliermondii isolat	607	98.9	98.9	535		8.2e-
EM_FUN:AY939792	Pichia guilliermondii strain	607	98.9	98.9	535		8.2e-
EM_FUN:EU568951	Pichia guilliermondii isolat	607	98.9	98.9	535		8.2e-
EM_FUN:EU568953	Pichia guilliermondii isolat	607	98.9	98.9	535		8.2e-
EM_FUN:EU568933	Pichia guilliermondii isolat	607	98.9	98.9	535		8.2e-
EM_FUN:EU568943	Pichia guilliermondii isolat	607	98.9	98.9	535		8.2e-
EM_FUN:EU568969	Pichia guilliermondii isolat	607	98.9	98.9	535		8.2e-
EM_FUN:EU568935	Pichia guilliermondii isolat	607	98.9	98.9	535		8.2e-
EM_FUN:EU568929	Pichia guilliermondii isolat	607	98.9	98.9	535		8.2e-
EM_FUN:AF209874	Debaryomyces hansenii var. f	607	98.9	98.9	535		8.2e-
EM_FUN:EU568945	Pichia guilliermondii isolat	607	98.9	98.9	535		8.2e-

APPENDIX II cont'd

Candida tropicalis IMI 398401 ITS sequence

SUBMISSION PARAMETERS			
Title	Sequence	**Database**	em_rel_fun
Sequence length	469	**Sequence type**	n
Program	Fasta	**Version**	35.04 Jan. 24, 2010
Expectation upper value	10.0	**Sequence range**	1-
Number of scores	50	**Number of alignments**	100
Word size	6	**Open gap penalty**	-14
Gap extension penalty	-4	**Histogram**	false

DB:ID	Source	Length	Identity%	Similar%	Overlap	E()
EM_GSS:AL441547	T3 end of clone XBD0AA002F06	1110	100.0	100.0	469	1.8e-120
EM_GSS:AL439197	T3 end of clone BD0AA003A11	1042	100.0	100.0	469	1.8e-120
EM_GSS:AL441098	T7 end of clone BD0AA016G07	1027	100.0	100.0	469	1.8e-120
EM_GSS:AL440045	T3 end of clone BD0AA009F01	982	100.0	100.0	469	1.8e-120
EM_FUN:EF190223	Candida tropicalis strain WC	556	100.0	100.0	469	2e-120

EM_FUN:EF192229	Candida tropicalis strain zh	556	100.0	100.0	469	2e-120
EM_FUN:EF200068	Candida tropicalis strain HK	555	100.0	100.0	469	2e-120
EM_FUN:EF216862	Candida tropicalis isolate 1	554	100.0	100.0	469	2e-120
EM_FUN:EF197999	Candida tropicalis voucher M	554	100.0	100.0	469	2e-120
EM_FUN:EF198020	Candida tropicalis strain w-	554	100.0	100.0	469	2e-120
EM_FUN:EF198007	Candida tropicalis strain WC	554	100.0	100.0	469	2e-120
EM_FUN:EU924133	Candida tropicalis strain CB	552	100.0	100.0	469	2e-120
EM_FUN:GQ376071	Candida tropicalis isolate U	551	100.0	100.0	469	2e-120
EM_FUN:FJ662410	Candida tropicalis isolate Z	533	100.0	100.0	469	2.1e-120
EM_FUN:AY939810	Candida tropicalis strain AT	526	100.0	100.0	469	2.1e-120
EM_FUN:EF190225	Candida tropicalis strain HK	523	100.0	100.0	469	2.1e-120
EM_FUN:FJ662409	Candida tropicalis isolate Z	523	100.0	100.0	469	2.1e-120
EM_FUN:EU266571	Candida tropicalis ATCC:6602	521	100.0	100.0	469	2.1e-120
EM_FUN:EF568042	Candida tropicalis	493	100.0	100.0	469	2.1e-120

	strain WM					
EM_FUN:AM117838	Candida tropicalis 18S rRNA	487	100.0	100.0	469	2.1e-120
EM_FUN:EF568038	Candida tropicalis strain WM	484	100.0	100.0	469	2.1e-120
EM_FUN:AB365316	Candida tropicalis genes for	480	100.0	100.0	469	2.1e-120
EM_GSS:AL439868	T7 end of clone BD0AA008D12	969	99.8	100.0	469	2.7e-120
EM_GSS:AL439664	T3 end of clone BD0AA007A08	994	98.9	100.0	469	1.2e-119
EM_FUN:FN652303	Candida tropicalis 18S rRNA	531	99.6	99.6	469	2e-119
EM_FUN:EU589208	Candida tropicalis strain AT	529	99.8	99.8	470	2e-119
EM_FUN:AY939801	Candida tropicalis isolate U	528	99.8	99.8	470	2e-119
EM_FUN:AF321539	Candida tropicalis 18S ribos	523	99.6	99.6	469	2e-119
EM_GSS:AL439205	T7 end of clone BD0AA003B04	1014	99.6	99.8	470	2.6e-119
EM_FUN:EU288196	Candida tropicalis 18S ribos	752	99.8	99.8	469	3.6e-119
EM_FUN:EF196807	Candida tropicalis voucher M	556	99.8	99.8	469	3.8e-119
EM_FUN:EF192214	Candida tropicalis strain zh	547	99.8	99.8	469	3.8e-119

EM_FUN:EF192215	Candida tropicalis strain HK	537	99.8	99.8	469	3.8e-119
EM_FUN:FJ515173	Candida tropicalis strain SN	508	99.8	99.8	469	3.8e-119
EM_FUN:EF568040	Candida tropicalis strain WM	483	99.8	99.8	469	3.9e-119
EM_FUN:EF568041	Candida tropicalis strain WM	483	99.8	99.8	469	3.9e-119
EM_FUN:DQ640769	Candida sp. N17 internal tra	534	99.6	99.6	469	1.2e-118
EM_FUN:EU121523	Candida tropicalis strain CI	529	99.6	99.6	469	1.2e-118
EM_GSS:AL440353	T3 end of clone BD0AA011G12	1009	98.3	99.4	469	2.9e-118
EM_GSS:AL441431	T7 end of clone XBD0AA002A06	1015	99.6	99.6	470	3.3e-118
EM_FUN:FN376412	Candida tropicalis partial I	507	99.4	99.4	469	3.7e-118
EM_FUN:DQ680841	Candida tropicalis strain 36	554	99.6	99.6	469	7e-118
EM_FUN:FN652304	Candida tropicalis 18S rRNA	529	99.6	99.6	469	7e-118
EM_FUN:EF568039	Candida tropicalis strain WM	482	99.6	99.6	469	7.1e-118
EM_GSS:AL440767	T3 end of clone BD0AA014F05	971	99.4	99.6	469	9.2e-118
EM_FUN:AF268095	Candida tropicalis 18S	529	99.1	99.1	469	1.2e-117

	ribos					
EM_FUN:GQ280297	Candida tropicalis isolate Z	535	98.9	98.9	470	1.1e-116
EM_FUN:AB467289	Candida tropicalis gene for	502	99.4	99.4	467	4.6e-116
EM_FUN:FJ697166	Candida tropicalis isolate Z	534	98.1	98.1	469	5.9e-116
EM_FUN:AB467293	Candida tropicalis gene for	502	99.1	99.1	465	8.7e-116
EM_FUN:AB467291	Candida tropicalis gene for	499	99.4	99.4	465	1.7e-115
EM_FUN:AB467290	Candida tropicalis gene for	498	99.4	99.4	465	1.7e-115
EM_FUN:EF151451	Candida tropicalis isolate F	519	98.3	98.3	469	6.6e-115
EM_FUN:L47112	Candida tropicalis 18S ribosomal	524	97.9	97.9	469	9.7e-115
EM_FUN:FJ011533	Candida tropicalis isolate S	513	99.6	99.6	456	2.7e-114
EM_FUN:AB467292	Candida tropicalis gene for	502	98.7	98.7	466	2.7e-114
EM_FUN:DQ666190	Candida tropicalis strain MB	527	98.9	98.9	456	8.2e-113
EM_FUN:GQ387379	Candida sp. SD 302 18S ribos	528	97.0	97.0	469	6.2e-112
EM_FUN:DQ666188	Candida tropicalis strain MA	524	98.9	98.9	452	1e-111

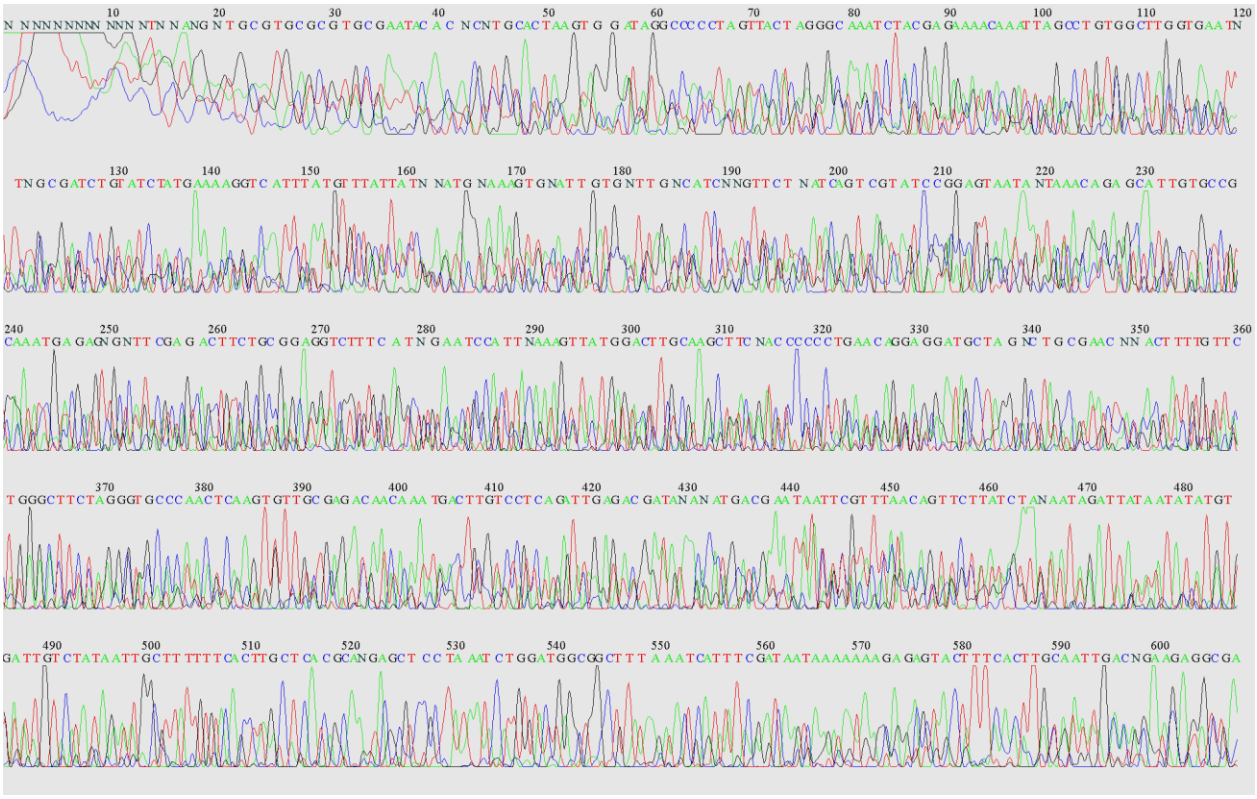
EM_FUN:AB437072	Candida tropicalis genes for	437	100.0	100.0	436	2.6e-111
EM_FUN:AB437074	Candida tropicalis genes for	437	100.0	100.0	436	2.6e-111
EM_FUN:AB437085	Candida tropicalis genes for	437	100.0	100.0	436	2.6e-111
EM_FUN:AB437078	Candida tropicalis genes for	437	100.0	100.0	436	2.6e-111
EM_FUN:DQ666193	Candida tropicalis strain Yf	521	98.7	98.7	452	3.2e-111
EM_FUN:AB467294	Candida tropicalis gene for	502	97.0	97.0	465	7.9e-111
EM_FUN:AB437048	Candida tropicalis genes for	437	99.8	99.8	436	8.1e-111
EM_FUN:AB437049	Candida tropicalis genes for	437	99.8	99.8	436	8.1e-111
EM_FUN:AB437054	Candida tropicalis genes for	437	99.8	99.8	436	8.1e-111
EM_FUN:AB437053	Candida tropicalis genes for	437	99.8	99.8	436	8.1e-111
EM_FUN:AB437062	Candida tropicalis genes for	437	99.8	99.8	436	8.1e-111
EM_FUN:AB437063	Candida tropicalis genes for	437	99.8	99.8	436	8.1e-111
EM_FUN:AB437064	Candida tropicalis genes for	437	99.8	99.8	436	8.1e-111
EM_FUN:AB437065	Candida tropicalis	437	99.8	99.8	436	8.1e-111

	genes for					
EM_FUN:AF287910	Candida tropicalis 18S ribos	520	96.8	96.8	468	2.2e-110
EM_FUN:AB305099	Candida tropicalis genes for	717	100.0	100.0	432	3e-110
EM_FUN:AB437050	Candida tropicalis genes for	436	99.8	99.8	436	4.8e-110
EM_FUN:AB437086	Candida tropicalis genes for	436	99.8	99.8	436	4.8e-110
EM_FUN:AB437073	Candida tropicalis genes for	436	99.8	99.8	436	4.8e-110
EM_FUN:AB437067	Candida tropicalis genes for	436	99.8	99.8	436	4.8e-110
EM_FUN:AB437052	Candida tropicalis genes for	436	99.8	99.8	436	4.8e-110
EM_FUN:AB437058	Candida tropicalis genes for	436	99.8	99.8	436	4.8e-110
EM_FUN:AB437051	Candida tropicalis genes for	436	99.8	99.8	436	4.8e-110
EM_FUN:AB437087	Candida tropicalis genes for	436	99.8	99.8	436	4.8e-110
EM_FUN:AB437046	Candida tropicalis genes for	436	99.8	99.8	436	4.8e-110
EM_FUN:AB437045	Candida tropicalis genes for	436	99.8	99.8	436	4.8e-110
EM_FUN:AB437066	Candida tropicalis genes for	436	99.8	99.8	436	4.8e-110

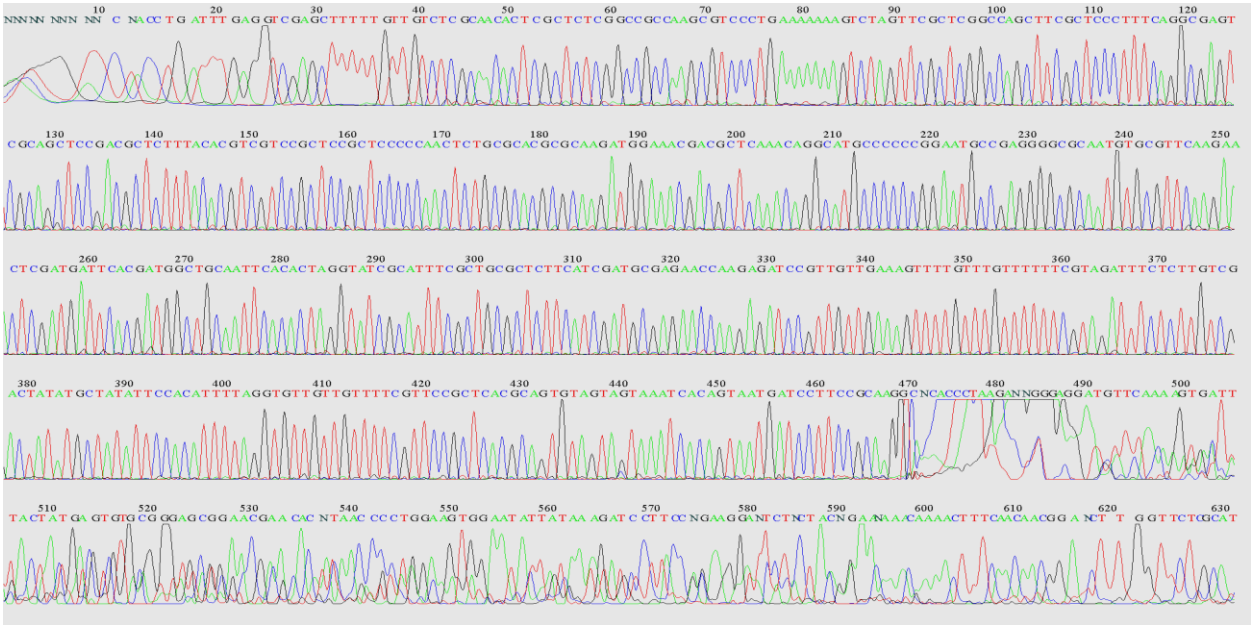
EM_FUN:AB437081	Candida tropicalis genes for	436	99.8	99.8	436	4.8e-110
EM_FUN:AB437055	Candida tropicalis genes for	436	99.8	99.8	436	4.8e-110
EM_FUN:AB437061	Candida tropicalis genes for	436	99.8	99.8	436	4.8e-110
EM_FUN:AB437060	Candida tropicalis genes for	436	99.8	99.8	436	4.8e-110
EM_FUN:AB437057	Candida tropicalis genes for	436	99.8	99.8	436	4.8e-110
EM_FUN:AB437082	Candida tropicalis genes for	436	99.8	99.8	436	4.8e-110
EM_FUN:AB437044	Candida tropicalis genes for	436	99.8	99.8	436	4.8e-110
EM_FUN:AB437047	Candida tropicalis genes for	436	99.8	99.8	436	4.8e-110
EM_FUN:AB437056	Candida tropicalis genes for	436	99.8	99.8	436	4.8e-110
EM_FUN:AB437075	Candida tropicalis genes for	436	99.8	99.8	436	4.8e-110
EM_FUN:AB437059	Candida tropicalis genes for	436	99.8	99.8	436	4.8e-110
EM_FUN:AB437068	Candida tropicalis genes for	436	99.8	99.8	436	4.8e-110
EM_FUN:AB437080	Candida tropicalis genes for	436	99.5	99.8	436	7e-110

APPENDIX III

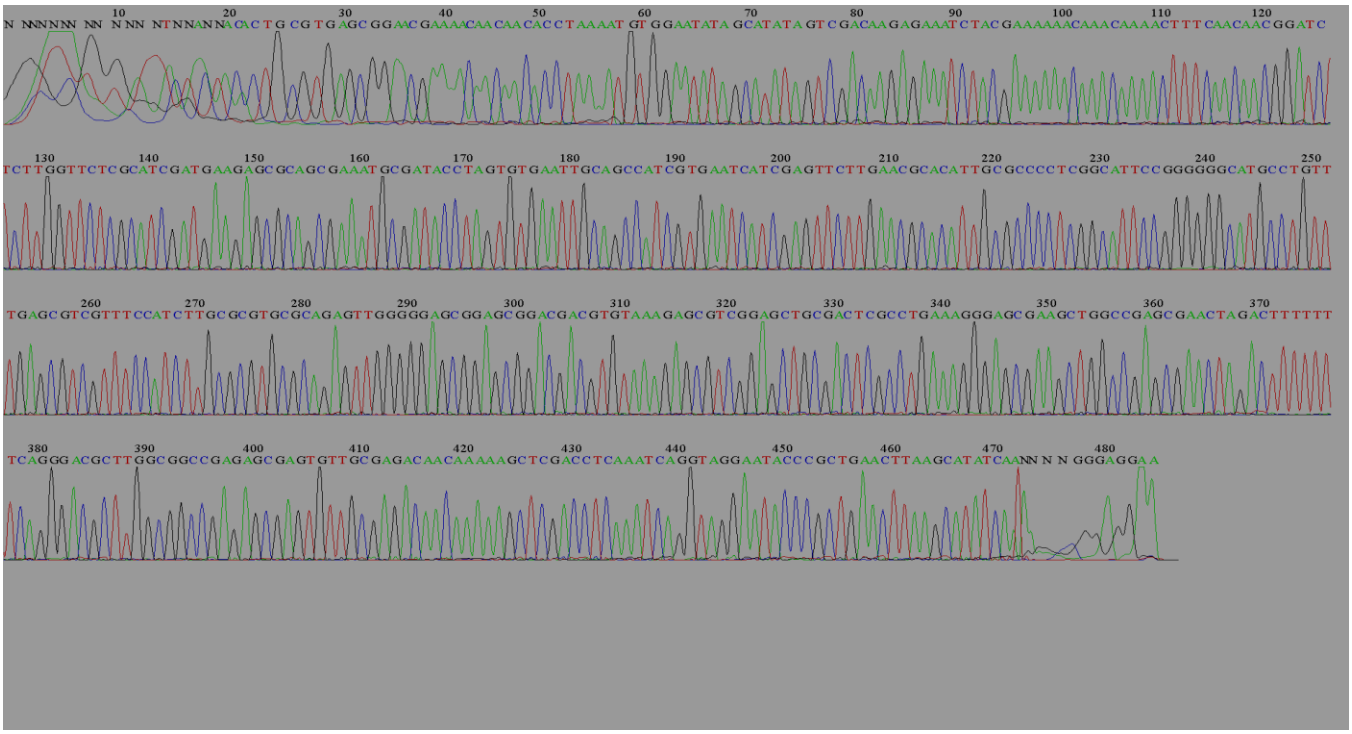
RAW DNA SEQUENCE DATA FOR YEAST ISOLATES



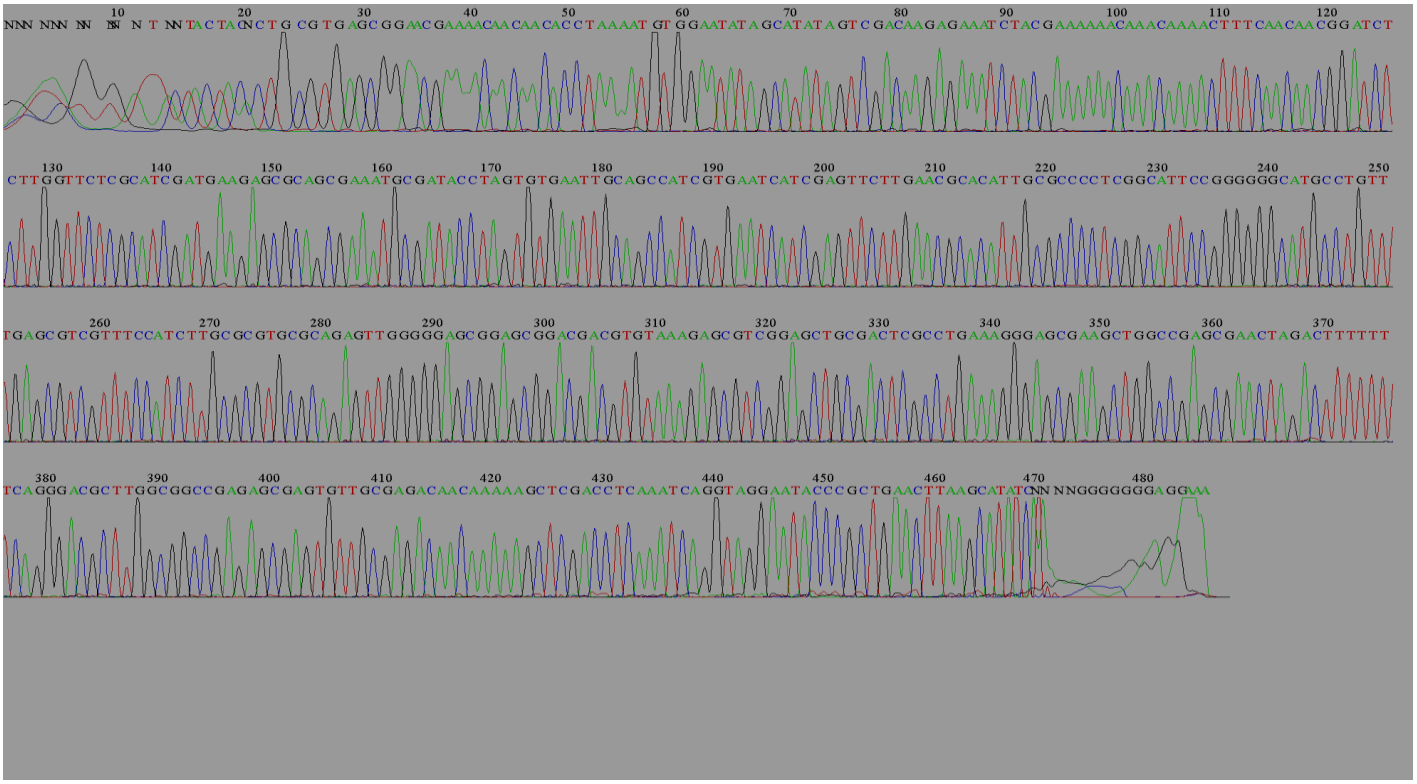
Chromatogram of *Shizosaccharomyces pombe*



Chromatogram of *Kluyveromyces marxianus*



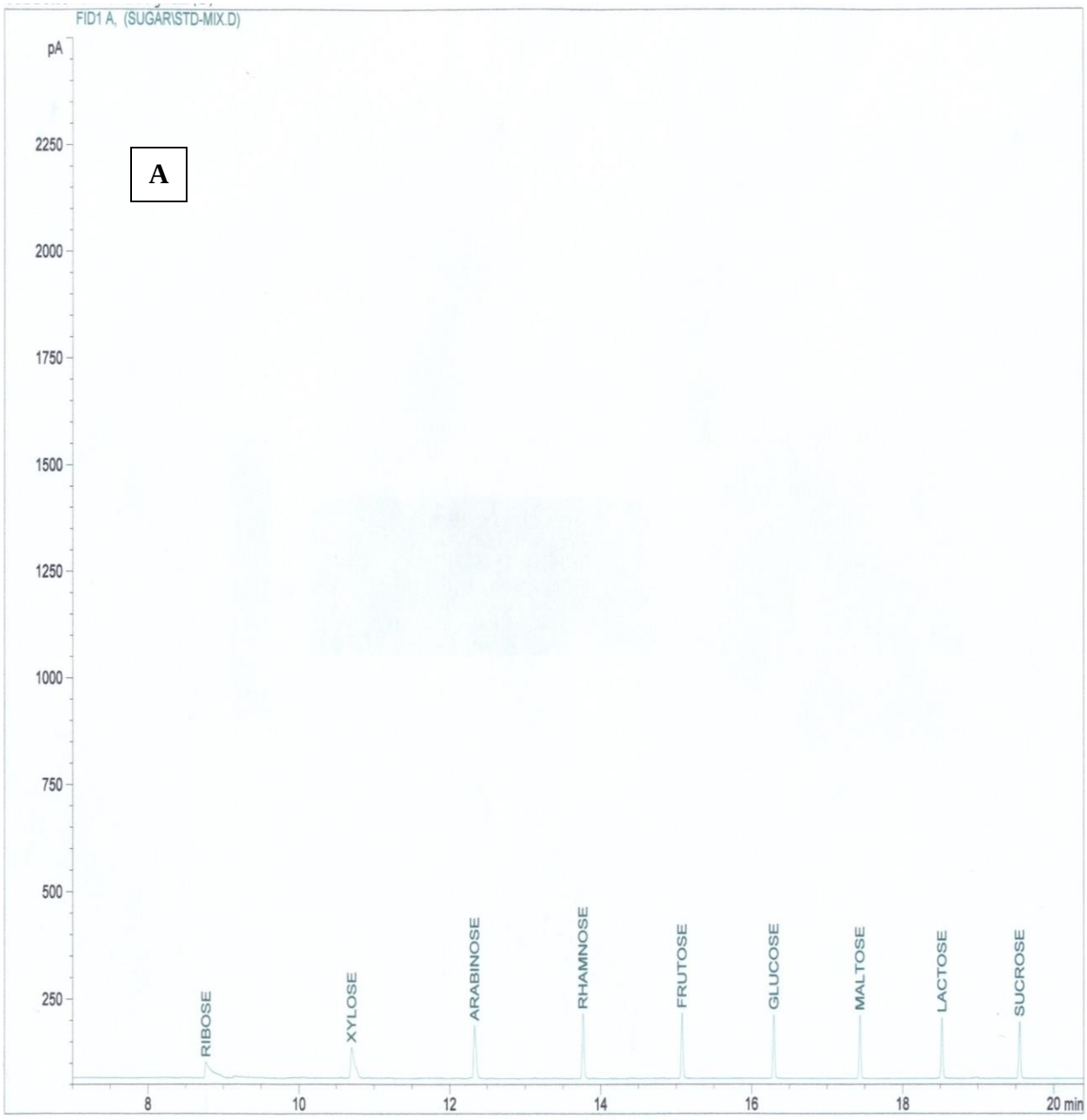
Chromatogram of *Saccharomyces cerevisiae* strain A

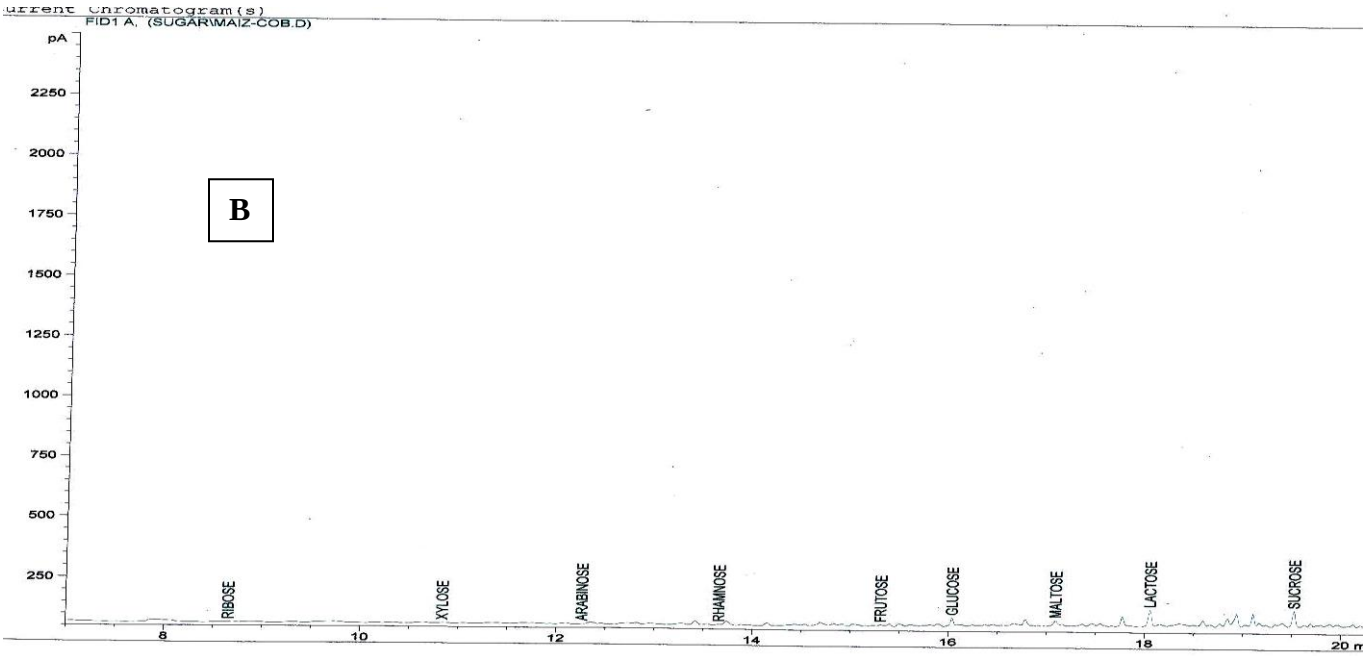


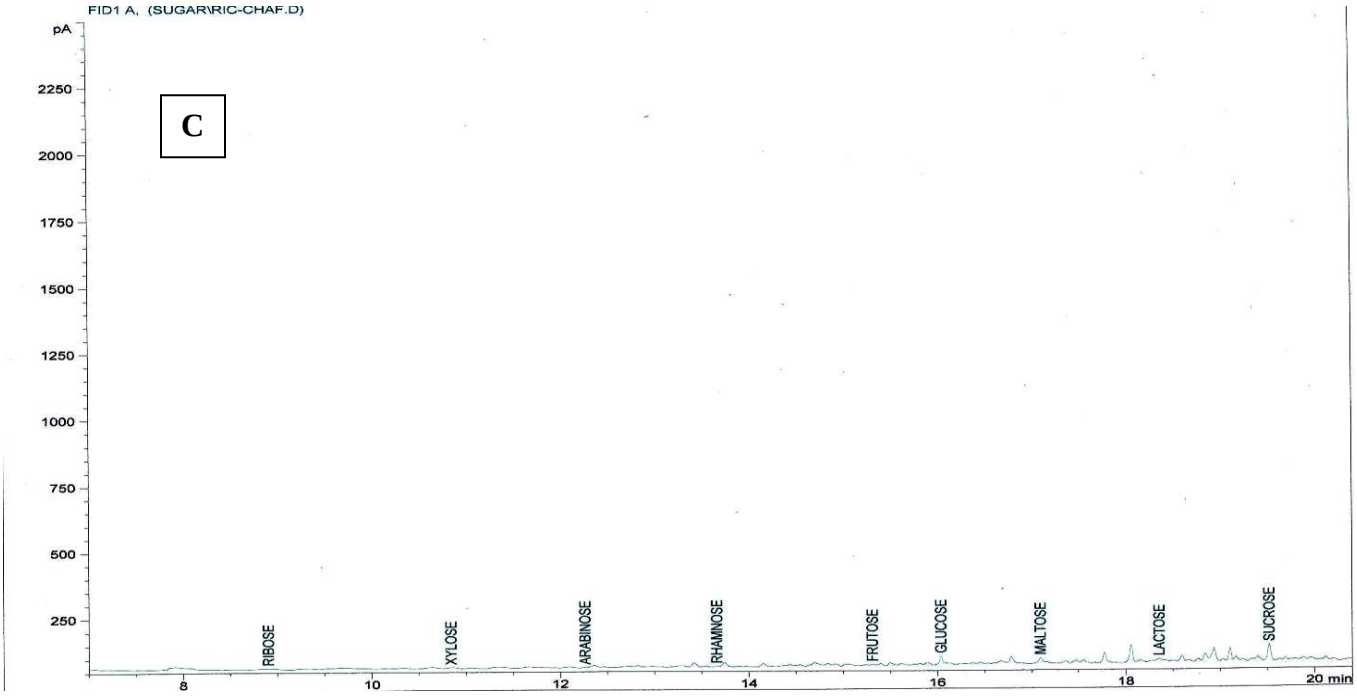
Chromatogram of *Saccharomyces cerevisiae* strain B

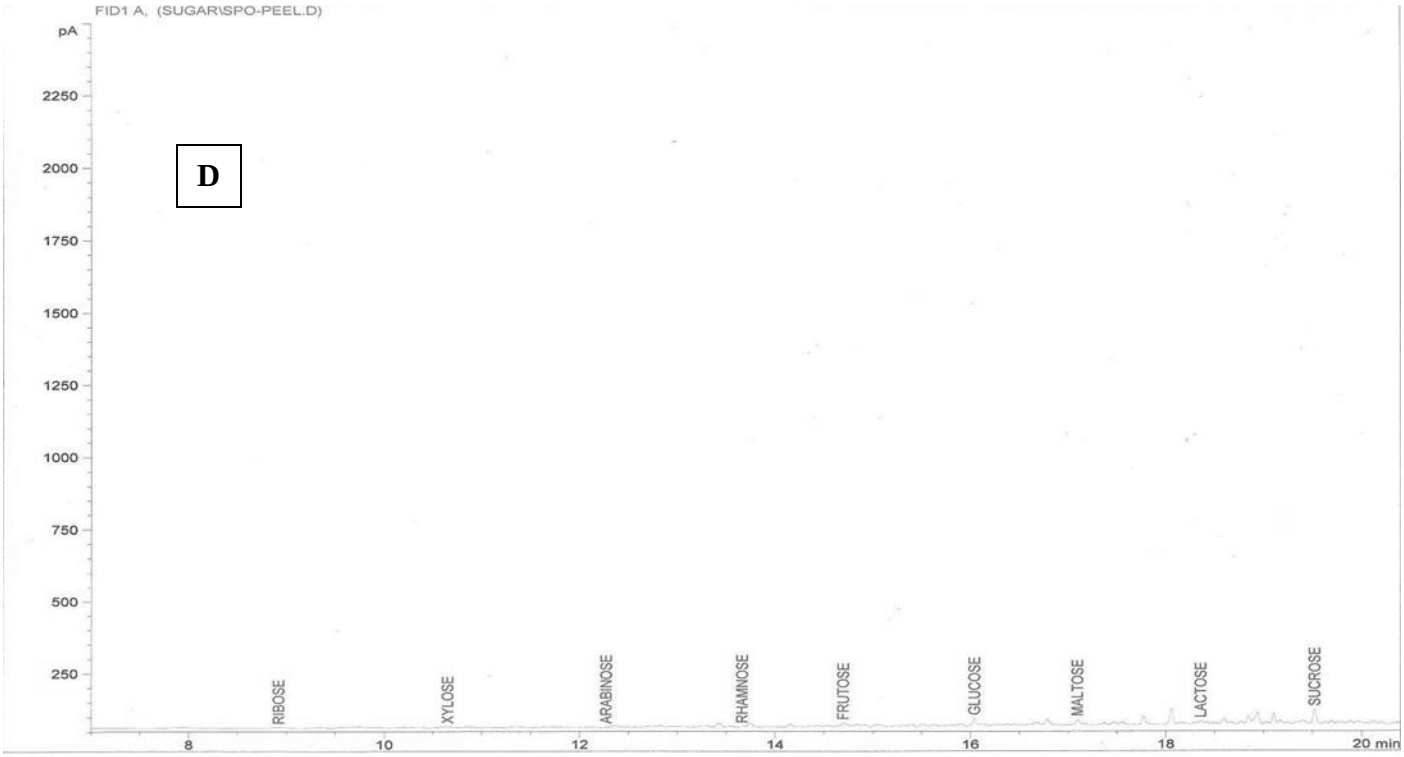
APPENDIX IV

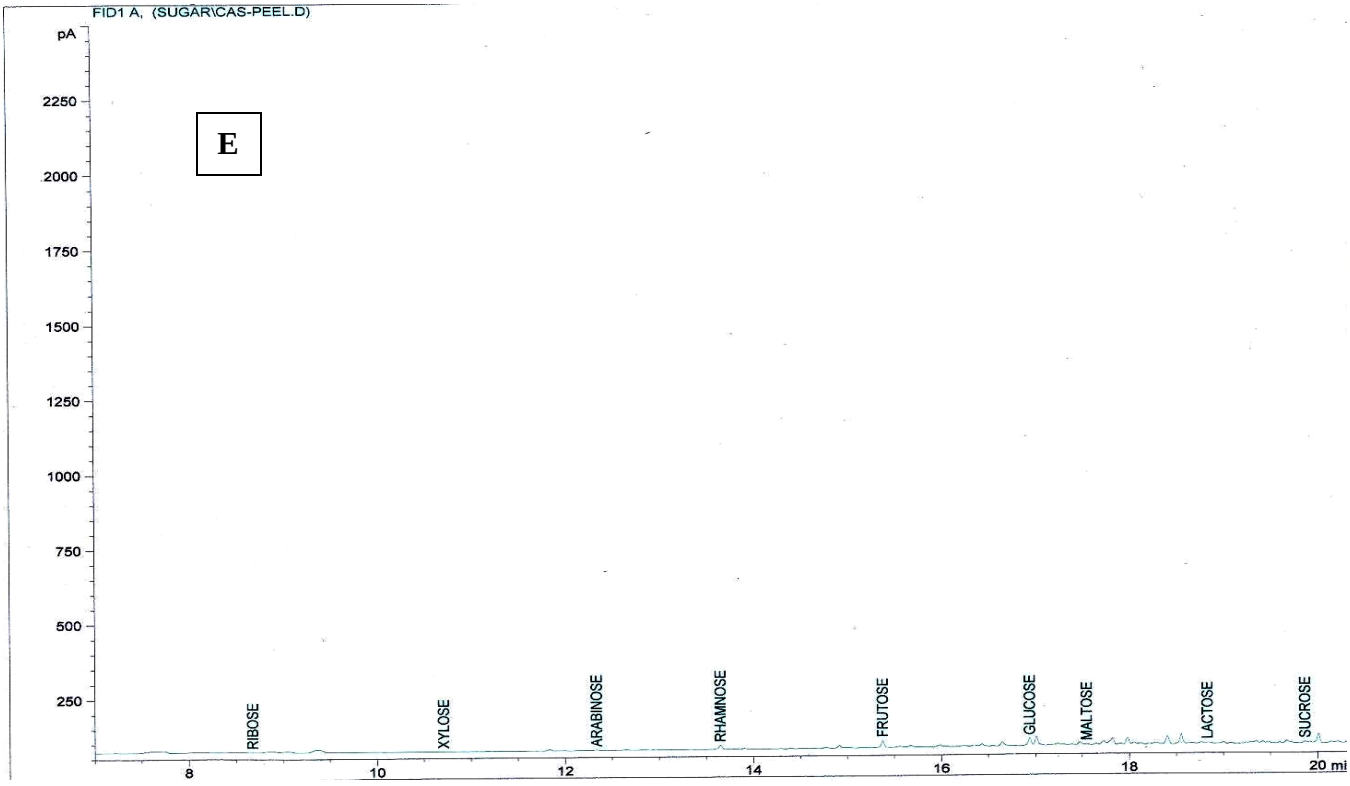
CHROMATOGRAM OF THE CONCENTRATION OF THE SUGAR IN THE PLANT ACID HYDROLYSATES




Current Chromatogram(s)
FID1 A, (SUGAR\MAIZ-COB.D)
pA
2250
2000
1750
1500
1250
1000
750
500
250
B
RIBOSE
XYLOSE
ARABINOSE
RHAMNOSE
FRUTOSE
GLUCOSE
MALTOSE
LACTOSE
SUCROSE
8
10
12
14
16
18
20 m


FID1 A, (SUGAR\RIC-CHAF.D)
pA
2250
2000
1750
1500
1250
1000
750
500
250
C
RIBOSE
XYLOSE
ARABINOSE
RHAMNOSE
FRUTOSE
GLUCOSE
MALTOSE
LACTOSE
SUCROSE
8
10
12
14
16
18
20 min

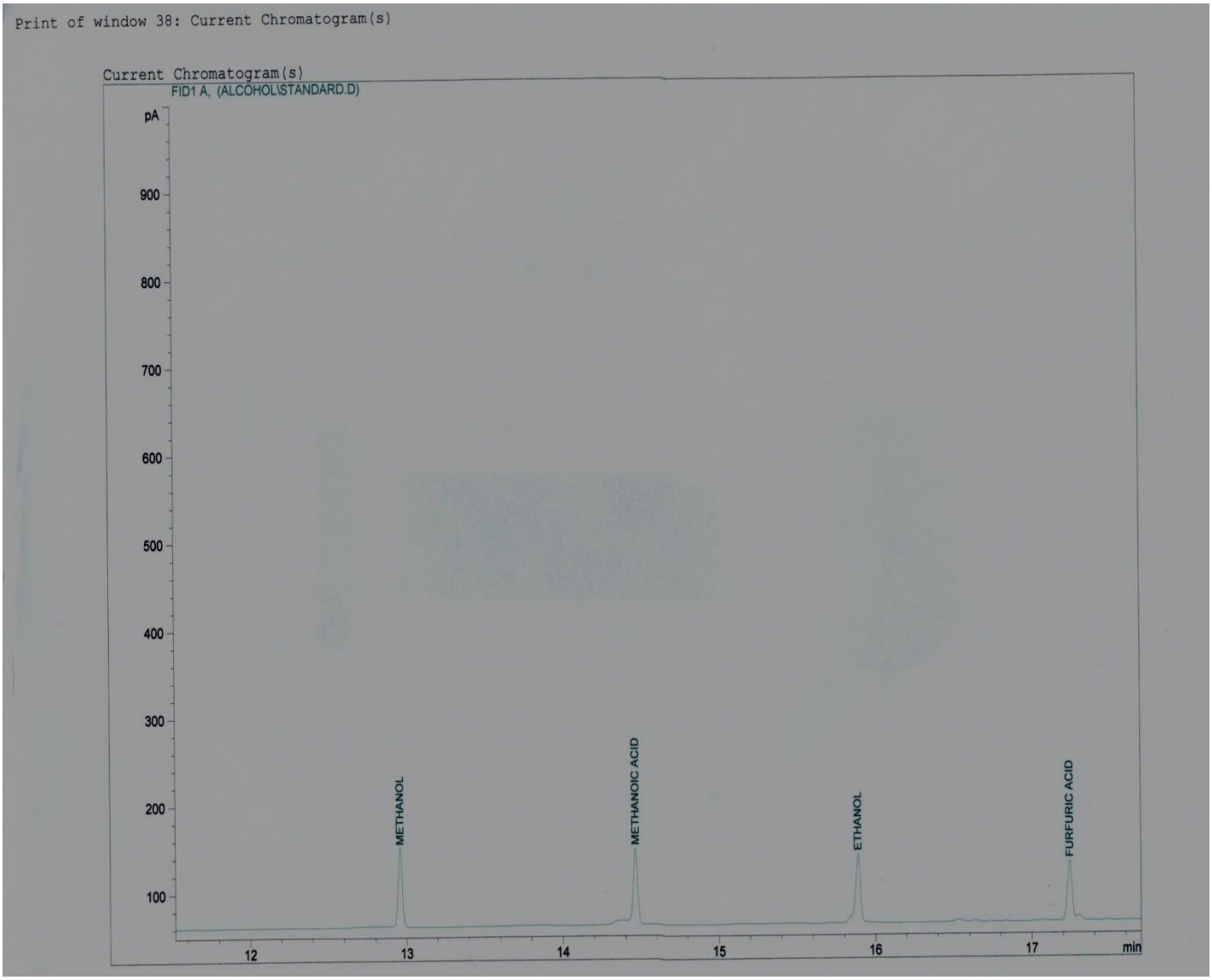

FID1 A, (SUGAR\SPO-PEEL.D)
pA
2250
2000
1750
1500
1250
1000
750
500
250
D
RIBOSE
XYLOSE
ARABINOSE
RHAMNOSE
FRUTOSE
GLUCOSE
MALTOSE
LACTOSE
SUCROSE
8
10
12
14
16
18
20 min


FID1 A, (SUGAR\CAS-PEEL.D)
pA
2250
2000
1750
1500
1250
1000
750
500
250
E
RIBOSE
XYLOSE
ARABINOSE
RHAMNOSE
FRUTOSE
GLUCOSE
MALTOSE
LACTOSE
SUCROSE
8
10
12
14
16
18
20 mi

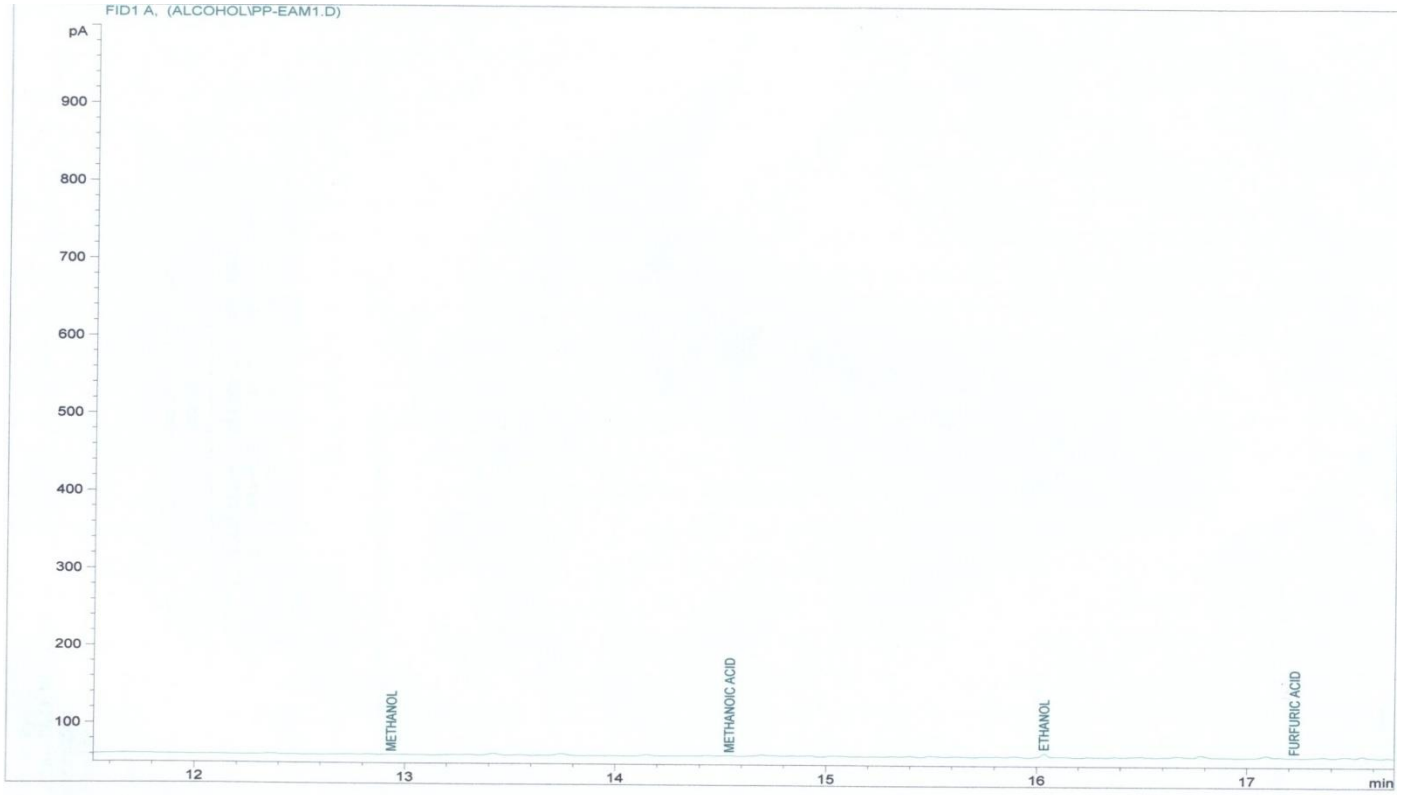
Gas chromatogram of Sugar profile of (A) standard used (B) maize cob (C) rice chaff (D) sweet potato peel (E) cassava peel (F) plantain peel showing various concentration of reducing sugar. Attenuation condition: Column: 0.0038 x 0.25µm, Oven temperature: Isothermal at 210°C, injector and detector temperatures: 250 and 325°C respectively, Hydrogen flow rate: 1.0ml/min, Detector: FID.

APPENDIX V

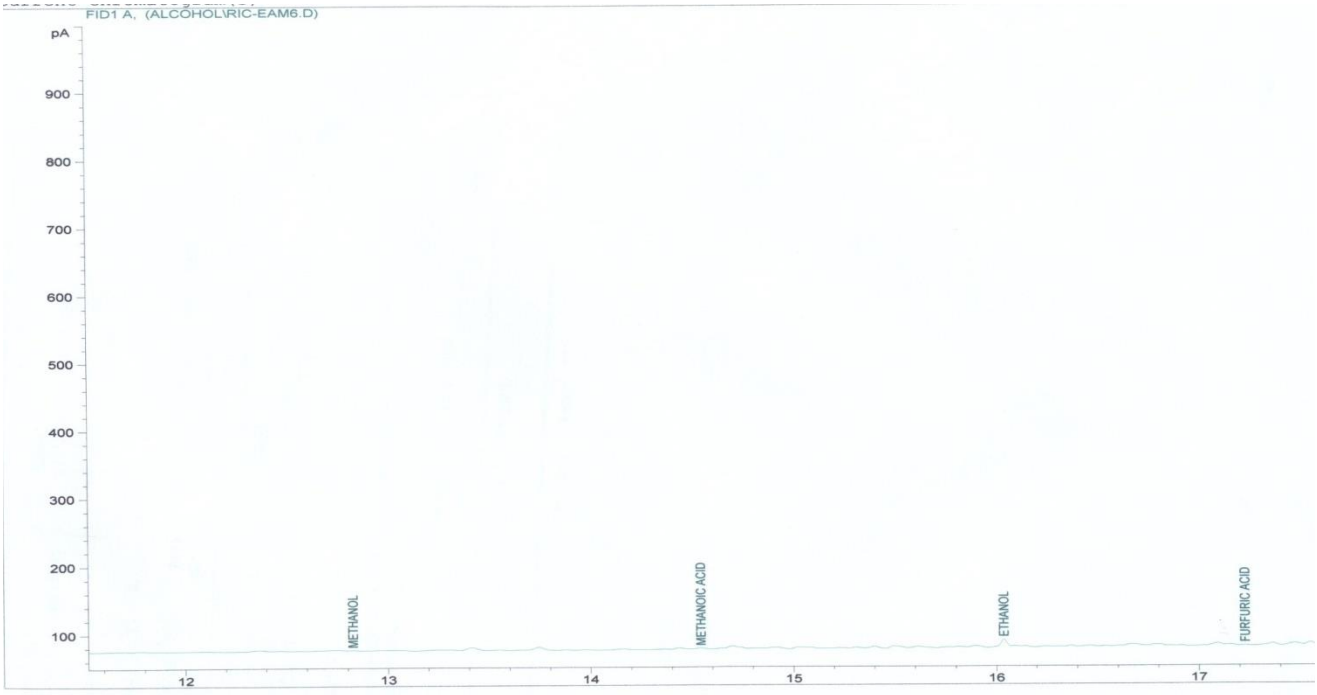
CHROMATOGRAM OF SOME BIOETHANOL PRODUCED



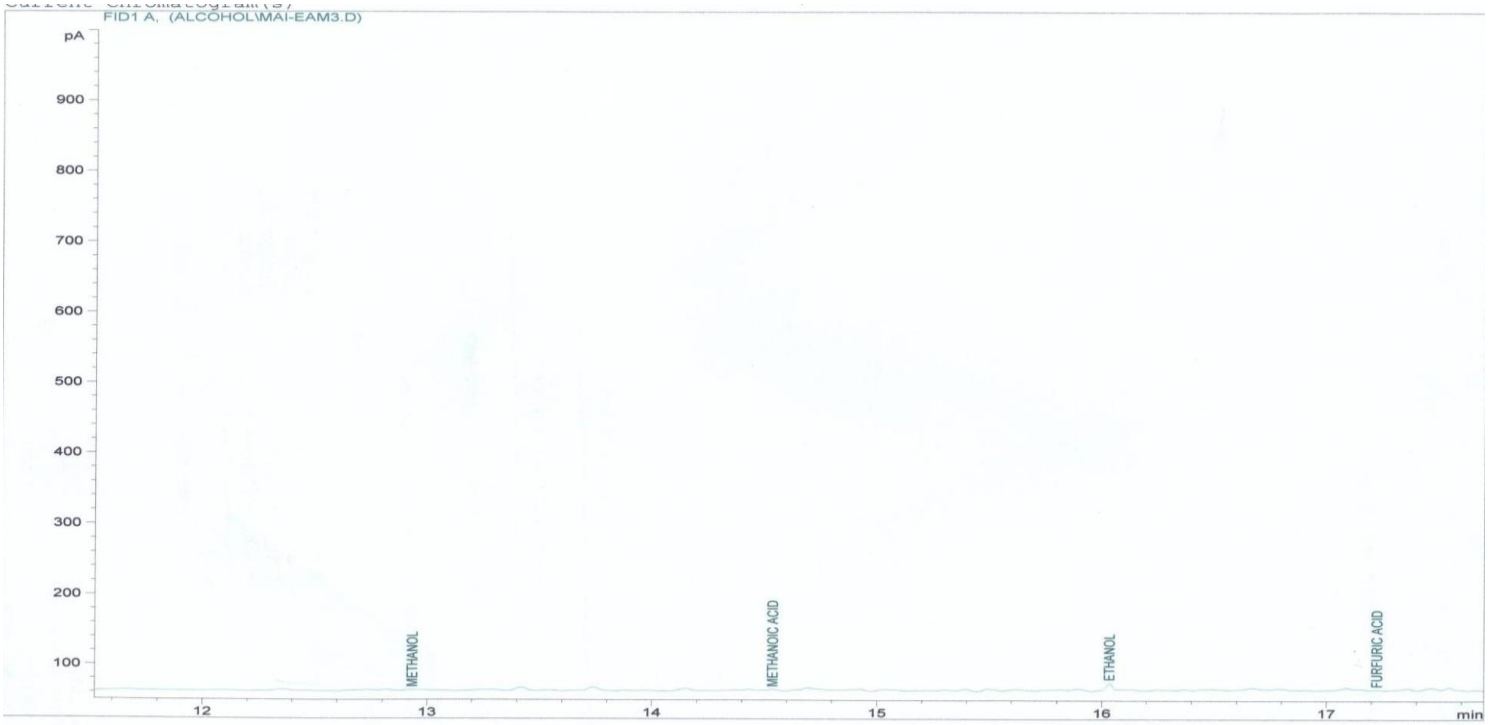
A= Standard used (methanol, methanol acid, ethanol, furfuric acid)



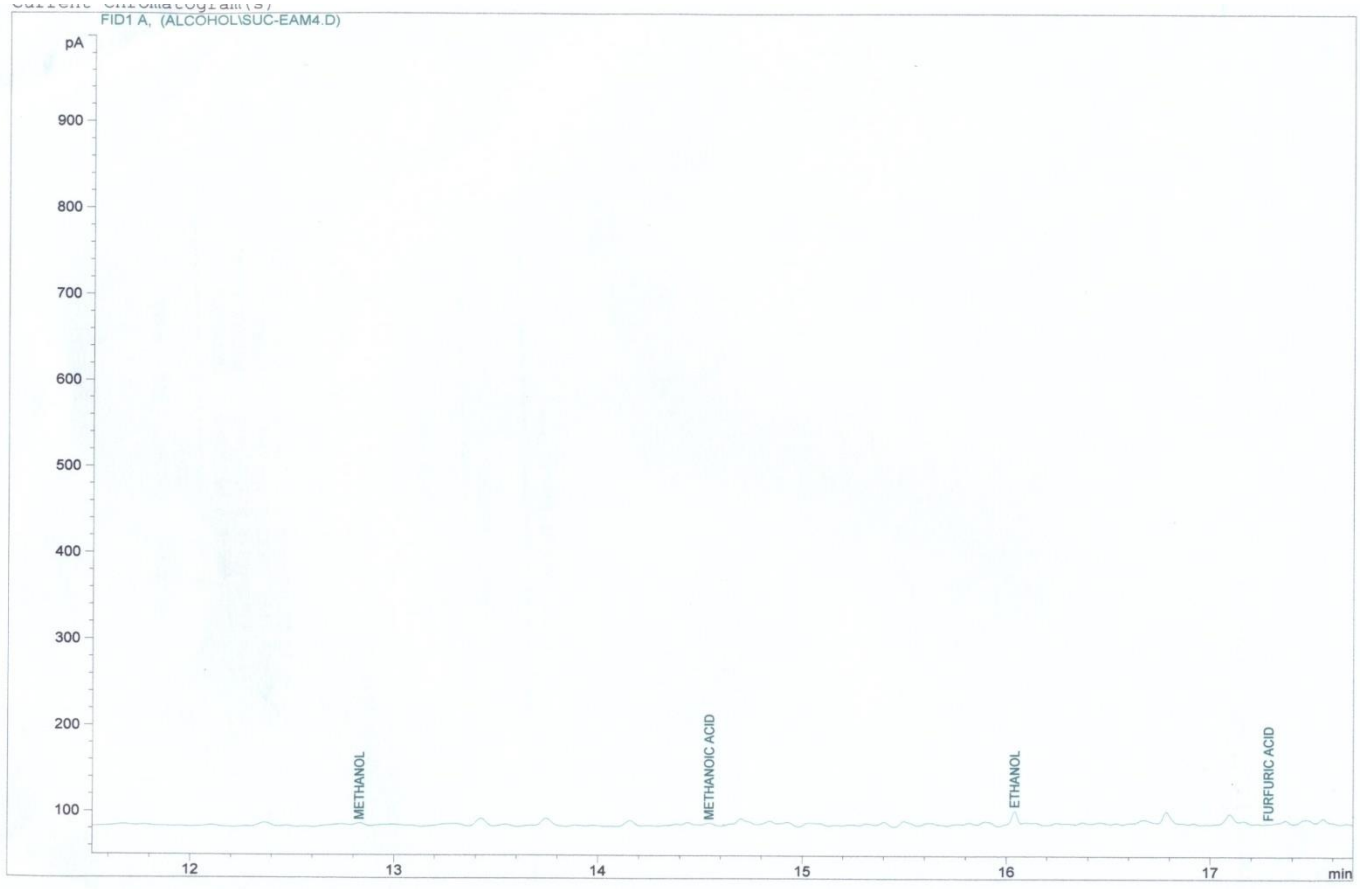
B= Plantain peel hydrolysate fermented with *S. pombe* (EAM 1)



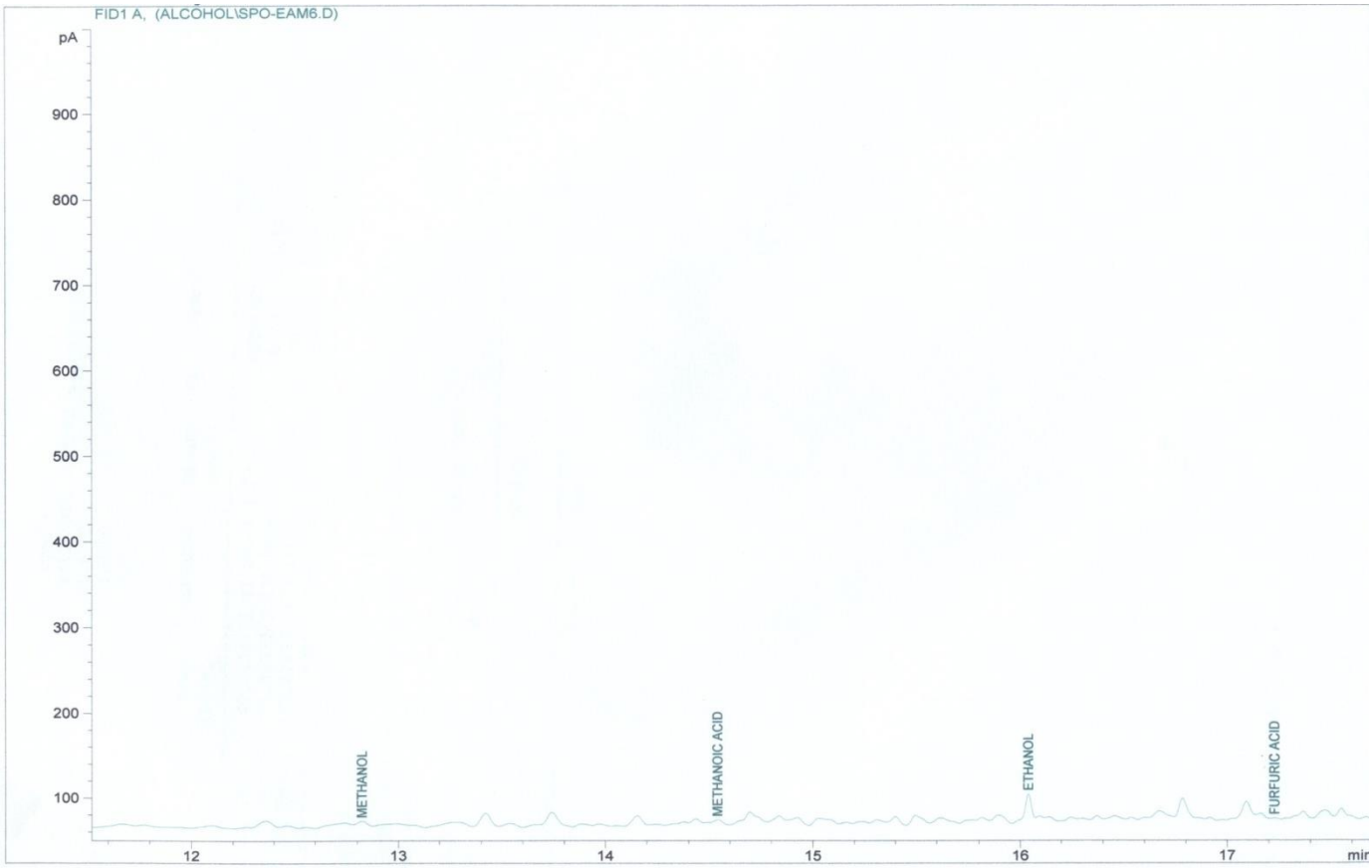
C= Rice husk hydrolysate fermented with *Saccharomyces cerevisiae* strain A (EAM 6)



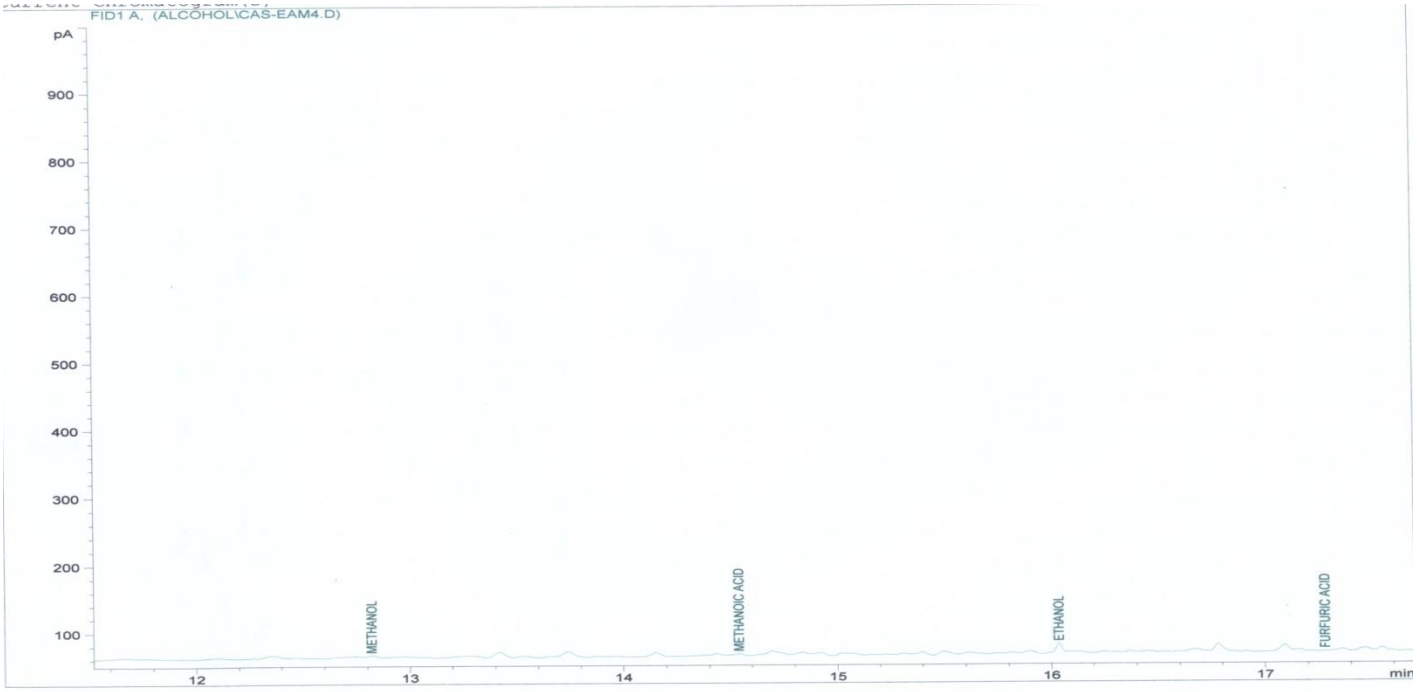
D= Maize cob hydrolysate fermented with *Pichia caribbica* (EAM 3)



E= Sugarcane chaff hydrolysate fermented with *Candida tropicalis* strain A (EAM 4)



F= Sweet potato peel hydrolysate fermented with *Saccharomyces cerevisiae* strain A (EAM 6)



G= Cassava peel hydrolysate fermented with *Candida tropicalis* strain A (EAM 4)

APPENDIX VI

CONSTITUENT OF THE LIQUID MEDIA USED IN THE GROWTH STUDIES

Malt Peptone Broth

Malt extract agar	3g
Peptone	2g
Distilled water	250ml

Potato Dextrose Agar Broth (Oxoid)

PDA	5g
Distilled water	250ml

Cassava Dextrose Broth

Cassava tubers	50g
Dextrose	10g
Distilled water	250ml

Carrot Dextrose Broth

Carrot	50g
Dextrose	10g
Distilled water	250ml

Millet Dextrose Broth

Millet grains	50g
Dextrose	10g
Distilled water	250ml

Crayfish Dextrose Broth

Crayfish	50g
Dextrose	10g
Distilled water	250ml

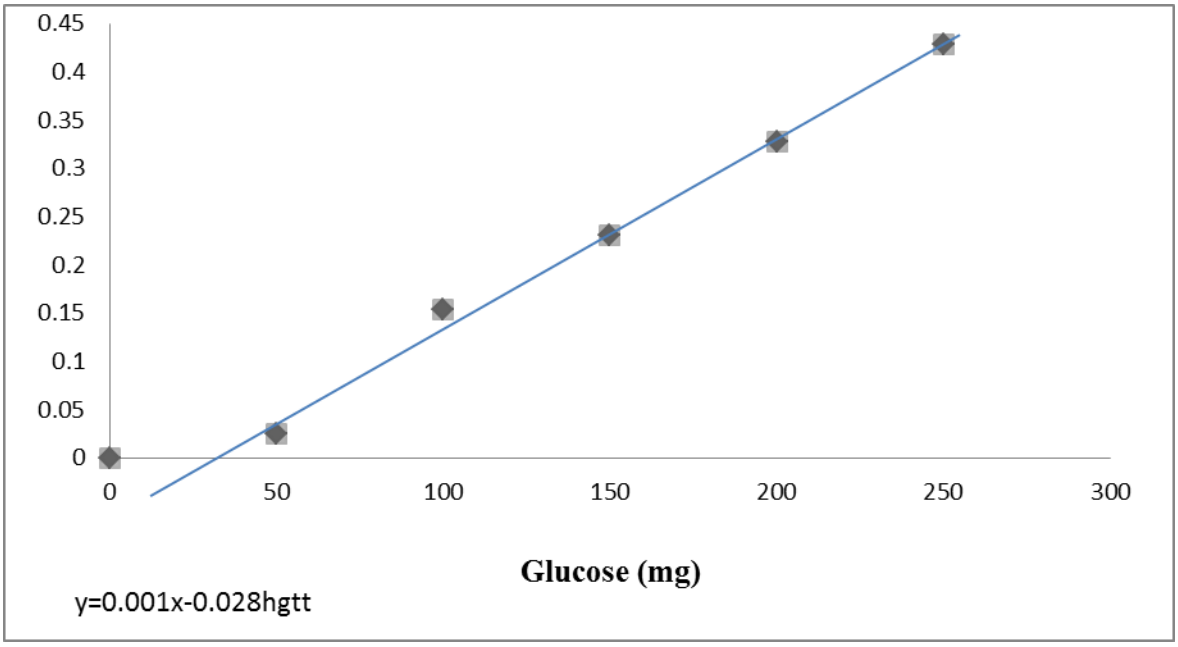
Maize Dextrose Broth

Maize seed	50g
Dextrose	10g
Distilled water	250ml

Sorghum Dextrose Broth

Sorghum seed	50g
Dextrose	10g
Distilled water	250ml

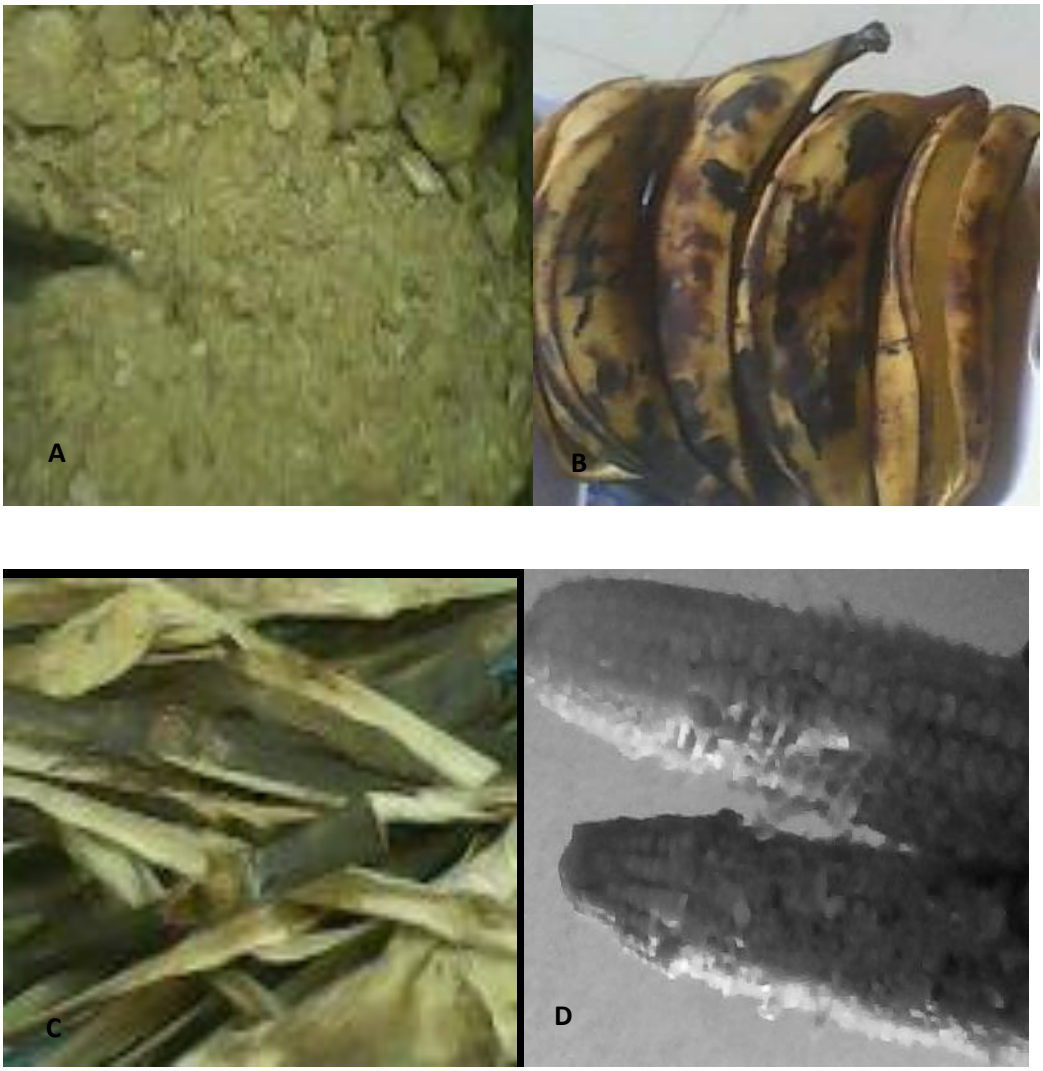
APPENDIX VII



STANDARD CURVE FOR REDUCING SUGAR ASSAY

APPENDIX VIII

SOME AGRO-WASTE USED IN THIS STUDY



A – Ground sweet potato peel

B – Plantain peel

C – Sugarcane chaff

D – Maize cob

APPENDIX IX

THE DISTILLATING EQUIPMENT USED IN THIS STUDY

I = Thermometer

II = Condenser

III = Water outlet

IV = Water inlet

V = Heating mantle

VI=Calibrated collecting cylinder