PRODUCTION OF BIOETHANOL FROM CASSAVA

PRESENTED

BY

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CERTIFICATION

This research work has been certified as meeting the requirement of the department of pure and applied chemistry of the Usmanu Danfodiyo University, Sokoto for the award of Bsc. Honour Degree in Applied Chemistry.

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DEDICATION

This research project is dedicated to God Almighty and my family.

ACKNOWLEDGEMENTS

I am profoundly grateful to the almighty God, who made it a reality for me to accomplish this great task and for this new level of academic achievement and for his protection love and care throughout the time of my BSc. Programme.

My sincere gratitude goes to my supervisor Dr. V. C Eze who stood by me throughout the course of this research work and for given me all the necessary guidelines to make this work a reality. I pray may God continue to bless him and him more wisdom, knowledge and understanding he requests to get to the top in his career. (Amen).

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ABSTRACT

Ethanol was produced using cassava as feedstock because of the ability of this plant to grow well even on poor soil. The concentration of reducing sugar was determined immediately after hydrolysis of the sample pH was also adjusted for the yeast to provide an enabling environment for the yeast for act in the fermentation process. The concentrations of reducing sugar obtained from the 0.6M hydrolysate are 9.094 mg/l, 5.922 mg/l, 5.127 mg/l, and for 0.8M hydrolysate are 5.140 mg/l, 5.294 mg/l and 4.988 mg/l and also for the 1M hydrolysate the concentration of the reducing sugar obtained are 5.127 mg/l, 7.796 mg/l and 6.375 mg/l all at varying temperature of 60°c, 80°c and 100°c and also at different time of 10, 30, and 60 minutes respectively. The result obtained for the final sugar concentration of the ethanol produced are 0.947 mg/l from the 1M hydrolysate, 0.907 mg/l for the 0.8M hydrolysate and 0.57 mg/l for the 0.6M hydrolysate. The bio ethanol obtained was dehydrated after the distillation process in order to obtain a pure product that is at least 98.5% to 99% pure. The final volumes of the bio ethanol produced from different acid concentrations are 51.5 cm^3 from the 0.8M, 48 cm^3 from 1M and 36 cm^3 from the 0.6M acid concentration. Hence, it was found that cassava root can be a great feed stock for the production of bio ethanol which give the percentage yield of 51.8%, 56.1% and 39.7% respectively.

CHAPTER ONE

INTRODUCTION AND LITERATURE REVIEW

1.0 Introduction

Ethanol is known as ethyl alcohol or fermentation alcohol. It is referred to one type of alcohols found in alcoholic beverages (Wyman, 2004). Due to the unstable price and the availability of crude petroleum, the fermentation has become an alternatives process to produce ethanol (Abella *et al*., 2007). Balat *et al*., (2002) had reported that almost 60% of the ethanol is produced by fermentation where the major world producers are Brazil and the US, which together account for about 80% of the world production. Mostly ethanol produced had been widely used in cars as a fuel alternative to gasoline.

Bio ethanol is easy to manufacture and process since the raw materials used as the feedstock is unlimited and cheaper. Major carbohydrate-containing substrates such as cane, corn are used for a feedstock in ethanol production and commonly available in tropical countries (Balat *et al*., 2008). Starchy substrate such as cassava could be exploited for ethanol production. The content of cassava composed of 95% starch and 2% moisture. Owing to its high carbohydrate content, tapioca becomes one of the most efficient sources of starch. This raw material has not yet been fully exploited in highly technical industries for ethanol production. Since the use of starch-based raw materials for ethanol production is not a common practice, it is important to determine the optimized conditions for starch processing in order to enhance the bio ethanol utilization (Aggarwal *et al*., 2001).

The hydrolysis of starch may be considered as a key step in substrate processing for bio ethanol production. The main role of this step is to effectively provide the conversion

of two major starch polymer components of amylase and amylopectin. Another crucial step would be the fermentation process that could subsequently be converted to ethanol by yeasts or bacteria (Olanbiwoninu *et al*., 2006). The parameters involve mainly pH, temperature, time etc. have to be evaluated and optimized in order to obtain a good yield of bio ethanol. (Wyman, 2004)

The earth experienced an increase in the mean temperature in the 19th century due to emission of greenhouse gases. Carbon dioxide has been the largest greenhouse gas emitted through combustion of fossil fuel as coal, oil and natural gases (Sun and Cheng, 2002). United States alone is responsible for 25% of global energy consumption and 25% of the world carbon dioxide emission. Researchers have shown that the deposit of crude oil in the earth crust is gradually depleting with time. This reason couple with the continuous hike in the pump price has driven global attention to the search for a renewable energy source to serve as transportation fuel and power industrial machines (Rogers, 2003). Bio ethanol produced from fermentation of sugars in plants has been discovered to be a perfect replacement for gasoline in some advanced countries (Bansal, 2007).

1.1 History of Cassava

Cassava (*Manihot esculenta*), sometimes also called manioc, is the third largest source of carbohydrates for human consumption in the world, with an estimated annual world production of 208 million tonnes. In Africa, which is the largest centre of cassava production, it is grown on 7.5 million hectare and produces about 60 million tonnes per year. It is a major source of low cost carbohydrates and a staple food for 500 million people in the humid tropics (Leen *et al*., 2007). The largest cassava market by far is in

Nigeria, responsible for 18% of world cassava production. Other important cassava producing countries are Brazil (upcoming), Indonesia, Thailand, Congo and Mozambique. Approximately 2% of world cassava is traded, mostly in the form of dried chips or pellets (Leen *et al.,* 2007).

1.2 Cultivation of Cassava

Major farming activities including land preparing, planting, fertilizing, weeding, and harvesting were covered in this stage (Nguyen *et al.,* 2007). Detailed information on fuel, fertilizers, and herbicides inputs was verified by field survey in the north eastern cultivation area of the country. The total cassava planting area in 2007 was 1.2 million hectare and production yield was 22.9 ton fresh roots per hectare (Pimentel, 1992). When comparing to India which had 0.24 million hectare of cassava planting areas, the production yield was 31.4 ton fresh roots per hectare which was 37% higher than production yield of Thailand (Office of Agricultural Economics, 2008). In traditional agriculture, the most common form of seedbed preparation for cassava planting is on mounts or on unploughed land (Adeoti, 2008).

Thereafter cassava may be planted on the flat, on ridges or in furrows. Flat plantings of cassava seem to produce higher yields of tuber than ridge or furrow plantings. However, flat planting is unsuitable on heavy clay soils, because the tubers tend to rot. Cassava is propagated vegetatively as clones. Generally, cuttings are taken from the mature parts of the stems, which give a better yield than those taken from the younger portion of the stems. The cuttings should have at least 3 nodes, which serve as origins of shouts and of roots (Leen *et al.,* 2007). Recent releases from agricultural breeding programmes include clones with resistance to many of the major diseases and pests.

Cultivar names are usually based on pigmentation and shape of the leaves, stems and roots. Cultivars may vary in yield, root diameter and length, disease and pest resistance levels, time to harvest, temperature adaptation. Storage root colour is usually white, but a few clones have yellow-fleshed roots. Each region has its own special clones. Most farmers grow several clones in a field. Cuttings produce root s within a few days and new shoots appear soon afterwards. Early growth is relatively slow, thus weeds must be continuing rolled during the first few months. (Yeshajaha, 1991)

1.3 Literature Review

Ethanol (CH_3CH_2OH) is a chemical compound which contains hydrogen, carbon and oxygen in its chemical structure. It is also known as ethyl alcohol or grain alcohol (U.S. EPA, 2008). It is a clear, colorless liquid with an agreeable odour (Bugaje, 2008). It is also referred to as the type of alcohol found in alcoholic beverages. Ethanol has a somewhat sweet flavor when diluted with water; a more pungent, burning taste when concentrated, it is more volatile than water, flammable, burns with a light blue flame, and has excellent fuel properties for spark ignition internal combustion engines (Wyman, 2004).

1.4 Historical Development of Bio ethanol as Fuel

The use of ethanol as fuel dates back to 1826, when Samuel Morey developed an engine that ran on ethanol and turpentine called camphene. Bio ethanol was used in Germany and France by the then incipient industry of internal combustion (IC) engines as early as 1894 (Afiuwa *et al,* 2007). Bio ethanol as fuel gained more prominence in 1908, when the Ford Motors in the USA developed the Henry Ford's model T vehicle

which was designed to use gasoline, ethanol (from corn) or a combination of both. The use of bio ethanol for fuel was widespread in Europe and the United States during this period. Brazil has utilized bio ethanol as transportation fuel since 1925. The potential of bio ethanol was ignored, especially after the World War II, because it became more expensive than petroleum-based fuel. The energy crisis of the 1970‟s then renewed interest in ethanol production for fuel and chemicals in both the USA and Brazil, where mass production of bio ethanol grown from corn and sugar cane started, respectively (Balat and Balat, 2009; Balat, 2009).

The United States is the world's largest producer of bio ethanol fuel, accounting for nearly 47% of global bio ethanol production. Brazil is the world's largest exporter of bio ethanol and second largest producer after the United States (Balat and Balat, 2009). Brazil produces her bio ethanol from sugarcane and cassava while the USA produces hers from corn (Naylor *et al*., 2007). China is also a leading contender in bio ethanol production, producing over I billion litres per year from wheat and corn, while France which is leading other European countries, produces over 200 million gallons of ethanol from sugar beets and wheat **(**Sperling and Cannon, 2004**).** Nigeria National Petroleum Cooperation (NNPC), to generate fuel ethanol from cassava and sugar cane. This policy thrust was designed with the aim of generating wealth and reducing environmental pollution (Kupolokun, 2006; Umar, 2006). At present, the Federal Government of Nigeria has agreed to the blending of 5 percent ethanol (E5) by composition with premium motor spirit (PMS) (Ezeobi 2008). The government adopted E5 because it believes that the level will not damage vehicles in Nigeria, although this proportion is expected to

increase to 10 per cent (E10) in the nearest future (Ugwuanyi, 2008). The core focus of the Nigeria bio fuel programme is to ensure the production of fuel ethanol domestically.

1.5 The Need for Bio Ethanol as Fuel

Bio ethanol being a bio fuel is produced from biological sources and has a lot of benefits which makes it a better energy source than fossil-based fuels.

1.6 Environmental Benefit of Bio ethanol

Carbon dioxide emission due to combustion of fossil fuels has become a major environmental concern. Carbon dioxide emission contributes greatly to green house effect, climate change and global warming. Bio ethanol is primarily seen as a good fuel alternative because the source crops can be grown renewably and in most climates around the world. In addition, the use of bio ethanol is generally CO_2 neutral. This is achieved because, in the growing phase of the source crop, CO_2 is absorbed by the plant and oxygen is released in the same volume that CO_2 is produced in the combustion of the fuel. This creates an obvious advantage over fossil fuels, which emit CO_2 as well as other poisonous emissions that have great negative impact on the environment (Cardona and Sanchez, 2007; and Hu *et al.*, 2008). Bio ethanol is bio-degradable, more environmentally friendly and less toxic than fossil fuel.

Also, bioconversion processes in general do not produce hazardous compounds, and if toxic solvents and chemicals are avoided in the processing stages, then fewer environmental pollutants are produced. In addition, biomass production and microbial conversion processes can be developed and used in a more distributed manner,

avoiding the need for transport of fuels via cargo ships or pipeline for long distances (Drapcho *et al.,* 2008).

Also, CO_2 from ethanol fermentation can be used to extract oils and nutraceutical compounds from biomass instead of using toxic organic solvents such as hexane (Walker *et al*., 1999). Bio ethanol, can be used in biodiesel production from biological oils in place of toxic petroleum based methanol traditionally used (Drapcho *et al.,* 2008).

1.7 Advantages of Bio ethanol as Fuel

Adding bio ethanol to gasoline increases the oxygen content of the fuel, which implies a less amount of required additive. The increased percentage of oxygen allows a better oxidation of the gasoline hydrocarbons with consequent reduction in the emission of CO and aromatic compounds (Umar, 2006). Wyman *et al* (1999) corroborated this by writing that “using bio ethanol blended fuel for automobiles can significantly reduce petroleum use and exhaust greenhouse gas emission”.

Bio ethanol has a higher octane number, broader flammability limits, higher flame speed and higher heat of vaporization (Yoosin and Sorapipatana, 2007). These properties allow for higher compression ratio and shorter burn time, which lead to theoretical efficiency advantages over gasoline in an internal combustion engine (Balat, 2009). Octane number is a measure of the gasoline quality for prevention of early ignition, which leads to cylinder knocking. An oxygenated fuel such as bio ethanol, with high octane number, provides a reasonable antiknock value (Balat and Balat, 2009).

It is believed that a given volume of ethanol could provide energy enough to drive about 75-80% of the distance as the same amount of gasoline, although it has only about two-third of the energy content (Adeoti, 2005).

Bio ethanol when related to MTBE (methyl tert butyl ether), which is also an oxygenator of gasoline, is not toxic and does not pollute ground water. Bio ethanol is most commonly blended with gasoline in concentrations of 10% bio ethanol to 90% gasoline, known as E10 and nicknamed “gasohol” (Oliveria *et al*., 2005). Bio ethanol can be used as a 5% blend with petrol under the EU quality standard EN228. Bio ethanol can be used at higher levels, for example, E85 (85% bioethanol) (Azmi *et al*, 2011).

1.8 Disadvantages of Bio ethanol as Fuel

Bioethanol has lower energy density than gasoline (bio ethanol has 66% of the energy that gasoline has). Also, the high oxygen content of ethanol and its ability to oxidize into acetic acid induce compatibility issues with some materials used in the engine, such as metals or polymers. In addition, ethanol leads to azeotropes with light hydrocarbon fractions and can lead to volatility issues, low flame luminosity, lower vapour pressure and high latent heat of vapourization (making cold starts difficult), miscibility with water (which can cause demixing issues when blended with hydrocarbons), toxicity to ecosystem (since its combustion in engines induces aldehyde emissions, which has negative impact on health) (Baks *et al.,* 2004). Though the use of bio ethanol as engine fuel has some disadvantages, its advantages as engine fuel far outweigh its disadvantages.

1.9 Feed stocks for Bio ethanol Production

Bio ethanol feedstock can be divided into three major groups:

(1) Starchy materials

(2) Sugar or sucrose-containing feed stocks and

(3) Ligno cellulosic biomass

1.9.1Starch Feed Stocks

The starch feed stocks are mainly; cereals, tubers and roots. Cereal grains are used mostly as food and feed. However because of their high starch content, they are also good feed stocks for conversion to bio fuels and other bio based products. Ethanol is the only bio fuel that has been produced commercially from these feed stocks in large quantities (Ayoola *et al.*, 2008). Cereals used as bio ethanol feed stocks include: wheat, sorghum, rice and oats. The United States and Canada are predominantly producers of bio ethanol derived from corn. Tubers and roots are potential feed stocks for ethanol production because of their high starch content. The two crops that have been given much attention are cassava and potato. Cassava is mainly used as feed stock in Brazil, Nigeria and China (Ayoola *et al*., 2008).

1.9.2 Sugar Feed Stocks

Main feedstock for ethanol production is sugar cane in form of either cane juice or molasses (by -product of sugar mills). About 79% of ethanol in Brazil is produced from fresh sugar cane juice and the remaining percentage from cane molasses (Wyman *et al*., 2004). Sugar cane molasses is the main feedstock for ethanol production in India,

cane juice is not presently used for this purpose (Ghosh and Ghose, 2003). Beet molasses are other source of fermentable sugars for ethanologenic fermentation. Nigeria and other tropical countries use sugar cane as main feed stock for ethanol production (Cardona and Sanchez, 2004). Sugar beet is the main feedstock for the production of bioethanol in European Union member states (Cardona and Sanchez, 2007).

1.9.3 Lignocellulosic Feed stocks

The three main components of lignocellulosic biomass are cellulose, hemicelluloses and lignin. Cellulose, which is an abundant component in plants and wood, come in various forms and a large fraction, comes from domestic and industrial wastes (Abella*et al*., 2007). Cellulose and hemicelluloses can be hydrolyzed with chemicals and or enzymes to monomeric sugars, which can subsequently become converted biologically to (Bamfort, 2008).

1.10 Cassava as a Preferable Feedstock for Bioethanol Production in Nigeria

Cassava (*Manihotesculenta*), also known as manioc, sagu, yucca and tapioca, is one of the most important tropical root crops (Betiku, 2010). It is a perennial woody shrub, with up to 32 per cent (fresh) starch content (Betiku, 2010), and has 70 percent moisture. Thus, on a dry basis it contains about 73 percent starch, which gives it a theoretical ethanol yield of 0.45 L/kg (Betiku, 2010). Cassava is the highest producer of carbohydrate with perhaps the exception of sugar cane. The yield in major producing regions varies between 6.4 and 17 tons/ha of fresh root, although under good conditions, yields could reach 90 tons/ha of fresh root (Bokanga, 1996).

Cassava has a comparative advantage over other feed stocks in ethanol production in that, it is a cheap substrate that is easily available in tropical countries (Amutha and Gunasekaran, 2001). It can be planted on marginal lands where other agricultural crops such as sugarcane, rice, wheat and corn do not grow well. It has a high tolerance to drought because it can survive even during the dry season when soil moisture is low, but humidity is high (Balat *et al*., 2009). Also it requires lower soil quality compared to sugar cane as it thrives better in poor soils than any other major plant (Nadir *et al*., 2009). Also, large area of little used land can be utilized for cultivation and fertilization is rarely necessary. Moreover, a cassava ethanol plant requires less complex processing equipment resulting in lower investments. This is due to the unique characteristics of cassava starch (Wang, 2002) and the low amounts of impurities which make the extraction of starch from the root relatively easy.

1.11 Physical and Chemical Properties of Ethanol

Ethanol posses the following in physical and chemical properties which are as thus:

1.11.1 Physical Properties of Ethanol

Ethanol like other alcoholic compounds posses the following physical properties.

- Ethanol is a volatile, colorless liquid that has a slant odor.
- Ethanol is a versatile solvent, miscible with water and with many organic solvents e.g. acetic acid, acetone etc.
- Ethanol burn with a smokeless blue flame that is not always visible in normal light.
- Ethanol is also miscible with light aliphatic hydrocarbons, such as pentane and hexane.

1.11.2 Chemical Properties of Ethanol

a. Deprotonation

Ethanol can behave as weak acids, this give it ability to undergo deprotonation reaction. The deprotionation reaction produces an alkoxide salt with either ni-butyllithum, sodium, potassium metal or strong base (Sodium hydride) (Lide and David, 2012).

$2R\text{-}OH + 2NaH \longrightarrow 2R\text{-}O^{-}Na^{+} + 2H_2\uparrow$ ……1

$2R\text{-}OH + 2Na \longrightarrow 2R\text{-}O^{-}Na^{+} + H_2$ ……2

$2CH_3CH_2\text{–}OH + 2Na \longrightarrow 2CH_3\text{-}CH_2\text{–}O^{-} + H_2\uparrow$ ……3

b. Esterification

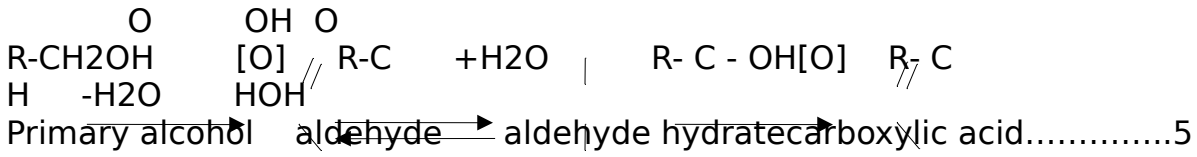
Simply means formation of an ester from alcohol and a carboxylic acid reaction, the reaction is usually performed at reflux with a catalyst of concentrated sulphuric acid (Lide and David, 2012).

$R\text{-}OH + R^1 - COOH \longrightarrow R^1\text{–}COOR + H_2O$ ……4

In order to drive the equilibrium to the light and produce a good yield of ester, water is usually removed by an excess of H_2SO_4. Ester may also be prepared by reaction of the alcohol with an acid chloride in the presence of a base such as pyridine (Lide and David 2012).

c. Oxidation

Ethanol ($R\text{-}CH_2\text{-}OH$) can be oxidized either to aldehydes (R-CHO) or to carboxylic acids ($R\text{-}CO_2H$) as shown below in equation 5 below



1.12 Significance of the Research

Increasing the use of bio ethanol for energy generation purpose is of particular interest because allow mitigation of greenhouse gases, bio ethanol is a potential substitute for current high pollutants fuels from conversional sources.

1.13 Aims and objectives

The aim of this research is to produces ethanol from cassava plant root. The objectives are:

i. To determine the concentration of reducing sugar in the cassava root.

ii. To determine the effect of different concentrations of hydrochloric acid solution and the effect of temperature and time on glucose yield in the ethanol produced.

CHAPTER TWO

MATERIALS AND METHODS

2.0 Materials Used

Table 2.1 Lists of Apparatus Used

The apparatus used and their manufacturers are in table 2.1

Apparatus	Model	Manufacturer
Beakers	Glass	pyrex, England
Conical flask	Glass	pyrex, England
Volumetric flask	Glass	pyrex, England
Measuring cylinder	Glass	pyrex, England
Autoclave		
Sample bottles	Plastic	pyrex,England
Mortar and Pestle	Wood Stirrer	Glass
Sieve		
Filter paper	Whattman paper	
Weighing Balance	Memmert	Galen, UK
PH meter		
Density bottle	Glass	pyrex, England
Thermometer	Glass	pyrex, England
Hot plate		
Heating mantle		
UV spectrophotometer	Model 721A	Mc. Jefferson's Comp. Ltd, USA
Distillation Apparatus		Galen, UK

2.1 Reagents Used

Table 2.2 Lists of Reagents Used

The reagents used with their chemical formula and manufacturers are shown in table2.2 below.

Reagents	**Formula**	**Manufacturer**
Tetraoxosulphate (VI) acid	H_2SO_4	Mayer and Bayer, England
Sodium hydroxide	NaOH	BHD Chemicals Ltd, England
Dinitrosalicylic acid	$C_7H_4N_4O_7$	BHD Chemicals Ltd, England
Crystalline phenol	C_6H_5OH	SDS Fine Chemicals Ltd
Sodium Sulphate	Na_2SO_4	Mayer and Bayer, England
Glucose	$C_6H_{12}O_6$	Mayer and Bayer, England
Peptone Water		Biotel
Yeast (Beaker's Yeast)		
Potassium Sodium tartrate	$KNaC_4H_4O_6.4H_2O$	Mayer and Bayer, England
Potassium dichromate	$K_2Cr_2O_4$	Mayer and Bayer, England

2.2 Sample Collection and Sample Treatment

2.2.1 Sample Collection

Harvested cassava plant was purchased from Sokoto North local Government Main market, Sokoto. The cassava was put in a polythene bag and brought to Chemistry Laboratory of Usmanu Danfodiyo University, where it was peeled.

2.2.2 Sample Treatment

The cassava which is the main feedstock for this analysis was peeled with a knife and sun-dried for a period of five days in order to remove the water content. It was later grinded into powdered form using mortar and pestle. The resulting powdered sample was then sieved so as to obtain a fine powder of homogenous sizes.

2.3 Acid Hydrolysis

Three different concentrations of 1M, 0.8M and 0.6M were prepared and 10g of the powered sample was added into each concentration. The mixture was soaked for two hours. The pH of each hydrolysate was adjusted using sodium hydroxide. The hydrolysate was filtered using filtered paper to remove the unhydrolysed materials and solid residue. Part of the filtrate was collected and analyzed for its reducing sugar.

2.4 Preparation of Reagents

2.4.1 Preparation of 1M H_2SO_4

This was prepared by measuring 5.43 cm^3 H_2SO_4 of known concentration with a measuring cylinder and 100 cm^3 of distilled water was added in a volumetric flask.

2.4.2 Preparation of 0.8M H_2SO_4

This was prepared by measuring 4.34 cm^3 H_2SO_4 of known concentration with a measuring cylinder and 100 cm^3 of distilled water was added in a volumetric flask.

2.4.3 Preparation of 0.6M H_2SO_4

This was prepared by measuring 3.26 cm^3 H_2SO_4 of known concentration with a measuring cylinder and 100 cm^3 of distilled water was added in a volumetric flask.

2.5 Preparation of 1% NaOH

The solution was prepared by weighting 1g of NaOH volumetric flask. It was dissolved in distilled water and made up to 100ml mark of the conical flask. The solution was stirred until completely dissolved.

2.6 Preparation of potassium sodium tartrate

The solution was prepared by weighting and dissolving 40g of potassium sodium tartrate into volumetric flask and made up to 100cm^3 marks.

2.7 Preparation of DNS Reagent

This was prepared by weighting 5g dinitrogen salicylic acid, 1g of crystalline phenol and 0.25 of sodium sulphate and all was dissolved in 1% NaOH and the whole mixture was diluted to 500cm^3 marks with distilled water in a volumetric flask.

2.8 Preparation of Glucose Standard

Glucose of 0.25g was weighed and dissolved in 500cm^3 of distilled water to prepare a 500 ppm stock solution. From this stock solution, 2$cm^{3,}$ 4cm^3, 6cm^3, 8cm^3, and 10 cm^3 were pipette into different request bottles and serial dilution made up 10cm^3 each with distilled water. The final concentrations were 100ppm, 200ppm, 300ppm, 400ppm, and 500ppm respectively.

From each of the individual concentration, 1cm^3 was taken and placed in a test tube: 1cm^3 of DNS solution and of distilled water was also added to each of the concentration.

The test tubes were covered with foil sheet and placed in a water bath. 1cm^3 of Rochelle salt solution was also added when the solution was warm. The intensity of each concentration was carried out using Uv spectrophotometer at 540 nm

2.9 Determination of Reducing DNS

Sample extract of 3 ml was put in test tubes and was equalized with 3 ml of distilled water; 3 ml of DNS reagent was added to the mixture. The mixture was treated at specific time and temperature to develop a yellow colour. 1 ml of sodium tartrate solution was added to the mixture the content in the test was still warm. The absorbance of the sample was recorded using Uv-vis spectrophotometer at 540nm

2.10 Activation of Yeast

The yeast was activated by weighting and dissolving 1.5 g of peptone water into a conical flask and filled up to 100 cm^3 marks with distilled water. The solution was covered with cotton wool and wrapped with foil paper sheet. It was then autoclaved to sterilize at 121°C for 15 minutes and then allowed to cool 2 g of yeast was measured and dissolved into the sterilized peptone water and the solution was incubated for 24 hours.

2.11 Fermentation

The fermentation was done 0.5 cm^3 of activated yeast into three different conical flask containing the samples. The conical flask were then covered with cotton wool and wrapped with foil sheet to avoid oxygen penetrating into the fermentation medium. The samples were all taken to the incubator and incubated at room temperature for 6 days.

2.12 Distillation

The ethanol which is produced from the fermentation process contains significant amount of water which has to be removed. This is achieved using fractional distillation set up. The ethanol boils at 78°C while water boils at 100°C therefore, when boiling the product ethanol turns into vapour state at its boiling point before the water. The ethanol

2.13 Determination of Ethanol Concentration

This was carried out by adding 3 cm^3 of potassium, dichromate, followed by 3 ml of the distillate ethanol] and equalized with distilled water. The mixture was heated using heating mantle for 15 minutes at 90°C to develop the light green colour. The absorbance of the sample was measured at 588 nm using Uv-spectrophotometer

2.14 Determination of pH

The pH was determined using an electronic pH meter, the pH electrode was dipped into the sample and the pH reading was recorded.

CHAPTER THREE

3.0 Result and Discussion

3.1 Results.

The results of the reducing sugar obtained from the hydrolyzed sample are shown in the tables below.

3.1.1 Concentration of reducing sugar using 0.6 M Acid Hydrolysis

The result in the table 3.1 below showed the percentage yield of reducing sugar using 0.6 M.

Time (min)	Temperature (°c)	Absorbance at 540nm	Concentration (Mg/l)
10	60	0.624	9.0961
30	80	0.406	5.922
60	100	0.353	5.127

3.1.2 Concentration of reducing sugar at 0.8 M acid hydrolysis

The result below showed the percentage yield of reading sugar using 0.8 M

Time (min)	Temperature (°c)	Absorbance at 540nm	Concentration (Mg/l)
10	60	0.353	5.140
30	80	0.370	5.294
60	100	0.351	4.988

3.1.3 Concentration of reducing sugar using 1.0 M acid hydrolysis

The result below showed the percentage yield of reducing sugar using 1.0M

Table 3.3 concentration of reducing sugar at 1.0M

Time (min)	Temperature (°c)	Absorbance at 540nm	Concentration (Mg/l)
10	60	0.353	5.127
30	80	0.534	7.796
60	100	0.439	6.395

3.1.4 Glucose yield of fixed Temperature and different Concentrations

The result in table 4 below shown that 500ppm which is the highest concentration has the highest absorbance and also showed a steady fall in the absorbance from 500ppm to 100ppm

Concentration (ppm)	Temperature (^{0}c)
100	90
200	90
300	90
400	90
500	90

3.1.5 Concentrations of Bio ethanol produced

Table 5 sugar concentration of bio ethanol produced

S/No	Sample	Absorbance (nm)	Concentration (mg/l)
1.	Ethanol from 1M acid	2.799	0.947
2.	Ethanol from 0.8M acid	2.659	0.907
3.	Ethanol from 0.6M acid	1.397	0.547

3.1.6 Volume of Bio Ethanol obtained

The different volumes of pure bio ethanol obtained from the different acid concentration are listed in the table 6 below.

Acid Concentration (M)	Volume of Hydrolysate (cm^3)	Volume of distillate (cm^3)	Percentage Yield (%)
1M	89.2	48	53.8
0.8M	91.8	51.5	56.1
0.6M	90.6	36	39.7

3.2 Discussion

The effect of acid concentration on bio ethanol production was carried out using 0.6M, 0.8M and 1M concentration of H_2SO_4 at different time and varying temperatures as shown in tables 1, 2 and 3 above. It was observed that the percentage concentration of reducing sugar changes from molar concentration with respect to time and temperature. The highest concentration for 0.6M was at 60^{o}C which has a concentration of 9.09 4mg/l for a time of 10mins as recorded in Table 1. The highest concentration for 0.8M was at 80°C which has a concentration of 5.294 mg/l for a time of 30min as recorded in Table 2 highest concentration for the 1m sample was at 80°C which has a concentration of 7.796 mg/l for a time of 30min and this is also the overall maximum concentration as recorded in table 3. This observation agrees with the research of Balat, 2009 because the concentration of reducing sugar at 80°c for a time of 30 minutes was found to be 8.696mg/l which is very close to the above result (7.796 mg/l). The sugar content in cassava tends to fluctuates between the various temperature and time.

The glucose concentration in Table 4 showed that the highest concentration at all conditions was 500ppm. The drop in the glucose concentration could be attributed to the fact that at higher concentration, glucose can be converted to levulinic and formic acid (Ghose, 1956), which lead to decrease in glucose yield. This suggests that highest glucose yield can be obtained at moderate acid concentration which also serves as the optimal pH condition for yeast to metabolize its substrate (Adams, et al., 1995).

The sugar concentration of the ethanol produced obtained at various acid concentrations was showed in table 5 above. It can be deduced from the table that the highest sugar concentration was obtained at 1M concentration of the acid 0.947(mg/l). This value is significantly in line with the previous result made by Usharani, 2009 with

the value of 0.865 mg/l. The sugar content in the cassava plant root tends to increase at acid concentration. However, the maximum concentration of reducing sugar obtained is directly proportional to the concentration of acid used for the hydrolysis. I.e. the sugar concentration increases as the acid concentration increases. From Table 6 above the percentage yield after distillation are 39.7% from the 36 cm^3 distillate, 56.1% from the 51.5 cm^3 and 56.1% from the 48 cm^3 distillate. This shows increase in the yield is directly proportional to the increase in the concentrations of the acid.

CHAPTER FOUR

4.0 CONCLUSION AND RECOMMENDATIONS

4.1 Conclusion

Ethanol with moderate percentage yields was produced from cassava using three different acid concentrations in the hydrolysis process. The main methods involved in the production are hydrolysis, fermentation, distillation and dehydration. With the exception of hydropower and nuclear energy stations, the major part of all energy used worldwide comes from crude oil. However, the sources of the energy from crude oil are limited and would definitely be exhausted in the near future. Therefore looking for alternative source of energy is of vital importance. In this work the absolute ethanol was produced from cassava using different concentrations and the product derived is of high quality which is a potential substitute for petroleum products. Cassava which is the main feedstock used is a rich in starch and can be cultivated on virtually any soil type even without any care therefore it's a very free crop for mass production of renewable energy.

4.2 Recommendations

This research has successfully proved that production of ethanol from cassava plant is a good venture that could reduce unemployment in Nigeria. Countries like the United States of America produce high amount of bio ethanol from corn and Brazil also produces high amount of bio ethanol from sugar cane annually. Nigeria can also utilize the advantage of the rich and fertile soil for mass cultivation of cassava which would generate enough feedstock for the production of bio energy.

Also bio ethanol processing technology needs more Improvement. Improving bio energy production should be stimulated in many aspects of scientific research fields. This is an

important move that would encourage the development of viable bio ethanol projects. And let's not forget the issue of climate change which is a serious threat to the human world, renewable energy source like bio ethanol is a great relief if properly utilized. Let us raise proper awareness about the benefits of bio energy, support bio ethanol research. As a result of this, I will recommend that;

i. Acid hydrolysis of cassava can be improved upon or other non-acid can be explored and used as an alternative to chemical methods to reduce toxicity.
ii. Bio ethanol should be produced on industrial scale so as to provide a lasting solution to the gradually depleting ozone layer a result of greenhouse gases emission.
iii. Instruments should be made available to carry out test on some fuel properties of bio ethanol such as flash point and vapor pressure in Nigeria research laboratories.
iv. More research should be conducted on the production of bio energy which are renewable energies.

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APPENDIX I

Preparation of acid concentrations used for the hydrolysis the standard acid solution is

18.4M

(1). 1M H_2SO_4

Using $C_1 V_1 = C_2 V_2$

Where;

$C_1 = 18.4$ $C_2 = 1M$

$V_1 = ?$ $V_2 = 100$

Therefore, $V_1 = \frac{C_2 V_2}{C_1}$

$= \frac{1 X 100}{18.4} = 5.43cm^3$

Therefore, $5.43cm^3$ of the known concentration was measured and diluted with $100cm^3$ of the distilled water.

(2). 0.8M

Using $C_1 V_1 = C_2 V_2$

$C_1 = 18.4$ $C_2 = 0.8M$

$V_1 = ?$ $V_2 = 100$

$= \frac{0.8 X 100}{18.4} = 4.35cm^3$

(3). 0.6M

Using $C_1 V_1 = C_2 V_2$

$C_1 = 18.4$ $C_2 = 0.6M$

V_1 =? V_2 = 100

$$= \frac{0.6\,X\,100}{18.4} = 3.26\text{cm}^3$$

APPENDIX II

The calculation of percentage yield

$$\% \text{ yield} = \frac{Volume\ of\ distillate}{volume\ of\ hydrolysate} \times 100$$

1. For 1M concentration

Volume of hydrolysate = 89.2ml

Volume of distillate = 48ml

Therefore,

$$\% \text{ yield } \frac{48}{89.2} \times 100$$

= 0.538 x 100

= 53.8%

2. For 0.8M concentration

Volume of hydrolysate = 91.8ml

Volume of distillate = 51.5ml

Therefore,

$$\% \text{ yield } \frac{51.5}{91.8} \times 100$$

= 0.561 x 100

= 51.6%

3. 0.6M concentration

Volume of hydrolysate = 90.6ml

Volume of distillate = 36.0ml

Therefore,

$$\% \text{ yield } \frac{36.0}{90.6} \times 100$$

= 0.397 x 100

= 39.7%