PRODUCTION OF BIOETHANOL FROM MOLASSES AND EFFLUENT TREATMENT OF SPENT WASH

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SUBMITTED TO THE DEPARTMENT OF BIOTECHNOLOGY AND APPLIED BIOLOGY, FACULTY OF NATURAL AND APPLIED SCIENCES IN PARTIAL FULFILLMENT FOR THE REQUIREMENTS OF THE AWARD OF BACHELOR OF SCIENCE, DEGREE IN BIOTECHNOLOGY.

JULY, 2017.

**APPROVAL PAGE**

This project has been presented and approved by Godfrey Okoye University, Enugu, in partial fulfillment of the requirement for the award of Bachelor of Science, degree in Biotechnology from the department of Biotechnology and Applied Biology, faculty of Natural and Applied Sciences.

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**DEDICATION**

I dedicate this project to God Almighty mu creator, my strong pillar, my source of inspiration, wisdom, knowledge and understanding. He has been the source of my strength throughout this program and on His wings only have I soared. I also dedicate this work to my aunty; Rev. Sis Mary Ambrose Amuche Ofoegbu who has encouraged me all the way and whose encouragement has made sure that I give it all it takes to finish that which I have started. God bless you.

**ACKNOWLEDGEMENTS**

My profound gratitude goes to God Almighty for his sustenance and good health throughout my four years pursuit for a certificate making my dream come through and for granting me a successful journey. I thank Mrs Ozorokonkwo Onyinye Cecillia, my supervisor who upon out of her tight schedule made out time to assist me in the course of this research. I cannot fail to acknowledge the efforts of my dean Prof Chidi Uhuegbu who worked so hard in ensuring the lecturers are up and doing, and never missed lectures. Also at this point I would like to thank my amiable Head of Department Dr Christy Onyia who had been not just HOD but a woman full of life, with words of encouragement to me. I thank my wonderful lecturers Mrs. Akaniro Ejim, Miss Chikezie, Mr Obih Chosen and all who contributed to the actualization of this degree. I also want to thank Mrs Ohaeri Chizaba and Mr Mba Fortunatus who assisted me during my research work. I can’t forget to thank my friends Njoku Emmanuel, Opara Eberechi and Ijeoma Chidinma for contributing to the success of this research work and for putting a trust in me. My caring coursemates Chidera Aneke, Dominic Okolo, Amara Okoh, Aniweze Cecille, Joshua Bassa, Chidiebere Ezeamama, Chidimma Eze and Desire Onyeagba are not left out, because they all made this research work look stress free because of their company and their contributions. I cannot fail to thank my parent (mother) Mrs Ofoegbu Angela Ada whom God used in actualising my dreams and also my Aunty Mrs. Akukwe Irene Nkeiru, Uncle Mr. Ofoegbu Okechukwu and my Aunty Mrs. Iheukor Ngozi for always believing in me. Finally, I wish to acknowledge my typist Kosi who helped in the arrangement and printing of this intellectual work.

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**ABSTRACT**

The use of ethanol in fuel blending has long been in use, and this help in controlling gas emissions but cannot be compared to bio-ethanol in control of greenhouse gas emissions. Bio-ethanol is known to adsorb CO2 they emit; it also improves rural economy. A lot of materials especially starchy crops are used in the production of bio-ethanol. This research work focuses on the production of bio-ethanol from molasses; a waste from sugar cane industries. Bio-ethanol is regarded as one of the most promising fuels from renewable sources. This study also looks at protecting the environment as the effluent of the bio-ethanol production can be treated before discharging to the open field. Four steps are involved in its production; dilution, fermentation, decantation and distillation. The molasses (700ml) was diluted with 300ml of water, after which yeast was added for fermentation process. Other reagents were also added to enhance the fermentation process and production of quality ethanol. The fermented molasses was distilled and to get bio-ethanol, the residue which is the spent wash was treated to reduce its contamination level in order not to affect lives when discharged to the environment. The results from the analysis gave 93.7% ethanol, 78.42oC boiling point value, 0.8301ml/l density, produced no offensive odour, completely miscible with water. Statistical analysis of untreated and treated spent wash reveals that it is statistically significant with p=0.032. These with other results gotten in this study are within the Nigerian Industrial Standard (NIS) and therefore have proven that molasses (waste) is a good substrate for bio-ethanol production which can be used for so many purposes.

**1.0 CHAPTER ONE**

**1.1 INTRODUCTION**

Transportation was one of the greatest challenges for society in the 21 century is to meet the growing demand of energy, heating and industrial processes, and to provide material for the industry in a sustainable way. Ethanol production, however, traces back as far as the days of Noah who was believed to have built himself a vineyard in which he grew grapes that he fermented into some sort of alcoholic beverages. Ethanol an important member of a class of organic compounds with general name ‘Alcohol’. During the early times, ethanol was used as a constituent of alcoholic beverages. With civilization and advancement in science and technology, the benefits derived from ethanol have continued to multiply. These include solvent, germicide, as anti-freeze, fuel and versatile intermediate for other organic chemicals. For these enormous advantages of ethanol, researchers have been geared towards the production of ethanol from various raw materials or feedstock. In addition, the environmental deterioration resulting from the over-consumption of petroleum derived products, especially the transportation fuels, is threatening the sustainability of human society (Bai *et al.,* 2008).The excessive consumption of fossil fuels particularly in large urban areas, has resulted in generation of high levels of pollution during the last few decades. In this scenario, fuel production as one of the renewable sources for the energy industry and the chemical industry may depend on biomass as an alternative source in the near future (Oliveria and Hira, 2009). All petroleum- based fuels can be replaced by renewable biomass fuels such as bio-ethanol, bio-diesel, bio-hydrogen, etc., derived from sugarcane, corn, switch grass, algae, etc (Sarkar *et al.,* 2011).

Ethanol is a volatile, flammable and colorless liquid that has a slight odor. In dilute aqueous solution it has a burning taste. Ethanol has been made since ancient times by the fermentation of sugars. All ethanol beverages and more than half of industrial ethanol is still made by this process. The only difference between bio-ethanol and synthetic ethanol is that synthetic ethanol come from fossil raw materials while bio-ethanol comes from contemporary materials but chemically they are both the same compound and the two is the isotopic composition of the carbon atoms.

Bio-ethanol is regarded as one of the most promising fuels from renewable sources. It is used for medicines, cosmetics and industrial materials, and its production is increasing every year (Cardona and Sanchez, 2007). With the increasing prices and global environmental concerns, bio-ethanol production has recently become a focus of attention (Bai *et al.,* 2008).

Generally, bio-ethanol feedback can be conveniently classified into three types which are sugar-based feedback, starchy materials and lignocelluloses biomass (Razmovski, 2012). Sugar-based feedback contains readily available fermentable sugars and can be an ideal substrate for ethanol production by direct fermentation. Direct fermentation of sugars has advantages in production costs of ethanol, compared to process that use starchy materials or lignocelluloses biomass as raw materials (Razmovski, 2012).

Molasses is a sweetener that is formed as a byproduct of the sugar-making process. Hence molasses is a solution of sugar, organic matter and inorganic matter in water. It is known as the final effluent (final molasses) and byproducts of sugar manufacture. Black strap molasses are molasses produced from raw sugar factories from cane or beet. Often, only the term molasses is used for molasses from a beet factory where as black strap is preferably used for cane molasses. Refinery (final) molasses (refinery black strap, barrel syrup), is derived mainly from cane refineries, while (high grade) white sugar is produced from raw sugar. Molasses contains around 40 percent of sugar content that is fermented by yeast during the ethanol process (Olbric, 2006).

 **1.2 STATEMENT OF PROBLEM**

High importance of ethanol from other countries for fuel blending, poor waste conversion of waste to other useful products.

**1.3 AIM**

To produce bio-ethanol from sugarcane molasses using yeast *(Sacchromyces cerevisiae).*

**1.4 OBJECTIVES**

* To recycle waste material (molasses) into bio-ethanol through fermentation process.
* To treat the waste product of fermentation (spent wash), making the waste environmentally friendly.

**2.0 CHAPTER TWO**

**2.1 LITERATURE REVIEW**

**2.2 ETHANOL**

Ethanol known as ethyl alcohol or grain alcohol is a flammable, colorless, mildly toxic chemical compound with a distinctive perfume- like odor, and the ethanol is found in alcoholic beverages (Prakasham, 2004).

Ethanol (EtOH), also called alcohol, or ethyl alcohol is the principal type of alcohol found in alcoholic beverages, produced by the fermentation of sugars by yeast, or by petrochemical processes. It is an addictive psychoactive drug (indeed one of the oldest and most common recreational drugs), causing a characteristic intoxication (drunkness). According to (Brust, 2010) the neurotoxicity of ethanol when consumed in sufficient quantity, act as a psychoactive drug. Ethanol is a commonly used substance among people all over the world with its intoxicating effects and potential for abuse.

Ethanol (an alcohol) is primarily made from corn grain starch. When added to petrol it reduces toxic emissions and increases the octane level of the fuel and so it can be compressed more before it spontaneously ignites good thing. It typically contains 7% to 4% water and this is referred to as hydrous ethanol. Anhydrous ethanol is ethanol that has been dehydrated to achieve at least 99% purity.

In common usage, it is referred to simply referred to as alcohol. Its molecular formula is variously represented as EtOH, CH3CH2OH or C2H5OH or as its empirical formula C2H6O (which it shares with dimethyl ether).

Natural energy resources such as petroleum and coal have been consumed at high rates over the last decades. The heavy reliance of the modern economy on these fuels is bound to end, due to their environmental impact and to the fact that they might eventually run out. Bio-ethanol is one of the most important renewable fuels contributing to the reduction of negative environmental impacts generated by the worldwide utilization of the fossils fuels (Cardona, 2007).

Sugarcane ethanol is an alcohol-based fuel produced by the fermentation of sugarcane juice and molasses. Because it is clean, affordable and low carbon bio-fuel, sugarcane ethanol has emerged as a leading renewable fuel for the transportation sector. Ethanol can be used in two ways; blended with gasoline at levels ranging from 5 to 27.5 percent to reduce petroleum use, boost octane ratings and cut tailpipe emission. Pure ethanol fuel is made up of 85 to 100 percent ethanol depending on country specifications and can be used in specially designed engines. Ethanol is high- octane fuel that helps prevent engine knocking and generates more power in higher compression engines, it also reduces global dependence on oil. Sugarcane ethanol is one more good option for diversifying energy supplies. Ethanol can be prepared by the fermentation of molasses.

**2.3 BIOETHANOL**

The improvement of living standard urges the hunt for sustainable energy in order to meet energy consumption across the world (Demirbas, 2010), the use of fossil fuel as the main energy resources caused the arising of worldwide problems such as environmental pollution and global warming (Hoekman, 2009; Kiran *et al.*,2014). These had led to the finding of environmentally friendly, renewable and sustainable energy by government, industries and energy sector (Shafiee and Topal, 2009) and Demirbas,(2009) . Among renewable energies, priority was given to liquid bio-fuel as it represents about 40% of the total energy consumption in the world Tan *et al.,*(2008). The use of liquid bio-fuels contribute to the reduction of greenhouse gas emissions, creation of job opportunities, regional development and supply security (Demirbas, 2009; Demibas and Balat, 2006).

Bio-ethanol is known as the most widely used bio-fuel in transportation sector and have a long history as alternative fuels. In 1984, Germany and France started to use bio-ethanol as a fuel in internal combustion engines (ICEs) (Liang, 2008).

The interest in using bio-ethanol has been increasing since the 1980s. After World War 11, the use of bio-ethanol was neglected due to its expensive production cost compared to petroleum fuel until the oil crisis in the 1970s (Demirbas, 2009).

Bio-ethanol is an alcohol made through fermentation processes, mostly from starchy crops such as corn, sugarcane, sweet sorghum. Cellulosic biomass, derived from non-food sources, such as trees and grasses, is also developed as a feedback for ethanol production. Ethanol can be used as fuel for vehicles in its pure form, but it is usually used as a gasoline additive to increase octane level and improve emissions. It is also produced from potatoes, rice, beetroot, recently grapes, banana, dates and some waste. Bio-ethanol is widely used in the USA (United State Of America) and Brazil. It is produced from the agricultural product such as corn, sugarcane, potatoes, rice, beetroot and recently using grapes, banana, dates and other waste have been used. it is also use for transportation which is now an increasing demand worldwide due to continuous depletion of fossil fuels, economic and political crises, and growing concern on environmental safety. Microorganisms such as yeast play an essential role in bio-ethanol production by fermenting a wide range of sugar to ethanol. As the main component in fermentation, yeast affect the amount of ethanol yield. Since thousands of year ago, yeast such as *S. cerevisiae* have been used in alcohol production especially in the brewery and wine industries. It keeps the distillation cost low as it gives a high ethanol yield, a high productivity and can withstand high ethanol concentration (Finore, 2012).

Fermentation of bio-ethanol can be carried out in batches, fed-batch, repeated batch or continuous mode. In batch process, substrate is provided at the beginning of the process without the addition or removal of the medium (Anyanti, 2013). It is known as the simplest system of bioreactor with a multi-vessel, flexible and easy control process. The fermentation process is carried out in a closed-loop system with high sugars and at the beginning and ends with high product concentration (Dash, 2014). There are several benefits of batch system these are; complete sterilization, requires no labors skills, easy to manage the feedstocks, can be controlled easily and flexible to various product specifications (Hristov,2011; Chaurasia and Jain, 2014).

However, the productivity is low and its needs are intensive and require high adaptable to start up. The presence of high sugar concentration in the fermentation medium may lead to substrate inhibition and results in the inhibition of cell growth and ethanol production (Cheng *et al.,* 2009).

Cell recycle batch fermentation (CRBF) is a strategic method for effective ethanol production as it reduce time and cost for inoculum preparation. The other advantages of repeated batch process are easy cell collection, stable operation and long-term productivity (Kida *et al.,*1991; Mohsenzadeh and Vazirzadeh, 2012).

 Sugar materials and immobilized yeast cells are used to facilitate cell separation for cell recycling (Liang, 2008; Kida *et al.,*1997).

Fed-batch fermentation is a combination of batch and continuous mode which involves the addition of substrate into the fermentor without removing the medium. It has been used to overcome the problem of substrate inhibition in batch operation. Volume of culture in fed-batch processes can vary widely but it must be fed properly at certain rate with the right component composition. Productivity of fed-batch fermentation can be increased by maintaining substrate at low concentration which allows the conversion of sufficient amount of fermentable sugars to ethanol (Chaurasia and Jain, 2014). This process has higher productivity dissolved oxygen in medium, shorter fermentation time and lower toxic effect of the medium components compared to other types of fermentation (Cheng *et al.,* 2009). However, ethanol productivity in fed-batch is limited by feed rate and cell mass concentration (Margaritis and Merchant, 1987).

Continuous operation is carried out by constantly adding substrates, culture medium and nutrients into a bioreactor containing active microorganism. Culture volume in continuous operation must be constant and the fermentation products are taken continuously from the media. Various types of products can be obtained from the top of the bioreactor such as ethanol, cells and residual sugar (Hristov *et al.,* 2011). The advantages of continuous system over batch and fed-batch system are higher productivity, smaller bioreactor volumes and less investment and operational costs (Chaurasia and Jain, 2014). At high dilution rate, ethanol productivity is increased while ethanol yield is decreased due to incompletely substrate consumption by yeasts (Cardona and Sanchez, 2008). However, the possibility for contamination to occur is higher than other types of fermentation (Chandel and Rudravaram, 2007). Moreover, the ability of yeasts to produce ethanol in continuous process is reduced due to long cultivation time.

Bio-ethanol decreases due to amount of fossil fuels, alternative energy sources need to be renewed, sustained, made efficient, cost effective, convenient and safe.

**2**.**4 FEEDSTOCK FOR BIOETHANOL PRODUCTION**

Bio-fuels originated from plant oils, sugar beets, cereals, organic waste and the processing of biomass. Biological feedstock that contain appreciable amounts of sugar or materials that can be converted into sugar, such as starch or cellulose can be fermented to produce bio-ethanol to be used in gasoline engines (Kumar *et al*., 2007).

2.5 **Classification of Bio-ethanol feedstock are ;**

* Sugar containing feedstock (e.g sugar beet, sweet sorghum and sugar cane).
* Starchy materials (e.g wheat, corn and barley).
* Lignocelluloses biomass (e.g wood, straw and grasses) (Kumar *et al*., 2007).

**2.5.1 SUCROSE-CONTAINING FEEDSTOCKS**

Feedstock for bio-ethanol is essentially comprised of sugar cane and sugar beet. Two-third of world sugar production is from sugar cane and one-third is from sugar beet (Kumar *et al.,*2007).These two types of sugar are produced in geographically distinct regions. Sugar cane is grown in tropical and subtropical countries, while sugar beet is only grown in temperate-climate countries. Brazil is the largest single producer of sugar cane with 27 per cent of global production and yield of 18 metric tons of dry sugar cane per-hectare (Kim and Dale, 2004).

**2.5.2 STARCHY MATERIALS**

Another type of feedstock that can be used for bio-ethanol production is starch-based materials (Sorapipatana, 2007). Starch is a biopolymer and can be defined as a homopolymer consisting only one monomer, d-glucose (Pongsawatmaint *et al.,* 2007). The chains of this carbohydrate must be broken down in other to obtain glucose syrup, which can be converted into bio-ethanol by yeast. This type of feedstock is the most used for bio-ethanol production in North America and Europe. Corn and wheat are mainly employed for these purposes (Cardona and Sanchez, 2007).

Starch consists of long chains of glucose molecules and can also be converted to fermentable sugar by a method called the hydrolysis technique*.* Hydrolysis is a reaction of starch with water, which is normally used to break down the starch into fermentable sugar (Sorapipatana and Yoosin, 2007). There are two types of hydrolysis which are enzymatic hydrolysis and acid hydrolysis. The hydrolysis of starch by amylases at relatively high temperatures is a process known industrially as liquefaction. The factors that affect the enzymatic hydrolysis of starch include substrates, enzyme activity and reaction conditions are temperature, pH, as well as other parameters (Neves, 2006).

**2.5.3 LIGNOCELLULOSIC BIOMASS**

Lignocelluloses biomass, such as agricultural residues (corn stover and wheat straw), wood and crops, is an attractive material for bio-ethanol fuel production since it is the most abundant reproducible resource on the Earth. Lignocelluloses biomass could produce up to 442 billion of bio-ethanol per year (Bohlmann, 2006). Thus, the total potential bio-ethanol production from crop residues and wasted –crops is 491 billion per year, about 16 times higher than the current world bio-ethanol production (Kim and Dale, 2004).

Lignocelluloses perennial crops (e.g short rotation copies and inedible grasses) are promising feedstock because of high yield of bio-ethanol, low costs, good suitability for low quality land which is more easily available for energy crops, and low environmental impacts (Balat *et al.,* 2008).

**Table 1: Different Feedstock for Bio-ethanol Production and their Comparative Production Potential.**

 Feedstock potential (L/ton) bio-ethanol production sugar cane 70

 Sugar beet 110

 Sweet potato 125

 Potato 110

 Cassava 180

 Maize 360

 Rice 430

 Barley 250

 Wheat 340

 Sweet sorghum 60

 Biomass (Bagasse and other cellulose). 280

 (Kumar et al., 2007)

**2.6 MOLASSES**

Molasses is defined as waste product of sugar industry of which further extraction of sugar is uneconomical, contains about 45-50 percent fermentable molasses into ethanol and its low price have made this raw material ideal for ethanol production. Molasses is generally used because it is rich in all salt except nitrogen which is normally employed in the actual growth of yeast cells. In years back there have been a jump in molasses price and limitation on molasses availability, and it have affected bio-ethanol production in molasses-based distilleries, (Nguyen and Gheewala, 2008). Molasses are the final effluent and by-product of sugar production. When sugarcane is mashed and boiled, cane syrup is created. A second boiling yields molasses and a third leaves blackstrap molasses. Molasses varies by amount of sugar, method of extraction and age of plant. Sugarcane molasses is agreeable in taste and aroma and is primarily used for sweetening and flavoring foods in Nigeria, U.S, Cananda and some other places while sugar beet molasses is foul smelling and unpalatable, so it is mainly used as an animal feed additive in Europe and Russia, where it is chiefly produced (Bitzer, Morris, 2014). Molasses is a defining components of fine commercial brown sugar. Sweet sorghum syrup may be colloquially called sorghum molassesin the southern United States (Rapuano, Rina,2014).

Similar products include treacle, honey, maple syrup, corn syrup and invert syrup.

2.7 **Types of molasses**

* light and Dark
* Blackstrap
* Sulphured and Unsulphured.

**2.7.1 LIGHT AND DARK MOLASSES**

This is produced by the first boiling of sugar cane (or sugar beet). This type is lighter in color and can be sulphured or un-sulphured. Sulphur Molasses treated with sulphur dioxide acts as a preservative. It is light in color and sweet in taste because only a small amount of sugar has been extracted. Light molasses is also known as sweet, Barbados, first or mild molasses. This type of molasses is commonly used as an ingredient in baking, marinades, rubs and sauces, or even as a topping on toast or oatmeal. When added, it can make cookies softer or breads crustier. Dark molasses, also known as *full* or *second* molasses, results after the second boiling and more sugar is extracted. It is darker in color, thicker and less sweet. It can still be used in a recipe that requires molasses. It is the ingredient commonly used in gingerbread cookies.

**2.7.2 SULPHURED AND UNSULPHURED MOLASSES**

These are types of molasses made from young sugar cane which are called sulphured molasses because of the sulphur dioxide that is added to keep the raw cane fresh until it is processed and to preserve the molasses byproducts produced from it. Unsulphured molasses is made from matured cane plants that have been allowed to ripen naturally in the field. Blackstrap molasses can be sulphured or unsulphured. The U.S, food and Drug Administration stated that sulphur dioxide is generally recognized as safe for use as a preservative except on meat or vegetables meant to be sold as fresh food.

**2.7.3 BLACKSTRAP MOLASSES**

Blackstrap molasses is the syrup produced after the third boiling. It is very thick and dark in color. It is also bitter in taste. Due to its bitter taste, it is not to be used as a substitute in

recipes. It can be found in many health food stores. Blackstrap molasses has the highest vitamin and mineral content than all the other types of molasses since it has been concentrated by the three different boilings. It is a good source of iron. According to the American Diebetic Association’s Complete Food and Nutrition Guide:1 tablespoon of blackstrap molasses contain 3.5 milligrams of iron, it also contains several other minerals and vitamins such as calcium, copper, magnesium, manganese, selenium, potassium, vitamin B6 and niacin.

**Table 2: MAIN COMPONENTS OF CANE BLACK STRAP MOLASSES**

 Composition Percentage (%)

Sugar 73.1

Sucrose 45.5

Invert sugar 22.1

Other 5.5

Glutamic acid and pryrollidine carboxylic acid 2.4

Other N 3.1

Organic acids 7.0

Pectin 2.7

Inorganic 11.7

K2O 5.3

N2O 0.1

CaO 0.2

MgO 1.0

SO2 + SO3  2.3

P2O5 0.8

Others 0.9

(RAPUANO, 2012).

**2.10 ECONOMIC IMPORTANCE OF MOLASSES**

**2.10.1 Nutritional value of molasses**

Molasses contains a number of essential minerals such as calcium, magnesium, manganese, potassium, copper, iron, phosphorus, chromium, cobalt and sodium. It is a good source of energy and carbohydrates and it contains sugar as well. In addition to this, it offers various vitamins such as nacin, vitamin B-6, thiamine and riboflavin. It is very low in both fat content and fiber.

2.8.2 **Health benefits of molasses**

Blackstrap molasses is a nourishing sweetener which contains a considerable amount of nutrients that are essential for the functioning of the body. Unlike refined white sugar, which only contains simple carbohydrates and other components like aspartame, which is not very helpful or healthy for human consumption.

2.8.3 **List of the health benefits of molasses are;**

* Antioxidant
* Source of iron during menstruation
* Reduces Obesity
* Better sexual Health
* Source of iron during constipation
* Source of iron during diabetes

2.8.3.1 **Antioxidant**

Blacksrap molasses contains the highest amount of antioxidants as compared to refined sugar and other readily available sweeteners. These antioxidants protect the body against the oxidative damage associated with cancer, cardiovascular disorders and degenerative diseases. This makes it a much better alternative to refined sugar.

2.8.3.2 **Source of iron during menstruation**

Molasses is a good source of iron and is very effective for menstruating women who are at major risk of iron deficiency due to blood loss. With no fat and very low calories, it is a better alternative for contributing iron content in the body as compared to other fatty sources like red meat. Iron prevents various disorders like menorrhagia which causes excessive blood flow for a longer duration during menstruation. The minerals such as magnesium and calcium that are present in it help to prevent the clotting of blood, relieve menstrual cramps and help in maintaining the health of uterine muscles. It is a healthy alternative, as compared to other medications for menstrual discomfort which might have certain side effects.

2.8.3.3 **Reduces obesity**

The polyphenol present in molasses have antioxidant effects which may prove effective in reducing obesity and manage weight gain. In the investigation conducted to assess the impact of molasses on a high-fat diet. It was evident that its extract helps in lowering the body weight and fat content by reducing the absorption of calories in the body (Barry *et al.,* 2004).

2.8.3.4 **Better sexual health**

Blackstrap molasses is rich in the trace mineral manganese, which helps in the healthy production of sex hormones. It also plays a vital role in the functioning of the nervous system, the prevention of blood clots and the production of energy from proteins and carbohydrates. A deficiency of manganese can lead to infertility, general fatigue and week bones.

2.8.3.5 **Source of iron during constipation**

According to Hansen *et al.,* (2011), molasses has been proven to be valuable in treating constipation. Routine milk and molasses enemas are as effective as sodium phosphate enemas given in the pediatric emergency department to cure constipation. It is also noteworthy that curing constipation with sodium phosphate requires an additional rectal treatment.

2.8.3.6 **Diabetes**

Blackstrap molasses help in stabilizing blood sugar levels. It has a low glycemic index and aids in slowing the metabolism of glucose and carbohydrates, which subsequently means less insulin production. This helps in preventing the accumulation of excess fats or lipids in the blood stream. It possesses a substantial amount of the essential trace element of chromium, which is valuable in relation to insulin action and maintenance of glucose tolerance in the body as well (Culpepper,2015).

There are other effective use of molasses such as animal feeds. It is a source of carbon and it is effectively used in horticulture to feed the microbes and boost the microbial activity of the soil. Beet molasses is also used to make bio-fertilizer. It is a beneficial sweetener that is rich in naturally available micro and macronutrients. Some people might develop allergic reactions due to a sensitivity towards the sulphite that is present in sulphured molasses. Unsulphured molasses is free of sulphur dioxide and safe to use in such cases. However, it is always advisable to obtain medical consent before considering it as a therapeutic remedy for various medical conditions.

Diluted molasses + yeast

Diluted molasses

Sugar cane (molasses)

 H2O H2OSO4 AND Penicillin

 Dilution Fermentation

Mixture of fermented wash + yeast

 Decantation

Fermented wash (spent wash + ethanol)

Spent wash

 Distillation

Ethanol

 Figure 1: FLOW CHART OF BIOETHANOL FROM SUGARCANE MOLASSES.

 (Salins, E.C and Sandy, 2009).

2.9 **The chemical reaction of ethanol from sugarcane molasses is showed below;**

 Zymase

C6H12O6  2CH3CH2OH + 2CO2

Fructose / Glucose Catalyst Ethanol

**2.10 EFLLUENT TREATMENT OF BIOETHANOL**

The main raw material of Distillery is Molasses and when it is distilled after fermentation to get alcohol is gotten, this ethanol is called bio-ethanol. The residual liquor remaining after the recovery of the alcohol is termed as “Spent wash” and discharged as waste. A sludge settling at the bottom of the fermenter is termed as “yeast sludge” which is also discharged as waste. If these wastes are directly (untreated) discharged either in river or on land they would cause severe pollution and the environment will not be suitable for people to live.

**2.11 PROCEDURES FOR EFFLUENT (SPENT WASH) TREATMENT FROM MOLASSES.**

* Anaerobic treatment i.e.: biogas generation.
* Aerobic treatment i.e.: secondary treatment.
* Classification unit with chemical treatment.
* Extended (secondary stage) aeration unit with chemical treatment.
* Sludge decanter (press leg dewatering machine).
* Classification by two M.S (Multivariate statiscal techniques) classifiers.
* Ferti irrigation in the zeal of zero discharge .
* Fountain with atomization technique.
* Flocculation unit with Resources Conservation Company (R.C.C) classifiers for chemical treatment.
* Recycling of treated trade effluent for cooling purpose.

**2.12 FACTORS AFFECTING BIO-ETHANOL PRODUCTION**

There are several factors which influence the production of bio-ethanol. Temperature, sugar concentration, pH, fermentation time, agitation rate, and inoculum size all , have an impact on fermentation process as well as ethanol yield. The growth rate of the microorganisms is directly affected by the temperature (Kanellaki *et al.,* 2003). High temperature which is unfavorable for cells growth becomes a stress factor for microorganisms, which is unfavorable for their growth. They produce heat-shock proteins in response to the high temperature and inactivate their ribosomes. In addition, microbial activity and fermentation process are regulated by different enzymes which are also sensitive to high temperature since it denatures their tertiary structure (McMeekin,, *et al.,* 2002). Moreover, enzyme which regulate microbial activity and fermentation process are sensitive to high temperature which can denature its tertiary structure and inactivates the enzymes. Thus, temperature is carefully regulated throughout the fermentation process.

**2.13 BENEFITS OF BIOETHANOL**

* Bio-ethanol has a number of advantages over conventional fuels. It comes from a renewable resources i.e crops and not from a finite resources and the crops it derives from can grow well in Nigeria like cereals, sugar beet and maize e.t.c.
* Another benefit of bio-ethanol over fossil fuels is the control of greenhouse gas emissions. The road transport network accounts for 22% of all greenhouse gas emissions but through the use of bio-ethanol, some of these emissions will be reduced as the fuel crops adsorb the CO2 they emit through growing. Also blending of bio-ethanol with petrol will help extend the life of the Nigeria’s diminishing oil supplies and ensure greater fuel security, avoiding heavy reliance on oil producing nations.
* By encouraging bio-ethanol’s use, the rural economy would also receive a boost from growing the necessary crops.
* Bio-ethanol is biodegradable and far less toxic than fossil fuels,the use of bio-ethanol in older engines help reduce the amount of carbon monoxide produced by vehicle thus improving air quality.
* Bio-ethanol is produced using familiar methods such as; fermentation and it can be distributed using the same petrol forecourts and transportation systems as before.
* The exhaust gases of bio-ethanol are much more cleaner. It undergoes complete combustion.
* The net effect of bio-ethanol use result in an overall decrease in the depletion of ozone layer in the atmosphere which is of important environmental concerns. The emissions produced by bio-ethanol combustion are less reactive with sunlight than those produced by burning gasoline has a low possibility of damaging the ozone.

**3.0 CHAPTER THREE**

**3.1 MATERIALS AND METHOD**

* Crude Molasses
* Water and ice-block
* Yeast (Sacchromyces cerevisiae)
* Urea
* Orthosphosphoric acid
* Tetraoxosulphate IV acid (H2SO4)
* Antibiotic penicillium
* Anti-form agent
* Methanobacterium Autotropicum
* Aluminium sulphate
* *Bacillus cereus*

**3.2 PROCEDURES FOR BIOETHANOL PRODUCTION USING BATCH FERMENTATION METHOD.**

**3.3 METHODOLOGY**

**3.3.1 DILUTION**

* 700ml of crude molasses was measured out into 1000ml glass measuring cylinder (pyrex) and 300ml distilled water was added to reduce sugar content.

**3.3.2 FERMENTATION**

* The diluted molasses was poured into a cylindrical vessel and 50g of yeast inoculum was added.
* 5g of urea and orthosphosphoric acid was added and mixed to generate nitrogen as yeast food.
* Tetraoxosulphate VI acid (H2SO4) was added gradually to reduce the pH to 4.5, thus the pH has to be reduced because the yeast produces acid during fermentation and also they grow very well at the given pH.
* 1ml of Antibiotic (penicillin) was added to prevent the growth of bacteria and 2g of anti-foaming agent was added to reduce foaming during fermentation.
* The mixture was then placed into an unplugged autoclave containing cold water covered and allowed to stand for 30hours for proper fermentation. An unplugged autoclave was used because anaerobic environment was needed for the fermentation process, cold water was used to control the temperature of the medium because it generates heat and this heat can stop the process by killing the yeast.

**3.3.3 DECANTATION**

* After 30hours the vessel i.e the vessel containing the fermented molasses was removed from the autoclave and was allowed to settle and decanted to separate the yeast sludge from the fermented molasses (ethanol and spent wash/ 7.65%).
* The percentage of ethanol in the fermented molasses was checked using alcohol meter and the reading was recorded.

**3.3.4 DISTILLATION**

* The ethanol was separated from the fermented wash by using distillation apparatus and ethanol was collected, leaving behind the spent wash.
* The percentage of ethanol was measured and recorded.

**3.5 PROCEDURE FOR TREATMENT OF SPENT WASH (EFFLUENT FROM MOLASSES DURING ETHANOL PRODUCTION).**

* After distillation of the ethanol, the spent wash left was very hot and was allowed to cool below 40oC using cold water and ice-block.
* After cooling below 40oC, the pH of the spent wash was adjusted to 6.8 using 1M of NaOH in drops.
* The spent wash was treated anerobically by adding 5g of *Methanobacterium autotropicum* and allowed to stand in autoclave for 48hrs in order to reduce the BOD (Biochemical Oxygen Demand) and COD (Chemical Oxygen Demand) and also SO4 and NO3.
* The anerobically treated spent wash was also treated aerobically by adding *Bacillus cereus penicullin* because they possess multiple enzyme systems and useful in waste water treatment, which reduce BOD (Biochemical Oxygen Demand) and silica and allowed to stand for 48hrs in order.
* It was allowed to settle and decanted.
* The decanted liquid was poured into aeration tank for oxidation of iron.
* 10g of aluminium sulphate was added to co-agulate some dissolved solids and also reduce the nitrogen content.
* The mixture was decanted and allowed to pass through 100g of packed activated carbon concurrently for one hour for further purification.
* The mixture was filtered using 0.5 microns of disposable microfiltre and the treated spent wash was tested.

**4.0 CHAPTER FOUR**

**4.1 RESULTS**

**TABLE 3: Molasses**

PARAMETER TESTED RESULT

 pH @ 200C 6.92

Sugar (as invert) 21.3

Sugar (Brix) 72.8

Sucrose 44.6

Inorganics % 10.2

Organics 16.16

Total volatile matters 32.4

Appearance/ colour dark reddish viscous liquid

Odour objectionable(unpleasant)

 ).

**Fig 2: Molasses (Crude Blackstrap molasses).**

TABLE 4: Result of analysis of produced bio-ethanol

 RESULT (Nigerian Industrial Standard)

 PARAMETERS ETHANOL Food $ drug standard)

 LIMIT

Volume of ethanol produced 75.50ml Not stated

% of ethanol produced 93.7% 92- 98.0

Boiling point 0C 78.40C 78.42(max)

Density ml/L 0.8301ml/L 0.789(min)

Miscibility with water completely miscible completely miscible

Color/ Appearance colourless liquid substance colorless liquid substance

Odour pleasant/ characteristics pleasant/ characteristics

Lead mg/L Not dictated Not dictated

Cadmium mg/L Not dictated Not dictated

Cupper mg/L Not dictated Not dictated



**Fig 3: 75.50ml of Bio-ethanol**

 TABLE 5: Analytical results of spent wash (untreated and treated)

 SPENT WASH RESULTS LIMIT(NIS)

PARAMETERS TESTED UNTREATED TREATED

pH at 200C 4.43 (0.002%) 6.4(0.39%) 6.0-9.0

Electrical conductivity 22960 (10.42%) 1100(68.04%) 1000

Total solids, mg/L 29330 (13.30%) 213.65 (13.21%) 500

Total dissolved solids mg/L 25120 (11.39%) 200.24(12.38%) 500

Total suspended solids mg/L 4210(1.90%) 13.41(0.83%) 25

Chemical oxygen demand (ppm) 87980 (39.91%) 43(2.66%) 90

Biochemical oxygen demand (w) 38000 (17.24%) 18(1.11%) 50

Total iron, mg/L 380 (0.17%) 0.07(0.0043%) 0.3(mix)

Total Acidity mg/L 12000(5.44%) 21.67(1.34%) 100

Ammonical Nitrogen mg/L 453.82(0.21%) 0.36 (0.02%) 1

Copper, mg/L 0.019(000000.9%) 0.008(0.00049%) 0.1

Lead, mg/L 0.20(00000.91%) 0.004(0.00023%) 0.05(max)

Total = 220438.469 1616.812

Appearance light brown liquid colourless colourless

Odour Objectionable Unobjectionable odourless

 (Unpleasant) (Pleasant)

χ2 = 0.233, df= 1, p=0.032, p<0.05 is significant, p>0.05 is not significant

**Fig 5: Electrical conductivity uh/cm of untreated and treated.**

**Fig 4: For pH at 200C of untreated and treated Effluent**

**Fig 6: Total solids mg/L of untreated and treated Effluent**

**N/B: 29330 of untreated was reduced by dividing by 100 to get 293.3 against 213.65.**

**Fig 7: Total dissolved solids mg/L of untreated and treated spent wash.**

**Note: 25120 mg/L of total dissolved solid mg/L was reduced by dividing by 100 to 251.2 against 200.24mg/L.**

**Fig 8: Total suspended solids mg/L of untreated and treated spent wash**

**NOTE: 4210mg\L of suspended solid was reduced by dividing by 100 to get 42.1 against 13.41.**

**Fig 10: Biochemical organic compound (w) of untreated and treated spent wash**

**Biochemical oxygen demand is 38000, Note: 38000 was reduced by dividing by 100 as 380 against 18ppl.**

**Fig 9: Chemical oxygen demand (ppm) of untreated and treated spent wash as 87980 against 43**

**Fig 11: Total iron, mg/L of untreated and treated spent wash**

**N/B: 380 was reduced by dividing by 1000 to get 0.38mg/L, by 380 against 0.07mg/L.**

**Fig 12: Total acidity, mg/L of untreated and treated spent wash**

**N/B: Total acidity mg/L of untreated spent wash was reduced by dividing 12000 by 100 to get 120 against treated as 21.67mg/L.**

**Fig 14: copper mg/L of untreated and treated spent wash**

**N/B: Copper mg/L of 0.019 against 0.008.**

**Fig 13: Ammonical Nitrogen mg/L of untreated and treated spent wash**

**N/B: Untreated spent wash as 453.82 against 0.36mg/L.**

**Fig 15: Lead mg/L of untreated and treated Effluent**

**N/B: Lead mg/L as 0.20 against 0.04**

**5.0 CHAPTER FIVE**

**5.1 DISCUSSIONS**

The composition of molasses from this study was in accordance with the findings of RAPUNANO, (2012), who recorded sucrose to be 45.5, invert sugar 22.1, organic 15.5, inorganic 11.7, while this research work recorded 44.6, 21.3, 16.16 and 10.2 respectively. The slight difference in the figures could be as a result of difference in temperature of the locations where the studies were performed. Pressure and pH of the blackstrap molasses used in both studies could also account for the difference in the readings. The molasses pH recorded in the study of Soweyah, (2007) was 5.5, as compared to that in this study which is 6.92. The difference in pH might be as a result of the nature of molasses used. The Brix sugar in the findings of RAMESHGGAVVA, (2011) was 87.6 as against the reading of this study which is 72.8, the difference from both findings could be from the difference in temperature at which both studies where performed, the experiment of RAMESHGGAVVA, (2011) was performed at 28oC, while that of this research was performed at20oC. The appearance, colour and odour both studies correspond. The volatile matter obtained in this work was32.4.Also the boiling point of bio-ethanol obtained in this research work was 78.420C, which is very close and related to the result gotten by Anand, (2015) who reported bio-ethanol boiling to be 78.300C.The volume of bio-ethanol obtained from this study was 75.50ml, produced from 700ml of molasses and diluted in 300ml of water. The % of bio-ethanol obtained from the produced bio-ethanol was 93.7%, which is within the Nigerian industrial standard (NIS-92-98.0% ethanol).The test for water miscibility of the produced bio-ethanol was observed to be completely miscible and in compliance with NIS. The density of the bio-ethanol obtained from this research work recorded 0.8301ml/L which is a little different from the study of RAMESHGGAVVA, (2011), who 0.791ml/L. The difference in both findings could be as a result of variations in quantity of molasses used in their researches and the ions tested in their research work were not detected as Lead, Cadmium and Copper.

The compositions of untreated and treated spent wash (effluent) were tested; pH of untreated spent wash was obtained as 4.43 at the temperature of 20oC which is very acidic and can affect lives when disposed to the environment. This was further treated with 1ml of NaOH in drops, and this adjusted the pH to 6.4 which made it alkaline in nature. The electrical conductivity of untreated spent wash was 22960 and after treating by passing it concurrently on 100g of packed activated carbon it was reduced to 1100 because of presence of *Bacillus cereus* during the aerobic treatment of the spent wash which helped in reducing the ions contained in it. The total solids of 22330mg/l was the amount of solids obtained but was later reduced to 213.65 after treatment carried out through anaerobic treatment by addition of *Methanobacterium autotropicum* which helped in reducing the BOD and COD contained in the solid. Total dissolved solids of untreated spent was obtained as 25120mg/l but after treatment it was obtained as 200.24mg/l as a result of aluminum sulphate added to it which helped in coagulating some dissolved solids and was later decanted. Total suspended solids (mg/L) of untreated spent wash of 4210mg/l were recorded after the removal of total dissolved solids from total solids. The effluent was treated by addition of *Bacillus cereus* which reduced the suspended solids to 13.41mg/l which is within the NIS limit for disposal. Chemical oxygen demand of untreated spent wash was 87980ppm which was treated during anaerobic treatment by addition of *Methanobacterium autotropicum* and was reduced to 43, total iron had a reading of 380mg/l , this was treated by pouring the decanted liquid into aeration tank for oxidation which reduced the value to 0.07. Total acidity of 12000 which was too high but was reduced after treatment by addition of alkaline (NaOH) in drops, Ammonical nitrogen of 453.82mg/l was treated and to 0.36, copper and Lead 0.019mg/l and 0.20mg/l respectively when treated during oxidation was reduced to 0.008mg/l and 0.004mg/l respectively. The organoleptic composition such as appearance and odour tested in this research work where found to be within the specifications of NIS. The statistical analysis by Two-way ANOVA of the untreated and treated spent wash revealed that there is statistical difference between the two parameters with a P value of 0.032 at 95% confidence interval.

**CONCLUSION**

The result gotten from this analysis have proven that (crude molasses) is a very good source for bio-ethanol production. Its high yield of fermentable sugar is also one of the characteristics that has confirmed molasses to be a substrate for production of bio-ethanol. The bio-ethanol produced is of high quality, yielding 93.7% alcohol and with Nigerian Industrial Standard (NIS) specification. Boiling point, density and other characteristics of the bio-ethanol being within the standard specification is also an evidence that the end product (bio-ethanol) is of high quality. Treatment of spent wash/effluent is very important as seen in this study, so as to reduce the waste a minimum bearable unit before discharging into the open environment. Having a data analysis that revealed a difference between the untreated and treated spent wash has justified the need of treating the spent wash before discharging to the environment.

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