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Abstract:

Nigeria is a rich nation in natural resources, which includes source of biofuel such as sweet potato starch etc. This starch can be converted to sugar through hydrolysis process in other to yield ethanol (biofuel). In this research work, the sweet potato peels was selected as a substrate for bioethanol production in the process, as it is rich in starch and cellulose. In this study, 5g, 10g, 15g, 20g and 25g each was weighed lactose and added to the Aspergillus Niger and Saccharomyces Cerevisae, the highest yield recorded when 20g of lactose was introduced. This achievement was observed after seven days, and the concentration of 91 ppm was highest for the sample containing 25g of lactose. The study also revealed that when lactose was added to fermentation medium together with enzyme Aspergillus Niger, the results obtained show that the 20g lactose represent a highest yield of biofuel production.
In summary, the increase and subsequent decrease in pH and specific gravity of each fermented sample indicated that the reaction (fermentation) was actually occurred, that is, the conversion of the substrate to products as well as the release of CO2 as the by-product took place.

Keywords: Aspergillus Niger, biofuel, starch and cellulose

1.0 INTRODUCTION

The energy that we produced from biofuels could be classified as *originally* from the sun. This solar energy was captured through photosynthesis by the plants used as feedstock (raw materials) for biofuel production, and stored in the plants' cells. Energy and environmental issues are the major concerns facing the global community today (Hu *et al.,* 2008). Renewable fuels (biofuels) such as bioethanol are becoming increasingly important due to

heightened concern for the greenhouse effect, depleting oil reserves and rising oil prices (Öhgren *et al.,* 2007). Ethanol, chemically known as ethyl alcohol, is a clear, colourless liquid, with an agreeable odour (Bugaje, 2008). Bioethanol can be utilized as oxygenator of gasoline, elevating its oxygen content, allowing a best oxidation of hydrocarbons and reducing the amount of aromatic compounds and carbon monoxide released into the atmosphere (Amira *et al*., 2012). Bioethanol is obtained from bioenergy crops and biomass which distinguishes it from those energy produced synthetically from petroleum source (Raposo *et al.,* 2009). Different countries use different bioenergy crops such as corn, cassava and sugarcane etc., for bioethanol production. Cassava and sugarcane are used mainly in Nigeria and Brazil (Mussatto and Teixcira (2010).

Biofuel as the name implies, is a form of fuel obtained from biological sources such as plant and animal products that can be used for heat, electricity and fuel. That is, a fuel made from biological matters, e.g. biodiesel, bioethanol, biogas and methane. They are emerging as major renewable, environmental friendly, and sustainable alternative to the conventional petroleum based fuels. Environmental concern and rising price of conventional liquid fossil fuels are creating a market gap for these emerging fuels. In Nigeria, biofuel are largely imported and being used as additives/ compliments to the conventional petroleum based fuels and mainly for transport sector of the economy, although use of fuels for process heat and electricity in industry and for domestic use are on the increase due to the deteriorating condition of the power sector in the country. The product is commonly known as bioethanol is part of the Brazil and United States pilot project. The Nigeria National Petroleum Corporation (NNPC) has already identified bioethanol 10% blend with PMS (E10) to be used by vehicles in Nigeria (Abila, 2010).

1.1 Problem Statement

Since late 1970s, renewable resources of bioethanol production has grown into huge industries and provides several billion gallons of ethanol for formulated gasoline in countries like Canada, Brazil, the United States of America (USA) etc. The annual production of ethanol in the United States was 3.4 billion gallons in 2005 and has been rising progressively (Lynd *et al*., 2003). This makes the fuel ethanol industry the fastest growing energy industry in the world. Rural areas in Nigeria are endowed with forest produce; cassava, sugar cane, rice, maize, crop residue, Jatropha seeds, animal wastes amongst others. Since 2005, research revealed that bioenergy reserves/potentials of Nigeria stood at: fuel wood (13,071,464

hectares), animal wastes (61 million tonnes per year), and crop residue (8.3 million tons). According Olanbiwoninu and Odunfa (2012), the country is poised for the production of biofuel from cassava, sugar cane, rice, maize and sorghum output. More so, cassava production has witnessed a phenomenal increase of 44.693 million tons since 2004 and is on the rise. To achieving more successes in the field of biofuel production taking into consideration the issue of food famine, there is need for continuous researches.

1.2 Significance of the Studies

The benefits of these studies are poised on the production of biofuel from farm produce like cassava, sugar cane, rice, maize and sorghum output, Jatropha seeds and animal wastes etc. More importantly, to diversifying the nations' economy through biofuel production, there is the need to extensively harness more robust ways of using farm produce with zero-famine.

2.0 Background Review

Biofuel is not new the term biofuel also refers to solid, liquid, or gaseous fuel derived from renewable raw materials. Biofuel have attracted increasing interest over the last few decades. As fuel made from locally grown renewable sources, they have been proposed as an alternative to expensive fossil fuels. When first demonstrating the engine bearing his name, Rudolf diesel ran is on peanut at world's fair in Paris in 1900 (Kewei *et al*., 2012).

Interest in both vegetable oils fuel for the internal combustion engine and plant material for ethanol production for transportation fuel was reported in several countries during 1920s and the year 1930s and also during the world war 2 where there serous fuel shortages, for example UK and Germany. In an interview in 1925, Henry Ford, the founder of Ford Motor Company, envisaged the processing of fruit and other plant into fuel for cars, the future of the fuel is going to come fruit like that sumac out of the road, or from apples, weeds, sawdust-almost anything. There is a fuel in every bit of vegetable matter that can be fermented. There is enough alcohol in one year's yield of an acre of potatoes to drive the machinery necessary to cultivate the field for hundred years (NYT, 1925).

Nowadays, energy has become a crucial factor for humanity to continue the economic growth and maintain high standard of living especially after the inauguration of the industrial revolution in the late 18^{th} and early 19^{th} century. In the past 30 years, the transportation sector has experienced a steady growth due to the increasing numbers of cars around the world. It has been estimated that the global transportation energy use is expected to increase by an

average of 1.8% per year from 2005 to 2035 (U.S EIA, 2010). Globally, the transportation sector is the second largest energy consuming sector after the industrial sector and accounts for 30% of the world's total delivered energy, of which 80% is road transport. It is believed that this sector is currently responsible for nearly 60% of world oil demand and will be the strongest growing energy demand sector in the future. Nearly all fossil fuel energy consumption in the transportation sector is from oil (97.6%), with a small amount from natural gas. Between 2006 and 2030, around three quarters of the projected increase in oil demand is expected to come from this sector (Abbas, 2010; Stephen and Michael, 2010; U.S EIA, 2010).

Under the Kyoto protocol, the government of Canada has committed to reduce the greenhouse gas emission by 6% from 1990 levels between 2008 and 2012. Ethanol blended gasoline has the potential to contribute significantly to reduce these emissions. It can also be used as a fuel for electric power generation, in fuel cells (thermo-chemical action) and in power cogeneration systems, and as a raw material in chemical industry. Bioethanol can be employed to replace octane enhancers such as methylcyclopentadienyl manganese tricarbonyl (MMT) and aromatic hydrocarbons such as benzene or oxygenates such as methyl tertiary butyl ether (MTBE) (Raimi *et al*., 2012).

Energy conversion utilization utilizes and access underlies many of the great challenges of our time, including those associated with sustainability, environmental equality, security and poverty. Biofuels are attractive alternative to current petroleum based fuels as they can be utilize as transportation fuels with little change to current technologies and have significant potential to improve sustainability and reduce greenhouse gas (GHG) emission. Research on improving biofuel production has been accelerating for both ecological and economic reasons, primarily for its use as an alternative to petroleum based fuels. Microbial fuel cells (MFCs) are also getting attention but they need huge improvement in technologies and also not sustainable for transport.

Biofuels could play an essential part in reaching targets to replace petroleum based transportation fuels with a viable alternative, and in reducing long term CO_2 emission, if environmental and economic sustainability are considered carefully they can be direct and immediate replacement for the liquid fuels used for transport and can be easily integrated into the logistic systems that are operating today. In recent years, the use of liquid biofuels in the

transport sectors has shown rapid global growth, driven mostly by policies focused on achievement of energy security, and mitigation of GHG emission (Sidra *et al*., 2013).

2.1 Aspergillus Niger

Aspergillus Niger is a famous and one of the most common species of the genes Aspergillus. It causes a disease called *mold* on certain fruit and vegetables such as grapes, onions and peanuts, and the common contaminant of food. It is everywhere in soil and most commonly reported from indoor environments, where its colonies can be confused with those of the stachybotrys which have also been called black mould (Samson, *et al*., 2001). World Health Organisation (WHO) supports the view that Aspergillus Niger can be cultured for industrial production of citric acid and glycolic acid that is safe for human consumption (Schuster *et al*., 2002). The organism is also explored for the production of enzymes like glycosidase, amylase, cellulose, pectinase, pectinase and protease. When Aspergillus Niger is cultured on sabourqud dextrose agar zapek dox agar or potato dextrose and incubated at 25^0C, they tend to produce spores within 7days (Verweij and Brandt, 2007).

2.2 Bioethanol

The principle fuel used as petroleum substitute is bioethanol and is mainly produced by sugar fermentation process, although it can also be produce by the chemical process of reacting ethylene with steam. The main source of sugar required to produce ethanol comes from fuel or energy crops and the plant. These fuel crops are normally grown specifically for energy use and they include maize, corn and wheat crops, waste straw, willow, sawdust, reed canary grass, cord grasses, Jerusalem artichoke, myscanthus, sorghum plant and algae which are basically spirogyra biomass as feed stock use in research. It is alternative fuels that can be used in ethanol engines and provide power similar to conventional ethanol fuel (Yue *et al*., 2008).

Bioethanol produced from pre-treatment and microbial fermentation of fungus has a great potential to become a sustainable transportation fuel in the near future. Bioethanol is a renewable and domestically produced liquid fuel that can help reduced the country dependence on foreign refined oil imports. Recent environmental and economic concerns have prompted resurgence in the use of bioethanol throughout the world (Thomsen *et al*., 2007).

In Brazil, biofuel production contains more than 25% energy and approximately 2.4 million cars in Brazil are able to utilized energy from pure alcohol. Since 1991, the European Community (EC) proposed a 90% tax reduction for the use of biofuel.

Bioethanol is usually obtained from the conversion of carbon-based feedstock. Ethanol is a colourless, volatile, flammable liquid that is an intoxicating agent in liquors. It is also used as a solvent called ethyl alcohol and has the chemical formula C_2H_5OH. Bioethanol from biomass sources is the principal fuel used as a petrol substitute for road transport vehicles. The high price of crude oil makes bioethanol fuel attractive14. Bioethanol is mainly produced by the sugar fermentation process although it can also be manufactured by the chemical process of reacting ethylene with steam. The reaction processes is summarised in Equation (1 - 5):

$$C_2H_6 + H_2O \longrightarrow C_2H_5OH \quad (1)$$

$$2C_6H_{10}O_5 + H_2O \longrightarrow C_{12}H_{22}O_{11} \quad \text{(liquefaction)} \quad (2)$$

$$C_{12}H_{22}O_{11} + H_2O \longrightarrow 2C_6H_{12}O_6 \quad \text{(saccharification)} \quad (3)$$

$$C_{12}H_{22}O_{11} + H_2O \longrightarrow C_6H_{12}O_6 + C_6H_{12}O_6 \quad \text{(fermentation)} \quad (4)$$

$$C_6H_{12}O_6 \longrightarrow 2C_2H_5OH + 2CO_2 \quad \text{(ethanol)} \quad (5)$$

The fermentation process is carried out at a temperature of between 25^{o}C and 30^{o}C and for the purpose of this research, 30^{o}C was used. This process involved the use of another fungi called saccharomyces cerevisae (commonly known as yeast), to yield ethanol and water which is then separated through a process known as fractional distillation; since both ethanol and water have different boiling points (Thomsen *et al*., 2007).

2.3 Distillation

For the ethanol to be usable as a fuel, water must be removed. Most of the water is removed by distillation. The purity is limited 95-96% due to the formation of a low-boiling water-ethanol azeotrope. This may be used as fuel alone but unlike anhydrous ethanol it is immiscible in petrol meaning it cannot be mixed i.e. E85. The water fraction is typically removed in further treatment in order to burn with in combination with petrol in petrol engines (Thomsen *et al*., 2007; Vasanthi and Meenakshisundaram (2012).

2.4 Dehydration

Currently, the most widely used purification method is physical absorption process using a molecular sieve, for example, ZEOCHEM Z3-03 (a special A3 molecular sieve for ethanol dehydration). Another method, azeotropic distillation, is achieved by adding the hydrocarbon benzene which also denatures the ethanol (to render it undrinkable for duty purposes). A third method involve the use of calcium oxide as a desiccant but unfortunately, as regard to this research none of the method is used due to cost and availability (Vasanthi and Meenakshisundaram, 2012).

3.0 Materials and Methods

3.1 Materials

Some of the important materials used in this study are funnel, pH meter, digital weighing balance, polythene hand gloves, beaker, cotton wool, conical flask, sieves, test tube, glass rod, Aspergillus Niger, lactose, sweet potato peel, distilled water and Saccharomyces cerevisiae etc.

3.2 Methodology

3.2.1 Collection of Sample (Sweet Potato peels)

Fresh sweet potato was collected from the local market of *Ngomari* bus stop Maiduguri, Nigeria. They are characterized by having bark and an inner light cream colour, the sweet potato was then washed thoroughly to remove dust and other debris, peeled off and chopped into small pieces. It then under the sun for 3day till all the moisture was removed, and then it was sieved through a steel mesh, the sweet potato flour was then stored in air tight container for further use. They are characterized by having bark and an inner light cream colour.

The treatment and drying of the sweet potato peels was achieved 4 days after the samples was been collected, washed and dried under the sun were all the moisture was completely removed. Figures 1 and 2 depict the pictorial representations.

3.2.2 Microorganisms

In this research study, Aspergillus Niger and Saccharomyces cerevisiae are the organisms used. They were both obtained from the stock culture of the Department of Veterinary Medicine, University of Maiduguri, Borno State.

3.3 Crushing and Grinding

Crushing and milling of dried sweet potato peel was carried out, grinding of big solid particles into smaller ones (0.4 – 0.6mm).

3.4 Liquefaction

In the liquefaction reactor, the starchy material usually gelatinized in order to dissolve the amylase and amylopectin present. Heat and enzyme (alpha-amylase) was added in this process to a temperature of about 90^{o}C which aid partial hydrolysis and conversion of the starch molecules to maltose, but this research only emphasized on the dried Sweet potato peel.

Fig 1: Sweet Potato samples

Fig 2: Sweet potato peels

Fig 3: Liquefied powdered sweet potato peels

3.5 Saccharification

Sweet potato peel was saccharified by enzymes produced by Aspergillus Niger (Amylase and cellulose). In first step the biomass was subjected to saccharification by Aspergillus Niger. Saccharification is the process of breaking down of a complex starch into a smaller form by enzymes which is Aspergillus Niger, The process was carried out for a period of seven days and he process was monitored every 24 hours. Figure 3 depicts the liquefied powdered potato peels.

3.6 Fermentation

The ethanol is produced by microbial fermentation of the sugar. The fermentation process were divided into five and transferred into another set of conical flasks and labelled correctly, covered, autoclaved at 121^0C for 15 minutes and allowed to cool. The flasks were inoculated with *Saccharomyces cerevisiae and* lactose of 5g, 10g, 15g, 20g and 25g separately to carryout fermentation for five (7) days. The pH of the hydrolysate containing *Saccharomyces cerevisiae* was adjusted to 4.5 and fermentation carried out at 25^0C (Raman and Pothiraj, 2008). The flasks were shaken at interval to produce a homogenous solution. The ethanol yield was determined after fermentation. Further details can be found from (Usman, 2017).

4.0 Experimental Results and Discussions

4.1 Results

The analytical results obtained from this experimental study are been described in Figures 4 – 17.

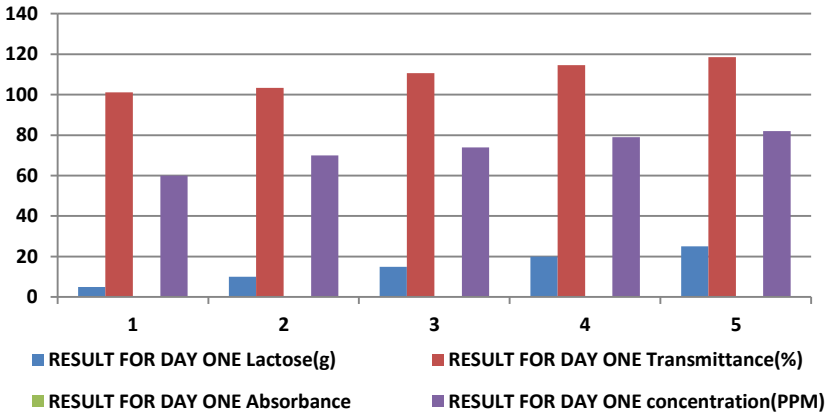

Fig 4: Kinetic analysis for day one (1st day): 18th April 2017

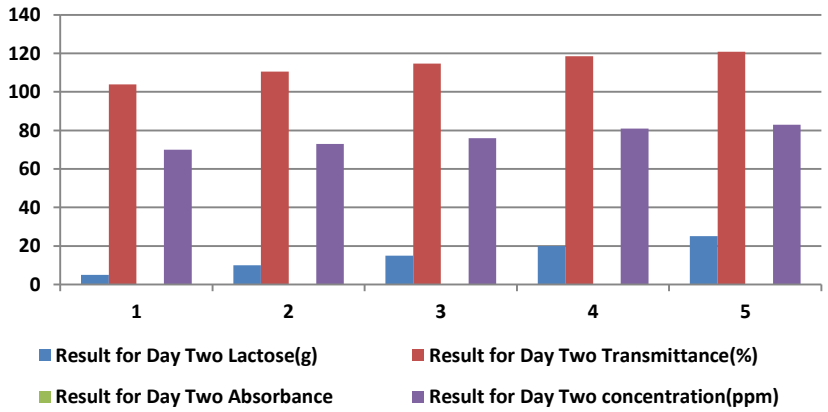

Fig 5: Kinetic analysis for day two (2nd day): 19th April 2017

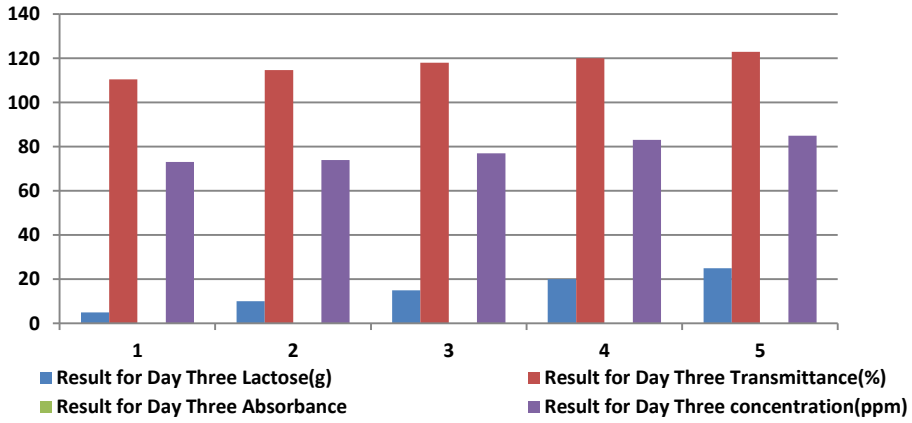

Fig 6: Kinetic analysis for day three (3rd day): 20th April 2017

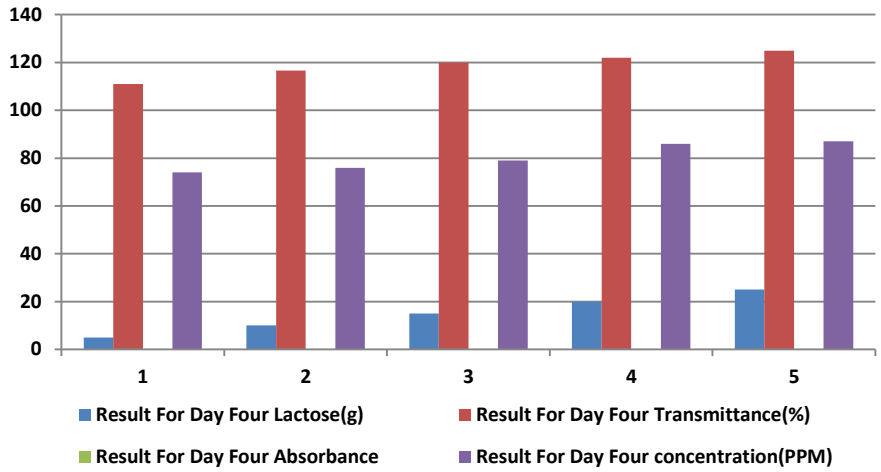

Fig 7: Kinetic analysis for day four (4th day): 21th April 2017

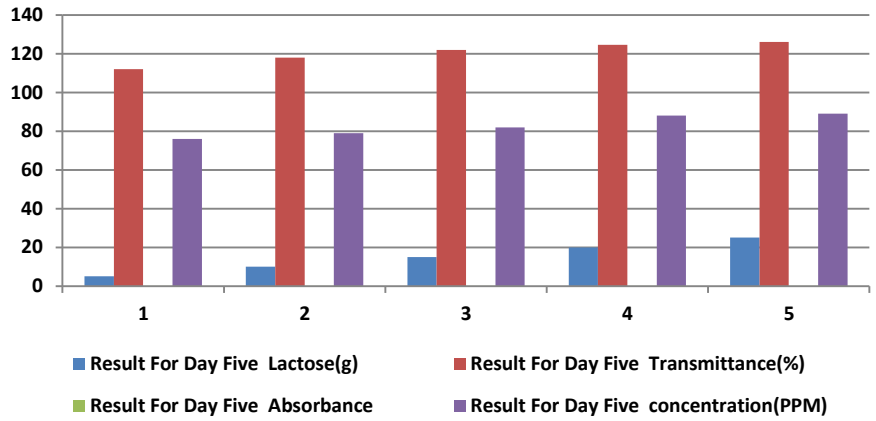

Fig 8: Kinetic analysis for day five (5th day): 22nd April 2017

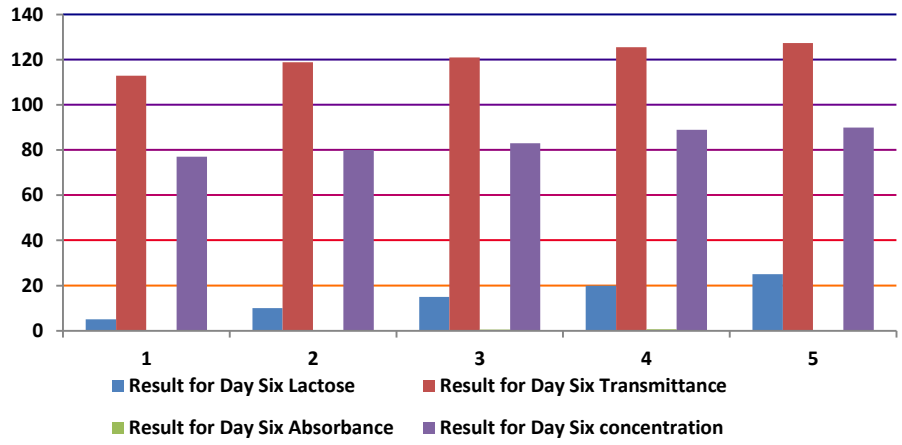

Fig 9: Kinetic analysis for day six (6th day): 23rd April 2017

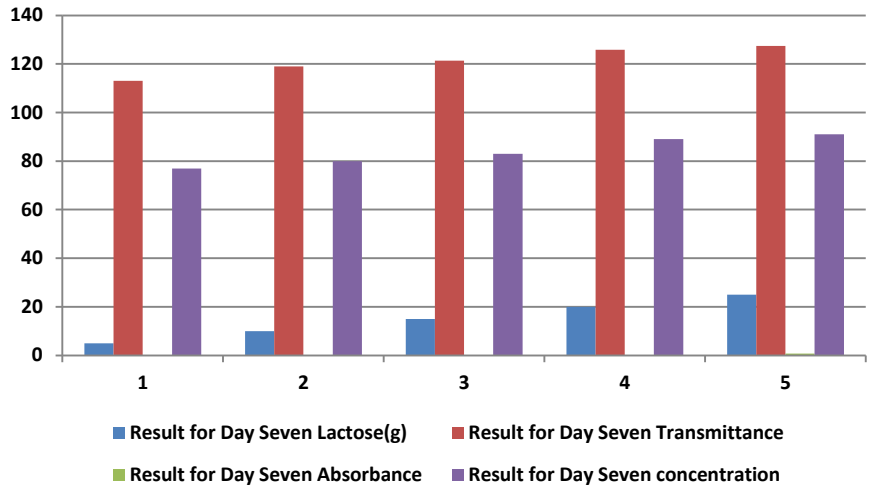

Fig 10: Kinetic analysis for day six (7th day): 24th April 2017

4.2 Discussions of Results

Figures 4 – 10 outlined the kinetic analysis for the bioethanol formation from day one to the seventh day. In each day, there was a progressive formation which is reflected on the absorbance, concentration and transmittance. In order to establish the severity of the bioethanol production

4.2.1 Aspergillus Niger

Since macro culture method was used to identify the organisms (Steinbach and Stevens, 2003). The organisms were sub cultured on scabrous dextrose agar plates to obtain distinct colonies. These were subsequently used to streak the surface of scabrous dextrose agar plate and incubated at 28^0C for three (3) days. The initial white coloration noticed on the colonies which later turns black at the top and with pale yellow colour at the bottom confirmed the presence of the organism to be Aspergillus Niger. This is also in accordance with the work of (Steinbach and Stevens, 2003). Aspergillus Niger was used as it is an enzyme source of cellulase and amylase for saccharification in this process because the potatoes can be converted into simple sugars, since pure commercially available enzymes are very expensive. This explained the fact that enzyme hydrolysis is a natural and ideal method for conversion of cellulose materials to sugars which could be used as a source of food, fuel or chemicals (Idris and Owofu, 2016) and (Martin and Thomsen 2008).

4.2.2 Saccharomyces cerevisiae

The Saccharomyces cerevisiae organisms were also identified physically on scabrous dextrose agar plates. Since the organisms was cultured on scabrous dextrose agar plate at 28^0C for six (6) days. The smooth white to creamy colour of the spherical colonies confirms the organisms to be Saccharomyces cerevisiae. Microscopic examination of the organism, a wet mount of the organism was prepared on a slide and fixed with a flame. It was viewed under microscope with oil immersion. The organisms showed oval shape organisms that are in clusters (Steinbach and Stevens, 2003).

Table 1: Physical and chemical properties of bioethanol produced

Chemical Formulae	C_2H_5OH	Units
Density	0.813	g/cm^2
Appearance	colour less liquid	-
Melting point	-114.14	^0C
Boiling point	78.24	^0C
Solubility in water	miscible	-
Vapour Pressure	6.01	kPa
Acidity	16.2	Pk_a
Basicity	-1.90	Pk_b
viscosity	1.40	mpa.s

Table 1 shows the chemical and physical properties of the bioethanol (biofuel) produced after distillation processes.

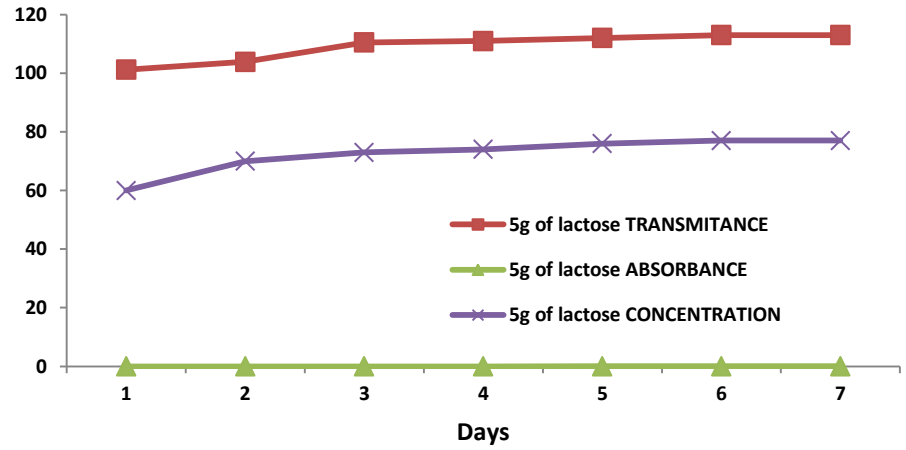

Fig 11: Profile for 5g lactose for seven days

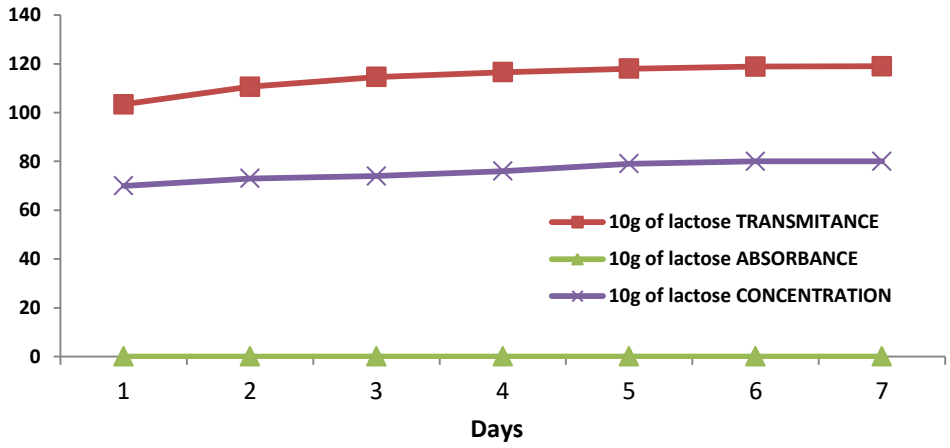

Fig 12: Profile for 10g lactose for seven days

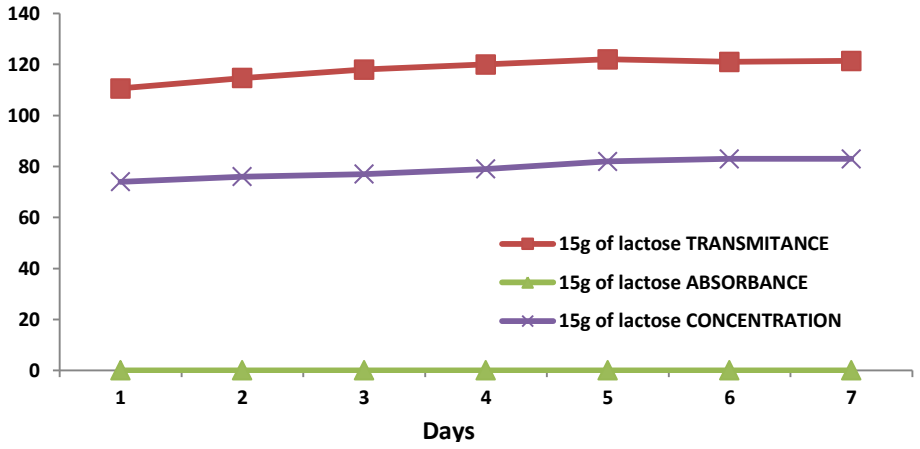

Fig 13: Profile for 15g lactose for seven days

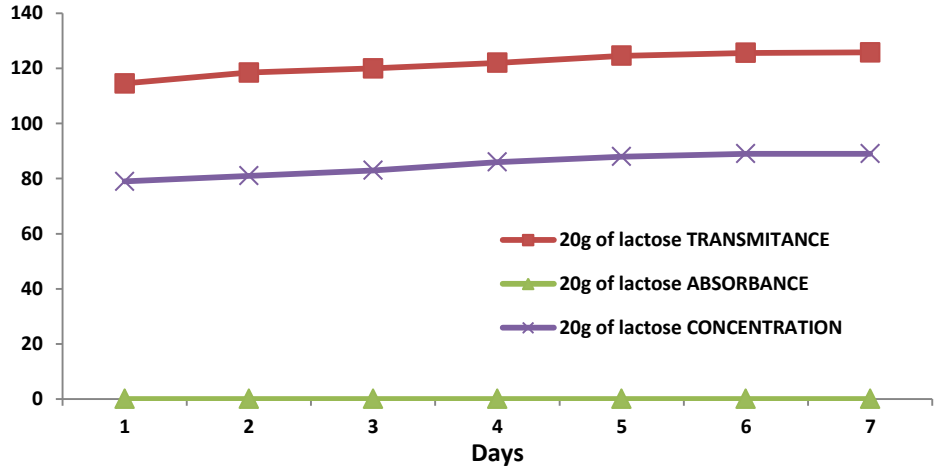

Fig 14: Profile for 20g lactose for seven days

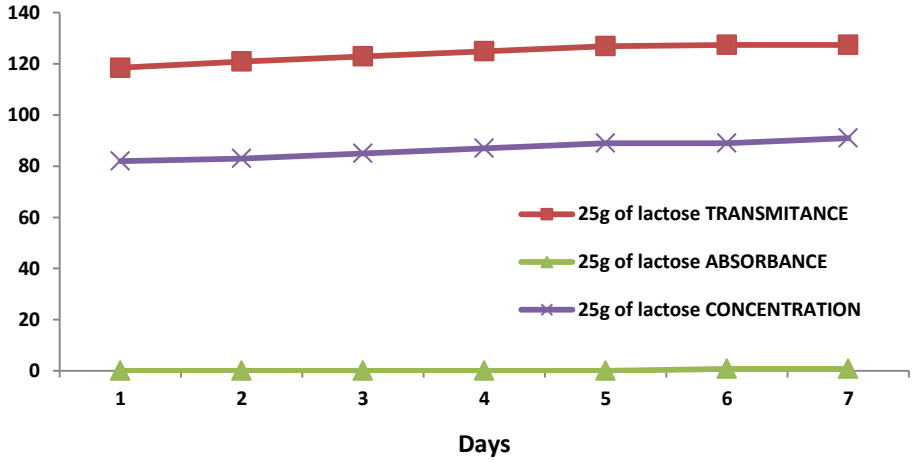

Fig 15: Profile for 25g lactose for seven days

Figure 11 – 15 respectively depicts the profiles for 5g, 10g, 15g, 20g and 25g lactose each for consecutive seven days yields. This achievement was recorded due to the addition of each (5g, 10g, 15g, 20g and 25g) weighed lactose to the *Aspergillus Niger and Saccharomyces Cerevisae*. These were the enzymes used for both saccharification and fermentation process respectively. In summary, the highest yield was recorded the was with 20g of lactose, after seven days it was recorded that the concentration was highest for the sample containing 20g of lactose as shown in Figures 15.

Table 2 depicts the concentration, mass, yield of the bioethanol produced. In this table, it is observed that the 20g of lactose have the highest yield of 43.09%.

Table 2: Summary of concentration, mass and yield produce after seven days

Lactose (g)	Concentration (ppm)	Mass (g)	Yield (%)
5	77	44.72	42.59
10	80	46.50	42.27
15	83	48.21	41.92
20	89	51.71	43.09
25	91	52.85	42.28

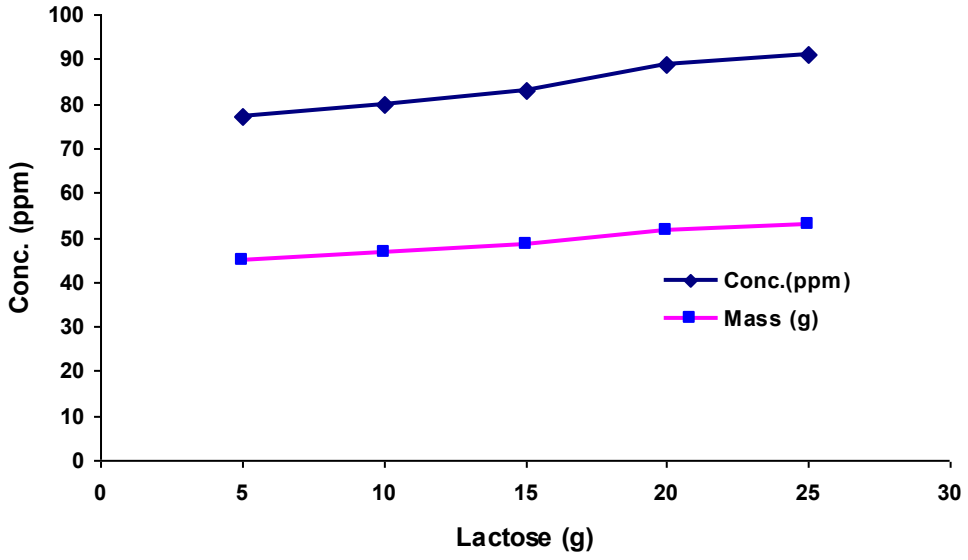

Fig 16: Profile for concentration and mass for lactose

In ordnance to the above analyses, Figure 16 depicts the concentration (ppm) in view with the mass (g), it was observed that there was uniform and progressive yield of the bioethanol produced. Figure 17 represents the percentage yield for biofuel production with respect to mass weight. This also shows a clear indentation that the bioethanol produced was highest at the 43.09% yield with the 20g lactose. The percentage significance error was incorporated in this figure which further identified the reasons why we may not need to introduce the 25g weight of the lactose if we must bent on high achievement of bioethanol production.

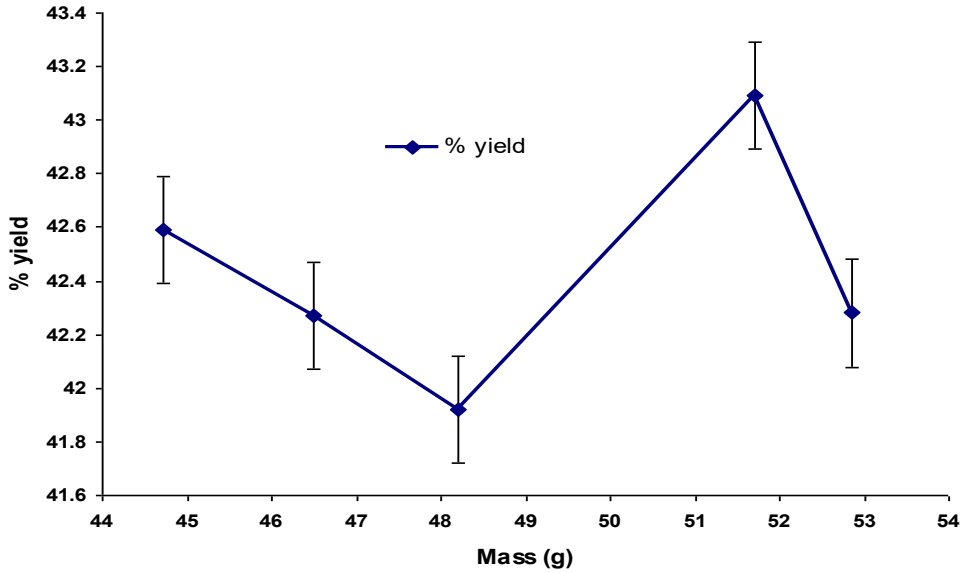

Fig 17: Percentage yield for biofuel production with respect to mass weight

5.0 Conclusions

In this work, when 5g, 10g, 15g, 20g and 25g each weighed lactose was added to the *Aspergillus Niger and Saccharomyces Cerevisae*, the highest yield recorded when 20g of lactose was introduced. This achievement was observed after seven days, it was recorded that the concentration of 91 ppm was highest for the sample containing 25g of lactose.

The study revealed that when lactose was added to fermentation medium together with enzyme *Aspergillus Niger*, the results obtained show that the 20g lactose represent a highest yield of biofuel production.

In addition, the increase and subsequent decrease in pH and specific gravity of each fermented sample indicated that the reaction (fermentation) was actually occurred, that is, the conversion of the substrate to products as well as the release of CO_2 as the by-product took place.

Recommendations

In this study, the following recommendations are:

1. Further experiment should be done but using different feedstocks e.g. Jatropha seeds, animal wastes etc. should be used.
2. Further work should be done on this experiment using other methods hydrolysis method.
3. A suggestion for a method of continuous process operation should be developed in order to reduce-error resulting from exposure to atmospheric condition.
4. Effects of temperature and pH on the yield of the ethanol should also be investigated.

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