Novelty Journals

ISSN 2394-9651

International Journal of Novel Research in Physics Chemistry & Mathematics
Vol. 10, Issue 3, pp: (1-19), Month: September - December 2023, Available at: www.noveltyjournals.com

Bioethanol Production from Low-Value Feedstocks: Wild Cocoyam, Waste Cassava Peels, and Waste Sugar Cane Molasses

Isah S[1]., Ahiakwo J[1], Odusina A[1]., Equere-Obong A[1]., George J[1]., Ojo E.M[1]., Udoh S[1]., Anwuchughum C[1]., Edward A[1]., Enahoro E[1]., Salami A[1].

[1]Caleb University, KM 15, Imota, Lagos State, Nigeria

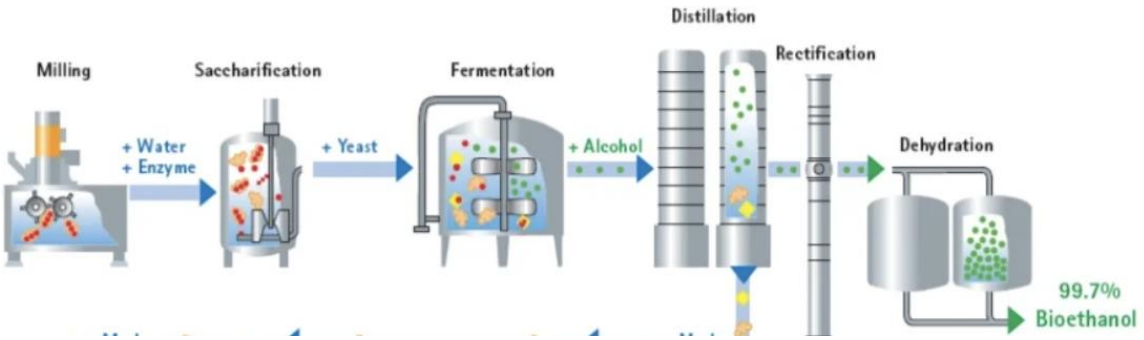
DOI: https://doi.org/10.5281/zenodo.8355346

Published Date: 18-August-2023

Abstract: **Bioethanol, produced by the anaerobic fermentation of carbohydrates, can be used as a renewable fuel, as vital ingredient in the production of beer, wine, or high-valued distillate alcoholic drink. Different plants have been installed in different parts of the world as carbon source to produce bioethanol. Feedstocks is a fundamental requirement for successful and efficient operations of these bioethanol manufacturing plants. One major challenge in choosing suitable feedstock is food versus fuel debate, that is, reducing to the barest minimum food crops serving as main source of food for human consumption. Thus, the focus of this review is to explore some crops rich in carbohydrate but less commonly consumed as food such as wild cocoyam, cassava peels and waste product of sugar refinery, sugar cane molasses as alternative feedstocks.**

In this review, the harvested wild cocoyam corms and cassava peels were washed, dried, ground and then made into a gelatinized solution to increase the surface area. The starch present in the slurry mixtures was then saccharified by the action of different hydrolytic enzymes, like alpha-amylase, protease, amylitic-TS, and amyloglucosidase. It was reported that the enzymatic hydrolysis of ground cocoyam and cassava was effective in yielding favorable levels of fermentable glucose. The saccharified wort was then inoculated with viable yeast strains to begin the fermentation process. On the other hand, sugar cane molasses considered highly rich in sugar content was converted to bioethanol using a gram negative, facultative anaerobic, rod shaped strain'' Zymomonas mobilis'' as the microorganism under anaerobic fermentation condition. The fermentation process varied for several days from 48 h to 168 h depending on the feedstock. Percent alcohol concentration produced from wild cocoyam sample was 12.90 % after 168 h of anaerobic fermentation, whilst sugar cane molasses recorded 9.3 % bioethanol content after 48 h of fermentation process. The percent alcohol recovered from waste cassava peel was 8.5 % after 96 h of fermentation.

Keywords: **Bioethanol, Anaerobic fermentation, wild cocoyam, molasses, and cassava peels.**



Novelty Journals

ISSN 2394-9651

International Journal of Novel Research in Physics Chemistry & Mathematics
Vol. 10, Issue 3, pp: (1-19), Month: September - December 2023, Available at: www.noveltyjournals.com

1. INTRODUCTION

The continuous increase in energy demand, the inevitable decline in the availability of fossil fuels, and the growing concerns about atmospheric pollution and climate change have sparked initiatives from governments to increase production of energy from alternative renewable sources. Biofuels are fuels derived directly from living matter and are getting a lot of attention as possible options for renewable fuel due to their clean burning nature. One such biofuel, bioethanol, is environmentally friendly and sustainable for use as an additive or substitute for petroleum fuel. It has been reported that ethanol reduces tailpipe carbon monoxide emissions by as much as 30%, toxics content by 13% (mass) and 21% (potency), and tailpipe fine particulate matter (PM) emissions by 50% (Zhang, *et al*., 2003.

Bioethanol can be produced from lignocellulose matter and starch-derived glucose in a biochemical process known as alcoholic fermentation. This process is brought about by the action of yeast that transforms the natural sugar present in any starchy material into alcohol with the evolution of carbon dioxide under controlled environmental conditions. The process is an anaerobic fermentation system catalyzed by enzymes produced by bacteria and fungi. Starchy materials are first hydrolyzed to fermentable sugars, and subsequently fermented with the required yeast species to produce bioethanol. The demand for bioethanol is high whereas the substrate for its production is limited and competitive. This review evaluated reports on the production of bioethanol from several species of wild cocoyam, waste cassava peels and waste product of sugar refinery known as sugarcane molasses.

Cocoyam is a stable root crop typically grown in tropical climates like West Africa (mainly Nigeria & Ghana). It serves as staple food for people in these regions. These plants possess high nutritional values and are proficient at supplying essential calories, vitamins, and minerals that they are considered the quintessential subsistent crops. Cocoyam is also cheap and available throughout the year; it thrives in infertile and difficult terrains that are not well suited for large-scale growing of most conventional staple crops. However, over 20 million tons are lost yearly due to inadequate storage and processing facilities. In Colombia, this plant does not have large crop areas, and the uses are mostly directed to animal feeding. Only a small portion of the production is directed to human food, sales, and the agroindustry; the leaves and the stem of the plant are the most used parts and the tuber is generally discarded. Cocoyam has been reported to contain 70-80% starch and 2-3 percent of protein. The average starch content of cocoyam is high when compared to other starchy feedstocks such as potatoes (15%) and yucca (18%). This composition makes it an excellent raw material for bioethanol production.

The success of cocoyam as a substrate for bioethanol production especially threatens food security in places where it is consumed. However, in Colombia where cocoyam is rarely used as human food, bioethanol production will not threaten food security. It is therefore imperative that research be turned to the use of non-food starchy items for the production of biofuels. One such non-food starchy item is wild cocoyam (*Caladium bicolor*) which is found in tropical regions and Asian countries– they look like the normal edible cocoyam *(Colocasia esculenta)* but can be differentiated by the red and white patches found on their leaves. *C. bicolor* is a non-human edible plant that grows along riverbanks, lakes, streams, brooks, oases, and shady areas in the tropics. Like normal cocoyam, they grow indiscriminately without being cultivated; their broad leaves suppress other weeds under and around them. All parts of *C. bicolor* are inedible – eating of its corm produces an intense irritation in the throat and may also irritate sensitive skin. The gainful use of *C. bicolor* as a substrate for biofuel production will bring about practical exploitation of this inedible abundant natural resource, which is wild and readily available. In addition, the use of this biomass will help solve the problem of the diminishing fossil fuel reserves and at the same time prevent food insecurity that can come about while using staple foods like normal cocoyam.

Experimental work was undertaken to assess the ethanol production potential of wild cocoyam corms. The initial step was to isolate the starch content and enzymatic hydrolysis to convert the starch into fermentable sugars. The sugars present in a healthy cocoyam are sucrose, maltose, glucose, and fructose; of these four, glucose is fermentable. Alcohol fermentation is brought about by the action of yeast, which transforms this sugar into alcohol. The process is an anaerobic fermentation process catalyzed by enzymes produced by bacteria and fungi. Because this reaction is enzyme-catalyzed, different reaction parameters, like pH and temperature, need to be optimized allowing for more efficient results. In one investigation conducted by Aisien, *et al.,* (2010), eighteen fungal strains were screened for their amylase production and fermentation capability. The best isolate was used to optimize reducing sugar production by varying the fermentation parameters – time, pH, substrate concentration, nitrogen source, and inoculum size. Eventually, under optimal conditions gotten from these varying experiments, the hydrolysate was further fermented. The fermented liquid was then distilled to yield ethanol.

Novelty Journals

ISSN 2394-9651

International Journal of Novel Research in Physics Chemistry & Mathematics
Vol. 10, Issue 3, pp: (1-19), Month: September - December 2023, Available at: www.noveltyjournals.com

The yields of bioethanol in different experimental investigations showed that wild cocoyam can be a feasible feedstock for ethanol production. The fermentation process is efficient taking as little as 5 days (120 h) for fermentation to be completed.

2. BIOETHANOL FROM WILD COCOYAM

Energy in all its forms is essential to humanity and is central to the improvement in people's quality of life. However, due to the progressive depletion of energy resources and the atmospheric pollution caused by the combustion of oil-derived fuels, research on renewable sources of energy is being encouraged (Zhang, 2003). In addition, the continuous increase in energy demand has sparked several initiatives from governments around the world to increase production of energy from renewable sources (Quintero *et al*., 2008). Consequently, biofuels are getting a lot of attention as possible options for renewable fuel: attracting interest as transport fuels to mitigate energy security concerns in many countries (WBA, 2013). Recently, Nigeria joined the league of biofuel users with the aim of generating wealth (Aisien *et al*., 2010), the Nigerian biofuel policy and incentives was released in 2007 with the aim of spurring a vibrant bioenergy sector development (NNPC, 2007).

Biofuels apply to solid, liquid, or gaseous fuel produced from biological materials (biomass), which can be used for the generation of power, heat, or fuel for automobiles (Agba, 2010; Bamikole, 2008). Some of the liquid biofuels are bioethanol, biodiesel, and biogas. Bioethanol is a plant-based liquid fuel that is a promising alternate energy source due to its clean burning nature and no detectable emission of pollutant gases such as NO_x or CO (Jarboe, 2009). It can be used as an additive or substitute to petroleum (Walker, 2011) and is one of the preferable liquid fuels due to its combustion properties (Galbe and Zacchi, 2002). Furthermore, bioethanol contributes to sustainable development and offers the opportunity to improve access to modern energy services for the poorest members of the society (Sathaye *et al*.2011).

Bioethanol is produced by a biochemical, alcoholic fermentation process brought about by the action of yeast through a process that transforms the natural sugar present in any starchy material into alcohol with the evolution of carbon dioxide under controlled environmental conditions (Miller and Litsky, 1976; Okafor, 1987; Saraswati, 1988; Saucedo *et al*.1998; Dubey, 2002; Okorondu *et al*., 2009). The process is an anaerobic fermentation catalyzed by enzymes produced by bacteria and fungi. Starchy materials are first hydrolyzed to fermentable sugars, and subsequently fermented with the required yeast species to produce ethanol (Kuboye and Akinrele,1971; Saraswati, 1988; Jaleel *et al*., 1988). See figure 1 for the hydrolytic process and biochemical equation.

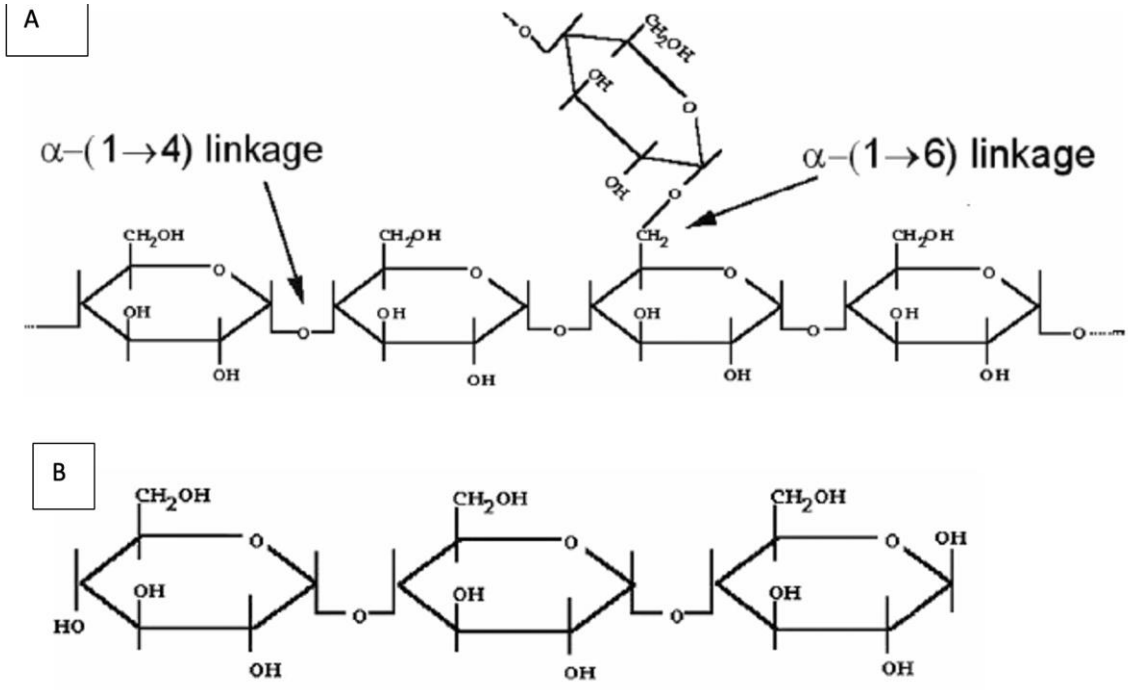


Fig 1: Glycosidic linkages of starch molecule before enzymatic hydrolysis (A) and after hydrolytic process (B)

$$C_6H_{12}O_6 + 2ADP + 2\ P_i \rightarrow 2C_2H_5OH + 2\ CO_2 + 2\ H_2O + 2\ ATP$$

Fig 2: The chemical equation for Emblen-Meyerhof-Parnas (EMP) pathway for conversion of sugar by yeast to bioethanol and carbon dioxide.

The global demand for ethanol is high whereas the substrate for its production is limited and competitive. Various investigations aimed at improving the processes employed in the production of bioethanol from different feedstock have been carried out (Okorondu *et al*., 2009), four of which are reported in this paper.

Cocoyam, whose origin is Central America, (Giacometti, 1992) is a plant crop found mainly in tropical climates – these regions have the highest production of cocoyam (Oke, 1998; Onwueme, 1994). Cocoyam serves as a root crop and vegetable in the diet of many rural dwellers in West Africa, where they are freely available (Okigbo, 1987). This plant is also cheap and available throughout the year, thriving in infertile and difficult terrains that are not well suited for large-scale growing of most conventional staple crops (Hodgkin, 2002). Its corms and tuber crops are well known for their high carbohydrate content; it has been reported that cocoyam has a starch percentage composition of 72% (Baum, 1982) with small size granules (Owusu, 2014), which makes it great for obtaining starch and starch-based compounds as value added products (USDA). However, its high carbohydrate level is yet to be fully harnessed in the industries (Nwufo and Fajola, 1998).

It has been established that bioethanol can be produced from lignocellulose and starch derived glucose, obtained from plant sources such as sugarcane, cassava, potatoes, maize, millets and cocoyam. The problem, however, with starch derived glucose is the fear that it would threaten food security and shorten natural agricultural resources (Koizumi, 2005). For example, cassava alongside sweet potato and yam have been classified as main starches that serve as staple foods for people in the world's hot and humid regions (Klass, 1998). The diversion of food resource to biofuel production may to a large extent have fueled the current food crises worldwide (Srinorakutara *et al*., 2008). It therefore becomes imperative that the searchlight be turned to the use of non-food starchy items for the production of fuel ethanol.

The use of the tuber of *Caladium bicolor,* considered unsuitable as food for humans, can help solve the problem of the diminishing fossil fuel reserve and at the same time not led to food insecurity that can come about while using staple foods. And in a country, such as Colombia, just a small portion of cocoyam production is directed to human food, sales, and agroindustry (Gómez,2002). Therefore, its use as raw material would not affect food security and presents a whole new opportunity for the production of biofuels. In Nigeria, where cocoyam is one of the stable root crops, over 20 million tons of cocoyam are lost yearly due to inadequate storage facilities (IITA, 2009). So, speedy conversion of the surplus harvest will reduce wastage and improve economic gains. Conclusively, the use of cocoyam in the production of bioethanol should be encouraged because of its high ethanol yield and low cost of production. This process is an encouragement to local farmers and will boost their economy.

2.1 Production process of bioethanol from wild Cocoyam

Production of bioethanol from wild cocoyam according to the method already described by Braide and Nwaoguikpe, (2011) is briefly described here. 200g of wild cocoyam cultivars was gelatinized by grating and thoroughly mixing with 100mL of water in a beaker. The mixture was then covered with aluminum foil and cooked for 30 minutes at a pressure and temperature of 10 psi and 108 °C. 900mL of water was added to the mixture before it was hydrolyzed in two stages. The first stage involved the use of *Amylitic-TS*, a bacterial alpha-amylase that broke down the starch in a liquification process. The pH and temperature were maintained at 6.0 and 95-100°C. The second stage involved the use of *AMG*, a fungal alpha-amylase that saccharified the starch, breaking it into oligomers. In this stage, the pH and temperature were adjusted to 5.4 and 60-64 °C to favor the activities of the enzyme. The action of these enzymes was stopped by heating the sample and then cooling it, while adjusting the pH to 4.2-4.5. This process also aided the sterilization of the sample. The initial brix level, specific gravity, and pH of the wort were measured and recorded.

The *Saccharomyces uvarum* yeast used in this study was obtained from consolidated breweries located in Awo-mamma in Imo State. The sterile liquor was inoculated with viable yeast in a flask and left to ferment for seven days at 30 °C. The brix level, pH, specific gravity, and percentage alcohol by volume were determined daily and recorded. Hydrogen ion concentration (pH) of the sample was determined with a uniscope pH meter. The specific gravity was calculated by standard

method while brix level of the sample was determined by hand refractometer method. Simple distillation was used in the determination of percentage alcohol in the sample.

The wild cocoyam plant used in the study was harvested from a farm in Oboloafor in Nsukka, Enugu State and identified as *Caladium bicolor* or "Florida Clown" by the Botany Department of the University of Nigeria, Nsukka. The unpeeled tubers were washed using sterile distilled water air-dried at room temperature. The tubers were then peeled, sliced into smaller pieces, and air-dried to a constant weight. The fleshy parts of the tuber where then ground to fine powders of the same size range.

The microorganisms used for this study were isolated from decayed *C. bicolor* and palm wine, eighteen pure cultures were obtained and preserved until required. The isolates were screened for their ability to hydrolyze starch using starch agar plate assay on standard media. After 72 hours of incubation, Gram's iodine reagent was used to qualitatively screen them – the development of purple/blue-black color indicates the absence of starch degradation while the presence of a clear zone around the colonies indicates secretion of amylase and hydrolysis of starch. Successful isolates where then quantitatively screened for their ability to produce amylase. Glucose content of samples obtained via centrifugation were analyzed by boiling with 1 mL of the Dinitro Salicylic Acid reagent for 10 minutes in a test tube. The color intensity of the tubes was then measured at 540 nm using a Colorimeter, absorbances were converted to glucose concentration using a prepared standard curve.

The best isolate was used to optimize reducing sugar production. For this part of the experiment, other parameters were varied and the reducing sugar production was compared. Some of the varied parameters include: substrate concentration, initial pH, nitrogen source, and inoculum size. The hydrolysate from the optimal conditions was then fermented using *Saccharomyces sp*. at pH 5.2 for 144 hours in an anaerobic environment. The fermented liquid was distilled to yield a maximum ethanol concentration and its concentration was determined by the boiling iodometric method.

In a related research by Gumel *et al.*, (2018) freshly harvested cocoyam, *Colocasia esculenta* corms were obtained from Yankaba Market in Kano State and identified by the Biological Sciences Department of Bayero University, Kano. The harvested corms were washed, peeled, sliced and then open dried for 7 days (168 h). The dried sample was milled to flour using an electric blender, oven dried and then stored in airtight polythene bags throughout the period of the experiments.

The experiment was carried out in two phases: with enzyme supplement and without enzyme supplement. 15g of the cocoyam flour was measured in triplicates for each group of the selected mashing temperatures (50°C, 60°C, 80°C). Distilled water was added to the samples to form slurry and stirred to homogenize. The samples without exogenous enzyme supplement were then cooked for 60 minutes, 90 minutes, and 150 minutes respectively. After mashing, the glucose and protein concentrations were determined and recorded. For the samples with enzyme supplement, three different cooking styles were employed using freshly prepared enzyme cocktail. The cocktail contained a total of 60 µL protease, 30 µL alpha amylase and 30 µL glucoamylase. The glucose and protein concentrations were determined after the mashing. The concentration of the glucose liberated in this study was determined using the glucose oxidase method. The principle is that glucose reacts with glucose oxidase enzyme to produce glucuronic acid and hydrogen peroxide. The hydrogen peroxide liberated subsequently reacts with amino phenazone and phenol in the presence of peroxidase to produce quinoneimine, which is a chromogen, and the intensity of the red-violet color formed is directly proportional to the concentration of the glucose.

The saccharified wort obtained was fermented with the viable yeast cells of *Saccharomyces cerevisiae*. Two fermentation processes were employed – Simultaneous Saccharification and Fermentation (SSF) and Separate Hydrolysis and Fermentation (SHF). The pH of the reaction was adjusted using sulfuric acid or sodium hydroxide. The conditions for the incubator shaker were set to be 32°C and 150 rpm for the temperature and rotations respectively. Samples were drawn at 24 hour, 48 hour and 72 hour intervals to monitor the progress of the fermentation. The concentration of ethanol was measured by using a working calibration curve of the absorption of ethanol at 192 nm using a UV/visible spectrophotometer and a Fourier-transform infrared spectrophotometer.

Application of Thermodynamic-Topological Analysis for bioethanol Production from Cocoyam (Xanthosoma sagittifolium), Serna-Loaiza et al., (2018).

The cocoyam plant species found in Colombia was determined to be *Xanthosoma sagittifolium*. Samples were collected in the rural zone of Manizales and washed and peeled to remove dirt. The samples were then wet grinded, and the resulting

mass was used for the following experiment. The quantitative determination of the starch content of the cocoyam species was performed by using sulfuric acid and phenol. The starch in the sample is converted to hydroxymethyl furfural, HMF, in an acidic medium. The resulting slurry was then reacted with phenol to produce a green solution with a maximum absorbance at 490 nm. The concentration of starch was determined using a standard curve obtained via a spectrophotometer. Enzymatic hydrolysis of the samples was performed using alpha-amylase and amyloglucosidase enzymes (0.01 – 0.2% w/w Liquozyme L 240 KNU/g Novozyme & 0.046–0.066% Spirizyme Fuel 750 AUG/g Novozyme Respectively). The sample was solubilized with water to a concentration of 67 grams per liter, then gelatinized at 71°C for 30 minutes. The fermentation of the hydrolyzed starch was carried out for 48 hours using *Saccharomyces cerevisiae* at 32 °C to produce bioethanol. The pH was maintained between 3.8 and 4.5. The glucose and ethanol levels were quantified using High Performance Liquid Chromatography.

The results from the first study, as shown in the table below, indicate that the pH of the broth decreased daily until the fermentation experiment was completed. Decreasing pH indicates increasing acidity which is optimal for the activity of the yeast used. The specific gravity and brix level, which is a measure of the amount of sugar present in a medium, of the broth also decreased as shown on table 1. That is, the sugar levels decreased as the yeast utilized them to produce bioethanol. The specific gravity of the broth decreased with an increase in alcoholic content from 0 to 12.9% of the fermenting broth.

Table 1: Percent alcohol, specific gravity, pH and brix level as a function of fermentation period

Period of Fermentation (days)	pH values (Mean ± SD)	Brix Level (TSS)	Specific Gravity	% alcohol produced
1	4.50 ± 0.2	15.0 ± 0.2	1.0000 ± 0.1	0.00
2	4.22 ± 0.2	7.5 ± 0.4	0.9991 ± 0.1	0.60 ± 0.1
3	4.18 ± 0.1	6.0 ± 0.3	0.9963 ± 0.3	2.50 ± 0.1
4	4.10 ± 0.2	4.2 ± 0.2	0.9955 ± 0.1	3.00 ± 0.2
5	4.06 ± 0.2	2.5 ± 0.1	0.9901 ± 0.2	7.00 ± 0.1
6	4.00 ± 0.4	2.1 ± 0.2	0.9850 ± 0.1	11.10 ± 0.2
7	3.82 ± 0.1	2.0 ± 0.1	0.9830 ± 0.3	12.90 ± 0.3

Screening results of the different fungal isolates from the **second study**, indicate that Org 2 (6.019 mg/mL) had the highest reducing sugar potential followed by Org 14 (5.728 mg/mL), and Org 11 (1.990 mg/mL) had the least potential to degrade starch from *C. bicolor*. Org 2, which was identified to be *Aspergillus sp.*, was selected for the second part of the experiment. Graphs plotted from the experimental results revealed that the maximum reducing sugar production occurred on day 6 (144 hours): there was a continuous decrease in residual starch concentration and an initial increase in the concentration of released reducing sugar. Varying the initial pH revealed that optimum reducing sugar production from the substrate was obtained at pH 5. The different substrate concentrations used ranged from 1%-5% w/v, and the experiment revealed that an increase in substrate concentration results in increase in reducing sugar concentration with 5% w/v substrate concentration giving the highest reducing sugar yield. For varying nitrogen sources, soya bean gave the highest glucose yield. Results of varying inoculum size revealed that inoculum size 2%, gave a maximum reducing sugar yield from *C. bicolor* tuber flour. The final experiment involves enzymatic hydrolysis of *Caladium bicolor* tuber flour using optimal conditions; pH 5, 5% substrate concentration, 0.5% soya bean as nitrogen source and 2% inoculum concentration revealed that the maximum reducing sugar yield occurred on Day 5 (6.989mg/mL). On inoculation with 2% *Saccharomyces sp.* (Org 19), ethanol was produced. A maximum ethanol yield of 0.485% was recorded on day 5 (120 hours).

In the third study, results for the mashed samples without exogenous enzyme supplementation indicated that the concentration of hydrolyzed glucose increases with a rise in temperature and increase in time. This may be attributed to increase in activity of the enzymes present in the substrate at higher temperatures. The highest concentration of up to 22.3g/100g was obtained in the samples mashed for 90 minutes at 80°C, while the lowest concentration of 9.7g/100g was recorded in samples mashed for 60 minutes at 50˚C. Three different batches of the enzyme cocktail treatments at different temperatures and time were used in order to optimize the most appropriate conditions for the hydrolysis of cocoyam starch to reducing sugars. The samples mashed with different enzyme supplementations named batches 1, 2 and 3 record the highest concentration of hydrolyzed glucose to be 86.8g/100g when the sample was mashed with batch 1 enzyme cocktail. This value was significantly higher than the values obtained from batch 2 and 3 (75.7g/100g and 76.2g/100g) respectively.

In addition, batch 1 liberated this highest glucose value at a lower temperature compared to the other 2 batches. Comparing these two experiments, it can be concluded that in order to attain optimal starch to glucose hydrolysis, exogenous enzymes supplementation is required; compare a maximum of 22.3g/100g to 86.8g/100g for non-exogenous enzymes and exogenous enzymes supplement respectively.

The concentration of Free Amino Nitrogen (FAN) was also monitored during the fermentation process. FAN serves as nitrogen content and is required for efficient yeast metabolism during fermentation. It affects the growth of yeast and subsequently, bioethanol production. The function of protease in the enzyme cocktail was to hydrolyze complex proteins present in the cocoyam to simple proteins or peptides for easy assimilation by the yeast. The concentration of FAN released by the hydrolysis process was high when analyzed and results showed that there was a drastic fall in this concentration after fermentation. In SSF, for example, the initial FAN concentration was 258.5mg/L and the final was 67.1mgL. It can be inferred from these results that no exogenous nitrogen source is required for effective bioconversion of cocoyam to bioethanol.

Following the hydrolysis of the biomass, the samples subjected to fermentation were considered for the two different fermentation protocols. The results obtained with Simultaneous Saccharification and Fermentation (SSF) had a significantly higher value of 398 L/ton, while Separate Hydrolysis and Fermentation (SHF) yielded 180.5 L/ton. This difference in the yield may be attributed to the fact that the SSF process has the advantage of continuous release of free sugar and subsequent fermentation while the SHF method released all free glucose before the commencement of fermentation. The presence of other required nutrients in the residue may have also contributed to effective fermentation of most sugars to bioethanol in SSF. The higher investment costs for two separate reactors for both hydrolysis and fermentation will make the SHF process more expensive compared to SSF. From the experiment, it was found that *Saccharomyces cerevisiae* produced bioethanol levels equating to 398 L/ton which compares favorably with yields from cassava 280 L/ton and corn 420 L/ton.

Results from another separate investigation revealed that the starch content of the harvested cocoyam tuber was 0.22 ± 0.017 grams of starch per gram of sample. For the starch enzymatic hydrolysis using S*accharomyces cerevisiae***,** a sample of a common starchy tuber (yucca – *Manihot esculenta*) was hydrolyzed and its glucose yield value was compared to that of a sample of *Xanthosoma sagittifolium*. The results are show in the table 2 below and corroborate the potential that cocoyam has for the harvest of fermentable sugars.

Table 2: Starch content per gram of sample

Sample	Glucose concentration [g/L]	Obtained yield [g Glucose/g Sample]
Cocoyam	19.24 ± 0.53	0.282
Yucca	23.76	0.335

3. PRODUCTION OF BIOETHANOL FROM WASTE CASSAVA PEELS.

Cassava is a major crop rich in starch and often employed for synthesis of starch and related products. During this process, large amounts of solid wastes called cassava pulps and peels are often produced. The main components include 60% starch and 30% cellulose fiber. Saccarification and ethanol fermentation is attempted on the cassava pulps and peels with arming yeast, some namely, a co-displaying α-amylase (α-AM), glucoamylase, endoglucanase, cellobiohydrase, and β -glucosidase. The novel yeast strain, possessing the activities of all enzymes, was able to produce ethanol directly from soluble starch, barley β -glucan, and acid treated Avicel. During fermentation, concentration of ethanol rises significantly while that of starch and cellulose fiber decreases.

A single step in bioethanol production under this category is the combination of raw cassava starch hydrolysis and fermentation. For the development of raw starch consolidated bioprocessing technologies, this method investigated optimum conditions and technical procedures to produce bioethanol from raw cassava starch in a single step. It successfully resulted in high yields and productivities of all the experiments from the laboratory, the pilot scale and the industrial scale-up. Yields of bioethanol were comparable with those in the commercial industries that use molasses (to be discussed on section 4.0) and hydrolyzed starch as the raw materials.

In this study, cassava peel wastes were used as a sole carbon source for bioethanol production using the process of fermentation and co-culture techniques. Production of bioethanol from cassava peels was examined using co-culture of Aspergillus niger and saccharomyces cerevisae.

Sulfuric acid solution with concentration of 2 %, 6 % and 10 %, was used to hydrolyze the substrates. Aspergillus niger and Saccharomyces cerevisiae were further used to ferment the substrates at 28 ^{o}C for 4 days. The fermented liquid was distilled at 78 ^{o} C and quantity of bioethanol produced was determined. These findings proved that 10 % H_2SO_4 concentrated acid pretreated sample resulted into maximum ethanol yield at pH 4.55, sugar content (15.5 %) and alcohol content (8.5 %) after 4 days. The study further revealed that bioethanol can be produced from cassava peels with maximum yield obtained using 10 % sulphuric acid for hydrolysis and Aspergillus niger and Saccharomyces cerevisiae for fermentation.

Bioenergy from renewable resources becomes an alternative replacing or supplement for fossil fuels. Bioethanol is one of prominent bioenergy which has some advantages such as high octane rating, and reduced pollution. It is a clean and renewable biofuel with major environment benefit, burning of the oxygenated fuel mixture results in more completely burnt fuel and thus, reduced emissions.

Bioethanol is obtained via a biochemical process of converting simple sugar into ethanol and carbon dioxide (CO_2) (Adrade *et al.*, 2004). It is a principal fuel that can be used as petrol substitute for vehicle (Pakula & Pentella 2005). Although it can also be manufactured by the chemical process of reacting ethylene with steam (Kroumov et al., 2006). The main sources of sugar required to produce bioethanol come from crops such as maize, cassava and cassava products, wheat crops, waste straw, guinea corn husk, rice husk, millet husk, sawdust, sorghum plant, sugar cane and sweet potato etc. (Kim & Dele, 2005; Balat et al., 2008). Sugar cane, as a raw material, is used for 60% of global ethanol production, while 40% of global production of ethanol comes from other crops. Cassavas are the main raw material of ethanol production in Nigeria whereas in Brazil, sugar cane is the major source (Nasidi et al., 2010). Saccharomyces cerevisiae, Zymomonas mobilis, Aspergilus niger and Schizo saccharomycespombe are microorganisms able to convert sugars to ethanol (Sean & Johann 2015).

The budding yeast Saccharomyces cerevisiae is used extensively for production of ethanol in the beverage industry. However, this yeast cannot fully utilize starchy and cellulosic materials to produce ethanol. The glucoamylase (AMG) from Rhizopus oryzae is an exo-type amylolytic enzyme that effectively severs both α-1,4-linked and α-1,6-linked glucose from starch. A flocculent yeast strain displaying two kinds of amylases which can convert soluble starch to ethanol under oxygen-limited conditions (Kondo et Al 2002).

The production method involves manufactured active dry yeast, and Saccharomyces cerevisiae, obtained from AB Mauri (Australia), used for this study. This yeast strain could produce the maximum ethanol yield exceeding 18% (v/v) or 12% (w/v) depending on fermentation procedures. Furthermore, it is extremely thermo-tolerant, and it has a wide range of fermentation temperatures from 25 to 40 °C. The active dry yeast inoculants were re-hydrated in distilled water at 40 °C for 20 min prior to inoculations into the single-step ethanol fermentations.

Typical laboratory instrument and chemicals required include weighing balance, conical flasks, beakers, measuring cylinder, spatula, funnels, filter paper, cotton wool, and aluminum foil, water bath, autoclave, distillation set-up, pH meter, refractometer. Sodium hydroxide (NaOH), sulfuric acid (H_2 SO_4), potassium dichromate ($K_2Cr_2O_7$), and ammonia.

3.1Samples Collection:

Cassava peels (CP) were collected from domestic wastes dump site located at Kawo area, Kaduna state, Nigeria. The samples were aseptically collected into a polythene bag and used for further analysis.

The cassava peels samples were washed thoroughly with distilled water to eliminate adhering soil and dust. The peels were sun dried and then grounded into powdered form using pestle and mortar. The grounded powder was then sieved through a 1mm screen to standardize the particle size range of 1mm. The sample was kept in a tightly close container at room temperature. The organisms used were Aspergilus niger and Saccharomyces cerevisiae and were collected from stock cultures of Microbiology Laboratory. The cultures were characterized and confirmed using morphological and biochemical methods described by (Holts et al., 2009; Oyeleke et al., 2012).

3.2 Preparation of cultures

The yeast strains used were Saccharomyces cerevisiae isolated from palm wine and commercial Baker's Yeast. The organisms were propagated (incubation conditions: 30 ^{o}C for 72 h) on potato dextrose agar medium (Oxoid, UK) to which 0.14 g/l streptomycin sulphate for inhibition of bacterial growth. Recovery of the yeasts was confirmed by the methods of Collins and Lynes (1989). The yeast cultures were transferred into broth medium and incubated on a shaker for 24 h at 28 ^{o}C after which they were ready to be used as inoculums in the cassava hydrolysates.

3.3 Different Methods to produce Bioethanol from Cassava pulp are as Follow: Starch extraction Starch extraction was by the method of Oyewole and Obieze (1995). Fifty (50) kg of cassava roots were peeled, washed in water, and grated with a commercial mechanical grater. The resultant pulp 116 was immediately sieved through a screen (25 mesh) and suspended in water. This separates the fibrous and other coarse root materials from the starch pulp. The starch pulp milk could sediment for 4 – 6 h before decanting the supernatant. The resultant thick starch cake at the bottom of the bowl was then pressed to remove the remaining water. The bright white coloured starch cake obtained was sun-dried for 72 h. The Cassava pulp was obtained, dried, and prepared at 60 °C for 3 days and then crushed with a 0.5 mm mesh screen.

Next, the cassava pulp (0.15 g) was re-suspended in H_2O and pretreated at 150 °C for 1 h. After pretreatment, the cassava pulp was used as the carbon source for fermentation medium. The fermentation medium contained the same components as the synthetic medium (SDC medium) except for glucose. After pre-cultivation in the SDC medium for 48 h, yeast cells were cultivated under aerobic conditions at 30 °C for 72 h in 50 ml of SDC medium with shaking at 100 rpm, collected by centrifugation for 5 min at 4,000 × g at 4 °C, and washed twice with distilled water. The ethanol fermentation was carried out at 30 °C under oxygen-limited conditions in butyl rubber-stoppered glass tubes with shaking at 100 rpm. The cassava fiber remaining in the cultivating medium was collected by centrifugation at 4,000 × g for 5 min and washed four times with hot distilled water. The insoluble sugar concentrations were detected by the phenol–sulfate method and determined by subtracting the yeast cell-derived sugar from the culture medium containing the yeast cells and cassava pulp. In the reference fermentations without addition of a carbon source and with 30, 33, and 50 g of yeast cell pellets and 0.5, 0.55, and 0.83 g, respectively, of ethanol was produced in 12 h probably from glucose carried over from SDC medium. Ethanol productivity is determined from the initial rate during the first 12 h of fermentation.

Production of bioethanol from cassava starch

In a separate investigation bioethanol was produced from cassava starch by Anyakorah et al. (1998). The steps involved in the production were: Gelatinization (50 kg cassava starch was stirred in 200 ml water at 80 ^{o}C until smooth gel was formed); Liquefaction (4 ml of α-amylase was added to gelatinized starch at 80 ^{o} C and incubated for 60 min); Saccharification (4 ml of aqueous solution of amyloglucosidase was added to liquefied starch.

Physico-chemical properties of samples: Moisture, ash and fibre contents of cassava starch samples determined according to (AOAC, 1990) including total titratable acidity (TTA) determined using 10 g of sample homogenized with 90 ml distilled water and expressed as the amount (ml) of 0.1 M NaOH to obtain a pH of 8.3. The pH of samples was determined with a combined glass electrode and a pH meter (Mettler-Toledo, Essex M3509 Type 340).

Total soluble sugars: The refractometer was employed to determine the percentage of total soluble sugar solids of the cassava hydrolysate after hydrolysis. This was carried out by placing a drop of cassava hydrolysate on the graduated hand refractometer glass slide and expressing the brix reading in percentage.

Dextrose equivalent analysis: The action of the enzymes on starch sample was determined by measuring the reducing sugar (calculated as dextrose equivalent) produced using the spectrophotometric (Spectonic 20D model) method of Bernfeld (1959).

Brix determination: The brix (%) was deter-mined using a hand refractometer according to AOAC (1990).

Determination of specific gravity of the filtrate: The brix table was used to determine the specific gravity of the filtrate according to AOAC (2000).

Yeast cell growth: The yeast growth determination was carried out using spectro-photometer by the method of Olasupo et al. (1996).

Total reducing sugar determination: The total reducing sugar determination was carried out using spectrophotometer (Spectonic 20D model) at a wavelength of 540 nm against dinitrosalicylic acid reagent with concentrations of glucose as standard curve (Miller, 1959).

Determination of sugar consumption: The amounts of sugar consumed were determined after fermentation by AOAC (1990). The initial amounts of sugar before fermentation and sugar concentration after fermentation were recorded and used to calculate the amounts of sugar consumed.

Ethanol yield determination: The ethanol yield was estimated according to AOAC (1990) by calculation using the formula: Ethanol yield =Ethanol produced /Sugar consumed x 100

pH Test

pH meter was first calibrated and was inserted separately into each of the filtrate. The readings were then taken as described by (Ademiluy et al., 2013).

Fermentation efficiency determination: The fermentation efficiency was determined using AOAC (1990) method and the data obtained from the sugar consumed and the initial sugar x 100%: Fermentation efficiency = Sugar consumed/ Initial sugar x 100

Sugar conversion efficiency: The ability of yeast to produce ethanol from available sugar is expressed as sugar conversion efficiency and it was determined by the method of De Macilla and Pearson (1984) and calculated using the conversion efficiency factor of 0.504.

Determination of the percentage alcohol of the filtrate: The brix table was used to determine the percent alcohol of the filtrate according to AOAC (2000).

FTIR analysis of treated and raw samples

Evaluation of chemical and structural changes that occurred with the different treatment was carried out on a scientific FTIR model 530, NARICT Zaria. 2mg of the untreated and treated samples was mixed with 250mg of dried KBr, pressed to pellets and scanned over the range 4000-600 cm^{-1} spectral resolution. The spectra (from KBr pellets) were used to evaluate the structural changes that occurred with different treatment (Himmelsbach et al., 2002; Pavia et al., 2005).

Confirmatory Test for Bioethanol Produced: Confirmatory test was carried out on the extracted bioethanol sample using potassium dichromate test as indicated by Caputi et al. (1959). 5 mL of the distillate sample was taken, and 2 drops of potassium dichromate was added into the distillate, heated in a water bath for 30 minutes.

Macroscopic and Microscopic Observation of Aspergillus niger

The macroscopic and microscopic identification of the organism was based on colony pigmentation and the structure of the conidial head as described by (Verweji et al., 2007). Also, Verweji et al. (2007) reported that colonies of A. niger are carbon black with a dark globular conidial head. Aspergillus niger strain was found to have good amylase production potential, which is important in the hydrolysis of starch, this agreed with the work of (Omemu et al., 2005) who reported that A. niger can be used for industrial production of ethanol, citric acid and gluconic acid because of its hydrolytic capacities in amylase production and its ability to have high tolerance to acidity thereby enabling it to prevent bacterial contamination. While

Saccharomyces cerevisiae, on the other hand is a yeast that is capable of withstanding stressful conditions and have high fermentation efficiency, effective sugar used, tolerance to high ethanol concentration, which are fundamental for industrial used (Andrietta et al., 2007).

Substrate Fermentation for bioethanol Production: Aspergillus niger and Saccharomyces cerevisiae were used to carry out fermentation of cassava peels at 28 ^{o}C, pH 4.5 and 20 g substrate for four days. From the results obtained, there was a gradual increase in ethanol yield because of higher concentration of sulfuric acid but a decrease in yield was observed with lower concentration of sulphuric acid indicating that under the condition of temperature (28 ^{o} C), pH (4.5) and substrate concentration (20 g); the maximum ethanol yield was obtained with 10 % H_2 SO_4, followed by 6 % and 2 % sulphuric acid treatment as shown in table 3.

Novelty Journals

ISSN 2394-9651

International Journal of Novel Research in Physics Chemistry & Mathematics
Vol. 10, Issue 3, pp: (1-19), Month: September - December 2023, Available at: www.noveltyjournals.com

Table 3: showing quantity of ethanol produced, sugar content, specific gravity, and percent alcohol of the filtrate

Serial no	Samples	Quantity of ethanol (g/ml)	Sugar content (%)	Specific Gravity	Percent Alcohol (%)
1	20 g CP + distilled H20	20.40	4.0	1.0166	2.0
2	20 g CP + 2% H2SO 4	23.45	8.3	1.0330	4.3
3	20 g CP + 6% H2SO 4	29.80	11.6	1.0467	6.1
4	20 g CP + 10% H2SO 4	37.35	15.5	1.0633	8.5

Pretreatment and hydrolysis of cassava peel sample: Cassava peel is one of lignocellulosic material, mainly contains carbohydrate such as cellulose and hemicellulose, and non-carbohydrate material lignin. Lignin should be reduced before fermentation of hydrolysate is carried out. Pretreatment of cassava peel was conducted by physical method which is autoclaving 20% cassava peel in water mixture at 121^{o}C for 1 hour. This physical method was chosen because it is easier and make pH alteration significantly.

Pretreated cassava peel was hydrolyzed using 1% sulfuric acid to produce a reducing sugar, such as glucose. In this experiment, hydrolysis was conducted in variation time 30, 45 and 60 minutes which produce different results. The highest reducing sugars was produced in 60 min hydrolysis time, (11.189%) compared with 30- and 45-min hydrolysis time, and the latter producing the least amount. The result of the hydrolysis process of cassava peel was prepared as many as 12 samples to be fermented at room temperature and pH 5. Bioethanol from each sample in the fermentation process was collected at the 2nd, 4th, 6th, and 8th day each sample. Results showed that the optimum time of hydrolysis process was 60 min. Determination of glucose from hydrolysis process was done by DNS method. Principle of this method was reducing sugars have the property to reduce many of the reagents during fermentation process by using durian isolate yeast which could convert glucose to bioethanol.

Composition of cassava hydrolysates from two cassava varieties

Cassava starch was hydrolyzed with α-amylase and amylo-glucosidase to yield cassava hydrolysate. Table 2 shows the analysis of the hydrolysates obtained from the two cassava varieties. The average yields of cassava hydrolysate for each sample at different enzyme concentrations were 66% for TMS 30572 and 66.3% for Idileru. The per cent total sugar soluble solid for the hydrolysates were 26% and 25% respectively. The pH of the hydrolysates from both varieties was 7.

Sugar conversion and ethanol production from cassava

The amounts of reducing sugars in the hydrolysates of TMS 30572 and Idileru during fermentation were monitored. There was a gradual and consistent decrease in per cent brix for both hydrolysates. Although the amounts of sugars consumed during fermentation by baker's yeast in both cassava varieties were higher, the ethanol conversion values and amounts of ethanol produced were lower. S. cerevisiae from palm wine was able to produce more ethanol from the two varieties more than the baker's yeast and TMS 30572 utilized more substrate for ethanol conversion during the fermentation more than Idileru variety.

The two yeast species used in this study were able to convert fermentable sugars into ethanol effectively. Ameh and (Okagbue *et al* 1987) and (Ezeogu and Emeruwa *et al.*, 1993) had observed that yeasts isolated from natural sources such as palm wine possess very high levels of ethanol and sucrose tolerance and may grow well in various substrates.

International Journal of Novel Research in Physics Chemistry & Mathematics
Vol. 10, Issue 3, pp: (1-19), Month: September - December 2023, Available at: www.noveltyjournals.com

4. PRODUCTION OF ETHANOL FROM WASTE SUGARCANE MOLASSES

This section aims to review the conversion of agro-industrial wastes such as sugarcane molasses to bioethanol. Sugarcane has received a lot of attention in recent years due to its availability and usefulness for production of bioethanol and because of the high sugar content it contains making it more useful for fermentation and other process. Bioethanol can be produced from various sources. Some plants containing cellulosic material can be used for bioethanol production. There are various plants utilizing sugarcane molasses as feedstocks to produce bioethanol. There has been worldwide research to produce ethanol from regional inexpensive substrates. A gram negative, facultative anaerobic, rod shaped strain'' Zymomonas mobilis'' was used as microorganism to produce bioethanol from sugar cane molasses using anaerobic fermentation. The review was conducted to investigate the optimized conditions for production of bioethanol through batch fermentation process. The fermentation unit was designed to determine the effect of process parameters such as fermentation temperature, pH, sugar concentration and supply of nutrients.

Ethanol (C_2H_6O) is a simple chemical compound known as simple alcohol. Ethanol is widely known for its use in intoxicating alcoholic beverages like beer, wine, or whiskey. Apart from being a good intoxicating agent, bioethanol (Wu et al., (2014)) has many other advantages such as being a common substance in most of the pharma and chemical synthesis industries to produce chemicals and medicines. It is also an active ingredient in preparation of high calorific value fuels like gasoline (Hansen et al., (2005). Furthermore, it has been widely used for making sanitizer and disinfectants to fight against harmful microorganism and pathogens (Assefa *et al.*, 2020; Thomson *et al.*, 2020). Among the biofuels, bioethanol is seeking audience day by day and widely produced as an important transportation fuel worldwide. Bioethanol can be used as substitute for gasoline in existing engines, or it can be blended, producing low emissions of greenhouse gases. Rubbing ethyl alcohol is also widely being used for its several applications. There are four kind of alcohol they include: pure ethanol, denatured ethanol, isopropyl alcohol and rubbing alcohol.

Sugarcane molasses is a dark viscous fluid, and rich in nutrients required by most microorganisms such as carbon, nitrogen, phosphorus, sodium, potassium, and non-nitrogenous compounds. They are highly demanded in industrial application (Mc Cambridge *et al.*, 2019) as well as in domestic applications (Adrian, M., & Ferguson, 1987; Market. *Et al.*, 2020). The utilization of sugar cane molasses for fermentation process is one of the oldest chemical process known to human. This practice is also widely used for bioethanol production. To produce ethanol from sugar cane molasses, urea was used as nitrogen source and added at different concentrations 0.15%, 0.5%, and 0.25% (w/v). Some experimental investigations conducted using four different sugar cane molasses concentration which was calculated as percentages of 10, 15, 20 and 25 (w/v). The pH of the mash was adjusted to 4.8 using concentrated sulphuric acid and 5% (w/v) baker's yeast was added. The fermentation process was conducted for 72 hours at 33 °C. During this process of bioethanol production, yeast is the primary choice for fermentation (Chandra & Panchal, 2003). Yeast is the most extensively used microorganisms for fuel ethanol fermentations due to their various uniqueness like high growth rates (anaerobically or aerobically), and capability to tolerate various stresses (Piskur et al., 2006).

Furthermore, it's found that if the source of carbohydrate is not simple it takes longer time for the fermentation to take place. To reduce the time, there is the need to apply enzyme or to carry out extra procedure to bring the carbohydrate source into a simpler form. Such simple carbohydrate is a good feed for microbe to consume and this may impact negatively on production costs. Therefore, selecting Sugarcane molasses as the raw material may act as a good alternative (Wu et al., 2020; Khoja et al., 2015). The Beet molasses is also a commonly used feedstock (Dodić *et al.*, 2009).

There are advantages in selecting sugarcane molasses which include: sugarcane molasses being a bye product of sugar (food sweeteners) refining, it is relatively inexpensive compared to the cost of other sources. Secondly it is available throughout the year therefore, there is no shortage of supply. Molasses is a bye products of sugar industry which can be utilized for bioethanol production, and as a result it contributes a huge flux in transportation sector as well as in energy sector. In today's automotive world where transportation is the necessity of life, fuel play an important role in the transportation industries. But since there is an increase in demand of automobile fuel the natural sources of fuel are being used up. Therefore, it is necessary to seek alternative for petroleum fuel before it's too late. Bioethanol is found to be very much efficient in running automobile after successful adjustment in its content (Zhang et al., 2020; Anderson et al., 2020; Li et al., 2020).

During fermentation of molasses different types of yeast can be used for the fermentation process and they include:

1. **Kluyeromyces Marxian**: This yeast has lot of importance regarding ethanol production since it increases the production of ethanol from molasses or any sugar content raw material significantly.

2. **Saccharomyces cerevisae**: is also capable of converting sugary material or carbohydrates into ethanol and increases the ethanol production and other fermentation process.

3. **Zymomonas mobilis** is not a common microorganism regarding production of ethanol from molasses, but it can be used to produce bioethanol than other type of yeast available. Zymomonas mobilis has many advantages due to its microbial activity as it improves the yield of ethanol than other yeast.

The focus of this section is to review alternative ways of improving bioethanol production from sugarcane molasses in batch fermentation process using Zymomonas mobilis. This yeast was chosen over other yeast strains because it has strength to uphold high content of sugar and tolerate higher temperature up to 40 °C.

Material requirements include Sugarcane molasses, H_2SO_4, Urea, yeast substrate, electronic balance, pH meter, Spectrophotometer heating mantle, autoclave and shaking orbital incubator

The production of bioethanol from molasses was described by Chandra & Panchal, 2003.

A bacto-peptone of 10 g/L, yeast extract 10 g/L, 15 g/L, glucose 20 g/L, distilled water of about 1000 mL was being cultured in a media of pH 7.00. The Media was autoclaved at 121°C for 45 minutes. The 250 mL molasses was measured into a conical flask for pre-fermentation with the sugar concentration of 7g/100 mL and inoculated with 5mL growth media.

The known amount of sugar cane molasses and grown media were mixed in fermenter and kept in shaker for anaerobic fermentation condition and kept for 48 h to investigate the effect of sugar concentration, pH, fermentation temperature, and supply of nutrients.

5 mL of fermented sample was taken and pinch of potassium dichromate and few drops of concentrated H_2SO_4 were added. The brownish color of sample was changed into green that indicated the presence of bioethanol.

Effect of pH

The samples were fermented using Zymomonas mobilis in different pH ranges from 4.0 to 6.0 to obtain the maximum yield of bioethanol by adding diluted sulphuric acid. The sugar concentration and temperature were kept constant. Anaerobic conditions were applied, and the fermented samples were analyzed after 48 hours. The maximum yield 7.9% (v/v) was achieved on pH 5.0 and fermentation efficiency was recorded 88%. The pH higher than 5.4 recorded a smaller amount of yield as shown on the table 4 below.

Table 4: Effect of pH on fermentation process using *Zymomonas mobilis* (ZM 424)

pH	Sugar Conc. (g/100 mL)	Fermentation temperature (°C)	Acidity	Ethanol yield (v/v) %	FE %
4	14	33	8.1	4.4	49
4.2	14	33	6.3	6.5	72
4.4	14	33	5.2	6.8	75
4.6	14	33	4.9	7.1	79
4.8	14	33	4.2	7.7	85
5	14	33	4	7.9	88
5.2	14	33	6.5	6.2	69
5.4	14	33	7.5	4.7	52
5.6	14	33	6.3	2.2	24
5.8	14	33	13.8	0.0	0

Effect of fermentation temperature

The samples were maintained in the optimum pH 5.0 for Zymomonas mobilis with the fermentation temperature ranging from 28 °C, 30 °C, 32 °C, 34 °C, 36 °C and 38 °C. The samples were kept for fermentation for about 48 h, after analyzing

the samples it was found that the optimum yield for bioethanol was achieved at 34 °C. At that temperature bioethanol yield was 8 % (v/v) along with the fermentation efficiency of 88.96 % The increase in temperature increased bioethanol yield till 34°C, further increase in temperature led to decrease in yield with increasing fermentation temperature (see table 5). The values may be varying because during fermentation the temperature increases due to exothermic reaction.

Table 5: Effect of the fermentation temperature on bacterial fermentation using *Zymomonas mobilis* (ZSM 424)

Fermentation Temperature (°C)	pH	Sugar Conc. (g/100 mL)	Acidity	EtoH yield % (v/v)	FE %
28	5	14	7.4	3.1	34.5
30	5	14	5.2	5.2	58
32	5	14	4.4	7.6	84.5
34	5	14	4.1	7.9	87.6
36	5	14	6.7	5.8	64.5
38	5	14	8.1	2.2	25

Effect of sugar concentration

Sugar molasses concentration was set from 10g/100 mL to 18g/100 mL by keeping the pH and temperature constant. Increasing the sugar concentration affected bioethanol production linearly and optimum production was attained at 16g/100 mL with 8.9 % (v/v) with the fermentation efficiency (FE) of 87 %, after this, the increase in concentration yielded lesser quantity.

Table 6: Effect of the sugar concentration on bacterial fermentation of *Zymomonas mobilis*

Sugar Conc. (g/100 mL)	Density of molasses	pH	Fermentation Temperature °C	Acidity	EtoH yield % (v/v)	FE %
10	1.079	5	33	6.2	4.70	73
12	1.097	5	33	5.9	6.2	80.4
14	1.116	5	33	5.2	7.8	86.7
16	1.135	5	33	4.4	8.9	87
18	1.155	5	33	9.4	5.5	50

Effect of nutrient

The effect of different nutrients in an anaerobic fermentation was investigated. Nutrients are quite good in the production of bioethanol from sugar cane molasses. The contribution of the Diammonium phosphate (DAP) and urea in the process were 1:1. Different nutrients can be supplied for the fermentation process, the most effective nutrients found are DAP and Urea. The yield increases with the addition of the nutrients in the fermentation process with a notable efficiency as shown on table 6. It was observed that the 2g/L dose was found as the best quantity to get the optimum yield of 9.3 % (v/v) with the fermentation efficiency 92.5%. The addition of nutrients was effective for higher sugar concentrations, and not more effective in low concentration of sugar.

Table 6: Effect of the nutrients (DAP) in the fermentation using *Zymomonas mobilis*

Nutrients (DAP) (g/L)	pH	Sugar Conc. (g/100 mL)	Fermentation Temperature °C	Acidity	EtoH yield % (v/v)	FE %
1	5	16	33	4.4	8.9	86.6
2	5	16	33	4.1	9.3	92.5
3	5	16	33	4.2	9.1	88.6

5. CONCLUSION

The use of cassava peel, wild cocoyam, and waste sugar cane molasses for bioethanol production as an alternative source of fuel provides a starting point for improvements in cultivation and adoption of these renewable agro-products as well as improving food security. Despite the ability to use cassava peels for ethanol production, the yield can be influenced by

several factors especially temperature, pH, time, and substrate concentration. The maximum ethanol yield of 8.5% was obtained after 96 h of fermentation using co-culture of Aspergillus niger and Saccharomyces cerevisiae carried out at 28 ^{o} C, pH 4.55 and 10 % substrate concentration. Saccharomyces cerevisiae strain from palm wine showed great potential for ethanol production from cassava, although more research is required to improve the efficiency of the process.

Similarly, bioethanol can be produced from the starch present in wild cocoyam due to the action of yeast cells like *Saccharomyces cerevisiae*, *Saccharomyces uvarum* and *Saccharomyces sp*. Initial gelatinization and grinding of the different cocoyam cultivars increased the surface area for enzyme hydrolysis and subsequent activities by microorganisms. The yeast strain used in the fermentation processes required an acidic medium which is favorable to break down sugar effectively and to produce bioethanol of an acceptable and wholesome quality. At the initial stage of the fermentation process, the yeast cells are dormant – consuming glucose available for their energy and development in the lag phase. Subsequently, their depletion becomes rapid, and the rate of carbon dioxide evolution is vigorous corresponding to an increase in alcohol production. This phase is characterized by rapid cell multiplication which is indicated by rapid fermentation. Eventually, the fermentation rate decreases as the sugar substrate present in the broth begins to decrease in concentration.

The high carbohydrate content of cocoyam indicates its promising potential as a very good source of starch for bioconversion to ethanol. Its ethanol yield is higher when compared to most of the already established feedstocks. The research efforts in this section of our review study opens a gateway towards the development of an efficient method for bioethanol production using different cocoyam species as a cheap source of raw material. In Colombia where starch material is not currently used for industrial applications or as human food, the use of cocoyam as raw material for ethanol production would not affect food security and presents a whole new opportunity for biofuel production. The cocoyam species, *Caladium bicolor*, which is unsuitable as food for humans has great potential as renewable raw material for the bioethanol industry. It is necessary to perform further research of this process, in terms of scale-up production. It should be noted that previous research reported loss of over 20 million tons of cocoyam lost yearly in Nigeria alone, due to inadequate storage facilities – cocoyam becomes perishable after harvesting. Hence, speedy conversion of the surplus harvest to alcohol will reduce wastage and improve economic gains.

Finally, the waste sugarcane molasses, a bye product of sugar refining, is a suitable feedstock for batch fermentation using Zymomonas mobilis in an anaerobic condition. The effect of different parameters such as pH, sugar concentration, temperature and nutrients supply have significant effect on bioethanol yield and efficiency. The parameters that yielded the highest bioethanol of 9.3% (v/v) and efficiency of 92.5% (v/v) in this study were 16g/100 mL sugar concentration, 2 g nutrients, pH 5.0 at fermentation temperature of 34°C

REFERENCES

[1] Agba, A.M. et al. (2010). "Developing the Biofuel Industry for Effective Rural Transformation in Nigeria." *European Journal of Scientific Research*, vol 40, pp 441–449.

[2] Aisien F.A., Faleye O. and Aisien, E.T. (2010). "Phytoremediation of heavy metals in aqueous solutions." *Leonardo Journal of Sciences,* vol 17, pp 37-46.

[3] Bamikole, M.A. and Babayemi, O.J. (2008). "Chemical Composition and *in sacco* dry matter Degradability of Residue and by-products of Palm Fruit Processing in the Rumen of Steers." *Animal Science Journal*, vol 79, pp 314-321.

[4] Baum, S.J. (1982). Introduction to Organic and Biological Chemistry. 3rd Edition, Macmillan Publishing Co. Inc, New York, USA, pp. 105-106, 294-296.

[5] Dubey, R.C. (2005). A Textbook of Biotechnology. 3rd Edition, S. Chand & Co., New Delhi, India, pp. 264-266, 339-354.

[6] Galbe, M. and Zacchi G. (2002). "A Review of the Production of Ethanol from Softwood." Applied Microbiology and Biotechnology, vol 59, pp 618-628.

[7] Giacometti, D., and León J. (1992). "La Agricultura Amazónica Caribeña in Cultivos Marginados: Otra Perspectiva de 1492." FAO Corporate Document Repository, pp 191-333. (in Spanish)

[8] Gómez, M.E. (2002) "Guía para El Cultivo y Aprovechamiento del bore Alocasia Macrorrhiza (Linneo) Schott" // Ed. Convenio Andrés Bello, pp 43.

[9] Hodgkin, T., Williams, J.T., Haq, N. (2002). "Managing Plant Genetic Diversity." Cabi Publishers.

[10] International Institute of Tropical Agriculture, IITA. (2009). Factors limiting Cocoyam Cultivation and Processing in Developing Countries. A paper presented at a workshop by Prof. Olumide Adebayo. IITA Ibadan, Nigeria.

[11] Jaleel S. A., Srikanta, S., Ghildyal, N. P. and Lonsane, B. K. (1988). "Simultaneous Solid Phase Fermentation and Saccharification of Cassava Fibrous Residue for Production of Ethanol." *Starch/Stärke*, vol 40, pp 55-58.

[12] Jarboe L. R., Shanmugam K. T., Ingram L. O. (2009). Ethanol in Encyclopedia of Microbiology, 3rd edition // M. Schaechter, Ed. Kidlington, Oxford, United Kingdom: *Academic Press*, pp 295-304.

[13] Klass, D. L. (1998). "Biomass for Renewable Energy, Fuels, and Chemicals." *Academic Press*, San Diego, CA.

[14] Koizumi T. (2015). "Biofuels and Food Security." *Renewable and Sustainable Energy Reviews*, vol 52, pp 829-841.

[15] Kuboye, A.O. and Akinrele, I.A. (1971). "Derivation of Table Vinegar from Fermented Palmwine." FIIRO Res. Rep. Technology, No 42. Lagos, Nigeria.

[16] Miller, B.M. and Litsky, W. (1976). Industrial Microbiology. 2nd Edition, McGraw-Hill, Inc., USA, pp. 120-135, 180-186.

[17] Nigerian National Petroleum Corporation, NNPC. Nigerian Biofuel Policy and Incentives, 2007.

[18] Nwufo, M.I and Fajola, A.O. (1998). "Production of Amylolytic Enzyme with the Corm Roots of Colocasia esculenta." *Acta Microbiologica Hungarica*, vol 4, pp 371.

[19] Okafor, N. (1987). Industrial Microbiology. 1st Edition, University of Ife Press Ltd., Ile-Ife, Nigeria, pp. 222-229, 254.

[20] Oke, O. L, Redhead, J. and Food and Agriculture Organization of the United Nations. (1998) "Roots, Tubers, Plantains, and Bananas in Human Nutrition." FAO Corporate Document Repository, pp. 24.

[21] Okigbo, B.N. (1987). "Roots and Tubers in Africa Food Crisis." International Development Research Centre, Canada, pp. 9-15.

[22] Okorondu S.I., Nedosa I.V., Wesley B., Akujobi C.O. (2009). "Ethanol Production from Cassava." *Current Topics in Biotechnology*, vol 5, pp 65-70.

[23] Onwueme, I.C, Charles, W.B. and Food and Agriculture Organization of the United Nations. (1994). "Tropical Root and Tuber Crops: Production, Perspectives and Future Prospects. FAO Corporate Document Repository, pp 126.

[24] Owusu-Darko, P.G., Paterson, A., Omenyo, E.L. (2014) "Cocoyam (Corms and Cormels) – An Underexploited Food and Feed Resource." *Journal of Agricultural Chemistry and Environment*, vol 3, No.1, pp 22-29.

[25] Quintero, J. A. et al. (2008). "Fuel Ethanol Production from Sugarcane and Corn: Comparative Analysis for a Colombian case." *Energy*, vol 33, pp 385-399.

[26] Saraswati, R. (1988). Paper delivered at a workshop on "Upgrading of Cassava & Cassava Waste by Appropriate Biotechnologies," pp 41-49.

[27] Sathaye, J. et al (2011). "Renewable Energy in the context of Sustainable Energy." IPCC Special Report on Renewable Energy Sources and Climate Change Mitigation (SRREN). Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA.

[28] Saucedo, G. et al. (1992). "Potentials of Cassava in Ethanol Production." *Applied Biochemistry and Biotechnology*, vol 36, pp 47-61.

[29] Srinorakutara, T. et al. (2008). "Strain improvement of Ethanol Fermenting yeast using Random Mutagenesis Technique." *Thai Journal of Biotechnology*, vol 8, No. 1, pp 42-44.

[30] United States Department of Agriculture. Germplasm Resources Information Network (GRIN).

[31] Walker, G.M. (2011). "Fuel Alcohol: Current Production and Future Challenges." *Journal of the Institute of Brewing*, vol 1, pp 3-22.

[32] World Bioenergy Association. Annual Report, 2013.

[33] Zhang C., Han W., Jing X., Pu G., Wang C. (2003). "Life cycle economic analysis of fuel ethanol derived from cassava in southwest China." *Renewable and Sustainable Energy Reviews*, vol 7, pp 353-366.

[34] Abd-Alla, M.H., Zohri, A.N.A., El-Enany, A.W.E. and Ali, S.M. (2015) Acetone–butanol–ethanol production from substandard and surplus dates by Egyptian native Clostridium strains. Anaerobe, 32, 77-86.

[35] Ali, M.M. (2010) Studies on production of ethanol and single cell proteins by local yeast strains. M.Sc. Thesis, Botany Department, Faculty of Science, Assiut University, Assiut, Egypt

[36] Bamforth, C.W. (2005) The science underpinning food fermentations, In: "Fermentation and Microorganisms", Blackwell Science Ltd Publishing (Ed.), 1, pp.78-84. Oxford, UK.

[37] Bazmi, A.A., Bhutto, A.W. and Ghauri, M. (2007) Ethanol fuel as feasible and desired option in Pakistan. ESDev, CIIT Abbott abad, Pakistan, 1, 247-258.

[38] Chandra, J. and Panchal, F. (2003) "Yeast Strain Selection". Bioprocess Technol. Series. Marcel dekker Pub. New York, pp. 349.

[39] Dodić, S., Popov, S., Dodić, J., Ranković, J., Zavargo, Z. and Mučibabić, J. (2009). Bioethanol production from thick juice as intermediate of sugar beet processing. Biomass Bioenerg, 33, 822-827.

[40] El-Gendy, N.S., Madian, H.R. and Abu Amr, S.S. (2013) Design and optimization of a process for sugarcane molasses fermentation by Saccharomyces cerevisiae using response surface methodology. Int. J. Microbiol. 2013, 1-9.

[41] Aditiya, H. B., T. M. I. Mahlia, W. T. Chong, Hadi Nur, and A. H. Sebayang. "Second generation bioethanol production: A critical review." Renewable and sustainable energy reviews 66 (2016): 631-653.

[42] Armstrong, Robert C., Catherine Wolfram, Krijn P. de Jong, Robert Gross, Nathan S. Lewis, Brenda Boardman, Arthur J. Ragauskas, Karen Ehrhardt-Martinez, George Crabtree, and M. V. Ramana. "Deconstructing Biomass [part of. The frontiers of energy]." Nature Tour, James M., Carter Kittrell, and Vicki L. Colvin. "Green carbon as a bridge to renewable energy." Nature materials 9, no. 11 (2010): 871 Energy 1, no. 1 (2016).

[43] Khoja, Asif Hussain, Muhammad Tahir, and Nor Aishah Saidina Amin. "Dry reforming of methane using different dielectric materials and DBD plasma reactor configurations." Energy Conversion and Management 144 (2017): 262-274.

[44] Uzair, Muhammad Ali, Ali Waqas, Asif H. Khoja, and Nisar Ahmed. "Experimental study of catalytic degradation of polypropylene by acid-activated clay and performance of Ni as a promoter." Energy Sources, Part A: Recovery, Utilization, and Environmental Effects 38, no. 24 (2016): 3618-3624.

[45] Zabed, H., J. N. Sahu, A. Suely, A. N. Boyce, and G. Faruq. "Bioethanol production from renewable sources: Current perspectives and technological progress." Renewable and Sustainable Energy Reviews 71 (2017): 475501.

[46] Khoja, Asif H., Ehsan Ali, Kashaf Zafar, Abeera A. Ansari, Azra Nawar, and Muneeb Qayyum. "Comparative study of bioethanol production from sugarcane molasses by using Zymomonas mobilis and Saccharomyces cerevisiae." African Journal of Biotechnology 14, no. 31 (2015): 2455-2462.

[47] Ansari, Abeera A., Asif Hussain Khoja, Azra Nawar, Muneeb Qayyum, and Ehsan Ali. "Wastewater treatment by local microalgae strains for CO2 sequestration and biofuel production." Applied Water Science 7, no. 7 (2017): 4151-4158.

[48] Cochran, S., & Anthonavage, M. (2015). Fatty Acids, Fatty Alcohols, Synthetic Esters and Glycerin Applications in the Cosmetic Industry. In: Lipids and Skin Health. Springer, CHAM. pp. 311-319

[49] Goswami, P., Chinnadayyala, S. S. R., Chakraborty, M., Kumar, A. K., & Kakoti, A. (2013). An overview on alcohol oxidases and their potential applications. Applied Microbiology and Biotechnology, 97(10), 4259–4275.

[50] Larsen, S. (1994). Alcohol use in the service industry. Addiction, 89(6), 733–741. https://doi.org/10.1111/j.1360-0443.1994.tb00959.x

[51] Rabenau, H. F., Kampf, G., Cinatl, J., & Doerr, H. W. (2005). Efficacy of various disinfectants against SARS coronavirus. Journal of Hospital Infection, 61(2), 107–111. https://doi.org/10.1016/j.jhin.2004.12.023

[52] Wu, J., Gao, M., & Taylor, D. H. (2014). Neuronal nicotinic acetylcholine receptors are important targets for alcohol reward and dependence. Acta Pharmacologica Sinica, 35(3), 311–315. https://doi.org/10.1038/aps.2013.181

[53] Ana M, Viorel T, Calin M (2012) Simulation of Fermentation Bioreactor Control for Ethanol Production. 11th International Conference on Development and Application Systems, Romaina. pp: 17-20.

[54] Aziz S, Memon H, Shah F, Rajoka I, Soomro S (2009) Production of ethanol by indigenous wild and mutant strain of thermotolerant Kluyveromyces Marxianus under optimized fermentation conditions. Pak Journal of Environmental Chemistry 10: 25-33.

[55] Bai F, Anderson W, Young M (2010) Ethanol fermentation technologies from sugar and starch feedstock's. Biotechnology Advances 26: 89-105

[56] Dai W, Word D, Hahn J (2014) Modeling and dynamic optimization of fuelgrade ethanol fermentation using fed-batch process. Control Engineering Practice 22: 231-241

[57] David R, Dochain R, Mouret A, Sablayrolles M (2010) Dynamical modeling of alcoholic fermentation and its link with nitrogen Consumption. Proceedings of the 11th International Symposium on Computer Applications in Biotechnology, Belgium. pp: 496-501

[58] Ghasem N, Habibollah Y, Ismail K (2004) Ethanol Fermentation in an Immobilized Cell Reactor using Saccharomyces cerevisiae. Bioresource Technology 92: 251-260

[59] Fan, Xing, Bo Chen, Xun Hu, Nilusha Sudasinghe, and Ya-He Zhang. "Characterization of Fossil and Renewable Fuels." International Journal of Analytical Chemistry 2018 (2018).

[60] Nawar, Azra, Asif Hussain Khoja, Naveed Akbar, Abeera Ayaz Ansari, Muneeb Qayyum, and Ehsan Ali. "Physical abrasion method using submerged spike balls to remove algal biofilm from photobioreactors." BMC research notes 10, no. 1 (2017): 666

[61] Thatoi, Hrudayanath, Preeti Krishna Dash, Sonali Mohapatra, and Manas Ranjan Swain. "Bioethanol production from tuber crops using fermentation technology: a review." International Journal of Sustainable Energy 35, no. 5 (2016): 443-468

[62] Vučurović, D., S. Dodić, S. Popov, J. Dodić, J. Grahovac, I. Tadijan, and Z. Zavargo. "Bioethanol as transport fuel." Tractors and Power Machines (2011).

[63] Sanchez, Oscar J., and Carlos A. Cardona. "Trends in biotechnological production of fuel ethanol from different feedstocks." Bioresource technology 99, no. 13

[64] A.O.A.C. 2000. Official Methods of Analysis of the Association of Official Analytical.

[65] Adesanya, O., Oluyemi, K., Josiah, S., Adesanya, R., Shittu, L., Ofusori, D., Bankole, M., Babalola, G. (2008). Ethanol Production by Saccharomyces cerevisiae from Cassava Peels Hydrosylate. International journal of Microbiology, 5(1):25-35.

[66] Adetunji, R.O., Youdeowei, P.K., Kolawole, O.O. (2015).Production of Bio-ethanol from Cassava Peels. Proceedings from International Conference on Renewable energy and power held at Atlanta, Georgia Vol 1.

[67] Adiotomre, K.O. (2015). Production of Bioethanol As An Alternative Source Of Fuel Using Cassava And Yam Peels As Raw Materials. Int. J. Innov. Sci. Eng. Technol. Res. 3(2):28-44.

[68] Ado, S.A., Olukotun, G.B., Ameh, J.B., Yahaya, A. (2009).“Bioconversion of cassava starch to ethanol in a simultaneous saccharification and fermentation process by cocultures of Aspergillus niger and Saccharomyces cerevesiae”. Science World Journal, 4(1):19-22.

[69] Ajay, K.S., Sanat, R., Yashab, K., Harison, M., Jyotsna, K., Jane, C., Pradeep, K., Pankaj, S. (2014). Bioethanol production from banana peel by simultaneous saccharification and fermentation process using Aspergillus niger and Saccharomyces cerevisiae. International Journal of Current Microbiology and Applied Sciences, 3(5):84-96.

[70] Aro, N., Pakula, T., Pentella, M. (2005). “Transcriptional regulation of plant cell wall Degradation by filamentous fungi”. Fems Microbiology revolution, 29: 719-739.

[71] Bernfeld, P. (1959). Amylases α and β. In: Colowick, S.P. and Raplan, N.A. (eds.) Methods in Enzymology Raplan Acad Press, New York, pp. 149 – 158.

[72] Collins, A. and Lynes, P. (1989). Microbiological Methods 6th Edition, Butler ad Tunner, Frome, Somerset Great Britain, pp. 233 – 241.

[73] De Mancilla, I. M. and Pearson, A.M. (1984). Increasing alcohol yield of selected yeast fermentation of sweet sorghum.Evaluation of yeast strains for ethanol production. Biotech and Bioengs. 26: 672 – 675.

[74] Dhamija, S.S., Bhaskar, D. and Gera, R. (1996). Thermotolerance and ethanol production are at variance in mutants of Schizosaccharomyces pombe. Biotechnology Letters 18 (11): 1341 1344.

[75] Dien, B.S., Cotta, M.A. and Jeffries T.W. (2003). Bacteria engineered for fuel ethanol production current status. Appl. Microbiol. Biotechnol. 63: 258 – 266.

[76] Ezeogu, L.I. and Emeruwa, A.C. (1993). Isolation of yeast from natural sources such as palmwine. Biotechnol. Lett. 15: 83 – 86.

[77] FAO (1984). Supply/Utillization accounts tape. FAO, Rome, Italy. http://www.fao.org.

[78] Gu Bi. and Ye Guozhen. (2002). Commercial -Scale production of ethanol form cassava pulp and wastewater from Guangxi University, Nanning, Guangxi, 53005 P.R. China.

[79] Ingram, L.O. and Buttke, T. M. (1984). Effects of alcohol on micro-organisms. Adv. Microb. Physiol. 25: 253 – 300.

[80] Kocchar, S. L. (1986). Tropical crops: A textbook of Economic Botany. Macmillan Publishers Ltd., London, p. 23.

[81] Leaky, C.L.A. and Wills, J.B. (1977). Food Crops of Lowland Tropics. Great Oxford University Press, Britain, p. 76.

[82] Alvira P, Ballesteros M and Negro MJ (2009). Bioresour. Technol. 101(13) 4851

[83] Antoni D, Zverlov V V and Schwarz WH (2007). Appl. Microbiol. Biotechnol. 77(1) 23

[84] Gray KA and Zhao L 2006 Curr Opin Chem Biol. 10(2) 141

[85] Muktham R, Bhargava SK, Bankupalli S and Ball AS 2016 Journal of Sustainable Bioenergy System 6 72

[86] Li H, Kim N, Jiang M, Won J and Nam H 2009 Bioresour. Technol. 100(34) 3245

[87] Dashtban M, Schraft H and Qin W 2009 Int. J. Biol. Sci. 5(6) 578

[88] Hahn-hägerdal B, Karhumaa K, Fonseca C, Spencer-martins I and Gorwa-grauslund MF 2007 Appl. Microbiol. Biotechnol. 74(5) 937

[89] Cardona CA, Quintero JA and Paz IC (2010) Bioresour. Technol. 101(13) 4754

[90] Krivorotova T and Sereikaite J (2014). Electronic Journal of Biotechnology 17 329

[91] Hermansyah, Novia, Sugiyama M and Harashima S 2015 Microbiology Biotechnology Letters 43(3) 241

[92] Kim TH, Taylor F and Hicks KB (2008). Bioresour. Technol. 99(13) 5694

[93] Qureshi AS, Zhang J and Bao J 2015 Appl. Biochem. iBotechnol. 175(6) 3173

[94] Prasetyo J, Naruse K, Kato T, Boonchird C, Harashima S and Park EY2011 Biotechnol. Biofuels 4(1) 35