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Optimization of fermentation conditions for Bioethanol production from Acid Hydrolysed Cassava fibres and Corn cobs using a Palmwine yeast

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Abstract

The optimization of some fermentation parameters for bioethanol production from cassava fibres and corncobs by a paimwine yeast was studied. The Malt Extract Agar was used for the isolation of the yeast from raffia palmwine using the spread plate technique. The yeast was used for the production of bioethanol from acid hydrolysed cassava fibres and corncobs at different fermentation time. Various inoculum sizes and substrate concentrations were employed to determine the optimum for bioethanol production. The effect of UV irradiation on bioethanol production by the palmwine yeast was also conducted. The result of the study revealed that the yeast strain isolated from the palmwine was *Saccharomyces cerevisiae* DBVPG6765. It was observed that maximum ethanol concentration of 8.0 %v/v was recorded after 72 h for corncobs, while 6.8 %v/v was recorded for cassava fibres after 96 h respectively. Optimum ethanol yield of 8 %v/v for corncobs and 6.8 %v/v for cassava fibres was observed at 1 %v/v inoculum size. The optimum ethanol yield of 8.3 %v/v for corncobs and 7.2 %v/v for cassava fibres was observed at a substrate concentration of 200g/l. The UV irradiated strain of the yeast, recorded 8.9 and 7.5%(v/v) of ethanol from corncob and cassava fibre respectively. The study has been able to establish that cassava fibre and corncob could be used as substrates for bioethanol production. It has also revealed that optimization of some fermentation parameters could enhance bioethanol yield.

Keywords: Bioethanol, *Saccharomyces cerevisiae,* palmwine, fermentation time, inoculum size, substrate concentration, UV irradiation

1. Introduction

Fossil energy resources has provided about 50% of total energy globally (Ohimain, 2010) and about 86% of total transportation fuel (Atadashi, 2011). Presently, it is depleting (Izah and Ohimain, 2013) due to increased population and economic growth (Daud *et al*., 2012). To avert the looming energy crisis, governments all over the world have encouraged the use of alternative sources of energy. Renewable energy sources provide several solutions to the challenge of conventional energy resources such as emission of pollutant gases into the atmosphere. The higher price of oil has attracted the greater attention to biofuels, especially bioethanol, biodiesel, biohydrogen, to list a few. Biofuels may be classified under the categories of first or second generation biofuels (Naik *et al.*, 2010). First generation biofuels are generally made from carbohydrates, lipids and oils or agro industrial wastes using conventional technologies. Second generation biofuels are generally derived from lignocellulosic biomass including cellulosic plant biomass such as the stalks, stems, wood. Many second generation biofuels such as biohydrogen, biomethanol and mixed alcohols are under development. Bioethanol can be used as a fuel, either pure or blended with gasoline (gasohol). In the United States, it is used as 10% solution in gasoline (E-10) while in Brazil it is used both blended (24% ethanol, 76% gasoline) and hydrated in flexible-fuel vehicles (Zabed et al., 2014). Others mixtures are E-15 (15% ethanol, 85% gasoline) and E-85 (85% ethanol , 15% gasoline). Using ethanol as a gasoline fuel additive as well as transportation fuel helps to alleviate global warming and environmental pollution. Bioethanol can also replace other additives, as octane boosters, in gasoline fuel, and ethanol–gasoline blend provides the highest brake power (Elfasakhany 2015). Other benefits come from using bioethanol as biofuel: it is totally basically non-flammable, non-toxic, biodegradable (Izah and Ohimain, 2013; Sameera *et al*., 2011), sulphur free and the products from its incomplete oxidation (acetic acid and acetaldehyde) are less toxic in comparison to other alcohols (Minteer, 2006).

Bioethanol is an example of liquid biofuel produced from renewable resources (Prasad *et al.*, 2009) through fermentation (Nzelibe and Okafoagu, 2007). The major pathway for bio-ethanol production from biomass is biochemical conversion through saccharification and fermentation (Balat, 2011). Bioethanol producing nations depend on sugarcane (Brazil), sugar beets (Europe), cassava (China, Thailand), sorghum (India, Philippines), Corn (USA). The production of bioethanol from these feedstocks could cause adverse effects due to its edibility. These challenges could be reduced through the use of non-edible grasses and wastes cellulosic/ lignocellulosic biomass such as switch grass (*Panicum virgatum), Miscanthus sp* giant reed (*Arundo donax*) and reed canary grass (*Phalaris arundinacea*) (Lewandowski *et al*.,2003), elephant grass (*Pennisetum purpureum*) (Ohimain, 2013), wild sorghum etc.

Lignocellulose materials are abundant renewable resource for the production of bio-fuel. The major components of lignocelluloses are cellulose, hemicellulose and lignin while the minor components are extractive liquid and ash.

Nigeria is the highest producer of cassava in the world, producing higher than Brazil, Thailand and Indonesia. Industrial and local processing of cassava to food and other product has led to

useful products (Mohammed *et al*, 2013). In Nigeria, corn is processed to a variety of diets including pap which is a major diet for weaning, and the capacity for corn production in Nigeria is high (Orji *et al*, 2016). Corn cobs form 30% of maize agro wastes (Zakpaa *et al*, 2009). These wastes end up polluting the surface and underground water (Mohammed *et al*, 2013). Most of the ethanol utilized in the country are imported, which involves spending huge amount of foreign exchange. Nigeria can explore the abundant agricultural wastes to produce enough ethanol for consumption and exportation.

In our previous study (published), it was possible to hydrolyse cassava fibres and corncobs using various concentrations of sulphuric acid and hydrochloric acid. The hydrolysed substrates were thereafter used for bioethanol production (Okpalla and Eleanya, 2020).

The aim of this work was to carry out optimization of bioethanol production from cassava fibres and corncobs using palmwine yeast.

2. Materials and Methods

Isolation of yeast from palmwine

A Sample of fresh palmwine from raffia palm was collected in a pre-sterilized container and immediately transported to the Department of Microbiology Laboratory at Chukwuemeka Odumegwu Ojukwu University, Uli Anambra State. The palmwine was allowed to stand for 12 h and thereafter 10 fold serial dilution was performed and aliquot of 0.1 ml of 10^{-3} of dilution was inoculated on plates of Malt Extract Agar (MEA) using the spread plate technique. The plates were incubated at 27 ^{0}C for 48 h. The isolated colonies with distinct morphological appearances were aseptically sub-cultured in freshly prepared agar plates and incubated at 27 ^{0}C for 48h. A distinct colony was selected from all the agar plates and pure culture of it was preserved in agar slant. The morphological characteristics were observed by adopting the method of Ogbo (2005).

Molecular characterization and identification of the palmwine yeast

The pure culture of the yeast was sucultured into fresh agar Malt Extract Agar plates and sent to Macrogen Inc. Seoul Republic of South Korea for molecular identification.

Inoculum preparation:

Two(2) loopfuls of the palmwine yeast were collected from the agar slant and inoculated into a test tube containing 2 %(w/v) glucose solution. The solution was incubated for 24 h under room temperature.

Effect of fermentation time on bioethanol production from hydrolysed corncobs and cassava fibres by the palmwine yeast

The effect of fermentation time on bioethanol production from hydrolysed corncobs and cassava fibres by the palmwine yeast was determined. Each of the hydrolysed corncobs and cassava

fibres (150g) was added into each cotton plugged vessels (4L size) containing 1 litre of sterile distilled water and pH was adjusted to 5.0. The fermentation medium was sterilized and thereafter one percent (v/v) of a 24h seed inoculum of the palmwine yeast was used to inoculate the medium. The fermentation was carried out at 27^0C for 168 h. At interval of 24 h, samples were taken from the medium and used for determination of ethanol, reducing sugar concentration and pH.

Effect of inoculum size on bioethanol production from hydrolysed corncobs and cassava fibres by the palmwine yeast

The effect of inoculum size on bioethanol production from hydrolysed corncobs and cassava fibres by the palmwine yeast was studied. Each of the hydrolysed corncobs and cassava fibres (150g) was added into each cotton plugged vessels (4L size) containing 1 litre of sterile distilled water and pH was adjusted to 5.0. The fermentation medium was sterilized and thereafter different inoculum sizes (0.5, 1, 1.5 and 2.0% v/v) of the palmwine yeast were used to inoculate the various media. The fermentation was carried out at 27 ^{0}C for 72 h in the case of corncobs, while 96h was used for cassava fibres. Experiments were performed in duplicate and samples were collected and used for the determination of ethanol.

Effect of substrate concentrations on bioethanol production by the palmwine yeast

The effect of substrate concentrations on bioethanol production from hydrolysed corncobs and cassava fibres by the palmwine yeast was determined. Various concentrations (100 – 200 g/l) of hydrolysed corncobs and cassava fibres were added into each cotton plugged vessels (4L size) containing 1 litre of sterile distilled water and pH was adjusted to 5.0. The fermentation medium was sterilized and thereafter one percent (v/v) of a 24h seed inoculum of the palmwine yeast was used to inoculate the medium. The fermentation was carried out at 27 ^{0}C for 72 h in the case of corncobs, while 96h was used for cassava fibres. Experiments were performed in duplicate and samples were collected and used for the determination of ethanol.

Effect of UV irradiation on Bioethanol production from hydrolysed corncobs and cassava fibres by the palm wine yeast

UV irradiation of palmwine yeast

The method of Singh and Sharma (2015) was adopted. The palm wine yeast (*Saccharomyces cerevisiae* strain DBVPG6765) culture was diluted by ten fold serial dilution method. Aseptically, 0.1 ml was collected from dilution 10^{-4} and spread plated on Malt Extract Agar Petri-dishes. The Petri-dishes were exposed to UV light at a distance of 55cm at various time

intervals (5, 10, 15, 20 and 25 min). The treated Petri-dishes were covered with dark paper and incubated in the dark at 27^0C for 3 days. A mutant strain of *Saccharomyces cerevisiae* strain DBVPG6765 developing on one of the agar plates was subsequently subcultured on malt extract agar and preserved in agar slant.

Bioethanol production by mutant of the palmwine yeast (*Saccharomyces cerevisiae* strain DBVPG6765)

The effect of fermentation time on bioethanol production from hydrolysed corncobs and cassava fibres by the mutant strain of the palmwine yeast was determined. Each of the hydrolysed corncobs and cassava fibres (150g) was added into each cotton plugged vessels (4L size) containing 1 litre of sterile distilled water and pH was adjusted to 5.0. The fermentation medium was sterilized and thereafter one percent (v/v) of a 24h seed inoculum of the mutant was used to inoculate the medium. The fermentation was carried out at 27 ^{0}C for 72 h in the case of corncobs, while 96 h was used for cassava fibres. At the end of fermentation, sample of the medium was collected and used for determination of ethanol.

Ethanol determination

The method of Izah and Ohimain, (2015) was adopted. The percentage ethanol yield was determined with this formula

$$\% \text{ Ethanol yield} = \frac{\text{Volume of distillate}}{\text{Volume of sample}} \times 100$$

Determination of Reducing Sugar

Reducing sugar was estimated using the method of Miller(1959). One millilitre of Dinitrosalicyclic acid (DNS) was added to 1 ml of the hydrolysates in test tubes and mixtures heated in a water at 100 ^{o}C for 10 min. The test tubes were cooled rapidly in tap water and volume adjusted to 12 ml with distilled water. A blank containing 1ml of distilled water and 1ml of DNS was prepared. The optical density (OD) was read against the blank in a spectrophotometer at 540 nm. The concentration of the reducing sugar was estimated from standard glucose curve.

pH determination

The pH was determined in-situ using pH meter (Hanna model 9605).

Statistical analysis

The data generated from the study were analysed using T test (unpaired, 2 tail).

3. Results

The yeast strain isolated from raffia palmwine was identified as *Saccharomyces cerevisiae* DBVPG6765

Figure 1 shows the effect of fermentation time on bioethanol production from corncobs by *Saccharomyces cerevisiae* DBVPG6765. Maximum ethanol yield 8.0 (%v/v) was recorded at 72 h and the yield reduced progressively with increase in fermentation time. It was noticed that both the pH and reducing sugar, decreased progressively till the end of the fermentation. . There was no significant difference in ethanol yield between cassava fibre and corn cob.

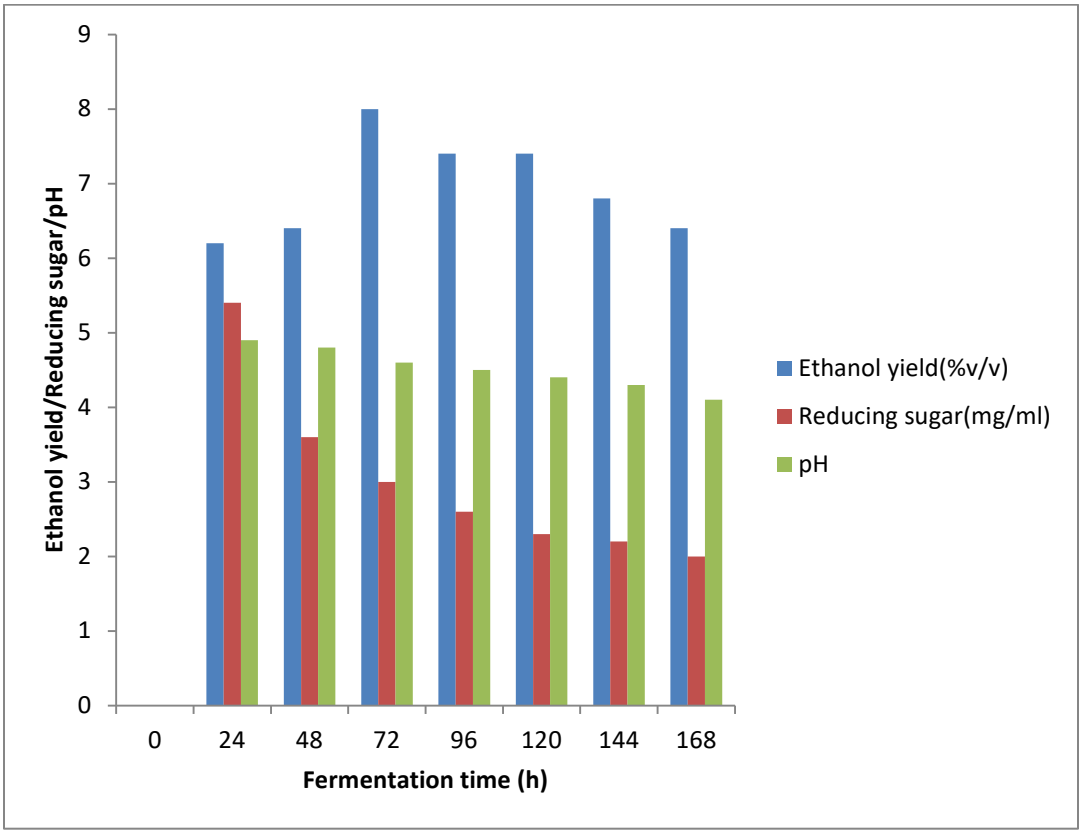

Figure 1: Effect of Fermentation Time on Bioethanol production from corncobs by *Saccharomyces cerevisae* DBVPG6765

The effect of fermentation time on bioethanol production from cassava fibre by *Saccharomyces cerevisiae* DBVPG6765 is as shown in Figure 2. Maximum ethanol yield 6.8 (%v/v) was recorded at 96 h and the yield reduced progressively with increase in fermentation time. It was noticed that both the pH and reducing sugar, decreased progressively till the end of the fermentation. There was no significant difference in ethanol yield between cassava fibre and corn cob.

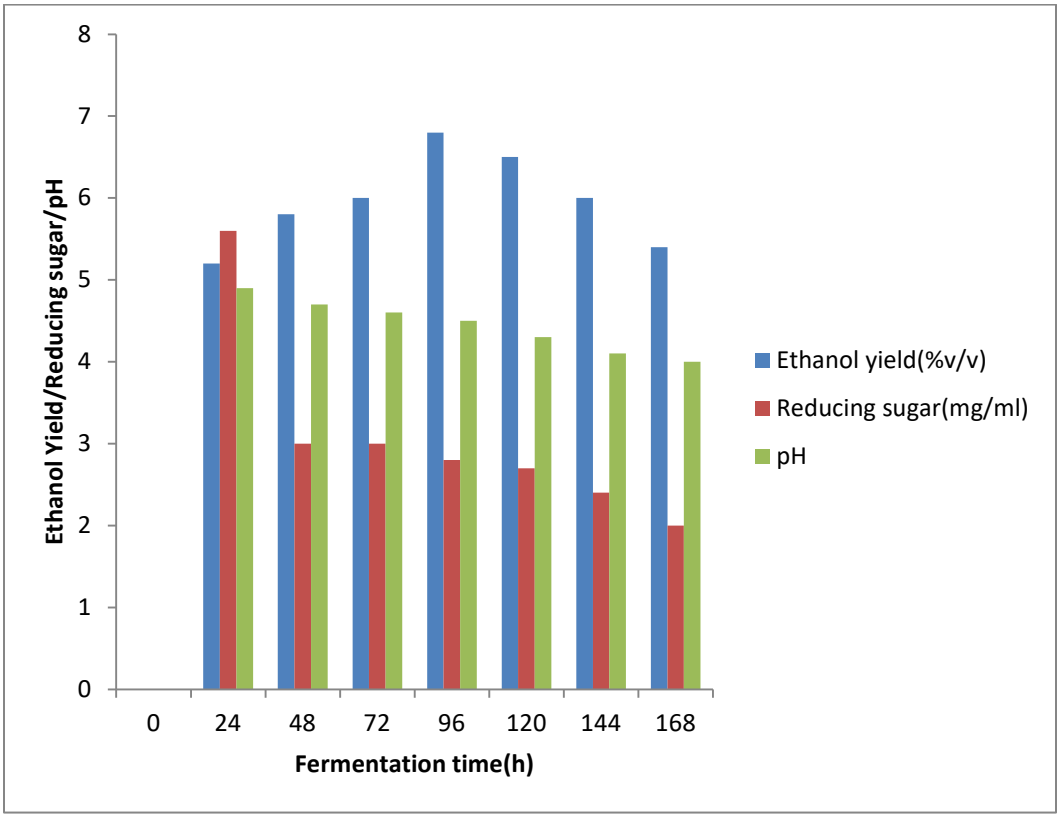

Figure 2: Effect of Fermentation Time on Bioethanol production from cassava fibres by *Saccharomyces cerevisae* DBVPG6765.

Figure 3 shows the results of the effect of inoculum size on bio-ethanol production from corncobs and cassava fibres by *Saccharomyces cerevisiae* DBVPG6765. Optimum ethanol yield of 8.0 and 6.8 (%v/v) was produced by 1(%v/v) inoculum size for both corncobs and cassava fibres respectively.

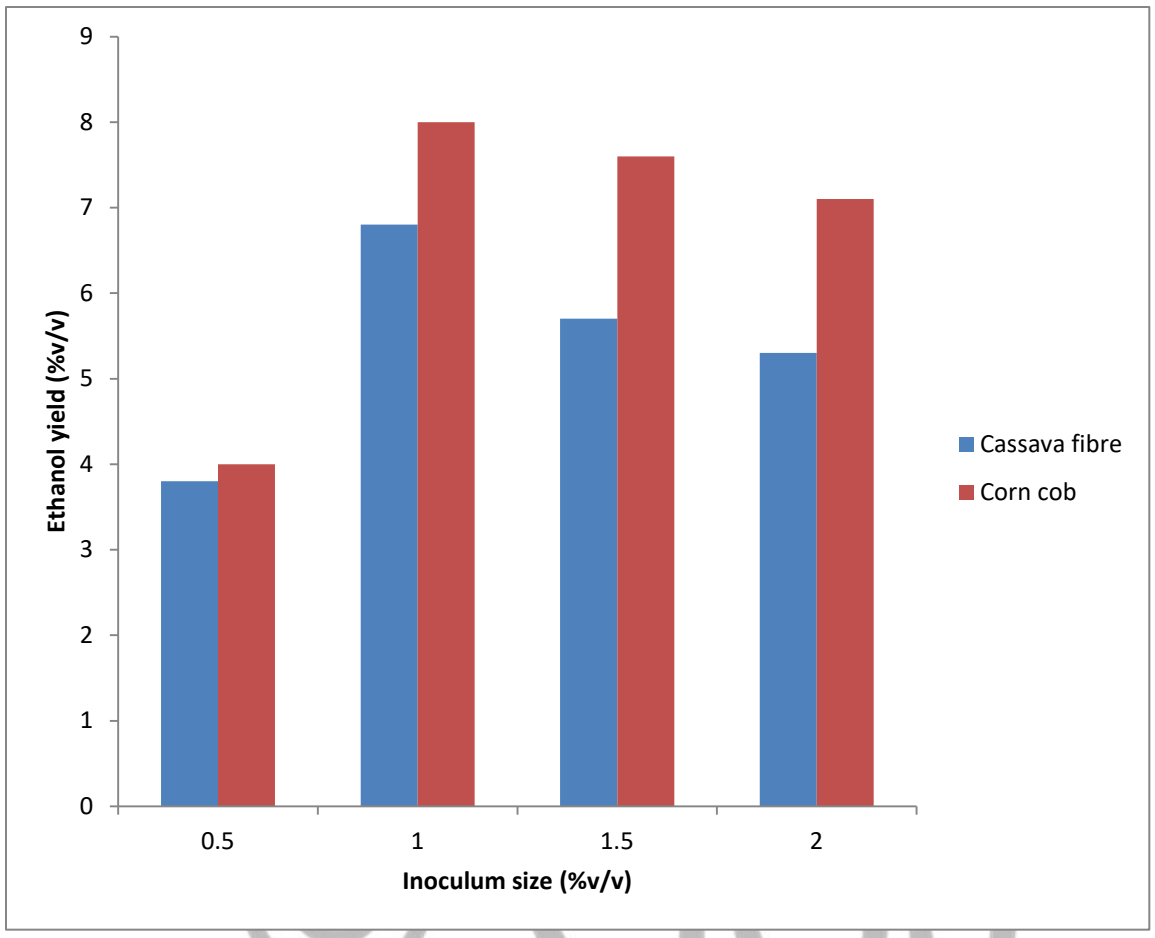

Figure 3: Effect of inoculum size on bio-ethanol production from corncobs and cassava fibres by *Saccharomyces cerevisiae* DBVPG6765.

The results of the effect of substrate concentrations on bioethanol production from corncobs and cassava fibres by *Saccharomyces cerevisiae* DBVPG6765 is shown in Figure 4.. Optimum ethanol yield of 8.7 and 7.3 % (v/v) was obtained using 200 g/l concentration for both corncobs and cassava fibres respectively.

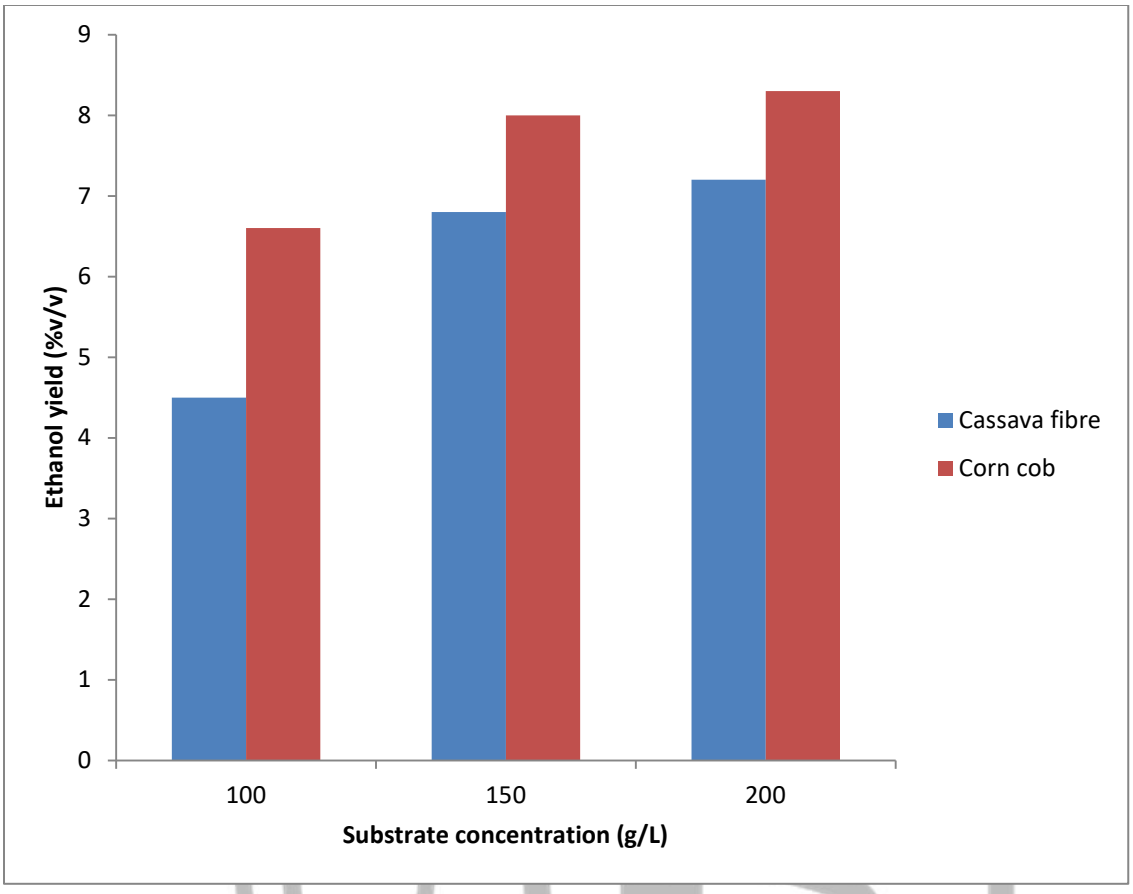

Figure 4: Effect of substrate concentration on bio-ethanol production from corncobs and cassava fibres by *Saccharomyces cerevisae* DBVPG6765.

Table 1 shows the results of the effect UV irradiation on *Saccharomyces cerevisiae* for bio-ethanol production from corncobs and cassava fibres. Ethanol yield of 8.9 and 7.5 (%v/v) was obtained from corncobs and cassava fibres respectively.

Table 1: Ethanol yield from corn cob and cassava fibre using the mutant strain of *Saccharomyces cerevisiae* DBGVG6765

Substrate	Ethanol yield (%v/v)
Corn cob	8.9
Cassava fibre	7.5

4. Discussion

In this research it was observed that the optimum ethanol production from hydrolyzed cassava fibres by the palmwine yeast was at 72 h. This was in contrast with the report of Akponah and Akpomie (2012), who observed enhanced ethanol yield from cassava effluent after 24 h. Again, it was observed in this study that reducing sugar concentration decreased progressively with increase in fermentation time, this is similar to the findings of Akponah and Akpomie (2012) and Akponah (2011). The progressive decrease in reducing sugar concentration could be due to the efficiency of the yeast in the utilization of reducing sugars. Decrease in reducing sugar concentrations beyond the time for maximum ethanol yield in both substrates could be attributed to increased production of aldehydes, phenols, lactic acid and an increased tolerance of the palmwine yeast to ethanol (Shyam *et al.*, 2011). According to the report of Archibong *et al*. (2016), yeasts isolated from natural sources such as palmwine possess a very high level of ethanol and sucrose tolerance that enables them to grow well in various substrates.

Maximum ethanol yield was obtained using 1% (v/v) inoculum size, while higher inoculum sizes produced lesser yield of ethanol as reported in this work. This observation is in contrast with the report of Bukhari and Loh (2015) and Udharayaraja and Narayanan (2012), who observed that 10%v/v inoculum size produced highest ethanol yield.

It was observed in the study that the ethanol yield increased with increase in substrate concentration. This finding corroborated the report of Shyam *et al*. (2011), who observed an

increase in ethanol yield with increase in substrate concentration (1-10%). However, a contradictory report was made by Magnaye *et al*. (2015), who observed that substrate concentration was inversely proportional to the percentage ethanol yield from elephant foot yam. Utilization of substrate during fermentation might have been influenced by various factors such as pectin, complex sugar, hemicelluloses, fiber and lignin.

5. Conclusion

The work has been able to establish that cassava fibre and corncob could be used as substrates for bioethanol production. It has also revealed that optimization of some parameters enhanced the yield of bioethanol produced. Further research is needed to study the optimization of other parameters for optimum bioethanol accumulation by *Saccharomyces cerevisiae.*

6. References

1. Abidin Z, E Saraswati and T Naid, 2014. Bioethanol production from waste of the cassava peel (*Mannihot esculanta*) by acid hydrolysis and fermentation process. Inter J PharmTech Res. 6(4): 1209–1212.
2. Agboola O and M Agboola, 2011. Nigeria's Bio-Ethanol: Need for Capacity Building Strategies to prevent Food Crises. Bioenergy Tech. 2(1): 87–89.
3. Akpan U G, AS Kovo, M Abdullahi and JJ Ijah, 2005. Production of ethanol from maize cobs and groundnut shells. AU J Technol. 9(2): 106–110.
4. Akponah E 2011. Production of ethanol from cassava (*Mannihot esculanta*) waste water using *Saccharomyces cerevisiae* and *Echerichia coli.* Nig J Biotechnol. 11(32): 8110–8116.
5. Akponah E and OO Akpomie, 2012. Optimization of bio-ethanol production from cassava effluent using *Saccharomyces cerevisiae.* Afri J Biotechnol. 11(32): 8110–8116.
6. Alam MZ, NA Kabbashi and SNIS Hussin, 2009. Production of bioethanol by direct bioconversion of oil palm industrial effluents in a stirred tank bioreactor. J Ind Microbiol and Biotechnol. 36: 801–808.
7. Archibong EJ, EC Obuboegbunam, UC Ewelukwa, VC Onuora, CC Ezemba, CB Okeke, and U Okafor 2016. Formulation of microbial growth media using brewers' spent grains (BSG) and growth rate assessment with three bacterial species. Eur J Exper Biol. 6(3): 19–24.
8. Association of Official Analytical Chemists 2003. Official methods of analysis of the association of official's analytical chemists, 17th edition. *Association of official analytical chemists, Arlington, Virginia,* 133 pp.
9. Atadashi IM, Aroua MK and AA Aziz 2011. Biodiesel separation and purification: a review. *Renewable Ener.* 36: 437 – 443.

10. Balat M, H Balat and C Oz, 2008. Progress in bioethanol processing. Progr. and Ener. Combustion Sci. 34: 551–573.
11. Balat M 2011. Production of bioethanol from lignocellulosic materials via the biochemical pathway: a review. Energy Conver Manag. 52:858–875

12. Bukhari N and S Loh, 2015. Optimization of fermentation conditions for bio-ethanol production from oil palm trunk sap by *Saccharomyces cerevisiae.* Malay J Microbiol. 11: 163–169.
13. Daud M, W Syafii and K Syamsu 2012. Bioethanol production from several tropical wood species using simultaneous saccharification and fermentation processes. Wood Res J. 3(2): 106 – 115.
14. Elfasakhany, A. 2015. Investigations on the effects of ethanol–methanol–gasoline blends in a spark-ignition engine: Performance and emissions analysis. Engin. Sci. and Technol. 18: 713–719.

15. Farrell AE, RJ Plevin, BT Turner, AD Jones , M O' Hare and DM Kammen 2006. Ethanol can contribute to energy and environmental goals. *Sci.* **311**(506): 1-23.
16. Ibeto CN, CO Okoye and AU Ofoefule 2014. Bio-ethanol production from thermally pre-treated corn chaff and cassava waste water. Inter J Pure Appl Chem. 4(2): 227–233.
17. Izah SC and EI Ohimain 2013. The challenge of biodiesel production from oil palm feedstock's in Nigeria. Greener J Biol Sci. 3(1), 1 - 12.

18. Izah SC and EI Ohimain 2015. Bio-ethanol production from cassava mill effluents supplemented with solid agricultural residues using bakers's yeast (*Saccharomyces cerevisiae*). *J Environ Treatment Techniq*. **3**(1): 47–54.
19. Jackson BA, NO Oladipo and MO Agaja 2013. Cassava: A Potential Crop for Industrial Raw Material in Nigeria. Inter J Life Sci, 3(3): 105–112.
20. Lewandowski I, JMO Scurlock, E Lindvall and M Christou 2003. The development and current status of perennial rhizomatous grasses as energy crops in the US and Europe. Biomas and Bioener. 25: 335 – 361.

21. Li Y, K Gao, S Tian, S Zhang and X Yang 2011. Evaluation of Saccharomyces cerevisiae Y5 for ethanol production from enzymatic hydrolysate of non-detoxified steam-exploded corn stover. *Bioresou Technol.* **102**(22):10548–10552,.
22. Magnaye RC, SS Alagar, CM Pagsinohin and YA Magnaye 2015. Evaluation of parameters of elephant foot yam (*Amorphallus spaeoniifolius*) for bio-ethanol production. Asia Pacific J Multidisciplinary Res. 3(4): 107–112.
23. Mohammed A, Oyeleke S B and EC Egwim 2013. Pretreatment and hydrolysis of Cassava peels for fermentable sugar production. *Asi. Jour. of Biochem.* 9(1): 65-70.

24. Minteer, S 2006. Alcoholic Fuels; Taylor & Francis: New York, NY, USA, 265p.

25. Naik SN, Goud VV, Rout PK and AK Dalai 2010. Production of first and second generation biofuels: A comprehensive review. Renew Sustain Energy Rev. 14:578-597

26. Neelakandan T and G Usharani 2009. Optimization and production for bioethanol from cashew apple juice immobilized yeast cells by *Saccharomyces cerevisiae*. American-Eurasian J Scientific Res. 4(2): 85–88.
27. Nzelibe HCN and UC Okafoagu 2007. Optimization of ethanol production from Garcinia kola (bitter kola) pulp Agro Waste. Afri J Biotechnol. 6(17): 2033-2037.
28. Ogbo FC 2005. Basic Microbiology: Fundamental and Techniques. Ugbene Abakpa Nike Enugu: Cresco Publisher.
29. Ohimain EI 2010. Emerging bio-ethanol projects in Nigeria: Their opportunities and challenges. Ener. Policy 38: 7161-7168.
30. Ohimain EI 2013. The potentials of elephant grass (*Pennisetum purpureum* Schumach) bagasse as fuel for power generation. Third Electrical Engineering National Conference tagged "Energy Sources for Power Generation" (ES4PG-2013) holding from July 21-23, 2013 at the Department of Electrical Engineering, University of Nigeria, Nsukka.
31. Okpalla J and LC Eleanya 2020. Utilization of reducing sugar from cassava fibre and corncob for bioethanol production. Glob Scientific Jour. 8(6): 516 - 531
32. Orji J, K Aleke, C Ejikeugwu, A Nwachi, M Ikechukwu, S Ehiu and E Ugbo 2016. Bioethanol production from corn cob hydrolysed by cellulase of *Aspergillus niger* using *Zymomonas mobilis* and *Saccharomyces cerevisiae* isolated from palm wine. Inter. Jour. Of Curr. Res. in biosci. and plant boil. 3(1):39- 45

33. Oyeleke SB and NM Jibrin , 2009. Production of ethanol from guinea corn husk and millet husk. Afri. Jour. of Microbiol. Res. 3(4), 147 – 152.
34. Prasad MP, S Rekha , M Tamilarasan and KS Subha, 2009. Production of Bioethanol using various agricultural raw materials by two step enzymatic process. Adv. Biotechnol. 1(3): 41 – 43.
35. Rabah AB, SB Oyeleke, SB Manga, and LG Hassan, 2011. Utilization of millet and guinea corn husks for bioethanol production. Afric. J. Microbiol. Res. 5(31), 5721-5724.
36. Sameera V., C Sameera and TY Ravi 2011. Current strategies involved in biofuel production from plants and algae. J Microbial and Biochem Technol. 120(4): 1948-5948.
37. Shyam KR, MI Ganesh, R Rajeswari and H Harikrishnan, 2011. Utilization of waste ripe banana, and peels for bio ethanol production. J Biosci Res. 2(2): 67-71.
38. Singh J and R Sharma 2015. Growth kinetic and modeling of ethanol production by wild and mutant *Saccharomyces cerevisiae* MTCC 170. Eur. J Experimental Biol. 5(4):1-6
39. Udhayaraja P and JS Narayanan, 2012. Optimization for production of bioethanol using sorghum stovar by *Saccharomyces cerevisae*. Inter J Pure and Appl Microbiol. **2**(4): 64– 67.

40. Zabed H, G Faruq , JN Sahu, MS Azirun, R Hashim and AN Boyce 2014. Bioethanol production from fermentable sugar juice. Sci. World Jour. 12, 957102.

41. Zhang M, P Shaukla, M Ayyachamy, K Permaul and S Singh 2010. Improved bio-ethanol production through simultaneous saccharification and fermentation of lignocellulosic agricultural wastes by *Kluyveromyces marximus* 6556. World J Microbiol Biotechnol. 26: 1041–1046.