Ethanol Production from Potato Peel Using *Saccharomyces Cerevisiae*

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Abstract
This research work aim to investigate the production of bio-ethanol from potato peel feedstock. Saccharomyces cerevisiae was used for the fermentation during the investigation. Fermentation was done over a period of 168 hours. The absorbance of the resulting solution after hydrolysis of the feedstock was measured at 540nm using spectrophotometer. There was optimum ethanol production after 72 hours. With the change in the concentration of yeast, the time required for the completion of fermentation decreased dramatically. Using a 2ml, 4ml, 6ml, 8ml yeast inoculum, maximum ethanol production was completely achieved in 1, 2, 3, 4, 5, 6, 7 days respectively. The maximum ethanol yield from waste potatoes was 2.1% (v/v) with a yeast growth of $8.9*10^{-5}$. The amount of ethanol content increased with the increase in substrate concentration. The result of this research may serve as a guide to bio technologist on how best to produce bio ethanol from potato peel waste feed stock and other vegetables to give maximum yield.

Keywords: Research, Bio-ethanol, ethanol, hydrolysis, absorbance, spectrophotometer and potatoes

Introduction

Recently, development of alternative environmental-friendly sources of energy (renewable energy) has become a global concern, partly due to the emission of anthropogenic greenhouse gases (GHGs) such as CO2 from the production and use of fossil fuels, and partly because of concerns about energy security. The Inter-Governmental Panel for Climate Change (IPCC, 1992) has described GHG emissions, especially CO2, as responsible for the increase in global temperature (global warming) from 0.3 to 0.6°C witnessed over the last 10 decades (IPCC, 2001). The evidence that CO2 emissions have increased significantly within a very short time (three years of the first assessment report) and are still increasing supports the broadly accepted scientific consensus that GHG emissions have a relationship with climate change. Global warming is believed to underlie at least some adverse climate effects such as droughts, floods and desertification, which are currently impoverishing the globe, especially the developing countries (IPCC, 2007). In addition, energy security, relating to increasing dependency on imported energy supplies, especially in the context of consistent increase in the price of fossil fuel recorded in the recent past, has made great impact in securing public support for the global development of renewable energy. Fossil fuels are finite and non-renewable, which means that development of alternative sources of energy is a necessity, not an option. Nevertheless, socio-economic and developmental pressures to promote rural development and boost rural economies by creating rural jobs through linking agriculture to energy production are major drivers of renewable energy (specifically biofuels) development especially in developing countries. Biofuels receive substantial attention as a substitute for fuel in the transport sector on a global scale, due to a combination of factors as listed above. In addition, biofuels offer the major current alternative energy source for the current infrastructure and physical capital dependent on the internal combustion engine.

Bioethanol is produced from cellulosic biomass, including agricultural and forestry residues, portions of municipal waste, and herbaceous and woody crops. Bioethanol has become important with its powerful economic, environmental and strategic attributes over the past few decades. However, sweet potato (Ipomoea batatas L.) represents an important biomass resource for fuel alcohol production, because of its chemical composition and high density of starch, compared to other forms of biomass, and thus premise as an alternative bioresource for the production of ethanol

through fermentation (Rosillo-Calle and Walter, 2006.). World bioethanol production and consumption is currently dominated by the USA and Brazil, accounting for 84% of the world´s production, with 50.3b and 23.7b litres (GL) respectively. Europe and China rank the 3rd and 4th largest bioethanol producers according to RFA (2013). The EU is leading global biodiesel production, with Germany as the major producer using rape seed, while Malaysia and Indonesia rank 2nd and 3rd respectively, using palm oil (RFA, 2008). Among developing countries, Brazil is leading bioethanol production using sugarcane as feedstock, with China following, using maize, wheat, sweet sorghum, sugarcane, sweet potatoes, and cassava. Others include India, Thialand, Indonesia, Colombia using a range of different feedstocks, as in China (RFA, 2008).

Currently, Nigeria, like other developing countries, is investing to become a bioethanol producer, even though she is the tenth largest crude oil exporter to the world market as of 2008 (EIA, 2011). The motivation to invest in renewable energy, specifically bioethanol and biodiesel production, is primarily to: (1) generate more energy that will help Nigeria meet her local energy needs and avoid becoming a net energy importer in future; (2) diversify her fossil oil-dependent economy and revitalize her agricultural sector by exploiting the link between agriculture and bioenergy, and (3) contribute to the global efforts to reduce GHG emissions (CO2) and possibly accumulate carbon credit points for investing in and implementing clean development mechanisms. However, ethanol production from waste potato is a relatively new topic and limited research has been conducted about the utilization of potato waste for ethanol production. Fadel (2000), showed that different wastes of potato industry can be a carbon source for yeast during alcohol fermentation by studying waste from potato chips industry (98.67% total carbohydrate) and different potato cultivations (starch content in a range of 11.2% to over 19.3%), respectively. Fadel also reported that the highest alcohol concentration (13.2% *v*/*v*) was achieved after 24 h at 34 °C in a medium contained 25% *w*/*v* glucose with initial pH level of 5, using 0.075% urea as the sole nitrogen source, 0.05% orthophosphoric acid; 8% inoculum size (*v*/*v*) and agitation rate of 100 rpm (Fadel, 2000). Therefore, this current study was undertaken for further investigation of optimum liquefaction and saccharification conditions specifically for production of biofuel such as ethanol from waste potato mash.

Statement of Problem

Energy security is a priority for most countries especially Nigeria and other third- World countries since it occupies the center piece for national economic development and growth as well as infrastructural development. Of course, the present Nigerian population figure and its growth rate translate into an increasing pressure on the national resources (e.g. utilization of fossil oil reserves), infrastructures and utilities (energy for example). Thus, it is important for the country to commence research into finding out safer and renewable means of generating power so as to meet up with the increasing power demand of the country. Bio-fuels example Bio-ethanol has pulled much wave over the past decade as an alternative means of Energy-renewable at that however; in Nigeria, there is limited literature available for innovative bodies and other development agencies regarding the potential of some biomass as feedstock in the synthesis of biofuels.

Aim/Objectives of the Study

The study was undertaken to determine the production of bioethanol from potatoes peel using baker's yeast.

The specific objective of the study includes:

I. Find out and describe ethanol production

II. To investigate the effect of yeast concentration on ethanol obtained from potato peels

III. To investigate the effect of substrate concentration on ethanol

Description of Sampling Site

The site of collection for this study was within Ogwashi – Ukwu town with coordinates of 6°10'59.06" N latitude and 6°31'27.72"E longitude located west of the state capital Asaba, which is the headquarters for the Local Government Area, Aniocha South Delta State with a population of about 50,234 people.

Collection and preparation of sample

Fresh potato sample was collected from a local market located in Ihiala Local Government area Anambra State Nigeria. The fresh sample was washed thoroughly to remove the dust and other debris and peeled off. It was washed with 5% potassium permanganate solution and mixed with

distilled water and ethanol and allowed to dry at room temperature. The dried substance was grinded into fine powdered form using blender and kept for further analysis.

Method/ procedures used

Sample preparation for hydrolysis

During the course of the hydrolysis, 1ml of hydrochloric acid (HCL) was measured into 99ml of distilled water and was made up to 100ml of the available solution. Before further analysis was carried out, 2% of hydrochloric acid (HCL) was measured into 98ml of distilled water. Further, 35% of hydrochloric acid (HCL) was measured into 97ml of distilled water and kept for further analysis. Also 4% of hydrochloric acid (HCL) was measured into 96ml of water and kept for further analysis Then, 20g of substrate was weighed into a conical flask with the addition of 100ml of solution in step the first step above. The resultant solution was sterilized at a temperature of 100^0C for 15 minutes and allowed to cool. Then 1ml of substrate was measured into 1% of Dini-tro-salicyclic Acid with the addition of 12ml of distilled water after which the solution was sterilize for 10 minutes and allowed to cool. Finally, the absorbance at 540nm was read using spectrophotometer.

Substrate fermentation

For optimal fermentation of the substrate, 50g was weighed using a weighing balance and put in 7 different containers labeled (a-g). Then 250ml of distilled water was added to each container A-G and kept for further analysis.

Sugar fermentation

During the fermentation of sugar, 1cube of sugar was dissolved into 50ml of water. After which the solution was sterilize at 100^0C and allowed to cool. Further, 1g of *Saccharomyces cerevisiac* {baker's yeast} was introduced to the solution and allowed to ferment for 24hours. Then**,** 5ml of the fermented solution was added to the container labeled A-G. Each of the containers was later distilled for ethanol extraction.

Distillation procedure to obtain ethanol

To obtain ethanol, container A to G was distilled for ethanol extraction over the course of 7 days using distiller. For the first day, a container was emptied into the distillation bottle and the spectrophotometric reading was taken as well. The same process was repeated for the next six days using the rest of the containers.

Effect of Yeast concentration on Ethanol

To determine the effect of yeast concentration on ethanol production, 50g of the substrate was measured into a container with addition of 250ml of distilled water. Further, 2ml of sugar solution was added and allowed to ferment for four days. Secondly 4ml ml of the sugar solution was added to another 50g of the substrate and allowed to ferment for 4 days. The same process was repeated using 6ml and 8ml. The readings were tabulated after distillation

Effect of substrate concentration on ethanol production

To determine the effect of substrate concentration on ethanol production, 25g of the substrate was measured into a container and kept. Then 50g of substrate was measured into a container and kept. Further, 75 g of substrate was measured into a container and kept. Then 5ml of sugar solution was added to each container and allowed to ferment for four days. Then the readings were tabulated after distillation.

Data Analysis

The data were presented in tables and charts and the means statistically analyzed using GraphPad Prism version 9.0.0 by adopting Analysis of Variance (ANOVA). Values less 0.05 are considered significant.

Presentation of Data

Table 1: Hydrolysis of potato peel for reducing sugar

HCl concentration (%)	Reducing sugar mg/ml
1	3.5
2	6.0
3	6.3
4	5.8

Table 2. Time course of ethanol production from potato peel using baker's yeast

Time (hours)	Reducing sugar(mg/ml)	Ethanol concentration (%v/v)	Yeast growth

0	4.2	-	$6.0 *10^{-5}$
24	3.9	0.9	$8.0*10^{-5}$
48	3.6	1.8	$8.5*10^{-5}$
72	2.5	2.1	$8.9*10^{-5}$
96	2.1	1.9	$9.4*10^{-5}$
120	1.5	1.7	$7.0*10^{-4}$
144	1.2	1.4	$5.6*10^{-4}$
168	0.9	1.3	$4.0*10^{-4}$

Table 3: Effect of yeast concentration ethanol production

Yeast inoculum size (%)	Reducing sugar (mg/ml)	Ethanol concentration %v/v
2 ml	2.3	1.6
4 ml	1.7	1.9
6ml	2.2	1.3
8ml	1.6	1.1

Table 4: Effect of substrate concentration on ethanol yield

Substrate concentration	Reducing sugar (mg/ml)	Ethanol concentration (%v/v)
25	2.4	1.4
50	2.1	2.2
75	2.6	1.6

Summary of Results

The result of the investigation showed that the fermented potato waste produced a significant amount of ethanol. The volumetric production of ethanol varied according to the variations in yeast concentration and that of substrate. There was maximum ethanol production at 72 hours of incubation this result is in line with other stated literatures which suggests that potato peel could be a good feedstock for ethanol production

Table 1 showed the results of absorbance at 540nm of the reducing sugar used during the course of this project work. The mean absorbance observed using 1%, 2%, 3%, 4%, of the Hcl and 1ml of Dinitrosalicylic acid (DNS) are 3.5 (mg/ml), 6.0(mg/ml), 6.3(mg/ml), 5.8 (mg/ml). From the result, 3% of the acid solution had the highest reading of 6.3(mg/m).

Table 2 showed the result of the time course of ethanol production from potato peel using baker's yeast. There were over three scenarios where the yeast had maximum growth. At 120 hours, there was $7.0* 10^{-4}$ yeast growth which gave 1.7 % (v/V) of alcohol. The absorbance of the reducing sugar at this time was 1.5 (mg/ml). At 72 hours, there was $8.9 * 10^{-5}$ yeast growth and the reducing had an absorbance of 2.5(mg/ml) with an ethanol production of 2.1 % (v/v) of ethanol. This was recorded as the maximal ethanol production. Of the highest yeast growth of $9.4 * 10^{-5}$ was at 96 hours with absorbance of 2.1 (mg/ml) and ethanol production of 1.9 %(v/v). However, the least yeast growth was at 0 hours with 0% (v/v) ethanol production. The absorbance at the time was read as 4.4mg/ml. The ethanol yield of inoculated samples was measured at 24 hours interval and was recorded up to 7 days of incubation. Different concentrations of yeast were used as inoculums for fermentation. The fermentation time decreased dramatically with increase in yeast (*Saccharomyces cerevisiae*) concentration. Increasing *Saccharomyces cerevisiae* inoculum from 2ml, 4ml, 6ml to 8ml gave a dramatic increase in the rate of ethanol production. This is not entirely in line with the study of Mohamed and Reddy (1986) who reported that the increasing *Saccharomyces cerevisiae* inoculums gave a dramatic increase in the rate of ethanol production from potato starch. Ocloo and Ayernor (2010) also reported that the time taken for the fermentation to be completed was affected significantly by the yeast concentration. The results obtained supported the fact that the speed of fermentation depends on the yeast concentration, the higher

the concentration, the shorter the fermentation period required to achieve maximum alcohol yield (Kordylas, 1990). Ueda *et al.* (1981) reported 5 days fermentation period for raw cassava root starch using 15% yeast suspension. Togarepi *et al.* (2012) reported increased production rate rapidly with the increase in the amount of yeast up to the yeast concentration of 8 g/20 g fruit pulp. Beyond that point the rates no longer significantly increased which is evident in the current study. At this point the substrate becomes limiting and increasing the yeast amount does not increase the rate of reaction.

Table 4.3 showed the result for the effect yeast concentration on ethanol production. It was observed that 2ml of yeast gave 1.6% (v/v) of ethanol with absorbance of 2.3(mg/ml) from the reducing sugar. The highest ethanol production was observed with 4ml of yeast with an absorbance of 1.7 (mg/ml). However the lowest volume of ethanol was 1.1 %(v/v) with a yeast concentration of 8ml.

Table 4.4 showed the result of substrate concentration on ethanol yield. The highest ethanol (2.2%v/v) was produced using 50g of substrate. The absorbance of the reducing sugar was 2.1 mg/ml. The lowest ethanol (1.4% v/v) was obtained using 25g of substrate. From the above result: Table 4.4. Showed the effect of substrate concentration on ethanol yield a fermentation that was done by *Saccharomyses cerevisiae* over the course of 4 days. The significant fluctuation in reducing sugar with increase in substrate concentration was reported by Chen *et al.,* (2007) in their study. The highest concentration of 50g produced the highest ethanol (2.2 v/v). This was reported in the study of Pan *et al.,* 2006.

Conclusion

There are many methods for production of bioenergy from the renewable sources but they are very expensive and complex methods. In comparison to other literature the methodology of this present thesis seemed the simplest for ethanol production. However, the result of this work showed the possibility of using potato peels waste as an economical source for bioethanol production. From this investigation, ethanol production can be achieved by fermentation of *Saccharomyces cerevisiae.* It was also show that the inoculum size and substrate concentration can affect the yield of bio ethanol this would serve as a guide to bio technologist on how best to produce bio ethanol for maximum yield.

Recommendations

Future studies could:

1. Investigate the feasibility of scaling up ethanol production from potato peels.
2. Identify and optimize yeast strains for improved ethanol production.
3. Evaluate the economic and environmental feasibility of producing ethanol from potato peels.

References

Abouzied, M., and C. A., Reddy. (1986). Direct fermentation of potato starch ethanol by cocultures of Aspergillus niger and Saccharomyces cerevisiae. *Applied and Environmental Microbiology* **52(5):** 1055-1059.

Amutha, R. and Gunasekaran, P., (2001). Production of ethanol from liquefied cassava starch using co-immobilized cells of Zymomonas mobilis and *Saccharomyces diastaticus*. *Journal of Bioscience and bioengineering* **92 (6):** 560-564.

Amutha, R and Gunasekaran, P. (2001). Production of ethanol from liquefied cassava starch using co-immobilized cells of *Zymomonas mobilis* and Saccharomyces *diastaticus*. *Journal of Bioscience and bioengineerin*g **92 (6):** 560-564.

Balat, M., Balat, H. and ÖZ, C. (2008). Progress in bioethanol processing. *Progress in Energy and Combustion Science* **34** 5**):** 51-573.

Chen, M, Xia L, Xue P., (2007) Enzymatic hydrolysis of corncob and ethanol production from cellulosic hydrolysate. *International Biodeterioration and Biodegradation*;**59**: 85-89.

Demirbas, M. F. and Balat, M., (2006). Recent advances on the production and utilization trends of bio-fuels: A global perspective. *Energy Conversion and Management* **47 :** 2371-2381.

E.I.A. (2011). *Nigerian Energy Profile*. Washington, USA: Energy Information Administration p 45.

Fadel, M. **(2000)**. Alcohol production from potato industry starchy waste. *Egypt Journal of. Microbiology* **35**: 273–287.

Ghobadian B, Rahimi H, Hashjin TT and Khatamifar M. (2008). Production of bioethanol and sunflower methyl ester and investigation of fuel blend properties. *Journal of Agriculture Science and Technology* **10**: 225- 232.

John, R. (2006). Bioethanol production from wasted crops and crop residues *Journal on the World watch Institute and center for American progress* **12 :** 30-42

KIM, S. and DALE, B. E. (2004). Global potential bioethanol production from wasted crops and crop residues. *Biomass and Bioenergy* **26 :** 361-375.

Kim, S. and Dale, B. E. (2004). Global potential bioethanol production from wasted crops and crop residues. *Biomass and Bioenergy* **: 26** 361-375.

Kordylas, J.M. (1990). Processing and preservation of tropical and subtropical foods. Macmillan Education Limited, Houndmills pp. 105- 107.

Kroumov,A.D., M´odenes, A.N. and Araujo, M.C. (2006). Development of new unstructured model for simultaneous saccharification and fermentation of starch to ethanol by recombinant strain. *Biochemical Engineering Journal* **28**: 243 255.

LAL, R. (1995). The Role of Residues Management in sustainable agricultural systems. *Journal of Sustainable Agriculture* **5 :** 51 - 78.

LAL, R. (2005). World crop residues production and implications of its use as a biofuel. *Environment International***: 31** 575-584.

Liimatainen, H, Kuokkanen T and Kaarianen, J. (2004). Development of bioethanol production from waste potatoes. Oulu University Press: Oulu. p. 123- 129.

Mohamed, M. R. and Reddy, C.A. (1986). Direct fermentation of potato starch to ethanol by cocultures of *Aspergillus niger* and *Saccharomyces cerevisiae*. *Applied and Environmental Microbiology* **10** 1055-1059. .

Narde, A. (2009); Distilleries in India. *Sugar India Year Book* **5:** 401-432.

NNPCRED,(2007). Official Gazette of the Nigerian Biofuel Policy and Incentives. Nigerian National Petroleum Cooperation Renewable Energy Development. Pp12.

Ocloo, F. C. K. and Ayernor, G. S.(2010). Production of alcohol from cassava flour hydrolysate. *Journal of Brewing and Distilling* **5**:15-21.

Pan, X.J. Gilkes, N. Saddler, J. (2006). Effect of acetyl groups on enzymatic hydrolysis of cellulosic substrates. *Holzforschung* **94**: 851-861.

Rosillo-calle, f. and walter, A. (2006). Global market for bioethanol: historical trends and future prospects. *Energy for Sustainable Development*, **10**, 20-32.

Rosillo-Calle, F. and Walter, A. (2006). Global market for bioethanol: historical trends and future prospects. *Energy for Sustainable Development,* **10,** 20-32.

Rothman, H., Greenshields, R. and Rosillo, C. F. (1983). Energy from alcohol: the Brazilian experience, University Press of Kentucky.

Roukas T. (1994). Solid-state fermentation of carob pods for ethanol production. *Applied Microbiology and. Biotechnology* **41**: 296–301.

Song, L., Ma, F., Zeng Y., Zhang, X. and Yu, H. (2013). The promoting effects of manganese on biological pretreatment with Irpex lacteus and enzymatic hydrolysis of corn stover. *Bioresource Technology* **135**: 89–92.

Srichuwong, S. M., Fujirama, X., Wang, T., Seyama, R., Shiroma, M., Arakane, N., Mukojima, K. and Tokuyasu, T. (2009). Simultaneous saccharification and fermentation (SSF) of very high gravity (VHG) potato mash for the production of ethanol. *Biomass and Bioenergy* **33**: 890-898.

Togarepi,E., Mapiye, C., Muchanyereyi, N. and Dzomba, P. (2012). Optimization of fermentation parameters for ethanol Production from ziziphus mauritiana Fruit Pulp Using *Saccharomyces cerevisiae* . *International Journal of Biochemistry Research and Review* **2**: 60-69.

Ueda, S., Zenin, C.T., Monteiro.,D.A. and Park, Y.K. (1981). Production of ethanol from raw cassava starch by a non-conventional fermentation method. *Biotechnology and Bioengineering* **23**: 291-299.

Wang,P., Singh, V., Xue, H., Johnston, D.B., Rausch, K. D. and Tumbleson M.E. (2007). Comparison of raw starch hydrolyzing enzyme with conventional liquefaction and saccharification enzymes in dry-grind corn processing. *Cereal Chemistry* **84***(1):* 10-14.