Production of Ethanol from Ripe Plantain Peel Hydrolysate by *Saccharomyces cerevisiae*

Cyprian E. Oshoma*, Ese Okojie and Bunmi B. Omorotionmwan

Department of Microbiology, Faculty of Life Sciences, University of Benin, P.M.B. 1154 Nigeria

Abstract: *Aim*: Nigeria is amongst the largest *Musa paradisiaca* (Plantain) producing countries and ripe plantain peels are discarded as waste thus polluting the environment. Utilization of this agricultural waste to useful products like ethanol will be a welcome development. The influence of pretreatment on plantain peels hydrolysate for ethanol production by *Saccharomyces cerevisiae* and the effect of media supplementation were studied.

Methodology: The pretreatment methods used before carrying out fermentation of the hydrolysate were acid, steam and alkali. Parameters analyzed in all the hydrolysates and during fermentation were cell number, pH value, ethanol, glycerol concentration and inhibitory compounds using standard procedures.

Results: The results showed that acid pretreatment had the highest cell number, glycerol and ethanol concentrations of 27.30 ± 2.47 x 10^6 cells/mL, 4.43± 0.15 mg/mL and 12.31± 0.08 mg/mL respectively. Alkali pretreated plantain peel hydrolysate had the least values of 12.25 ± 1.77 x 10^6 cells/mL, 3.81 ± 0.10 mg/mL and 7.50 ± 0.21 mg/mL for cell number, glycerol and ethanol concentrations, respectively. There was a significant difference in ethanol production when comparing the acid pretreatment to the others ($P < 0.05$). The acid hydrolysate was optimized by supplementing the media and results showed that the cell number, glycerol and ethanol concentration slightly increased.

Conclusion: It was observed that acid hydrolysate of plantain peels can be utilized economically as a cheap substrate for bioethanol production and the yield can be enhanced through media supplementation.

Keywords: Plantain peel, pretreatment, fermentation, *Saccharomyces cerevisiae*, ethanol.

1. INTRODUCTION

Global reliance on fossil fuels for different energy uses is improving daily [1]. In recent times, research has been focused on generating alternative sources of energy due to the effect of fossil fuels on global warming as well as global depletion trends [2], in a bid to supplement the inevitable shortage of world's energy supply. In some developing countries, it is necessary to have other sources of energy, such as those derived from lignocellulosic biomass including agricultural residues [3]. Many countries have mandate on ethanol blend with gasoline to be used as fuel [1] hence, there is an increase in the need for ethanol to reduce fuel imports, boosting rural economies and improving air quality as ethanol derived from biomass is one of the major liquid transportation fuel that does not contribute to the greenhouse gas effect [2, 4]. Nigeria is amongst the largest producing countries of *Musa paradisiaca* (plantain) and ripe plantain peels are commonly discarded as waste contributing to environmental pollution [5]. According to Ayanwale *et al*. [6] reported that 2011 annual plantain production was 2.103 million tons. Presently, due to the fast growing societies and demand for ready to eat foods by the non-farming urban populations, the consumption of plantain has risen greatly in Nigeria. For people in most regions, plantain is their staple food more so a delicacy and ideal snack for people in other ecologies [5]. Huge wastes in the form of ripe peels are generated from industries that process plantain, however, in order to reduce the hazards caused by illegal dumping of these wastes, their utilization as fermentation substrates to produce valuable metabolites such as ethanol is a welcome development [7]. The utilization of ripe plantain peels as a substrate is attributed to its high carbohydrate content, source of vitamins and minerals (particularly sodium, potassium, calcium but low in nitrogen and magnesium) and low fat content [8]. Nevertheless, the nutrient deficiency of plantain peels can be improved by media supplementation. Thus, it is important to study the waste management and utilization of ripe plantain peels for ethanol production. Here, the influence of pretreatment on plantain peels hydrolysate and media supplementation on ethanol production by *Saccharomyces cerevisiae* was studied.

2. MATERIALS AND METHODS

2.1. Sample Collection and Preparation

Ripe plantain peels were collected from roasted plantain sellers in Benin City, Edo State. The peels were collected in bulk, cut into small pieces and washed with tap water until clean and dust free. It was sun dried for 14 d to remove the water content. After

*Address correspondence to this author at the Department of Microbiology, Faculty of Life Sciences, University of Benin, P.M.B. 1154 Nigeria;
Tel: +2349099727664; E-mail: cyprian.oshoma@uniben.edu

ISSN: 1814-8085 / E-ISSN: 1927-5129/19

drying, they were ground in electric grinder to get fine powder and were kept in a desiccator [9].

2.2. Yeast Propagation and Preparation of Inoculum

The yeast *Saccharomyces cerevisiae* was isolated from Cassava whey which was kept in the laboratory for 3 d. The whey was 10-fold serially diluted then plated onto the medium containing- Yeast extract (10.0 g); Peptone water (20.0 g); D-Glucose (20.0 g); Agar (15.0 g); Distilled water (1000 mL) and Chloramphenicol (0.03 %). The plates were incubated at room for 48 h. Colonies suggestive of yeasts were preliminarily identified by microscopic examination for cell shape and budding formation. The species was identified as *S. cerevisiae* by studying morphological, biochemical and physiological characteristics [10, 11]. The yeast propagation was carried out using yeast extract peptone D-glucose (YPD) medium (w/v): yeast extract (1 %); peptone (2 %); and glucose (2 %); pH (5.0). A single colony of *S. cerevisiae* was aseptically inoculated into 10 mL of YPD broth and incubated at 30 ^{o}C for 48 h. The 10 mL culture was transferred aseptically into 100 mL of YPD broth and incubated at 30 ^{o}C for 48 h, and was finally transferred aseptically into 400 mL of YPD broth and cultured for 48 h at 30 ^{o}C [9, 12]. At the end of the yeast propagation, the culture was transferred into twelve 10 mL centrifuge tubes and centrifuged at 3500 rpm for 5 min. The supernatant was discarded and pellets were washed twice with sterile distilled water and centrifuged each time of washing.

2.3. Pretreatment of Substrate for Fermentation

The milled plantain peels sample was weighed into 1 L conical flask containing distilled water at a ratio of 10 % (w/v). The substrate was hydrolyzed using steam pretreatment at a temperature of 121 ºC for 15 min and designated as steam pretreated plantain peel (SPP) medium. Acid hydrolysis of the substrate was carried out by using of 1 % (v/v) concentrated H_2SO_4 and autoclaved at 121 ^{o}C for 15 min designated as acid pretreated plantain peel (APP) medium while alkali hydrolysis of the substrate was carried out by using 3 M NaOH and autoclaved at 121 ^{o}C for 15 min designated as alkali pretreated plantain peel (AlPP) medium. After autoclaving, samples were allowed to cool to room temperature and filtered using muslin cloth. The pH of the hydrolysates was adjusted to 5.0 using 1.0 M H_2SO_4 and 1.0 M NaOH and was used as a fermentation medium for the production of ethanol [13].

From the filtrate hydrolysate of each pretreated methods, 700 mL was transferred into 1 L bioreactor bottles. The various hydrolysates were autoclaved at 121 ^{o}C for 15 min and media were prepared in triplicates.

2.4. Fermentation of Plantain Peel Hydrolysates

Fermentation was carried out using three hydrolyzed Plantain peel media according to modified method of Oshoma *et al*. [3]. The prepared inoculum size of 10^6 cells/mL of yeast suspension were aseptically transferred into each of the bottles. The bottles were sealed and equipped with a bubbling CO_2 outlet. All bottles were incubated at room temperature of 28±2 ^{o}C on an orbital shaker at a speed of 120 rpm for 11 d. Samples were collected for determination of total cell number using methylene blue staining method and pH values. At the same time samples were withdrawn and centrifuged (4000 rpm for 5 min). The supernatant was transferred into a tube and frozen at -20 ^{o}C. These were analyzed for concentrations of furfural, acetic acid, glycerol and ethanol using Gas Chromatography. All fermentations were carried out in triplicate.

The effect of supplementation on ethanol production using the pretreated hydrolysate with the highest ethanol yield was investigated. The media used were designated as supplemented plantain peel (SPP) medium with composition Magnesium chloride (0.1 g), Calcium carbonate (0.2 g), Ammonium sulphate (0.2 g), Manganese sulphate (0.0016 g) and Ferrous sulphate (0.001 g) made up to 1 litre with pretreated plantain peel hydrolysate. The second, Unsupplemented Plantain peel (UPP) medium had only the 1 litre pretreated plantain peel hydrolysate. The third uninoculated supplemented plantain peel (USPP) medium. The pH was adjusted to 5.0 using 1.0 M H_2SO_4 and 1.0 M NaOH [8] and 700 mL was transferred into I L bioreactor bottles. The various media were autoclaved at 121^{o}C for 15 min. The medium in each flask was inoculated with 500 uL of *S. cerevisiae* inoculum (10^6 spores/ mL). The media were left to ferment on an orbital shaker at 120 rpm at temperature of 28 ± 2 ^{o}C for 7 d. At the end of fermentation media were analyzed for cell number, pH, concentrations of furfural, acetic acid, glycerol and ethanol

2.5. Analytical Techniques

2.5.1. Total Cell Number of Yeast Analysis

The total cell number was determined with a haemocytometer according to the method of Sami *et al*.

[14]. Methylene blue 0.01 % (w/v) was dissolved in sodium citrate 2 % (w/v) solution. Yeast broth at various sampling time point was diluted using sterile water. The cell suspension was mixed with methylene blue solution in a ratio 1:1. The solution was examined microscopically and total cells counted using Neubauer haemocytometer (depth 0.1 mm, area 0.0025 mm^2, Marienfield, Germany) and compound light microscope (Zeiss, Oberkocken, Germany) at ×40 objective lens.

2.5.2. Gas Chromatography Analysis

The concentrations of furfural, acetic acid, glycerol and ethanol were analyzed using headspace Gas Chromatography according to the modified method of Wang *et al.* [15]. The GC cycle time was set at 13.5 min with a constant helium flow rate of 3 mL/min. The injection port temperature was maintained at 90 °C with a 5:1 split injection of the headspace and a septum purge flow of 3 mL/min. The initial GC oven temperature of 35 °C was held for 2 min and thereafter ramped at 25°C/min to a final temperature of 250 °C, which was held for 8.4 min.

2.5.3. Statistical Analysis

Analysis of variance and mean difference were calculated using SPSS version 20. Graphs and charts were constructed using Microsoft Excel 2007 version [16]. Statistically, differences were considered significant when $p < 0.05$.

3. RESULTS AND DISCUSSION

It was observed that the cells in the various pretreated hydrolysate had a normal growth curve as the cell number reading continued to rise, peaking at day 7 with acid having the highest cell number (27.30 ± 2.47 x 10^6 cells/mL), followed by steam (16.00 ± 2.12 x 10^6 cells/mL) and alkali having the lowest (12.25 ± 1.60 x 10^6 cells/mL) afterwards dropping till day 11 (23.30 ± 1.77 x 10^6 cells/mL, 11.50 ± 2.07 x 10^6 cells/mL, 9.50 ± 1.78 x 10^6 cells/mL for acid, steam and alkali hydrolysate, respectively) as presented in Figure **1**. The variation in cell growth in the various hydrolysates could be as a result of the varying concentration of fermentable sugars released during various pretreatment, as the fermentation process is significantly dependent on the effectiveness of sugar transporters of *S. cerevisiae* cells on translocating different sugars across the cell membrane on which they grow on [7].

The pH changes per day during the fermentation process are presented in Table **1**. Generally, the pH ranged from 5.0 on day 0 to 5.25 on day 7. The acid and steam hydrolysate had a lower pH values (5.0 - 4.95) compared to alkali hydrolysate which had an increase in pH range of (5.0 - 5.25). Alterations in the pH of the environments can be conducive for the biocatalyst resulting in improved metabolic activity and performance or such alterations could be limiting and achieve undesired effects [17]. The pH is critical to the physiology and metabolism of microorganisms. In this study, there decline in pH level from an initial pH of 5.00 ± 0.00 on day 0 to 4.97 ± 0.00 and 4.85 ± 0.05 for acid and steam pretreated hydrolysates on day 11 respectively. This is as a result of increasing metabolic rates evident in high cell counts and metabolite yields hence, the pH of the medium decreases. Yeast grows well in acidic conditions which help to control

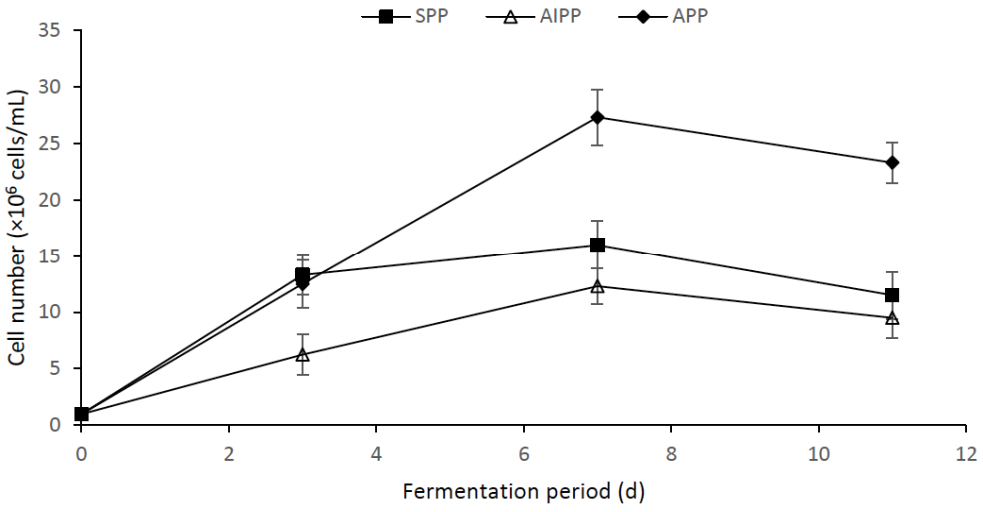

Figure 1: Cell number in various pre-treated hydrolysates during fermentation. (SPP - steam pretreated plantain peel medium, APP - acid pretreated plantain peel medium and AlPP – alkali pretreated plantain peel medium).

Table 1: Changes in pH of various pre-treated hydrolysates during fermentation

Pretreatment	Fermentation period (D)			
	0	3	7	11
APP	5.00 ± 0.00	5.05 ± 0.05	4.95 ± 0.05	4.97 ± 0.00
SPP	5.00 ± 0.00	4.95 ± 0.05	4.95 ± 0.05	4.85 ± 0.05
AlPP	5.00 ± 0.00	5.15 ± 0.05	5.25 ± 0.05	5.45 ± 0.05

contamination from competing microbes while promoting relatively high ethanol productivity [18].

The glycerol concentration obtained during fermentation on day 0 and 7 from the three pretreated hydrolysates is shown in Figure **2**. During the fermentation period, glycerol concentration of the three hydrolysates were found to increase gradually from the first day to the seventh day with the acid pretreated hydrolysate having the highest concentration of 4.43 ± 0.15 mg/mL, followed by steam pretreated hydrolysate 4.12 ± 0.16 mg/mL, while the least was obtained from alkali pretreated hydrolysate 3.81 ± 0.10 mg/mL. In *S. cerevisiae*, glycerol plays important roles in physiological processes such as combating osmotic stress, managing cytosolic phosphate levels and maintaining the NAD^+/NADH redox balance by reoxidizing surplus NADH, formed in the synthesis of biomass and secondary fermentation products, to NAD [19]. The amount of glycerol produced by the yeast depends on its population and the stress level it is experiencing in its environment [20]. Hence, alkali pretreated media setups with higher osmotic stress levels due to excessive salt production and the presence of unutilized reducing sugars had the lowest glycerol concentration on day 7. This also had the lowest cell concentration. Acid pretreated media with higher cell concentration and sugar consumption rate produced more ethanol and glycerol compared to steam pretreated medium due to ethanol toxicity.

Figure **3** shows the concentration of ethanol in the fermentation setup. On day 7 the acid plantain peel hydrolysate had the highest yield (12.31 ± 0.08 mg/mL) followed by the steam hydrolysate (9.68 ± 0.51 mg/mL) and least was the alkali hydrolysate (7.50 ± 0.21 mg/mL). The observations from this study showed that acid, steam and alkali pretreatments successfully produced fermentable sugars from ripe plantain peel for ethanol production of which acid pretreatment having the highest fermentation efficiency produced the highest amount of ethanol. This is as a result of a relatively high favorable condition for the yeast cells in the acid pretreated hydrolysate to adequately utilize sugars present for ethanol productivity.

Acetic acid, an inhibitor formed primarily by hydrolysis of acetyl groups of hemicellulose had low concentration on day 0 and increased on day 7 in the course of fermentation (Figure **4**). Acid hydrolysate had the highest concentration of acetic acid on day 7 and the least was from alkali plantain peel hydrolysate and

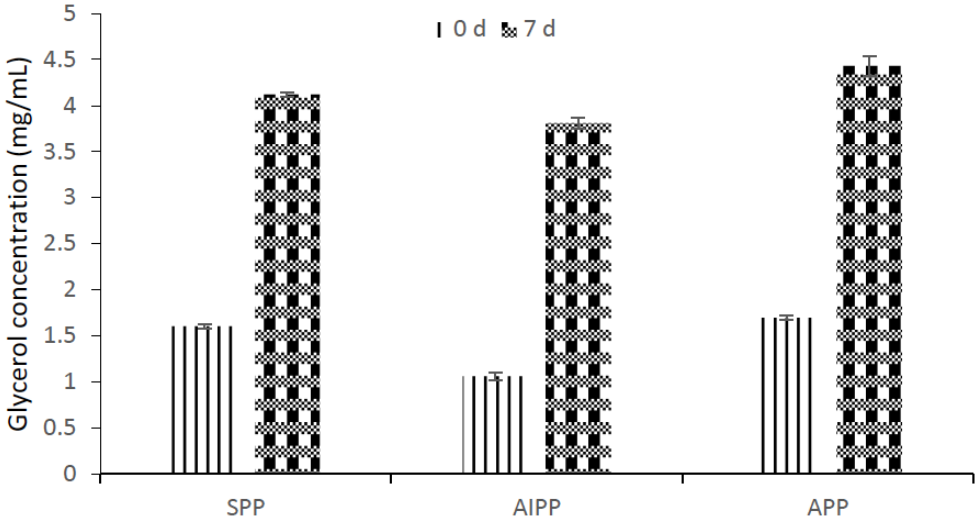

Figure 2: Glycerol concentration (mg/mL) in various pre-treated hydrolysates on day 0 and 7. (SPP - steam pretreated plantain peel medium, APP - acid pretreated plantain peel medium and AlPP – alkali pretreated plantain peel medium).

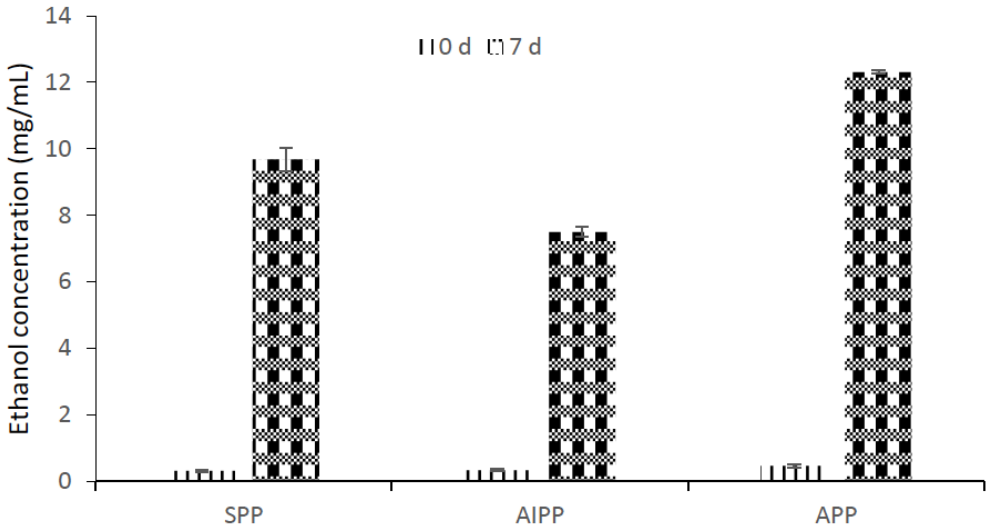

Figure 3: Ethanol concentration (mg/mL) in various pre-treated hydrolysates on day 0 and 7. (SPP - steam pretreated plantain peel medium, APP - acid pretreated plantain peel medium and AlPP – alkali pretreated plantain peel medium).

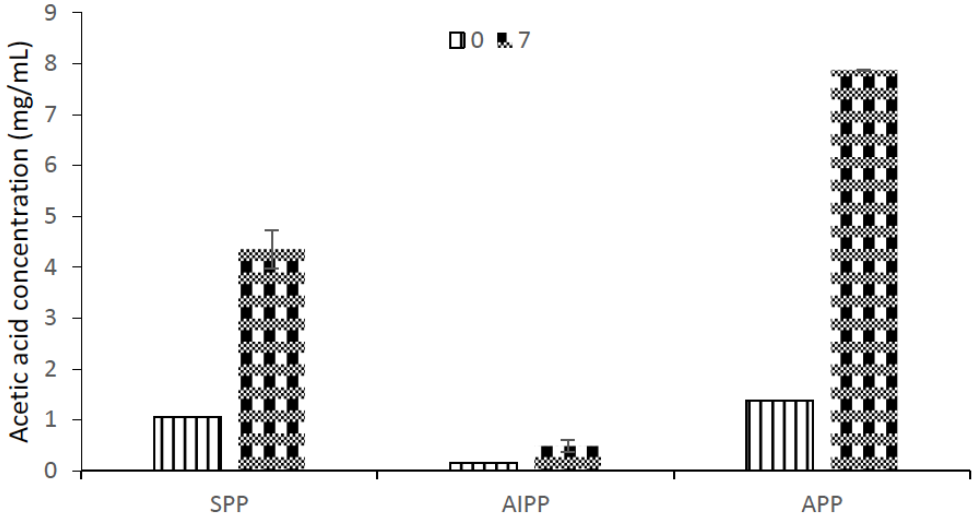

Figure 4: Acetic acid concentration (mg/mL) in various pretreated hydrolysates on day 0 and 7. (SPP - steam pretreated plantain peel medium, APP - acid pretreated plantain peel medium and AlPP – alkali pretreated plantain peel medium).

the values were 7.87 ± 0.02 and 0.48 ± 0.04 mg/mL respectively. Furfural is an inhibitor produced from decomposition of the pentose sugars. Its concentration reduced slightly on day 7 from the initial concentration on day 0 during the course of fermentation (Figure **5**). Acid plantain peel hydrolysate had the highest furfural concentration and alkali plantain peel hydrolysate had the least on day 7, the values were 1.180 ± 0.001 mg/mL and 0.104 ± 0.01 mg/mL respectively.

Production of inhibitors has become the bane of ethanol fermentation in recent times. These inhibitors result from the pretreatment method employed. The results obtained shows that alkali pretreatment happens to produce the least amount of inhibitors. However, a significant disadvantage of alkaline pretreatment is the neutralization reaction that takes place in the medium thereby yielding salts which are inhibitory to microbial growth. In addition, the treatment of large amount of salts has become a challenging issue for alkaline pretreatment [21], and can be a limiting factor for the cell growth. It was observed that the concentration of furfural reduced in all pretreated hydrolysates on day 7, this is because the yeast cells are able to carry out *in-situ* detoxification of furfural converting it to its corresponding alcohol less toxic [22]. Acetic acid has a minimal toxic effect on *S. cerevisiae* and its effect is attributed to the undissociated form. The acid dissociation in the yeast cytosolic cell leads to a decrease in the intracellular pH. The decrease in the

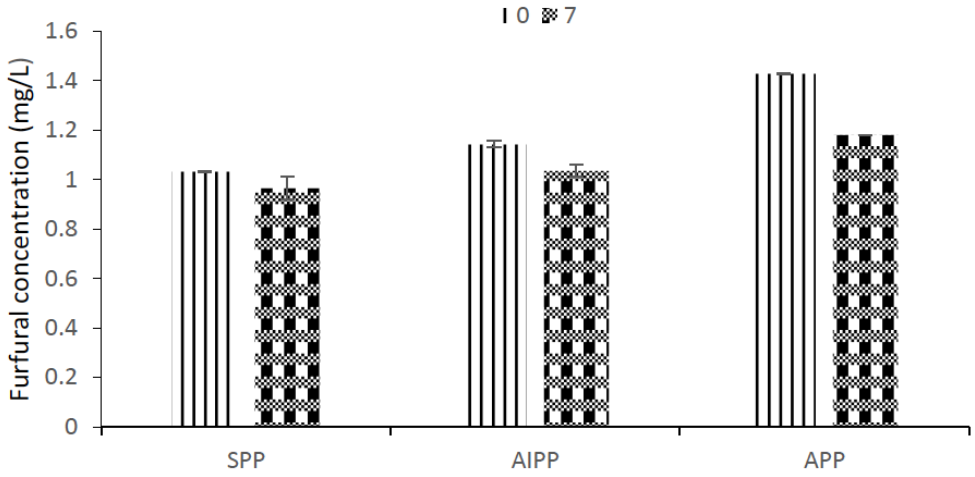

Figure 5: Furfural concentration (mg/mL) in various pretreated hydrolysates on day 0 and 7. (SPP - steam pretreated plantain peel medium, APP - acid pretreated plantain peel medium and AlPP – alkali pretreated plantain peel medium).

intracellular pH t may lead to increased ethanol yield but will reduce biomass formation as a consequence of the cell's attempt to maintain a constant cytosolic pH by pumping out protons through the plasma membrane ATPase [23, 24].

The results from the various pretreated media showed that acid pretreated media had the highest yield of ethanol which was then optimized by supplementing the media to get a higher yield of ethanol. The cell number, ethanol produced and other parameters analyzed in the supplemented and unsupplemented media after 7 d of fermentation is presented in Table **2**. Supplemented acid hydrolysate had the highest cell number, ethanol and glycerol concentration of 79.0 ± 5.66 x 10^6 cells/mL, 14.96 ± 0.26 mg/mL and 6.96 ± 0.60 mg/mL as compared to the unsupplemented acid hydrolysate (40.8 ± 7.42 x 10^6 cells/mL, 12.43 ± 0.09 mg/mL and 6.33 ± 0.13 mg/mL) respectively. In comparing the media for cell number, ethanol and glycerol produced showed a significant difference statistically (P < 0.05). Other agro-wastes biomass been reported to have ethanol concentration of 4.64 g/L from jackfruit rinds [7] and 7.4 g/L from coffee pulp [25].

Optimization of acid pretreated hydrolysate with mineral supplements was employed in this study. These supplements that are constituents of salts which supports the cell in providing nitrogen for biomass yield, buffering the medium pH and managing osmotic pressure. Maintaining the initial pH of 5.0, the same trend of gradual decrease with time was observed and by day 7, supplemented acid pretreated hydrolysate had a pH value of 4.45 ± 0.05 which is as a result of increasing metabolic rates evident in high cell counts and metabolite yields. Yeast grows well in acidic conditions which help to control contamination from competing microbes while promoting relatively high ethanol productivity [18].

The fermentable sugars from the Plantain peel hydrolysate were supplemented with minerals for *S. cerevisiae* growth thereby producing ethanol. Enzymatic activity and ethanol production of *S.*

Table 2: Summary of cell number, ethanol and inhibitors results under each hydrolysate after seven (7) d of fermentation

Parameters	Hydrolysate		
	SPPH	UPPH	USPPH
Cell number (x 10^6 cells/mL)	79.0 ± 5.66	40.8 ± 7.42	0
Ethanol (mg/mL)	14.96 ± 0.26	12.43 ± 0.09	0.44 ± 0.11
Glycerol (mg/mL)	6.96 ± 0.60	6.33 ± 0.13	0.95 ± 0.09
Acetic acid (mg/mL)	8.05 ± 0.10	7.72 ± 0.18	1.41 ± 0.08
pH	4.45 ± 0.05	4.65 ± 0.05	5.05 ± 0.05
Furfural (mg/mL)	1.08 ± 0.01	1.16 ± 0.07	1.18 ± 0.12

cerevisiae were reported to be superior to other yeast strains using different agricultural waste [26]. Agricultural wastes are usually low in some key nutrients (such as nitrogen) essential for optimal yeast growth and stress tolerance [27, 28]. Supplementation of plantain peel hydrolysate with nitrogen source and other trace elements for yeast metabolism improve the growth thus, increase in ethanol productivity. The low cell number observed from UPP medium could be due to nutrient limitation particularly nitrogen sources required for yeast growth and ethanol formation [29]. Therefore, media supplementation with nitrogen source other trace elements are necessary for yeast cell growth during fermentation, this in turn imparted the cell tolerance to stresses such as ethanol and inhibitory compounds [30].

Supplemented acid pretreated media had higher cell concentration and produced more glycerol as compared to unsupplemented media. This is because of the increased metabolic activities and stress level in the media due to the salts present in the hydrolysate as the organisms strive to survive as well as ferment the substrate [31]. It was also observed that the ethanol yield increased in the supplemented acid hydrolysate. In contrary, Boudjema *et al*. [32] noted that supplementation of cheese whey with glucose produced an ethanol with concentration of 17.06 g/L which is slightly above the concentration reported in this investigation.

4. CONCLUSION

This study suggests that ripe plantain peel biomass can be utilized using acid pretreatment method for ethanol production by *Saccharomyces cerevisiae* with a pH of the fermentation medium ranging from 4.5-5.0. However, supplementation using inorganic nitrogen sources and mineral components can be used to enhance cell growth, sugar utilization and ethanol production. Considering the urgent and simultaneous need for energy coupled with the condition that these need be met without compromising the environment, ethanol production from ripe plantain peel is a viable option, holding great potential for acquiring a sustainable system.

ACKNOWLEDGEMENTS

The authors are grateful to the Director, National Centre for Energy and Environment (Energy Commission of Nigeria), University of Benin, Benin City, Nigeria for providing laboratory facilities.

CONFLICT OF INTEREST

The authors declare no conflict of interest in this work

AUTHORS' CONTRIBUTIONS

CEO designed the experimental plan and supervised the whole study. EO carried out all the experiments, analyzed the data and wrote the manuscript. BBO contributed in the preparation of the manuscript and data analysis.

REFERENCES

[1] Waghmare AG, Arya SS. Utilization of unripe banana peels waste as feedstock for ethanol production. Bioethanol 2016; 2(1): 146-156. https://doi.org/10.1515/bioeth-2016-0011

[2] Patel H, Patel A, Surati T, Shah G. Potential use of banana peels for the production of fermented products. Int J Environ Sci Develop 2012; 9(1): 1-7.

[3] Oshoma CE, Greetham D, Louis EJ, Smart KA, Phister TG, Powell C, Du C. Screening of Non-*Saccharomyces cerevisiae* Strains for Tolerance to Formic Acid in Bioethanol Fermentation. Plos One 2015; 10(8): 0135626. https://doi.org/10.1371/journal.pone.0135626

[4] Tesfaw A, Assefa F. Current Trends in Bioethanol Production by *Saccharomyces cerevisiae*: Substrate, Inhibitor Reduction, Growth Variables, Coculture, and Immobilization. Int. Scholarly Research Not 2014; Article ID 532852, 11 pages. https://doi.org/10.1155/2014/532852

[5] Akinyemi SOS, Aiyelaagbe IOO, Akyeampong E. Plantain (*Musa* spp.) cultivation in Nigeria: a review of its production, marketing and research in the last two decades. Acta Horticulture 2010; 548: 211-219. https://doi.org/10.17660/ActaHortic.2010.879.19

[6] Ayanwale AB, Fatunbi AO, Ojo M. Innovation Opportunities in Plantain Production in Nigeria. Guide Book 1. Forum for Agricultural Research in Africa (FARA), Accra Ghana 2016.

[7] Suhas VB, Arun P, Naveen S, Ashok K. Production of Bioethanol from fruits rinds by Saccharification and fermentation. Int J Sci Res Eng Technol 2013; 2(6): 362-365.

[8] Ojure MA, Quadri JA. Quality evaluation of noodles produced from unripe plantain flour using xanthan gum. Int J Res Rev Appl Sci 2012; 13(3): 740-752.

[9] Ignale S, Joshi SJ, Gupte A. Production of Bioethanol using agricultural waste: Banana pseudo stem. Brazil J Microbiol 2014; 45(3): 885-892. https://doi.org/10.1590/S1517-83822014000300018

[10] Kreger van Rij, NJW. The Yeasts-A taxonomic study. Third revised and enlarged edition. Elsevier, Amsterdam 1984

[11] Boekhout T, Kurtzman CP. Principles and methods used in yeast classification, and overview of currently accepted genera. In: Wolf K, editor. Non-conventional Yeasts in Biotechnology: A Handbook. Springer Verlag, Berlin, Heidelberg 1996. https://doi.org/10.1007/978-3-642-79856-6_1

[12] Nuwamanya E, Chiwona-Karltun L, Kawuki RS, Baguma Y. Bioethanol production from non-food parts of Cassava (*Manihot esculenta* Crantz) AMBIO 2012; 41: 262-270. https://doi.org/10.1007/s13280-011-0183-z

[13] Vaitheki S, Deepa B. A Comparative Study on the Production of Bioethanol from Individual and Mixed Fruit Wastes. Imperial J Interdiscipl Res 2016; 2(5): 652-657.

[14] Sami M, Ikeda M, Yabuuchi S. Evaulation of alkaline methylene blue staining method for yeast activity determination. J Ferment Bioeng 1994; 87: 212-216. https://doi.org/10.1016/0922-338X(94)90292-5

[15] Wang M, Choong Y, Su N, Lee M. A Rapid Method for Determination of Ethanol in Alcoholic Beverages Using Capillary Gas Chromatography. J Food Drug Analy 2003; 11(2): 133-140.

[16] Ogbeibu AE. Biostatistics: A Practical Approach to Research and Data Handling (2nd edition). Mindex Publishing Company, Edo, Nigeria 2015; p. 285.

[17] Clarence SY, Sunny EI, Emmanuel IU. Temperature optimization for bioethanol production from corn cobs using mixed yeast strains. J Biol Sci 2010; 10: 103-108. https://doi.org/10.3844/ojbsci.2010.103.108

[18] Benerji DSN, Anyanna C, Rajini K, Rao BS, Banerjee DRN. Studies on physico-chemical and nutritional parameters for the production of ethanol from Mahua Flower (*Madhuaindica*) using *Saccharomyces cerevisiae* 2090 through submerged fermentation. J Microb Biochem Technol 2010; 2(2): 46-50. https://doi.org/10.4172/1948-5948.1000022

[19] Scanes KT, Fang HY, Wang ZX, Chen DZ, Jin HR. Glycerol production by a novel osmotolerant yeast *Candida glycerinogenes*. Appl Microbiol Biotechnol 2001; 55: 686-692. https://doi.org/10.1007/s002530100596

[20] Taherzadeh MJ, Adler L, Liden G. Strategies for enhancing fermentative production of glycerol-A review. Enzyme Microbiol Technol 2002; 3(1): 53-66. https://doi.org/10.1016/S0141-0229(02)00069-8

[21] Zheng Y, Pan Z, Zhang R. Overview of biomass pretreatment for cellulosic ethanol production. Int J Agric Biol Eng 2009; 2: 51-68.

[22] Ask M, Maurizio B, Valeria M, Lisbeth O. The influence of HMF and Furfural on Redox-balance and Energy state of Xylose-utilizing *Saccharomyces cerevisiae.* Biotechnol Biofuels 2013; 6: 1-13. https://doi.org/10.1186/1754-6834-6-22

[23] Almeida JRM, Modig T, Peterson A, Hahn-Hagerdal B, Liden G, Gorwa-Grauslund MF. Increase tolerance and conversion of inhibitors in lignocellulosic hydrolysates by *Saccharomyces cerevisiae*. J Chem Technol Biotechnol 2007: 82(4): 340-349. https://doi.org/10.1002/jctb.1676

[24] Jonsson LJ, Alriksson B, Nilvebrant N. Bioconversion of lignocellulose: inhibitors and detoxification. Biotechnol Biofuels 2013; 6(16): 1-10. https://doi.org/10.1186/1754-6834-6-16

[25] Kefale A, Redi M, Asfau A. Potential of bioethanol production and optimization test from agricultural waste: The case of wet coffee processing waste (pulp). Int J Ren Energy Res 2012: 2(3); 446-450.

[26] Akponah E, Akpomie OO. Optimization of bio-ethanol production from cassava effluent using *Saccharomyces cerevisiae*. Afri J Biotechnol 2012; 11(32): 8110-8116. https://doi.org/10.5897/AJB12.3920

[27] Kapu NS, Piddocke M, Saddler JN. High gravity and high cell density mitigate some of the fermentation inhibitory effects of softwood hydrolysates. AMB Express 2013; 3: 15. https://doi.org/10.1186/2191-0855-3-15

[28] Linde M, Galbe M, Zacchi G. Steam pretreatment of acid-sprayed and acid soaked barley straw for production of ethanol. Appl Biochemist Biotechnol 2006; 129-132: 546-562. https://doi.org/10.1007/978-1-59745-268-7_44

[29] Hamadi S, Muruke MH, Hosea KMM. Optimization of fermentation parameters for production of ethanol from Coffee Pulp Waste using *Pichia anomala* M4 Yeast isolated from Coffee environment in Tanzania. Int J Environ Sci 2014; 3 (4): 255-262.

[30] Yue G, Yu J, Zhang X, Tan T. The influence of nitrogen sources on ethanol production by yeast from concentrated sweet sorghum juice. Biomass and Bioenerg 2012; 39: 48-52. https://doi.org/10.1016/j.biombioe.2010.08.041

[31] Tomas-Pejo E, Ballesteros M, Oliva JM, Olsson L. Adaptation of the xylose fermenting yeast *Saccharomyces cerevisiae* F12 for improving ethanol production in different fed-batch SSF processes. J Ind Microbiol Biotechnol 2010; 37: 1211-1220. https://doi.org/10.1007/s10295-010-0768-8

[32] Boudjema K, Fazouane-Naimi F, Hellal A. Optimization of the bioethanol production on Sweet Cheese Whey by Saccharomyces cerevisiae DIV13-Z087C0VS using Response Surface Methodology (RSM). Rom Biotechnol Let 2015: 20(5): 10814-10825.

Received on 07-11-2019 Accepted on 21-11-2019 Published on 02-12-2019

https://doi.org/10.29169/1927-5129.2019.15.06