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PRODUCTION OF BIOETHANOL FROM PLANTAIN PEELS USING *Aspergillus* spp. AND *Saccharomyces* spp.

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ABSTRACT

The study investigated the production of bioethanol from ripe and unripe plantain peels. Aqueous extracts of plantain peels were obtained and inoculated with Saccharomyces cereviae and Aspergillus spp. The set-ups were incubated for a period of eight days. The physicochemical properties (reducing sugar, cell density, pH, temperature and ethanol yield) of the set-ups were assessed at two day interval for a period of eight days during the fermentation period. There was a consistent decrease in the amount of reducing sugar with values between 0.412 to 0.078 g/mol. There was a corresponding increase in ethanol yield with values between 0.30 to 7.80 ml. Cell density increased between 0.221 to 0.789 g/mol. pH decreased with values between 6.22 to 3.85 as the period of fermentation increased. Temperature increased with values between 27 to 34^{O}C in the course of fermentation. The highest ethanol yield was obtained in the set-up containing ripe plantain peel and Saccharomyces cerevisae with a final yield of 44 ml compared to the set-ups containing ripe plantain peel, Aspergillus and Saccharomyces with a final yield of 42 ml, unripe plantain peels, Aspergillus and Saccharomyces with a final yield of 32 ml, unripe plantain peels and Saccharomyces with a final yield of 30 ml, ripe plantain peel with a final yield of 15 ml, and unripe plantain peel with a final yield of 9 ml. Ethanol can be produced efficiently by controlled fermentation technique from plantain peel waste using Saccharomyces cerevisiae and Aspergillus spp.

Key words: Bioethanol, *Saccharomyces cerevisiae*, *Aspergillus* spp., Plantain Peel and Fermentation

INTRODUCTION

Bioethanol can be used as biosolvent in the laboratory, pharmaceutical, cosmetic, medical and biomedical industries (Kimberly *et al*., 2010). It has also been found useful, as an alternative fuel for engines. In view of the rising demand for ethanol, there has been increasing interest in searching for alternative sources for its production. (Kimberly *et al*., 2010). Biofuel has been gaining momentum in terms of research and development. Since there are various factors such as recent rise in oil prices, increase in demand of fossil fuel, depletion of mineral oil reserves , demand of the energy increases with the increase of the world population and urbanization. The negative impacts of fossil fuel on the

environment and the unstable oil market are the major factors that lead to the constant search for alternative fuels (Hossain *et al*., 2011). Efforts are more concentrated on using cheap and abundant raw materials (Chand and Venkateswar, 2009) for ethanol production and several forms of biomass resources exist (starch or sugar crops, weeds, oils plants, agricultural, forestry and municipal wastes) but of all biomass, cellulose based resources represent the most abundant global source (Ashiru, 2005). No other sustainable option for production of transportation fuels can match ethanol made from lignocelluloses biomass with respect to its dramatic environmental, economic and infrastructure advantages. The lignocellulosic materials include agricultural residues, municipal solid wastes (MSW), pulp mill refuse, switch grass and lawn, garden wastes (Pimentel and Patzek, 2005). Simultaneous saccharification and fermentation of lignocelluloses to alcohol by Baker's yeast and a thermorolerant *K. marxianus* using wastes as substrate has been reported (Itelima, 2013), while simultaneous saccharification and fermentation of yam peel to ethanol by culture of *Aspergillus niger* and *Saccharomyces cerevisiae* was reported by Jimoh *et al*., (2009). Ethanol an important biofuel, having high calorific value has the added advantage of being less polluting than most sources of energy that are in use today. Reports available suggest that previous natural substrates for ethanol production via saccharification include sugarcane bagasse, wheat straw, corn and softwood (Sharma and Mishra, 2015). Banana peels has also been a useful substrate in the production of bioethanol. Banana is one of major constitute of the principal food resources in the world and occupy the fourth world rank of the most significant foodstuffs after rice, corn and milk (INIBAP, 2002). Most of the fruit peels/residues are dried, ground, pelletized, and sold to the feed manufacturers at a low price which is not considered a highly viable proposition (Sharma and Mishra, 2015). As per the FAO statistics, India is the largest producer of banana in the world and accounts for nearly 30% of the total world production of banana. Though banana peel is a fruit residue, it accounts for 30-40% of the total fruit weight (Emaga *et al*., 2008) and contains carbohydrates, proteins, and fiber in significant amounts. Banana peels are readily available agricultural waste that is under-utilized as potential growth medium for yeast, despite their rich carbohydrate content and other basic nutrients that can support yeast growth (Brooks, 2008). Since banana peels contain lignin in low quantities, it could serve as a good substrate for production of value-added products like ethanol (Sharma and Mishra, 2015). This study is therefore aimed at producing ethanol from plantain peels using *Saccharomyces cerevisae* and *Aspergillus* sp.

MATERIALS AND METHOD

Sample Collection/Preparation

Ripe and unripe plantains were obtained from Choba market. The plantain peels were removed and used as substrate as described below.

Extraction / pretreatment of substrate

Fifty grams of dry milled peel was homogenized with 50ml of 100°C boiled water and was allowed to stand for 24hours after which the liquid was extracted from the mixture using clean muslin cloth, the extract was heat sterilized as a pretreatment method.

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Microorganisms
Saccharomyces cerevisiae and *Aspergillus* spp. were isolated and maintained in Potato Dextrose agar.

Media Preparation: Sabouraud Dextrose Agar (SDA) and Sabouraud Dextrose Broth were prepared according to manufacturer's instruction.

Development of inocula: Yeast inoculum **(***Saccharomyces cerevisae*) and *Aspergillus* spp. were developed and scaled up according to the methods of Ogbonna *et al*. (2013)

Experimental set-up

1. Unripe plantain peel + *Aspergillus* and *Saccharomyces* spp.(set A)
2. Unripe plantain peel + *Saccharomyces* spp.(Set B)
3. Ripe plantain peel + *Saccharomyces* spp.(Set C)
4. Ripe plantain peel + *Aspergillus* and *Saccharomyces* spp.(Set D)
5. Unripe plantain peel (Control) (Set E)
6. Ripe plantain peel (Control). (Set F)

Fermentative production of bioethanol by Simutaneous saccharification
For fermentative production of bioethanol using the shaking method, co-cultures of yeast (*Saccharomyces cerevisiae)* and *Aspergilus* spp. were employed. Five milliliters of 0.87 optically dense *Saccharomyces cerevisiae* culture and 3 days old sample of *Aspergillus* spp (5ml), were introduced into 25ml of crude plant extract, sealed with aluminum foil, placed on a shaker at room temperature for eight days. Forty eight hours samples were withdrawn from the culture broth and taken for the estimation of bioethanol produced according to the methods of (Ogbonna and Okoli, 2010).

Physicochemical Analysis:
Temperature Determination
The temperature range was monitored by inserting a unicon thermometer.

pH range
The pH was determined using a labtech digital pH meter (Photic 20).

Determination of cell density
Cell density was measured using a UV spectrophotometer.

RESULTS
Physicochemical properties obtained during the fermentation process
The physicochemical properties obtained at 2-day intervals for a period of eight (8) days during the fermentation period are shown in tables1, 2, 3 and 4 below. There was a consistent decrease in the amount of reducing sugar with values between 0.412 and 0.078. There was a corresponding increase in ethanol yield with values between 0.30ml and 7.80ml. Cell density increased between 0.221 and 0.789. pH decreased with values between 6.22 and 3.85 as the period of fermentation increased. Temperature increased with values between 27 and 34^0C in the course of fermentation. The results of the total ethanol yield after the period of fermentation reveals that higher ethanol yield (3.60 and 7.80)ml was obtained in the set-up containing ripe plantain peel and *Saccharomyces* with a final yield of 44ml compared to the set-ups containing ripe plantain peel, *Aspergillus* and *Saccharomyces* (3.20 and 7.20)ml with a final yield of 42ml, unripe plantain peels, *Aspergillus* and *Saccharomyces* (3.12 and 2.58)ml with a final yield of 32ml, unripe plantain peels and *Saccharomyces* (3.01 and 7.00) with a final yield of 30ml, ripe plantain peel (0.80 and 1.20)ml with a final yield of 15ml, and unripe plantain peel (0.30 and 1.08)ml with a final yield of 9ml.The values of yields proves to be statistically significant at $p< 0.5$

Table 1: Physicochemical properties fermentation process on day 2

S/N	Set-up	Reducing sugar	Ethanol yield	Cell density	pH	Temperature (^{O}C)
1	Set A	0.148	3.12	0.394	5.01	27
2	Set B	0.134	3.01	0.402	5.54	28
3	Set C	0.212	3.60	0.451	5.81	30
4	Set D	0.180	3.20	0.409	6.22	29
5	Set E	0.412	0.80	0.370	4.88	26
6	Set F	0.402	0.30	0.221	4.86	28

Unripe+Asp+sacc =Set A; Unripe+sacc =Set B; Ripe+sacc =Set C; Ripe+Asp+sacc =Set D; Ripe (Control)=Set E; Unripe (Control)=F; Sacc = *Saccharomyces* sp.; Asp = *Aspergillus* sp.

Table 2: Physicochemical properties of fermentation process on day 4

S/N	Set-up	Reducig sugar	Ethanol yield	Cell density	pH	Temperature (^{O}C)
1.	Set A	0.124	4.63	0.633	4.91	30
2	Set B	0.129	3.50	0.543	5.66	30
3	Set C	0.108	5.00	0.648	5.32	31
4	Set D	0.139	4.90	0.581	6.91	32
5	Set E	0.387	1.03	0.384	4.72	28
6	Set F	0.349	0.54	0.277	4.17	28

Table 3: Physicochemical properties of fermentation process on day 6

S/N	Set-up	Reducing sugar	Ethanol yield	Cell density	pH	Temperature (^{O}C)
1.	Set A	0.112	5.87	0.681	4.45	30
2	Set B	0.128	4.20	0.611	4.82	31
3	Set C	0.099	6.50	0.678	5.04	31
4	Set D	0.118	6.00	0.595	5.88	33
5	Set E	0.285	1.15	0.501	4.66	29
6	Set F	0.232	1.00	0.357	4.00	30

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Table 4: Physicochemical properties obtained of fermentation process on day 8

S/N	Set-up	Reducing sugar (g/mol)	Ethanol yield (ml)	Cell density (g/mol)	pH	Temperature (^{O}C)
1	Set A	0.98	6.58	0.728	4.22	32
2	Set B	0.102	7.00	0.639	4.46	33
3	Set C	0.078	7.80	0.789	4.88	34
4	Set D	0.104	7.20	0.598	5.21	34
5	Set E	0.277	1.20	0.510	4.50	31
6	Set F	0.233	1.08	0.400	3.85	30

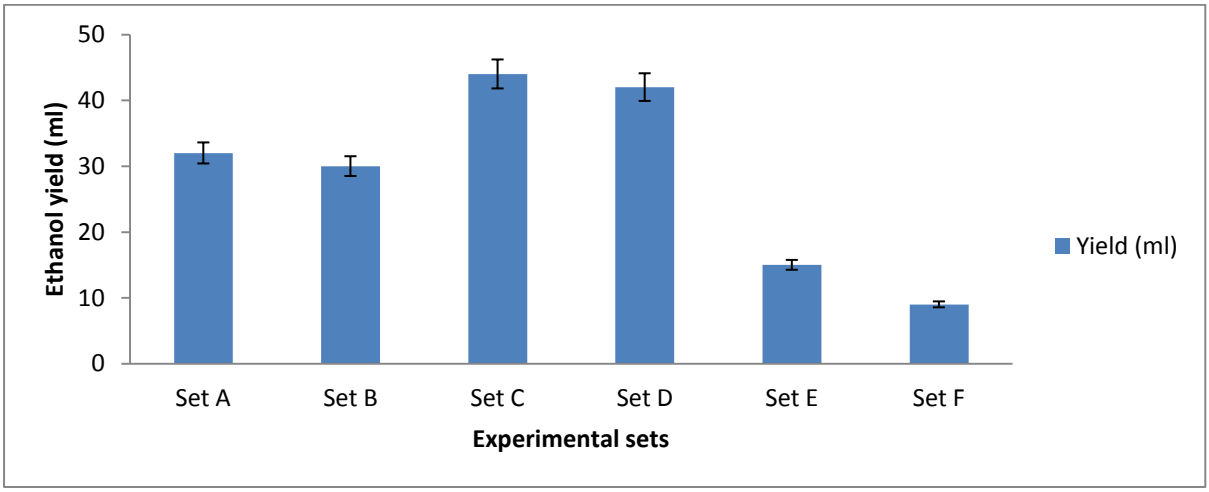

Fig. 1. Cumulative yield of ethanol

DISCUSSION

Results obtained from the study showed that reducing sugar was higher in the ripe plantain peels compared to the unripe with 0.212 to 0.412 g/mol and 0.134 to 0.402 g/mol respectively. This suggests that breakdown of complex polysaccharides may have been taking place during ripening. The control set-ups had the highest amount of reducing sugar with 0.412 and 0.402 g/mol for ripe and unripe plantain peels respectively. This result suggests that indigenous microorganisms that posses the enzyme systems to break down complex polysaccharides could have been present in the control peels.

There was a corresponding increase in ethanol yield as reducing sugar decreased in all set-ups for the period of fermentation.

This is similar to the study of Ali *et al*. (2014) who observed that as the ethanol yield increases, reducing sugar concentration decreases. In another study, results showed that in all the substrates, the concentration of the reducing sugar decreased gradually as the fermentation period and ethanol yield increased (Itelima *et al*., 2013). Ethanol yield was observed to increase as the fermentation period increased for all set-ups.

This result corresponds with the findings of Itelima *et al*. (2013). In their study, the ethanol yield of the 3 substrates used were found to increase gradually from the first day to the seventh day with the pineapple peel having the highest yield of 8.34% (v/v), followed by banana peel 7.45% (v/v), while the least was obtained from plantain peel 3.98% (v/v). There was a corresponding increase in the cell density as the fermentation period increased. The same observation was reported by Itelima *et al*. (2013). The gradual increase in cell densities in all set-ups from the day one to the eighth day of the fermentation periods suggests that substantially, more carbon was utilized for ethanol production. There was a decrease in pH from day 2 to day 8 (final day) of fermentation. These results are similar to the results obtained from the study of Ali *et al*., (2014). They observed that as ethanol production increased pH decreased from 4.2 to 3.5. It is common knowledge that one of the products of fermentation is organic acids. Production of acid in the course of sugar fermentation could have lowered the pH in the reactors. A higher ethanol yield (3.60 to 7.80 ml) was obtained in the set-up containing ripe plantain peel and *Saccharomyces* compared to the set-ups containing ripe plantain peel, *Aspergillus* and *Saccharomyces* (3.20 to 7.20 ml), unripe plantain peels , *Aspergillus* and *Saccharomyces* (3.12 to 2.58ml), unripe plantain peels and *Saccharomyces* (3.01 to 7.00 ml), ripe plantain peel (0.80 to 1.20), and unripe plantain peel (0.30 to 1.08 ml). Many authors have relied on *Sacchromyces* spp. for the production of bioethanol (Bhatt and Shilpa 2014; Udhayaraja and Narayanan 2012; Ali *et al*., 2014). Co cultures of *Aspergillus* and *Saccharomyces* have also been used in the production of bioethanol (Rath *et al*., 2014; Singh *et al*., 2014).

This study has revealed that plantain peel can serve as a suitable substrate for the production of ethanol. Furthermore, ethanol can be produced efficiently by controlled fermentation technique from ripe plantain peel waste using *Saccharomyces cerevisiae* and the ripe peel has a higher prospect to produce higher yield of ethanol compared to unripe peel and enzymatic pretreatment methods with crude enzymes are necessary to delignify wastes in order to obtain higher yield of reducing sugar and corresponding ethanol yields.

REFERENCES

Ali, N., Ubhrani P., Tagotra M. and Ahire M. (2014). A Step Towards Environmental Waste Management And Sustainable Biofuel (Ethanol) Production From Waste Banana Peelings. *American Journal of Engineering Research*. 3 (5): 110 – 116.

Ashiru, A. W. (2005). Production of Ethanol from Molasses and Corn Cobs Using Yeast and A Mould," *The Book of Abstract of the 29th Annual Conference and General Meeting*

Scientia Africana, Vol. 16 (No. 1), June 2017. Pp 1-8
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(Abeokuta, 2009) on Microbes As Agents of Sustainable Development Organized by Nigerian Society for Microbiology (NSM) University of Agriculture, Abeokuta, from 6-10th November, pp. 22.

Bhatt, S.M. and Shilpa (2014). Bioethanol Production from Economical Agro Waste (Groundnut Shell) in SSF Mode. *Research Journal of Pharmaceutical, Biological and Chemical Sciences*. 5 (6) :1210-1219.

Brooks, A.A. (2008). Ethanol production potential of local yeast strains isolated from ripe banana peels. *African Journal of Biotechnology*. 7(20): 3749- 3752.

Chand, P. and Venkateswar L. R. (2009). Thermotolerant yeasts for Bio-ethanol Production using Lignocellulosic substrates. *Yeast Biotechnology*: *Diversity and Applications*. 111, 551-588.

Emaga, T.H., Robert C., Ronkart S.N., Wathelet B. and Paquot M. (2008). Dietary fibre component and pectin chemical features of peels during ripening in banana and plantvarieties. *Bioresource Technology*. 99 : 4346-4354.

Hossain, A.B.M.S., Ahmed S.A., Ahmed M.A., Faris M.A.A., Annuar, M.S.M., Hadeel M., and Norah H. (2011). Bioethanol Fuel Production from Rotten Banana as an Environmental Waste. *Management and Sustainable Energy*. 5(6): 586-598.

INIBAP, (2002) Net Working Banana and Plantain: INIBAP Annual Report 2001, Montpelier, France.

Itelima, J., Onwuliri F., Onwuliri E., Onyimba I. and Oforji S. (2013). Bio-Ethanol Production from Banana, Plantain and Pineapple Peels by Simultaneous Saccharification and Fermentation Process. *International Journal of Environmental Science and Development.* 4 (2): 213 – 216.

Jimoh, S.O., Ado S.A and Ameh J.B. (2009). Simultaneous Saccharification and Fermentation of Yam peel to Ethanol by Co-culture of *Aspergillus niger* and *Saccharomyces cerevisae*. *Biol. and J. for the Tropics.* 6 (1): 96-100.

Kimberly, R.L., Catherina A.B. and Martin.L. (2010). Ethanol from waste banana peelings, Technical Report, The Department of Science and Technology at School of Engineering Institution, Philippines.p17.

Ogbonna JC, Mashima H, Tanaka H (2001). Scale up of fuel ethanol production from sugar beet juice using loofa sponge immobilized bioreactor. *Biores. Technol*. 76:1-8.

Pimentel, D. and Patzek T. W. (2005). Ethanol Production Using Corn. Switch grass and wood; Biodiesel Production using Soybean and sunflower," *Natural Resources Research*. 14 (1): 65-76.

Rath, S., Singh A.K., Masih H., Kumar Y., Kiran J., Singh P. and Mishra S.K. (2014). "Bioethanol production from waste potatoes as an environmental

waste management and sustainable energy by using cocultures *Aspergillus niger* and *Saccharomyces cerevisiae*. *International Journal of Advanced Research*. 2 (4): 553-563.

Sharma, P. and Mishra A.A. (2015). Biofuel Production from Banana Peel by Using Micro Wave. *International Journal of Science, Engineering and Technology*. 3 (4): 1015 – 1018.

Singh, A.K., Rath S., Kumar Y., Masih H., Peter J.K., Benjamin J.C., Singh P.K. and Singh P. (2014). Bio-Ethanol Production from Banana peel by Simultaneous Saccharification and Fermentation Process using cocultures *Aspergillus niger* and *Saccharomyces cerevisiae*. *Int.J.Curr.Microbiol.App.Sci*. 3 (5): 84-96.

Udhayaraja, P. and Narayanan J.S (2012). Optimization For Production Of Bioethanol Using Sorghum Stovar BY *Saccharomyces cerevisiae*. *International Journal of Research in Pure and Applied Microbiology*. 2 (4): 64-67.