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Bioethanol Production from Pineapple (*Ananas Cosmosus)* and Watermelon (*Citrullus Lanatus)* Peels

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Abstract:- This research was on bioethanol production from pineapple peels and watermelon peels. Watermelon peels and pineapple peels were obtained from different fruit vendors, washed, oven-dried and trounce to powder. *Aspergillus niger* was obtained from contaminated soil sample while *Penicillium* was obtained from fresh palm wine and both samples were cultured using potato dextrose agar. Forty-two plates of 1000ml container were used, twenty-one plates containing pineapple peels powder and the other twenty-one plates containing watermelon peels powder and 300ml of water was added to make up 1:6 ratios in each plates. 2ml of *Aspergillus niger* and *Penicillium* were added respectively to make the mixture loose and the pH of each plates were adjusted to different levels 4.5, 5.5 and 6.5). Data were collected on density, volume yield of ethanol and ethanol concentration, this was subjected to a two-way analysis of variance (ANOVA). The result obtained showed that watermelon peels and pineapple peels are capable of producing ethanol, but pineapple peels produced highest percentage ethanol of (11.11±0.39 and 10.19±0.31). Nevertheless, the pH that is best of optimal production was 4.5 with an average productivity of (32.91±0.7). Finally, the effect of *Aspergillus niger* was more prominent in the research as pineapple fermented with *Aspergillus niger* recorded the average density of 12.40±0.5, volume yielded of 110.45 and ethanol concentration in percentage of 11.11±0.39. Furthermore, when *Aspergillus niger* was fermented with watermelon the following results were obtained, average density of 10.47±0.37, 103±1.59 for the average volume yielded and 10.17±0.31 for the average ethanol concentration in percentage. In conclusion the different levels of the pH and The microorganism has effects on the quantity of ethanol produced.

Keywords:- *Bioethanol, Aspergillus Niger, Penicillium Niger, Pineapple Peels, Watermelon Peels, Biofuel, Ethanol.*

I. INTRODUCTION

Large amount of wastes is generated every year from the industrial processing of agricultural raw materials. Most of these wastes are used as animal feed or burned as alternative for elimination. However, such wastes usually have a composition rich in sugars, minerals and proteins, and therefore, they should not be considered "wastes" but raw materials for other industrial processes. (Solange *et al.*, 2012). The burning of petroleum-based fossil fuels in machines and vehicles accompanied by evolution of dangerous gases has been a major cause of increase in environmental problems. This, thus call for an alternative source of fuel which can be obtained by focusing attention to greener technology in the production of bio-fuel using various renewable materials. The production of bio-fuel for domestic and external uses following advancement of biotechnology has been one of the most prominent features of the 20th century which has greatly moved to the 21st century (Mushimiyimana *et al.*, 2015) leading to the elimination of the use of fossil fuel which has expressed high deleterious effects on the environment. Bio-fuel is a fuel whose energy is obtained through a process of biological carbon fixation. In order words can be said to be a hydrocarbon that is made from a living organism. Ethanol (ethyl alcohol, grain alcohol) is a clear, colorless liquid with a characteristic, agreeable odor. In dilute aqueous solution, it has a somewhat sweet flavor, but in more concentrated solutions it has a burning taste. Ethanol is a group of chemical compounds, whose molecules contain a hydroxyl group, –OH, bonded to a carbon atom and are generally called alcohol. It has the chemical formula of: C_2H_5OH. Ethanol melts at –114.1°C, boils at 78.5°C, and has a density of 0.789 g/ml at 20°C. It has the lowest combustion rate when compared to other fuels such as methanol, methane, propane, natural gas and hydrogen. It emits low carbon dioxide, low unburned hydrocarbon and brings about Sulphur (iv) oxide (SO_2) free exhaust. In addition to these properties, it has a high-octane number of 120 which improves its performance when compared to 87-98 in the case of gasoline (Purohit *et al.*, 2012). Ethanol is used for numerous purposes which include but not limited to fuel cell for stationary power and also as an automobile fuel by itself or mixed with gasoline which forms gasohol, manufacture of alcoholic drink, ethanoic acid, solvent for paint, preservation of specimen, fluid in thermometer and lots more. Ethanol can be obtained through a process called fermentation. Fermentation is the breakdown of complex forms of carbohydrate into glucose and conversion of glucose to ethanol and carbon (iv) oxide (CO_2), (Azevedo *et al.,* 2000). There are five (5) main types of fermentation, these includes alcohol fermentation. Microorganisms are living organism that can be seen only with the aid of a magnifying glass. They cloud be unicellular having only one cell or multicellular having two or more cell. They are different types of microorganism which include; bacteria, fungi, virus, protist (Fuerst, 2014). In fermentation, numerous microorganisms such as yeast, fungi and bacteria are used to achieve maximal results. However, the most successful and commonly used for this process are *Saccharomyces cerevisiae, Penicillium sp, Aspergillus niger, Echerichia coli, Zynomonas mobilis.* These organisms can be extracted from palm wine or palm sap and from contaminated soil respectively. They are essential in the fermentation process as they serve as catalyst in the reaction. *Saccharomyces cerevisiae,* commonly called baker's yeast is a single celled eukaryote that is frequently used in scientific research especially fermentation. It is considered to be the top fermenting yeast because as yeast flocculate or clump together they attach to the carbon dioxide that is produced. It is also called the top-fermenting yeast as it is characterized by a warm temperature of 59 to 60^o F required for optimal fermentation (David, 2015). It can be extracted from palm wine or gotten directly from baking powder. *Penicillium* is one of the largest and most important genera of microscopic fungi, with over 400 described species distributed worldwide (Visagie *et al.,* 2014). Blue mold" is a common term used to describe several species of *Penicillium* that cause postharvest decay of important fruit crops because visible sporulation on infected fruits is blue-green in color (Jurick *et al.,* 2016)

II. MATERIALS AND METHODS

The research was conducted in the Department of Genetics and Biotechnology while the laboratory work was done at the Chemistry Laboratory, Department of Chemistry, Cross River State University of Technology. Watermelon peel and pineapple peel were obtained from different fruit vendors around Calabar metropolis. The watermelon rind and pineapple peel were thoroughly washed with tap water and oven dried at 75^0C for 48 hours and then trounce to powdered using food processor. Contaminated soil was gotten from a mechanic workshop within the Calabar metropolis and a freshly tapped palm wine was bought from palm wine vendor at Atimbo. The experimental design was a 2 x 3 factorial in a Completely Randomized Design (CRD).

Factor 1: The fungi species *Aspergillus niger* and *Penicillium sp*
Factor 2: pH levels, 4.5, 5.5 and 6.5

➢ *Fermentation*

Fermentation was carried out in a 1000ml container containing 50g powdered pineapple peels and watermelon peels in 300ml of distilled water respectively. The pH of the medium was adjusted to 4.5, 5.5 and 6.5 in different sludge, using one normal HCl and NaOH. The mono culture fermentation was inoculated with 2ml of freshly harvested inoculums of *Aspergillus niger* and 2ml inoculums of *Penicillium sp* respectively for four days in other for fermentation to take place.

➢ *Distillation*

After fermentation, the broth was filtered. The supernatant was collected and fed into a round bottom flask and optical density was taken, an anti-bombing was added as a precautionary measure. Then the supernatant was placed on a hot plate and was allowed to boil. The boiling temperature of ethanol is 78^0C hence distillation was carried out above that temperature which facilitated the evaporation of ethanol. The vapor was collected and allow to condense by means of the circulation of cold water around the column. The distillate was recovered in a conical flask at the other end of the column. The distillate was redistilled to obtain ethanol in its purest form.

➢ *Statistical Analysis*

Data collected was subjected to two-way analysis of variance in a Completely Randomized Design and significant means was separated using the least significant difference (LSD) at 5% probability level.

III. RESULTS AND DISSCUSSION

The effect of pH on the yield of ethanol from pineapple peels and watermelon peels, using *Aspergillus sp.* and *Penicillium sp.* as fermented is recorded in Tables 1 and 2. The cumulative effect of microorganism and bioethanol production is recorded in Table 3.

➢ *Density of Hydrolysate*

Density which is the weight of ethanol in a mixture (in this case the hydrolysate measured in kg/m^3) was observed to be high at the pH of 4.5 at every sample mixture, however, it recorded highest in the mixture of pineapple peels fermented with *Aspergillus* (13.76±0.13) with significant difference (P>0.5) in the mean as recorded in Table 1. This was closely followed by (13.2±0.01) recorded in the fermentation of pineapple peels with *Aspergillus sp* at pH 5.5. It was also observed that as the pH level increased, the density of the hydrolysate decline because at the pH of 6.5 density recorded ranged between 9.17±0.04 to 7.03±0.01 and at the control at pH of 7.0, the density recorded was 6.51±0.29 for pineapple peel and 6.01±0.12 for watermelon peels, and as pH decrease so did the hydrolysate density increase. Table 2 which shows the cumulative effect of pH on the bioethanol production revealed that the pH 4.5 gave off the highest mean density was significant difference (P>0.05) mean ± SE value of (32.34±2.05) this was followed by pH 5.5 (30.07±1.97) and then pH 6.5 (25.99±1.29). The density of the control recorded the least with 6.25±0.18.

Waste +microorganism	pH	Density (g/ml)	Volume Yield (v)	Ethanol Conc. ($^{v}/_{v}$) (%)
Control (Pineapple peel + water)	7.0	$6.51^{j} \pm 0.29$	$90.67^{e} \pm 0.6$	$7.18^{l} \pm 0.36$
Pineapple peel + *Penicillium sp.*	4.5	$11.70^{c} \pm 0.03$	$105.00^{c} \pm 5.0$	$11.14^{c} \pm 0.16$
	5.5	$10.55^{d} \pm 0.06$	$103.00^{d} \pm 0.88$	$10.24^{e} \pm 0.23$
	6.5	$9.17^{e} \pm 0.04$	$100.67^{e} \pm 0.33$	$9.20^{ij} \pm 0.32$
Pineapple peel + *Aspergillus sp.*	4.5	$13.76^{a} \pm 0.13$	$114.7^{a} \pm 0.67$	$12.06^{a} \pm 0.02$
	5.5	$13.02^{b} \pm 0.01$	$112.00^{b} \pm 2.73$	$11.65^{b} \pm 0.33$
	6.5	$10.40^{d} \pm 0.16$	$104.67^{cd} \pm 1.67$	$9.63^{g} \pm 0.31$
Control (watermelon peel + water)	7.0	$6.01^{k} \pm 0.12$	$84.67^{g} \pm 0.88$	$7.09^{l} \pm 0.18$
Watermelon peel + *Penicillium sp.*	4.5	$9.20^{f} \pm 0.13$	$85.9^{f} \pm 2.52$	$10.05^{f} \pm 0.30$
	5.5	$8.37^{g} \pm 0.04$	$90.3^{e} \pm 1.45$	$9.04^{j} \pm 0.10$
	6.5	$8.02^{i} \pm 0.02$	$83.00^{g} \pm 0.58$	$8.43^{i} \pm 0.03$
Watermelon peel + *Aspergillus sp.*	4.5	$8.60^{d} \pm 0.07$	$90.57^{e} \pm 0.61$	$10.63^{e} \pm 0.03$
	5.5	$8.14^{h} \pm 0.07$	$90.21^{e} \pm 1.20$	$9.29^{h} \pm 0.08$
	6.5	$7.03^{i} \pm 0.01$	$90.61^{e} \pm 4.23$	$8.78^{k} \pm 0.15$
LSD		0.21	1.96	0.17

Table 1:- Effect of pH Microorganism Plants and the Production of Ethanol
Mean with the same superscript along the vertical arrays indicate the significant difference ($P>0.05$)

pH	**Volume yield (v)**	**Density (g/ml)**	**Ethanol Conc. ($^{v}/_{v}$) (%)**
Control	$87.67^{c} \pm 1.43$	$6.25^{d} \pm 0.18$	7.14 ± 0.18
4.5	$297.1^{a} \pm 11.45$	$32.34^{a} \pm 2.05$	$32.91^{a} \pm 0.7$
5.5	$296.91^{a} \pm 9.22$	$30.07^{b} \pm 1.97$	$30.16^{b} \pm 1.02$
6.5	$284^{b} \pm 8.53$	$25.99^{c} \pm 1.27$	$27.03^{c} \pm 0.45$
LSD	3.92	0.47	0.35

Table 2:- Mean Effect of pH Level on Bioethanol Production Parameters
Mean with the same superscript along the horizontal arrays indicate the significant difference ($P>0.05$)

Microorganism	**Density (g/ml)**	**Volume Yield (v)**	Ethanol Conc. ($^{v}/_{v}$) (%)
Aspergillus niger + Pineapple peels	$12.40^{a} \pm 0.51$	$110.45^{a} \pm 1.77$	11.11a±0.39
Aspergillus niger + Watermelon peels	$7.93^{d} \pm 0.24$	$90.46^{c} \pm 1.28$	$9.57^{c} \pm 0.28$
Penicillium sp + Pineapple peels	$10.47^{b} \pm 0.37$	$103^{b} \pm 1.59$	$10.19^{b} \pm 0.31$
Penicillium sp + Watermelon peels	$8.53^{c} \pm 0.18$	$86.41^{d} \pm 1.37$	$9.17^{d} \pm 0.25$
LSD	0.41	3.92	0.04

Table 3:- Cumulative Effect of Microorganism on Bioethanol Production
Mean with the same superscript along the horizontal arrays indicate the significant difference ($P>0.05$)

➢ *Volume Yield of Ethanol (ml)*

Table 1 which displays the interaction between all factors considered showed that pineapple peels fermented with *Aspergillus niger* recorded the highest volume yield of ethanol with mean ±SE of 1147±0.67 after distillation at the pH of 4.5, this was followed by 112.00±2.73 at pH 5.5. However, in the case of watermelon peel, the highest volume yield of ethanol was 90.57 when fermented with *Aspergillus niger* at pH 4.5. The result thus showed a striking similarity between the control group of pineapple peels and watermelon peel fermented with *Aspergillus niger* as they were no significantly different (P<0.05). Also, there was no significant difference in the volume yield of pineapple peel fermented with *Aspergillus niger* at pH 6.5 (104.6±1.67) with that fermented with *penicillium sp* at 4.5 pH (105±5) and pineapple peels fermented with *Penicillium sp* at 5.5 pH was not significantly different from pineapple peels fermented with *Aspergillus niger* at pH 6.5 (103.00±0.88 and 104.67±1.6). Table 2 however showed that pH of 4.5 and 5.5 gave off the highest volume yield with no significant difference in their means (P<0.05) (297.1±45 and 296.91±9.22) and pH of 6.5 recorded the average volume yield of 284±8.53 in ml.

IV. DISCUSSION

The use of the lignocellulisic raw materials such as fruits, vegetable wastes, agricultural residue and much more for the production of biochemical is a good way to convert environmental pollutant into useful materials for maximal production of ethanol (Mohammed *et al.,* 2018). However, pineapple and watermelon peel the discarded as waste due to the low awareness of either its nutritional or environmental values was used as the substrate for this study. 50g of the flour was dissolved in 300ml of distilled water to bring about the ethanol yield which ranged from 8.43% to 12.06% with the most effective pH of 4.5. An interesting observation is how both plant substrates gave off different ethanol percentage when subjected to different pH levels and microorganisms, this is observed in Table 3 where the highest density, volume yield and percentage concentration of ethanol was gotten from the pineapple peels. This tremendous productivity can be attributed to the high sugar content of 14% in pineapple peel. Watermelon peels on the other hand gave off very minute quantities of ethanol when compared to pineapple and this also can be attributed to the low sugar content of 6% in watermelon, this is because the higher the sugar, the more glucose will be available for microorganisms to work on during fermentation and the more the end product. Furthermore, various parameters affect the microorganism and their interaction which in turn affect ethanol yield. One of those very import factor which was studies was the pH. From the mean ± standard error tables above, it can be observed that as the pH was reduced, the amount of ethanol produced was higher and as the pH increased, the amount of ethanol produced reduced. The highest yield was recorded at pH 4.5 (11.65%0.53) and the least was at pH 7.0 (7.09%±0.18) of the control group. This high productivity of low pH is due to the fact that ethanol is best obtained at lower pH which are acidic. In an acidic solution, the growth of harmful bacteria which could hinder fermentation is retarded and so fermentation is optimal. (Kourkoutas *et al.,* 2004).

V. CONCLUSION

At the end of the study, it was concluded from the result that both watermelon peels and pineapple peels are capable of producing ethanol, but pineapple peel produced the highest percentage ethanol (11.11±0.39 and 10.19±0.31). Furthermore, the pH that is best of optimal production is that of 4.5 with an average productivity of (32.91±0.7). Finally; the effect of *Aspergillus niger* recorded the average density concentration in percentage of 11.11±0.39 and when fermented with watermelon the following results were obtained 10.47±0.37 for average density, 103±1.59 for the average volume yield and 10.17±0.31 for the average ethanol concentration in percentage.

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