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OPTIMIZATION OF BIOETHANOL PRODUCTION FROM BANANA PEELS: AN ALTERNATIVE ENERGY SOURCE

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ABSTRACT

The environmental issues associated with fossil fuels have resulted to increase in research interest globally on alternative and renewable energy sources such as bioethanol that are sustainable and environmentally friendly. In Nigeria, the use of fruit wastes such as banana peels for bioethanol production is yet to be harnessed effectively despite their rich carbohydrate content. This study is aimed at optimization of bioethanol produced from banana peels. The milled sample was subjected to pretreatment, hydrolysis, fermentation and distillation processes to produce bioethanol. The hydrolysis and fermentation process were optimized using classical optimization technique of one factor at a time to determine the effect of their parameters on the yield of glucose and bioethanol respectively. The results obtained indicated that maximum glucose yield of 42.14±0.92% was obtained at optimum factor conditions of 2% acid concentration,116^{o}C temperature and 25minutes hydrolysis time while the maximum bioethanol yield of 44.68±0.82% was obtained at optimum factor conditions of 6% yeast concentration, 5.5 pH, 35^{o}C temperature and 3 days fermentation time. The bioethanol produced was characterized for fuel properties such as boiling point, flash point, kinematic viscosity, refractive index, density using ASTM methods and the results obtained revealed that they conform to the standard. These findings suggest that banana peels is a good and sustainable feedstock for bioethanol production in Nigeria. Due to its relative abundance and availability for large scale production it should not be discarded in our environment as this is also a means of generating waste from wealth.

Keywords: Bioethanol, Optimization, Pretreatment, Hydrolysis, Fermentation

INTRODUCTION

The rise in the price of petroleum (fossil fuels) and the environmental issues associated with its use has led to search for alternative fuels that are renewed, environmentally friendly and sustainable. Recently, there is a growing interest in the production of bioethanol from biomass materials which is one of the cheapest liquid fuel alternatives to non-renewable fossil fuels [1].Bioethanol is an organic compound with the general name ‘alcohol’ and its use by humans dates back to prehistoric times when it was largely consumed as beverage [2]. It is currently used as an alternative source of fuel because it is biodegradable, made from renewable sources, has high octane number and is less toxic when compared to conventional petroleum-based fuels [3]. Bioethanol fuel is mainly produced by fermentation of sugar containing crops although it can be manufactured by chemical process of reacting ethylene with steam [4] as can be seen from the equation of reaction below:

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$C_6H_{12}O_{6(s)} \longrightarrow 2C_2H_5OH(aq) + 2CO_2(g)$ ..(1)

$C_2H_4(g)+H_2O(g) \longrightarrow C_2H_5OH(g)$…….(2)

Approximately, 80% of world supply of ethanol is through the fermentation of sugar and starch containing crops which is known as first generation bioethanol or by product from industries based on such crops [5]. Bioethanol has been extensively acknowledged to be a perfect substitute to petroleum although the product is still commercially unavailable especially in the developing countries such as Nigeria. This can be attributed to the production of bioethanol from edible feed stocks such as starch and sugar which has led to their increase in price thereby creating competition between food and fuel and resultant increase in price of bioethanol in the global market [5,6]. This problem can be solved by the use of non-edible feedstock such as lignocellulose substrates, agricultural wastes, forest wastes, municipal wastes for bioethanol production [7]. Bioethanol can be produced from mainly biomass materials but the potential of using these materials as feedstock for large scale production especially in developing countries like Nigeria depends on their cost, abundance, carbohydrate content and the ease with which they can be converted to ethanol [8]. Enormous quantities of agro-industrial residues are generated throughout the world from processing of raw agricultural materials for food. Also, food processing industries produce large amount of waste materials and their disposal have become an environmental concern especially when they are not property disposed [9]. Banana peels are not considered very useful and are therefore dried, ground, pelletized and sold to feed manufacturers at a low price. Although it is a fruit residue, it accounts for 30 – 40% of the total fruit waste and contains carbohydrates, proteins and fiber in significant amounts. Since banana peels contain lignin in low quantities it could serve as a good substrate for the production of value added product like bioethanol [10,11]. The use of banana peel in bioethanol production contributes greatly to the conversion of biomass waste into wealth thereby enhancing waste management, cost efficiency and environmental sanitation.

The production of bioethanol from banana peels involves four basic steps such as pretreatment, hydrolysis, fermentation and distillation. Pretreatment which is the first step is aimed at breaking the rigid structure of the lignocellulosefor easy access to the lignin, cellulose, and hemicellulose molecules [11].

Hydrolysis of the pretreated sample which if the next step is aimed at converting the cellulose and hemicellulose to glucose chains which is followed by fermentation with yeast such as *saccharomyces cerevisiae* to produce bioethanol.

The final stage is the distillation process which is aimed at obtaining the pure bioethanol

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Studies have been conducted on the production of bioethanol from maize wastes and discarded Newspapers [12], sugar cane waste and maize waste [13], apple, Kiwi fruit, Peach wastes [14] among others,

The main process variables which affect the yield of bioethanol are yeast concentration, pH, temperature, time and concentration of acid. Thus the aim of this study is to optimize the concentration of bioethanol production from banana peels by optimizing the hydrolysis and fermentation process.

MATERIALS AND METHODS

Sample Collection

Fresh banana peels were obtained from banana purchased from Eke Market Afikpo. Plastic bags was used to collect the sample and transported to the Chemistry Laboratory, AkanuIbiam Federal Polytechnic, Unwana for further analysis.

Sample Preparation

The banana peels were cut into smaller sizes (between 3 to 4cm) using a knife after it was rinsed with distilled water. The peels were dried in an oven to produce easily crushable materials for 72 hours at 60^{o}C and ground into fine powder using an electric grinding machine. The powdered sample was then sieved to a particle size of 1mm, stored in sealed plastic containers and kept at room temperature until the next stage of analysis [4]

Sample Pretreatment

The pretreatment of the sample was carried out using steam to reduce cellulose crystalinity and promote the porosity of the material for easy hydrolysis. The steam pretreatment method was carried out according to the method described by Wondale [15]. Batch analysis using 50g of the powdered sample was carried out by dissolving the sample in distilled water at a ratio of 10:1 (vlw). The mixture was transferred into a 1000ml conical flask, covered with aluminum foil and autoclaved. The autoclave's temperature was adjusted to a temperature of 121^{o}C and the pre treatment carried out for 15 minutes. The sample was allowed to cool after the pre treatment and the soluble component separated from the non soluble component and stored for further analysis. The non-soluble component was used for acid hydrolysis step.

Hydrolysis

Dilute acid hydrolysis using H_2S0_4 was carried out on the non soluble fraction of the sample to break down the cellulose and hemicellulose polymers into fermentable sugars that will be used to produce bioethanol [16]. The pretreated sample was used for the hydrolysis in batches at different factor conditions of Acid concentration (1.0-3.0%), Temperature (100 – 132^{o}C) and Time (10 – 30 minutes) to determine the optimal factor conditions that will give maximum yield of glucose and their effect on the yield. After hydrolysis, 1M NaOH was used to adjust the pH until it reached 7 [17]. In order to eliminate the non fermentable lignin, the solid particles were separated from the sugar rich liquid by filtration and added to the previously filtered solution from

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pretreatment process. The solution was stored at room temperature for further analysis.

Fermentation Process

Fermentation was carried out under anaerobic condition according to the method described by Wondale [15]. The fermentation media was first prepared before the fermentation process to enable a conducive environment for yeast growth and to supply the required amount of nutrients [18].

The fermentation media was prepared using dextrose sugar (10g), urea (1.0g), yeast extract (0.2g), MgSO4. $7H_2O$ (1.0g) and distilled water to make up 100ml.The samples were then mixed with the fermentation media in the ratio of 1:10 and added into the reactor using separating funnel. The conical flask was properly covered to maintain anaerobic condition with an outlet provided for the release of CO_2 as it turns lime water milky. Triplicate fermentation broths of the same composition were prepared and incubated under the same condition. The fermentation process was carried out initially at varying yeast concentration of (2 – 10%), temperature of 30^{o}C for 72 hours at pH 5.5. The pH of the fermentation was adjusted below 5.5 to accommodate yeast growth by adding the required amount of 4M NaOH and 2.5M HCl [1]. Subsequent fermentation process was carried out at various conditions of pH (4.0 – 6.0), temperature (20 - 40^{o}C), time (1 – 5) days to determine the optimum factor conditions and their effect on the yield of bioethanol. After fermentation, the sample were taken out and distilled.

Filtration and Distillation of Bioethanol Produced

The samples were filtered using muslin cloth to separate the solid substrate from the liquid. The resulting mixture of bioethanol, water and other impurities was then transferred into the distillation flask and placed on a heating mantle fixed to a distillation column enclosed in a tap running water in order to obtain pure bioethanol. The distillate was collected at 78.5^{o}C (boiling point of ethanol) for 3 hours with another flask fixed at the other end of the column [4]. Distillation is the method used to separate two liquids based on their difference in boiling points.

Quantitative Estimation of Bioethanol Produced

The amount of bioethanol produced was estimated quantitatively using specific gravity method as described by Geirwyr [19] and Aleme *et al* [20]. A 25ml Pycnometer (specific gravity bottle) was cleaned and dried first and weighed and the weight noted as Wo at 20^{o}C. The bottle was filled with bioethanol and reweighed at 20^{o}C to give W_1. The bioethanol sample was substituted with water after washing and drying the bottle and weighed at 20^{o}C to give W_2. Using these observations, specific gravity was calculated and the percentage of bioethanol in the distillate was estimated from the relationship between the specific gravity and the proportion of the ethanol in alcohol solution using AOAC Table. The specific gravity of the sample was obtained using the following equation:

Specific gravity = $\frac{W_1-W_0}{}$

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W_2-W_0

Where W_0 = Weight(g) of the empty bottle

W_1 = Weight(g) of bottle + Sample (Bioethanol)

W_2 = Weight(g) of bottle + water

Statistical Analysis

The results were obtained in triplicates and expressed as Mean ± Standard deviation. Data obtained from the study were subjected to one – way analysis of variance (ANOVA) at 5% level of significance ($P<0.05$) using SPSS version 23.0 software.

Optimization of Hydrolysis Process

The effect of the variables on the yield of reducing sugar produced from banana peels was studied to determine the optimal factor conditions that gave the maximum yield. The hydrolysis process parameters studied include acid concentration, temperature and time. The results are presented as mean ± standard deviation at n = 3 for $P < 0.05$. The acid concentration was varied from 1.0 to 3.0 % at a step increase of 0.5 % while the other parameters were kept constant in order to determine the optimal acid concentration that will give the maximum yield.

The effect of hydrolysis temperature on the yield of reducing sugar from potato peels was determined by varying the temperature from 100 – 132^{o}C at step increment of 8^{o}C while keeping other parameters constant. The hydrolysis time was varied from 10 to 30 minutes with step increase of 5 minutes and other parameter kept constant in order to determine its effect on reducing sugar yield.

Optimization of Fermentation Process

The effect of fermentation process variable on the yield of bioethanol production from banana peel hydrolysate was investigated in order to determine the optimum factor conditions that gave the maximum bioethanol yield. The fermentation process variables studied were: yeast concentration, pH, temperature and time. The results are presented as mean ± standard deviation at n = 3 for $P < 0.05$. The concentration of yeast was varied from 2 to 10% at a step increment of 2% in order to determine its effect on the bioethanol yield while keeping the other parameters constant. To determine the effect of pH on the yield of bioethanol the pH was varied from 4.0 to 6.0at a step increment of 0.5 while keeping other parameters constant. The effect of fermentation temperature on the yield of bioethanol was studied by varying the temperature from 20 – 40^{o}C at a step increment of 5^{o}C while keeping other parameter constant. The fermentation time was varied from 1 to 5 days with step increase of 1 day while keeping the other parameters constant.

RESULTS AND DISCUSSION

Optimization of Acid Concentration

The result of the effect of acid concentration on the reducing sugar yield is shown in Table1. From the result obtained, increase in acid concentration increased the glucose yield till it reached the maximum value of 29.03±1.21% at optimum acid concentration of 2% when it started decreasing with further increase in the concentration of acid.

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The decreased glucose yield with high acid concentration results from the fact that low acid concentration is conducive for glucose production during hydrolysis. However, the use of high acid concentration for hydrolysis results to browning and charring of the hydrolysates as a result of the degradation of monomeric sugars (xylose, glucose) to fufural and 5 HMF which leads to decrease in glucose yield [4,21,22]

Optimization of Temperature

The result of the effect of temperature on the yield of reducing sugar is presented in Table 2 and it was observed that reducing sugar yield increased with increase in hydrolysis temperature till it attained a maximum value of 28.22±0.89% at optimum hydrolysis temperature of 116^{o}C when it started decreasing with further increase in temperature. The results obtained indicated that extreme temperature has unfavorable effect on the conversion of sugar from the substrates due to the degradation of the simple sugar which result in the formation of fufural and 5 HMF that are toxic for *Saccharomyces cerevisiae* in fermentation [21].

Optimization of Hydrolysis Time

The result of the effect of time on the reducing sugar yield presented in Table 3 reveals that increase in hydrolysis time increases the yield of reducing sugar till it reached the maximum value of 42.14 ±0.91% at optimum time of 25 minutes. On exceeding the optimum time, the reducing sugar yield reduced with further increase in time. The decrease in yield on exceeding the optimum time is attributed to the decomposition of glucose to degradation product (Fufural and 5HMF). Therefore maximum yield of reducing sugar was obtained at optimum 2% acid concentration, 116^{o}C temperature and 25 minutes hydrolysis time.

Table 1: Reducing Sugar Yield from Hydrolysis of Banana Peels at Different Concentration of Acid

Acid Concentration (%)	***Glucose yield (%)***
1.0	25.41± 0.55
1.5	27.27 ±1.28
2.0	29.03 ± 1.2
2.5	26.32 ± 0.79
3.0	24.28 ± 0.79

Table 2: Reducing Sugar Yield from Hydrolysis of Banana Peels at Different Temperature

Temperature (^{o}C)	***Glucose Yield %***

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100	2.1.16 ±0.96
108	24.72± 0.73
116	28.22± 0.89
124	25.29 ±0.86
132	23.08 ±1.04

Table 3: Reducing Sugar yield from hydrolysis of Banana peels at Different Time

Time (Minutes)	***Glucose yield (%)***
10	30.50± 0.74
15	35.20± 0.90
20	37.26 ±0.94
25	42.14± 0.91
30	34.18 0.93

Optimization of Fermentation Yeast

The result obtained for effect of yeast concentration on the bioethanol yield is presented in Table 4 which shows that the yield of bioethanol is increased with increase in yeast concentration till it reached a maximum value of 26.20 ± 1.05% at an optimum yeast concentration of 6%. On exceeding 6% optimum concentration of yeast, the bioethanol yield decreased and this may be due to the inoculums' size not having significant effect on the bioethanol yield but rather on the rate of sugar consumption [22]. Above the optimum yeast concentration, the cells grow rapidly resulting in rapid consumption of the glucose to produce bioethanol at a reduced fermentation time [22].

Optimization of pH

The result of the effect of pH on bioethanol yield is shown in Table 5 below which revealed that bioethanol yield increased with increase in pH till it attained the highest value of 30.50 ± .0.77% at an optimum pH of 5.5 when it started to decrease as the pH increased further. The optimum pH for bioethanol production using *saccharomyces cerevisiae* has been reported to be 4.0 to 5.5. On exceeding pH of 5.5, the yeast is denatured and the catalytic activities reduced which results to the decrease in the bioethanol production [22].

Optimization of Fermentation Temperature

The result of the effect of temperature on the yield of bioethanol is as shown in Table 6 which revealed that bioethanol yield increased with

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increase in fermentation temperature until it attained the maximum value of 37.15 ±1.08% at optimum temperature of 35ºC. On exceeding the optimum temperature, the bioethanol yield decreased. One of the characteristics of microorganism is that temperature has a stimulating effect on them over narrow range [22]. According to Egbosiuba *et al* [6] fermentation process above 45ºC could lead to the death yeast of cells which enhances reduction in their activity thereby decreasing the yield of bioethanol produced while lower temperature slows them down. The results is similar to that obtained by Duhan *et al* [23] who studied the effect of temperature on bioethanol yield and obtained maximum yield at 35ºC.

Optimization of Fermentation Time

The result obtained is presented in Table 7 which showed that bioethanol yield increased with increase in fermentation time until it attained the highest value of 44.67±0.82% at an optimum time of 3 days. On exceeding the optimum fermentation time, the bioethanol yield started decreasing. The optimum time of 3 days is similar to the result obtained by Phisalaphong *et al* [24].

Table 4: Bioethanol Yield Banana Peel Hydrolysate at Different Concentration of Yeast

Yeast extract %	*Bioethanol yield %*
2	23.64 ±0.77
4	24.70 ±0.72
6	26.20 ±1.05
8	24.60 ±0.74
10	22.46 ±0.75

Table 5: Bioethanol Yield from Banana Peel Hydrolysate at Different pH

pH	*Bioethanol yield*

ISSN 0795 - 22066

4.0	27.09±0.77
4.5	28.14 ±0.91
5.0	29.30 ±1.01
5.5	30.50 ±0.77
6.0	26.73 ±0.75

Table 6: Bioethanol Yield from Banana Peel Hydrolysate at Different Fermentation Temperature

Temperature (^oC)	***Bioethanol yield %***
20	34.23 ± 0.92
25	34.28 ± 0.94
30	35.06 ± 1.04
35	37.15 ± 1.08
40	35.27 ± 0.95

Table 7: Bioethanol Yield from Banana Peel Hydrolysate at Different Fermentation Time

Time (days)	***Bioethanol Yield***
1	39.14±0.93
2	40.19±0.88
3	44.67±0.82
4	42.21±0.83
5	40.23±1.06

Physicochemical Properties of Banana Peel Bioethanol

The physicochemical properties o the bioethanol produced were compared with America Society for Testing and Materials and the result presented

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in Table 8. From the result, the density of bioethanol produced is 0.79±0.04 which is within the ASTM standard limit for bioethanol. Density is the ratio of mass to volume of the fuel which greatly affects the ignition quality of the fuel. It has significant impact on the fuel consumption as the fuel introduced into the combustion chamber is determined numerically [22].

Viscosity is another very important property of fuel. The biofuel should neither be too viscous nor too thin. The kinematic viscosity of the bioethanol produced was determines as 1.41± 0.57 mm^2/s and is therefore within ASTM limit. High kinematic viscosity of biofuels results in poor atomization and incomplete combustion which gives rise to cocking of injector tops [22] on the other hand, very low viscosity fuel produces very subtle spray which cannot be properly transferred into the combustion chamber thereby forming a fuel rich zone that give rise to sooth formation [25].

Flash point measures the degree of flammability of the fuel and the flash point value of 13.7± 1.51^oC obtained from the bioethanol produced is within the ASTM limit. The pH value of 7.33± 0.05 obtained is within the ASTM limit of bioethanol. The neutral pH implies that the bioethanol produced is of good quality. According to Okoh [26], bioethanol with pH below 6.5 may contribute to failure in fuel pump and fuel injector as a result of corrosion while pH above 9.0 may negatively impact the plastic parts in the system. The water content of the produced bioethanol is 1.2 ± 1.26% which is within the ASTM Standard limit for bioethanol. High water content predisposes the oxidation of the fuel as well as being the major cause of corrosion in storage tanks when stored for a very long period. The water content obtained therefore has little or no effect on the degradation of the fuel or on the corrosion in the tanks during storage. The boiling point of bioethanol was evaluated as 78.65± 0.47^oC when is within ASTM Standard Limit for bioethanol. Refractive index indicates the state of purity of a substance. The refractive index of bioethanol produced is 1.36± 0.01^oC and is within the ASTM Standard.

Table 8: Physicochemical Properties of bioethanol produced from banana peels compared to ASTM Standard

Parameter	*Unit*	*Banana Peel*	*ASTM Standard*
pH		7.33±0.05	6.5-9.0
Density	$glcm^3$	0.79±0.04	0.794

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Refractive index (°C)	1.362±0.01	1.357 – 1.44
Boiling point (°C)	78.65±0.47	78.5
Kinematic viscosity (mm^2/s)	1.41±0.57	1.2 – 1.5
Flash Point (°C)	13.7±1.51	<14
Water content (°C)	1.21±1.26	0-3

CONCLUSION

This study focused on the pretreatment and hydrolysis of banana peels to produced glucose and subsequent fermentation of the sugar to bioethanol. The result obtained for hydrolysis and fermentation process indicated that acid concentration, temperature, and time of hydrolysis affects the optimum yield of glucose significantly as the yield of 42.13± 0.92% was obtained at optimum factor condition of 2.0% acid concentration temperature of 116°C an time of 25 minutes while the maximum bioethanol yield of 44.68± 0.08 % was obtained at optimal factor condition of 6% yeast concentration, pH of 5.5, 35°C temperature and 3 days fermentation time. Also the physicochemical properties of bioethanol produced when compared to ASTM standard specification for bioethanol were all within Limits. This demonstrates that banana peel wastes can be converted into value added and eco friendly product such as bioethanol through hydrolysis and fermentation process. This will offer better waste management process options thereby solving the problem of waste disposal and mitigating of environmental pollution challenge.

RECOMMENDATION

Biomass wastes such as banana peels are rich in carbohydrate content and are in abundance in Nigeria. They are renewable sources hence bioethanol production from them holds tremendous potential in terms of meeting the energy needs and promoting environmental benefits. It is recommended that the use of these wastes as alternative sources of producing bioethanol should be encouraged to help alleviate the problem of waste disposal and environmental pollution thereby boosting the economy of Nigeria.

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