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Effect of Particle sizes on Percentage yield and fuel Property Characterization of Bio-Ethanol Derived from Cadaba Farinosa Forskk Shrub in Northern Nigeria.

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ABSTRACT: *This paper present the effect of particle size on the percentage yield of ethanol produced from Cadaba farinosa forssk shrubby simultaneous saccharification and fermentation processes. The physico-chemical properties and fuel quality were also determined. Sample was collected from Bayara area of Bauchi State. The material (Cadaba farinosa forskk shrub) was sun dried, chipped, pulverized and sieved into smaller varying particle sizes of 200, 300 and 400µm respectively.The highest sugar concentration and bioethanol yield of Cadaba farinosa forskk plant was found to be 59% and 0.85L when particle size of* 200*µmand 1300g of the material was used.The specific gravity (S.G) was 0.789, kinematic viscosity is 34.52mm*^{2}*/s, calorific value is 29.5 MJ/kg, flash, pour and cloudpoints were found to be* 11.4℃, −8℃ *and* − 6℃ *respectively. Also the boiling point was found to be*78.3℃*.The physico-chemical properties of Cadaba Farinosa Forssk bio-ethanol were within the set standards for industrial ethanol and are found to be within the limits of ASTM and EN standards.The experimental results obtained in this work showed that both the conversion and fermentation processes were optimal andthe high ethanol yield of Cadaba farinosa forskk plant is an indication that the shrub is a good source of bioethanol.*

KEYWORDS: *Bioethanol, Fermentation, % percentage yield, Saccharomyces cerevisiae, Cadaba Farinosa Forskk.*

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I. INTRODUCTION

Bioethanol is an alcohol made by fermentation, mostly from carbohydrates produced in sugar or starch crops such as corn or sugarcane [1]. Cellulosic biomass, derived from non-foodsources such as trees and grasses, is also being developed as a feedstock for ethanol production. Ethanol can be used as a fuel for vehicles in its pure form, but it is usually used as agasoline additive to increase octane and improve vehicle emissions. Bioethanol is widelyused in the USA and in Brazil. Current plant design does not provide for converting the lignin portion of plant raw materials to fuel components by fermentation [2]. Bioethanol fuel is simple to use, biodegradable, non-toxic and essentially free of sulphur and aromatics [4]. Bioethanol fuels are virtually inexhaustible, and domestically produced from agricultural resources. It is also oxygenated, thereby providing the potential to reduce particulate emissions in compression-ignition engines [5]. Bioethanol can be used directly on its own as in hydrous ethanol (95% purity) or as an anhydrous ethanol (99.5% purity) blended with gasoline [6].

Researchers have increasedover the years on how to extract ethanol in commercial quantity from non-edible agricultural products[7]. Eventually, the success in this field of research will go a long way to increase theprospects of ethanol as an essential biofuel component.Promising new technologies are beingdeveloped that uses enzymes to break down celluloseand release the plants sugar for fermentation intoethanol.

1.1 Cadaba farinosa forskk Plant

Cadaba farinosa forskk is a slender shrub with strongly furrowed stem. The fruit is oblong and cylindrical shaped with contractions 5cm long and densely farinose. The interior of the fruit is orange-red when mature. The seeds are the size of a millet grain, comma-shaped, shiny, dark brown, and arranged in a single layer within the fruit[8].

Cadaba farinosa forskk has many active phytoconstituents such as non-tannin phenolicskaempferol,new spermidine alkaloid Cadabicine, L-Stachydrine and 3-hydroxystachydrine, 3-hydroxystachydrine [9], three novel spermidine alkaloids one Capparisine and an aromatic acid, ά,ß-Dihydroferulic acid,novel sesquiterpenoid Cadabicine methyl ether, Cadabicine diacetate and besides a sisquiterpene and cadibicilone [10].According to [11],it also contains 3(4- formylphenoxy) -4-methoxybenzaldehyde, Methyl cinnamate, methyl ferulate ether, ether of p-cinnamic acid-m- ferulic acid, Thiazolidine compound. According to [9], it also shows the presence of quercetin, isoorientin, hydroxybenzoic acid, syringic acid, vanillic acid and 2-hydroxy-4-methoxy benzoic acid.

Fig. 1: Fresh Dangarfa Tree (Cadaba farinosa forskk)

The oral administration of leaf extracts at dose 1000 mg/kg led to a significant blood glucose reduction. Phytochemical analysis of alcohol extract revealed the presence of terpenoids, flavonoids, steroids, proteins, alkaloids, gums, sugars and saponins, but negative result was observed in aqueous extract except terpenoids, flavonoids, proteins, furans, gums and sugars. Their product has the following uses [12].

i. The young leaves are edible and are also used in spicing and flavouringfood.
ii. Flowers, leaves and fruits are relished by all livestock as fodder, except horses anddonkeys, particularly during the dry season. Camels are particularly fond of them and are the main consumers, since other species find it difficult to reach the foliage. Buffalo, black rhino and hartebeasts also seek the foliage. The fodder has high protein content, 30%, and a digestibility in vitro value of 78%. C. farinosa also has high ash content.
iii. Serves as biomass fuel.
iv. Crushed leaves mixed with millet-flour are used as medicine against coughs.
v. Other products: The alkaloids cadabicine and cadabicine diacetate have been isolatedfrom C. Farinosa stem bark.

1.2 Bioethanol Extraction

Bioethanol is one of the most important renewable fuels due to the economic and environmental benefits of its use. The use of bioethanol as an alternative motor fuel has been steadily increasing around the world for the number of reasons [3].

1. Fossil fuel resources are declining, but biomass has been recognized as a major reasons World renewable energy source.
2. Greenhouse gas emissions is one of the most important challenges in this century because of fossil fuel consumption, biofuels can be a good solution for this problem.
3. Price of petroleum in global market has raising trend.

4. Petroleum reserves are limited and it is monopoly of some oil-importing countries and rest of the world depends on them.
5. Also known petroleum reserves are estimated to be depleted in less than 50 years at the present rate of consumption. At present, in compare to fossil fuels, bioethanol is not produced economically, but according to scientific predictions, it will be economical about 2030.

Therefore, this research was focused on accessing the potential yield of ethanol fromCadaba farinosa forskk shrub, characterization of bioethanol produced and also determined its fuel qualities.

II. MATERIALS AND METHODS

2.1 Preparation of the Materials

The material was sun dried, chipped and then pulverized and sieved into smaller varying particle sizes of 200, 300 and 400µm (refer to plate II) to ensure the faster diffusion of the biocatalyst (i.e. yeast) on the substrate of Cadaba farinosa forskk plant. In this case, Cadaba farinosa forskk shrub was cut to smaller sizes, and then grinded to smaller sizes using mortar and pestles to increase their surface area. The average particle size is very important for the yield of ethanol. Smaller particles sizes are an essential requirement to facilitate easy water absorption, gelatinization, saccharization, fermentation of the substrate and higher bioethanol yield. In addition, smaller or finer particle sizes enhances substrate density, ensures easier transportation, and cheaper haulage costs.

Fig. 2: Crushed Dangarfa Tree(Cadaba farinosa forskk)

2.2 Conversion of Sucrose to Glucose

To convert sucrose in the Cadaba farinosa forssk substrate at 200µm, 300µm, and 400 µm particle size to glucose for the production of ethanol. 1300g of 200µm particle size of Cadaba farinosa forssk was mixed with 1650g of diluted sulfuric acid, and was heated to 100^{o}C for 6h. The hydrolyzed material was soaked in water and drained, several times; the solid residue was dewatered, and soaked in 40% sulfuric acid for 4 hours. The material was then dewatered, dried and re-mixed with 40% sulfuric acid and maintained at 100^{o}C for 3h. The content was filtered to remove solid and recover the sugar/acid solution. The sugar/solid solution was neutralized using Sodium Hydrogen Phosphate. The procedure is repeated for substrate with particle sizes of 300 µm and 400 µm respectively.

2.3 Preparation of Yeast Culture

200g each of sugar and Saccharomyces cerevisiae (yeast) flour were respectively added to 250ml of water. The solution was heated to 100^{o}C for 45minutes, and then cooled to room temperature. 20g of dried yeast was added to the mixture. The yeast culture was maintained in the refrigerator at 20^{o}C until when it is required for experimental use.

2.4 Fermentation and Distillation of the Sample

The wort (Cadaba farinosa forskk) was filtered, mixed and diluted with water of 250ml to adjust the initial sugar concentration.Sulfuric acid was also added to adjust the pH to 5.0. Seven (7) airtight fermentation set-ups were made. The activated yeast culture was added and the entire wort was equally distributed into the seven air tight fermentation set up. The entire set up was kept at room temperature 28±2^{o}C until when the wort

ceased from bubbling and the yeast cake had sunk to the bottom. The fermented wort was immediately distilled, and benzene was added to obtain 95% ethanol.

2.5 Determination of Sugar and Ethanol Concentrations

The concentration of sugar in the wort was determined at a 12-hour interval using sugar automatic analyzer (Model YP-2378 P1). The thin plastic sample tubing from the glucose analyzer was inserted into the wort sample tube. The sugar analyzer was switched on, the sample key was pushed. The sugar concentration was displayed within a minute (i.e. 60 seconds), and the value was recorded. Every 12 hours, sample of the fermented wort from the 200 µm, 300 µm and 400 µm Cardoba farinose forskk particle sizes was taken for ethanol concentration analysis and compared for their bioethanol yield. The fermented wort was distilled and the specific gravity of the fermented wort was determined with a hydrometer. The biomass concentration was determined using spectrophotometer (Model 63 SPEC-nm) at a 12hours interval and the results from different particle sizes are presented in Figure 4. A small quantity of the sample was taken and placed in the spectrophotometer. The absorbance of the sample was taken at a wavelength of 630nm using water as a blank.

2.6 Characterization of Bio-ethanol from Cadaba farinosa forssk shrub

The following tests were employed for the characterization of the bio-ethanol.

2.6.1 Determination of density

The density of the bioethanol was determined by employing the ASTM D97-93 standard methods (ASTM, 1993). In this case, the weight of a small empty bottle was determined using an electronic weighing balance. The bottle was then filled to the brim with the bioethanol sample and the weight of the bottle and sample determined. The density was calculated using the formula below.

Density (ρ) = $\frac{w_2 - w_1}{v}$ … (2)

w_2 = weight of bottle and Ethanol, w_1 = weight of empty bottle and v = volume of the sample.

2.6.2 Determination of Specific Gravity

An improvised specific gravity bottle was washed and rinsed with acetone dried in the oven. The bottle was cooled at room temperature in a desiccators and the weight of the empty bottle determined using an electronic weighing balance. The weight of bottle filled with water was recorded, then the water was poured out and the bottle rinsed with acetone and dried in the oven. The specific gravity was calculated using equation 3 below:

Specific gravity = $\frac{w_3 - w_2}{w_1}$… (3)

w_3= weight of bottle and Ethanol, w_2= weight of empty bottle and w_1= weight of equal volume of water.

2.6.3 Kinematic Viscosity

The viscosity of the bio-ethanol was taken using the U-tube viscometer (Ostwald viscometer**)**, the ethanol in the lower bulb was sucked to a point above the top white ring mark of the second bulb of the viscometer. The bio-ethanol meniscus was adjusted by releasing the thumb till it is at the same level with white ring mark on top of viscometer second bulb. The bio-ethanol was then allowed to flow and a stopwatch was used to take the time interval of the flow. The time for the ethanol to pass the second ring mark was recorded. Similar procedure was repeated for water and the time recorded.The viscosity was calculated from the results obtained.

v = $\frac{T_s - T_w}{T_w}$ … (4)

v = Kinematic Viscosity (mm^2/s), T_S = Time to run samples and T_W = Time to run water.

2.6.4 Calorific value measurement

The sample was poured in the capsule. A fuse wire was inserted in the sample which was then placed in the bomb. The bomb was then charged with oxygen from the oxygen cylinder after which it was covered. The calorimeter bucket was then filled with 2 liters of water. The bomb was then lowered partially in the bucket. The two ignition lead wires were then pushed into the terminals of the bomb head. The wires were oriented away from the stirrer shaft so that they do not tangle. The calorimeter cover was closed. The calorimeter takes over the running of the test and the calorific value of sample was read.

2.6.5 Flash Point Determination

A 150ml conical flask with a branch opening and a cork with an opening to allow the entrance of the thermometer were fitted into the flask and the thermometer put in place. The tip of the thermometer was immersed in such a way that it does not touch the bottom of the flask. The set up was place on a hot plate. The flaskwas filled with 5ml of bio-ethanol and was heated at slow constant rate on the hot plate. The flash point was taken at the lowest temperature when an application of the test flame caused the vapour leaving the flask opening to ignite.

2.6.6 Pour Point Determination

A portion of the bio-ethanol was poured into a test tube and the mercury point of the thermometer with calibration below 1^{o}C, was inserted in the test tube. The set up was inserted in a beaker containing ice and left to solidify. When the solidification was confirmed, the test tube was removed and tilted and closely observed till it started to flow. The lowest temperature at which the oil was observed to flow was recorded as the pour point.

2.6.7 Cloud Point Determination

A portion of the bio-ethanol was poured into a test tube and the mercury point of the thermometer with calibration below 1^{o}C, was inserted in the test tube. The set up was inserted in a beaker containing ice. The bio-ethanol was observed closely. After some time, the bio-ethanol was observed to form a cloud of gel. The temperature was taken as cloud point and was recorded.

III. RESULTS AND DISCUSSION

3.1 Percentage Ethanol Yield

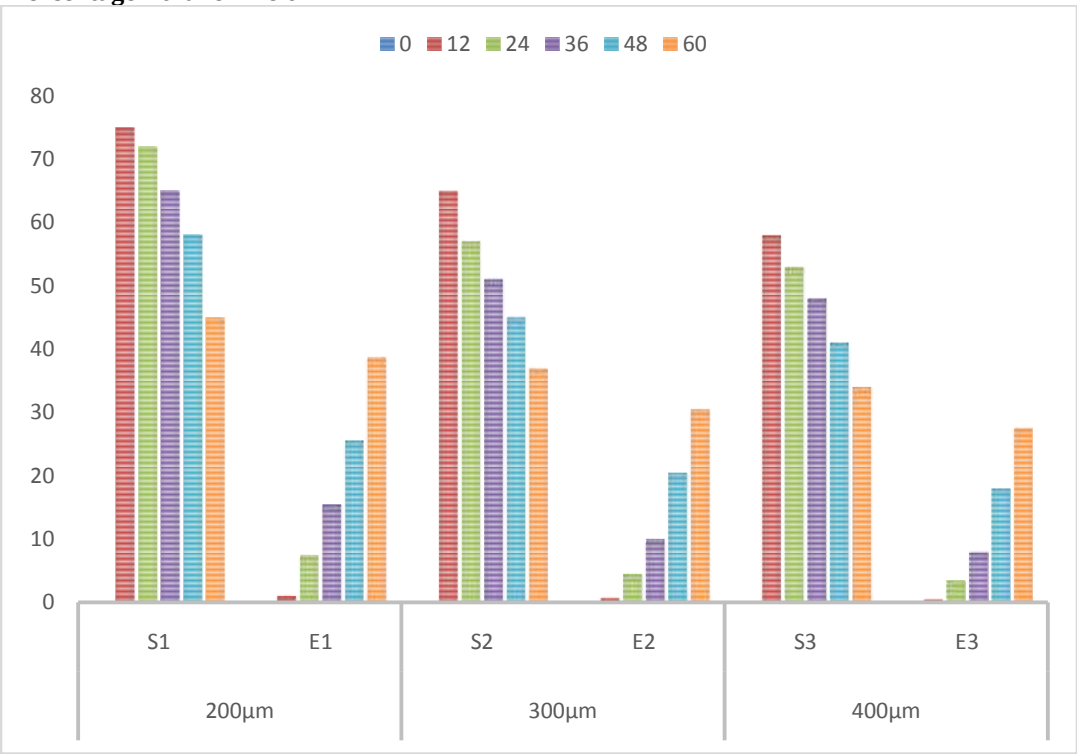

Fig. 3: Ethanol and Sugar Concentrations from Cadaba farinosa Forskk Substrate of Particle Size 200 µm, 300 µm and 400 µm at different Time Interval

The common factors that affect ethanol yield are the Temperature, PH and the substrate.

However, Fig. 3 showed that when thetemperature increased, the maximum fermentation time wasshortened, but a much higher temperature inhibited thegrowth of cells and then the fermentation significantlydeclined. In this study, cell growth and ethanol productiondeclined considerably at 50℃, which showed the inhibitioneffect on cell growth at higher temperatures.

The highest sugar concentration and bioethanol yield of Cadaba farinosa forskk plant was found to be 59% and 0.85L when particle size of 200µm was used as shown in Figure 3. This means that substrate of 1300g and containing 40%-60% fermentable sugar can produce a maximum ethanol yield of about 0.93 liters and a minimum yield of 0.65 liters. The high ethanol yield of Cadaba farinosa forssk plant is an indication that the

shrub is a good source of bioethanol since the yield of 0.85L of ethanol from 1300g of the material is well within acceptable range of 40%-60%.

1.3 Bioethanol Properties

Pure bioethanol is a polar solvent that is water-soluble and has a 11.4℃ flash point from Table 1. The bioethanol has a density of 789, which indicates that it is heavier than air. Consequently, ethanol vapors donot rise, similar to vapors from gasoline, which seek lower altitudes. The bioethanol has a specific gravity of 0.789, which indicates it is lighter than water but since it is water-soluble (it be consideredhydrophilic). It will thoroughly mix with water. The boiling point is78.3℃. Ethanol is less toxic than gasoline or methanol. The kinematic viscosity, the calorific values were also found to be 34.52mm^2/s and 29.5MJ/kg respectively. The pour and cloud points were also Found to be -8 and -6 respectively. Pour and cloud points become important for heavier fuels in the higher boiling range. Thus, the pour-ability of bioethanol is not a problem but it was specified in the guideline of fuel properties standard.

Bioethanol is bio-fuel substitute of gasoline; i.e. it is ethanol obtained from biomass (not from fossil fuels), and used as a gasoline blend.Interestingly, ethanol and some bioethanol blends can conduct electricity while gasoline does not and is considered an electrical insulator.

Table 1: Summary of the Properties of Tested Sample

Fuel Property	Bio-ethanol
Density at 20^{o}C (kg/m^3)	789
Specific gravity	0.789
Kinematic Viscosity at 40^{o}C (mm^2/s)	34.52
Calorific Value (MJ/kg)	29.5
Flash Point (^{o}C)	11.4
Pour Point (^{o}C)	-8
Cloud Point (^{o}C)	-6

IV. CONCLUSION

Ethanol was produced from Cadaba farinosa Forskk by means Simultaneous saccharification and fermentation processes. Ethanol can be produced from Cadaba farinosa Forskk in reasonable quantity. The highest sugar concentration and bioethanol yield of Cadaba farinosa forskk plant was found to be 59% and 0.85L when particle size of 200μmand 1300g of the material was used, which is an indication that the particle sizes have influence in the percentage yield.The physico-chemical properties of Cadaba Farinosa Forskk bio-ethanol produced were within the set standards for industrial ethanol and are found to be within the limits of ASTM and EN standards.The experimental results obtained in this work showed that both the conversion and fermentation processes were optimal and thatthe high ethanol yield of Cadaba farinosa forskk plant is an indication that the shrub is a good source of bioethanol.

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