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Production and Physicochemical Analysis of Bioethanol from Groundnut Shells Substrate and *Schizosaccharomyces Pombe* Yeast Specie

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Abstract: The production of bioethanol from groundnut shells using *Schizosaccharomyces pombe* as the fermenting yeast was studied. The pretreatment and hydrolysis process successfully convert the lignocellulosic components of groundnut shells into fermentable sugars, generating a reducing sugar concentration of 213.83 mg/dL. Fermentation generated 65.00mL of bioethanol with a percentage yield of 21.67% portraying the potential of groundnut shells as a second generation feedstock for bioethanol production. Physicochemical results of the produced bioethanol revealed a density of 0.789 g/cm³, kinematic viscosity of 1.14 cSt, and pH of 7.2, that conform to the ASTM standards, but the boiling point (69°C) was below the ASTM reference value (78.37°C) signifying the presence of lighter volatile compounds. The flash point (14°C) and fire point (21°C) confirmed the fuel's safety and combustibility. Overall, the results highlight groundnut shells as a promising, low cost feedstock for bioethanol production, though further optimization of pretreatment and fermentation processes is required to enhance yield and fuel quality.

Keywords: *Bioethanol, biomass, fermentation, groundnut shells, schizosaccharomyces pombe.*

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I. INTRODUCTION

Biofuels generated from biomass include biodiesel, biogas, and bioethanol. These fuels could help decrease carbon emissions, create job opportunities, rise region development and improve energy security [1, 2, 3]. The interest in using bioethanol has been growing since the 1980s and many countries have believe bioethanol is a promising alternative fuel. Bioethanol is the most common biofuel used in transportation sector and has been utilised as an alternative fuel for a very long time [2]. By 2030, the number of vehicles is predicted to be 1.3 billion and it is anticipated to rise to 2 billion by 2050 [1, 4]. The forecast from 2010 to 2040 shows that the world's oil consumption will increase by 26.2%. Thus, there is a very high possibility that the growing energy needs might be met by using renewable energy sources. Therefore, significant interest within the scientific community about the investigation of renewable and sustainable energy sources

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is on the rise[1].

Various feedstocks are available and used for bioethanol production. The first generation feedstock such as corn and sugarcane has been extensively exploited. However, controversy over the use of first generation feedstocks has rise because they are essential food sources. Thus, interest has been move to lignocellulosic biomass i.e second generation feedstock which is mainly of agricultural waste products [5]. *Schizosaccharomyces pombe* is a strong yeast with good fermentation proficiency and ethanol tolerance, making it an appropriate choice for bioethanol production [6, 7]. Furthermore, previous studies such as that conducted by 8 has conclude that *Schizosaccharomyces pombe* can be used as an alternative to *saccharomyces cerevisiae*.

This study aim to produce bioethanol from groundnut shells using *Schizosaccharomyces pombe* as a fermenting yeast specie and evaluation of the final product to assess its quality and suitability as an unconventional fuel. Transforming groundnut shells biomass into bioethanol is an Eco-friendly means to handle agricultural waste and helps create renewable and sustainable energy sources. Groundnut shells are readily available agricultural waste products that are usually discarded or underutilized to their full potential. Using them as bioethanol feed-stock adds value to waste materials and reduce pollution. The use of *schizosaccharomyces pombe* yeast specie as opposed to the commonly used *saccharomyces cerevisiae* gives insight about it suitability and proficiency for bioethanol production using groundnut shells as feedstocks. Understanding the yeast fermentation potential aids in enhancing bioethanol yield and advancing process viability.

II. MATERIALS AND METHODS

A. *Materials*

Groundnut shells were used as feedstocks for bioethanol production. The feedstock was chosen because they are abundant, inexpensive agricultural waste rich in lignocellulosic material (cellulose and hemicellulose). Groundnut shells also do not compete with food crops and aids in waste management and sustainable energy production. The yeast specie *Schizosaccharomyces pombe* was obtained from Microbiology Department of Usmanu Danfodiyo University Sokoto, Sokoto State, Nigeria.

B. *Methods*

1) *Bioethanol Production*

- Pretreatment:
  First, the feedstock (groundnut shells) was sorted manually to take off dirt, stones and other unwanted substances. This was followed by drying the clean groundnut shells for five days at room temperature to reduce their moisture content. Subsequently, the size of the feedstock was reduce by grinding to fine powder using pestle and mortar and then sieved through 36 μm mesh. The resulting fine powdered was used as the main feedstock for the production of bioethanol in this study.

- Alkaline Hydrolysis:
  Hydrolysis was done with 1% dilute sodium hydroxide (NaOH). Thirty grams (30g) of the powdered groundnut shell were added to each of the three different conical flasks. The flasks were covered with cotton wool and aluminum foil after been treated with 300 mL of 1% NaOH. The mixtures were heated in a water bath for an hour at 60-70 °C. Then kept for 24 hours before filtration to eliminate solid residue from the liquid. The residual solid residue was washed repetitively with distilled water till a neutralize pH was obtained and then filtered again. The filtrate was analyzed for reducing sugar concentration.

- Fermentation:
  Schizosaccharomyces pombe yeast specie was added to each of the three conical flasks containing the filtrate from alkaline hydrolysis. The flasks were capped with cotton wool and wrapped in aluminium foil, then secured with a masking tape. The samples were shaken and incubated for five days at 35°C.

- Distillation of the Fermented Broth:
  Simple distillation was used to purify the produced ethanol. The fermented mixture was placed in a round bottom flask connected to a condenser. The flask was heated using a heating mantle and distillation was continued until the vapor temperature reached approximately 78°C. The distillates was collected in a receiving flask. All experiments runs were conducted in triplicate, hence, results are presented as mean values.

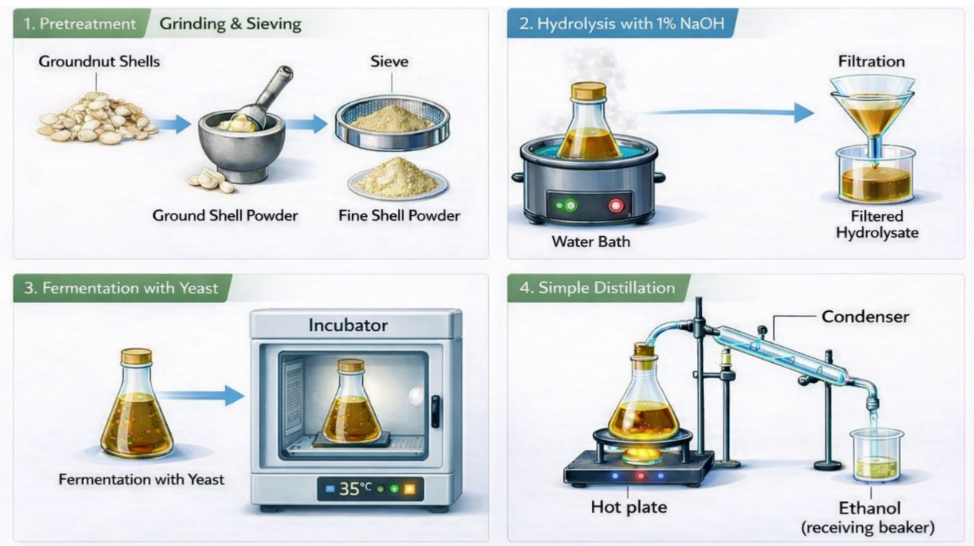

Fig. 1. Schematic presentation of bioethanol production process

C. Analysis and Characterization of the Produced Bioethanol

- Determination of Reducing Sugar:
  Two drops of acidified 0.1M potassium dichromate ($K_2Cr_2O_7$) were mix with 2ml of the distillate and heated slowly in a water bath for a few minutes. The test tube's contents became green, implying that there is either primary or secondary alcohol.

- Determination of the Quantity of Bioethanol Produced:
  A measuring cylinder was used to measure the distillate. The quantity of ethanol generated was recorded in ml.

- Determination Percentage yield:
  The percentage of the ethanol produced was calculated using the formula below.

$$Percentage\ yield = \frac{\text{Actual yield}}{\text{Theoretical yield}} \times 100 \quad (1)$$

- Physiochemical Characterization:
  The density, pH, boiling point, flash point and fire point of generated bioethanol were determined according to ASTM standard D4052, D1293, D1078, D93 and D92 respectively. The kinematic viscosity at 35°C was estimated using the following formula:

$$\nu = \frac{\mu}{\rho} \quad (2)$$

  Where: ν means kinematic viscosity, μ is dynamic viscosity (0.00090 Pa·s at 35°C) and ρ is density of the generated bioethanol.

III. RESULTS AND DISCUSSIONS

Figure 2 and Table 1 shows the major parameters that describe the production of bioethanol from groundnut shells using *Schizosaccharomyces pombe* as the fermenting yeast. Figure 2 values are approximated to zero decimal point. The pretreatment and hydrolysis processes has successful break down the lignocellulosic components of groundnut shells into fermentable sugars. This is confirmed by the reducing sugar concentration of 213.83 mg/dL. Reducing sugar concentration is an important parameter in bioethanol production because it determine the amount of substrate available for microbial fermentation [9].

The fermentation process using *schizosaccharomyces pombe* generated 65.00 mL of bioethanol, illustrating the yeast's capacity to metabolise the released sugars and convert them into ethanol. This also indicate the suitability of *schizosaccharomyces pombe* for fermenting sugars that come from agricultural waste, like groundnut shells.

The percentage yield of 21.67% do not represent the maximum theoretical yield. In fact, better yield could be obtained. However, it demonstrates the potential of converting groundnut shells to ethanol using *schizosaccharomyces pombe* yeast. The moderate conversion efficiency that gave birth to percentage yield of 21.67% might be caused by many factors including but not limited to partial hydrolysis, length of hydrolysis, presence of inhibitory compounds, fermentation conditions, type of feedstock and yeast metabolic limitations [10, 11, 12]. It is very significant to note that ethanol yield for second generation bioethanol feedstocks such as groundnut shells is substantially variable and feedstock dependent [12].

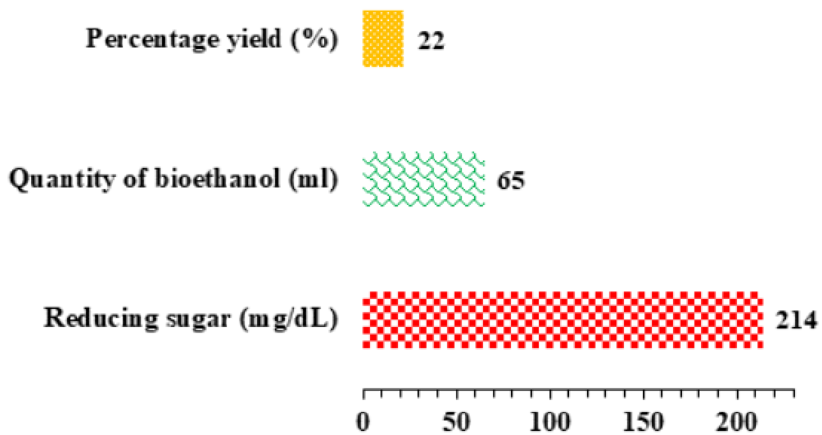

Fig. 2. Concentration of reducing sugar, quantity and percentage yield of bioethanol produced from groundnut shells and *schizosaccharomyces pombe* yeast specie.
Note: Values are rounded to zero decimal point.

S/N	Parameter	Value
1	Concentration of reducing sugar (mg/dL)	213.83
2	Quantity of produced bioethanol (ml)	65.00
3	Percentage yield (%)	21.67

Table 1. Concentration of reducing sugar, quantity and percentage yield of bioethanol produced from groundnut shells and *schizosaccharomyces pombe* yeast specie.

Table 2 represent the physicochemical properties of bioethanol produced from groundnut shells and schizosaccharomyces pombe yeast specie at neutral a pH, temperature of 35°C and five days fermentation period in comparison to ASTM standard specifications. One of the most significant property of a fuel is it density. This is because it affects production, transportation and distribution processes. Density also influences every process that happened in the internal combustion engine [13]. The measured density of 0.789g/cm³ conforms with the ASTM standard specification as depicted in table 2. The kinematic viscosity (1.14cSt) is negligibly lesser than the ASTM standard specification (1.2cSt). The decrease in kinematic viscosity could be attributed to the high temperature usually above 35°C in Nigeria. Previous studies has shown that as the temperature of fuel increases it kinematic viscosity decreases [14, 15]. The pH value (7.2) of the generated bioethanol sits well within the ASTM standard range of 6.5-9.0. This neutral pH implies that the generated bioethanol is chemically stable and doesn't have any major acidic or basic impurities. A neutral pH is vital for storage and engine performance. Bioethanol that is extremely acidic or alkaline can corrode metal parts and reduce the durability of the fuel system [16].

No.	Parameter	Unit	Experimental Bioethanol Value	ASTM Reference Value
1	Density	g/cm^3	0.789	0.789
2	Kinematic Viscosity	cSt	1.14	1.20
3	pH	—	7.2	6.5-9.0
4	Boiling Point	°C	69.00	78.37
5	Flash Point	°C	14.00	$\geq$12
6	Fire Point	°C	21.00	-

Table 2. Physicochemical properties of bioethanol produced from groundnut shells and schizosaccharomyces pombe yeast specie compared with ASTM standard specifications

Lighter volatile compounds such as water, methanol and other fermentation by products can reduce the overall boiling point of ethanol. The low boiling point could also mean partial purification during the distillation process

[17, 18]. Although this might not stop the bioethanol from being used as a fuel, it indicate that the bioethanol could be a mixture rather than exclusively pure ethanol. Such observe phenomenon is expected in bioethanol generated from fermentation prior to final alteration.To safeguard bioethanol quality, water and other contaminants in it should be considered when assessing energy content and combustion characteristics [19]. This is because the presence of lighter volatiles can affect engine performance [20, 21].

A measure that determined the flammability of a fuel is called flash point. Flash point is the lowest temperature a fuel ignites when exposed to an ignition source. Flash point determine the degree of hazards when traveling or storing a fuel [22, 23]. The flash point 14°C as shown in table 2 is within the ASTM standard range of $\geq$ 12°C. Meaning the produced bioethanol has satisfactory fulfil the safety and handling condition for bioethanol fuel. This also means the bioethanol fuel is sufficiently volatile to ignite under normal circumstances, while remaining safe for storage and transport [22, 23].

The fire point can be define as the temperature which the bioethanol can sustain continuous combustion for a minimum of 5 seconds [24]. The fire point value of 21 °C was expected because ethanol is a low molecular weight alcohol, with low boiling point and readily vaporize forming flammable vapours that support ignition . Fire point is usually 5 to 10 °C higher than flash point [25]. Flash and fire point are the key parameters that determine the safety of a fuel when designing its transportation [24].

IV. CONCLUSION

Generally, the results show that groundnut shells are promising, available and inexpensive feedstock for bioethanol production using *schizosaccharomyces pombe* yeast specie. But further studies to optimized processes such as pretreatment and fermentation conditions is required to improve ethanol quantity, yield and standard fuel specifications.

V. FUNDING

None.

VI. CONFLICT OF INTEREST

The authors declare no conflict of interest.

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