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Bioethanol production from Jatropha seed cake via dilute acid hydrolysis and fermentation by *Saccharomyces cerevisiae*

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Abstract

The interest in using *Jatropha curcas* L. as a feedstock for the production of bio-diesel is rapidly growing. Available literatures holds [promise for the simultaneous wasteland reclamation capability and oil yields of the plant hence fueling the *Jatropha* bio-ethanol hopes. This research investigated the bioconversion of cellulose from press cakes of Jatropha oil seeds, which is a byproduct from a biodiesel plant, into ethanol by using the methods of acid pretreatment, hydrolysis and fermentation by *Saccharomyces cerevisiae*. The process includes the pretreatment method of the finely ground cellulosic solid oilseed cake with dilute sulphuric acid and heating the mixture at a high temperature to break the crystalline structure of the lignocellulose to facilitate the hydrolysis of cellulosic component by dilute acids. About 63.33% ethanol was recovered as confirmed by the infra-red spectroscopy and the investigated physicochemical parameters show that the produced bioethanol holds promise for its use as a possible candidate for replacement for petroleum diesel.

Keywords: Bioethanol; Bio-fuel; *Jatropha curcas*; Physic nut

1. Introduction

*Jatropha curcas*is a large and fast-growing shrub belonging to the Euphorbiaceae family. Their average height is about 2 - 3 m and under special conditions, it can grow up to 5 m. The shrub has small yellow-green flowers, and the fruit is a capsule with three smooth, slightly flattened and elliptical black seeds, within which is found the white kernel, tender and rich in oil [1,2].The exploration of *J. curcas* seeds for biofuel generation is on the rise since the use of its seeds as animal or human feed is restricted due to its toxicity and the presence of antinutritional factors, such as phorbol esters, phthalates, lectins, saponins and trypsin inhibitors hence requiring a detoxifying treatment before its consumption [3-5].

The dependence on petroleum as the energy source comes at a high cost and the world petroleum production has perhaps reached its peak [6]. Experts suggested that existing oil and gas reserves would only last for a few additional years [7]. Besides emissions from the burning of these fuels such as CO_2 CO, NOx and sulfur containing residues are the principal causes of global warming [8]. This has stimulated the widespread search fora cheap and an eco- friendly alternative source [9]. Biofuels offer a partial solution to many of these problems [10]. The feed stocks for biofuel production are produced by domestic agriculture products, which mean that biofuel production occurs domestically [10]. In order to meet the rising energy demand and diminishing petroleum reserves; fuels such as biodiesel and bioethanol, are in the forefront of alternative technologies under development. Accordingly, the viable alternative for the compression-ignition engines is biodiesel [7].

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Biodiesel is a mono alkyl ester of fatty acids derived from vegetable oils and animal fats; it is a clean and renewable fuel [11,12]. It is a liquid which varies in colour between golden and dark brown depending on the production feedstock [13]. Biodiesel is one of the most attractive biofuel because of its biodegradability, higher flash point (150^{o}C), reduced exhaust emissions, miscibility in all ratios with petroleum diesel, compatibility with the existing fuel distribution infrastructure and inherent lubricity [14]. Other attractive features of biodiesel includes; non flammability, non toxic, reduced petroleum imports, low sulphur content,domestic production and oxygenating potentials [15-17].This study sought to chemically characterise the bioethanol produced from *J. curcas* seeds cultivasted in Sokoto State North Western Nigeria.

2. Material and methods

2.1. Collection and preparation of *Jatropha curcas* nuts

Clean *Jatropha curcas* seeds were procurred from retailers at Sokoto central market, Sokoto State Nigeria. The nuts were properly dried after which the seeds were obtained. The seeds were crushed before being subjected to extraction of the residual oil with ethyl ether with a solid-liquid ratio of 1:5. After drying at 60 °C for 48 hours, the seed cake was crushed and sieved through a 0.5 mm mesh sieve according to the procedure outlined by [18].

2.2. Pretreatment

The procedure outlined by Mohit *et al*. [19] was adopted. Equal volume of 0.5% sulfuric acid was added since a higher yield is obtained with a catalyst like H_2SO_4. The mixture was heated under pressure at a temperature of 125 - 130 C and 25 psi pressure for 1 hour. The mixture was again dried with hot air at 30-35^{o}C.

2.3. Hydrolysis

The substrate was hydrolyzed by adding dilute acid to the pretreated material twice the volume of 2%, 3% and 5% H_2SO_4 before ensure a uniform mixture by thorough shaking. The mixture was poured in glass bottles and sealed to prevent vaporization of acid due to heat. The mixture was kept at a temperature of 55^{o}C for 3 days. Regular mixing of the mixture was done to prevent precipitation.

2.4. Isolation of the microorganism

The procedure described by Snehal *et al*. [20] was adopted with slight modification. Procured *Sachromyces cerevisiae* TMB3400 was maintained on malt extract-glucose-yeast extract-peptone (MGYP) medium with the composition 0.3 g% malt extract, 1.0 g% glucose, 0.3 g% yeast extract, 0.5 g% peptone and 2.0 g% agar. The pH was maintained within the 6.4 - 6.8 range. The biomass was obtained by cultivating the yeast cells in MGYP broth medium and 1 x 10^7 cells were used for inoculation into fermentation media. During the fermentation process biomass obtained was deflocculated by washing 2-3 times with sterile normal saline (0.9% NaCl). The enzyme broth was filtered using coarse filter paper and the filtrate obtained was centrifuged at 10,000 rpm for 15 min. The crude enzyme extract obtained was analyzed for various enzyme activities. Cellulolytic enzymes obtained are used for carrying out saccharification and saccharified hydrolysate utilized for ethanol production.

2.5. Fermentation

Anaerobic batch fermentation of 200 ml broth media consisting of pretreated and hydrolyzed Jatropha seed cake was carried out in order to convert the released sugars into ethanol with the conversion process being accomplished by the enzymes released by *Saccharomyces cerevisiae*. The pH of the solution was maintained at approximately 4.2 to accommodate yeast growth by the addition of the required amount of 4 M NaOH solution. The volume of the broth was made up to 200 ml by the addition of the required amount of distilled water. The hydrolyzed material was sterilized by autoclaving at 120˚C, 15 psi pressure for 30 min before inoculating with the microorganism. The fermentation was carried out in a closed conical flask at a temperature of 32^{o}C with occassional agitation. The cornical flasks were properly sealed to maintain anaerobic condition with an outlet provided for the release of CO_2. The other end of the outlet was dipped in lime water to confirm the release of CO_2 as it turns lime water milky. Triplicate fermentation broths of same composition were prepared and incubated in the same conditions. The fermentation was continued for 6 days and samples were taken from each of the broths on each alternate day for analysis to get triplicate results [19].

2.6. Fractional distillation

The fermented broth was transferred into a round-bottom flask fixed to a distillation column with a running tap water through the column. A conical flask was fixed to the other end of the distillation column to collect the distillate. A heating

mantle with the temperature adjusted to 78.3°C was used to heat the round-bottomed flask containing the fermented broth for each group. The distillate collected was measured using measuring cylinder [21]. The percentage bioethanol yield was calculated according to the expression proposed by Gunasekaran and Kamini [22].

$$\text{Bioethanol Yield (\%)} = \text{Volume of Bioethanol Produced} / \text{Volume Sample Used}$$

2.7. Qualitative test for ethanol

The 2 cm^3 of acetone was added to a test tube containing 4 drops of the fractionated bioethanol. 2 drops of chromic acid were then added. The test tube was fitted with a tight cork with the mixture shaken vigorously to observe any colour change [23,24].

2.8. Density determination

The procedure described by Emtron [25] was employed. The volume of the bioethanol distillate from each fermentative organism with reference to the original mass of the sample utilized was employed to determine the density of the produced bioethanol according to the expression:

Density (g cm^3) = Mass of Sample (g) / Volume of ethanol produced (cm^3)

2.9. Infrared spectroscopy

The produced bioethanol distillate was subjected to IR spectroscopy to establish the presence or otherwise of important functional groups to confirm or otherwise if ethanol was actually produced [26].

2.10. Physiochemical Analysis

The physiochemical properties of the produced biodiesel namely pH, flash oint, pour point, cloud point, specific gravity, viscosity, refractive index, moisture content and conductivity were performed using standard procedures [27,28].

3. Results and discussion

3.1. Biochemical composition of *Jatropha curcas*

The biochemical composition of *Jatropha curcas* seed in comparison with the findings of other researchers is presented in Table 1.

Table 1 Biochemical composition of *Jatropha curcas* seed.

Properties	**Present Study w/w%**	**Eboibi *et al*. [29]**	**Aranisola *et al*. [28]**
Ash	3.77	3.50	3.25
Protein	23.67	24.00	23.20
Crude fibre	21.20	20.00	23.12
Moisture content	4.11	5.00	4.03

It was found that the obtained data were within the range of previous research investigations [28,29].

3.2. Percentage yield

The percentage yield of bioethanol produced using S. cerevisiae as the fermentative organism is 63.33%. This is a confirmation of the findings and report of Gunasekaran and Chandra [30] which shows that S. cerevisiae facilitates ethanol production by ensuring the continuous fermentation of pentose sugars which are normally found in hemicelluloses biomass.

3.3. Qualitative test for ethanol

The change in colour of the mixture forming a blue-green precipitates within few second of adding drops of chromic acid confirms the presence of ethanol.

3.4. Density determination

The density of the produced bioethanol using S. cerevisiae fermentative organism is 0.898 g/cm3. The produced bioethanol is within the acceptable density of 0.8033 g/cm3 for the standard ethanol.

3.5. Physicochemical characterization of the produced bioethanol

The physiochemical properties of the Jatropha biodiesel are shown in Table 2.

Table 2 Physiochemical properties of bioethanol obtained *Jatropha curcas* seed.

Property	Present study	Eboibi *et al*. [28]	Aranisola *et al*. [27]	ASTM Biodiesel standard
pH	5.69	-	-	6.50 – 9.00
Flash point (^oC)	152.00	146.00	170.00	>130.00
Pour point (^oC)	3.00	2.00	6.00	-
Cloud point (^oC)	7.00	8.00	3.00	-
Specific gravity	0.87	0.92	0.88	0.86 – 0.90
Viscosity ($mm^2 s^{-1}$) at 24^oC	2.40	2.65	5.64	1.90 - 6.00
Refractive index	1.49	1.46	-	-
Moisture content	0.04	0.05	0.00	<0.03
Conductivity (µs/cm)	41.90	-	-	-

The reported pH value of 5.69 falls below the acceptable limit. Fuel pH grade ethanol below 6.5 may contribute to failure in fuel pump and fuel injectors as a result of corrosion wear while a pH above 9.0 may negatively impact plastic parts in the fuel system. While the values of the flash point, viscosity and specific gravity obtained from the analysis of the Jatropha biodiesel were found to be within the range of the standard biodiesel, that of moisture content were slightly below and above the bioethanol standard respectively. Viscosity is the measure of material resistance to flow, higher viscosity materials flows with great difficulty and a material with less viscosity flow more easily. Viscosity is important to diesels and biodiesels because it has impacts on the operation of some engine components such as the fuel pump. The viscosity of the biodiesel produced (2.40 mm^2/s) is lower than that reported by Aranisola *et al*. [28] and Eboibi *et al*. [29], however it is within the specified range of the ASTM standard (1.9mm^2/s to 6.0mm^2/s). Other parameters tested confirm the biodiesel produced from this study to meet the criteria for acceptable standards.

3.6. Infrared spectroscopy confirmation of the produced bioethanol

The IR spectrum reveals the presence of the following functional groups with frequencies: C-O (1043.7 and 1084.7 cm^{-1}), C-H (2903.6 and 2981.9 cm^{-1}) and O-H (3347.1 cm^{-1}) which is a confirmation of the broad and intense O-H peak in the 3650-3200 cm^{-1} region while that of the C-O stretch is seem in the region of 1300-1000 cm^{-1} and C-H stretch observed in the (2800-3000 cm^{-1}) region.

4. Conclusion

It can be concluded that Jatropha oil seed cake has a capability to undergo acid hydrolysis and fermentation for production of bio-ethanol. The result of biodiesel characterization shows that the fuel fulfills most of the ASTM standard so can be used as a possible candidate for replacement for petroleum diesel.

Compliance with ethical standards

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Disclosure of conflict of interest

The author declare that there is no conflict of interest to disclose.

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