

Rhizoclonium grande Bioethanol in Biofuel Production by Transesterification of *Jatropha curcas* Oil

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Abstract

There is a need for an alternative sustainable fuel based on renewable sources and efficient carbon dioxide scrubbers. Alkyl esters prepared by transesterification of vegetable oils from various plants have been evaluated. In this work the potential of sustainable macroalgae bioethanol in biofuel production by transesterification of *Jatropha curcas* oil was assessed. *Rhizoclonium grande* macroalgae was collected from Shimoni shores in Kwale, Shelly beach in Mombasa, Jamvi la Wageni in Mtongwe Likoni, English point near Kenya Marine and Fisheries Research Institute (KMFRI) Mombasa, dried and processed to obtain 5.36% ± 0.355% v/w bioethanol using *Aspergillus niger* for hydrolysis and *Saccharomyces cerevisiae* for fermentation. *J. curcas* seeds were collected from contracted farmers of Energy Africa in Shimba hills, Coast region. Oil from the seeds was machine cold pressed and solvent extracted using n-hexane giving 44% - 53%v/w yield. The physicochemical properties of the *J. curcas* oil were investigated. Transesterification of *J. curcas* oil was carried out using bioethanol from the algae with 63% - 70% FAEF yield. Characterisation of the bioethanol and ethyl esters was done using GC-MS. Physicochemical and fuel properties of the biofuel were investigated at Technical University of Mombasa (TUM), Jomo Kenyatta University of Agriculture and Technology (JKUAT), Government Chemist and Kenya Pipeline Laboratories in Mombasa. There was significant difference in physicochemical and fuel properties observed in density, calorific value, kinematic viscosity, pour point and cloud point between the *Jatropha* oil and *Jatropha* fatty acid ethyl esters (JAT FAEF) samples. No significant difference observed in the physicochemical and fuel properties between the JAT FAEF and standard biodiesel samples. This was according to statistical analysis of data done

using STATA/SE 13.0 and Xlstat at 95% confidence level ($P < 0.05$) two-tailed. From the findings bioethanol from *R. grande* biomass used in transesterification has a potential to improve the sustainability, physicochemical and fuel properties of biofuel from *J. curcas* a non-food crop. The effect of the use of bioethanol and its byproduct on shelf life of the biofuel can further be investigated.

Keywords

Jatropha curcas, *Rhizoclonium grande*, Biofuel, Physico-Chemical Properties and Fuel Properties

1. Introduction

There is a need for non-food crops biomass such as *J. curcas* L. (**Plate 1(a)** and **Plate 1(b)**) and carbon dioxide scrubbers such as algae for alcohol biomass source in biofuel production. Algae can produce up to 30 times more energy per acre than land crops such as soyabean hence more sustainable [1]. *J. curcas* seeds husked (**Plate 2(a)**) and dehusked (**Plate 2(b)**) constitute 53% - 57% non-edible oils [2] [3].

Lower alcohols such as methanol, ethanol, and butanol are the most frequently employed in transesterification of plant based oils [4] [5] [6]. The preference of the alcohol is based on cost and performance. Ethanol may be preferable to methanol because of its dissolving power for oils and less toxicity. Ethanol when

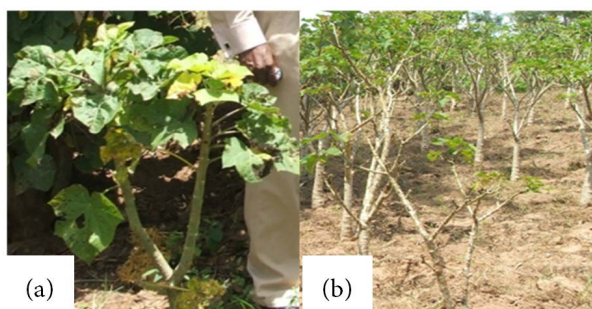


Plate 1. *J. curcas* crop (a) young; (b) mature.

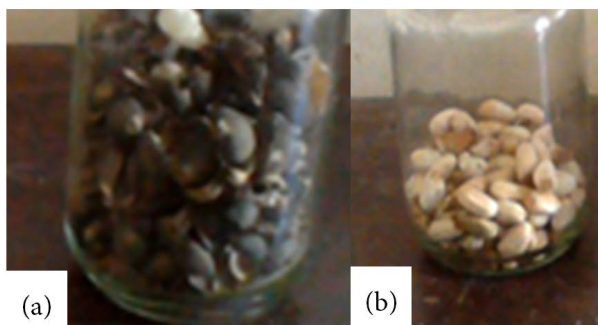


Plate 2. *J. curcas* seeds (a) husked; (b) dehusked.

used for the transesterification of vegetable oils produces ethyl esters rather than methyl esters. The extra carbon atom provided by the ethanol molecule slightly increases the heat content and the cetane number of the biodiesel [7].

Potassium and sodium hydroxide are the most commonly used homogeneous alkali catalysts in the transesterification of oil with very low free fatty acid content. Alkali metal catalysts are found to be more effective than acidic catalyst [8].

A biofuel from *Jatropha curcas* oil and bioethanol from algae biomass would improve sustainability since algae can be easily cultured and easily produced. There is scanty information on renewable sources of ethanol for transesterification apart from edible sources of carbohydrates and lignocelluloses that may require complex pretreatment to remove lignin. Biodiesel has advantages such as increased lubricity and reduction of exhaust emissions than petroleum based diesel [9].

This work sought to assess physicochemical and fuel properties of fatty acid ethyl esters obtained from transesterification of *Jatropha curcas* L. oil using bioethanol from algae *Rhizoclonium grande*. The performance of the biofuel was compared with standard biodiesel. This work was an attempt to unveil a more sustainable, cheap and quality biofuel.

2. Materials and Methods

2.1. Sample Collection and Pretreatment

J. curcas seeds were collected from Shimba hills, Kwale County which is one of the major *Jatropha* growing areas. The farmers contracted by the out grower scheme Energy Africa were randomly sampled by snow balling and the seeds were bought in 50 kilogram gunny polypropylene bags. The husked seeds were transported to Technical University of Mombasa (TUM) where they were dehusked using a locally fabricated dehusker. The dehusked seeds were weighed and preserved in polythene bags in an air conditioned room at temperature 25°C ready for extraction.

Macroscopic and filamentous marine algae species *R. grande* was randomly collected from different sampling sites namely Shimoni shores in Kwale County South Coast, Shelly beach in Likoni South Coast, Jamvi la Wageni in Likoni South Coast, English point near Kenya Marine and Fisheries Research Institute (KMFRI) Mombasa North coast and Tudor Creek near Kenya Meat Commission along Makupa Causeway. The macroalgae and filamentous species, identified by a taxonomist from KMFRI as *R. grande*, was collected and put in 5kg polythene sample bags. The samples were kept in cold boxes and transported to Technical University Laboratories for further treatment. The algae biomass was dried under a shade for three days then it was ground to powder using Hamilton Beach commercial blender.

The algal biomass was extracted with hexane to remove the oil component. The extracted material was collected and dried at 80°C to remove trapped hexane. The dried *R. grande* biomass was sieved through sieve of mesh number 18

equivalent to 1mm pore. The fine powder obtained was used for saccharification and fermentation experiments.

2.2. Saccharification of the Algal Biomass

The de-oiled [10] and dried fine powder (150 g) of marine algae *R. grande* were weighed and formed the biomass for ethanol production. The fungi *Aspergillus niger* for saccharification was obtained from Microbiology Laboratory Kenyatta University. The fungi was cultured and maintained on potato dextrose agar medium at 30°C.

Mycelial mat of *A. niger* was weighed (0.85 g) on analytical balance and added to the biomass, 75 ml of distilled water was added and homogenised using a sterile blender and then transferred into stainless steel trays. The biomass was allowed to start decaying to rupture the thin cell walls and release fermentable carbohydrates by *A. niger*. The saccharification was carried out for a period of six days at 30°C.

2.3. Spectrophotometric Analysis of Reducing Sugars Produced During Saccharification

The reducing sugar level was followed by use of UV-Visible spectrophotometry Miller's Dinitrosalicylic method [11]. The process was monitored every 24 hrs for sugars released by Millers method of glucose estimation using standard calibration graph. 100 mg of glucose in 100 ml of distilled water was prepared as the reducing sugar standard stock solution. 0.2, 0.4, 0.6, 0.8 and 1 ml from the stock solution in 10 ml of solution in distilled water were further prepared and used as the standard solutions for calibration. Similarly sample solutions were prepared by 2 g of sample biomass in 100 ml distilled water followed by filtration.

2.4. Fermentation of Saccharised Algal Biomass

The fungi *Saccharomyces cerevisiae* for fermentation was obtained from Agrochemical and Food Company Limited. The yeast *Saccharomyces cerevisiae* was cultured and maintained on yeast extract, peptone and dextrose (YPD) agar media at 30°C. After 6 days 10% of the brewers' yeast was added to the fermentable solution. The mixture was allowed to ferment for 4 days at room temperature 30°C.

2.5. Separation of Algal Bioethanol by Fractional Distillation

The bioethanol produced was then separated from the fermentation solution by fractional distillation method [12]. Ethanol boils at 78.4°C while water boils at 100 °C. When the mixture was heated, ethanol which is the most volatile component concentrated to a greater degree in the vapor phase and left the liquid. The distillate formed an azeotrope, comprising of mainly 96% bioethanol and 4% water at 78.2°C and the mixture is more volatile than pure ethanol. For this reason, ethanol was further purified by dehydration using anhydrous magne-

sium sulphate [13].

2.6. Extraction and Analysis of Oil from *Jatropha curcas* Dehusked Seeds

J. curcas oil (JO) was extracted from dehusked seeds using fabricated cold oil press and n-hexane as the solvent and also industrial blender for grinding. The oil was allowed to settle then filtered using vacuum filtration. For the solvent extracted oil from the cake the solvent was recovered by vacuum evaporator model ROVA-3L at 50°C with a negative pressure 450 mmHg and reused. The amount of oil was measured using a 100 ml measuring cylinder and kept in a refrigerator at 4°C and later analysed for oil physicochemical properties using ASTM procedures and Association of Official Analytical Chemists (AOAC), official methods [14].

2.7. Transesterification of *Jatropha Curcas* Oil and Analysis

Dry bioethanol prepared from marine algae *R. grande* L. biomass was used for transesterification [15]. Dry potassium hydroxide was reacted with the bioethanol to produce the ethoxide ions mixture in a ratio of 1:10. The mixture was added to *J. curcas* L. oil in a ratio of 3:10 ethoxide ion solution from the bioethanol to oil preheated at 65°C with constant stirring using a mechanical stirrer at 200 rpm for two hours to produce fatty acid ethyl ester biofuels [16] [17]. The ethyl esters were then separated from the glycerol by centrifugation using centrifuge Model Neofuge 15R at 10,000 rpm for 15 minutes followed by decantation, washing with distilled water and drying. Any bioethanol residues blends with the biodiesel [18]. The ethyl esters from *J. curcas* L oil were analysed by GC-MS analysis [19] to ascertain composition. The crude fatty acid ethyl esters (FAEE) were then analysed for physicochemical and fuel properties using ASTM D6751 standard test methods which were compared with those of standard biodiesel.

2.8. Data Analysis

Three independent replicates were used per analysis and the results were expressed as mean values \pm standard deviation of mean. Analysis of variance (ANOVA) followed by Post-Hoc test ($P \leq 0.05$) was used for comparison and separation of mean. Exploratory data analysis and standard statistical analyses were done using the statistical software package STATA, version SE 13 (STATA/SE13 © 1985-2013 StataCorp LP).

3. Results and Discussion

3.1. Fermentable Sugars from Algae Biomass

After 6 days the amount of reducing sugar present in 100 g algae biomass was 47.82 g. Generally the conversion level for *Rhizoclonium grande* algae biomass of 19.01% (Table 1) is similar to that reported for spirogyra biomass a similar fi-

lamented green algae. The amount of carbohydrates in some algae can be 40% - 60% [20]. This is sufficient for fermentation.

3.2. Bioethanol Yield from *R. grande*

Shelly beach algae recorded the highest bioethanol production 5.83% and Jamvi la Wazee the lowest 5.00%. Devedra and Rackesh, 2013 recorded 6 g/100 g biomass from algal biomass. The difference in conversion rate in *R. algae* may be due to the greater toughness of the cell wall that makes it harder for the fungi to rupture. On average *R. grande* produced $5.36\% \pm 0.355\%$ v/w of bioethanol (Table 2).

3.3. GC-MS Spectra of Bioethanol

GC-MS spectra of bioethanol from *R. grande* biomass after fractional distillation and drying using anhydrous magnesium sulphate showed the main component is ethanol $R_t = 1.965$ with some small quantities of methanol $R_t = 1.852$. Main m/z values of ethanol are 18.1, 31 and 45 and methanol m/z 18.1 and 31.

3.4. *Jatropha curcas* Oil from Dehusked Seeds

The % mean mass of *J. curcas* dehusked seeds from different contracted farmers in an out grower scheme Energy Africa in Shimba hills, Kwale County ranged from 58.679% to 64.544%. This can be attributed to difference in soil texture, nutrients and pH. The volume of *Jatropha* oil extracted from *J. curcas* using

Table 1. Reducing sugars produced during saccharification of algae biomass.

Day	Absorbance of 2.5 g/l biomass	Concentration of reducing sugars (mg/l) from 2.5 g/l biomass	Amount of reducing sugars per 100 gram biomass	% conversion to reducing sugars
0	0.284	720.25 ± 0.19	28.81	-
1	0.402	1174.54 ± 0.28	46.96	18.17
2	0.403	1176.23 ± 0.22	47.04	18.24
3	0.406	1178.04 ± 0.21	47.12	18.31
4	0.406	1178.38 ± 0.29	47.13	18.33
5	0.409	1195.03 ± 0.19	47.80	18.99
6	0.409	1195.58 ± 0.26	47.82	19.01

Table 2. Amount of bioethanol produced after fermentation of algae biomass.

Sample	Biomass (g)	Bioethanol (ml)	% v/w yield of bioethanol
Algae-Jamvi	150	7.50	5.00
Algae-Tudor	150	8.10	5.40
Algae-Shelly	150	8.75	5.83
Algae-Shimoni	150	7.80	5.20
Mean \pm SD		8.04 ± 0.534	5.36 ± 0.355

hexane after cold pressing or grinding by blender from five sampling fields in Shimba Hills. The amount of *Jatropha curcas* oil obtained by cold press followed by hexane extraction was lower 330 ± 1.00 ml ($44\% \pm 0.130\%$) than that obtained by blender followed by hexane extraction ($51\% - 53\%$) (**Table 3**). This may be attributed to loss during pressing by evaporation and wetting of the cold press. *Jatropha curcas* kernels have about 50% oil [21]. The oil recovery in mechanical expeller is about 85%, while more than 95% recovery of oil could be achieved when extracted by solvent method. The mean yields using blender and hexane solvent were similar to those reported 40% - 60% [22] [23] [24].

3.5. Physicochemical Properties of *Jatropha curcas* Oil

The oils extracted from *Jatropha curcas* was analysed [25] for physicochemical and thermodynamic properties using AOCS, AOAC and ASTM standard test methods which include: Density kg/dm^3 ASTM D1298 at 20°C , Kinematic Viscosity at 40°C (cSt) ASTM D445, Refractive index (AOAC Method 920.212), Acid value (ISO Method 660:2009), Iodine value (AOAC Method 920.159) and Saponification value (AOAC Method 920.160). The results were recorded (**Table 4**). The density and kinematic viscosity of the *Jatropha curcas* oil (JO) is more than the standard biodiesel. The high density, refractive index and kinematic viscosity may be due to the difference in molecular weight of fatty acids in the oil. The high kinematic viscosity confirms that the oil require transesterification to reduce the viscosity. It also indicates the relative difficulty of pumping the oil. The flash point is higher than for petroleum based diesel (**Table 4**). From the flash point values it implies that the oil (JO) has no storage or handling problem without serious fire hazards. The heating value and cetane number are almost similar to petroleum diesel. These properties are mainly dependent on the elements present in the oils namely carbon, hydrogen and oxygen. Iodine value is more in *Jatropha* oil but lower than 120 hence can readily be used as biofuel feedstock. The use of iodine value of a maximum of 120 can serve to restrict certain vegetable oils as biodiesel feedstocks especially when oxidative stability specification parameter are not included in the analysis [26].

Table 3. % yield of *Jatropha* oil per gram of biomass from each sampling site.

Sampling field	<i>Jatropha</i> biomass (g)	<i>Jatropha</i> oil produced (ml) (mean \pm SD)	%v/w yield of <i>Jatropha</i> oil per gram biomass (mean \pm SD)
JOFA ^a	750	330 ± 1.00	44 ± 0.130
JOFA ^b	750	390 ± 2.00	52 ± 0.267
JOFB ^b	750	400 ± 1.00	53 ± 0.135
JOFC ^b	750	380 ± 2.00	51 ± 0.267
JOFD ^b	750	390 ± 5.00	52 ± 0.667
JOFE ^b	750	385 ± 4.36	51 ± 0.581

^aSample obtained by cold press followed by hexane; ^bSample obtained by blender followed by hexane.

Table 4. Physicochemical properties of jatropha oil.

Jatropha oil Sample	Physicochemical oil Properties (mean \pm SD)					
	Density kg/m ³ ASTM D1298 at 20°C	Refractive index (AOAC Method 920.212)	Kinematic Viscosity at 40°C (cSt) ASTM D445	Acid Value mg KOH/g (ISO Method 660:1996)	Iodine Value mg/g (AOAC Method 920.159)	Saponification Value mg/g (AOAC Method 920.160)
JOFA ^b	917 \pm 1.12	1.497 \pm 0.012	34.12 \pm 0.16	36.58 \pm 0.12	106 \pm 1.0	194.5 \pm 0.1
JOFB ^b	916 \pm 1.03	1.496 \pm 0.010	33.96 \pm 0.13	36.57 \pm 0.16	106 \pm 1.0	195.2 \pm 0.3
JOFC ^b	916 \pm 0.19	1.496 \pm 0.011	34.10 \pm 0.09	36.40 \pm 0.13	107 \pm 0.0	195.6 \pm 0.6
JOFD ^b	916 \pm 1.10	1.496 \pm 0.012	34.09 \pm 0.12	36.46 \pm 0.18	108 \pm 0.0	195.8 \pm 0.2
JOFE ^b	916 \pm 1.02	1.496 \pm 0.015	35.20 \pm 0.11	36.49 \pm 0.15	106 \pm 1.0	194.9 \pm 0.1

3.6. Ethyl Esters Yield from *Jatropha curcas* Oil

The percentage yield of the ethyl esters was highest in oil from JOFC (70%) and the least was that from JOFE (63%) (Table 5). The values are lower than those in literature [27] for *Jatropha* oil methyl esters of >90%. The relatively lower yield values of alkyl esters compared to methyl esters could be attributed to difficulty experienced in separation and isolation of alkyl esters and glycerol [28]. In this case the separation of ethyl esters was improved by centrifugation.

3.7. GC-MS Spectra of *Jatropha curcas* Ethyl Esters

The main components of *Jatropha curcas* ethyl esters are Ethyl hexadecanoate (palmitic acid ethyl ester) (C16:0) Rt 11.21(4), Ethyl octadecanoate (C18:0) Rt 12.302(5) and Ethyl 9(Z)-octadecenoate (C18:1) Rt 12.368(6). Others are ethyl-decanoate (C10:0) Rt 6.467(1), ethyl dodecanoate (C11:0) Rt 8.143(2), ethyl nonadecanoate (C19:0) Rt 9.651(3) and ethyl eicosanoate (C22:0) Rt 13.619(7) in smaller quantities (Figure 1).

3.8. Physicochemical and Fuel Properties of Ethyl Esters from *Jatropha curcas* Oil

Jatropha ethyl esters from all the sample sites had physicochemical and fuel properties (Table 6) acceptable by ASTM D 6751 (Table 7). The mean values obtained are comparable with those obtained for *Jatropha curcas* L. methyl esters from various publications. According to Rajas' findings on analysis of fatty acid methyl ester (FAME) produced from *Jatropha curcas* the properties obtained were kinematic viscosity (4.82 mm²/s), flash point (128°C), specific gravity (0.84 g/ml), calorific value (42.80), pour point (−2°C) and cloud point 8°C [29]. Properties of the FAEE biodiesel obtained with bioethanol in this case were comparable to the FAME and ASTM standard biodiesel [30] (Table 7) showing that they were within the specifications.

Data on physicochemical and fuel properties of the biofuel JO FAEE investigated showed that there was no significant difference ($P < 0.05$) in density, calorific value, kinematic viscosity, pour point and cloud point with those of standard biodiesel samples. Flash point was within the allowable limit of 130°C minimum

Table 5. %yield of ethyl esters from *Jatropha curcas* oil from different sites.

Jatropha oil sample	Volume of <i>Jatropha</i> oil (ml)	Mass of Potassium hydroxide (g)	Volume of dry ethanol (ml)	Mean \pm SD Volume of ethyl esters (ml)	Mean \pm SD %yield of ethyl esters
JOFA ^a	100	3	30	65 \pm 1.5	65 \pm 1.5
JOFB ^b	100	3	30	67 \pm 1.0	67 \pm 1.0
JOFC ^b	100	3	30	70 \pm 0.6	70 \pm 0.6
JOFD ^b	100	3	30	64 \pm 2.0	64 \pm 2.0
JOFE ^b	100	3	30	63 \pm 0.0	63 \pm 0.0

Table 6. Physicochemical and Fuel Properties of ethyl esters from *Jatropha* oil.

Ethyl ester (FAEE) Sample	Sampling farm site	Physicochemical and Fuel Properties (mean values)							
		Density kg/m ³ ASTM D4052/D1298 at 20°C	Calorific Value (KJ/Kg) ASTM D4809	Kinematic Viscosity at 40°C (cSt) ASTM D445	Flash point (°C) ASTM D93	Cetane Index ASTM D4737	Pour Point (°C) ASTM D97	CFPP ASTM D6371	Cloud Point (°C) ASTM D2500
FAEE1	SFA	865.0	41.36	5.350	182	63.3	-10	-2	-4
FAEE2	SFB	865.9	40.84	5.405	183	62.4	-9	-2	-3
FAEE3	SFC	868.4	40.62	5.426	183	63.2	-9	-2	-4
FAEE4	SFD	887.3	41.05	5.432	183	63.4	-8	-1	-2
FAEE5	SFE	886.2	40.56	5.430	182	63.2	-8	-2	-3

Table 7. Biodiesel Specifications and test methods of ASTM D6751-07b.

Properties	Limits	Units
Flash point	130 min.	°C
Kinematic viscosity at 40°C	1.9 - 6.0	mm ² /s
Water and sediment	0.050 max	% volume
Density at 15°C	875 - 900	kg/m ³
Cloud point	report	°C
Pour point	-10 to 12	°C
Acid value	0.80 max	mg KOH/g
Distillation temperature, 90% recovered	327 max.	°C

despite the $p > 0.05$. This is an advantage making it safe in use. There was a significant difference ($P > 0.05$) in density, calorific value, kinematic viscosity, pour point and cloud point between JO FAEE and JO samples which justified the need for transesterification. Statistical analyses of data were obtained using scientific data analysis softwares (STATA/SE 13.0 and Xlstat) at 95% confidence level ($P < 0.05$) two-tailed.

4. Conclusion

Jatropha curcas FAEE produced by transesterification using bioethanol from *R.*

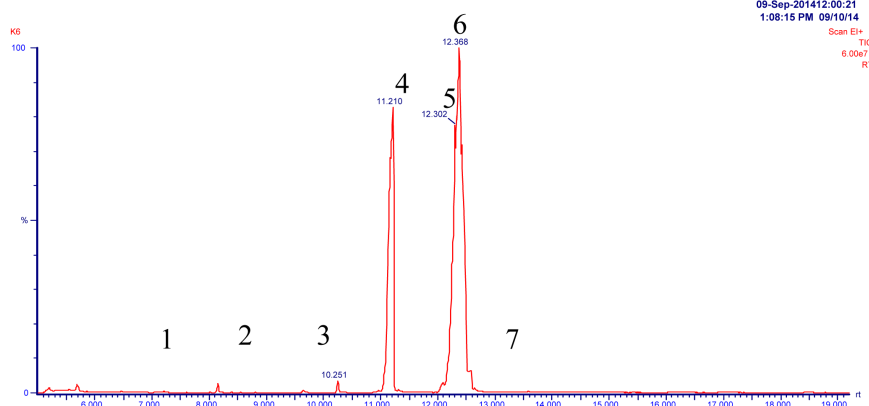


Figure 1. GC-MS spectra of *Jatropha curcas* ethyl esters.

grande biomass has desirable physicochemical and fuel properties making it a potential quality biofuel. Bioethanol from *R. grande* biomass has a potential to improve sustainability of the renewable energy.

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