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ORIGINAL ARTICLE

Fruit Biomass Derived Bio-ethanol: Its Physical, Chemical, Biochemical Mechanical, properties and Bio-energy Generation

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ABSTRACT
Bioenergy from biomass derived bioethanol is biodegradable, non-toxic and does not cause environmental pollution . Fruits waste can be reused for bioethanol production. Hence, it can reduce pollution and waste material, thus, assists in waste disposal management and reducing global warming. The aim of the study was to produce bioethanol and get the optimization from waste rambutan. Rambutan fruit waste biomass was used at different condition of fermentation using yeast (Saccharomyces cerevisiae). The optimum condition of bioethanol yield was having 4 g of yeast at 30°C by following pH 5.8 for two days of incubation. Glucose content reduced after fermentation due to the conversion of glucose to ethanol and carbon dioxide in case of all parameters. The chemical content, viscosity and acid values of the bioethanol produced were within ASTM (American Society for Testing and Materials) standard specifications with less hazardous chemical content in produced bioethanol. Moreover, the engine test result showed that greenhouse gas emission like hydrocarbon (HC), NOx, SO_x, CO2 and CO content in E5 and E10 were significantly lower in bioethanol than in 100% gasoline tested in 2 stroke engine. Thus, it can potentially be used as good biofuel for petrol engine purposes.
***Key words**: Bioethanol, fruit waste, emission, renewable energy, global warming.*

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INTRODUCTION

Bio-energy generated from biodiesel or bioethanol, is currently becoming an important issue all over the world. Global warming is being increased mainly by the combustion of petroleum which needs to be reduced. Excessive engine emission (NO_X, CO_2, CO, HC etc.) is one of the factors to increase greenhouse gas as well as global warming. Biofuel is one of the significant components to reduce greenhouse gas [2-7]. Biothanol is biodegradable, non-toxic and does not cause environmental pollution if spilt [8]. Bio-ethanol is usually obtained from the conversion of carbon based feedstock. Agricultural waste feed stocks are considered renewable because they get energy from the sun using photosynthesis [8].

There are many advantages of bioethanol as energy source. Firstly, bioethanol is considered as carbon neutral. This is because of the carbon dioxide they release when burnt is equal to the amount that the plants absorbed. Therefore, they don't contribute to global warming. And for the same reason bioethanol causes less pollution than fossil fuels [11, 15, 17, 18]. Biofuel increases farm income, reduce energy costs and promote further rural development while pleasing the environmental community and offering jobs in rural areas [9]. Production of biofuel replaces the usage of high price petroleum. A lot of fruit wastes, causing some environmental problems in waste management and they have no economic value [8].

That's why this fruit have been chosen for the experiment. Therefore, this study was investigated to produce bioethanol from waste rambutan fruit biomass as non-food materials which could be an attempt to reduce the emission, waste and pollution caused by fruit waste material. In Addition bioelectricity could be successfully produced using bioethanol based fuelcell. The objectives of the research were:

1. To produce bioethanol fuel effectively from waste rambutan fruit.

2. To determine the optimization of bioethanol produced and its physical, biochemical, mechanical and chemical properties

MATERIALS AND METHODS

Raw material collection

Rotten rambutan fruits were obtained from commercial open market, Jalan Pantai Dalam, Kuala Lumpur in Malaysia (Figure 1). The mango fruits selected included those having physical defects as well as the unsold rotten fruits. The microorganisms employed were commercial dry yeast.

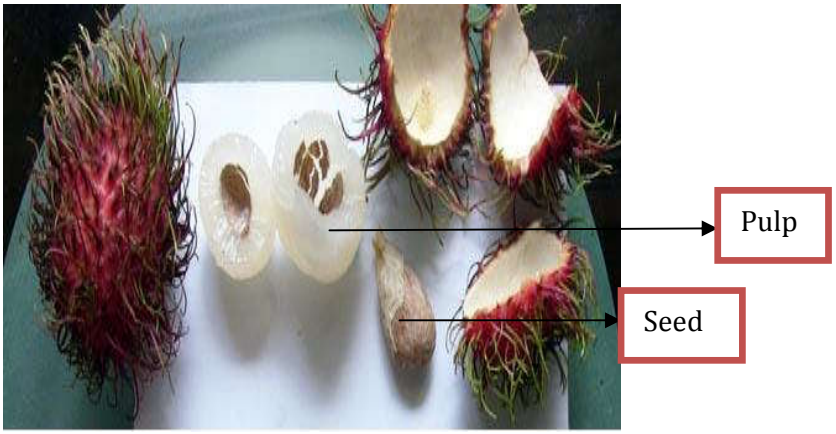

Figure 1. Rambutan biomass/rotten was used in the experiment.

Pre-fermentation treatment of samples

The mangoes collected were washed under running tap water for five minutes to remove dust and reduce the number of contaminating microorganisms, particularly fungus which normally grow on the skin of the rotten fruits. After washing, the mangoes were peeled using fruit peeler. The remaining mango pulp were cut and chopped into small pieces and then ground until it became liquefied. This mango mash was used for subsequent fermentation process.

Rehydration of yeast

Before adding to the mango mash for fermentation, the dried yeast was rehydrated to recover its activity and viability. Rehydration process was done by adding clean filtered tap water to the yeast at 37 ^{0}C to 40 ^{0}C for 15 minutes. The rehydrated yeast must be used immediately after rehydration process.

Enzymes : Cellulase

Cellulase from *Aspergillus niger* (C1184-25KU) purchased from Sigma-Aldrich® was used in the experiments. The cellulase enzyme catalyzes the hydrolysis of endo-1,4-β-D-glycosidic linkages in cellulose, lichenin, barley glucan, and the cellooligosaccharides cellotriose to cellohexaose, as well as cleaving intact glycosaminoglycan from a core peptide by hydrolyzing the xylosyl serine linkage.

α-Amylase

Amylase is an enzyme that catalyses the breakdown of starch into sugars. Amylase derived from *Aspergillus niger* (C1184-25KU) purchased from Sigma-Aldrich® purchased from Sigma-Aldrich® was used in the experiments.

Yeast

Two types of *Saccharomyces cerevisiae* yeast strains were used in this research: commercial baker's yeast (Mauri Pan brand) and *Saccharomyces cerevisiae* Type II (YSC2-1KG) purchased from Sigma-Aldrich® were used in the experiments. All experiments used commercial baker's yeast, except for fermentation experiment using different yeast strains that used both commercial baker's yeast, *Saccharomyces cerevisiae* Type II yeast.

Fermentation Parameters

Amount of yeast (2, 3 and 4g/l)

The amount of yeast used (2, 3 and 4g/l). The fermentation method was the same method described below. The yeast was used in the fermentation.

Incubation time (1, 2 and 3 days)

The time for incubation was to be for 1 day (24 h), 2 days (48h) and 3 days (72h). The fermentation method was the same method mentioned as below. The time of incubation was the only factor to be changed.

Different enzymes

Yeast, amylase and cellulose were used in the fermentation. The fermentation method was the same method stated below.

Fermentation

The simplified fermentation reaction equation for the carbon sugar or glucose is:

$C_6H_{12}O_6$ —> 2 CH_3CH_2OH + 2 CO_2 (glucose —> ethanol + carbon dioxide

Fermentation was done by using yeast, *Saccharomyces cerevisiae,* blended rambutan then poured into 500ml Schott bottles. It was incubated at 30^{0}C for 2 days (Figure 2a) for all parameters except different temperature parameter. After 2 days, the samples were taken out from the incubator and filtered with clean folded cheese cloth and filter paper (Figure 2).

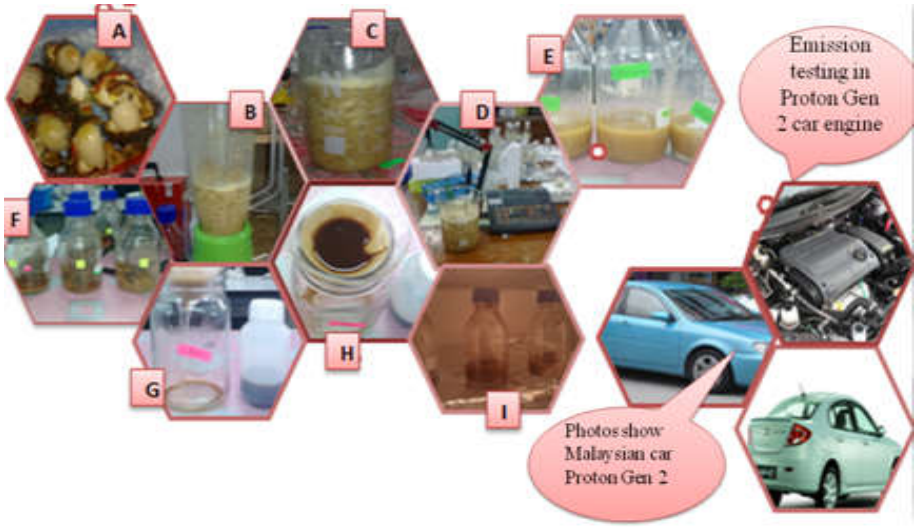

Figure 2a. Bioethanol production process, a. rotten rambutan, b. in the blender, c. after blender, d. Ph measuring e. before fermentation , f. after fermentation, g. filtration, h. bioethanol yield, i. during filtration. Figure2b. Fuel cell for bioelectricity. Bioethanol based fuelcell and electricity production from rambutan biomass

Filtration

For all experiments, the rambutan samples were taken out from the incubator, they were filtered by pouring the sample into a beaker with filter paper Whatman® Grade No. 1 for a certain time. The total soluble solid and pH values of the filtrate after fermentation were measured.

Bioethanol and glucose content determination

Bioethanol yield determination (%)

Ethanol concentration was determined according to the method of Williams and Darwin (1950). 100 ml of potassium dichromate reagent solution was prepared by dissolving 1 g of potassium dichromate in concentrated (6N) sulfuric acid. The prepared solution was shaked for homogeneity of mixture solution. On the other hand, saturated s-Diphenylcarbazide solution was prepared by dissolving 1 g of s-Diphenylcarbazide to 1 ml of 95% ethanol and the supernatant was collected. The 1 ml of ethanol solution was added to the glucose sample into a capped test tube. The test tube was covered with a piece of paraffin film to avoid loss of liquid due to evaporation. The mixture was then heat up using water bath at 90ºC for 5-15 minutes until it looks like red-brown color. The mixture was then added with 1 ml of a 40% potassium sodium tartrate (Rochelle salt) solution to stabilize the color. The ethanol absorbance values were measured at 575 nm after cooling to room temperature in a cold water bath. Vaccum evaporator was used to separate the bioethanol from water and other liquid at 70 ^{0}C.

Glucose content

Glucose content was determined according to the method of Miller [10].

Viscosity and acid value analysis

Viscosity analysis of sample fermented at different temperatures was measured at the Faculty of Engineering, University of Malaya, Malaysia. For viscosity test, the samples were put in the beaker and heated up at 40°C and then measured by using viscometer. The viscometer was set with the rpm of 30. Then the spindle with the size of 63 was used. Acid value was measured by titration meter.

Element Analysis

Bioethanol from the fermentation of the rotten dates at different pH of rotten dates and different concentrations of yeast was analyzed by using Multi-element Oil Analysis (MOA) Spectrometry.

Engine test

Three types of blending fuels were used in this study. The first one was pure petrol fuel (called E0). The second one was bioethanol blended with petrol fuel containing 5% bioethanol (E5) and the third one was bioethanol blended with petrol fuel containing 10% bioethanol (E10) [For engine starting, (Gen 2 car engine) 2 litres petrol (synergy, 95), were needed. For E5, it was produced 100 ml and for E10, it was produced 200ml bioethanol]. The multi cylinder hydra spark ignition engine with injection system was used. The tests were performed at 2000 rpm and the test fuels were gasoline (E0) and gasoline ethanol blends E5 and E10, the numbers following E indicate percentage of volumetric amount of ethanol. Fuel consumption was measured using Ohaus GT 8000 model (Gen 2 proton, made by Malaysia) and exhaust emissions were measured using Sun MGA 1200 model emission tester.

Bioelectricity determination

Bioelectricity was determined representing m volt versus time using bioethanol based fuelcell Bioenergy kit and Horizon, renewable energy monitor (Horizon fuel cell Technologies).

RESULTS

The optimum bioethanol yield from waste rambutan was carried out at 2 days having pH 5.8, at 30^0C, with 4g/l yeast concentration for rotten condition. Fig 3 shows the bioethanol production was higher in 2 days than in 1 day and 3 days. Because 2 days are the optimum for fermentation and yeast can activate more at 2 days. It was also observed that a concentration of 4g/l gave the highest amount of bioethanol produced (Fig. 4). With enzymatic hydrolysis yeast was more effective than amylase and cellulose for producing ethanol (Fig.5). Glucose (%) was reduced after fermentation in case of 1, 2, and 3 days. Because, reducing sugar or glucose was broken down and converted to the bioethanol. The glucose content was lower in 2 days than other days (Table 1). In addition, viscosity and acid value readings obtained were well within the ASTM standards (Table 1). Similarly, the metal element content in the bioethanol produced some followed the ASTM standards and rest showed higher value (Fig. 6). Here Fe, u and Pb shoed the value about zero, this is good for engine. Other showed higher value, because of rambutan contains much nutrients. As shown in (Table 2) fuel consumption and greenhouse gas emissions was significantly lower in bioethanol fuel (5% and 10%, E5 and E10 fuel) than in natural fuel (100% gasoline) when tested in the Malaysian proton car engine.

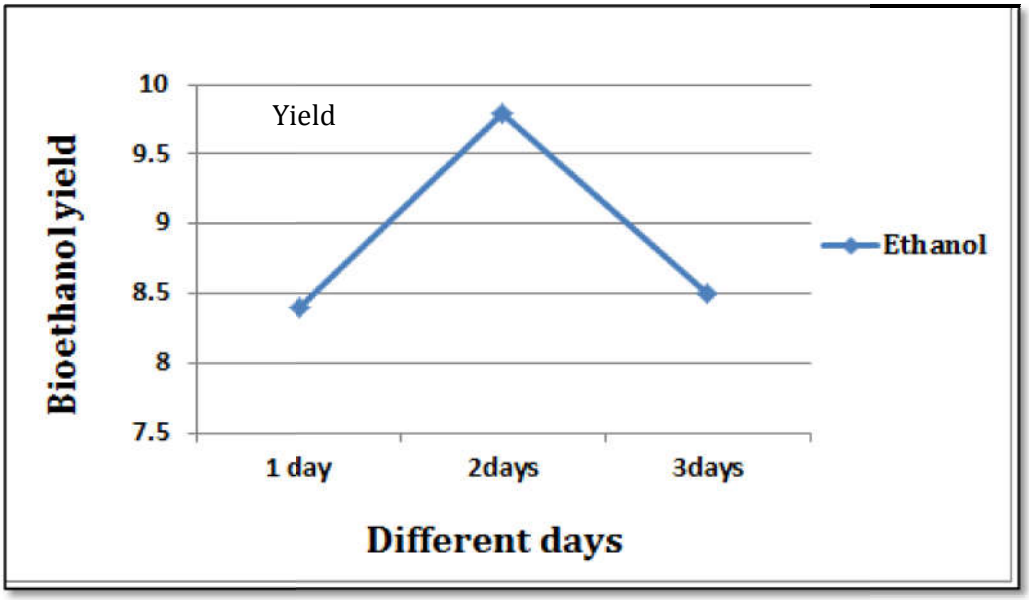

Figure 3. Bioethanol Yield (%) [v/v] at different days

DISCUSSION

Optimum amount of yeast produced the maximum amount of bioethanol. Increasing the yeast amount resulted in decline in fermentation yield which was in accordance to results reported earlier [12, 13]. The higher amount of yeast produced lowest ethanol percentage. The higher concentration of yeast exceeded

the ratio of suitable yeast to sugars condition caused the high competition of yeast in insufficient supply of sugars. As the consequence, higher amount of glycerol produced by the yeast cells as the glycerol was a major byproduct of ethanol fermentation by *Saccharomyces cerevisiae*. Concomitant with increased glycerol synthesis, decreased levels of ethanol occurred [11]. However, increased quantities of other by-products such as acetaldehyde and acetate had also been observed in other researches [12] and in the case of wine production a number of these products were considered unfavorable. These induced alterations to the metabolism of yeast cells seem to be related to a redox imbalance created by the increased flux of carbon towards the formation of glycerol. So, to prevent the loss of raw material in ethanol production by anaerobic yeast cultures, glycerol formation had to be reduced [1].

Chemical (metal) analysis

From LUBE Oil Analysis by Multielement Oil Analyzer (MOA), the MOA spectrometry value showed the metals content (Fe, Pb, Al, Cu, Mn etc.) were within ASTM (American Society for Testing and Materials) standard specification, thus could potentialy used as a good biofuel. From Figure 10, the hazardous chemical contents such as Pb, Al and Cu not included in this bioethanol produced, while Fe, Mn, B, V, Zn, Si and etc. were in very low amount, so this bioethanol produced was eco-friendly biofuel.

Viscosity and acidity test

From the result obtained in table 1, it could be seen that the bioethanol produced from fermentation of waste rambutan were at the range of ASTM standard considered, which were within 1 to 5 centistroke. This would give an indication that ethanol produced from rambutan fruit biomass was suitable as a possible biofuel substitute. As in advantage, low viscosity value was good for engine used and reduced problem of corrosion to the engine. Impure ethanol might have other components which lead to increasing in viscosity. However, the viscosity obtained was still maintained under ASTM standard, which indicated best result for this ethanol produced.

From the result, the acid values measured were almost the same for all fermentation except in day 3. The results obtained were in the best range and under ASTM standard specification.

Engine performance and emission test

The ethanol produced from this experiment were tested by generated the Proton Gen 2 multicylinder engine for 1 hour at 2000rpm (60km/hour). The hydrocarbon content for fuel consumption (ml/sec) was measured at 100% gasoline, E5 (A blend of 5% bioethanol/95% gasoline) and E10 (A blend of 10% bioethanol/90% gasoline). From figure 11, the hydrocarbon content in E5 and E10 were significantly lower than in 100% gasoline. This showed that the fuel burned more completely in E5 and E10 compared to in 100% gasoline, so fewer unburned hydrocarbon resulted. This observation strongly believed the ethanol produced from rambutan waste had highly potential as renewable energy. Recently, it was possible with certain engine modifications, to run on pure ethanol and Brazil operates almost 50% of their vehicles on pure ethanol while a 10% blend requires no engine modifications at all [15].

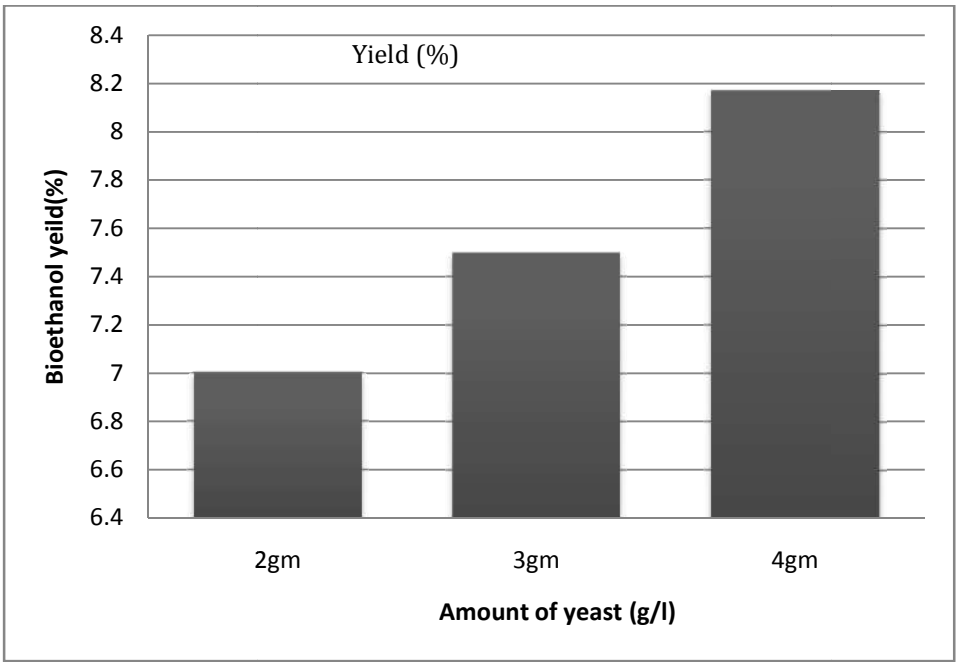

Figure 4. Bioethanol Yield (%) [v/v] at different yeast concentration.

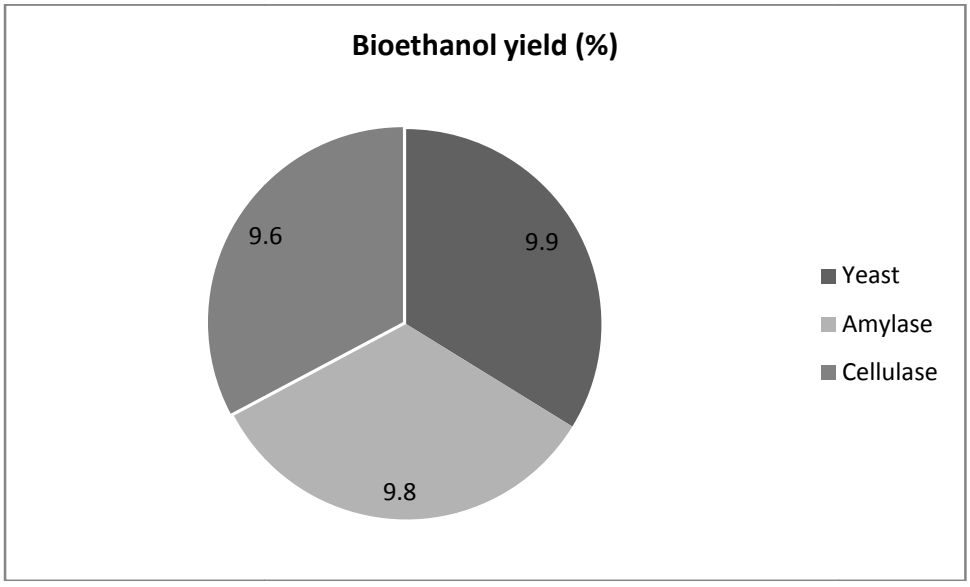

Figure 5. Bioethanol Yield (%) [v/v] at different enzymes.

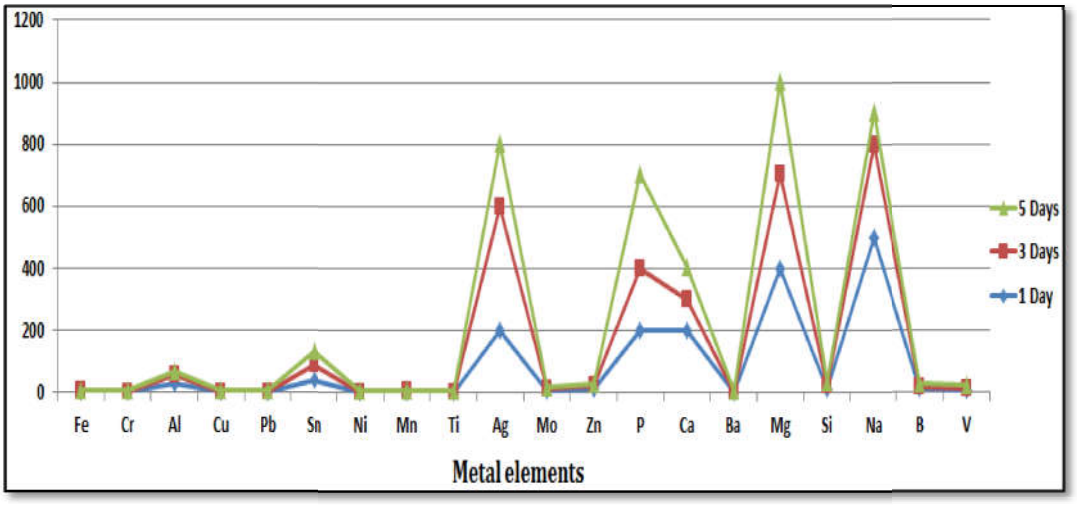

Figure 6. Metal content (%) [v/v] at different days

Table 1. Determination of viscosity and acid value in produced bioethanol at different days.

Days of incubation	Glucose content (%)	Visccosity (Cst)	Acid value (mg KOH/g)
1	4.6a	2.23a	0.38a
2	3.0b	1.58b	0.36a
3	3.2b	1.62b	0.48b

Table 2: Engine emission determination

	Engine performance (ml/sec)	HC (Emission, PPM)	NOx (Emission, PPM)	SOx (Emission, PPM)	CO2 (Emission, PPM)	CO (Emission, PPM)
Petrol (Gas)	1.6	80	51	40	17	9
5% ET + 95PET (gas)	1.5	55	40	30	16	8.5
10% ET + 90PET (gas)	1.56	50	33	25	14	7.5

CONCLUSION

From the results it can be concluded that bioethanol can be produced from waste rambutan as the substrates. The bioethanol from the waste rambutan was also safe for use in the petrol engine as it did not contain any unwanted metal elements. Its use led to lower fuel consumption and reduced emissions of environmentally unfriendly gases, namely CO2, CO, SO2, HC and NOX. That is why, it can be proved that this bioethanol from waste rambutan fruit biomass was of good quality which generated bio-power using fuel cell.

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