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Fruit Bio-Waste Derived Bio-Ethanol Production and Bioelectricity Generation as Renewable Energy

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Abstract: Bio-waste from food industry is a significant component to pollute the environment. Bioproducts like biomaterials, biofuel can be produced from biowaste and pollution can be reduced. The study was conducted to evaluate the quality of bioethanol production and investigate the bioethanol for bioelectricity generation using rambutan fruit biowaste. Bioethanol yield was the highest in 2 days incubation period of fermentation. The highest bioethanol yield was found at 30°C having pH 5.0 compared to the 28 and 35°C. With enzymatic hydrolysis, yeast exhibited more effective as compare to the amylase and cellulose for producing bioethanol. However, the glucose content was lowest in the bioethanol produced at 2 days incubation period. In addition, viscosity and acid value were exhibited well at 2 days incubation period followed by the ASTM standards. Similarly, the metal element contents (P, Ca, Si, Fe, Cu and Pb) were found satisfactory within the limit of the ASTM standards. The Si, Cu and Pb showed the value zero which was of good for energy generation. Bioelectricity represented by Voltage (mV) using bioethanol was monitored in fuel cell and found the Voltage (269.23 and 233.84 mV) at 100% ethanol and 100% bioethanol. The rotation of the fan in the fuel cell was observed and exhibited 510 and 450 RPM at 100% ethanol and 100% bioethanol. Voltage decreased while incubation time increased in bioethanol based fuel cell. Therefore, it was found from the results that bioethanol produced was of good quality where conversion rate was 97.2% and optimized at 30°C having pH 5.0, 2 days incubation period and 4g/l yeast concentration as well as bioelectricity represented by voltage (mV) was generated successfully using the bioethanol produced and fuel cell as an innovative technology in the field of renewable energy.

Keywords: Fruit Biomass, Chemical Element, Bioethanol, Bioelectricity Generation, Fuel Cell

Introduction

An alternative energy fuel like biodiesel or bio-ethanol is currently promising as a superlative factor all over the world. Global warming is being escalated by the combustion of petroleum product which necessary to be considerably reduced. Excessive engine emission (NO_X, CO_2, CO, HC etc.) is one of the key components to increase greenhouse gas emission as well as global warming. Biofuel is one of the significant components to reduce greenhouse gas emission (Hossain *et al*., 2008; Hossain and Fazliny, (2010), Hossain *et al*., (2011); Demirbas, (2008). Bioethanol is non-toxic, biodegradable and does not cause environmental pollution as compared to the fossil fuel (Hossain *et al*., 2010). Bio-ethanol is produced by the conversion of carbon based feedstock coming after fermentation technology. Agricultural waste feed stocks are considered as renewable components because of getting energy from the sun using photosynthesis (Hossain *et al*., 2011). Sugar based fruit such as rambutan, date, grapes etc. are popular and the most famous and highly grown in tropical countries such as Malaysia, Saudi Arabia (Hossain *et al*., 2011).

There are many advantages of biofuel as bioenergy source. Firstly, biofuel is considered as carbon neutral. This is due to the release of carbon dioxide while burning which is equal to the amount that the plants absorb. Therefore, they don't contribute to the increasing of the global warming. For the same reason biofuel is less polluting the environment than fossil fuel. Biofuel

encourages farm income, reduce energy costs and promote further rural development while pleasing the environmental community (Licht, 2008). Production of biofuel replaces the usage of high price petroleum. A bulk of fruit wastes causes some environmental hazards in waste management process and they have no economic value (Hossain and Boyce, 2009; Hossain *et al*., 2008). That's why this fruit has been chosen for the present study. Therefore, this study was investigated to produce biofuel as bioethanol from waste rambutan fruit biomass as non-food materials which could be an attempt to reduce the green house gas emission, bio-waste and pollution. The objectives of the research were undertaken:

1. To produce good quality of bioethanol fuel effectively from waste rambutan fruit
2. To determine the optimization of produced bioethanol
3. To observe the power (mV) in generating electricity using bioethanol based fuel cell

Materials and Methods

Raw Material Collection

Rotten rambutan fruits were collected from commercial open market, Jalan Pantai Dalam, Kuala Lumpur in Malaysia (Fig. 1). The mango fruits were selected including physical defects and unsold rotten fruits. The commercial dry yeasts were used as microorganisms.

Pre-Fermentation Treatment of Samples

The collected rambutan fruits were washed by using tap water for five minutes to remove dust and reduce the contaminating microorganisms, particularly fungus which normally grows on the skin of the rotten fruits. The rambutan fruits were peeled after washing. The remaining rambutan pulp was cut and chopped into the small slice and then ground until it became liquid state. This rambutan mash was utilized for subsequent fermentation process.

Rehydration of Yeast

The dried yeast was rehydrated to recover its activity and viability before adding to the rambutan mash for fermentation. Rehydration process was conducted using clean filtered water with yeast at 37°C for 15 min. After rehydration process, the rehydrated yeast was used immediately.

Enzymes

Cellulase

Cellulase from Aspergillus Niger (C1184-25KU) collected from Sigma-Aldrich was utilized in the study. The cellulase enzyme catalyzed the hydrolysis of endo-1,4-β-D-glycosidic linkages in cellulose, lichenin, barley glucan and the cellulose-oligosaccharides cellotriose to cellohexaose, as well as cleaving intact glycosaminoglycan.

α-Amylase

Amylase enzyme catalysed the breakdown of starch into sugars. Amylase derived from *Aspergillus Niger* (C1184-25KU) collected from Sigma-Aldrich was used in this study.

Yeast

Saccharomyces cerevisiae yeast strains were used in this study. Commercial baker's yeast as *Saccharomyces cerevisiae* Type II (YSC2-1KG) collected from the same sources as other enzymes and were used in this study.

Fermentation Parameters

Temperature (28, 30 and 35°C)

The temperatures maintained were 28, 30 and 35°C. The fermentation technology was the same method stated below.

Incubation Time (1, 2 and 3 days)

The incubation period was 1 day (24 h), 2 days (48 h) and 3 days (72 h). The fermentation technology was the same technology introduced as below.

Different pH

The pH 4, 5 and 6 were used. The fermentation technology was the same technology mentioned as below.

Different Enzymes Concentration

Different types of enzymes such as yeast (4 g/L), amylase (4 g/L) and cellulase (4 g/L) were utilized in the fermentation. The fermentation method was the same method introduced below.

Fermentation

Fermentation is a series of chemical reactions that alter the sugars to the ethanol. The fermentation reaction is occurred by using yeast or bacteria, which feed on the sugars. Ethanol and carbon dioxide are produced when the sugar is consumed. The simplified fermentation reaction equation for the carbon sugar or glucose is shown below:

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

Fermentation process was carried out by using yeast, *Saccharomyces cerevisiae*, mixing with blended rambutan then poured into the 500 mL Schott bottles. It was incubated at 30°C for 2 days (Fig. 2a) for all parameters excluding the different temperature parameter. After 2 days, the samples were departed from the incubator and filtered with clean cheese cloth and then filter paper (Fig. 2b).

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Fig. 1: Photograph shows the waste rambutan biomass used in the study for bioethanol production

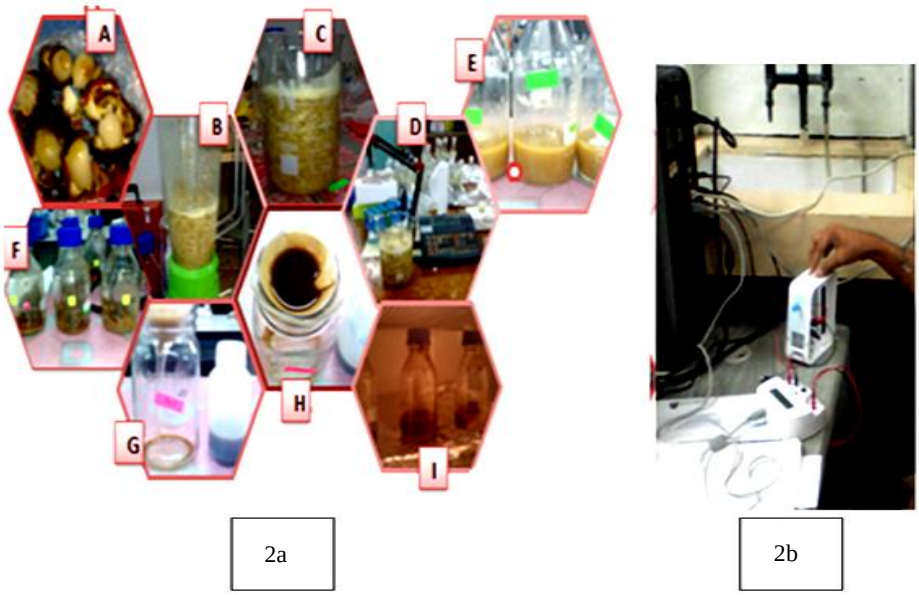

Fig. 2: Photograph shows the bioethanol production process, a. Rotten rambutan, b. In the blender, c. After blender, d. pH measurement e. Before fermentation, f. After fermentation, g. filtration, h. Bioethanol yield, i. During filtration. Fig. 2b. Fuel cell for bioelectricity. Bioethanol based fuel cell and electricity generation from rambutan biomass

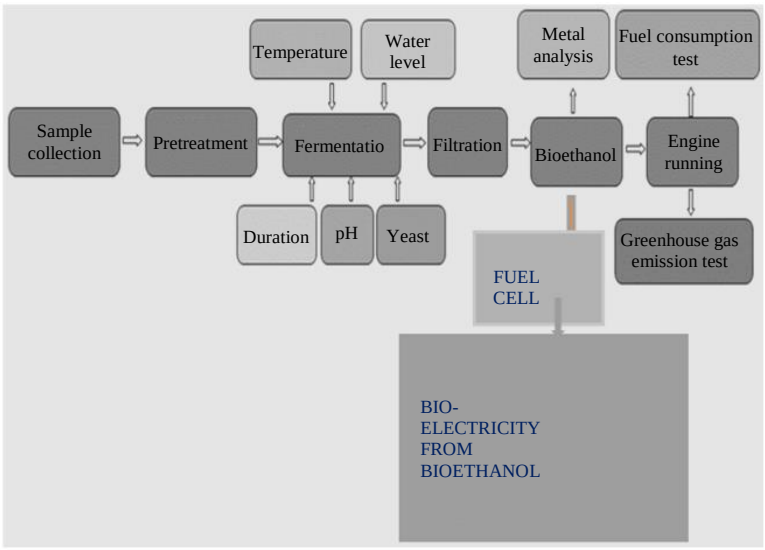

Fig. 3: Design shows the bio-ethanol production process and energy flow chart

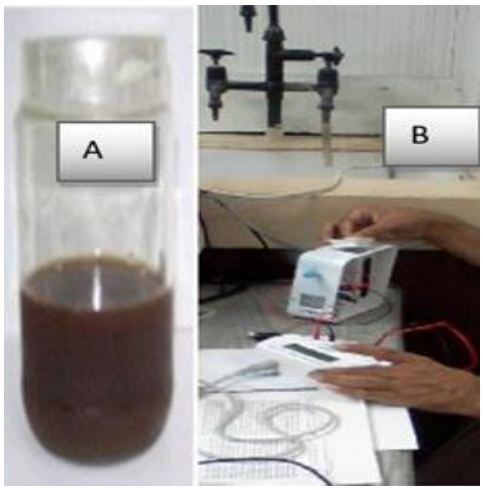

Fig. 4: Photograph shows the bioethanol (A) and methodology for bioelectricity generation (B)

Filtration

For all studies, the rambutan samples were removed from the incubator and 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 were measured after fermentation.

Bioethanol Separation

Bioethanol was separated from water and other liquid by vacuum evaporator at 70°C after filtration.

Glucose Determination by GC-FID

The ground samples were filtered and extracts were evaporated to dryness employing a rotary evaporator. The residues were removed in 10 mL of 80% ethanol and kept in the freezer until analysis. Al aliquot of 20 µL sample was taken into the vial and dried them by using dryer. Then, 40 µL pyridine including TPB (1,3,5 tri-phenyl benzene) 1 mg/mL as an internal standard, 40 µL HMDS (hexamethyl disilazane) and 40 µL TMCS (chlorotrimethylsilane) were added to the dried samples. The vials were incubated at 60°C for 30 min. One µL of the trimethylsilated sample was injected into a gas chromatograph (GC-FID). The GC condition was as follows: Column temperature: 150-265°C at the increment rate of 10°C/min. The GC was equipped with a glass column (2.6 mm × 2 m) peaked with 1.5% Se-30 coated on Chromosorb WAW DMCS (80-100 mesh). nitrogen was utilized as carrier gas at the flow rate of 30 mL/min.

Bioethanol Determination by GC

Sample Preparation

One hundred microliters of bioethanol samples were mixed thoroughly with 10 µL of is. The samples were diluted to 500 µL with the Triton X-100 and acetonitrile solution, centrifuged and 0.5 µL of the supernatant were directly injected into the GC.

Gas Chromatography Properties

The GC used was a Thermo Finnigan Model Focus GC equipped with a FID. The injection port of the chromatograph was installed with a glass liner (5-mmi.d.) suitable for the split analysis, to prevent the contamination of the GC column. The analyses were completed under the following chromatographic conditions: Column, CPWax57CB (WCOTF used Silica), 25 m × 0.25 mm i.d., DF = 0.2 µm, from Varian (Palo Alto, CA). The temperature of the FID was 220ºC and the injector temperature was 220°C. The oven temperature was designed to 40°C (for 2 min), followed by an increase of 5ºC/min until 200 248°C. The carrier gas was helium with a flow of 1.5 mL/min. The injection of B and VH was performed by means of a 10 µL Hamilton syringe (Model 701 RN) with a removable needle (needle gauge 22S), cleaned under vacuum between each injection with the Triton X-100 and acetonitrile solution. Moreover, the injection of CCM and U was completed by means of a 5-µL SGE syringe (Model 5F-GP) cleaned under vacuum between each injection with de-ionized water. The volume of injection was 0.5 µL with a split ratio of 100 and a split flow of 120 mL/min for B and VH; and a split ratio of 60 and a split flow of 90 mL/min for U and CCM.

Viscosity and Acid Value Analysis

Viscosity analysis of fermented sample 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 at 40°C and then determined by utilizing 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.

Chemical Element Analysis

Bioethanol from the fermentation of the rotten rambutan at different pH of rotten rambutan and different concentrations of yeast was analyzed by using Multi-element Oil Analysis (MOA) Spectrometry at Tribology Laboratory, Faculty of Engineering, University of Malaya, Malaysia. The 5 mL of each sample were used for analysis.

Bioelectricity Determination using Fuel Cell

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

Bioenergy kit, water and ethanol and Horizon renewable energy monitor were used to create electricity. Fan blade was attached to bioenergy kit motor slowly and carefully. Purging valve was opened by pushing the switch to the right side. It was made sure that turbine connection of the container to the fuel cell was securely attached. Samples (100% Ethanol (15 mL), 100% bioethanol (15 mL), 50% bioethanol (7.5 mL), 30% bioethanol, 20% bioethanol, 100% Dis. Water/0% ethanol. It was remarkable to mention that with all samples certain quantity of water was added usually for this fuel cell it was 1:8.5 = Ethanol: Water), (Horizon fuel cell technologies). Ethanol or bioethanol samples were poured into the ethanol container. The lid of the container was put back. When the solution started dripping outside of the tube then the purging valve was closed by pushing the switch to the left side. It was waited for 5-10 min before connecting the wires to the motor. After that two crocodile clips were attached to the motor to the two terminal plates of the fuel cell current collector which were located in the upper part of the fuel cell. The Renewable Energy Monitor was connected to the fuel cell. The Rotation (RPM) and Voltage (mV) were displayed and recorded. After using the individual sample, every time 'spent fuel' (previous sample) was replaced with new fuel or sample and begun with other sample after taking some gap.

Results

The optimum bioethanol yield from waste rambutan was carried out at 2 days having pH 5.0, at 30°C, with 4 g/L yeast concentration for rotten condition. Fig 5 showed the bioethanol production was higher in 2 days than 1 day and 3 days. Because, 2 days were the optimum for fermentation and yeast might activate more in 2 days than 1 and 3 days. Bioethanol production from waste rambutan fruits at different temperatures showed that maximum yield occurred at 30°C as compared to the 28 and 35°C (Fig. 6). Bioethanol yield was highest at pH 5.0 (Fig. 7). With enzymatic hydrolysis yeast was more effective than amylase and cellulose for producing bioethanol (Fig. 8). Figure 9 showed the conversion peak of bioethanol. The highest conversion rate was 97.2% found in the 2 days incubation. Glucose (%) was reduced after fermentation in case of 1, 2 and 3 days (Table 1). 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 obtained were well within the ASTM standards (Table 1). Similarly, the metal element content in the bioethanol produced some followed the ASTM standards (Table 1). Fe, u and Pb showed the value zero, these were of good quality bioethanol and suitable for energy generation. Bioelectricity as represented by voltage (mV) using bioethanol was monitored in fuel cell and found the Voltage (269.23 and 233.84 mV) at 100% ethanol and 100% bioethanol (Table 2). The rotation of the fan in the fuel cell was observed and exhibited 510 and 450 RPM at 100% ethanol and 100% bioethanol. Voltage decreased while incubation time increased in bioethanol based fuel cell (Fig. 10).

Discussion

Optimum concentration of yeast produced the maximum yield of bioethanol. Increasing the yeast concentration resulted in decline in fermentation yield which was in accordance with the results reported earlier (Sharma *et al*., 2007; Reddy and Reddy, 2005). 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 bioethanol fermentation by *Saccharomyces cerevisiae*. Concomitant with increased glycerol synthesis, decreased levels of bioethanol occurred (Prior *et al*., 2000). However, increased quantities of other by-products such as acetaldehyde and acetate had also been observed in other researches (Reddy and Reddy, 2005) and in the case of wine production a number of these products were considered as 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 (Cronwright *et al*., 2002). Therefore, to prevent the loss of raw material in bioethanol production by anaerobic yeast cultures, glycerol formation had to be reduced (Albers *et al*., 1996).

Chemical (Metal) Analysis

The MOA spectrometry value showed the metal contents (Fe, Pb, Si, Cu, P, Ca) were under the ASTM (American Society for Testing and Materials) standard specification and bioethanol was used as a good biofuel. The hazardous chemical contents such as Pb, Si and Cu were not existed in this bioethanol produced. Hossain and Al-Eissa (2013) reported that produced bioethanol was found of good quality from date palm fruit while showing the metal contents like Pb, Si and Cu not existed.

Viscosity and Acidity Test

The viscosity of the bioethanol produced was an important when considering the spray characteristics of the fuel within the engine, since the change in spray could greatly convert the combustion properties of the mixture. From the result obtained in Table 1, it was observed that the bioethanol produced from fermentation of waste rambutan were at the range of ASTM standard considered, which were within 1 to 5 centistoke. This might have an indication that bioethanol produced from mango was

suitable as a possible biofuel substitute. As in advantage, low viscosity value was of good quality for engine utilized and reduced problem of corrosion to the engine. Glycerol was a major byproduct of bioethanol fermentation by *Saccharomyces cerevisiae*. Thus, the yeast cells produced glycerol under anaerobic and glucose-repressing growth conditions in order to function to help maintain a cytosolic redox state conducive to sustain glycolytic catabolism (Albers *et al*., 1996). Therefore, with the higher glycerol content could cause higher viscosity to the solution. In addition, the bioethanol produced from this experiment was not being purified or distillated. So, impure bioethanol might have other components which led to increasing in viscosity. However, the viscosity obtained was still maintained under ASTM standard, which indicated best result for this bioethanol produced.

Table 1 also showed the result of acid value test from samples fermented at different concentration of yeast. From the result, the acid values measured were almost the same for all fermentation except in 3 days incubation. The results obtained were in the best range which was around 0.5 mg KOH/g and under ASTM standard specification.

Discussion on Electricity Generation

The highest electricity production [Voltage (mV)] was found in the samples of 100% Ethanol (15 mL), 100% bioethanol (15 mL), 50% bioethanol (7.5 mL) than in 30% bioethanol (4.5 mL), 20% bioethanol (3 mL), 100% dis. water/0% ethanol or bioethanol. Voltage was the highest at 100% ethanol. The 2nd highest was found at 100% bioethanol (Table 3). In case of all concentrations it showed declining trend from initial to final. Table 2 showed the average voltage was higher with higher concentration of ethanol and bioethanol and gradually decreased with lower concentration. When the fan started to run from faster to slower, it was meant that ethanol or bioethanol inside the fuel cell chamber, was turned into the acetic acid. Ethanol or bioethanol turned into the acetic acid during the reaction took place at anode side of the fuel cell and pH of the solution was changed (which tested by pH paper and turned to the paper red color). The chemical reaction took place at the anode side showing the acetic acid is formed as hydrogen protons departed from the ethanol or bioethanol molecule and water molecule. These hydrogen protons crossed the fuel cell membrane then supplied the electrons and formed the electricity that was able to rotate the fan. That is why, it was proved that this bioethanol from waste rambutan fruit biomass was of good quality which generated bioelectricity using fuel cell. Sumathi *et al*. (2007) and Hamelinck and Faaij (2006) supported our research and they stated that bioelectricity was produced by gasification and pyrolysis of biomass. Therefore, it might be concluded that an innovative result was found using fuel cell Fig. **3** and 4.

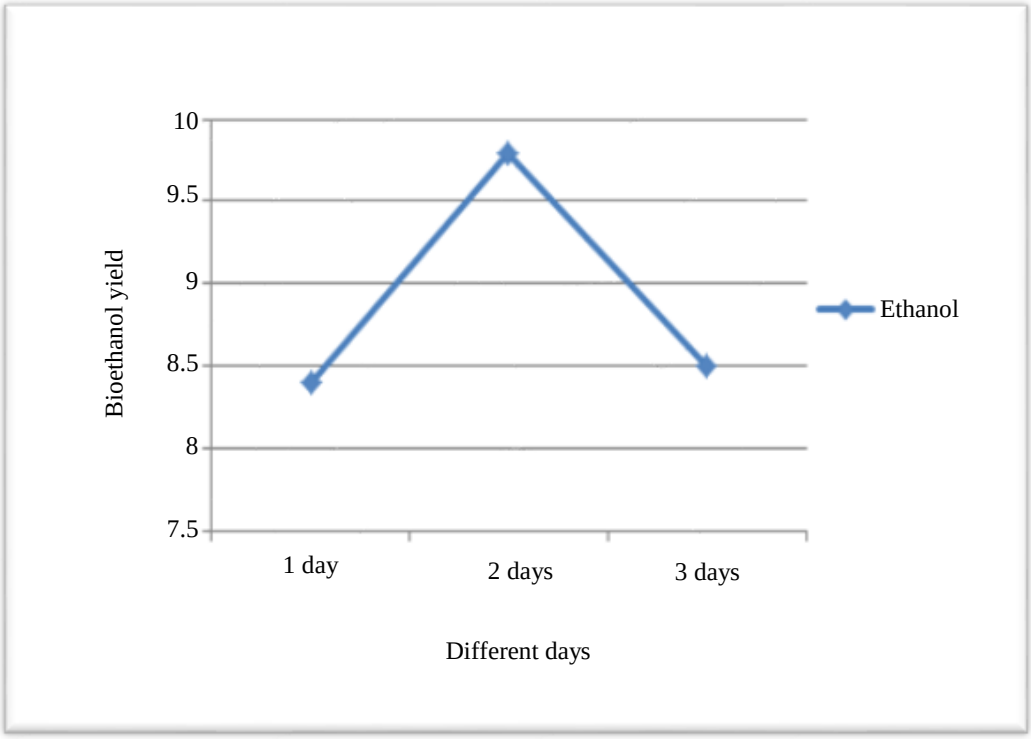

Fig. 5: Shows bioethanol Yield (%) at different days

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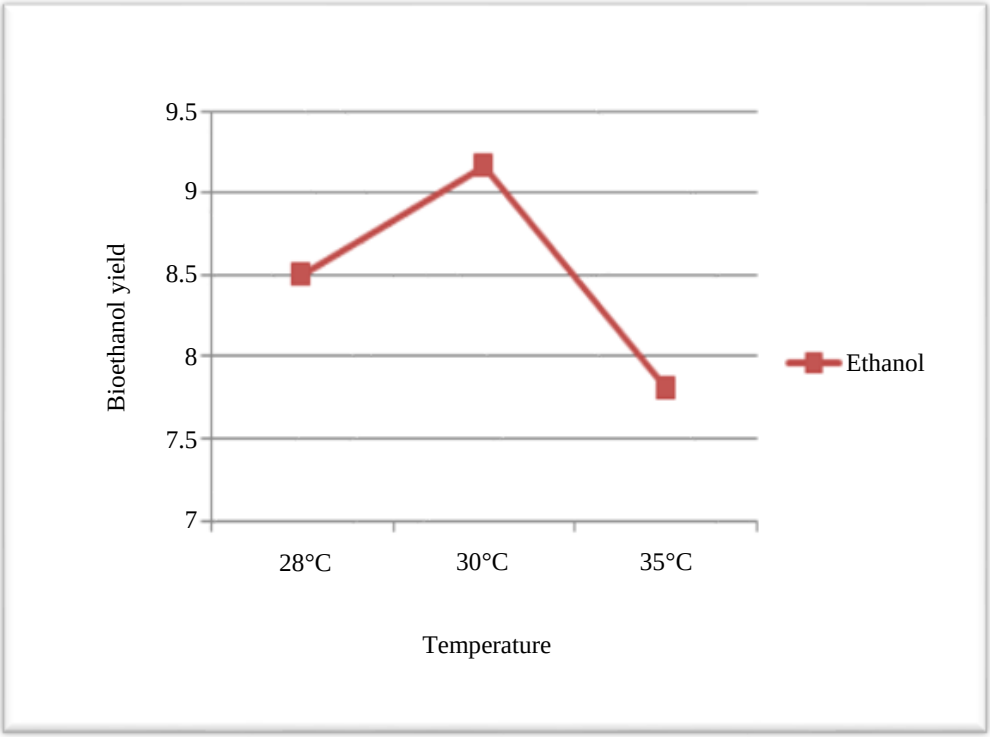

Fig. 6: Shows Bioethanol Yield (%) at different temperatures (°C)

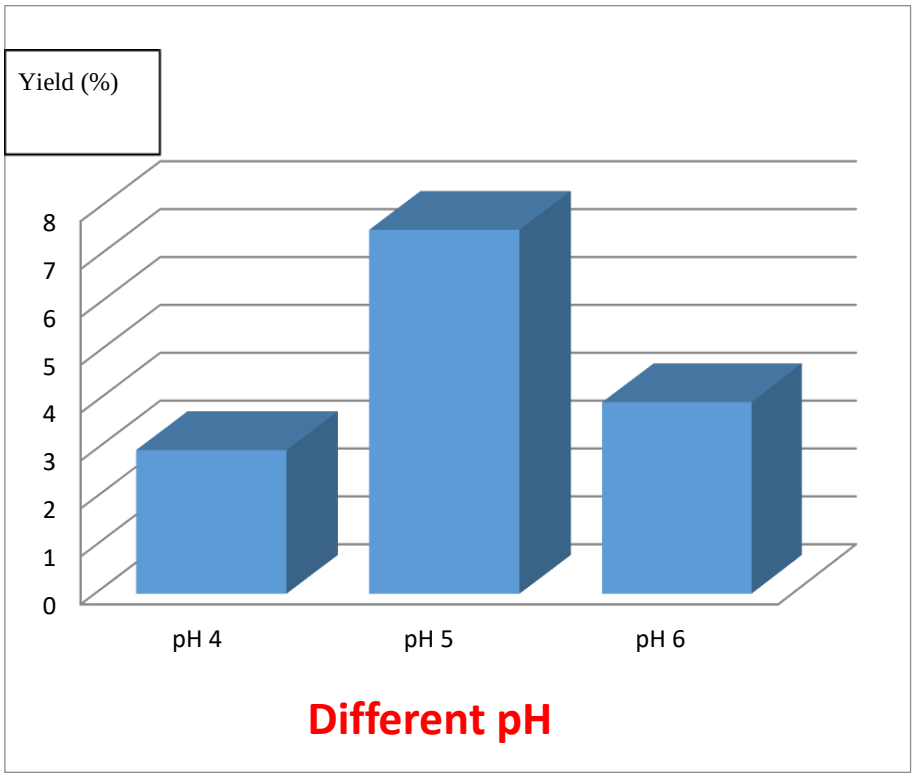

Fig. 7: Bioethanol Yield (%) [v/v] at different pH

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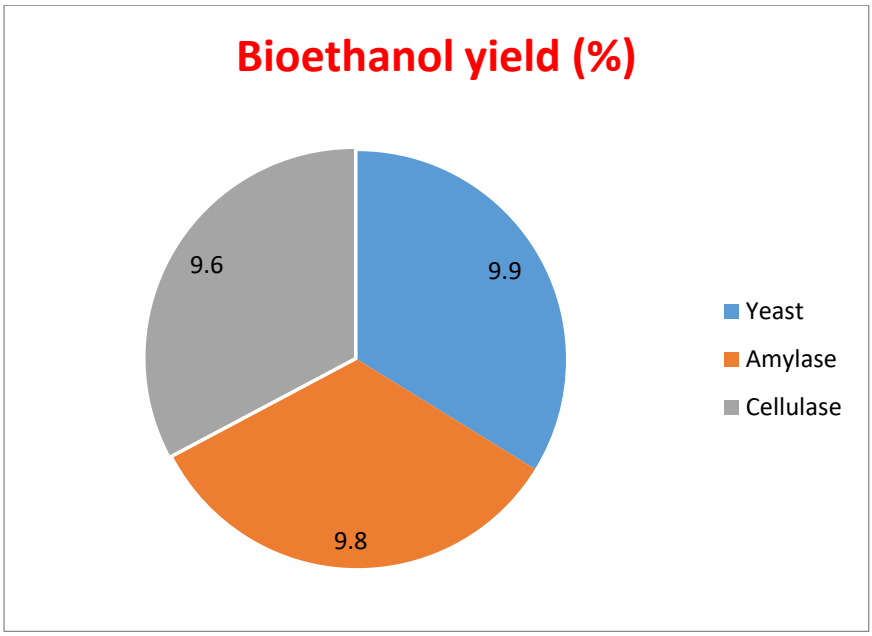

Fig. 8: Shows bioethanol Yield (%) at different enzymes

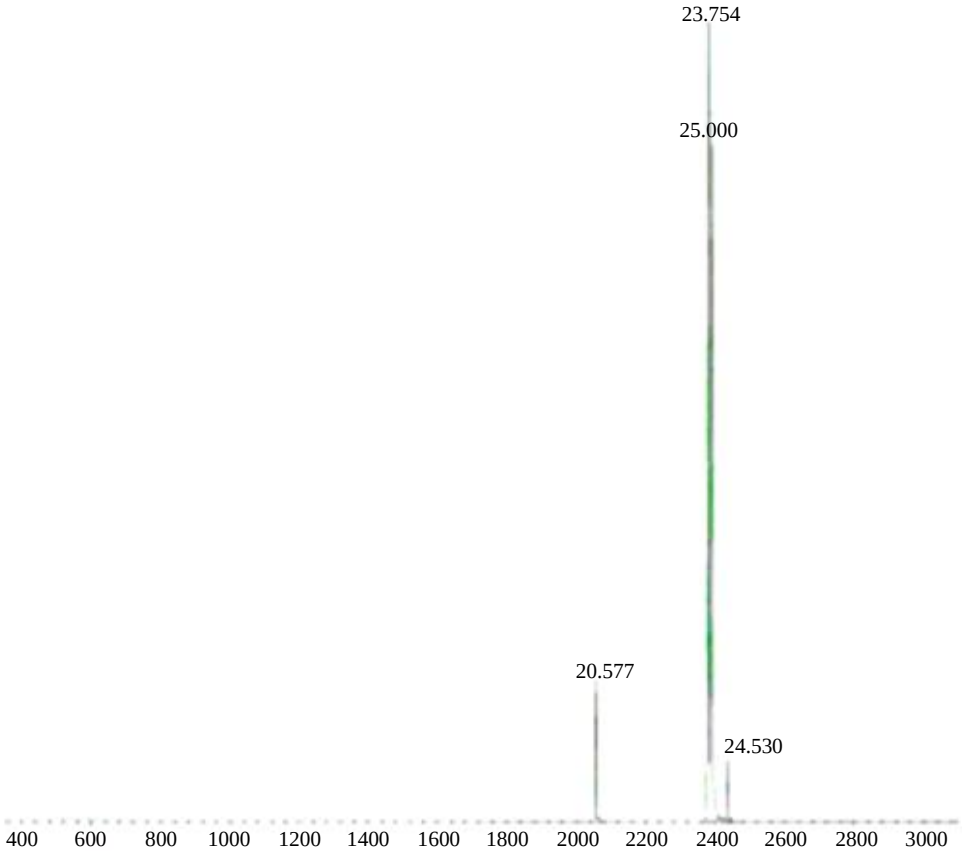

Fig. 9: Peak shows the bioethanol conversion (Peak) percent

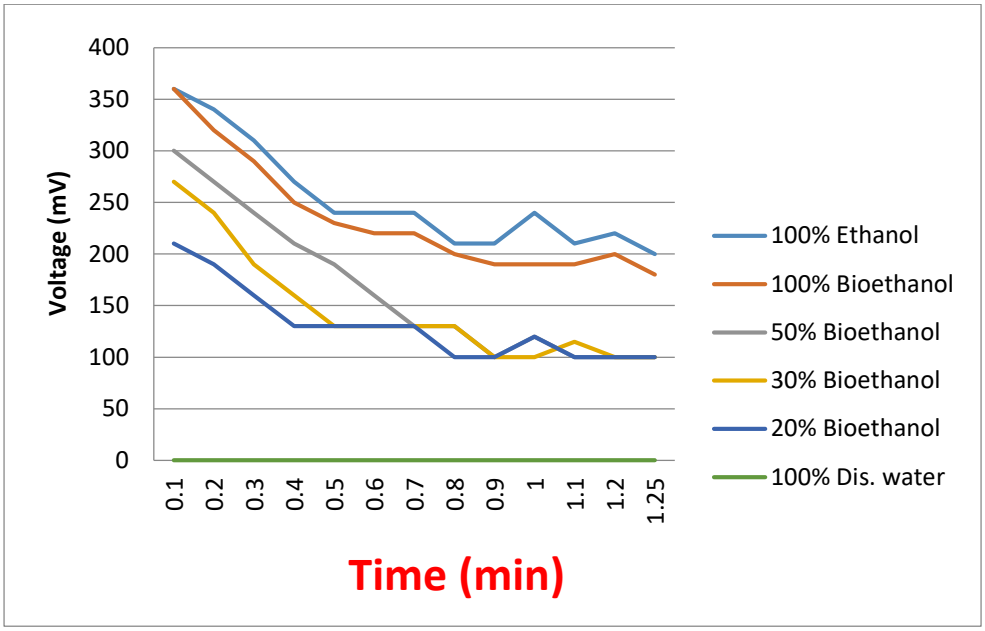

Fig. 10: Bioelectricity generation as represented by Voltage (mV) using bioethanol in fuel cell at different ethanol and bioethanol percentage

Table 1: Measurement of viscosity and acid value in produced bioethanol at different days

Days of incubation	Bioethanol conversion (%)	Glucose content (%)	Viscosity (Cst)	Acid value (mg KOH/g)
1	80.1a	4.6a	2.23a	0.38a
2	97.2b	3.0b	1.58b	0.36a
3	82.0a	3.2b	1.62b	0.48b

Table 2: Determination of chemical element in bioethanol

	Chemical element (PPM)						
Days of incubation	Cu	Pb	Fe	Si	P	Ca	ASTM standard value
1	0	0	0.20	0	4.0	5.0	[0-5 PPM]
2	0	0	0.04	0	3.8	4.2	
3	0	0	0.12	0	4.0	4.6	

Table 3: Bioelectricity as represented by Voltage and RPM determination using bioethanol in fuel cell.

Ethanol concentration	Average voltage (mV)	Fan rotation (Initial RPM)
100% Ethanol	269.23	510
100% Bioethanol	233.84	450
50% Bioethanol	165.38	395
30% Bioethanol	147.36	320
20% Bioethanol	123.57	285
100% Dis. Water/0% ethanol	0.00	000

Conclusion

From the studies, it can be concluded that bioethanol can be produced from waste rambutan as the substrates and safe for the bioenergy generation as it did not contain any unwanted metal elements. The optimum bioethanol was found at 30°C for 2 days fermentation having pH 5 from rambutan biomass. Bioelectricity was generated successfully by using ethanol based fuel cell from biomass. That is why, it has been proved that produced bioethanol from waste rambutan fruit biomass was of good quality which generated bioelectricity using fuel cell and it can be an innovative research study.

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Author's Contributions

ABM Sharif Hossain: Supervision the review data collection work, partially review writing and editing, final draft checking.

Musamma M Uddin: Literature review collection, Writing - original draft.

Ethics

This article is original and contains unpublished material. The corresponding author confirms that all of the other authors have read and approved the manuscript and no ethical issues involved.

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