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Bioethanol Production from Mango Waste (*Mangifera indica* L. cv chokanan): Biomass as Renewable Energy

[1]Mohammed Saifuddin, [2]Mohammad Moneruzzaman Khandaker, [1]ABMS Hossain, [2]Nashriyah Binti Mat and [1]Amru Nasrulhaq Boyce

[1]*Institute of Biological Sciences, Faculty of Science, University of Malaya, 50603 Kuala Lumpur, Malaysia*
[2]*School of Plant Sciences, Faculty of Agriculture, Biotechnology and Food Science, Universiti Sultan Zainal Abidin, Tembila Campus, 22200 Besut, Terengganu, Malaysia.*

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ABSTRACT

The exploration of biomass fuels encourages the reduction of world atmospheric pollution and global warming. In addition, the depletion of non renewable energy sources such as fossil fuels induces the development of technologies to harness new and renewable energy sources. Abundant of fruit waste can be re-utilized in the bioethanol production. Hence, it can reduce pollution and waste materials, thus helps in waste disposal management. This study was investigated to evaluate the feasibility of the utilization of mango waste, *Mangifera indica* L. cv Chokanan to produce bioethanol *via* fermentation by yeast, *Saccharomyces cerevisiae.* The highest production of bioethanol yield could be obtained from mango pulp in the yeast concentration of 3 g/L at the temperature of 30°C that yielded 15 % (v/v) of ethanol. The ethanol production increased with the increase of fermentation time until five days of incubation. Total soluble solid (TSS), glucose and pH were reduced after fermentation. The trace elements (Pb, Al, Cu, Ca and Mg), viscosity and acid values of the bioethanol were found to be within the ASTM (American Society for Testing and Materials) standard, specifications with less hazardous element. Furthermore, the engine test showed that hydrocarbon, NO, CO and CO_2 content were significantly lower in E5 (5 % bioethanol with 95 % gasoline) and E10 (10 % bioethanol with 90 % gasoline) than in E0 (100% gasoline) having less fuel consumption. The results showed that bioethanol fuel can be produced from mango waste and can be used in petrol engine in combination with pure petrol fuel, and fuel consumption can be reduced by using mango waste.

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INTRODUCTION

The need for energy is increasing continuously, because of rapid increases in industrialization and vehicles. The basic sources of this energy are petroleum, natural gas, coal, hydro, and nuclear. The increasing concern of fuels as well as the escalating social and industrial awareness leads to exploration for the clean renewable fuels (Razif *et al*., 2009). Therefore, bioethanol produced from renewable energy sources or non-edible feedstocks, such as sugar and starch materials, is believed to be one of these options, and it is concurrently being practiced in waste management (Reddy and Reddy, 2005). Thus, bioethanol has become important in recent times as the world researches to find an alternative energy as a suitable substitute for fossil fuels and to reduce greenhouse gas emissions. Apart from being the alternative energy, bioethanol has been shown to be less polluter compared to nitrous oxide, carbon monoxide and sulphur dioxide. It is considered to be non-toxic and biodegradable, as well as obtainable from renewable sources (Fazliny and Hossain, 2010).

Scientists are doing research to convert food waste or inedible parts of fruits like peels and seeds into bioethanol. Although the idea is not new, it has gained considerable attention in recent years due to the escalating price of petro-fuel throughout the world. The fruits processing industry disposes of around million tones of seeds and peel waste each year, but many researchers have shown that it is possible to convert these wastes into bioethanol (Fazliny and Hossain, 2010; Hossain *et al*., 2008). Mango peel and seed have several characteristics that make them potential feedstocks for bioethanol production. They have high cellulose and hemicelluloses contents that can be readily hydrolyzed into fermentable sugars (Carla *et al*., 2010). In terms of chemical composition, ethanol is a common molecule in biological systems, being the end product of

Corresponding Author: Mohammad Moneruzzaman Khandaker, School of Plant Sciences, Faculty of Agriculture, Biotechnology and Food Science, Universiti Sultan Zainal Abidin, Tembila Campus, 22200 Besut, Terengganu, Malaysia.
E-mail, moneruzzaman@unisza.edu.my, Tel: 09-6993450, Fax: 09-6687850

metabolism (fermentation). The ethanol content of ripening fruit is 0.7%, and 85% of its dry mass is sugar (Francisco *et al*., 2004).

Ethanol can be produced from biomass by the hydrolysis and sugar fermentation processes (Fazliny and Hossain, 2010). In this study, bioethanol was produced by using yeast fermentation and enzyme hydrolysis from mango biomass. The cellulose and the hemi cellulose portions were broken down (hydrolyzed) by yeast and then the fermented sugar was converted into bioethanol. The lignin presents in the biomass is normally used as a fuel for the ethanol production plants boilers. It has been reported that bioethanol can be used as a fuel for cars in its pure form, but it is usually used as a gasoline additive to increase octane and improve vehicle emissions. Bioethanol can be blended with gasoline in varying quantities to reduce of the consumption of petroleum fuels, as well as to reduce air pollution. It has been also stated that bioethanol up to 5-20% can be blended with conventional fuel without any engine modifications (Fazliny and Hossain, 2010). Therefore, the objective of this present study was to determine the impacts of mango biomass as a renewable bioethanol resource, measuring the engine emission rate and optimizing the variables which affect the bioethanol production.

MATERIALS AND METHODS

1.1. Raw material and microorganism:

Rotten and waste mango fruits, *Mangifera indica* L. cv *Chokanan* were collected from selected farms, located at Pantai Dalam, Kuala Lumpur, Malaysia. Fruits were kept until fully rotten and soften in cupboard, at room temperature. Yeast (*Saccharomyces cerevisiae* Type II) was obtained from the ABO laboratory, University Malaya, Malaysia and was subjected to rehydration process with the addition of 10 % distilled water and warmed at 40 °C in water bath for 15 minutes.

1.2. Sample preparation and measurements:

The rotten mango fruit was hand peeled and seeded off manually. Fruit pulp and peel were cut into small cubes/pieces and pulverized separately. The 100 g of sample was filled into 500 mL Schott bottle. Total soluble solid (TSS) value of sample before fermentation was taken by using refractometer according to the methods described in Khandaker *et al.* (2011& 2012). The sample pH value was measured by using pH meter (Hanna) and set to be at pH 5. This pH was standardized to all samples tested.

1.3. Fermentation:

Prepared yeast (2 g/L) was poured into 500 mL Schott bottle containing 100 g of slurry sample and was shaken well. Batch fermentation of sample was conducted in the incubator for 120 hours. The experiments for all parameters tested were done in triplicates.

1.4. Fermentation at different temperatures, pH, yeast concentration and incubation time:

The fermentation method was similar to method stated above, except of changes in the temperature of 23, 30 and 35 °C, *pH* of 4, 5 and 6 and yeast concentration of 1, 3 and 5 g/L, respectively.

1.5. Filtration:

After 120 hours of incubation, samples were taken out from incubator and then were filtered by filter paper. The pH and total soluble solid (TSS) values were measured after fermentation.

1.6. Ethanol yield and reducing sugar content determination:

Bioethanol yield was determined by the measurement of ethanol absorbance at 575 nm wave length, after conducting ethanol assay following the Dichromate Colorimetric Method (William and Darwin, 1950) using spectrophotometer. The absorbance values were compared to the ethanol standard graph and the percentage of ethanol had been calculated. Glucose content was determined by DNS method (Miller, 1959) and the absorbance taken from each samples was compared to the standard curve of reducing sugar to calculate the sugar content. The content of reducing sugars was determined by 3, 5-dinitrosalicylic acid. A standard curve was drawn by measuring the absorbance of known concentrations glucose solutions at 450 nm. The DNS reagent was consisted of 1% dinitrosalicylic acid, 0.2% phenol, 0.05% sodium sulphite and 1% sodium hydroxide. To measure glucose content, 3 mL of unknown glucose solution was filled into a test tube, followed by addition of 3 ml of DNS reagent. The test tubes were then heated in boiling water bath for 15 minutes. Exactly 1 mL of 40% potassium sodium tartrate solution was then added prior to cooling. All test tubes were cooled and then its absorbance was measured at 450 nm wave length.

1.7. Metal content of bioethanol:

Different amount of yeast samples were analyzed to quantify the elemental content by using multielement oil analyzer (MOA II).

1.8. Viscosity and acid value analysis:

Acid value was measured according to Fazliny and Hossain (2010). 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 at 30 rpm. Then the spindle with the size of 63 was used.

1.9. Engine test:

Engine emission of produced bioethanol was tested by first generating it using the Proton Gen 2 multicylinder engine for 1 hour at 2000 rpm (60 km/hour). The hydrocarbon, CO, CO_2, NO_X SO and fuel consumption (mL/sec) was measured for E0 (100% gasoline), E5 (a blend of 5% bioethanol with 95% gasoline) and E10 bioethanol (a blend of 10% bioethanol with 90% gasoline).

RESULTS AND DISCUSSION

As shown in Fig. 1, mango pulp showed the highest bioethanol yield of 15% (v/v) followed by mixture at 13% (v/v) and peel at 11 % (v/v). It is well documented that pulp contained the highest amount of starch, which would then be converted into reducing sugars after ripening. So, this huge amount of reducing sugars would generate more bioethanol. According to Reddy and Reddy (2006), there are three types of sugar namely glucose, fructose and sucrose, and the major sugar is sucrose in mango fruits.

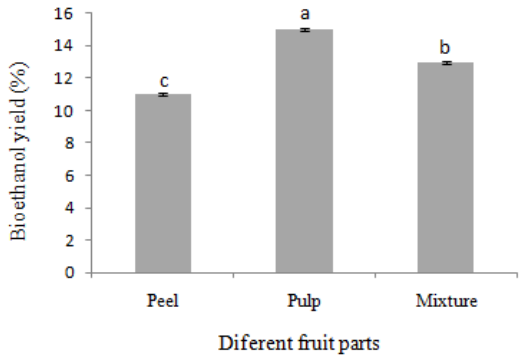

Fig. 1: Bioethanol yield determination at different fruit parts. Mean ± S.E. are significantly different by ANOVA ($P < 0.05$).

During ripening process, the starch and sucrose would be degraded into fructose and glucose. So, after undergoing ripening stage, the amount of reducing sugars in mango would increase. This promoted the high production of bioethanol from mango *via* fermentation, economically. Whereas, peel contained low sugars, which lead to lower production of bioethanol, while the mixture contained an intermediate content of sugars in between pulp and peel. The amount of produced ethanol was mainly dependent on the amount of fermentable sugar present in sample. All the sugars available were used up by *Saccharomyces cerevisiae* Type II as the amount of residual reducing sugars was detected by the dinitrosalicylic colorimetric (DNS) method in the filtrate after fermentation. Interestingly, in this experiment, mango peel had been used because it was totally a waste, and had no economical value. So, an advantage of converting this waste into important product was observed.

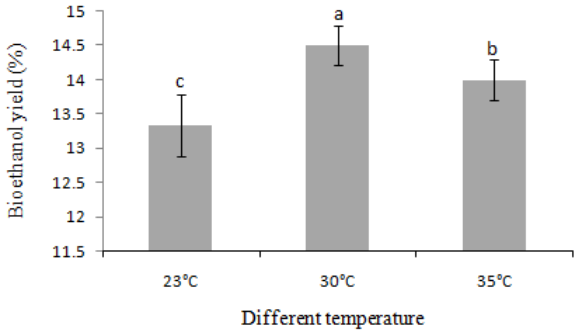

Fig. 2: Bioethanol yield determination at different temperature. Mean ± S.E. was significantly different by ANOVA ($P<0.05$).

Temperature had a profound influence on the rate of alcoholic fermentation of mango waste. Yeast reactions were particularly sensitive to small changes in temperature. Maximum bioethanol concentration produced was 14.5% (v/v) at the temperature of 30°C and the lowest was obtained at 23°C (1 %) (Fig. 2). The results showed that the bioethanol yield was increased with the increase of temperature from 23 °C to 30 °C within 120 hours of incubation times and this result was in harmony with the result obtained by Reddy and Reddy (2009). Sujit *et al*. (2009) also reported that the bioethanol concentration, bioethanol productivity and fermentation efficiency increase at the increase of temperature between 25-30°C, decrease gradually from 30 to 35°C, and drastically decreased above 35°C (Table 1). There were several reasons contributing to the lower production of ethanol yield due to changes in temperature. One of the points highlighted was the feature and characteristic of yeast itself as it functions in fermentation process. Yeast is a single-celled fungus which made up of protein and initiated fermentation by secreting certain enzymes such as zymase or invertase. From temperature of 23°C to 30°C, the production of bioethanol increased. This observation was mainly due to the kinetic theory. The kinetic theory stated that with the increasing temperature, the rate of reaction would also increase due to the increase of speed of the particles. So, the rapid particles movement induced particle collisions, thus the reaction rate could be higher until certain range of temperature. But from Fig. 2, the production of ethanol was slightly lower at 35°C as compared to 30 °C. This might be due to a particular correlation between yeast and temperatures in their metabolic activity.

Table 1: Relationship between yeast and temperature in fermentation (Jerry and Marsha, 2001).

Temperature	Activity
-20°C (-4 F)	Loss of Fermentation Capacity
< 20°C (68 F > 40°C (104 F)	Growth Rate Significantly Reduced
20°C (68 F) - 27°C (81 F)	Most Favorable Range For Yeast to Multiply
26°C (79 F)	Optimum multiplication of Yeast Achieved
27°C (81 F) - 38°C (100 F)	Optimum Fermentation Range
35°C (95 F)	Optimum Fermentation Temperature
> 60°C (140 F)	Yeast cells Die

Similar research was done by Sharma *et al*. (2007) on bioethanol production from kinnow waste and banana peels *via* fermentation. They stated that the production of ethanol at above temperature 30°C is declining. Decline in ethanol yield at increased temperature might be due to the inactivation of yeast that is involved in ethanol production pathways (Sharma *et al*., 2002; Sanchez *et al*., 2004; El-Abyad *et al*., 1992).

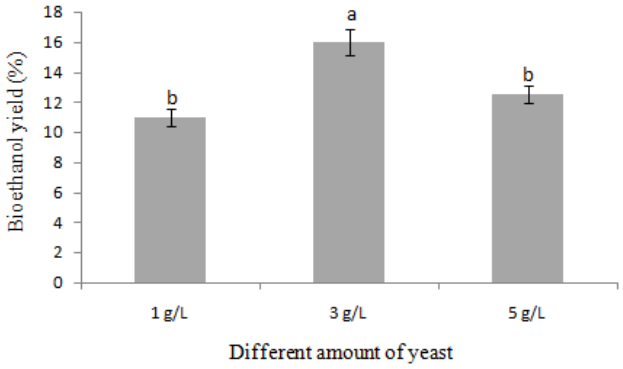

Fig. 3: Bioethanol yield determination at different amount of yeast. Mean ± S.E. was significantly different by ANOVA ($P<0.05$).

Data presented in Fig. 3 show that as the concentration of yeast increases, the yield of bioethanol increased up to 3 g/L and then it decreases. The bioethanol production increases with the increased of yeast concentration (Akin-Osanaiye *et al*., 2008). Increasing the amount of yeast beyond the 3g/L resulted in a decline in fermentation which was in accordance with the results reported by Sharma *et al*. (2007) and Reddy and Reddy (2005). The higher concentration of yeast exceeded the ratio of suitable yeast to sugar condition causing the high competition of yeast in insufficient supply of sugars. However, there is a limit to yeast content above which yeast cells might not function well to produce bioethanol (Alan and Barnett, 2007). Another reason might be due to a decrease in porosity, lower oxygen interaction and low aeration inside the solution (Ray *et al*., 2008).

The samples were kept in anaerobic condition for a period of five days and fermented solution was analyzed at first, third and fifth day to evaluate the maximum yield of bioethanol. From Fig. 4, it was shown that during the fermentation process, the bioethanol production increases with the increase of incubation time. At the day 5 of incubation period, the production of bioethanol (16.2% v/v) was enhanced followed by 3 days and 1 day with 14.5% and 12.8% (v/v) bioethanol, respectively.

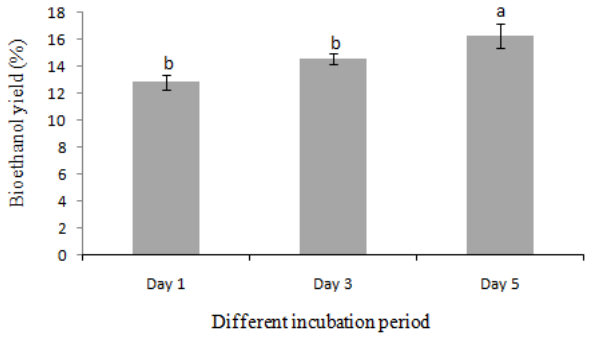

Fig. 4: Bioethanol yield determination at different incubation period. Mean ± S.E. was significantly different by ANOVA (P<0.05).

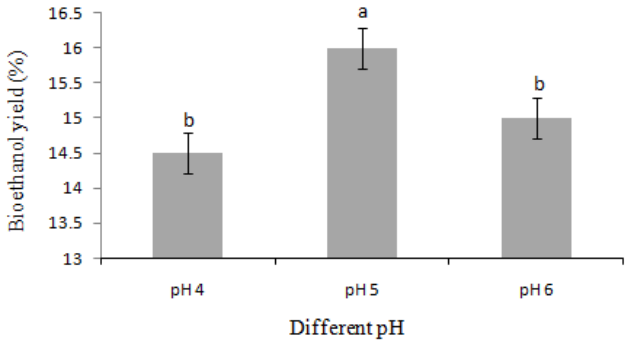

Fig. 5: Effect of pH on bioethanol yield (%). Mean ± S.E. was significantly different by ANOVA (P<0.05).

There is a suitable pH value for yeast action on fermentation process. As shown in Fig. 3, bioethanol production reached a significantly higher level at pH 5.0. It had been reported that the initial pH affected the levels of the alcohols produced due to the metabolic pathway which might change with pH. Different composition of fruits preferred a certain pH for its fermentation progress. In this study, mango feedstocks indicated that the yeast was better adapted to utilize the substrate at pH of 5.0.

Table 2: Total soluble solid (TSS) measurement at different treatments. Mean ± S.E. was significantly different by ANOVA (P<0.05).

Treatments	Parameters	TSS Before	 After
Temperatures (^{0}C)	23	12.5	4.4
	30	12.5	4.0
	35	12.5	4.1

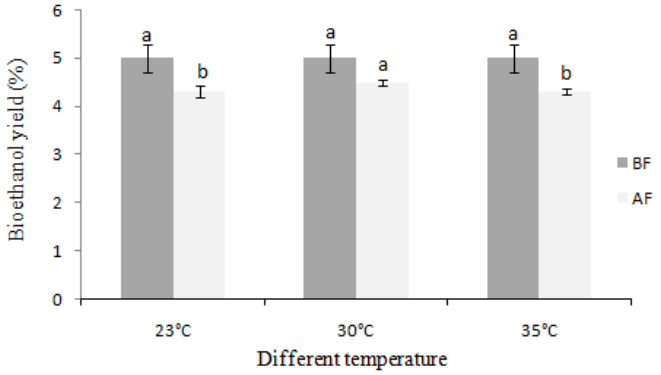

Fig. 6: pH measurement at different temperature. Mean ± S.E. was significantly different by ANOVA (P<0.05). BF = Before fermentation & AF = After fermentation.

1.10. Sample analysis:

Total soluble solid (TSS) was estimated before and after the fermentation process. From Table 2, the results show that the TSS was decreased after fermentation from 12.5 to about 4 in all parameters. Even though in the post-harvest storage, TSS content of storage fruit and flower decreases with advancement of time (Khandaker *et al.*, 2010). Sugars are the major soluble solids in mango; therefore, TSS was considered for the estimation of the sugar content in the bioethanol. The refractometer measured the refractive index, which indicated the amount of light beam would be refracted when it passed through the sample solution which is then be correlated with TSS (Verma *et al*., 2000). The reduction of TSS after fermentation was mainly due to the utilization of sugars by yeast to produce bioethanol.

From Fig. 6, the pH values were reduced after fermentation. The pH values decreased to 4.42-4.31, due to the production of carbonic acid, the carbon dioxide that dissolved in water and other organic acids.

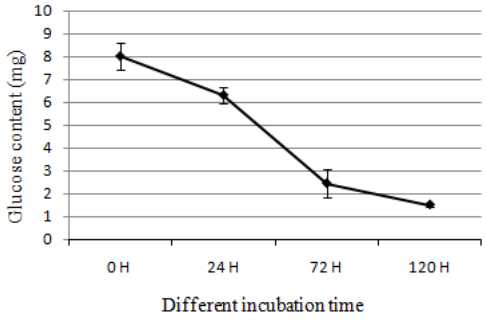

Fig. 7: Glucose content (mg) measurement in different incubation times (hours). Mean ± S.E. was significantly different by ANOVA ($P<0.05$).

Glucose content was determined by the DNS method (Miller, 1959) and the absorbance taken from each samples was compared to the standard curve of reducing sugar to calculate the sugar content. The glucose concentration had been evaluated from samples of fermentation of mango pulp, at 30°C, in pH 5 for 0, 24, 72 and 120 hours of incubation time. From Fig. 7, the reducing sugars measured were decreased as the fermentation going on, and the bioethanol produced increased. From fermentation, glucose had been utilized by yeast to produce bioethanol and carbon dioxide.

1.11. Chemical analysis:

Multielement Oil Analyzer (MOA) was used for the identification of the metallic element and quantities in the bioethanol sample. Results showed that the metal contents (Fe, Pb, Al, Cu, Mn, Zn, Ca, Mg, Si, Sn, B and V) were maintained within ASTM (American Society for Testing and Materials) standard specification, thus could potentially be used as a good biofuel. From Table 2, the hazardous chemical contents such as Pb, Al and Cu were not included in this bioethanol produced, while Fe, Mn, B, V, Zn, Si Ca, Mg, Si, Sn, B and V were a in very low amount. Therefore, this bioethanol produced was an eco-friendly biofuel. The Mg and Ca contents had quite a big different among the different yeast amount. This was due to error that occurred mainly because of the improper handling of samples.

Table 3: Metallic elemental content.

Chemical	MOA Spectrometry Value of Chemical Content		
	Amount of Yeast (g/L)		
	1	3	5
Fe	0.5	1	1
Pb	0	0	0
Al	0	0	0
Cu	0	0	0
Mn	14	6.5	17
Zn	7	8	5.5
Ca	8.5	11.0	8.2
Mg	14.8	15.2	16.3
Si	15.5	11.5	10.5
Sn	4.2	4.1	4.5
B	2	2	2
V	4.5	4.5	5

Table 4: Viscosity and acid value for fermentation at different temperature.

Amount of yeast (g/L)	Viscosity value (cst)	Acid Value (mg KOH/g)

1	1.01	0.40
3	1.09	0.50
5	3.85	0.45

1.12. Viscosity and acidity test:

The viscosity of the bioethanol produced was important when considering the spray characteristics of the fuel within the engine, since the change in spray could greatly alter the combustion properties of the mixture. From the results obtained in Table 4, it could be seen that the bioethanol produced from fermentation of mango pulp at the temperature of 30 ℃ with different amount of yeast were in the range of considered ASTM standard, which were within 1 to 5 centistroke. This would give an indication that bioethanol produced from mango was suitable as a possible biofuel substitute. As in advantage, low viscosity value is good for engine utilization and would reduce problem of corrosion to the engine. The samples from fermentation at 1g/L and 3 g/L showed a slightly increase in viscosity values which were 1.01 cst and 1.09 cst, respectively. In contrast, the viscosity value from fermentation in 5g/L yeast amount had a higher value which was 3.85 cst. The increased of viscosity value in fermentation with 5 g/L yeast was mainly due to the presence of higher glycerol in the solution. However, the viscosity obtained was still maintained under ASTM standard, which indicated best result for this ethanol produced. Table 4 also shows the result of acid value test from samples fermented at different amount of yeast. From the result, the acid values were almost the same for all fermentation in 1 g/L, 3 g/L and 5 g/L of yeast with acid value of 0.40, 0.50 and 0.45 mg KOH/g of samples, respectively. The results obtained were in the range about of 0.5 mg KOH/g and under ASTM standard specification.

Table 4: Fuel consumption and greenhouse gas emission analysis.

Emission (ppm) and Fuel consumption (ml/sec)	Standard or 100% gasoline: E0	5% ethanol & 95% gasoline: E5	10% ethanol & 90% gasoline: E10
CO_2	9	8.1	8.1
CO	6.2	6.0	6.0
SO_X	902	335	280
NO_X	66	27	22
HC	75	35	27
Fuel consumption	1.8	1.4	1.3

1.13. Engine test:

Table 4 showed that the amount of emission from engine test of E0, E5 and E10 fuel. The effects of different volumetric percentages of bioethanol–gasoline blends, ranging 0, 5 and 10%, on engine emissions were tested on petrol engine (Gen-2 proton engine). The present study showed that the variations of the NO_X, CO_2, CO and HC emissions were depended on the blending ratio. The E0 (100% gasoline) produced higher carbon dioxide than E5 (5% bioethanol + 95% gasoline) and E10 (10% bioethanol + 90 % gasoline) blending. The oxygen content in the blending fuel favours conversion of the CO produced during combustion into CO_2. The E5 produces higher NO_X emissions than the E10 blending. This is due to the faster flame speed and higher peak temperature in the combustion chamber of E5. As it is well known that the NO_X formation is a strong function of peak chamber temperature, blending gasoline also appears to be a good choice for lower HC emissions. The chemical structure of the bioethanol-gasoline blend, with higher presence of carbon and hydrogen is less favourable for HC formation (Al-Hasan, 2003; Mongi *et al*., 2005). The results taken from the present experiment have confirmed that the engine performance was improved with less green house gas emissions by E10 bioethanol (Rodrigo and Jose, 2010; Lan-bin *et al*., 2010). Due to proper fuel injection in time, more fuel can be in contact with the relatively cool cylinder, and sufficient time for complete fuel combustion. This is probably because bioethanol has higher cetane number, leading to faster evaporization and auto ignition (Changming *et al*., 2009). This observation strongly showed that the bioethanol produced from mango waste had a high potential as renewable energy.

2. Conclusion:

The results of this study showed that the bioethanol production through fermentation of mango waste was the highest in percentage, from mango pulp in yeast concentration of 3 g/L at temperature of 30°C after five days of incubation. The glucose content, total soluble solid (TSS) and pH values were reduced after fermentation, due to conversion of glucose into bioethanol. The engine test results showed that the hydrocarbon, NO, CO and CO_2 contents were significantly lower in E5 and E10 than in 100% gasoline. The findings of this work encourage the production of bioethanol from mango waste as the alternative fuel to provide a renewable energy. Besides, it can also be used efficiently as environmental recycling for waste management. In addition, green house gaseous emissions could be reduced by using this technology.

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