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Full Length Research Paper

Bioethanol fuel production from rotten banana as an environmental waste management and sustainable energy

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Banana waste is discarded due to the imperfection during grading process. Banana biomass can be used as raw material to produce bioethanol. In this study, fermentation of banana waste was conducted using *Saccharomyces cerevisiae*, Type II under anaerobic condition. Production of bioethanol was determined and the effects of various operating conditions which included different temperatures, shaking period, rotten and fresh banana fruit and saccharification method were observed. Overall, the fermented banana fruit waste produced 4.1 to 7.1% bioethanol. The bioethanol yield from mixture of rotten banana fruit increased with increase fermentation period. It is also increased with yeast concentration, using 35% of water at 35°C. The optimum shaking hours for fermentation was 6 h at pH 5.8. Combination of enzyme (pectinase and cellulase) produced higher bioethanol than enzyme alone. Viscosity and acid value of the produced bioethanol followed the ASTM (American Standard for Testing Materials) and EN (European Norms) standards. Fermented banana treated with mixture of enzymes was the best method used for higher bioethanol production. The results showed that, utilization of mixture (skin and pulp) of rotten fruit was more suitable for bioethanol production as renewable energy which could reduce the cost of the initial process. In addition, it did not compete with the consumer food supply and could avoid the overloaded waste for compose, as well as could be used as fuel in the normal petrol engine. In addition to that, energy could be produced from waste banana fruit as environmental recycling process.

Key words: Bioethanol, rotten banana, yeast, enzyme, temperature.

INTRODUCTION

Combustion of the fossil fuels at the current rate would contribute to the environmental crisis globally (Chandel et al., 2007). The increase in demand of fossil fuels combined with depletion of this reserves mineral oil has led to the development of eco-friendly concepts (Demirbas and Demirbas, 2007) . In addition, demand of the energy increases with the increase of the world population and urbanization (Demirbas, 2008) and thus, development of bioenergy as alternative energy might help to reduce these problems.

Bioenergy can be defined as energy obtained from biomass, which is the biodegradable fraction of products, waste and residues from agriculture like vegetables and animal origin, forestry and related industries and also, from the biodegradable fraction of industrial and municipal waste (FAO, 2008). Different forms of bioenergy can be produced from a wide range of biomass sources, for example, agricultural residues (Hossain et al., 2008; Hossain and Fazliny, 2010).

There are many countries that use waste biomass as option rather than use food supply for energy production, like Zimbabwe and Australia. In Zimbabwe, some researches have been conducted on energy production from crop residues. The gross energy consumption was

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about 44% in Zimbabwe which came from waste biomass (Jingura and Matengaifa, 2008). Meanwhile, banana waste has been used to produce biogas using fed-batch digestion in Australia (Biopact, 2008).

In Australia, approximately 30% of the harvested bananas are rejected at the packing shed (Clarke et al., 2007). Banana waste that have been discarded due to the imperfections are normally dumped as a huge masses of wastes, which ultimately cause contamination of water source as well as can affect the environment and health of living microorganisms (Tock et al., 2009). Thus, to avoid the environmental problem due to the decomposition of waste, it is usable to make energy from banana waste as biofuel production source.

In order to develop the new technologies and improve the available technologies regarding the biofuels production, it is essential to address the challenges and opportunities of biofuels in the context of food security and sustainable development needs (FAO, 2008). Akin-Osanaiye et al. (2005) stated that, ethanol production by fermentation faces competition with ethanol production from petroleum-based products. However, as the values of the petrochemical were increased, fermentation of ethanol received more attention (Ahmeh et al., 1988). Since renewable materials (waste) are cheaper, sometimes nothing to pay that is why it is easily available and more economical.

Currently, a combination of pectinases, cellulases and hemicellulases as macerating enzymes has been used in the extraction and clarification of fruit and vegetable juices (Galante et al., 1998; Grassin and Fauquembergue, 1996). The efficiency of biomass conversion to ethanol depends upon the ability of the microorganism used in the process to utilize the diverse carbon sources and amount of fraction present in biomass (Prasad, 2007).

The objectives of this study were to investigate the influence of different temperatures, shaking hours and water content on bioethanol production by using rotten banana mixture (pulp and peel). In addition, to investigate the proper yeast concentration and enzymes for fermentation from banana mixture (skin and pulp) and to know the standard properties (viscosity, pH and metal content) of bioethanol for the use in petrol engine

MATERIALS AND METHODS

Raw materials

The banana wastes (rotten) were bought from the market around Petaling Jaya, Kuala Lumpur, Malaysia.

Enzyme

The enzymes used in this experiment included pectinase and cellulase.

Cellulase

Cellulase used was bought from BioChemika with Fluka no. 22180, off-white powder derived from culture of *Aspergillus niger*. This enzyme also known as 1,4- (1,3:1,4)- -D-Glucan 4-glucanohydrolase. Cellulase preparation had 0.3 units activity per mg which 1 U corresponds to the amount of enzymes which liberates 1 mol glucose from carboxymethylcellulose per minute at pH 5.0 and 37°C. Optimum temperature and pH are 60°C and 5.8, respectively.

Pectinase

Pectinase derived from culture of *Rhizopus* sp., with Sigma no. P4300, supplied in the crude powder form. This enzyme also known as Macerozyme R-10, Poly-(1,4- -D- galacturonide) glycanohydrolase or polygalacturonase. Pectinase enzyme had activity of 400 to 800 units per gram solid which one unit would liberate 1.0 mole of galacturonic acid from polygalacturonic acid per min, having pH 4.0 at 25°C.

Yeast

Yeast was derived from culture of *Saccharomyces cerevisiae* Type II. Only approximately 10% would autolyze in aqueous buffer at 37°C and fast dried to yield 90% active, viable yeast in a convenient solid form (Sigma).

Preparation of samples

900 g of rotten banana were thoroughly washed with distilled water, cut using a sterile knife and were blended using a sterilized automatic juice blender. The banana mash was then, dispensed into the nine set of sterile schott bottle already labeled according to the dates for each sample analysis. 25 ml of water were added into the schott bottle containing banana mash. The pH of the banana mash was 5.0. After that, total soluble solids of banana mash were taken.

Fermentation

The 3 g/l of yeast, *S. cerevisiae* was added into each set and all of the bottles were closely air tightened to ensure they were made air-tight to provide an anaerobic condition and placed in incubator at 30°C±2. The dry active yeasts were rehydrated in water bath at 40°C, by using clean water and allowed taking to room temperature before adding into the banana mash. Fermentation was carried out for 3 days. After fermentation, the clean sterile cotton cloth was used to sieve the product from the residue. Extract was collected in nine different sterile plastic containers. The obtained raw bioethanol was then kept in room temperature to measure pH and total soluble solid (TSS). The same method was repeated as mentioned earlier for the following parameters. Fermentation was done at different temperatures like 23, 30 and 35°C were used to incubate for the hydrolysis reactions. The bioethanol yield was also measured from the fermentation of the rotten banana mash by using different shaking hours, 0, 3 and 6 h. In addition to that, the bioethanol yield was determined by using different amount of water content, 0, 15, 25 and 35% added to the rotten banana mash. Other parameters by applying the different enzymes saccharification method were done. The rotten banana mash was treated with 0.3 ml of pectinases at 40°C in water bath for 2 h. The optimum treatment time was used to hydrolyze the banana mash (Cheirsilp and Umsakul, 2007). These macerated mashes were heated at 90°C for

5 min to stop the activity of enzymes and pH was readjusted to 5.0. Subsequently, the pectinase- hydrolyzed mash was treated with 0.3 ml of cellulases at 60°C for 2 h. The other set of banana mash was treated with 0.3 ml of cellulases alone at 60°C. Then each set of the mashes was allowed to come to room temperature before the fermentation process was carried out. Untreated banana mash was used as a control for ethanol production.

Analytical assay

pH, total soluble solids, bioethanol yield, viscosity and element content were analysed.

pH

The changes of pH in all fermentations were determined by pH meter (model Hanna instruments). The pH was checked before and after the fermentation process.

Total soluble solid (TSS)

Total soluble solids content in all fermentations were determined by using Atago digital refractometer (Tokyo, Japan) with a scale ranging between 0 and 30% brix unit. The results were reported as % brix. Total soluble solid content was checked before and after fermentation process.

Viscosity

Viscosity of the produced bioethanol was determined by using viscometer.

Elemental analysis

Metel contents like P, Ca, Mg, Fe, Pb, Cu, etc. were analyzed by using multi-element oil analysis (MOA) spectrometry.

Bioethanol concentration

Ethanol concentration was determined according to the method of Williams and Darwin (1950). The 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 shaking 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 the capped test tube. The test tube was covered with a piece of paraffin film to avoid the loss of liquid due to evaporation. The mixture was then heat up using water bath at 90°C for 5 to 15 min 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.

Glucose estimation

Glucose content was determined according to the method of Miller (1959). The 1% of dinitrosalicylic acid reagent solution was prepared by adding 10 g of dinitrosalicylic (DNS) acid, 2 g of phenol, 0.5 g of sodium sulfite, 10 g of sodium hydroxide and mixed; followed by 1 L of water and mixed well. 3 ml of DNS reagent was added to 3 ml of glucose sample in a lightly capped test tube. The mixture was then incubated in water bath for 5 to 15 min at 90°C until the red-brown color appeared. Then, 1 ml of a 40% potassium sodium tartrate (Rochelle salt) solution was added to stabilize the color. The absorbance values of the reducing sugar was measured using spectrophotometer at 575 nm, after cooling to room temperature in a cold water bath.

Statistical analysis

Data were recorded as means ± standard deviations and analyzed by STATGRAPHICS Plus 3.0. One-way analysis of variance was carried out to test for any significant differences between the means values. P- values less than 0.05 were considered statistically significant. All analyses were performed in triplicate

RESULTS

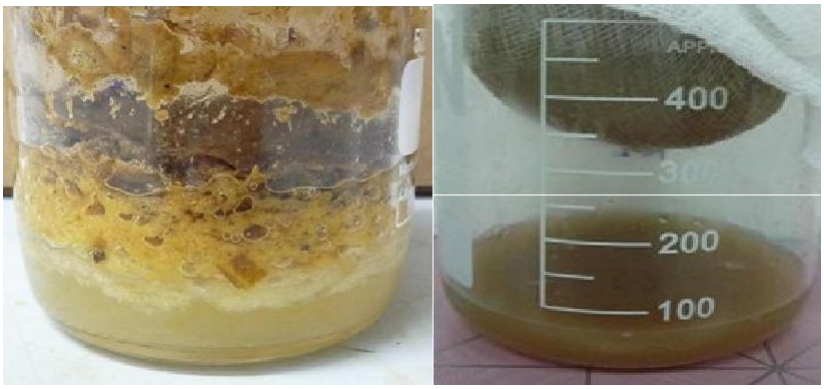
Comparison of concentration of bioethanol was shown in Figure 1. Fermentation at temperature 35°C showed the highest concentration of bioethanol, compared with fermentation at 30 and 23°C with reading of 6.21, 5.88 and 5.39% (v/v), respectively. The concentration of bioethanol increased as the temperatures increased (Figure 1).

Reading of the total soluble solid and pH measurement of banana mash treated with different temperature were shown in Table 1. Total soluble solid of the banana mash before fermentation were slightly higher than those after fermentation. The highest total soluble solid was occupied by banana mash treated at temperature 23° followed by 30 and 35°C. The pH measurements of banana mash before fermentation were higher than pH measurement of fermented mash after fermentation. The concentration, total soluble solid values and pH of bioethanol can be considered as significantly difference based on ANOVA method at $p < 0.05$.

The concentration of bioethanol at different shaking hour was shown in Figure 2. The fermented banana mash that have been shaken for a long time produced higher bioethanol with 6.55% (v/v), followed by 3 h of shaking period (6.35%) and fermentation of banana mash without shaking, only produce 5.86% of bioethanol. The concentration of bioethanol increased as the time of the shaking process was increased. Based on the data, the values of the total soluble solid for the fermented banana mash were lower than before fermentation (Table 2). From the pH results, measurements of the pH before fermentation were higher than after fermentation. The pH of the fermented banana mash without shaking exhibited the lower value than those that have been shake for 3 and 6 h. There were significant differences in this parameter in the concentration of bioethanol and there was no significant difference on pH measurement between fermented banana shake with 3 and 6 h. The result of the bioethanol produced from fermented banana

Rotten banana **Cut banana** **Ground banana mash**

Sample before fermentation **Incubator** **Fermented banana**



Layer of fermented banana **Filtered bioethanol**

Figure 1. Shows different stages of fermentation.

mash showed that the concentration of bioethanol produced range from 4.33 to 6.36% (Figure 3). The lowest volume of 4.33% (v/v) was produced from the fermented banana mash without water, while the highest volume of 6.36% (v/v) produced from the fermented banana mash treated with 35% of water. The fermented banana mash treated with 15 and 25% of water, produced 5.37 and 5.86% of bioethanol concentration,

Table 1. Effect of different temperature treatment on the concentration of bioethanol, total soluble solid and pH of banana mash. Different superscript letters in each column indicate statistically significant differences ($p < 0.05$).

Parameter (°C)	Total soluble solids (TSS) (˚Brix) ± S.D		pH ± S.D	
	Initial	After	Initial	After
23	17.20 ± 0.20	15.47 ± 0.42^{c}	5.00 ± 0.00	3.85 ± 0.03^{b}
30	17.33 ± 0.31	13.67 ± 0.12^{b}	5.00 ± 0.00	3.74 ± 0.04^{a}
35	17.00 ± 0.00	11.53 ± 0.31^{a}	5.00 ± 0.00	3.97 ± 0.02^{c}

SD = Standard deviation.

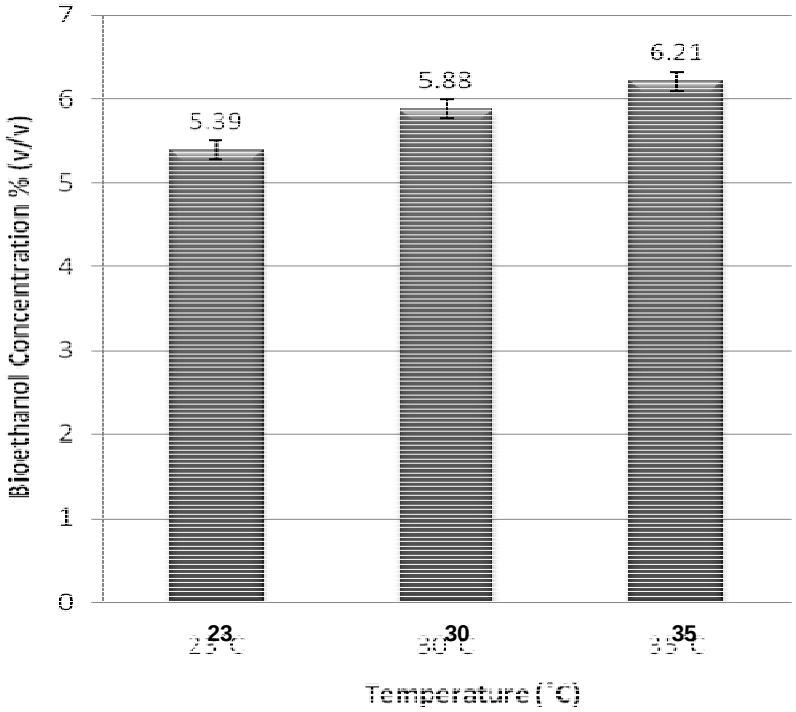


Figure 2. Comparison of bioethanol concentration from fermented banana (*M. acuminata*) mash using different temperature treatment.

Table 2. Effect of different shaking hour treatment on the concentration of bioethanol, total soluble solid and pH of banana mash. Different superscript letters in each column indicate statistically significant differences ($p < 0.05$).

Parameter (shaking hour (h)	Total soluble solids (TSS) (˚Brix) ± S.D		pH ± S.D	
	Initial	After	Initial	After
0	17.00 ± 0.00	10.00 ± 0.20^{a}	5.00 ± 0.00	4.03 ± 0.02^{a}
3	17.00 ± 0.00	10.47 ± 0.31^{b}	5.00 ± 0.00	4.25 ± 0.06^{b}
6	17.00 ± 0.00	10.07 ± 0.12^{ab}	5.00 ± 0.00	4.27 ± 0.09^{b}

SD= standard deviation.

respectively. The concentration of bioethanol increased as the amount of water was increased. However, different amount of water applied to the banana mash only gave slight difference on the concentration of bioethanol when 15 to 35% amount of water was used. According to the data, the values of the total soluble solid for the fermented banana mash were lower than before fermentation (Table 3). Among the fermented banana

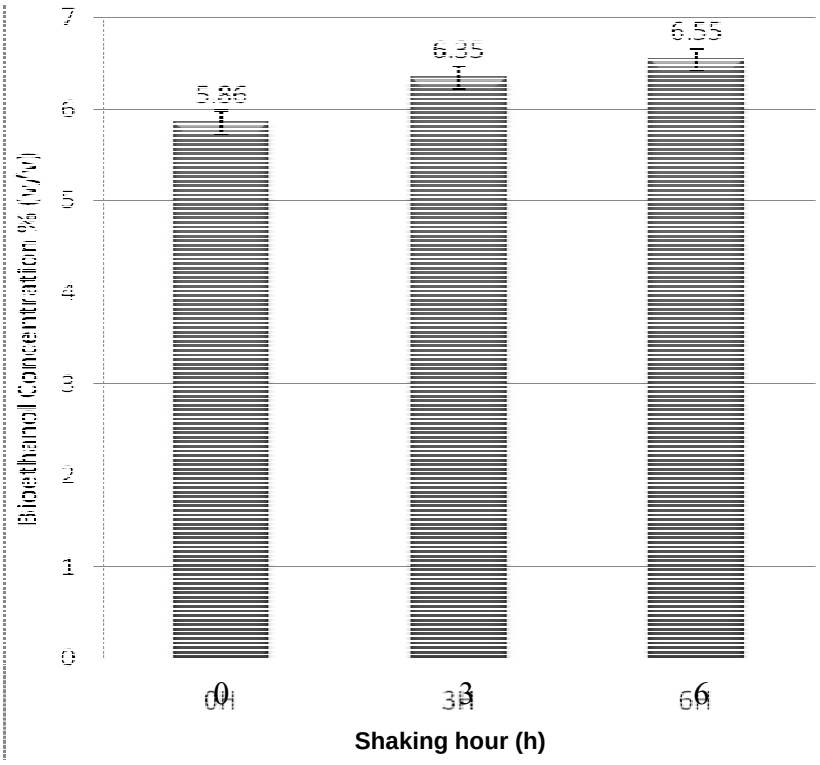


Figure 3. Comparison of bioethanol concentration from fermented banana (*M. acuminata*) mash using different shaking hour.

Table 3. Effect of different amount of water treatment on the concentration of bioethanol, total soluble solid and pH of banana mash. Different superscript letters in each column indicate statistically significant differences ($p < 0.05$).

Parameter (amount of water (%))	Total soluble solids (TSS) (˚Brix) ± S.D		pH ± S.D	
	Initial	After	Initial	After
0	17.33 ± 0.31	15.47 ± 0.12^{c}	5.00 ± 0.00	3.89 ± 0.09^{a}
15	17.33 ± 0.42	13.60 ± 0.20^{b}	5.00 ± 0.00	3.94 ± 0.03^{ab}
25	17.20 ± 0.00	8.20 ± 0.20^{a}	5.00 ± 0.00	4.03 ± 0.02^{b}
35%	17.07 ± 0.12	8.27 ± 0.12^{a}	5.00 ± 0.00	4.19 ± 0.01^{c}

SD= standard deviation.

mash with different amount of water, fermentation of banana mash without water exhibited the higher value of total soluble solid, followed by fermentation with 15, 35 and 25% of water. From the pH results, measurements of the pH before fermentation were higher than after fermentation. After fermentation, fermented banana mash with 35% of water had the highest pH measurement among the others, followed by fermentation without water with 15 and 25% of water.

Comparison of the bioethanol concentration of banana mash using rotten and fresh banana fruit were shown in Figure 4. From the plotted graph, the highest concentration of bioethanol was produced from the fermentation that had been used rotten banana fruit with 5.79% (v/v), followed by fresh banana fruit with 4.12% (v/v) of bioethanol. Before fermentation, the values of total soluble solid of fresh banana fruit were higher than rotten banana fruit, same as after fermentation (Table 4). For both rotten and fresh banana fruit, the value of total soluble solid after fermentation were lower than after fermentation, as sugar content of banana mash was used by yeast to do the fermentation. From the pH measurement, after fermentation, fresh banana mash exhibited the lower value than before fermentation. The concentration, total soluble solid and pH of bioethanol can be considered as significantly difference at $p < 0.05$.

The result of bioethanol produced from fermented banana mash showed that, the concentration of

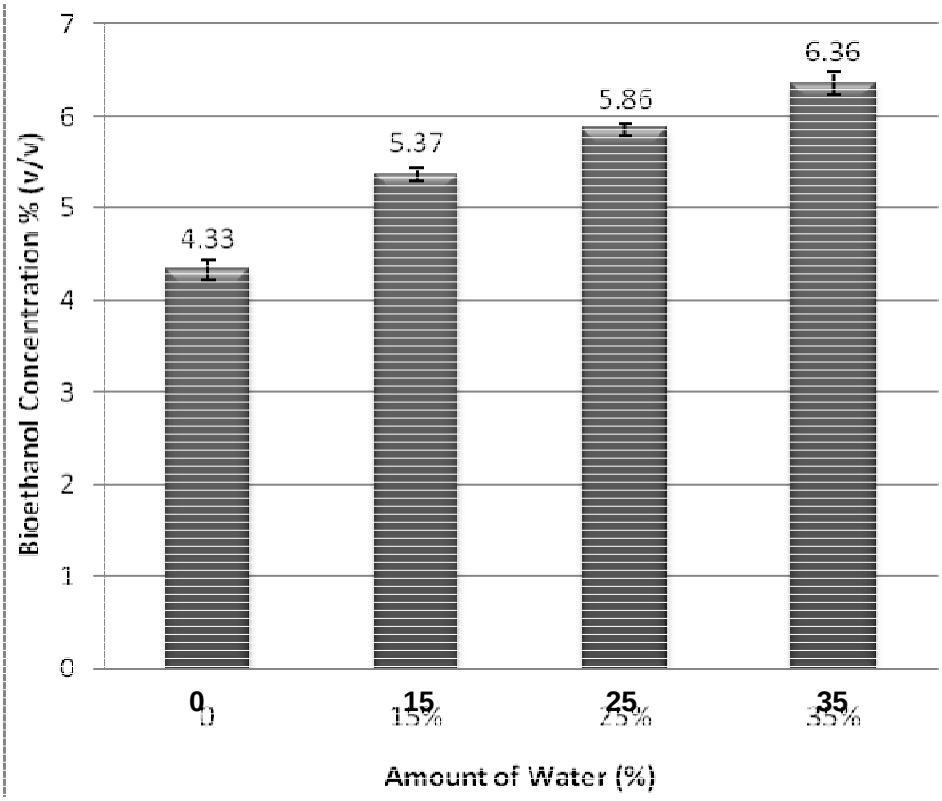


Figure 4. Comparison of bioethanol concentration from fermented banana (*M. acuminata*) mash using different amount of water.

Table 4. Effect of rotten and fresh *M. acuminata* on the concentration of bioethanol, total soluble solid and pH of banana mash. Different superscript letters in each column indicate statistically significant differences ($p < 0.05$).

Parameter	Total soluble solids (TSS) (˚Brix) ± S.D		pH ± S.D	
	Initial	After	Initial	After
Rotten	19.13 ± 0.12	13.27 ± 0.12[a]	5.00 ± 0.00	3.85 ± 0.01[b]
Fresh	20.40 ± 0.40	14.27 ± 0.42[b]	5.00 ± 0.00	3.76 ± 0.05[a]

bioethanol produced range from 5.84 to 7.08% (Figure 5). The lowest volume of 5.84% was produced from the fermented banana mash treated with conventional method (without enzyme), while the highest volume of 7.08% (v/v) was produced from the fermented banana mash treated with mixture of enzymes saccharification method. Banana mash treated with cellulase produce 6.64% (v/v) of bioethanol, while by using pectinase saccharification method, the result was 7.03% (v/v) of bioethanol.

The values of total soluble solid of banana mash were higher before fermentation than after fermentation (Table 5). Among the different enzyme saccharification method, after fermentation, the fermented banana mash treated with conventional method exhibited the highest value of total soluble solid. From the pH measurement, after fermentation, fermented banana mash treated with the cellulase saccharification method exhibited the higher pH value among the other saccharification method. The concentration of bioethanol, total soluble solid and pH values of bioethanol can be considered as significantly difference based on ANOVA method at $p < 0.05$

Glucose analysis

Table 6 showed the standard curve for glucose prepared by using DNS method with different glucose concentrations. The concentration of the glucose in the samples was obtained from the standard graph of glucose concentration. The glucose concentration was analyzed by using DNS method and the absorbance

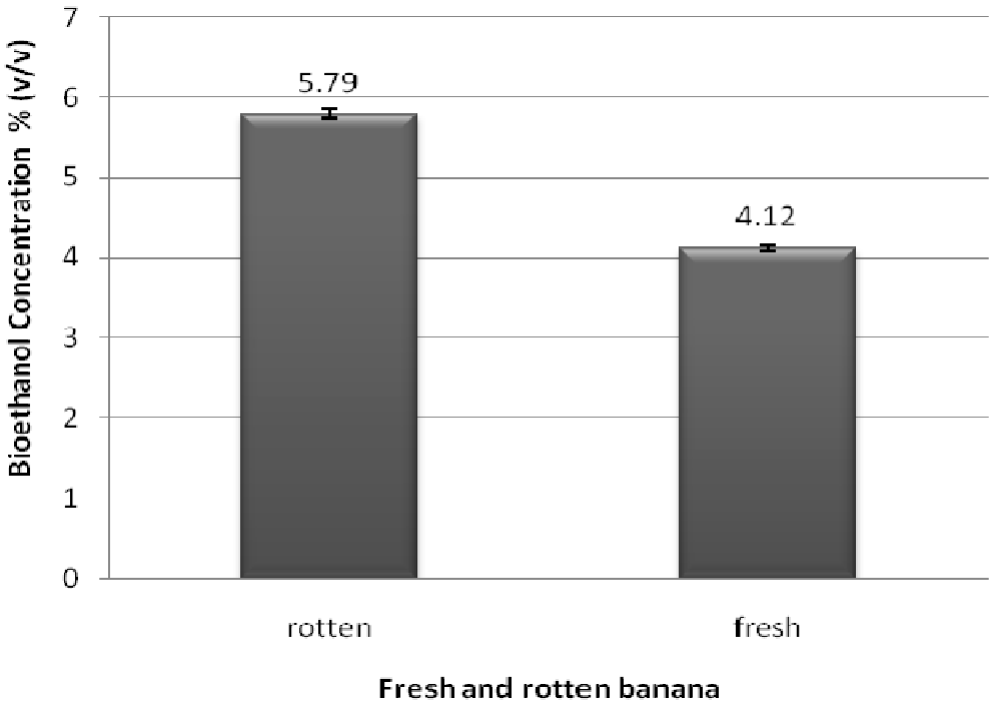


Figure 5. Comparison of bioethanol concentration of fermented banana (*M. acuminata*) mash using rotten and fresh banana fruit.

Table 5. Effect of different enzymes treatment on the concentration of bioethanol, total soluble solid and pH of banana mash. Different superscript letters in each column indicate statistically significant differences ($p < 0.05$).

Parameter	Total soluble solids (TSS) (˚Brix) ± S.D		pH ± S.D	
	Initial	After	Initial	After
Without enzyme	15.00 ± 0.00	6.00 ± 1.00^{b}	5.00 ± 0.00	4.77 ± 0.06^{a}
Cellulase	15.00 ± 0.00	7.00 ± 0.00^{b}	5.00 ± 0.00	4.11 ± 0.00^{b}
Pectinase	15.00 ± 0.00	4.00 ± 1.00^{b}	5.00 ± 0.00	4.89 ± 0.02^{a}
Pectinase with cellulase	15.00 ± 0.00	7.67 ± 1.15^{a}	5.00 ± 0.00	4.07 ± 0.01^{c}

Table 6. Glucose concentration of fermented banana mash treated with different fermentation period.

Time (days) (hour)	Glucose concentration % (w/v)	Bioethanol concentration % (v/v)
0	13.00 ± 0.00	0.00 ± 0.00
1 (24)	3.620 ± 0.08	5.51 ± 0.12
3 (72)	3.284 ± 0.04	5.86 ± 0.07
5 (120)	0.537 ± 0.14	6.09 ± 0.04

readings were taken using spectrophotometer. The data and the figure of the comparison of glucose concentration of fermented banana mash treated with different fermentation period were shown in Table 6. There was a rapid decreased of glucose concentration from 0 to 24 h (day 1) of fermentation period, as the glucose utilized by yeast cells to produced bioethanol. However, from 72 day 3) to 120 h (day 5), there was only slight increment of the bioethanol concentration from 5.86 to 6.09% (v/v). The large amounts of glucose utilized at the initial stage caused the rapid bioethanol production within 24 h, where 5.51% (v/v) of ethanol was produced. The highest

Table 7. Effect of different concentration of yeast treatment on the viscosity of bioethanol.

Concentration of yeast (g/l)	Viscosity (cSt)
2	1.46 ± 0.22[a]
3	1.36 ± 0.21[a]
4	1.46 ± 0.22[a]

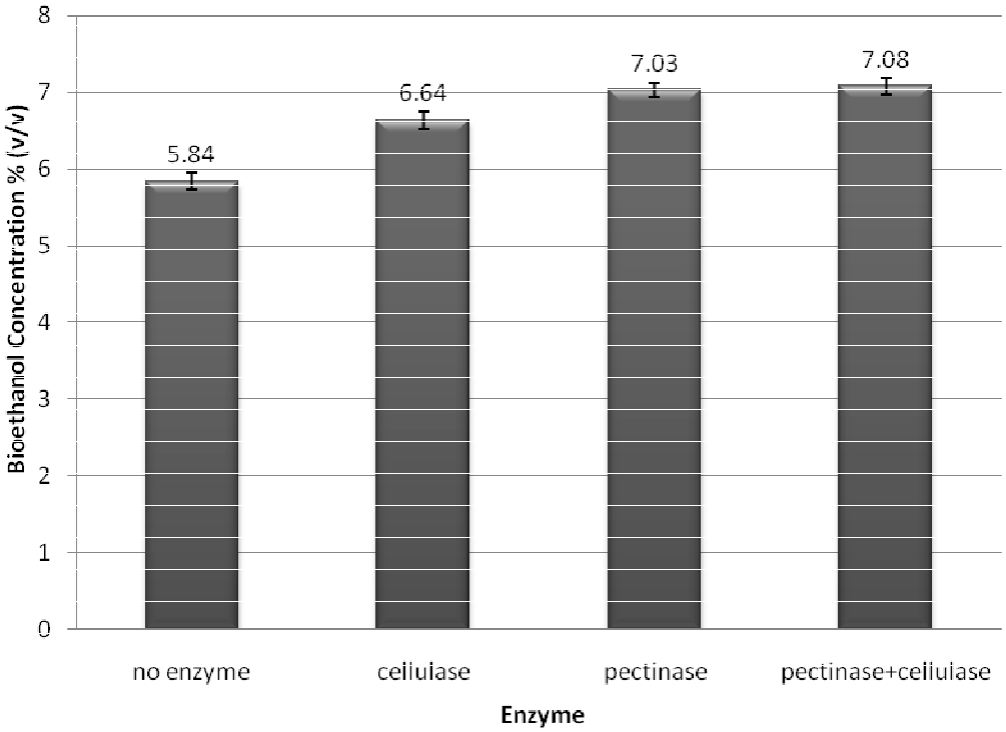


Figure 6a. Comparison of bioethanol concentration from fermented banana (*M. acuminata*) mash using different enzyme treatment.

bioethanol production and the lowest glucose concentration were observed at 120 h where concentration of bioethanol was 6.09% (v/v), while glucose concentration was 0.537% (w/v).

Table 7 has shown the comparison of the viscosity of bioethanol within the treatments. The highest viscosity values were occupied by fermented banana mash using 2 and 4 g/l of concentration of yeast with value of 1.46 cSt. Fermentation of banana mash by using 3 g/l of yeast has the value of 1.36 cSt. The effects of using different concentration of yeast on the viscosity of bioethanol of banana mash are shown in Table 4. There were no significantly different among 2, 3 and 4 g/l of yeast in viscosity values.

Figure 6a and b showed the comparison between the values of the element that existed in the bioethanol. The result of the element analysis from fermented banana mash showed that, the value of bioethanol element range from 0 to 280 ppm. Most of the elements followed the ASTM standard that is better for engine use. Among the elements existing in the bioethanol, argentum (Ag) had the highest value of 407 ppm, found in banana mash treated pH 6, while banana mash that had been treated with pH 4 and 5, the values of argentum (Ag) were 231.5 and 0 ppm. Chromium (Cr), aluminium (Al), cuprum (Cu), plumbum (Pb), nickel (Ni), titanium (Ti), molybdenum (Mo) and barium (Ba) having the smallest value of 0 ppm for banana mash that have been treated with all of the three different pH 4, 5 and 6. Moreover, few of the elements present in the bioethanol have the fluctuated values when pH increased. These elements included manganese (Mn), phosphorus (P), magnesium (Mg) and calcium (Ca). Values of the manganese were 14, 15 and 14 ppm for the pH 4, 5 and 6, respectively. In addition, the values for phosphorus were 111.5, 122 and 11 ppm in the increasing of pH. Besides that, the values of boron

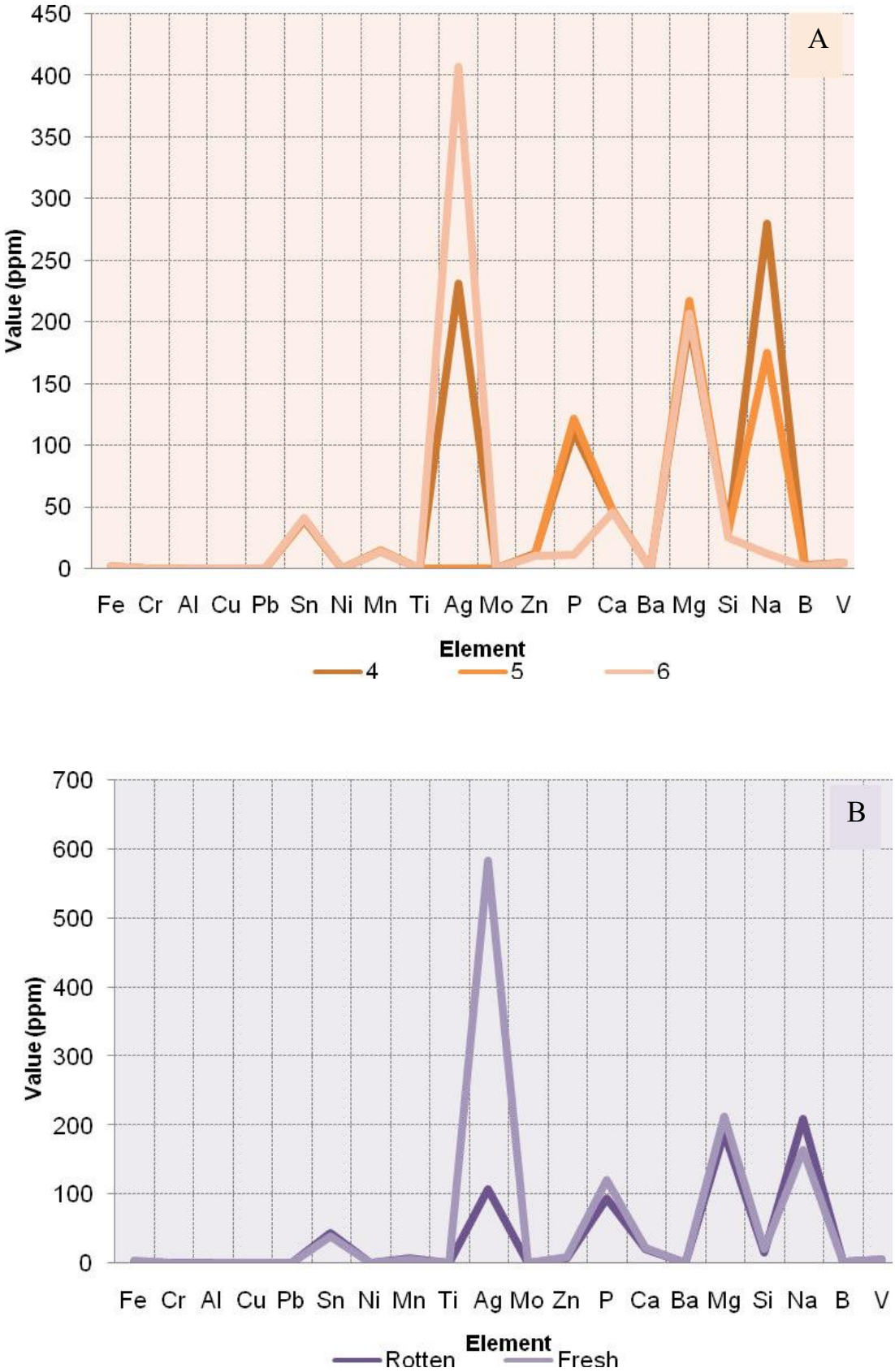


Figure 6b. (A) Comparison of element of *M. acuminata* bioethanol for different pH of banana mash. pH: 4, 5 and 6; (B) Comparison of element of *M. acuminata* bioethanol for rotten and fresh banana mash.

were 2.5, 2 and 2 ppm, while the values for vanadium were 4.5, 4.5 and 5 ppm for the pH 4, 5 and 6, respectively.

DISCUSSION

Currently, researchers have great interests on the production of ethanol by using biomass. Banana (*Musa acuminata*) wastes are examples. In this study, banana wastes (*M. acuminata*) were chosen because it consists of useful sugars and monomers of sugars that could be fermented to produce ethanol and found suitable to be used as alternative energy source (Chandel et al., 2007).

As the temperature of the fermented banana mash increased, the concentration of bioethanol also increased. The highest temperature (35°C) of fermented banana mash gave the highest concentration of bioethanol, followed by 23 and 30°C. From the data, the concentration, total soluble solid and pH values of bioethanol exhibited the significantly difference based on ANOVA method at $p < 0.05$. The production of ethanol was low at temperature of 23°C. This might be due to the inappropriate temperature condition contributing to the lack of metabolic activity which consequently, gave an effect on the diffusion of substrate and product (Alain et al., 1987).

The optimum shaking hour was at 6 h. However, based on the data analysis, after 3 h of shaking process, there were no significant differences between shaking at 3 and 6 h. The pH values of bioethanol exhibited the significant difference between bioethanol produced from the fermentation of banana mash without shaking and fermented banana mash treated with 3 h of shaking process. The amount of water that had been used gave an effect on the production of ethanol. The highest concentration of bioethanol was produced from the fermented banana mash treated with the highest volume of water 35 followed by 25 and 15%. The concentration of bioethanol increased as the amount of water increased. Total soluble solid of bioethanol only exhibited the significant difference between fermented banana mash without water and the fermented banana mash treated with 15 and 25% of water. The fermented banana mash treated without enzyme saccharification gave the lowest concentration of bioethanol followed by banana mash treated with cellulase, pectinase and the highest concentration was produced from the fermented banana mash treated with mixture of enzymes saccharification method.

The concentration of bioethanol after treatment with pectinase was higher than that of the control. This happened because of the breaking down of pectin molecules by pectinase enzyme, subsequently causes a reduction of water holding capacity and released more free water from the system. After treatment with pectinase, cellulose enzyme was used to hydrolyze the cellulose bridge. In this study, the optimum heat treatment was used to ensure the effective enzyme saccharification process. This is necessary in order to reduce the contamination by bacteria, as growth of bacteria can be prevented through applying the heat. In addition, at the initial fermentation step, large amount of reducing sugar produced could be converted rapidly to alcohol (Ki et al., 1988). Chua et al. (1984) proved that, heating of the mash for 5 min at 75 to 85°C was sufficient for getting the almost complete saccharification process.

During pretreatment of banana mash before fermentation, banana mash were treated with 0.3% pectinase with pH of 5, incubated at 40°C for 2 h and 0.3% of cellulase, incubated at 60°C for 2 h. Leng (2008) had conducted experiment and stated an optimum condition with a pectinase concentration of about 0.3% of the substrate volume, at pH 4.5 to 5 and incubated at 40°C for 2 h and got the positive result. In addition, there was no significant difference in the volume of banana extracted juice by applying the higher pectinase concentrations (0.0125%) and up to 0.1% (w/w). A maximum hydrolysis of banana mash was achieved by pectinase for 2 h (Cheirsilp and Umsakul, 2008). Studies by several researches reported that, enzyme sachharification method produced the higher yields of fruit juices and vegetables products (Sreenath et al., 1994; Czukor and Nyarady, 1999; Demir et al., 2000 and Will et al., 2000). The banana juice obtained was turbid, very viscous, grey in colour and tends to settle during storage and therefore, needs further processing such as enzyme treatment in order to produce clarified banana juice (Lee et al., 2006). Thus, in this study, parameter of different enzyme saccharification was used. After addition of enzyme, the banana mash was incubated in water bath. Hot water extraction was used to extract banana juice. This method often used to maximize juice yield, colour and flavor extraction (Mc Lellan, 1996) as heat can be used to breakdown the pulp of banana fruit. Heating in water bath also had been used as it can simultaneously inactivate enzymes in the juice (Luh and Woodroof, 1975) before addition of yeast.

The highest viscosity values were exhibited by fermented banana mash using 2 and 4 g/l of concentration of yeast. Ghobadian et al. (2008) reported that, the viscosity of pure ethanol had the lowest value (1.10 cSt). The use of the enzymes would reduce the viscosity values and facilitate in liquefying of the non-soluble polysaccharides present in the cell walls (Grassin and Fauquembergue, 1996). The dilution of the medium is necessary to reduce the osmotic pressure (Panchal et al., 1980). Elemental analysis of the bioethanol produced from fermented banana treated with different concentration of yeast, different pH treatment and using rotten and fresh banana was analyzed by using multi-element oil analyzer (MOA). The values of the element obtained were varied among different parameters. This might be due to the source of raw material because even

though the species for the banana that had been used during the experiments. The highest value was from bioethanol of fermented banana mash treated with 2 g/l of yeast and bioethanol produced from fermentation of banana mash using rotten banana fruit that had the lower value of silicon. Silicon might come from samples.

The element of zinc (Zn) had the values of 7 to 13 ppm, while calcium (Ca) had the values of 19.5 to 52 ppm and the values of magnesium (Mg) were higher, ranging form 189.5 to 264 ppm for the three different parameters; different concentration of yeast, different pH treatment and fermentation using rotten and fresh banana fruit. Zinc (Zn), ferum (Fe), magnesium (Mg) and calcium (Ca) are an additive element found in the bioethanol. These elements were not harmful though it was the higher range, while lead (Pb) of bioethanol obtained from three different parameters had no value (0 ppm). This is a good sign of bioethanol as lead can affect the engine emission.

Studies by Saint' Pierre et al. (2005) on the determination of trace elements using ET- ICP-MS (electrothermal vaporization inductively coupled plasma mass spectrometry) method got the positive results on the limitation of these elements. Another research done by Oliveira et al. (2002) using ETAAS (electrothermal atomic absorption spectrometry) method got result a bit higher than other researches. From the metal analysis, some of the element present in bioethanol shows ASTM standard, while some did not follow the standard. However, it can be used for petrol engine because of not having harmful element for the engine.

Conclusion

Banana fruit waste could be used to produce bioethanol effectively. It can be concluded that, produced bioethanol from banana biomass was of good quality and can be used in the engine for transportation purpose with producing less emission. In addition to that, it can be used as environmental recycling process for waste management.

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