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Original Article

Reducing Sugar Estimation and Bioethanol Production from Banana, Pineapple and Mango Fruit Wastes

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Abstract - *The exploration of biomass fuels encourages the reduction of world atmosphere pollution and global warming. In addition, the depletion of non-renewable energy sources such as fossil fuels induces the developing technologies to harness new and renewable energy sources. Abundant fruit waste can be reused in bioethanol production. Hence, it can reduce pollution and waste material, thus helping manage waste disposal. This study was carried out to evaluate the feasibility of using different fruit wastes (mango, banana and pineapple) to generate bioethanol through fermentation bioprocess using yeast. The highest production of bioethanol yield was found in mango waste than in pineapple and banana at the concentration of 3 g/l yeast at the temperature of 30°C. The bioethanol production increased and then decreased with the increasing fermentation time until five days of incubation. Reducing sugar content (glucose) total soluble solid (TSS) and pH values reduced after fermentation in the case of all fruit wastes. The viscosity and acid values of the bioethanol produced were within ASTM (American Society for Testing and Materials) standard specifications. Thus it can be concluded that bioethanol potentially be used as of good quality as an antiseptic and biofuel.*

Keywords - *Bioethanol, Fruit waste, Viscosity, Renewable energy.*

1. Introduction

Bio-energy 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 [1-3]. Different forms of bioenergy can be produced from a wide range of biomass sources, for example, agricultural residues, municipal waste, forest waste, fruit and vegetable waste, and kitchen waste [4].

Infrared radiation (IR) carries greenhouse gases (GHG) such as water vapor, CO_2, ozone, and methane, delaying its eventual escape into space. The atmosphere is warm by this mechanism and, in turn, emits IR radiation, with a portion of this energy could warm the surface and lower the atmosphere. As a result, the earth's temperature could maintain about 30^0C higher than it would be without atmospheric and reradiation of IR energy [5]. However, problems may arise when the atmospheric concentration of GHG increase. One of the causes of those problems is because of the burning of fossil fuels. When burnt, it could increase the net of CO_2, NO_X, SO_X etc. Energy consumption worldwide has increased 17-fold in the last century as an effect of the burning of fossil fuels, mainly used in the transportation sector, causing primary atmospheric pollution by the oxidation of CO_2.SO_2, NO_X emission [6]. Combusting fossil fuels at the current rate would contribute to the global environmental crisis [7]. The increase in demanding fossil fuels combined with the depletion of these mineral oil reserves has led to the development of eco-friendly concepts [8]. In addition, energy demand increases with the increase of the world population and urbanization [9]; thus, bioenergy development as alternative energy might help reduce these problems. Different fruit wastes have been used to produce biogas using fed-batch digestion in Australia [5,10].

In order to develop new technologies and improve the available technologies regarding biofuel production, it is essential to address the challenges and opportunities of biofuel in the context of food security and sustainable development needs [1]. It was stated [11] that ethanol production by fermentation faces competition with ethanol production from petroleum-based waste products. However, as the values of the petrochemical were increased, ethanol fermentation received more attention [12]. Since renewable materials (waste) are cheaper, there is sometimes nothing to pay for, making them easily available and more economical [13].

Biofuels benefit more since they come from renewable resources, sustainability, reduced greenhouse gas emission, regional development, social structure and agriculture and security supply [13, 9]. Pineapple waste has the potential for recycling to get valuable raw material and convert it into useful and higher value products, food or feed after biological treatment and even as raw material for other industries [9]. One example of raw material is pineapple waste, converted to bioethanol [14-15]. The objectives of this study were to investigate the influence of different temperatures, shaking hours, and enzyme concentration on bioethanol production by using rotten banana, mango and pineapple waste. In addition, to know the standard properties (viscosity and acid value) of bioethanol.

2. Review of Literature

It was stated that biofuel, produced from pineapple and date wastes, had shown the potential achievement for recycling to get valuable and higher bioproducts [9].

It was reported that fruit waste materials were used as the feedstock of bioethanols which were cheaper and sometimes nothing to pay, which is why it was easily available and more economical [13].

It was observed that the increased viscosity value in fermentation with 5 g/l using different fruit wastes was mainly due to the presence of higher glycerol in the solution [14].

Many scientists also reported that glycerol was a major byproduct of ethanol fermentation by Saccharomyces cerevisiae. Therefore, 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 sustaining glycolytic catabolism[15].

Bioethanol was produced using different fruit wastes like mango, apple and rambutan. Fruit wastes were one of the examples of the cheapest raw materials converted to bioethanol using fermentation bioprocess [17].

3. Materials and Methods

3.1. Experiment 1

The banana waste (rotten) was bought from the market, Pantai dalam, Kuala Lumpur. The enzymes used in this experiment included pectinase and cellulase. Cellulase used was bought from BioChemika with Fluka No. 22180, **an** off-white powder derived from the culture of *Aspergillus niger*. Yeast derived from the culture of *Saccharomyces cerevisiae* Type II. Only approximately 10% would autolyze in an aqueous buffer at 37°C and fast-dried to yield 90% active, viable yeast in a convenient solid form (Sigma).

3.1.1. Preparation of Samples

The 1000 g of rotten bananas were thoroughly washed with distilled water, cut using a sterile knife, and blended using a sterilized automatic juice blender. The banana mash was then dispensed into the nine sets of sterile schott bottles labeled according to each sample analysis's dates. 25 mL of water was added to the schott bottle containing banana mash. The pH of the banana mash was 5.0. After that, the total soluble solids of banana mash were taken.

3.1.2. Fermentation

The 3 g/l of yeast, *Saccharomyces cerevisiae,* was added into each set, and all of the bottles were closed to ensure they were made air-tight to provide an anaerobic condition and placed in an incubator at 30°C. The dry active yeasts were rehydrated in a water bath at 40°C using clean water and allow taking to room temperature before being added to the banana mash. Fermentation was usually carried out for 3 days (in the parameter of 0, 3 and 6 hours as well as 0, 2, 3 and 5 days). After fermentation, the clean, sterile cotton cloth was used to sieve the product from the residue. The extract was collected in nine different sterile plastic containers. The obtained raw bioethanol was then taken at room temperature to measure pH and total soluble solids (TSS). The bioethanol yield from the fermentation of the rotten banana mash by using different shaking hours, 0, 3, and 6 hours. The bioethanol yield by using a certain amount of water added to the rotten banana mash. The various water content of 15% was used. The bioethanol was yielded by using rotten and fresh banana mash.

Samples obtained from different fermentation processes were determined by the changes in pH, total soluble solids, bioethanol concentration and residue weight, viscosity and elemental analysis. The changes in the pH of all fermentations were determined (measured by pH meter model HANNA instruments). The pH was checked before and after the fermentation process. The total soluble solids content of all fermentations was determined by using Atago digital refractometer (Tokyo, Japan). Ethanol concentration was determined by an ethyl alcohol refractometer (Japan). Glucose content was determined by a digital glucose refractometer.

3.2. Experiment 2

The sample preparation was the same as Expt 1 except for raw materials. Here sample was used as pineapple waste. Fermentation was done by using the following parameters: temperatures were 28, 30 and 35^0C. Ethanol yield was determined by the ethyl alcohol refractometer (Japan) mentioned above, the same as Expt 1. Glucose content was determined by the digital glucose refractometer mentioned above, the same as Expt 1.

3.3. Experiment 3

The sample preparation was the same as Expt 1 mentioned above except for raw materials. Here sample was used as rotten mango waste. Fermentation was done by using the following parameters: 1, 3, and 5 g/l yeast concentrations. Ethanol yield was determined by an ethyl alcohol refractometer (Japan), the same as Expt 1. Glucose content was determined by a digital glucose refractometer, the same as Expt 1. Viscosity was determined by viscometer, and acid values were determined by mgKOH/g.

4. Results and Discussion

4.1. Experiment 1

The concentration of bioethanol at different shaking hours is shown in Figure 1. The fermented banana mash that was shaken for 6 hours produced higher bioethanol with 6.55% (v/v), followed by 3 hours of shaking period (6.35%) and fermentation of banana mash without shaking, which only produced 5.86% of bioethanol. The concentration of bioethanol increased as the time of the shaking process increased. Based on the data, the values of the total soluble solid for the fermented banana mash were lower than before fermentation (Table 1). The pH results showed that pH measurements before fermentation were higher than after. The pH of the fermented banana mash without shaking exhibited a lower value than those that had been shaken for 3 and 6 hours. There was a significant difference in this parameter in the concentration of bioethanol and no significant difference in pH measurement between fermented banana shake with 3 hours and 6 hours.

4.1.2. Glucose Determination

Table 2 shows glucose prepared by using a digital refractometer. There was a rapidly decreasing trend of glucose concentration from 0 to 24 hours (day 1) of the fermentation period as the glucose utilized by yeast cells to produce bioethanol. However, from 72 hours (day 3) to 120 hours (day 5), the bioethanol concentration slightly increased from 5.86 to 6.09%. The large amounts of glucose utilized at the initial stage caused rapid bioethanol production within 24 hours, producing 5.51 % of ethanol. The highest bioethanol production and the lowest glucose concentration were observed at 120 hours, where bioethanol concentration was 6.09 % while glucose concentration was 0.537 %.

4.2. Experiment 2

The bioethanol production percentages were shown at different temperatures for 28°C, 30°C and 32°C using rotten pineapple wastes fermented with yeast, *Saccharomyces cerevisia*e (Table 3). It was observed that the maximum ethanol yield production at a temperature of 30°C with 8.7%, followed by 32°C with 7.42% and at room temperature, 28°C produced 7.2% of ethanol yield, the lowest among the parameters. Hence, the yeast strain *Saccharomyces cerevisiae* performed better at 30°C than at other temperatures.

4.3. Experiment 3

According to the parameter as stated in Table 4, the results of bioethanol production from mango waste at different concentrations of yeast (1, 3 and 5 g/l) are shown in Table 3. The bioethanol production was linear to the concentration of yeast. As the increasing of concentration of yeast, a higher percentage of bioethanol yield was produced. The 1 g/l concentration of yeast produced 7.81% of ethanol yield, 3 g/l concentration produced 7.96 %, and 5 g/l concentration produced the highest ethanol yield with 8.11%. Based on the results in Table 4, the pH values for all concentrations of yeast were reduced after the fermentation. For TSS values, all concentrations of yeast showed a reduction of TSS after fermentation. Before fermentation, all yeast concentrations had the same TSS value, which was 12.8; after the fermentation, 1 g/l concentration reduced to 3.93 while 3 g/l and 5 g/l concentrations reduced to 4.

Reducing sugar content was determined by the DNS method [16] (Fig. 2), and the absorbance taken from each sample was compared to the standard sugar reduction curve to calculate the sugar content. The sugar reducing sugar concentration was evaluated from samples of fermentation of mango pulp at 30°C, pH 5 for 0, 24,72 and 120 hours of incubation. From Figure 2, the reducing sugars measured were decreased as the fermentation went on, and the ethanol produced increased. From fermentation, the sugars were utilized by yeast to produce ethanol and carbon dioxide.

Table 5 also shows the result of the viscosity and acid value test from samples fermented at different amounts of yeast. From the result obtained in Table 5, it was seen that the viscosity from bioethanol produced from the fermentation of mango pulp at the temperature of 30°C with different amounts of yeast was in the range of ASTM standards considered, which were within 1 to 5 centistroke.

From the result, the acid values measured were almost the same for all fermentation in 1, 3 and 5 g/l of yeast with an acid value of 0.40, 0.50 and 0.45 mgKOH/g of samples, respectively. The results obtained were in the best range, around 0.5 mgKOH/g and under ASTM standard specification.

From the above results, it can be discussed that all fruit waste produced a good quality of bioethanol. It was reported [17] that the ethanol concentration obtained at the range of 22.10 to 35.10 (g/L) at 25°C, 27.17 to 46.60 g/L at 30°C and 27.17 to 40.32 g/L at 32°C. It was observed [18] that ethanol concentrations of about 91.60% can get at temperatures of 27°C at pH

3.4 on pineapple juices. However, the ethanol concentration from 28°C only at 48.71%, about half of the reported by researchers [19]. The experiment at 28°C produced the lowest yield compared to the other parameters, which are 30°C and 32°C. This is because when at low temperatures, all metabolic functions' reaction rates slowed down, reducing the substrate and product diffusion rates for higher ethanol yields. However, this statement is not support experiments carried out at 30°C and 32°C where the ethanol yield obtained at 30°C was much higher than 32°C. From table 3.4, the pH values of the fermented pineapples for all samples of temperatures parameters were decreased gradually, which where the input of pH for 28°C, 30°C and 32°C was 5.55, 5.56 and 5.57, respectively but reduced to 4.30, 4.31 and 4.39 respectively. The total soluble solid (TSS) also decreased during the fermentation period; the initial TSS was 11.1 for all temperatures of 28°C, 30°C and 32°C and reduced to 3.83, 4 and 4.2, respectively. The residue for 28°C, 30°C and 32°C was 23.43 g, 23.89 g and 23.05 g. It was reported that the ethanol concentration obtained ranged from 22.10 to 35.10 (g/L) at 25°C, 27.17 to 46.60 g/L at 30°C and 27.17 to 40.32 g/L at 32°C.

This would give an indication that ethanol produced from mango was suitable as a possible biofuel substitute. As an advantage, the low viscosity value was good for the engine used and reduced the corrosion problem of the engine. The samples from fermentation at 1 g/l and 3 g/l showed a slightly increased viscosity value of 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 viscosity value in fermentation with 5 g/l was mainly due to the presence of higher glycerol in the solution. Glycerol was a major byproduct of ethanol 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 sustaining glycolytic catabolism[20-22]. So, the higher glycerol content could cause higher viscosity to the solution [23-25].

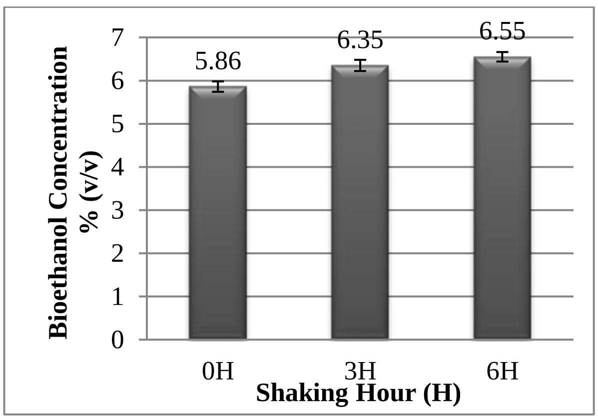

Fig. 1 Comparison of bioethanol concentration from banana mash using different shaking hours.

In addition, the ethanol produced from this experiment was not being purified or distillated. So, impure ethanol might have other components which lead to increased viscosity. It was reported that pure ethanol had less viscosity, so our results showed similar results[26-27]. However, the viscosity obtained was maintained under ASTM standards, indicating the best result for this ethanol produced.

Table 1. Effect of different shaking hour treatments on the concentration of bioethanol, total soluble solid and pH of the banana mash

Parameter (Shaking hour (H))	Total Soluble Solids (TSS) ± SD		pH ± S.D	
	Initial	After	Initial	After
0	17.00 ± 0	10.00 ± 0.20[a]	5.00 ± 0	4.03 ± 0.02[a]
3	17.00 ± 0	10.47 ± 0.31[b]	5.00 ± 0	4.25 ± 0.06[b]
6	17.00 ± 0	10.07 ± 0.12[ab]	5.00 ± 0	4.27 ± 0.09[b]

Different superscript letters in each column indicate statistically significant differences ($p < 0.05$).

Table 2. The glucose concentration of fermented pineapple waste was treated with different fermentation periods.

Days	Glucose concentration % (w/v)	Bioethanol Concentration % (v/v)
0	13.0 ± 0	0
1	3.62± 0.08	5.51 ± 0.12
3	3.28 ± 0.04	5.86 ± 0.07
5	0.53 ± 0.14	6.09 ± 0.04

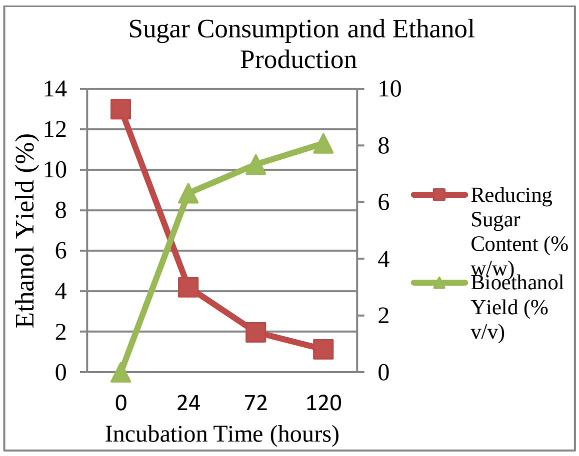

Fig. 2 Comparison of bioethanol yield and reducing sugar content from fermented mango using different shaking hours.

Table 3. Bioethanol yield, pH, total soluble solid (TSS) and glucose content in different Temperatures in mango waste. The same letters (a, a) showed no difference at the 5% significance level by the Least significant difference (LSD) test.

Tem	Bioethanol yield, (%)	pH		Total soluble solid (TSS)		Glucose (%)
		Before	After	Before	After	
28°C	7.2b	5.55a	4.30	11.1a	3.83a	3.67a
30°C	8.7a	5.56a	4.31	11.1a	4a	4.43a
32°C	7.42b	5.57a	4.39	11.1a	4.2a	3.78a

Table 4. pH, total soluble solid (TSS) and glucose content using different concentrations of yeast. The same letters (a, a) showed no difference at a 5% significance level.

Pr (g/L)	Bioethanol yield, (%)	pH		Total soluble solid (TSS)		Glucose (%)
		Before	After	Before	After	
1	7.81b	5.67a	4.74a	12.8a	3.93a	3.9a
3	7.9b	5.71a	4.69a	12.8a	4.0a	4.05a
5	8.8 a	5.58a	4.97a	2.8a	4.0a	4.13a

Table 5. Determination of viscosity and acid value.

Amount of yeast (g/l)	Viscosity value (cst)	Acid Value (mgKOH/g)
1	1.01±0.01	0.40±0.001
3	1.09±0.02	0.50±0.002
5	3.85±0.04	0.45±0.001

5. Conclusion

It can be concluded that bioethanol from bananas, pineapple and mango can be produced having good quality. The highest bioethanol was produced in the mango fruit waste. In the case of all fruit waste, reducing sugar was reduced while bioethanol production increased. Therefore, mango waste was the best feedstock regarding yield, compared to pineapple and banana waste. It can be recommended that this potential energy generation can govern the replacement of historic energy depletion.

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