COMPARISON OF BIOETHANOL YIELD PRODUCED FROM *MORINGA OLEIFERA* SEEDS HUSK REMOVED MANUALLY AND MECHANICALLY

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ABSTRACT

Moringa oleifera is a plant which benefits to mankind from its root until leaves. In this research, the seeds husk of *Moringa oleifera* were used to compare the bioethanol yield produced from seeds husk removed manually and mechanically. About 30g of *Moringa oleifera* seeds husk was used for each sample and 450 ml of water was added. Pre-treatment was started by adding NaOH up to pH of 11-12. The samples then left for 24 hours, and the pH was adjusted to 4.5, 5, and 5.5 using 0.5M H_2SO_4. The samples were located at autoclave for 2 hours at 120°C. The samples collected from autoclave are left to be cold at room temperature. Then, the *Saccharomyces cerevisae* (Baker's yeast) was added with different dose of 1g, 5 g, and 10 g in a closed conical flask at temperature of 32°C and located at shaking incubator with agitation rate of 200 rpm for 72 hours. On a 12 hour basis, the samples were collected and tested using HPLC to determine the bioethanol yield. The results showed that the concentration of bioethanol produced from *Moringa oleifera* seeds husk that is removed mechanically is higher than that removed manually with a concentration of 15.5058 gm/L and 13.4365 gm/L, respectively. As a conclusion, both method results the best yields at same fermentation time of 12 hours. Therefore, it can be concluded that both methods are good in producing bioethanol with the same concentration, and it is better to use the mechanical method because the manual method is very tough and time consuming which make it not economical.

Key words: *Moringa oleifera*, seeds husk, fermentation, bioethanol, hydrolysis.

Introduction:

In view of continuously rising petroleum costs and dependence upon fossil fuel resources, considerable attention has been focused on alternative energy resources. Production of ethanol or ethyl alcohol (CH_3CH_2OH) from biomass is one way to reduce both, the consumption of crude oil and environmental pollution (Lang et al., 2001). Primary consideration involves the production of ethyl alcohol from renewable resources and determination of the economic and technical feasibility of using alcohol as an automotive fuel blended with gasoline (Goldstein, 1981). Ethanol represents an important, renewable liquid fuel for motor vehicles. The use of gasohol (ethanol and gasoline mixture) as an alternative motor fuel has been steadily increasing around the world for a number of reasons. Domestic production and use of ethanol for fuel can decrease dependence on foreign oil, reduce trade deficits, create jobs in rural areas, reduce air

pollution, and reduce global climate change carbon dioxide buildup. Ethanol, unlike gasoline, is an oxygenated fuel that contains 35% oxygen, which reduces particulate and NOx emissions from combustion. Ethanol is the most widely used liquid biofuel. It is an alcohol and is fermented from sugars, starches or from cellulosic biomass. It is used as a renewable energy fuel source as well as for manufacture of cosmetics, pharmaceuticals and also for the production of alcoholic beverages. Ethyl alcohol is not only the oldest synthetic organic chemical used by man, but it is also one of the most important (Taherzadeh, 1999). Bioethanol is made from biomass source and it is categorized as a domestic liquid fuel product that is harmless to the environment and human being. Production of ethanol can be made as long as the raw materials of biomass contain sugar like cellulose or starch that can be converted into simpler molecules of sugar. Traditional biomass is estimated to contribute around 11% of the world's energy demand (Abbas and Assumable, 2010). Globally, there are an interest on utilization and specifications of bioethanol energy sources that urged the studies and researches on its costs and efficiencies in terms of industrial processing or a pilot plants (Tanaka, 2006).

The top ten bioethanol producers in the world are: USA, Brazil, Europe, China, Canada, Thailand, Argentina, India, and rest of the world with a production of 14,300, 6,190, 1,445, 635, 510, 310, 160, 155, 865 million of gallons/year, respectively (Renewable Fuels Association, RFA, 2014).

This research was carried out to find a new alternative energy sources (bioethanol) from biomass. The main purpose of this experiment is to compare the bioethanol yield from *Moringa oleifera* seeds husk removed manually and mechanically from seeds.

MATERIALS AND METHOD

Materials

The chemicals used in this experiment are H_2SO_4 and NaOH, which is obtained from Universiti Malaysia Pahang labs. The yeast *Saccharomyces cerevisiae* was bought from supermarket, and *Moringa oleifera* seeds husk was obtained from Kuantan area (the husk was removed manually), and the mechanically removed seeds husk was obtained from Mitomasa Sdn. Bhd., Kuala Lumpur.

Sample preparation

The *Moringa oleifera* seeds husk was dried in the oven for about 24 hours at temperature 80°C to remove the moisture. The seeds husk is then cut into small pieces.

Pre- treatment

Nine samples of 30g each is prepared from of *Moringa oleifera* seeds husk and placed in conical flask and 450 ml of water. Pre-treatment was started by adding NaOH until pH reached 11-12 to have maximum extraction of cellulose which would increase ethanol production. The samples then left for 24 hours, and the pH was adjusted to 4.5, 5, and 5.5 for a set of three samples each by using 0.5M H_2SO_4. The flasks then covered and wrapped in aluminium. The flasks then covered and wrapped with aluminium foil in order to avoid side reactions and samples were located at autoclave for 2 hours at 120°C.

Fermentation

The samples collected from autoclave are left at lab until the temperature is cooled down. Then, the *Saccharomyces cerevisae* (Baker's yeast) was added with different dose of 1g, 5 g, and 10 g in a closed conical flask at temperature of 32°C with agitation rate 200 rpm using shaking incubator. On a 12 hour basis, the samples were collected and tested using HPLC to determine the bioethanol yield.

RESULTS AND DISCUSSION

The concentration of ethanol was obtained using high performance liquid chromatography (HPLC) by using REDEX-ROA column with 0.005 N sulphuric acid as mobile phase.
The mobile phase used was 0.005N of sulphuric acid and used ethanol with different concentration as standard solution such as 5mL/L, 10mL/L, 15mL/L 20mL/L and 25mL/L. the results of ethanol standards are presented in Table 1. And the calibration curve is drawn in Figure 1, with R^2 equal to 0.9984.

Table 1: Ethanol standard

Concentration (ml/L)	Area {(nRIU), $\times 10^5$}
5	3.35
10	6.37
15	9.83
20	12.4
25	15.8

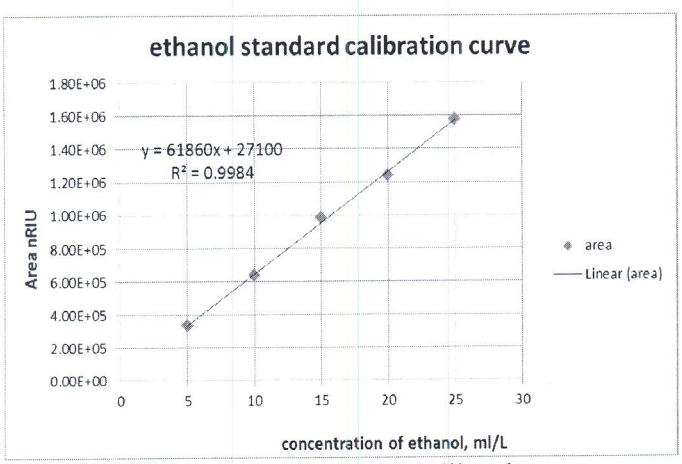

Figure1: Ethanol standard's calibration curve

1. Manually removed *Moringa oleifera* seeds husk

The samples of 2 mL were filtered using membrane filter with size of 0.02 μm and applying vacuum to get pure sample before injecting to HPLC. After filtration, the samples were distillate using rotary evaporator to separate the water and ethanol.

The results are shown in Table 2 for the manually removed *Moringa oleifera* seeds husk.

Table 2: Ethanol yield for the manually removed *Moringa oleifera* seeds husk at different time

Time (hours)	pH	Mass of yeast (g)	Concentration (g/L)
12	4.5	1g	0.633689
24	4.5	1g	0.419512
36	4.5	1g	0.430569
48	4.5	1g	0.0
60	4.5	1g	0.0
72	4.5	1g	0.0

According to the results from Table 2, which shows the best yield obtained was at 12 hours fermentation time. Therefore, it is better to choose this time for comparison with other parameters involved in this study which is the pH value and the mass of yeast. The results are shown in Table 3.

Table 3: Ethanol yield with different pH and mass of yeast at 12 hours of fermentation time.

Time (hours)	pH	Mass of yeast (g)	Concentration (g/L)
12	4.5	1	0.633689
12	4.5	5	12.22795
12	4.5	10	13.43652
12	5.0	1	0.548416
12	5.0	5	0.600307
12	5.0	10	1.006903
12	5.5	1	0.000000
12	5.5	5	0.516602
12	5.5	10	1.048610

The results show that the best yield is 12.23 g/L when the pH was 4.5 and yeast used is 5 g with fermentation time of 12 hours.

2. Mechanically removed *Moringa oleifera* seeds husk

According to the results obtained for manually removed *Moringa oleifera* seeds husk at different parameters. The results shown in Table 3, shows that the best yield was obtained at pH 5, 10g yeast, and fermentation time of 12 hours for mechanically removed *Moringa oleifera* seeds husk.

Table 3: Ethanol yield with different pH and mass of yeast at 12 hours of fermentation time.

Time (hours)	pH	Mass of yeast (g)	Concentration (g/L)
12	4.5	1	0.000000
12	4.5	5	0.457759
12	4.5	10	0.740780
12	5.0	1	0.000000
12	5.0	5	7.758164
12	5.0	10	15.50580
12	5.5	1	0.000000
12	5.5	5	0.449046
12	5.5	10	0.752667

CONCLUSION

It was observed in this study that bioethanol yield produced from *Moringa oleifera* seeds husk removed manually and mechanically methods are giving little difference in yield of (13.436518) using 10g/L of yeast and pH 4.5 for manually removed husk and (15.50580g/L) by adding 10g/L yeast at pH 5. Both method results the best yields at same fermentation time of 12 hours. Therefore, it can be concluded that both methods are good in producing bioethanol with the same concentration, and it is better to use the mechanical method because the manual method is very tough and time consuming which make it not economical.

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