

Possibility of Producing Ethanol from *Moringa Oleifera* Pod Husk

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Abstract – *Moringa oleifera* is a plant with various benefits to mankind from its root until leaves. From food to biofuel applications, all parts are useful for daily uses. In this research, the pod husk was examined to determine the possibility of producing ethanol. The pod husks were dried and used in two forms; one is grinded into powder, and the other is cut within 5 x 5 x 2 mm. About 10 grams of *Moringa oleifera* pod husk was put into a conical flask and added with distilled water up to 250 mL. The pre-treatment was made by adding an alkaline solution, NaOH, where the pH of the sample was adjusted to (4.5, 5.0, and 5.5) using H₂SO₄. The samples were autoclaved at a temperature of 120°C for 2 hours before the samples were cooled to room temperature (25±/2°C). Baker's yeast (*Saccharomyces cerevisiae*) was prepared with different concentrations (1g, 5g, and 10g) and added to the samples for fermentation process that took place in the incubator shaker at a temperature of 36°C, for a period of 72 hours. The bioethanol concentration was measured using High Performance Liquid Chromatography (HPLC) with a refractive index detector and REZEX ROA-Organic Acid HPLC Column using 0.05 N H₂SO₄ as the mobile phase. The bioethanol produced from *Moringa oleifera* pod husk was 8.400 g/L using 1g/L yeast, and the fermentation took place at pH 4.5 for the sample size of 5 x 5 x 2 mm. The results showed that *Moringa oleifera* pod husk can be introduced as a new material for bioethanol production in Malaysia and other tropical countries where this tree is available. Copyright © 2015 Penerbit Akademia Baru - All rights reserved.

Keywords: Biomass; Bioethanol, *Moringa oleifera* pod husks, *Saccharomyces cerevisiae*, fermentation.

1.0 INTRODUCTION

The need for fossil fuels is increasing rapidly whereas the fuel resources are depleting. Therefore, finding alternatives for fossil fuels is of great importance. Many researchers have worked on finding energy alternatives, in which some of these are biofuels such as biodiesel and bioethanol. Bioethanol can be produced from lignocellulosic biomass.

Ethanol production by fermentation of sugars has been practiced since the old days. Nowadays, all beverage ethanol and most industrial ethanol are still using this conventional method. Zymase, an enzyme from yeast, turns raw material i.e. simple sugars into ethanol and carbon dioxide. This fermentation reaction process can be represented with a simple equation:



Bioethanol is made from biomass of plants and it is categorized as a domestic liquid fuel product that is harmless to the environment. 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 a simpler molecule of sugar.

Production of ethanol from various source of materials have been developed gradually from time to time in order to support and replace the production of conventional fuels like diesel and petroleum. Besides that, ethanol has its own characteristics, which make them feasible and marketable, nowadays.



Figure 1: *Moringa oleifera* pods



Figure 2: *Moringa oleifera* tree

Many studies have been published on bioethanol production from crops such as: wheat [1] and corn [2]. Other biomass that are available and abundant can also become raw materials for bioethanol production; for example, microalga *Chlorella vulgaris* [3], oil palm fronds [4-5], *Yarrowia lipolytica* Po1g biomass [6], *Gracilaria* biomass [7], empty fruit bunches of oil palms [8-9], oilseed rape straw [10-11], wheat straw (*Triticum vulgare*), [12-13], bamboo [14-15], *Posidonia oceanica* residues (which can cause pollution) [16], macrophytic green alga *Ulva fasciata* Delile [17], Potato peel [18], waste papers [19], pine wood chips [20], wild cassava (non-edible) *Manihot glaziovii* [21], rye straw (*Secale cereale*), oat straw (*Avena sativa*) and corn stover (*Zea mays*) [13].

In addition, some plant husks can also be used to produce bioethanol such as coconut husk [22-23], rice husk [24] and for this research work, *Moringa oleifera* pod husk (Fig. 1) from the *Moringa oleifera* tree (Fig. 2) will be introduced as a new raw material to produce bioethanol. The *Saccharomyces cerevisiae* was used as fermentation agent.

2.0 METHODOLOGY

Generally, the composition of lignocellulosic biomass feedstock consists of cellulose, hemicelluloses, lignin and ash as shown in Fig. 3.

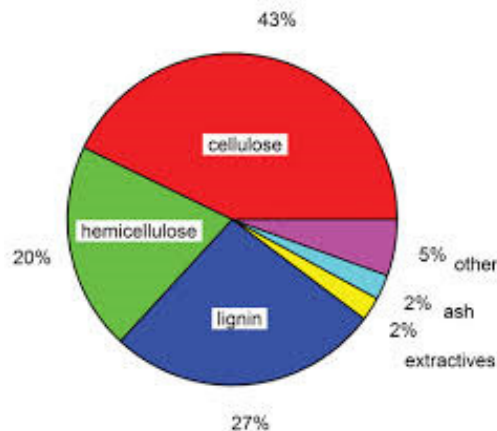


Figure 3: Composition of lignocellulosic biomass

2.1 Preparation of *Moringa oleifera* pod husk

Good quality *Moringa oleifera* pods were selected from Gambang, Pahang, Malaysia. The seeds were removed from the pods and the collected husks were allowed to completely dry. The pod husks were used with two sizes; half is grinded using a domestic blender (National, MX-896TM) to turn into powder, and the other half is cut into 5 x 5 x 2 mm.

2.2 Pre-treatment of sample

Pre-treatment helps in breaking the crystalline structure of the lignocellulose by removing the lignin and exposing the cellulose and hemicellulose molecules, which facilitate the hydrolysis of the cellulose. The next step is hydrolysis, which can be affected by the porosity of lignocellulosic biomass, cellulose fiber crystallinity, lignin and hemicellulose contents [25]. Depending on the biomass material, either physical or chemical pre-treatment methods may be used. Chemical pre-treatment of cellulosic materials is done by using chemicals such as dilute acid, alkali, organic solvent, ammonia, sulphur dioxide, carbon dioxide or other chemicals to make the biomass more digestible by the enzymes. It is required to change the cellulosic biomass structure so that the enzymes can access the cellulose more easily [26], as shown in Fig. 4 [25].

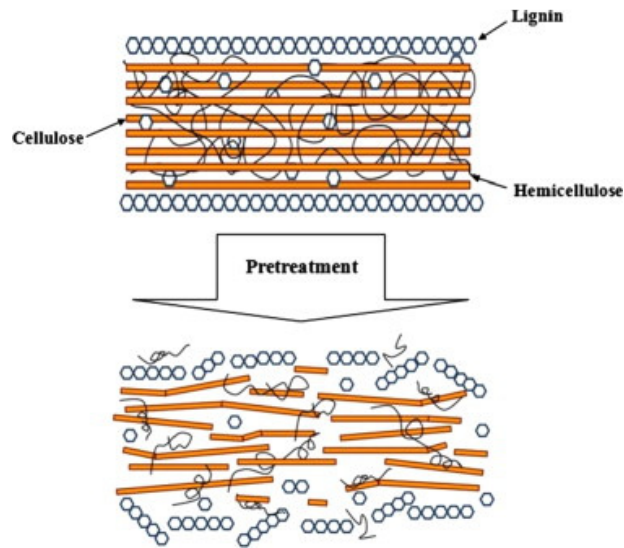


Figure 4: Pre-treatment of biomass

About 10 grams of powdered and large size *Moringa oleifera* pod husks were put into a conical flask, where distilled water was added up to 250 ml. The pre-treatment was made by adding 2M of sodium hydroxide (NaOH) alkaline solution until the pH was between 11 and 12 for maximum extraction of cellulose, which would influence the production of ethanol. The flasks were covered and wrapped with aluminum foil as shown in Fig. 5 to ensure that no side reactions occur and was left for 24 hours.

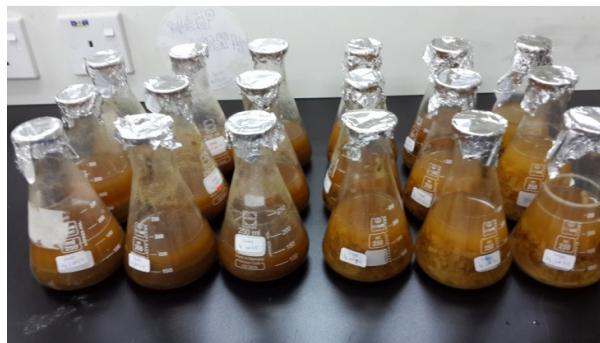


Figure 5: Samples ready for autoclave

2.3 Hydrolysis

Hydrolysis includes the processing step that converts the carbohydrate polymers into monomeric sugars [26]. The samples were autoclaved at a temperature of 120°C for 2 hours [23]. After the autoclave process, the samples were cooled down until approximately of room temperature (25+/-2°C) before adding *Saccharomyces cerevisiae* (baker's yeast). The yeast was prepared by mixing the yeast powder with warm water to enhance the growth of yeast before it undergoes the fermentation process [23]. Three doses of 1g, 5g and 10g yeast were used. The pH of the samples was adjusted to 4.5, 5 and 5.5 by adding H₂SO₄, before adding the yeast.

2.4 Fermentation

The fermentation of the samples took place in the incubator shaker at an agitation rate of 150 rpm with temperature of 36°C and fermentation time of 72 hours. The samples were collected for analysis to measure ethanol concentration at 72 hours by using a syringe and then put into vials that were later stored in the chiller. The fermentation process was done for the different size samples, pH values and yeast concentrations following the same procedure with a fixed temperature of 36°C and time of 72 hours.

2.5 Analysis of Ethanol

The samples taken from each flask was kept in vials and stored in the chiller to ensure no other reactions occurred. The chiller is set at 2°C. The samples were then filtered using a 0.22µm Nylon filter to ensure no impurities contaminated the samples.

High Performance Liquid Chromatography (HPLC) was used for analysis (Model: Agilent 1200 Series), together with Refractive Index Detector and REZEX ROA-organic acid HPLC column. Other conditions that needed to be considered were 0.5 mL/min flow rate, temperature of 60°C, and injection volume of 10µL.

The solvent for the mobile phase (0.05 N sulphuric acid, H₂SO₄) and the ethanol standard for analysis and samples were filtered using 0.45µm vacuum filter, which have all gone through a degasifying process before being analyzed.

For the analysis, standard ethanol was used to find the concentration of the bioethanol produced in this study. Five dilutions from the stock ethanol standard solution were prepared to draw the calibration curve.

In order to get the exact concentration of produced ethanol, the standard is prepared by diluting 99.4 % of ethanol stock solution with water for five different concentrations of 2, 4, 6, 8, and 10 ml/L. The analysis of the multi dilutions from the standard ethanol solution creates a standard calibration curve, which is used as a reference to calculate the concentration of ethanol content in the samples, according to HPLC response. The data for standard calibration is shown in Fig. 6.

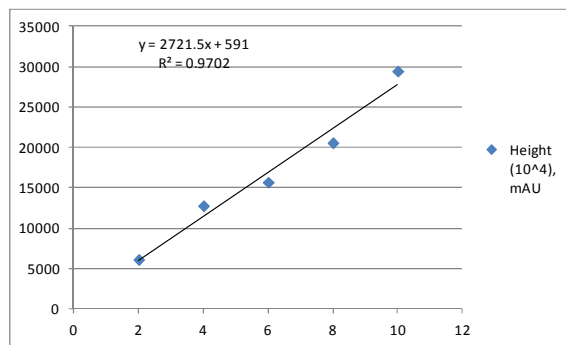


Figure 6: Calibration curve for ethanol standards

The samples were diluted 10 times before being injected into HPLC. From the calibration curve, it shows that $y=2721.5x+591$; where y is the peak height X 104 mAU; x is the volume of ethanol in 250 mL sample and $R^2=0.9702$. To convert the unit of ethanol produced to g/L, the x value needs to be multiplied by the dilution factor (10) and density of ethanol (0.789 g/L). The result summary for all the parameters is tabulated in Table 1.

Table 1: Summary of ethanol concentration

pH	Crushed sample			Powdered sample		
	Yeast concentration					
	1	5	10	1	5	10
4.5	8.400	8.311	8.110	7.890	7.802	7.678
5.0	8.334	8.034	8.030	7.840	7.756	7.691
5.5	8.264	8.145	8.010	7.810	7.755	7.654

3.0 RESULTS AND DISCUSSION

3.1 Effect of the sample size

One of the parameters studied in this research is the size of the sample [23]. Two different sizes of sample were prepared which are the powdered sample and 5 x 5 x 2 mm sample. As shown in Table 1, the bioethanol produced from the powdered sample is less than that produced from larger sized pod husks. Grinding might have affected the structure of lignocellulos thus reducing its porosity, which is important to allow the enzymes to access into the biomass during the fermentation process. Therefore, it is better not to grind the husk but to crush it instead into a large size.

3.2 Effect of pH value of the sample

One of the parameters that might affect the fermentation process is the pH value of the sample [5]. The pH value chosen for this study were 4.5, 5, and 5.5. The bioethanol produced at these pH values showed that as the pH increases, the bioethanol yield will decrease for both sample sizes.

3.3 Effect of yeast concentration

This research has considered increasing the yeast concentration of the sample for fermentation process. Using 1, 5, and 10g/L of yeast concentration showed that when more yeast is added, it will produce less bioethanol as shown in Table 1.

4.0 CONCLUSION

This preliminary study using *Moringa oleifera* pod husks revealed that it can be introduced as a new raw material for bioethanol production. The best results of the produced bioethanol is 8.400 g/L, obtained at pH 4.5, yeast concentration of 1g/L, and sample size of 5 x 5 x 2 mm. Since the *Moringa oleifera* pod husks can be considered as a zero value waste, more research should be focused towards it to study the optimum conditions for bioethanol production and the different pre-treatment methods. It can replace corn and wheat whereby both have food values. There is no need to grind the husk, by which this is considered to be an economical advantage of the process. It will become a good economical resource for any developing tropical countries, in which this tree can be planted and grown easily.

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