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Possibility of Producing Ethanol from *Moringa Oleifera* Pod Husk

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Abstract – *Moringa oleifera is a plant which benefits to mankind from its root until leaves. From food to biofuel, all parts are useful to human being's daily life. In this research, the pod husk was examined to determine the possibility of producing ethanol. The pod husk were dried and used with two sizes; one is grinded to get powder, and the other is cut within 5 x 5 x 2 mm. About 10 grams of Moringa oleifera pod husk was put into conical flask and distilled water was added up to 250 mL. The pre-treatment was made by adding an alkaline solution of NaOH, the pH of the sample was adjusted to (4.5, 5.0, and 5.5) using H2SO4. The samples were autoclaved at temperature of 120oC for 2 hours then, the samples were cooled to room temperature (25+/-2oC). Baker's yeast (Saccharomyces cerevisae) was prepared with different concentrations of (1g, 5g, and 10g) and added to the samples for fermentation process which took place in the incubator shaker at temperature of 36oC, and for a period of 72 hours. The bioethanol concentration was measured using High Performance Liquid Chromatography (HPLC) with 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 ferementartion 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.*

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

1.0 INTRODUCTION

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

Ethanol production by fermentation of sugars has been created since the old days. Nowadays, all beverage ethanol and most of the industrial ethanol is still using the conventional and traditional method. Zymase, an enzyme from yeast, turns the raw materials which is simple sugars into ethanol and carbon dioxide. This fermentation reaction process can be represented by the simple equation:

$$C_6H_{12}O_6 \rightarrow 2CH_3CH_2OH + 2CO_2$$

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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 made them feasible and marketable nowadays.

Figure 1: *Moringa oleifera* pods

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Figure 2: *Moringa oleifera* tree

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Many studies have been published for producing bioethanol from crops such as: wheat [1], corn [2]. Some other biomass which is available and abundant can be the raw materials for bioethanol production, and as an example of this biomass material is; microalga Chlorella vulgaris [3], oil palm fronds [4-5], Yarrowia lipolytica Po1g biomass [6], Gracilaria biomass [7], empty fruit bunches of oil palm [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 cereal), oat straw (Avena sativa) and corn stover (Zea mays) [13].

In addition, some plant husk can 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 Moringa oleifera tree (Fig. 2) will be introduced as a new raw material to produce bioethanol. The Saccharomyces cerevisae was used as fermentation agent.

2.0 METHODOLOGY

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

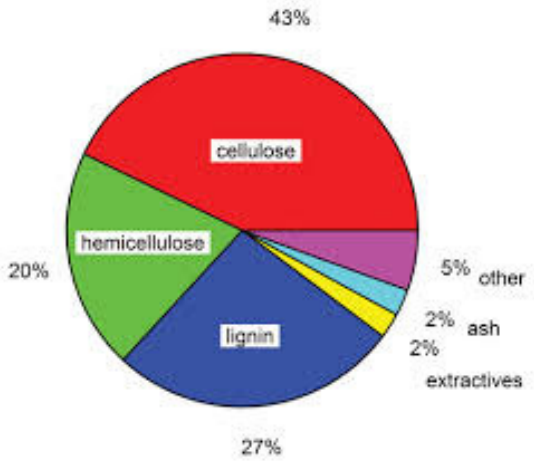

Figure 3: Composition of lingocellulosic biomass

2.1 Preparation of Moringa oleifera pod husk

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

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2.2 Pre-treatment of sample

Pre-treatment deals with breaking the crystalline structure of the lignocellulose and removes the lignin to expose the cellulose and hemicellulose molecules to facilitate the cellulose hydrolysis. The next step is hydrolysis, which can be affected by porosity of lignocellulosic biomass, cellulose fiber crystallinity, lignin and hemicellulose content [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 to cellulose easily [26], as shown in Fig. 4 [25].

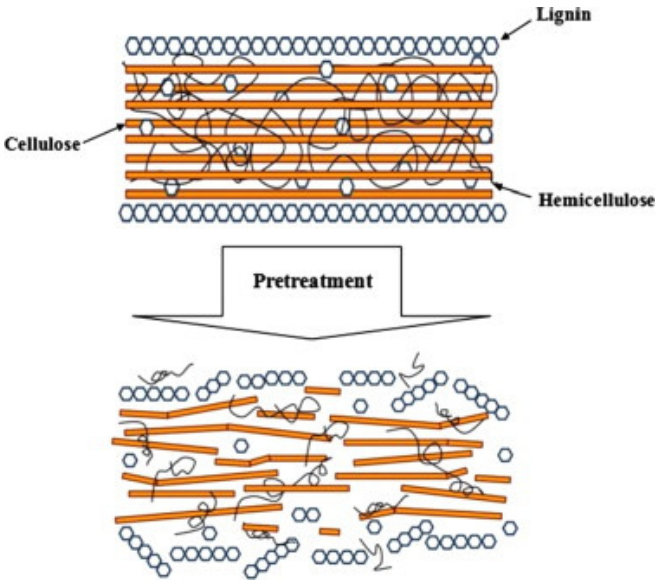

Figure 4: Pretreatment of biomass

About 10 grams of powdered and large size *Moringa oleifera* pod husk was put into conical flask and distilled water was added up to 250 ml. The pre-treatment was made by adding an alkaline solution of 2 M of sodium hydroxide (NaOH) until the pH was between 11 and 12 in order to have 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 left for 24 hours.

Figure 5: Samples ready for autoclave

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2.3 Hydrolysis

Hydrolysis includes the processing step that converts the carbohydrate polymers into monomeric sugars [26]. The samples were autoclaved at temperature of 120°C for 2 hours [23]. After the autoclave process, the samples were cooled down until it is almost the same with room temperature (25+/-2°C) before adding *Saccharomyces cerevisae* (baker's yeast). The yeast was prepared by mixing the yeast powder with warm water in order to enhance the growth of yeast before undergoes fermentation process [23]. Three doses used were 1g, 5g, and 10g. The pH of the samples was adjusted before adding the yeast with 4.5, 5, and 5.5 by adding H_2SO_4.

2.4 Fermentation

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

2.5 Analysis of Ethanol

Sample taken from each flask by syring was kept in vial and stored in chiller to ensure no other reactions occur. The chiller is set at 2°C. Samples then filtered using 0.22µm Nylon filter to ensure the sample with have no more impurities.

High Performance Liquid Chromatography (HPLC) was used for analysis (model Agilent 1200 Series), with Refractive Index Detector, and REZEX ROA-organic acid HPLC column. Other conditions need to be considered are; 0.5 mL/min flow rate, temperature of 60ºC, and injection volume of 10µl.

The solvent for mobile phase (0.05 N sulphuric Acid, H_2SO_4), and the ethanol standard for analysis, and samples were filtered using 0.45µm vacuum filter, and all gone through degasifying process before being analyzed.

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

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

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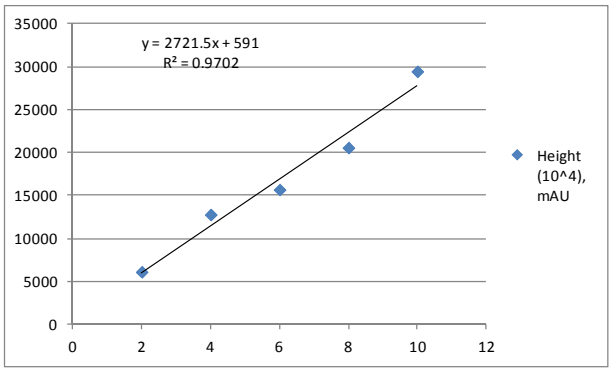

Figure 6: Calibration curve for ethanol standards

The samples were diluted 10 times before injecting to HPLC. From calibration curve, it is shown that y=2721.5 x+ 591, where: y, is the peak height X 104 mAU, x, is the volume of ethanol in 250 mL sample and R2= 0.9702. To convert the unit of ethanol produced to g/L, need to multiply the x value by dilution factor (10) and density of ethanol (0.789 g/L). The results summary for all parameters is tabulated at Table 1.

Table 1: Summary of ethanol concentration

	Crushed sample			Powder sample		
	Yeast concentration					
pH	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 Sample Size

One of the parameters studied in this research is the size of sample [23]. Two different sizes of sample are prepared which are the powdered sample and 5 x 5 x 2 mm sample. As shown in Table 1, that the bioethanol produced from powdered sample is less than that produced from larger size pod husks. Grinding might affect the structure of lingocellulos and reduces the porosity which is important for good access of enzymes to the biomass during fermentation process. Therefore, it is better not to grind the husk and just crush it to larger size.

3.2 Effect on pH value of sample

One of the parameters that might affect the fermentation process is the pH value of sample [5]. The pH value chosen for this study were 4.5, 5, and 5.5. The bioethanol produced at these pH values shown that the as pH increase the bioethaol yield will be decreased for both size of sample.

3.3 Effect of yeast concentration

The yeast concentration added on the sample for fermentation process was considered in this research work. Using 1, 5, and 10g/L of yeast concentration showed that the more yeast added will produce less bioethaol as shown in Table 1.

4.0 CONCLUSSION

This preliminary study for using Moringa oleifera pod husks revealed that it can be introduced as a new raw material for bioethanol production. The best results of bioethanol produced is 8.400 g/L obtained at pH 4.5, yeast concentration of 1g/L, and size of sample 5 x 5 x 2 mm. Since the Moringa oleifera pod husks considered as a zero value waste, it can be considered for more research to study the optimum conditions for bioethanol production and different pretreatment methods. It can replace corn and wheat which both has food value. There is no need to grind the husk and this is considered as an additional economical value in the process. It will be a good economical resource for developing tropical countries where this tree can plant and grow easily.

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