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Exploratory Studies on the Technical Viability of Bioethanol Production from Fermented Sago (Metroxylon sagu) Starch

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Abstract: ***This study was conducted to assess the viability of production of bioethanol from Metroxylon sagu Rottb. The experimental setup included different levels of yeast and molasses in the substrate. There were nine treatments examined in this experiment. The YIM1 (50g yeast, 150g molasses), Y2M1 (100g yeast, 150g molasses), Y3M1 (150g yeast, 150g molasses), Y1M2 (50g yeast, 200g of molasses), Y2M2 (100g yeast, 200g molasses), Y3M2 (150g yeast, 200g molasses), Y1M3 (50g yeast, 250g molasses), Y2M3 (100g yeast, 250g molasses), Y3M3 (150g yeast, 250g molasses). Experiment was monitored by their brix, temperature and time of fermentation. The results shows that higher the brix measured, has high yield of ethanol produced, among the treatments Y2M3 (100g yeast, 250g of molasses) gained higher yield of ethanol with 40.33% produced during first distillation. On the other hand, the lowest yield was Y3M1 (150g yeast, 150g molasses) with 17% gained of distillate from the mixture and has longer fermentation period. Using Analysis of Variance, RCBD, the level of significance was determined.***

Keywords: Sago Starch, Bioethanol, Fermentation, Alcohol Distillation

1. INTRODUCTION:

The research on development of renewable and sustainable fuels has gained traction in recent years due to the issue on the shortage of petrochemical fossil fuels and environment pollution. Furthermore, it has an increasing demand as a legally mandated additive to commercial gasoline. Bioethanol is obtained from biomass and bioenergy crops and have been assigned as a feasible alternative to gasoline fuel [1]. Brazil is a perfect example of this technology wherein huge numbers of automobiles use either Gasohol (76% gasoline and 24% ethanol) or pure ethanol. In the Philippines, gasoline contains at least 10% ethanol as mandated by the Biofuels Act of 2007. Ethanol replaces lead as the main octane-enhancing additive in gasoline [2].

Ethanol is commonly sourced from crops such as sugarcane, corn, and sweet sorghum. However, the food versus fuel paradox raises concern about maintaining a balance between the of bioethanol and food staples. Hence, there is a clear need to explore nonconventional

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sources of bioethanol. Sago palm is a largely untapped bioethanol source that pose huge potential for commercial production. The palm is by far the most important economics species and is now grown commercially in Malaysia, Indonesia, Philippines, and New Guinea to produce sago starch and/or conversion to animal food or fuel ethanol [3].

Ethanol production from sago starch (raw or hydrolyzed) has been proven to be feasible as substrates for production of ethanol for biofuel [4]. Starch is a polysaccharide composed of the chains of glucose residues bound with a glycosidic linkage, but the structures formed are spatial in character. Sago starch was utilized as an alternative substrate for glucose production, which will be used as feed stock for bioethanol production. Sago pith is a starchy lignocellulosic by-product generated from pith of sago palm after starch extraction process. Sago Pith contains 58% starch, 23% cellulose, 9.2% hemicellulose, and 4% lignin [5]. There are several challenges in utilizing sago starch from pith for the extraction of glucose and subsequent alcohol fermentation. Sago starch contains more amylopectin than amylose making it difficult to dissolve in water. Hence, pretreatment is necessary to break the complex molecular bonds in starch and allow it to be digested anaerobically by yeast during fermentation. The hydrolysis of hemicellulose decomposed hexose sugar units to produce simpler compounds of ß-Dglucose and D-galactose. This is achieved through gelatinization that opens the structure of lignocellulose so that the cellulose can be hydrolyzed.

This research investigated the technical viability of extracting bioethanol from sago pith starch using heat treatment hydrolysis and anaerobic yeast fermentation. This is an exploratory study that tackles the possibility of coming up with an upscaled process for bioethanol production from sago. Hence, the research team utilized commercially available substrates and microorganisms to mimic industrial applications.

2. METHODOLOGY

Preliminary experiment on yeast selection

The study compared two strains of commercial Saccharomyces cerevisiae to determine which produced higher alcohol yields. Yeast A is an active yeast, while Yeast B is an instant yeast. The active yeast is activated by mixing it with water at 30-40℃ prior to inoculation in the fermentation bottle, while the instant yeast is added directly to the fermentation bottle. The experimental set-up for the yeast selection preliminary experiment is shown in Table 1. The results of the preliminary experiment showed that active yeast yielded higher alcohol content. Hence, it was chosen as the fermenting microbe in the main experiment.

Table 1. Experimental set-up for yeast selection

SET-UP A ACTIVE YEAST	SET-UP B INSTANT YEAST
50 g Active yeast	50g Instant yeast
150g Hydrolyzed Starch	150g Hydrolyzed Starch
150g Molasses	150g Molasses
1.5L of Distilled Water	1.5L of Distilled Water

Starch hydrolysis and fermentation

Sago starch was hydrolyzed according to the method conducted Azlin et al. [6]. A total of 150g starch was mixed with 500 mL water and subjected to heat hydrolysis using an electric

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stove for 30 minutes. The starch is heated and mixed until it became gelatinized. Fermentation is conducted after the starch hydrolysis. The total soluble solids (TSS) brix prior and after fermentation were measured with a handheld refractometer as a basis for representing the fermentable sugars contained in the samples after the hydrolysis.

The fermentation set-up contained 150g hydrolyzed sago starch, 1.5L distilled water and varying concentrations of active yeast (50g, 100g and 150g) and molasses (150g, 200g, 250g). The molasses was added as a substrate that would facilitate the growth of yeast since it is composed of simple sugars in glucose. The distilled water added in the fermentation set-up was preheated to 40℃ to provide a suitable environment for microbial growth. The temperature inside the fermentation bottle was monitored every 30 minutes. The fermentation was concluded after the bubbling inside the fermentation bottle stopped.

Distillation

The distillation was done using a simple distiller's pot heated using an electric stove. The temperature of the distilling column is cooled down using a DC pump that conveys water in the jacket to maintain the temperature under 80℃. The distillation apparatus is equipped with thermoregulator that controls the function of the pump. The ethanol concentration of the distillate is measured using an alcohol hydrometer.

Experimental Design

The experiment was laid out in a two-factor Randomized Complete Block Design (RCBD) with yeast and molasses as the independent variables. A total of nine treatment combination were used in this study as shown in Table 2. The Statistical Tools for Agricultural Research software to conduct the Analysis of Variance (ANOVA) and determine the Least Significant Difference between treatment means.

Table 2. Two-factor treatment combinations used in the study

Factor A(Yeast Weight)	Factor B (Molasses Weight)		
	M1 (150g)	M2 (200g)	M3 (250g)
Yeast 1 (50g)	Y1M1	Y1M2	Y1M3
Yeast 2 (100g)	Y2M1	Y2M2	Y2M3
Yeast 3 (150 g)	Y3M1	Y3M2	Y3M3

3. RESULTS AND DISCUSSION

Fermentation of sago starch

Several factors affect the efficiency and rate of conversion of glucose into ethanol during anaerobic fermentation. The rate of ethanol conversion is dependent on the concentration of the substrate [7]. This concept is an important factor in the decision to add different concentrations of molasses to facilitate the growth of yeast in preparation for alcohol conversion of the sago starch. The glycosidic linkage that bounds the glucose chains in starch makes it difficult to digest for ordinary yeast [8]. This was addressed using heat treatment and adding the molasses to allow the population of yeast to multiply fast. The importance of heat hydrolysis cannot be understated. Preliminary experiments conducted involved comparing the

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alcohol conversion between heat treated and non-heat treated sago starch substrates. There preliminary experiments showed that heat treatment hydrolysed and decomposed the polysaccharide bonds in the sago starch and allowed the yeast to digest and convert the simple sugars into ethanol. Hence, the final protocol of this study involved the heat hydrolysis prior to fermentation of the substrates.

The data in Figure 1 shows that the highest rate of sugar conversion was observed in the Y2M3 (100g yeast, 250g molasses) treatment. Generally, treatments with higher substrate concentrations also yield higher sugar conversion. The addition of molasses provided for a substrate that will support the exponential growth phase of the yeast. Since sago starch is composed of complex sugar bonds, it would be difficult for the inoculated yeast to consume it rapidly during the exponential growth phase. The glucose content in molasses present the readily available substrate for yeast digestion and stimulated the rapid microbial growth in the medium.

Figure 1. Sugar Conversion at varying concentrations of yeast and molasses

Table 3 summarizes the fermentation time of sago starch across varying concentrations of yeast and molasses substrate. The data shows that Y3 (150g of Yeast) has longest fermentation time, while treatments with Y1 (50g of Yeast) produced the shortest fermentation time. Comparison of treatment means using Least Significant Difference showed that the fermentation time of treatments with Y3 is significantly different than Y1. Thus, the amount of yeast inoculated in the substrate enhances the fermentation time. The results agreed with the findings of Subashini [2] that ethanol fermentation and recovery are dependent on the amount of substrate used and the efficiency of yeast to convert the reducing sugar to ethanol.

Table 3. Sago starch fermentation time (h) among the different treatments

YEAST	MOLASSES	Mean

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	M1 (150g)	M2 (200g)	M3 (250g)	
Y1 (50g)	15.00	14.87	16.46	15.44b
Y2 (100g)	15.43	17.43	18.85	17.24ab
Y3 (150g)	19.00	18.33	18.80	18.71a

*Means with the same letter superscripts are not significantly different using Least Significant Difference

Comparison among means show significantly longer fermentation time in Y3 (150g of molasses) compared to the other yeast treatments. The amount of yeast in the substrate directly affects the fermentation time. On the other hand, the analysis of variance showed that the amount of molasses did not have a significant effect on the fermentation time.

Alcohol yield of the distillate

Figure 2 shows the alcohol content of the distillate of the different treatments. The alcohol was obtained using simple distillation and the purity was measured using a hydrometer. The results show higher alcohol yields in the treatments containing 250g molasses. These treatments also had higher sugar to alcohol conversion rates because of the added glucose content in the molasses. The highest yield was treatment Y2M3 (100g of Yeast and 100g of Molasses) garnering 40.33% of distillate and the lowest was Y3M1 (150g of Yeast and 150g of Molasses) with yield of 17% of distillate. Based on the result, it was observed that all M1 (150g of Molasses) has lower yield of distillate regardless of the amount of yeast added.

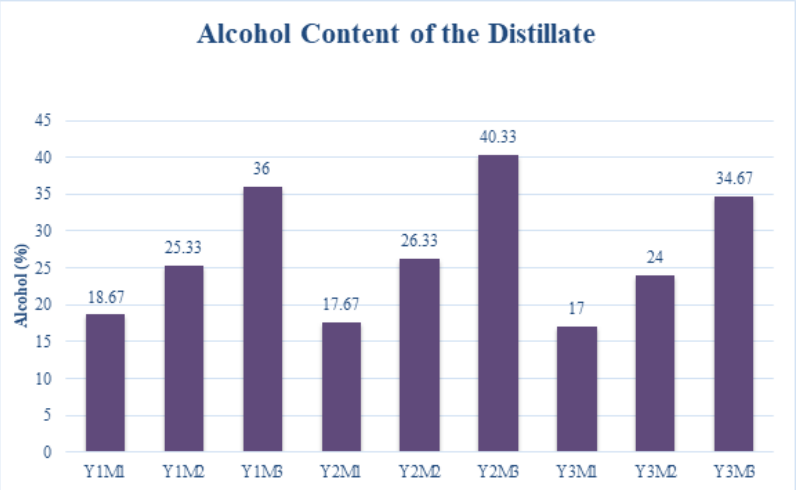

Figure 2. Alcohol content of the distillate

4. CONCLUSION

This study was able determine to the technical viability and develop a protocol to produce bioethanol from sago starch using heat hydrolysis and anaerobic fermentation. The results show that the amount of yeast and molasses in the sago starch substrate influence fermentation time, sugar conversion, and alcohol yield. The fermentation time is directly influenced by the yeast content as faster fermentation time were recorded in samples with

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high yeast content. Sugar conversion respectively, result shows that the more sugar consumed by the yeast it has a high potential yield of alcohol from distillate. The results ofshow that higher sugar conversion and alcohol yields were obtained in Y2M3 (100g yeast, 250g molasses).

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