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Processing and Economic Analysis of Rain Tree (*Samanea saman*) Pods for Village Level Hydrous Bioethanol Production

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***Abstract*—**Biofuel is one of the renewable energy sources adapted by the Philippine government in order to lessen the dependency on foreign fuel and to reduce carbon dioxide emissions. Rain tree pods were seen to be a promising source of bioethanol since it contains significant amount of fermentable sugars. The study was conducted to establish the complete procedure in processing rain tree pods for village level hydrous bioethanol production. Production processes were done for village level hydrous bioethanol production from collection, drying, storage, shredding, dilution, extraction, fermentation, and distillation. The feedstock was sundried, and moisture content was determined at a range of 20% to 26% prior to storage. Dilution ratio was 1:1.25 (1 kg of pods = 1.25 L of water) and after extraction process yielded a sugar concentration of 22 ^{0}Bx to 24 ^{0}Bx. The dilution period was three hours. After three hours of diluting the samples, the juice was extracted using extractor with a capacity of 64.10 L/hour. 150 L of rain tree pods juice was extracted and subjected to fermentation process using a village level anaerobic bioreactor. Fermentation with yeast (*Saccharomyces cerevisiae*) can fasten up the process, thus producing more ethanol at a shorter period of time; however, without yeast fermentation, it also produces ethanol at lower volume with slower fermentation process. Distillation of 150 L of fermented broth was done for six hours at 85 °C to 95 °C temperature (feedstock) and 74 °C to 95 °C temperature of the column head (vapor state of ethanol). The highest volume of ethanol recovered was established at with yeast fermentation at five-day duration with a value of 14.89 L and lowest actual ethanol content was found at without yeast fermentation at three-day duration having a value of 11.63 L. In general, the results suggested that rain tree pods had a very good potential as feedstock for bioethanol production. Fermentation of rain tree pods juice can be done with yeast and without yeast.

***Keywords*—**Fermentation, hydrous bioethanol, rain tree pods, village level.

I. Introduction

PRODUCTION of bioethanol in the Philippines was boosted by the implementation of Republic Act 9367 commonly known as Biofuel Act of 2006 that mandates a 5% to 10% blend to gasoline fuel in order to mitigate the adverse effects of greenhouse gas emission in the environment. To reduce the net greenhouse gas emissions to the atmosphere, bioethanol has been recognized as a potential alternative to petroleum derived transportation fuels [1]. According to the forecast of the Department of Energy, demands for the bioethanol in the Philippines shall drastically increase along with the steady growth in registration number of automobile and accelerating blending ratio of bioethanol to the gasoline [2].

Continuous production of bioethanol is mainly dependent on the availability and volume of feedstock in a certain location. Also, different feedstocks require different handling processes for the production of bioethanol. There is a lot of feedstock available in the country such as sugarcane, sweet sorghum, corn and others. Aside from being a seasonal crop, the cost of production and the food versus fuel debate are the main constraints on using those feedstocks for the production of bioethanol. Lignocellulosic feedstock is also considered as a promising source of bioethanol; however, it needs additional technology before the sugar can be fermented that in return leads to higher production cost. The simplest way to produce ethanol is the sugar to ethanol production. Thereby, biomass that contains six – carbon sugars is used which can be fermented directly to ethanol. Although fungi, bacteria, and yeast microorganisms can be used for fermentation, the specific yeast Baker's yeast (*Saccharomyces cerevisiae*) is frequently used to ferment glucose to ethanol [3]. Baker's yeast is widely used in ethanol production due to its high ethanol yield and productivity, no oxygen requirement, and high ethanol tolerance.

Rain tree pods are a possible feedstock suitable for bioethanol production because it contains an appreciable amount of fermentable sugar and has a large volume of production during its fruiting season. Rain tree fruit is a promising source of bioethanol that does not compete on the food sector and on the space needed to plant cash crops. Rain tree pods are sessile indehiscent, six to eight inches long and half to one inch broad, flattened, containing 10 to 12 seeds embedded in a sugary edible pulp and yields up to 275 kilos of pods per year which can be obtained from 15 years old trees [4]. In the Philippines, rain tree pods have been commonly utilized as a feed for ruminant animals since they contain high amount of protein. However, it was observed that most of the pods are unutilized and remain to the ground until they are rotten. In places where rain tree pods are planted along roadside and school vicinities, the pods fallen on the ground were pounded, and as a result, become sticky and invite flies when rotting, and those make the rain tree pods completely a waste.

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The general objective of the study is to establish a complete procedure of processing rain tree pods for village level hydrous bioethanol production. Specifically, the study aims to establish procedure on the collection, drying, storage, and preparation of rain tree pods for bioethanol production; evaluate data on fermentation of rain tree pods in terms of reducing sugar, pH and temperature; determine the ethanol yield from distillation of fermented broth using rain tree pods as feedstock; and, conduct economic analysis on the production of hydrous bioethanol for village level operation using rain tree pods as feedstock.

Since there is no complete process in harnessing the potential of rain tree pods as a feedstock, processing of the feedstock was done to establish the protocol for the complete extraction of hydrous bioethanol at a village scale. Sourcing of new available feedstock that will utilize a low-cost village level anaerobic bioreactor, and village scale reflux distiller will contribute a lot in pursuing ethanol production.

Gathering of data throughout the production process was done to provide the necessary information and condition needed in establishing the processes needed in bioethanol production using rain tree pods as feedstock. Information that was established in the study is a breakthrough in searching for the potential feedstock that may help the country in searching for possible alternative energy source. Also, possible by-products along the production process may provide a useful revenue through feedstock for animal feed and power generation. The fuel that will be derived from the feedstock through fermentation and distillation process is useful in powering agricultural machines that operate on a spark ignition engine. Bioethanol can be blend in gasoline fuel which improves its octane rating, thus making it a better fuel. The main product (bioethanol) can also be used for the production of alcoholic beverages, solvent for cosmetics and perfumes, fuel cells, and as a fuel for cooking burners. The study included the identification of the possible by products from the hydrous bioethanol production process using rain tree pods as feedstock and their possible uses. However, the study did not include the utilization of the main (bioethanol) and by-products of the production process.

II. Materials and Methods

A. Drying and Storage of Rain Tree Pods

Drying process can reduce the moisture content of the feedstock, thus, prolonging its shelf life. The collected samples were sun dried to prevent mold growth, seed germination, and rotting of the sample prior to storage. After drying, the rain tree pods were stored in a dry area. The polyethylene sacks were served as storage media for the dried pods. They were stored in a dry area under ambient condition. Moisture content of the samples was determined.

B. Shredding, Dilution and Extraction

Existing biomass shredder available was used in reducing the size of the rain tree pods. Since the sugar present in the pods was difficult to extract, dilution was done for ease of extracting the juice. Water was used as solvent in dilution process. Shredded rain tree pods were soaked in water for a period of three hours at various dilution ratio to determine the proportions of mixing pods with water in order to produce a sugar concentration of 30%. Since there was no available rain tree pod juice extractor, simple device was fabricated for ease of extraction. The design of the device was based on 6 to 8 kg per loading of samples, utilized hydraulic jack for pressing action, and was capable of extracting the juice present in the sample. The extracted juice was placed in a 200 L drum, then it was transferred into the bioreactor prior to fermentation process.

C. Compositional Analysis of Rain Tree Pods

Rain tree pods were analyzed using High Performance Liquid Chromatography (HPLC) method for determining its sugar profile (fructose, glucose, sucrose, maltose). Samples were taken from the collected rain tree pods and were analyzed at the First Analytical Service and Technical Laboratories (F.A.S.T.) prior in determining the sugar profile of the rain tree pods used in the study.

D. Yeast Fermentation

Village level anaerobic bioreactor available at Central Luzon State University- Affiliated Renewable Energy Center was used. The bioreactor had an actual capacity of 320 L of broth per loading. However, only 150 L rain tree pods juice was loaded in the bioreactor, and the remaining 170 L served as space for gas. The juice extracted from rain tree pods ranged from 22 ^{0}Bx to 24 ^{0}Bx prior to loading in the bioreactor. The amount of yeast (baker's yeast) needed for fermenting 150 L of broth was activated before pouring it in the bioreactor. The mixture was continuously stirred for 1 hour at aerobic condition to ensure the activation of yeast before anaerobic fermentation commences. Data collection was done to establish the actual conditions in the fermentation of rain tree pods juice. The data collected from each sample were the following: reducing sugar, pH, and, temperature.

E. Distillation

After fermenting the feedstock, distillation was the next stage. Village scale reflux distiller capable of fermenting 150 L of broth per batch was used in the distillation. 150 L of fermented broth was distilled for a period of six hours utilizing firewood as source of heat. The ethanol extracted from the feedstock after passing thru the distillation process was collected and was placed in a container.

F. Statistical Analysis

Relevant data were gathered, recorded, organized, tabulated, and analyzed statistically using Factorial experiment in Completely Randomized Design with three replications per treatment. Comparison among treatment means was done using Duncan's Multiple Range Test. Two factors were considered, amount of yeast as Factor A (no yeast, with yeast-1.47 g/L) and days of fermentation as Factor B (three days and five-day fermentation). The duration of agitation was set to

five minutes [5], and eight-hour agitation interval was used [6].

III. Results and Discussions

A. Established Procedure for the Collection, Drying, Storage and Preparation of Rain Tree Pods

Production of bioethanol at a village level using rain tree pods as feedstock was done in the study. Below is the summary of the procedures done in the study:

1. We determined the location wherein clean rain tree pods were obtained (not pounded by vehicle/person, free from sand particles, not eaten by insects). Manual collection of rain tree pods was done in the study. Mean collection capacity of an individual was 283.20 kg/day.
2. We collected the pods and placed them in polyethylene sacks.
3. Feedstock was dried at moisture content range of 20% to 26%.
4. Placed the dried feedstock using polyethylene sacks and stored at a dry place. Placed dunnage at the floor to avoid contact on the feedstock. Bags were piled properly to maintain quality and allowing space for the circulation of convective air currents that provided a medium for heat dissipation.
5. 130 kg of rain tree pods was shredded using available biomass shredder. Reduction of size was necessary for ease of extracting the sugar present in the pods.
6. Placed 125 kg of shredded pods in a 200 L drum. Dilution ratio is 1:1.25. 1 kg of shredded pods was mixed with 1250 mL of water. The pods were diluted for a period of three hours to allow the migration of sugar.
7. Rain tree pods juice was extracted using available rain tree pods juice extractor.
8. The initial sugar concentration, pH and temperature of the fermentation broth (juice) were determined using handheld refractometer, pH meter, and thermocontroller, respectively.
9. The extracted 150 L juice was fermented using available bioreactor. The extracted juice was loaded using water pump into the bioreactor. Yeast (*Saccharomyces cerevisiae*) and additives were pitched into the fermentation broth. The bioreactor was sealed to allow the anaerobic fermentation of the fermentation broth in order to produce bioethanol.
10. After fermentation, distillation was the final process. The fermentation broth was unloaded using water pump. The fermentation broth was loaded into the village level reflux distiller. Six hours of distillation time was allotted for distillation of 150 L fermented broth.

B. Compositional Analysis of Rain Tree Pods

The sugar profile (fructose, maltose, glucose, sucrose) of rain tree pods were analyzed using HPLC method at the First Analytical Service and Technical Laboratories (F.A.S.T. Lab.). The purpose of the analysis was to determine the potential carbohydrate content of the feedstock which is directly related to the fermentable sugars that were converted to ethanol. Table I shows the sugar profile of the rain tree pods.

TABLE I
Sugar Profile of Rain Tree (*Samanea saman*) Pods

Sugar Profile	Results (%)
Sucrose	9.13
Fructose	11.3
Glucose	11.2
Maltose	0

The result of the analysis revealed that the rain tree pods contained about 9.13% of sucrose, 11.3% of fructose, 11.2% of glucose, and 0% of maltose. Sucrose is a disaccharide composed of $_D$-glucose and $_D$-fructose linked by an α-1-4 glycosidic bond. In the initial stages of fermentation, sucrose is rapidly hydrolyzed into glucose and fructose by the action of periplasmic enzyme invertase, prior to sugars transported across the cell membrane. Growth of *Saccharomyces cerevisiae* on a medium consisting of a mixture of glucose and fructose results in preferential uptake of glucose [7]. Also, during fermentation glucose was approximately utilized twice the rate of fructose. Sucrose, glucose, and fructose indicated the sugars available in the rain tree pods which were converted in the production of bioethanol through anaerobic fermentation.

C. Fermentation

Fermentation process is significantly affected by several factors in order to produce maximum potential of the feedstock to produce ethanol which includes temperature, method of fermentation, reducing sugar and pH of the fermentation broth. The whole process of fermentation that was conducted using juice of rain tree pods as feedstock monitors the variation of pH, reducing sugar, and the temperature of the fermentation broth.

D. Reducing Sugar

Reducing sugar of rain tree pods juice was an indicator of the fermentable sugars that were converted into ethanol through fermentation process. The average reducing sugar as influenced by days of fermentation and amount of yeast is shown in Table II. The highest reducing sugar of 11.67 ^{0}Bx was obtained for five-day fermentation period and with the presence of yeast (1.47 g/L). On the other hand, the lowest reducing sugar was observed for three-day fermentation period without the addition of yeast on the fermentation process.

TABLE II
Reducing Sugar (^{0}Bx) as Affected by Amount of Yeast and Days of Fermentation

Amount of Yeast	Days of fermentation, days		Mean
	3	5	
Without yeast (0 g/L)	9.67	10.83	10.25^y
With yeast (1.47 g/L)	10.67	11.67	11.17^z
Mean	10.17^a	11.25^b	10.71

Means not sharing the same letter, in a row or column, differ significantly by DMRT at 5% level of significance.

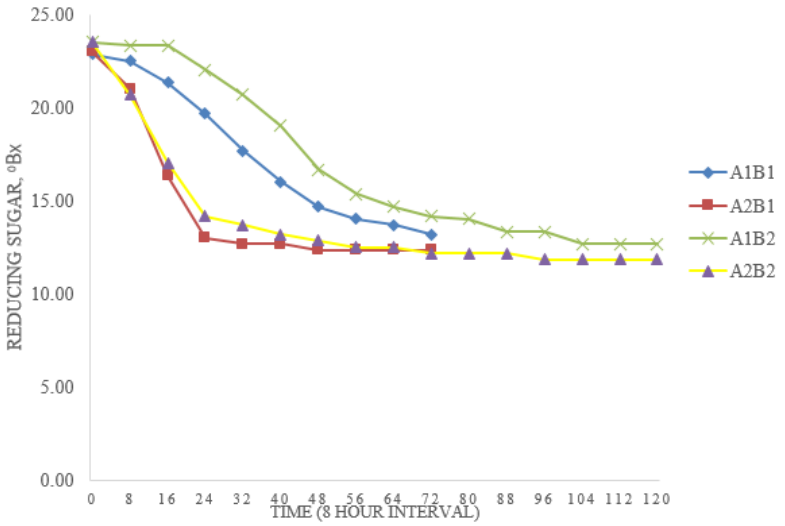

Fig. 1 Mean reducing sugar of the fermentation broth for every treatment combination

Analysis of variance showed that the amount of yeast and days of fermentation had significant effects on the reducing sugar of the fermentation process. However, the interaction of the two factors had no significant effect on the fermentation process in terms of reducing sugar which signified that both factors act independently with each other.

Comparison among means on the reducing sugar was higher for fermentation broth with yeast compared to fermentation broth without the addition of yeast with means equal to 11.17 ^{0}Bx and 10.25 ^{0}Bx, respectively. The capability of the yeast to convert the carbohydrates into ethanol and carbon dioxide when added to the fermentation broth resulted in higher and faster reduction of sugar. Fermentation process without the addition of yeast resulted in longer fermentation period and lower reducing sugar. Yeast (Baker's yeast) could fasten up the fermentation reaction to produce ethanol. However, it is established that an apparatus as complicated as yeast cell is not required to institute the fermenting process. Rather, the carrier of the fermenting activity must be regarded as dissolved substance, undoubtedly a protein. This is called zymase [8].

Comparison among means on the reducing sugar revealed that five-day fermentation (11.25 ^{0}Bx) and three-day fermentation (10.17 ^{0}Bx) were significantly different from each other. Higher reduced sugar was observed at five-day fermentation because the fermentation of the sugar was done, while at three days fermentation, reducing of sugar was still happening. It indicated that, at five-day fermentation, the stationary phase of yeast growth was reached, while at three days fermentation, the yeast growth was still happening. When all the sugar was used up and the ethanol concentration rose up to the maximum level, the yeast growth stopped and the stationary phase started [9].

Fig. 1 shows the trend of the reducing sugar of each treatment combination as affected by days of fermentation and amount of yeast added. It reveals that for A_1B_1 and A_1B_2, fast sugar reduction was between 0 to 56 hours and after passing on that point, slow fermentation happened. However, for A_2B_1 and A_2B_2, Fig. 1 illustrates that the fast fermentation rate happened between 0 to 24 hours and also after passing on that time, slow fermentation happened.

E. Temperature of the Fermentation Broth during the Fermentation Process

Temperature of the fermentation broth was observed to determine the variation of temperature along the fermentation period and to observe the effect of yeast addition in the variation of temperature of the fermentation broth. Fermentation process started when temperature of the fermentation broth start to increase. The increase in temperature of the fermentation broth up to its maximum point resulted in the peak reduction of sugar. The sudden increase in temperature was brought by the yeast activity which signified that fermentation process started. The sudden decrease of temperature of the fermentation broth indicated that fermentation was nearly at end. The amount of heat evolved was related to the stoichiometry for growth and product formation, whereas the rate of heat evolution was related to the microbial activity [10]. The highest temperature was 32.10 °C for without yeast fermentation; however, for treatment combination that with the aid of yeast (1.47 g/L),the maximum temperature was 35.17 °C. It signified that yeast (*Saccharomyces cerevisiae*) affected the fermentation process resulting in an increase change of temperature thus, reduction of sugar. However, for treatment combination without the addition of yeast, the increase or decrease of temperature was slower and lower compared to fermentation broth that had yeast.

F. pH Level of the Fermentation Broth during the Fermentation Process

The highest mean value of pH level of the fermentation broth was obtained from three-day fermentation with yeast of 1.47 g/L added on it and the lowest mean pH value was obtained from five-day fermentation with yeast of 1.47 g/L

added on it, which was equal to 5.30 and 4.93, respectively. For without yeast fermentation, the descending value of pH level started from 0 to 64-hour period. The decreasing value in pH was slower compared to fermentation broth with yeast. Also, after it reached the maximum point of reduction, the change of pH was unstable, but the pH values were ascending. The variation of pH at the latter part of fermentation signified the end of fermentation. The reduction of pH of with yeast fermentation was observed from 0 hour to 24 hours, which imparted that the fastest reduction of sugar happened in this period as related to pH. However, it suddenly increased after 24 hours and had an unstable ascending value, which signified that fermentation was slowing down.

G.Actual Ethanol Content

Distillation was done to know the actual ethanol content of the fermented broth. Actual ethanol content of the fermented broth was obtained using a village level reflux distiller which was capable of producing 95% bioethanol. The average actual ethanol content as influenced by the days of fermentation and amount of yeast was shown in Table III. The highest volume of ethanol recovered of 14.89 L was obtained for five-day fermentation with the addition of yeast equal to 1.47 g/L. In contrary, the lowest volume of ethanol recovered of 11.63 L was attained from three-day fermentation without yeast added.

TABLE III
ACTUAL ETHANOL CONTENT (L) OF THE 150 L FERMENTATION BROTH

Amount of Yeast	Days of Fermentation, Days		Mean
	3	5	
Without yeast (0 g/L)	11.63	13.87	12.75[y]
With yeast (1.47 g/L)	13.79	14.89	14.34[z]
Mean	12.71[a]	14.38[b]	13.55

Means not sharing the same letter, in a row or column, differ significantly by DMRT at 5% level of significance.

Analysis of variance revealed that both factors had significant effect on the actual ethanol content of the fermented broth. Nevertheless, the interaction between the two factors showed that there was no significant effect on the actual ethanol content of the fermented broth. It signified that both factors could act independently with each other and could not affect the actual ethanol content of the fermented broth.

Comparison among means revealed that the actual ethanol content was highest for fermentation broth added with yeast compared to fermentation broth without yeast added with means equivalent to 14.34 L and 12.75 L, respectively. *Saccharomyces cerevisiae* was used extensively in batch fermentations to convert sugars to ethanol for the production of beverages and biofuels. *Saccharomyces cerevisiae* was capable of very rapid rates of glycolysis and ethanol production under optimal conditions [11].

Comparison among means revealed that the actual ethanol content was highest at five-day fermentation period compared to three-day fermentation period with a mean equal to 14.38 and 12.71, respectively. Five-day fermentation period finished the attenuative stage and started the conditioning phase as observed in the conduct of the study, while three-day fermentation was still undergoing the attenuative stage. During the attenuative phase, the yeast is vigorously converting the sugar to carbon dioxide, alcohol and other byproducts during the attenuative stage. As observed in the study, especially in three-day fermentation without yeast, fermentation was still happening, which resulted in lower recovery of ethanol during the distillation. However, at five-day fermentation, the fermentation process was completed resulting in higher ethanol recovery.

H.Ethanol Yield of Rain Tree Pods

Table IV shows the ethanol yield of the treatment combinations used in the study. It shows that the highest ethanol yield per kilogram of rain tree pods based on initial sugar concentration was obtained from A_1B_2 and A_2B_2, and the lowest ethanol yield was obtained from A_1B_1 with a value equal to 143.84 L per ton and 139.76 L per ton, respectively. Based on the actual volume of ethanol recovered after distillation, it revealed that the ethanol yield was highest at A_2B_2 at 119.09 L per ton. This could be explained by the fact that A_2B_2 had longer fermentation period and with yeast added on it. However, the lowest ethanol yield was obtained from A_1B_1 with a value equal to 93.07 L per ton of rain tree pods. This was probably because A_1B_1 had shorter fermentation period and without yeast added before the fermentation.

TABLE IV
AVERAGE ETHANOL YIELD (L/TON OF FRUIT) OF RAIN TREE PODS

Treatment Combination	Average Sugar Concentration, ^{0}Bx	Average Ethanol Yield (L/ton of Rain Tree Pods)	
		Based on initial sugar concentration	Based on the actual volume of ethanol recovered after distillation
A1B1 (without yeast, 3-day fermentation)	22.83	139.76	93.07
A2B1 (with yeast, 3-day fermentation)	23	140.80	110.35
A1B2 (without yeast, 5-day fermentation)	23.5	143.84	110.96
A2B2 (with yeast, 5-day fermentation)	23.5	143.84	119.09

The highest ethanol yield of rain tree pods based on the actual volume of ethanol recovered after distillation (119.09 L/ton) was grander than sugar beet (110 L/ton) and sugarcane (70 L/ton). It revealed that rain tree was a good source of bioethanol with high volume of ethanol produced compared to other feedstocks.

J. Economic Analysis

Economic analysis was done for the whole village level hydrous bioethanol production using rain tree pods as feedstock. The economic analysis was done from the collection of rain tree pods, drying of collected rain tree pods, shredding of dried pods, dilution and extraction of the shredded pods, fermentation of extracted juice, and distillation of the fermentation broth. Table V shows the summary of the cost of production of bioethanol using rain tree pods as feedstock along the various processes. The recommended treatment combination in the study was five-day fermentation

with yeast addition. The cost involved in the conversion of rain tree pods into bioethanol was Php 46.20 per liter. Based on the Sugar Regulatory Commission, the bioethanol price index as of April 2016 was about Php 59.98 per liter using molasses and sugarcane as feedstock. The released bioethanol price index was based from the large-scale bioethanol production facility in the Philippines.

TABLE V
COST OF PRODUCTION OF BIOETHANOL USING RAIN TREE PODS AS FEEDSTOCK ALONG THE VARIOUS PROCESSES, PHP/LITER

Process	With Yeast- 5 Days Fermentation
1. Collection, Php/L	6.38
2. Drying, Php/L	1.68
3. Shredding, Php/L	1.68
4. Dilution and extraction, Php/L	8.74
5. Fermentation, Php/L	11.76
6. Distillation, Php/L	15.96
Total, Php/L	46.20

Economic analysis revealed that benefit cost ratio, net present value, and internal rate of return were 2.40, Php 2,012,031.82, and 35.45%, respectively based on five-year life span which suggested that the utilization of rain tree pods as feedstock for village level hydrous bioethanol production was financially viable.

K. Main and by Products of the Production Process

Along the production process of producing bioethanol using rain tree pods as feedstock, various by products were produced. It included the seeds and pulp of the rain tree pods after extraction process. Along the distillation process, stillage was produced which contained a sugar concentration of 9 to 10 ^{0}Bx after distillation. Those by-products can be further processed to recover more value from the feedstock.

The main product of the production process was the hydrous bioethanol which can be used as fuel or blend for spark ignition engines, fuel for cooking burners, alcoholic beverages, and others.

IV. CONCLUSION

Rain tree pods have a good potential as feedstock for bioethanol production. The sugar concentration used in the study ranged from 22% to 24%. The reducing sugar for without yeast fermentation at 3-day period was 9.67 ^0B_x, 10.67 ^0B_x for with yeast fermentation at three-day period, 10.83 ^0B_x for without yeast fermentation at five-day period, and 11.67 ^0B_x for with yeast fermentation at five-day period. Higher peak temperature was observed at fermentation with yeast compared to without yeast fermentation with a value of 35.17 °C and 32.10 °C, respectively. The ethanol yield from distillation of fermented broth using rain tree pods as feedstock was 11.63 L for without yeast fermentation at three-day period, 13.79 L for with yeast fermentation at three-day period, 13.87 L for without yeast fermentation at five-day period, and 14.89 L for with yeast fermentation at five-day period. Six-hour distillation was done. The economic analysis on the production of bioethanol using rain tree pods as feedstock showed that it was financially viable.

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