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Fed-batch Alcoholic Fermentation of Palm Juice (*Arenga pinnata* Merr) : Influence of the Feeding Rate on Yeast, Yield and Productivity

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ABSTRACT

In North Sulawesi has been known a very potential crop for yielding bio ethanol, is palm crop. Palm crops more productive than cane in yielding sugar and biofuel per hectare. Its productivity about 4 - 8 times compared to cane, and its sugar rendement 14%, while cane only 7%. Based on this research were obtained the tapping process should be conducted by unique sequential steps in order to reach pH 8 and sugar concentration (brix) 12 – 14 % of juice. Alcohol is a chemical was produced from crops like cassava, corn, sago and was usually named as bio ethanol. Cassava, corn and sago is food crops which is ordinary to be planted by people almost in all Indonesia region, but in North Sulawesi Province were very potential crops and have some excellences compared to other crops in yielding bioethanol, that is palm crops. The tap is a good substance for alcohol fermentation due to its high sugar and low ash contents.

The objective of this study to support procurement of energy alternative.and production of bioethanol 99.5% The process of alcohol fermentation was conducted in a bottle glass under an aerobic conditions and at room temperature. The substances of fermentation process consist of 500 ml arenga pinnata sap and starter, solution of NPK and the bread yeast can be added when necessary. The fermentation process was carried out in several treatment methods and the treatment was based on the fermentation subctances used. The differences between the treatment methods: P-1, P-2, P-3 and P-4 are in the percentages of palm juice volume (%): 86, 90, 75 and 75, starter volume (%): 14, 10, 25 and 25 and NPK addition (%): 0.4, 0.4, 0.4 and 0.4 and 5% bread yeast. The fermentation of arenga pinnata sap was conducted batch system in room temperature during 24 hours incubation. Fermentation activities are monitored by some perameter such as microbial population, alcohol content, sugar content.

The result shows that the palm juice is a good feedstock for fermented bioethanol. The best yield is obtained by fermentation of feedstock containing 25% starter, 0.4% NPK solution, and 5% bread yeast and bio ethanol concentration is about 12% (89% bioethanol content of feedstock). By using of *Saccharomyces serevisiae* was obtained ferment liquor with alcohol rate from 12% - 14% and purity about 35 – 42 %.

By using of reflux distillation and purification fuel grade bioethanol by absorbtion technique with zeolite and CaO was resulted the purity of bioethanol 90% - 96% until 99.5%. The highest purity was 96% by maintaining the column temperature of 76^0C while purity of 93% was of $76,5^0$C. The flow rate of bio ethanol also was from 0.6 liter to 1 liter /hour by varying column temperature from 76 to 78^oC and bioethanol 99.5 % : 100 liter per day.

Keywords- *Absorbtion technique; Activation zeolite and CaO; Fermentation; Palm juice; Reflux distillation.*

I. INTRODUCTION

Arenga Pinnata Merr belongs to family *Arecaceae. Arenga pinnata* palm has been reported to have ethanol yields ranging from 6480 to 20,000 liters/ha, which make it several time more productive then the sugarcane. The palm can be tapped 4 years after planting, and will yield for 50 or more years (by comparison, the oil palm has a maximum life of 15 years). Traditional methods of harvesting are intriguing, and include slapping and kicking the tree on a daily basis to encourage the flow of sap [Anonimous,1999, Anonimous,2006, Anonimous and Peter&Sivasothi,1999].

The sap as tapping products from the fruit of arenga pinnata palm has high sugar contents and very sweet taste with colorless liquid. Thus the arenga pinnata can be use as feedstock for alcohol fermentation. The sap is collected by cutting the fruit at its point of attachment to the stalk. During this tapping process, this fruit secrete the sap. It's almost colorless and has very sweet taste. From six different palm samples were analyzed in the Philippines, the best quality of the sap in grams per 100 cubic centimeters containing 18.00 total solid, 17.00 sucrose, 0,48 ashes, and trace of reducing sugars. The alcohol production from nypa palm sap should be above 6% of the sap. In favorable condition it would be 7%. It seen that 9,300 gallons of the sap would produce about 650 gallons of alcohol, which would be the annual yield per acre [Anonimous,2007, Gibbs,1911].

In Malaya, Dennett, 1927 gives the real maximum yield per spathe as 0.1025 gallons. Base on of two spathes in tapping per palm, 200 palms per acre and 340 tapping days per annum,this gives a yield of 13,940 gallons of sap per acre per annum. He gives the mean alcohol content as 10 per cent by volume, so that the mean yield per acre per annum would be 1,394 gallons. He also gives the real minimum mean yield as 1,270 gallons. In "An Outline of Malayan Agriculture", Grist gives the theoretical yield of absolute alcohol as over 1,100 gallons per acre per annum [Dennett,1927, Hitchinson,1941].

Fermentation is one of several methods in alcohol fermentation, biochemical activities of microorganisms is usually used under

anaerobic condition (Bailey and Ollis,1997). During fermentation, feedstock changed to alcohol. Sucrose content of feedstock is broken down to glucose, and glucose fermentation produces alcohol [Prentis,1990]. Basically, fermented alcohol comes from glucose conversion. The yeast *Saccharomyces serevisiae* is usually used as supporting microbes for fermentation.

The alcohol fermentation is started with glycolysis process. The glucose compounds are broken down to pyruvic acid via Embden-Meyerhof pathways. The pyruvic acid is then converted to acetaldehyde and carbon dioxide by microbes. Finally, this acetaldehyde was converted to ethanol (see **figure 1**).

Indonesia is one of tropical countries has large of arenga pinnata palm forest, this palm has a very high sugarich sap yield that can be tapped continuously from the trees inflorescence. The arenga pinnata sap is a good substance for fermented ethanol production, however, the fermentation study with Indonesia arenga pinnata sap is limited. In conection with this conditions, an fermentation study was conducted with arenga pinnata palm from North Sulawesi. This palm was used as feedstock of productions of fermented alcohol.

The objective of this study to support procurement of energy alternative.

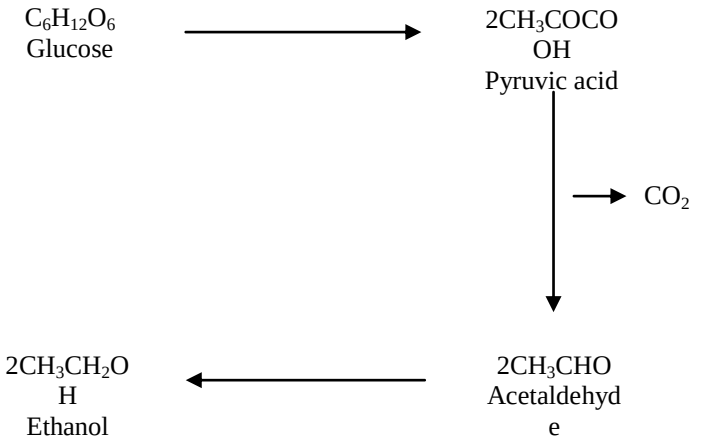

Figure 1: Reaction Sequences for the Alcohol Fermentations

Table 1.Treatment Methods for Alcohol Fermentation Process

Treatment method	Volume of sap (%)	Volume of starter (%)	NPK additional (%)	Microbes
P-1 (control)	90	10	0,2	*S.cerevisiae*
P-2 (control)	90	10	0,4	*S cerevisiae*
P-1	86	14	0,4	*S cerevisiae*
P-2	90	10	0,4	*S.cerevisiae* 5% bread yeast
P-3	75	25	0,4	*S.cerevisiae*
P-6	75	25	0,4	*S.cerevisiae* 5% bread yeast

Tabel 2: The Analysis Data of *Arenga pinnata* Palm Sap (Before Fermentation)

Analysis	Sample 1	Sample 2	Sample 3
Glucose (%)	26,25	26,30	26,19
Total sugar (%)	57,50	58,00	56,90
Sucrose (%)	29,12	30,12	29,17
Ash content (%)	0,53	0,55	0,42
pH	4,06	4,11	4,09

II. EXPERIMENTAL

A. Material

The alcohol fermentation was conducted in laboratory scale. The sap as feedstock was collected from a *Arenga pinnata* palm forest in North of Sulawesi, by means of cutting the fruit at its point of attachment to the stalk.

B. Microbes

Saccharomyces cerevisiae and the bread yeast were used as microbial culture in this work. *S. cerevisiae* growing in the *Arenga pinnata* palm sap was applied as starter for fermentation process. In order to support the microbial growth, the NPK solution was used as nutrition addition.

C. Treatment Methods

The process of alcohol fermentation was conducted in a bottle glass under anaerobic conditions and at room temperature. The substances of fermentation process consist of 500 ml *Arenga pinnata* palm sap and starter. The solution of NPK and the bread yeast can be added when necessary.

The fermentation process was carried out in several treatment methods. The treatment was based on the fermentation substances used. The differences between the treatment methods are in the percentages of sap volume, starter volume and NPK addition. The treatment methods for the fermentation process are show in **Table 1**.

The fermentation of *Arenga pinnata* palm sap was conducted batch system in room temperature during 24 hours incubation. Fermentation activities are monitored by some parameter such as microbial population, alcohol content, sugar content, further more, the fermented sap is distillated at 78^{o}C to separate alcohol from water.

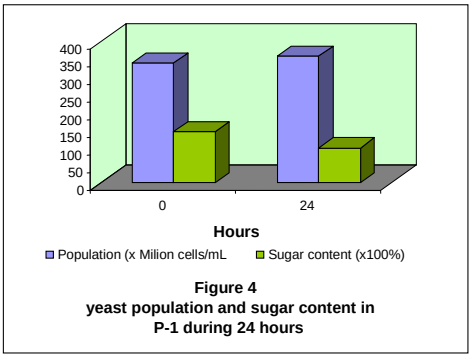

Figure 4
yeast population and sugar content in P-1 during 24 hours

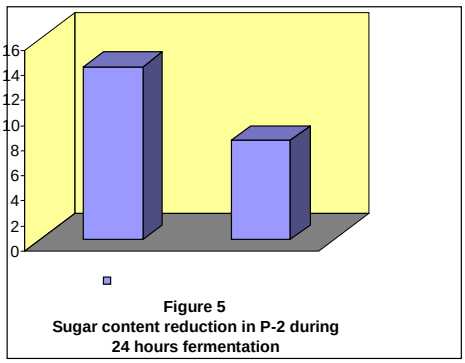

Figure 5
Sugar content reduction in P-2 during
24 hours fermentation

RESULT AND DISCUSSION

A. Feedstock

The tapping from fruit of *Arenga pinnata* palm that produces the sap is a good substance for alcohol fermentation due to its high sugar and low ash contents. The sugar content of *Arenga pinnata* palm is capable as feedstock for alcohol fermentation. The content of sugar is almost 60%. The total sugar is dominated by sucrose and glucose compounds. During process fermentation, the sugar was converted to alcohol compounds by microbial activities. The characteristic of the sap is acid with pH value is approx 4 (see **Table 2**).

B. Alcohol Fermentation of Treatment

The success of alcohol fermentation depends on the three important factors such as the quality of microbial cultures and feedstock as well as the condition of the biogical process. *Saccharomyces cerevisiae* was used as microbial cultural on this work. *Saccharomyces cerevisiae* that grew in the *Arenga pinnata* sap was used as starter for fermentation process. P-1 contains *Arenga pinnata* palm sap with 10% starter and 0.2% NPK. During fermentation process, the population of yeast is around 3 to 4 x 10^8 cells/ml (see **Figure 4**). The amount of the yeast cell is sufficient enough to support fermentation process of alcohol. Usually, minimum cell population of yeast for alcohol fermentation process is about million cells/ml.

The analysis result sugar content in *Arenga pinnata* sap before fermentation process is around 14.4%. After 24 hours incubation, 25% of sugar content was reduced, therefore 10.8% of sugar content was still remain in the sap (see **Figure 4**).

When addition NPK solution as a nutrition was increased to 0.4% (P-2), the sugar reduction also increases. During 24 hours incubation, the sugar content reduction is approx 37.5%. The result of fermentation activity is shown in **Figure 5**. The treatment of P-3 is almost similar with P-2. The difference between them is on the starter addition. The starter for P-3 is increased to 14%. However after 24 hours of fermentation the reduction of sugar increased slightly. The reduction of sugar is 43% (see **Figure 6**).

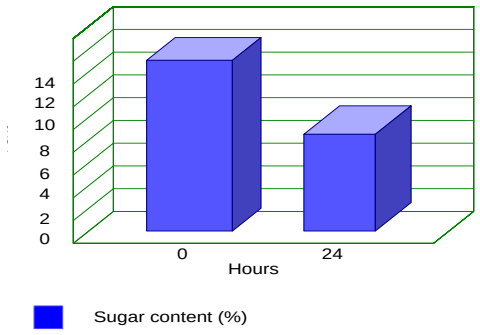

Figure 6
Sugar content reductions in P-3 during
24 hours fermentation

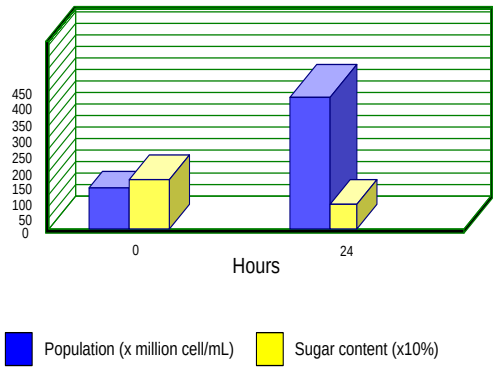

Figure 7
Yeast Population and Sugar Content Reduction in P-4
during 24 hours Fermentation

P-4 is P-2 with 5% bread yeast addition. In P-4,the population of yeast increases significantly. During 24 hours fermentation, the population increase from 0.9 to 4 x 10^8 cells/ml. The capability of microbes to reduce sugar content is also improved. As a result during 24 hours incubation, the sugar reduction is approx 58% (see **Figure 7**)

In P-5 the starter addition was increased to 25%. The reduction of sugar is good by treatment. The sugar reduction obtained is up to 79% during 24 hours incubation (see **Figure 8**)

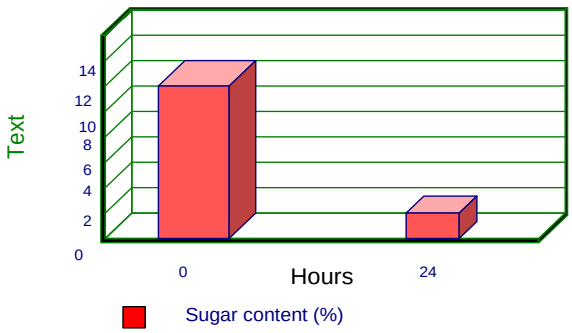

Figure 9
Reduction of sugar content in P-6
during 24 hours fermentation

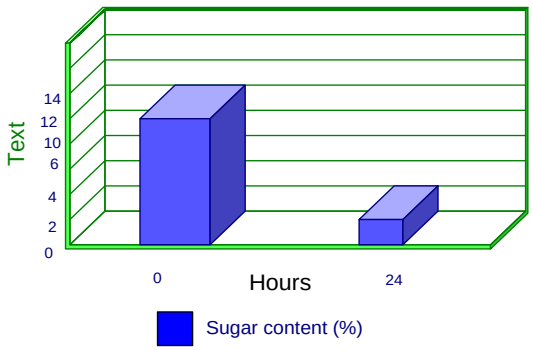

Figure 8
reduction of sugar content in P-5
during 24 hours fermentation

P-6 is P-5 with 5% bread yeast addition. P-6, the fermentation process is apparently improve and after 24 hours incubation the sugar reduction increasing up to 87.5% (see **Figure 9**) Figure10 shows the value of sugar reduction in *Arenga pinnata* sap during fermentation process in P-1 to P-6. The yeast activities are capable to reduce sugar content in *Arenga pinnata* sap from 25% to 89%.

During fermentation process in P-3 to P-6, some of sugar content in arenga pinnata palm sap converted to alcohol. The percentage of alcohol content and volume yield in P-3 to P-6 shown in **Figure 11**.

The lowest yields of alcohol come from P-3, which is round 8 ml with 58% alcohol content, The best yield of alcohol produced by P-6 is about 30 ml with 89% alcohol contents. This is equal with 11% of feedstock (see **Figure 12**)

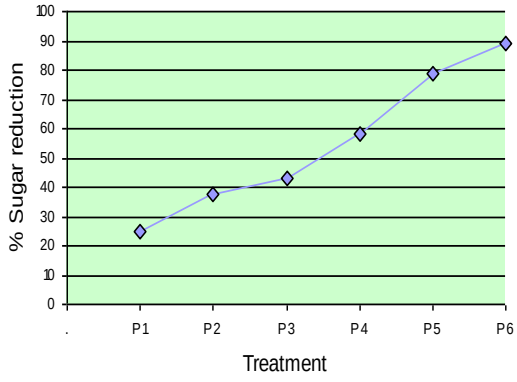

Figure 10
Curve of sugar content reduction during
24 hours fermentation in P-1 to P-6

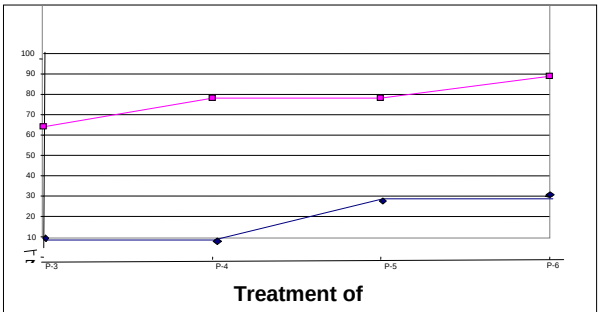

Figure 12
Percentage of Alcohol Production and Alcohol Content during 24 hours Fermentation in P-3 to P-6

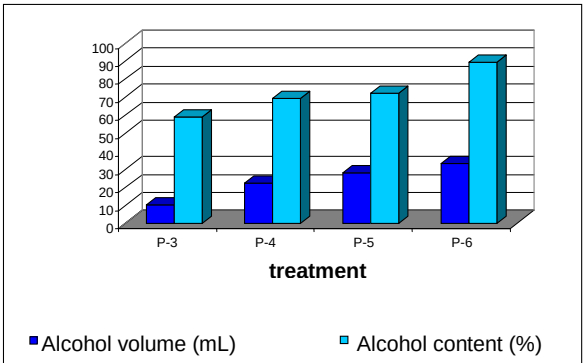

Figure 11
Alcohol Content and Volume of Alcohol Production in P-3 to P-6 during 24 hours Fermentation

Table 3: Test Result of the Alcohol of Fermentation Product

Characteristics	Product-1	Product-2	Test methods
Ethanol %(w/w)	85.24	86.05	GC
Methanol %(w/w)	0.07	0.07	GC
n-Propanol %(w/w)	.05	0.05	GC
Acetaldehyde % (w/w)	0.03	0.03	GC
Iso Amyl Alcohol % (w/w)	0.1	0.1	GC
Acidity as Acetic Acid % (w/w)	0.07	0.07	ASTM D. 1613
SG at $15^{O}C$	0.8233	0.8233	Picnometer

The Specification of Alcohol

The best yield of fermentation from the *Arenga pinnata* sap is fermented alcohol with 89% alcohol content. The content of alcohol is dominated by ethanol (more than 85%). The impurities also obtained in this product alcohol compounds such as methanol, n-propanol, and iso-amil alcohol are very low.

Specification of fermentation alcohol; shown in **Tabel 3**. The specific gravity at 15^{o}C is about 0.8233.

III. CONCLUSION

The sugar content in the sap of *Arenga pinnata* palm is more than fifty per cent. Consequently the arenga pinnata palm sap is sufficient to be used as alcohol fermentation feedstock. In alcohol production using arenga pinnata sap as the feedstock, the best yields was obtained when the fermentation was treated with 25% of starter, 0.4% NPK and 5% bread yeast. The yield of fermented alcohol is approx 12% (89% alcohol content) of feedstock

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