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The Effect of Ammonium Sulphate, Calcium Chloride, Magnesium Chloride, pH and Temperature on Ethanol, Carbon Dioxide and Fermentation Efficiency from Aren sap

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ABSTRACT

This study was aimed at determining the impact of ammonium sulphate $(NH_4)_2SO_4$, calcium chloride ($CaCl_2$), magnesium chloride ($MgCl_2$), pH, and the temperature on the production of ethanol, carbon dioxide (CO_2), and the fermentation efficiency of *Arenga pinnata* sap in laboratory scale. The sap of *Arenga pinnata* (locally called *Nira Aren*, Aren sap) was harvested from mature individual of *Arenga pinnata* trees from traditional orchards in Tomohon, North Sulawesi Province, Indonesia. The results of the experiment showed that the addition of 2.25 gr of $(NH_4)_2SO_4$ was able to perform the highest production of ethanol, CO_2, and efficiency. The addition of 12 mg of $CaCl_2$ resulted in the highest production of ethanol, CO_2, and the fermentation efficiency. Among the amount number of $MgCl_2$, the highest production of ethanol, CO_2 and fermentation efficiency occurred in 24 mg of $MgCl_2$. This study concludes that pH contributes significantly to the ethanol production, CO_2, and the fermentation efficiency. The highest production occurs in the fermentation process with pH 5 and at 35° C..
KEYWORDS: Aren sap fermentation, biofuel, $(NH_4)_2SO_4$, $MgCl_2$, $CaCl_2$, pH, temperature

INTRODUCTION

Recently, energy crisis leads the global community to explore opportunities to produce and provide alternative energy. It is particularly relevant with the vision of global community to achieve sustainable development. Scholars point out that in the recent decade, the world needs of energy increase significantly [1] [2]. More than 80% of the world energy needs are supplied by fossil fuels. Many countries rely heavily on fossil energy, including Indonesia. World Bank reported that the fossil consumption in Indonesia was considered high (c.a. 65.57%) in 1999. Scholars assume and estimate that the average of fuel consumption growth during 2006-2020 is 4.6%. Unfortunately, the increase in energy consumption is not accompanied by adequate energy production. Currently, the production of fuel oil and gas sector has declined due to a dwindling source in the layer of the earth. In addition to limited resources, long and intensive uses of fossil-fuel energy have been identified to contribute to negative impacts towards the environment. Significantly, global consumption of fossil fuel has produced CO_2 which becomes the ultimate gas to trigger global warming. Nowadays, increase of CO_2 in atmosphere and the global warming phenomena become the crucial problems in the world [3] [4]. Attempts to shift the use of energy resources from fossil fuels to alternative fuels become an intensive discussion. Among the significant resolution, the development of bio-ethanol from biomass becomes a potential approach to implement. It is particularly relevant in order to meet future energy needs. Bio-ethanol is a potential alternative energy source which is produced from a fermentation process. The use of bio-ethanol, both in industry and domestic, has many advantages. Bio-ethanol as an energy is considered environmentally friendly, and it is one of the renewable energy source [5] [6].

The demand and production of bio-ethanol in the world have increased every year. In 2000, it was reported that global demands of bio-ethanol reached 300 billion litres. The desire to meet sustainable life in biosphere has increased the bio-ethanol consumption. Scholars believe that there are benefits of bio-energy development. First, it is a renewable energy that can be produced from plant biomass. The abundance of biomass in biosphere, therefore, provides opportunities to produce bio-ethanol in a large scale [6] [7] [8]. Secondly, there will be foreign exchange savings due to the reduction of imports of oils. It is particularly important in many countries without natural resources to produce fossil fuels, or in countries where fossil fuel resources decline. There are potentials of bio-ethanol development in Indonesia due to the fact that this country is rich of raw materials for bio-ethanol production. It encompasses sugarcane stalk, lingo-celluloses material, cassava, maize grain, sweet potato tuber, palm sap, and woods [9]. The uses of sugarcane stalk, cassava, maize grain, and sweet potato tuber,

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however controversial due to their materials, are known as food sources. Using such materials potentially leads to food crisis, particularly in developing countries.

Arenga pinnata is one of the potential resources in the bio-ethanol production. This species is native to Indonesia and abundant in many places. This species is not used for daily food and, therefore, provides opportunities to process and produce bio-ethanol. The plant grows in all parts of Indonesia, from the low lands up to the mountains. The content of sugar in Arenga palm sap is around 6-16% [10]. This fact indicates that sap of *A. pinnata* can be considered as one of the potential sources for bio-ethanol production. In Indonesia, one of the centre for *A. pinnata* population is located in Tomohon, North Sulawesi Province. According to Pontoh (2009), the sugar content of such a palm in Tomohon can reach 11 to 16%, meaning that the species becomes a potential raw material for bio-ethanol production [11]. Nevertheless, the potential of such a material has rarely been explored and discussed. This study was aimed to examine the effect of ammonium sulphate $(NH_4)_2SO_4$, calcium chloride ($CaCl_2$), magnesium chloride ($MgCl_2$), pH and the temperature on the production of ethanol, carbon dioxide (CO_2), and fermentation efficiency of *A. pinnata* sap.

MATERIALS AND METHODS

Materials

The *Arenga pinnata* sap (locally called *Nira Aren*, Aren sap) was collected from several *A. pinnata* orchards in Tomohon, North Sulawesi Province. The mature individual of *A. pinnata* was selected from several traditional orchards in Tomohon. The process of tapping was carried out by cutting the blossom's spadix of the mature *A. pinnata,* and sap liquid was collected in the sterilized 10-liter bottle. The tapping was carried out at 6 pm to 6 am. The collection of Aren sap was done with a help from local inhabitants. The sap which was collected in bottles were, then, transferred to laboratory for next experiment steps.

Method

The Aren sap was boiled at 100ºC by using an electric boiler. During the boiling process, a sample was taken periodically and its sugar content was observed by using Brixt meter. The boiling was stopped when the sugar content had met 20%. The samples were cooled in temperature rooms. After cooling, the liquid sap with 20% sugar was prepared for fermentation. The fermentation was performed at 500 ml bottle. There were five experimental groups being treated differently from one to another. The first group was Aren sap fermentation with the addition of some amount of $(NH_4)_2SO_4$, the second group was Aren sap fermentation with the addition of some amount of $MgCl_2$, and the third group was Aren sap fermentation with the addition of $CaCl_2$ in different amounts. The fourth and fifth were fermentation with different application of pH and temperature (Table 1). Each of the bottles contained 300 ml Aren sap. In this experiment, Baker's yeast *Saccharomyces cereviceae* (locally called *ragi*) was employed for the fermentation process. Fermentation process was stopped after 32 hours.

Table 1 Different concentration treatments of $(NH_4)_2SO_4$, $MgCl_2$, $CaCl_2$, pH and temperature in the fermentation of A. *pinnata* sap

Treatments	$NH_4)_2SO_4$ (gr)	$MgCl_2$ (mg)	$CaCl_2$ (mg)	pH	Temperature (ºC)
I	0.75	12	12	3.5	25
II	1.5	24	24	4	30
III	2.25	36	36	4.5	35
IV	3.0	48	48	5	40
V	3.75	60	60	5.5	

The impact of differences in the amount of $(NH_4)_2SO_4$, $MgCl_2$, $CaCl_2$ as well as pH and temperature in the fermentation were analysed by using ANOVA.

RESULTS AND DISCUSSION

The fermentation of Aren sap to produce ethanol was the anaerobic process that converted carbohydrate into Alcohol and CO_2. In this fermentation process, however, several conditions have been reported to affect the fermentation process and its results.

3.1. The effect of $(NH_4)_2SO_4$

The $(NH_4)_2SO_4$ is sources of Nitrogen for microbes in fermentation process and its existence has been considered crucial. In this experiment, the variation amount of $(NH_4)_2SO_4$ provides different amount on ethanol and CO_2 as a fermentation product (Table 2). Nevertheless, the ANOVA analysis shows that there was no significant difference among the $(NH_4)_2SO_4$ treatments to the production of ethanol and fermentation efficiency, but there was a significant difference on the CO_2 production among the $(NH_4)_2SO_4$ treatments (Table

3). Nitrogen plays an important role in fermentation. Lack of nitrogen affects the low rate of fermentation process and, therefore, affects the fermentation process [12]. The deficiency of Nitrogen is often modified by adding supplementary nitrogen [13] [14]. The fermentation efficiency ranged from 87.99 to 92.84%. According to Jacques et al. (1999), the fermentation efficiency in industry was recorded about 90-93% [15]. It could be said that the addition of $(NH_4)_2SO_4$ about 0.75 gr., 1.50 gr., 2.25 gr., 3.00 gr., and 3.75 gr., was able to perform good efficiency rates in Aren sap fermentation.

Table 2 The effect of different amount of $(NH_4)_2SO_4$ in Aren sap fermentation product

$(NH_4)_2SO_4$	Ethanol (%)	CO_2 (litre)	Efficiency value (%)
0.75 gr	11.98	9.36	87.99
1.50 gr	12.03	11.18	88.37
2.25 gr	12.64	13.58	92.84
3.00 gr	12.44	10.03	91.39
3.75 gr	12.30	12.10	90.41

Table 3 ANOVA analysis on the impact of $(NH_4)_2SO_4$ different amount to ethanol and CO_2 production and fermentation efficiency

Variables	F_{test}	Sig F
Ethanol	2.864	0.060
CO_2	250.913	0.000
efficiency	2.856	0.061

3.2. The Effect of $CaCl_2$

$CaCl_2$ amount in Aren sap fermentation affects the ethanol and CO_2 production as well as its efficiency (Table 4). The amount of CaCl2 significantly produces ethanol in ranges 12.27 to 12.54%. In similar table, the impact of amount of $CaCl_2$ in CO_2 production varied. Scholars point out that high calcium level significantly controls the microbial growth in the fermentation process [16][17] and, therefore, affects the fermentation process. There is a critical amount of $CaCl_2$ in order to meet the optimum condition for microbes in fermentation process. According to Zing et al. (2010), adequate calcium chloride in fermentation has a significant impact on the ethanol production [17]. The ANOVA test confirms that different amount treatments of $CaCl_2$ provide significant value to ethanol production, CO_2 and efficiency value (Table 5). The value of efficiency ranged from 90.18 to 92.12 and such a result confirms that the addition of $CaCl_2$ 12 mg., 24 mg., 36 mg, 48 mg and 60 mg provides good efficiency in Aren sap fermentation. Overall, such efficiency value was recorded in range of the fermentation efficiency in the industrial fermentation (c.a. 90-93%) [15].

Table 4 The impact of different $CaCl_2$ amount on ethanol and CO_2 production and fermentation efficiency

$CaCl_2$	Ethanol (%)	CO_2 (litre)	Efficiency value (%)
12 mg	12.54	12.50	92.12
24 mg	12.33	12.20	90.54
36 mg	12.35	10.40	90.71
48 mg	12.32	11.28	90.45
60 mg	12.27	9.88	90.18

Table 5 ANOVA on the impact of different amount of $CaCl_2$ on Arenga sap fermentation

Variables	F_{test}	Sig F
Ethanol	5.067	0.009
CO_2	109.613	0.000
Efficiency	4.990	0.009

3.3. The Effect of $MgCl_2$

The impact of different amount of $MgCl_2$ on fermentation product and fermentation efficiency is given in Table 6. The $MgCl_2$ contributed significantly to the ethanol production. In this research, ethanol production ranged from 12.29 to 12.57% depending on the amount of MgCl2. Scholars point out that $MgCl_2$ has a positive impact on fermentation process. Principally, the inorganic salt $MgCl_2$ has been reported by Kang et al. (2010) to affect the hydrolysis in xylan of miscanthus straw in the biofuel production [18].

Table 6 The impact of different $MgCl_2$ amount on ethanol and CO_2 production and fermentation efficiency

$MgCl_2$	Ethanol (%)	CO_2 (litre)	Efficiency value (%)
12 mg	12.29	10.65	90.28
24 mg	12.57	11.83	92.34
36 mg	12.55	11.60	92.21
48 mg	12.48	11.58	91.63
60 mg	12.46	11.28	91.52

There were also significant different on CO_2 production as well as fermentation efficiency (Table 7). The amount of CO_2 which was released as a natural process of Aren sap fermentation is crucial in global climates changes. In such issues, however, a biotechnology approach to find efficient fermentation which released low CO_2 becomes crucial [19]. The addition of $MgCl_2$ in Aren sap fermentation contributes to efficiency achievement which was recorded to range from 90.28 to 92.34. These values are similar with the industrial fermentation efficiency which was recorded at 90-93% [15].

Table 7 The ANOVA analysis on the impact of $MgCl_2$ in Aren sap fermentation

Variables	F_{test}	Sig F
Ethanol	3.788	0.025
CO_2	6.173	0.004
Efficiency	3.802	0.025

3.4. The Effect of pH

Through the statistical analysis, pH was found to contribute significantly to the ethanol and CO_2 production as well as the fermentation efficiency (Table 8 and 9).

Table 8 Impact of pH in Aren sap fermentation product

pH	Ethanol (%)	CO_2 (litre)	Efficiency value (%)
3.5	11.85	9.28	87.03
4.0	11.88	11.98	87.29
4.5	12.00	9.50	88.19
5.0	12.64	12.18	92.84
5.5	12.31	10.48	90.47

There was no significant ethanol production in pH 4.00–4.50. The highest ethanol production was produced in pH 5.00. In such level of pH, the fermentation was able to produce ethanol as much as 12.64%. It is similar with the finding of Hwang et al. (2004) in that they found that the production of bio-ethanol below 4.5 may be influenced by the ability of microorganism to digest carbohydrates and to convert it to ethanol [20]. The lowest efficiency (87.03%) occurred in pH 3.5, while the highest efficiency (92.84%) occurred in pH 5.0.

Table 9 ANOVA of the contribution of pH in Aren sap fermentation

Variables	F_{test}	Sig F
Ethanol	30.262	0.000
CO_2	24.787	0.000
efficiency	30.262	0.000

3.5. The Impact of Temperature

The level of temperature shows significant impact on the Aren sap fermentation (Table 10). This experiment shows that temperature significantly affects the ethanol production. There were significant differences on the impact of temperature to CO_2 production and efficiency (Table 11). The lowest efficiency was reached in the temperature of 40˚C in which the efficiency was calculated at about 80.78%. The highest efficiency was obtained in 35˚C in which the efficiency was calculated at about 88.13%. According to Gaur (2006), temperature plays an important role in the microbe's metabolism during the fermentation process. Fermentation commonly occurs in 25-35˚C. The temperature of 40˚C could decrease the microbe's viability to convert glucose to ethanol [21].

Table 10.The impact of temperature on the Aren sap fermentation

Temperature	Ethanol (%)	CO_2 (litre)	Efficiency value (%)
25°C	11.74	10.83	86.13
30°C	11.83	11.65	86.79
35°C	12.00	12.98	88.13
40°C	11.00	9.53	80.78

Table 11 The impact of temperature

Variables	F_{test}	Sig F
Ethanol	4451.952	0.000
CO_2	57.442	0.000
Efficiency	3240.368	0.000

Overall, the amount of ammonium sulphate $(NH_4)_2SO_4$, calcium chloride ($CaCl_2$), and magnesium chloride ($MgCl_2$) contributes to Aren sap product's profiles. These chemical compounds, however, are essential to support microbes in the fermentation process to produce ethanol and CO_2. Another aspect such as pH and the temperature are also known to affect Aren sap fermentation. In order to enhance bioethanol product from *A. pinnata*, however, there are still intensive experiment needed. It is especially related to the implementation of biotechnology [19].

CONCLUSION

The importance of fermentation technology in Aren sap is evident in the present study that greatly contributes to bioethanol production. As far, the fermentation technology for Aren sap is rare, and this study significantly provides some basic information on the fermentation technology in laboratory scale. This study concludes that the highest production of ethanol, CO_2 and the efficiency rate is obtained in the fermentation with 2.25 gr of $(NH_4)_2SO_4$ and occurs in 12 mg of $CaCl_2$ and in 24 mg $MgCl_2$, while at the same time requires the fermentation process with pH 5 and the temperature of 35°C.

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