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Article

Study the Effect of pH on the Fermentation Anaerobic-Aerobic Siwalan (Borassus flabellifer L.) Sap to produce Acetic Acid

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Abstract. The purpose of this research is to study the effect of ethanol fermentation aerobic pH on acetic acid product. Anaerobic fermentation uses saccharomyces cereviceae to produce ethanol, and aerobic fermentation uses acetobacter aceti for acetic acid production. In aerobic ethanol fermentation using pH 3; 3.5; 4 and 5. The concentration of ethanol was analyzed using GC ULTRA Scientific Gas Chromatography, DSQ II detector, and MS 220 column. Acetic acid produced from the aerobic fermentation process was analyzed using an alkalimetric method. Anaerobic fermentation uses Saccharomyces cereviceae with 1-day log phase, while aerobic fermentation uses acetobacter aceti with a 5 day log phase. Fermentation using saccaromyces cerevisiae within 24 hours in order that reduction sugar could stably decrease, optimum ethanol could be got at optimum ph 6 which could decrease 55 percent of reduction sugar concentration to produce 8,20583 %v/v ethanol. Fermentation acetic acid content using acetobacter aceti observed in 3 days at pH 6 and 30 ^{0}C will produce 6,659 g/l also shows that ph 4-6 at 30 ^{0}C will produce 6,605 g/l acetic acid. Aerobical fermentation of acetic acid in 3 days shows that ph 4-6 is highly affected by temperature at 30 ^{0}C. Statical analysis shows, in ethanol production pH and fermentation time give significant effect, but interaction from two factors has no significant effect.

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1. Introduction

Siwalan (Borassus flabellifer L.) is a type of palm plant that has multipurpose advan- tages. In Tuban planting area of siwalan is 1.193 hectares. Nira siwalan is one of the most widely used products of siwalan tree. Inside the siwalan sap has a high sugar content about 10.15 g / liters [1]. High sugar content can be utilized in the manufacture of fermented foods, besides siwalan sap used for the manufacture of sugar, tuak (alcoholic beverages), vinegar, and brown sugar [2]. Siwalan sap can be made into more economical products, such as siwalan sap as raw material for making food grade ethanol. However, until now the biggest utilization of siwalan sap for fresh drink and alcoholic beverage with a maximum storage capacity of 3 days.

In tapping siwalan sap, special handling is needed, this is because the sap contains certain sugar levels such as fructose, sucrose, and glucose. If the sap is stored for too long, the sugar in the palm is fermented into alcohol and acetic acid due to the presence of enzymes and microorganisms such as glucokinase enzyme fofoglukoisomerase, phosphor frutokinase enzyme, aldolase enzyme, enolase enzyme, pyruvate kinase enzyme and acetobacter acetic can accelerate the process of fermentation of sap into alcohol and acetic acid. Therefore, after the tapping of the sap from the tree must be processed immediately, the minimal processing time is 90 minutes after the siwalan sap was taken from the tree [2].

To overcome spontaneous fermentation in siwalan sap could be done by heating pro- cess to inhibit the performance of microorganisms. In a study conducted by [2] the maximum ethanol and acetic acid levels produced by spontaneous fermentation were 4.358% and 4%. So that further treatment is needed to increase the ethanol content and to produce acetic acid with high purity.Utilization siwalan sap to produce useful product and have a longer shelf life is to process siwalan sap into acetic acid. Acetic acid is traditionally used as a food preservative, for health and beauty, solvents, flavor enhancers in the food industry, as raw material for making chemicals.

Acetic acid is one of the industrial products that is highly needed in Indonesia, the import of acetic acid reached 31.613.115, 200 tons with the value of 14.945.208, 41 US dollars [3]. Preparation of acetic acid can be done in two ways, ie synthetically/chemically and microbiologically or fermented, but fermentation is preferred, because it is cheaper, more practical and the small risk of failure. In the fermentation of acetic acid from the liquid substrate is generally only done two stages of fermentation namely alcohol fermentation and acetic acid fermentation. Alcohol fermentation is carried out if the ingredients used are rich in sugar content [3].

Some research results related to fermentation of vinegar, among other [4], using cassava skin substrate with concentration of aceti 10%, yielding 2.58% acetic acid, Juniawati using banana peel extract, and also coconut water each was given a combination treatment of *S. cerevisae* 15% and *A. aceti* 10% to produce a total acid of about 4%; Periadnadi, 1985 used fermented palm juice for 14 days with a dose of *S. cerevisiae* 7.5 ml/100 ml yielding a 12.3% alcohol content . Based on the description is known that the concentration and duration of yeast and bacterial fermentation, and the type of substrate effect on the resulting acid content. This research is to study the effect of ethanol fermentation aerobic pH on acetic acid product, in the utilization of siwalan sap to a more useful product and has a longer storage time is by processing the siwalan sap into acetic acid by fermentation process using bacteria *saccharomyces cereviceae* to convert siwalan sap into ethanol and *acetobacter aceti* to convert ethanol into acetic acid.

Experimental Section

Material And Methods

Siwalan sap was purchased from Tasikmadu Tuban, East Java – Indonesia. Siwalan sap analyzed by HPLC Agilent 1100 series with refractive index detector in ULFPP Airlangga University Surabaya to analyzed the glucose, fructose, and sucrose content. Acetic acid produced from the aerobic fermentation

process was analyzed using an alkalimetric method based on neutralization reaction and NaOH (1N) as standard solution carried out in the Department of Industrial Chemical Engineering, Vocational Faculty, ITS Surabaya.

Preculture of Cell for Ethanol Production

S.cerevisiae was obtained from Indus- trial Chemical Engineering Department, Faculty of Vocational, ITS Surabaya, Indo nesia. 180 ml of sterile Siwalan sap; 1 (g/l) of KH_2PO_4; 0.5 (g/l) of $MgSO_4.7H_2O$; 1 (g/l) of $(NH_4)_2SO_4$; and 10 (g/l) of yeast extract. The cultures were incubated at 30°C and 125 rpm and drown every single hour for the measurement of cell growth using Hemacytometer. By plotting the number of cell against a time, the log phase for culture growth could be determined. The cell number as the variables were designed in the range of log phase, thus in time of the cell number was achieved, 1 of ml culture were inoculated to 100 ml of sterile Sugar palm juice. The culture growth was monitored during the fermentation process by counting the number of cell in the sample for every 8 hours for 48 hours. The reducing sugar formed was analyzed in aliquots by the 3.5-dinitrosalicylic acid reagent using a visible spectrophotometer detector (Cecil CE 1011) with a wavelength of 540 nm [5].

Ethanol Fermentation process

The sterile sugar palm juice was mixed with the log phase starter in incubator shaker at 32 ^{0}C and 125 rpm for 24 hours *(Saccharomyces cerevisiae)*. Inoculum concentration (cell/ml per sugar concentration) has used as variable for ethanol fermentation with inoculum concentration 5000000; 10000000; 15000000 cell/ml. The ethanol concentration from fermen ted broth was analyzed using Gas Chromatography Scientific GC ULTRA, detector DSQ II, and coloumn MS 220.

Acetic Acid Fermentation process

Broth of ethanol fermentation was added with acetobacter aceti. The addition of acetobacter aceti is 10% of the total volume of broth. Fermentation of acetic acid was carried out by aerobic process with passing air into the fermented broth. Before fermentation acetic acid by acetobacter aceti to determine time of fermentation we used preculture of cell *acetobacter aceti*. 180 ml of sterile broth from ethanol fermentation; 1 (g/l) of KH_2PO_4; 0.5 (g/l) of $MgSO_4.7H_2O$; 1 (g/l) of $(NH_4)_2SO_4$; and 10 (g/l) of yeast extract. The cultures were incubated at 30°C and 125 rpm and drown every single hour for the measurement of cell growth using Hemacytometer. By plotting the number of cell against a time, the log phase for culture growth could be determined. The cell number as the variables were designed in the range of log phase, thus in time of the cell number was achieved, The culture growth was monitored during the fermentation process by counting the number of cell in the sample for every 8 hours for 120 hours. The reducing sugar formed was analyzed in aliquots by the 3,5-dinitrosalicylic acid reagent using a visible spectrophotometer detector (Cecil CE 1011) with a wavelength of 540 nm[5].

Result And Discussion

Preparation of Siwalan Sap

Siwalan sap is obtained from tapping siwalan trees in Tuban, East Java, Indonesia, filtered and sterilized at 121°C and 15 psi for 15 minutes. the sap content of palm sap was analyzed using High Performance Liquid Chromatography (HPLC). The results of High Performance Liquid Chromatography (HPLC) can be seen in Table 1.

Table 1. Siwalan sap content from HPLC analysis

Sugar name	Concentration (%b/v)
Glucose	2 %
Sucrose	1 %
Fructose	2 %

Sugar content in siwalan sap was quite low, because the sugar content of siwalan sap is influenced by varieties of siwalan plants, plant age, plant health and soil. The sweet taste of siwalan sap was reduced. So that the type of soil on the siwalan plantation can affect the sweetness level of the siwalan sap. Other causes of sugar content in siwalan sap is siwalan sap had not done pre-treatment process, so that the siwalan sap will be spontaneously fermented and effect the decrease of sugar content in siwalan sap.

Ethanol Fermentation Process.

Ethanol production from fermentation siwalan sap using *saccharomyces cereviceae* has inoculum concentration for variable. *Saccharomyces cereviceae* growth analysis by applying counting chamber system to detect a well-done fermentation. From Figure 1 can be seen saccharomyces cereviceae growth curve which show a cell growth of 426666666,7 cell/ml within 24 hours and at this stage balance growth or logphase with a constant growth is faced by the microorganism.

pH effect on ethanol production

Detecting pH effect upon batch fermentation by comparing pH performance in conversion of sugar into ethanol process by residual sugar analysis by DNS system applying acid reagent 3-5 dinitrisalisilate to detect reducing sugar content. Reducing sugar curve fermented by s,cereviciae for each ph can be see in figure 2. In Figure 2 sugar concentration curve which is declining during microorganism development, which shows that on batch fermentation reducing sugar within medium is used to produce carbon for *saccharomyces cereviceae* to synthesize energy through ethanol fermentation, highest cell growth at ph 6 is 15200000000 cells/ml sample.

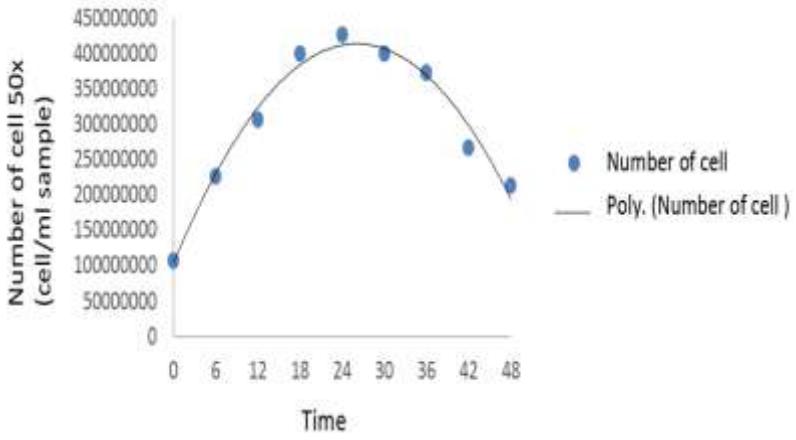

Figure 1. *Saccharomyces cereviceae* growth curve

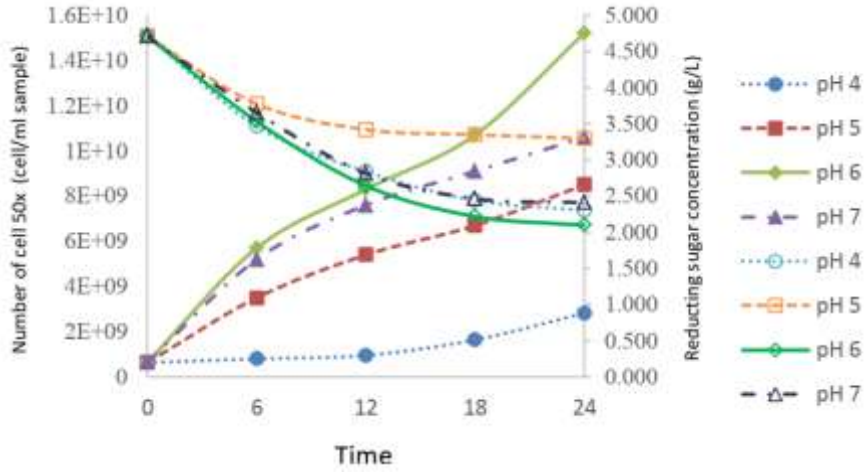

Figure 2. Reducing sugar as a function of time in batch fermententation using *Saccharomyces Cereviseae*

In this reasecarh unaerobic fermentation for 24 hours is taken to make reduction sugar decline steadily, From this research shows ph 6 can decrease 55 percent of reducting sugar concentration converted into ethanol of 8,20583 percent v/v through analysis of chroma- tography scientific gas *GC ULTRA* dengan *detector DSQ II* dan *column MS 220.* (Universitas Surabaya).

Acetic acid fermentation and pH effect in the fermentation

Acetic acid was form from ethanol, using acetobacter aceti to convert ethanol to acetic acid. Time of fermentation using log phase from *acetobacter aceti*. From this research log phase acetobacter aceti in 120 hours or 5 days (data not shown), however the other study mentioned that time of fermentation of *acetobacter aceti is* 72 – 80 hours [7]. Analyze acetic acid using acidi alkalimetri method with pp indicator [9]. In this study pH and temperature effect was analysis. From this research acetic acid form in 120 hours at pH 4; 5; 6; 7 with two different temperature 25^{o}C and 30^{o}C.

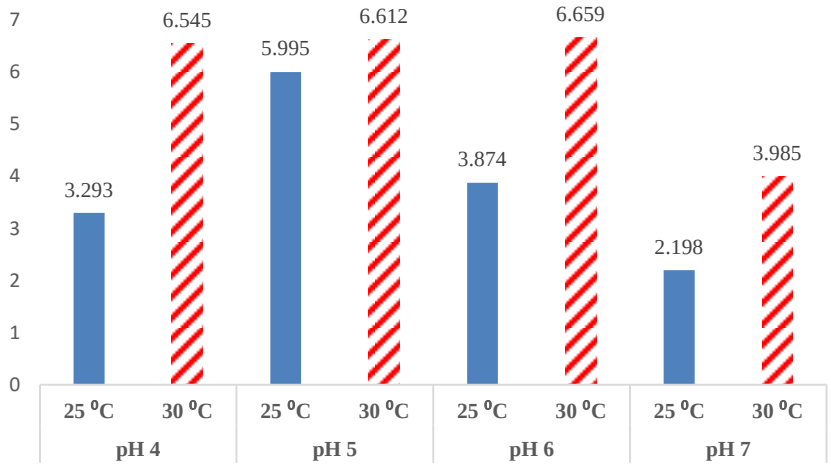

Figure 3. pH and Temperature Effect on the Acetic Acid Concentration

Figure 3 shows pH and temperature effects toward acetic acid produced, highest acetic acid concentration was on pH 6 and temperature 30^{o}C which is 6,659 g/l, this is in accordance with [8] stating that acetic acid was optimum at pH 5,5 and 30^{o}C, higher than this will cause *acetobacter aceti* damaged cells, lower than this will cause inactive *acetobacter aceti*, thus fermentation will not happen.

Statistical Analysis for Ethanol and Acetic Acid Production

Ethanol production

Producing alcohol from sugar fermentation uses 2 factors, pH with four levels pH 4, pH 5, pH 6 and pH 7, the second factor is time of fermentation with five levels are 0, 6, 12, 18 and 24 hours. following are test on effec t of experimantal factors, first is testing interaction effect on ph factor and observation time, if an unsignificant result, next test will be on similarity effect of ph and observation time factors, hypothesis test for the existance of interaction effect of ph factor and observation time, anova chart test statistic, the test is against ho, meaning that at least there is one hour of different orservation it can be detected from the test on parametre in this case factors like ph and observation time will have a significant effect but interraction between these two factors will not have any effect on acetic acid formation. Interraction of ph 4 and 5 and 7 but in the test the interaction has no significant effect toward produced ethanol percentage, it could be seen that ph 6 will produce higher ethanol percentage for all factor levels of observation time in comparison to all other, thats why ph 6 more suggested due to higher ethanol percentage produced.

Acetic Acid Production

From the analysis we can conclude that variable temperature give P-value = 0,036 < α (α = 0,05), this is indicating that temperature has effect for % acetid acid concentration, meanwhile pH has P-value pH = 0,103 > α, this is indicating that pH has no effect in acetid acid production. From figure 4 shows temp 30^{o}C will produce higher acetic acid concentration percentage than of 25^{o}C while pH 4 and 6 will produce the same result at 30^{o}C except pH 7 which will decline sharply.

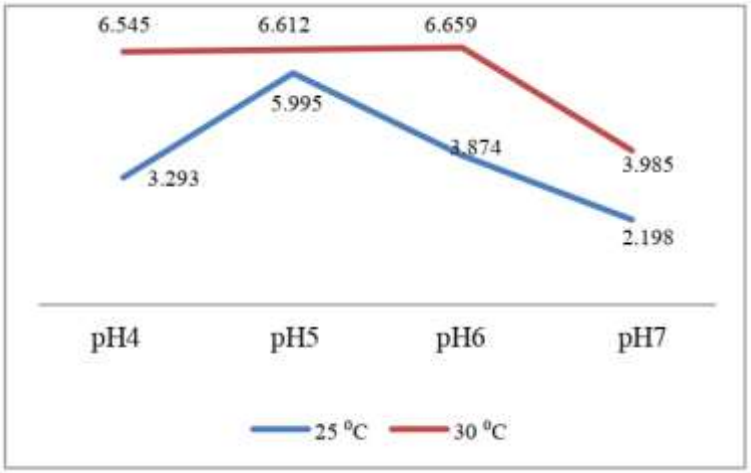

Figure 4. Plot % Acetic Acid Concentration Based On Temperature and pH

Conclusion

Palm juice can be unaerobic fermented produce acetic acid by adding *sacca- romyces cerevisiae* and aerobic fer- mented by adding *acetobacter aceti*, first fermentation using *saccaromyces cerevisiae* within 24 hours in order that reduction sugar could stably decrease, optimum ethanol could be got at optimum ph 6 which could decrease 55 percent of reduction sugar concentra- tion to produce 8,20583

%v/v ethanol using analysis of gas chromatography scientific GC ultra and detector dsq II and column ms 220. Fermentation acetic acid content using *acetobacter aceti* observed in 3 days at ph 6 and 30 ^{0}C will produce 6,659 g/l also shows that ph 4-6 at 30 ^{0}C will produce 6,605 g/l acetic acid. Aerobical fermentation of acetic acid in 3 days shows that ph 4-6 is highly affected by temperature. Statistical analysis shows, in ethanol production pH and fermentation time give a significant effect, but not significant for the interaction both of the two factors. Statistical analysis from acetic acid production is tem- perature giving a significat effect to acetic acid production ($P_{value} = 0{,}036 < \alpha$), while pH has no significant effect to acetid acid production ($P_{value} = 0{,}103 > \alpha$).

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