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Optimization of Initial pH and Total Sugar Concentration Variables on Citric Acid Production from Pineapple Waste with *Aspergillus niger* Yeast by Using Response Surface Methodology

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Abstract

Citric acid can be produced from pineapple waste by using fermentation process. This process is done in bubble column reactor with Aspergillus niger yeast. The objective of this research is to find the optimum conditions of initial pH and total sugar concentration. The optimization method used was response surface methodology. This research was carried out at a temperature of 30 ^{0}C, spore concentration of 1.23 x 10^9 spore/ml, total volume 2.0 liter, flow rate of air 58.07 cc/sec and a 5% antifoam concentration. The fermentation process lasted 7 days and the citric acid concentration was analyzed by High Pressure Liquid Cromatography (HPLC) method. Statistica 6 software was used for the data treatment. The mathematical model for the optimization citric acid fermentation in bubble column reactor is $Y = 54.507 + 2.985 X_1 - 8.987 X_1^2 - 2.581 X_2 - 15.446 X_2^2 - 7.989 X_1 X_2$. The parameter of Y is citric acid yield, X_1 is a coding initial pH and X_2 is a coding total sugar concentration. The results has given an initial pH optimum 3.61 and total sugar concentration 19,285% w/v with optimum an yield of 55.03 % .

Keywords: Bubble column bioreactor, Citric acid fermentation, Initial pH, Total sugar concentration, Response surface methodology

1. Introduction

The pineapple (*Ananas comosus, L. Mer*) is a tropical fruit which is widely produced across Indonesia and thus having a wide range of developmental prospects. Pineapples are utilized mainly in the food industry to be canned. The portion of the fruit that can processed and consumed is only about 53% of the whole, while the rest is the pineapple husk waste. To increase the economic value of pineapple husk waste, it can be used as raw material in citric acid fermentation. The results of analysis done by Sasaki et al. (1992), has shown the existence of carbohydrates such as glucose, fructose and sucrose, and has potential if use as raw material in fermentation processes

Citric acid is a naturally occurring compound that can be readily found in many kinds of plants, especially fruits. From the year 1993 citric acid has been produced in Indonesia using fermentation. This process has many advantages because the raw materials are cheap, it requires low operating temperature and pressures and it is energy efficient. Citric acid was produced by using raw material of tapioca industry waste, dried cassava, sugar palm flour and molasses (Judoamidjojo et al., 1992), solid discard and bagasse of fruits (Kumar et al., 2003) and generally use continuous stirred tank bioreactor (Rehm and Reed, 1985). Widayat et al. (2006) have used solid waste of pineapple as raw material on citric acid fermentation process. The result of this research shown sterilization by filter is the best method for air sterilization and followed thermal sterilization, and chemical sterilization.

Citric acid is an organic acid which have function of vital importance in beverages industry, industrial of pharmacy, industrial of cosmetic, metal industry and various other chemical industry. More or less 70% citrate used at beverages industry, 12% for the industry of pharmacy and 18% for other industry (Scraag, 1988). Citric acid is one of the most commonly used acids in the food and pharmaceutical industries on account of its high solubility, palatability and low toxicity. Millions of ton per year needed to fulfill industrial requirement. In Indonesia, amount of requirement of citrate estimated to reach 100.000 ton per year. Therefore, do not be surprised if this product assign value commercial very is profiting.

The objective of this research is to obtain the optimum initial sugar concentration and pH level using the response surface methodology. This research is done using a bubble bioreactor. This type of reactor has been widely used. The advantages of this reactor are that it has favourable mass and heat transfer characteristics and low operational an maintenance costs because it is more compact has few moving parts. Also, the addition and removal of microorganisms can be easily done. The variables that influence citric acid production are the conditions of the medium, sugar concentration, pH, oxygen supply, microbial nutrition and temperature. Antifoam is also needed in citric acid production to eliminate any foaming that occurs. If not dealt with, the formation of foam will result in infection through the wetting of the outlet air filters. Very large loss of volume will occur as aresult of changes in stirring patterns in the fermen- tation process.

Apergillus niger is predominantly used to produce citric acid.This is because it is easy to handle, economic, the substrates used are cheap and it produces high yields. The concentration of the microbe (*Asp. niger*) alone is important in the citric acid fermentation process, this has to do with the effective amount of microbes needed in the breakdown of carbohydrates and produce various organic compounds such as alcohol, piruvic acid, oxalic acid, etc. Thus, by using high amounts of *Asp. niger* spores, it is hoped that the amount of citric acid will be correspondingly large.

2. Methods

Waste pineapple materials (pineapple cusp and husk) are obtained from markets in the city of Semarang. The pineapple dilution analysis is done in order to find the content of sugars such as like glucose, fructose and sucrose by Loft Schoorl method. The ions were analyzed by the Absorption Atomic Spectroscopy (AAS) method. The strain of yeast used in this study was *Aspergillus niger* obtained from Biology Laboratory, Biology Department of Diponegoro University. All chemicals used were analytical grade. The preparation of inoculum medias starts with the transferring of the spores from the yeast to a Potatoes Dextrose Agar (PDA) medium. After the yeast has grown in the PDA medium, the media was observed for amounts of spores in the inoculums.

Table 1. Experiment design with central composite design

X_1	X_2	Initial pH	Total sugar concentration (% v/v)
0.000	-1.414	3.5	12.93
1.000	-1.000	4.0	15.00
1.000	1.000	4.0	25.00
0.000	0.000	3.5	20.00
-1.000	1.000	3.0	25.00
0.000	0.000	3.5	20.00
-1.000	-1.000	3.0	15.00
-1.414	0.000	2.8	20.00
0.000	1.414	3.5	27.07
1.414	0.000	4.3	20.00

Notes.
1. Compressor
2. Valve
3. Piping
4. Output valve
5. Rotameter
6. Buble bioreactor
7. Degaser
8. Membrene filtration

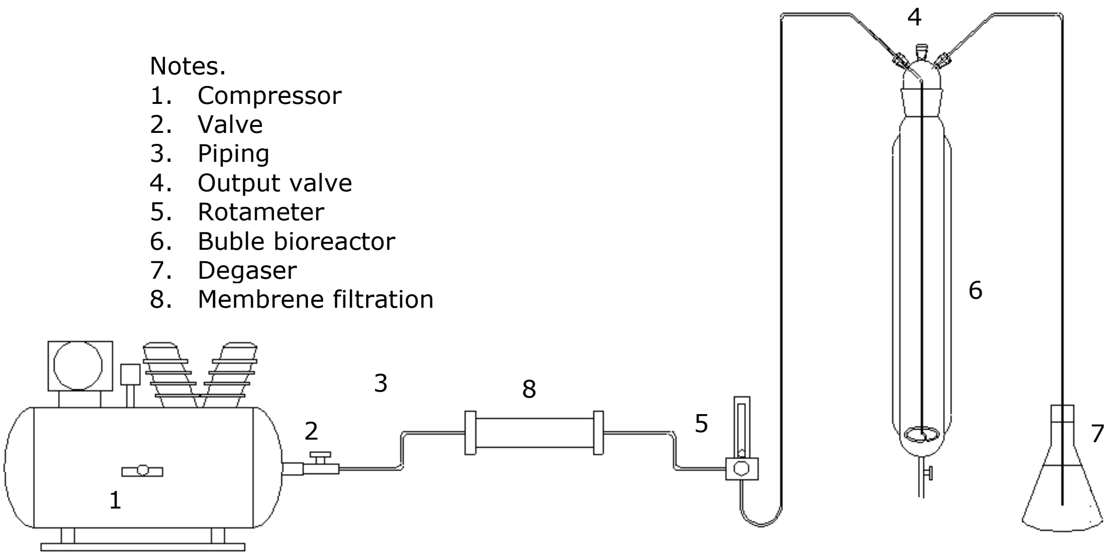

Figure 1. Equipment for experiments

The bioreactor used was a bubble column reactor with an inside diameter of 6,9 cm, external diameter of 7 cm and a height of84 cm. The Organic acid content was measured using HPLC (Waters TM 600). A 250 mm X 4.6 mm ID Spherisob Octyl column (Waters) with UV detector (210 nm) was used. The eluent used was H_2SO_4 0,005 M dan metanol 0,005 M with a 9: 1 ratio. Flow rate of eluent is 1 ml per minute at a temperature of 30°C. The air was sterilized with using mechanical method. The air is flowing to membrane filter. The membrane filter used was a hollow fiber type of acetate cellulose with model [illegible] 3 [illegible]0 mark of Baxter®. The equipment for experiment are shown in figure 1.

The experiment design used in this research like shown in Table 1. X_1 and X_2 are a coding for initial pH variable and total sugar concentration (% v/v). The correlation of X_1 and X_2 with initial pH and variable and total sugar concentration (% v/v) like shown in equation 1 and 2. Variabel response in this research are citric acid concentration, malic acid concentration and oxalix acid concentration.

$$X_1 = \frac{T - 3.5}{0.5} \quad (1)$$

$$X_2 = \frac{C\ total\ sugar - 20\ \%(v/v)}{5\ \%(v/v)} \quad (2)$$

Table 2. The results of experiments citric acid fermentation process

No	X_1	X_2	Yield of citric acid from observed
1	-1.000	-1.000	27.948
2	-1.000	1.000	29.568
3	1.000	1.000	22.843
4	1.000	-1.000	53.179
5	0.000	0.000	47.522
6	0.000	0.000	61.492
7	0.000	1.414	23.158
8	0.000	-1.414	17.452
9	1.414	0.000	35.122
10	-1.414	0.000	31.322

3. Results and discussion

In this experiment, citric acid is obtained from pineapple husk from fermentation process by *Aspergillus niger* yeast. The results of yield citric acid like shown in Table 2. The data in second and third column are a coding of variables process and the fourth column is yield of citric acid. The data from experiment runs 1-6 is then treated with Statistica with Box, Hunter and Hunter design. The results are calculated using equation 3.

$$Y = 33.3845 + 4.6265\,X_1 - 7.179\,X_2 - 7.989\,X_1 X_2 \quad (3)$$

Where Y is yield of citric acid, Y calculated from mass of citric acid divided by the mass of total sugar. Equation 3 has parameters or constant values 4.6265 and -7.179 for X_1, X_2 respectively. The parameter X_1 shows that the influence of initial pH levels to citric acid yield is positive. It means that an increase in pH levels will result in an increase in yield. Total sugar concentration has more influence than initial pH. Total sugar concentration has a negative influence on citric acid yield. The comparing value of parameter total sugar with parameter of initial pH is -1.552. Coefficient of interaction initial pH and total sugar concentration variables value negative. This value was higher than value in coefficient on single variable. This means that equation 3 is shown to be a non-linear mathematical model. So, the analysis supported a curvature check by ANOVAs /variance. The data analyzed ANOVAs by using Statistica 6 software. The results of analysis are shown in Table 3. The data for F is higher than value of probability (p), for curvature check. This shows that the mathematical model is a non linier equation and that equation 3 is a non-linear model. The mathematical model in equation 3 is a square equation.

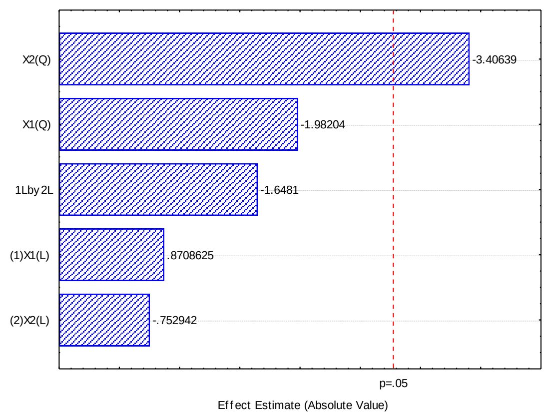

Figure 2. Pareto Diagram for Citric Acid Yield Analysis

The data in Table 2 (row 1-10) was analyzed by using Statistica 6 software. The process analysis uses central composite design or response surface methodology. The results include a Pareto chart and a ANOVAs Table, as shown in Table 4 and figures 2-5. From the data processing results we get a Pareto chart which illustrates how much influence variables (and variable interactions) have on the overall process.

Figure 2 shows that square of total sugar concentration has the most significant effect followed by the square of initial pH variable. The parameter square of total sugar concentration exceeds the minimum limit of p = 0.05 where p is the minimum effect estimate that a variable needs to have influence over response. Therefore we can say that to achieve optimum conditions, the accurate total sugar concentration and initial pH is needed. The results support the ANOVAs analysis.

In Table 4, it is shown that the value of F (in fifth column) was higher than value of p (sixth column). It means that the mathematical model (equation 4) can be used for optimization. The data from the observations were used to validate equation 4 as a mathematical model. The results of validated models are shown in figure 3. The analysis found an MS Residual value of 93.99.

Table 3. The results of analysis of variance 1-6 data from experiments

	Sum of Square	Degree of freedom	Mean of Square	F value	P value
Curvatr.	594.880	1	594.8800	6.096303	0.244983
(1)X_1	85.618	1	85.6180	0.877409	0.520800
(2)X_2	206.152	1	206.1522	2.112638	0.383643
1 by 2	255.296	1	255.2965	2.616267	0.352513
Error	97.580	1	97.5804		
Total SS	1239.527	5			

Table 4. The results of analysis of variance for all experiments

	Sum of Square	Degree of Freedom	Mean of Square	F value	P value
(1)X_1 (L)	71.282	1	71.282	0.75840	0.432975
X_1 (Q)	369.237	1	369.237	3.92849	0.118524
(2)X_2 (L)	53.285	1	53.285	0.56692	0.493373
X_2 (Q)	1090.611	1	1090.611	11.60353	0.027117
1L by 2L	255.296	1	255.296	2.71622	0.174678
Error	375.959	4	93.990		
Total SS	1877.825	9			

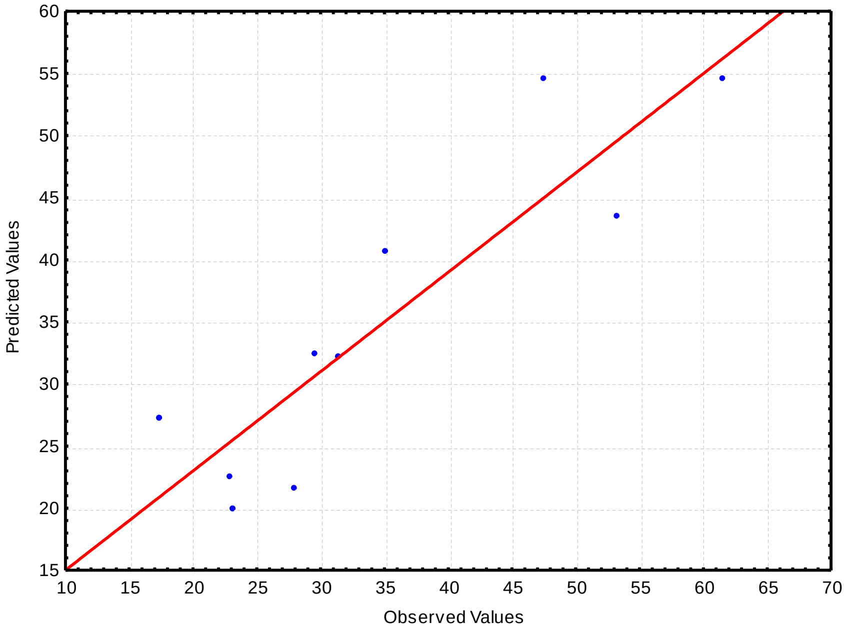

Figure 3. Correlation Graph for observed data versus predicted data for citric acid yield

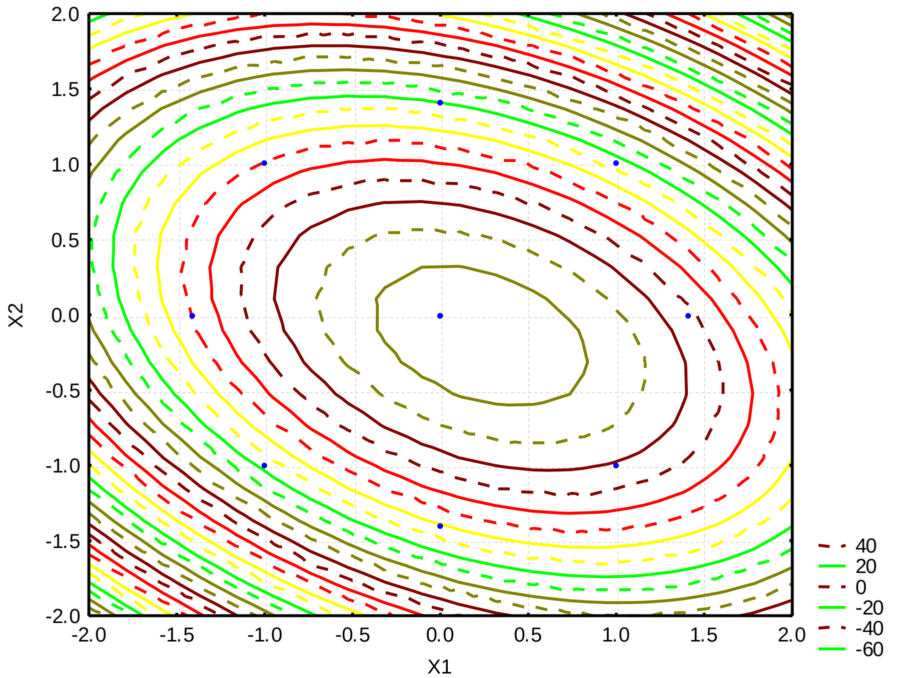

Figure 4. Contour graph for citric acid optimization

Figure 3 shows that all observed data corresponds closely to predicted data (from model). Therefore the model can be considered representative of the process of citric acid production using solid pineapple waste. So, equation 4 was then plotted to a contour graph and a surface response graph. The results of plotting are shown in Figure 4.

$$Y = 54.507 + 2.985\,X_1 - 8.987\,X_1^2 - 2.581\,X_2 - 15.446\,X_2^2 - 7.989\,X_1 X_2$$

Figure 4 shows a contour graph for citric acid optimization. The graph represents a simple maximum. The maximum condition for citric acid yield can be found from this graph. The area with colored dark red represents the maximum condition.

Table 5. Critical value for X_1 and X_2 variables

	Observed	Critical	Observed
X_1	-1.414	0.229	1.414
X_2	-1.414	-0.143	1.414

The value of maximum condition is shown in Table 5. The critical values for X_1 variable and X_2 variable are 0.229; -0.143 respectively. The X_1 is coding for initial pH variable, and the value of initial pH is 3.61. The X_2 is a coding for total sugar concentration variable, and the value of total sugar concentration is 19.285 %w/v. These conditions are the optimum conditions for citric acid production from pineapple solid waste with bubble column bioreactor and *Aspergillus niger* yeast. The optimum yield of citric acid is 55.03.

4. Conclusion

The research on the optimization of initial pH and total sugar concentration variables on citric acid fermentation process from pineapple solid waste with bubble column reactor and *Aspergillus niger* was done in a laboratory. The optimization process uses response surface methodology. The analysis and data calculations were done by using Statistica 6 software. The following conclusions can be taken from the research: The mathematical model for optimization is:

$$Y = 54.507 + 2.985\,X_1 - 8.987\,X_1^2 - 2.581\,X_2 - 15.446\,X_2^2 - 7.989\,X_1 X_2$$

The critical values for X_1 variable and X_2 variable are 0.229; -0.143 respectively. The X_1 is coding for initial pH variable, and the value of initial pH is 3.61. The X_2 is a coding for total sugar concentration variable, and the value of total sugar concentration is 19.285 %w/v. The yield optimum of citric acid is 55.03.

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