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Utilization Tofu Dregs as a Source of Nitrogen in Fermentation of Tuber Ganyong (*Canna edulis* Kerr.) by *Saccharomyces cerevisiae*

Rudi Kartika, I Putu Sukanadi, Arika Sukma Prasetya, Dedy Irawan

Abstract: This study was combined of tofu dregs powder as a source of nutients with enzimatic hyrolyzed tuber ganyong powder (*Canna edulis* Kerr.) in the ethanol fermetation process using *Saccharomyces cerevisiae* has been performed. This study also purpose to determine the concentration of tofu dregs powder and fermentation time was used to produce the optimum ethanol content. The enzymatic hydrolysis method carried out through liquification phase with α-amylase and saccharification phase with gluco-amylase. Furthermore the process of fermentation stage with the variation of the time (130 hours, 154 hours and 178 hours) using the *Saccharomyces cerevisiae* with the addition of tofu dregs powder as a nutrient at concentration variation (1 % (w/v), 2 % (w/v) and 3 % (w/v)). The results of the research, the highest ethanol content was found in the addition tofu dregs powder of 2 % (w/v) with fermentation time of 178 hours. The results of ethanol content obtained from density methods was 85 % and from gas chromatography methods was 84,451 %.

Index Terms: Ethanol, Fermentation, Tofu Dregs, Tuber Ganyong (Canna edulis Kerr.), Hydrolysis.

1 Introduction

Global demand for energy continues to increase due to increasing human population and increasing industrial development in developing countries. fossil fuels are still the main energy such as oil, coal and natural gas. the effects of greenhouse gases in the Earth's atmosphere have increased rapidly due to the use of fossil fuels from last centuries to the present. consequently the depletion of world energy supplies and unstable oil markets spurred researchers to search for alternative fuels. Ethanol has long been considered as a suitable alternative to fossil fuels either as a sole fuel in cars with dedicated engines or as an additive in fuel blends with no engine modification requirement when mixed up to 30% [1].

The development of bioethanol is an appropriate step in the face of the depletion of reserves oil world. The process of making of bioethanol itself is divided into 4 stages, namely preparation, pretreatment, hydrolysis and fermentation. Process technology of biofuel developing 2nd generation directs to the raw material of non-food which organic waste biomass conversion can be chosen. Waste organic is having lignocellulose contains what it contains 3 main-compliments, cellulose (30 - 50 % weight), hemicellulose (15-35 % weight) and lignin (13-30 % weight). The important production process of biofuel from organic waste is hydrolysis and fermentation.Hydrolysis of organic waste will be resulting reduction glucose [2].

To produce bioethanol using starchy materials should be initiated through the process of breaking starch into simple sugars or glucose by means of acid or enzymatic hydrolysis. Currently ethanol production has been widely used, by utilizing various sources of raw materials. But in the ethanol fermentation process is not only necessary source of carbohydrates (carbon), but also necessary nutrients that support the growth of microorganisms in the fermentation process. The common nutrients needed are nitrogen, phosphorus, sulfur and a small fraction also required vitamins such as biotin and riboflavin [3].

The development of bioethanol required nutrients, tofu dregs is known to have high level protein it content of 22.2%, so it can be used as a source of nitrogen for the formation of enzymes and high lipid level can be used as carbon sources, so that the microorganism will produce more enzymes and carbon required for microbial growth [4].

Ganyong (Canna edulis Kerr) is an herbaceous plant originating in South America. Ganyong is a potential plant as a source of carbohydrates. Rhizoma or tuber when grown can be eaten by processing it first or to be taken away [5].

From the above description, this study aims to combine the tofu dregs as a source of nitrogen with ganyong tuber that have been hydrolyzed enzymatically in the fermentation process using Saccharomyces cerevisiae and to search optimum time of fermentation and percentage of optimum dregs.

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2 Material and Methods

2.1 Breeding of Khamir *Saccharomyces cerevisiae* Making Media Agar

Weighed as much as 9.75 g Potato dextrose agar (PDA) and dissolved into 250 mL of aquades by homogenising. It was sterilized using an autoclave for 15 minutes at 121 °C and cooled in a reaction tube of 15 mL at room temperature and stored in the refrigerator until required.

2.2 Regeneration of Yeast

Taken *Saccharomyces cerevisiae* parent. It was then cultured on agar medium in a sterilized reaction tube, for approximately 24 hours at a temperature of 30 °C.

2.3 Making of Ganyong Flour

Ganyong tuber samples were cleaned of skin, washed and grated using electric coconut grater. Then dried under sun heat for ± 1 day and oven it at temperature 105 °C until dry. Furthermore, the dried sample is mashed using a blender, sieved and sterilized using an autoclave.

2.4 Hydrolysis Process

Liquification Process-The grinded ganyong tuber, as much as 1800 g is dissolved into 6500 mL of distilled water and arranged pH between 6-6.5. A 3.0 mL alpha-amylase was added and stirred at 80-90 °C for 1 hour. The liquification result is then cooled to ± 55 °C.

Saccharification Process-The result of the liquification process, rearranged the pH between 4-5 and added 3 mL of gluco-amylase. It is then stirred at 50-60 °C for 1 hour until it does not produce blue in the iodine test. It is then cooled to a temperature of ± 34 °C.

2.5 Fermentation of Saccharification Results by *Saccharomyces cerevisiae*

The sample result of the saccharification process was put in into 12 sterilized fermented containers. Then added the tofu dregs flour, to the fermentation bottle as much as 1% (w/v), 2% (w/v) and 3% (w/v) while homogenized. Furthermore, *Saccharomyces cerevisiae* added 2 ose on each bottle of fermentation and sealed with fermentation bottle using cotton and aluminum foil. Fermentation is carried out for 130 hours, 154 hours and 178 hours. Keep maximum temperature 35 °C with optimum pH 4-6.

2.6 Purification Process

Evaporation Process-Filtering of samples of fermentation obtained and inserted into a round bottom flask. Then put the flask on the evaporator that has been provided. Set the temperature to 60 ° C with pressure of 175 mbar for 2 hours. The results obtained are continued to the distillation stage.

Distillation Process-The resultant sample of the resulting evaporation process is put into the round bottom flask of the designed destillator. Further heated and set the temperature of 78 °C for 2 hours. The distillation process is carried out for 4 times. The result of distillation obtained was analyzed ethanol content.

2.7 Analysis of Ethanol Levels

Type Weight Method

The pycnometer is carefully cleaned using acetone, then dried and weighed initially. The pycnometer is filled with cooled water that has been cooled to below the experimental temperature (± 15 ° C) carefully to full and allowed to reach the experimental temperature (20 °C). The pycnometer containing the aquadest was immediately weighed and weighed [6].

$$\text{Density} = \frac{\text{weight of alkohol}}{\quad}$$

Gas Chromatography Method

The 2 µL distillate samples were injected by gas chromatography at column conditions: RTX-wax; detector: FID 1; temperature: 45 °C column, 200 °C detector and 145 °C injector with 70 kPa pressure; mobile phase: helium gas; stationary phase RTX-wax column (fused silica). Measurements are done at the Laboratory of Biochemistry FMIPA Mulawarman University.

3. RESULT AND DISCUSSION

3.1 Volume of Distillation Results

The distillation for the purification of the fermentation results was performed 4 times using simple distillation, so that the results obtained as table 1.

Table 1
Distilation Results

Fermentation Time (hour)	Concentration of Nutrition (%)	Destilation Volume phase I (mL)	Destilation Volume phase II (mL)	Destilation Volume phase III (mL)	Destilation Volume phase IV (mL)
130	Blank	7,5	5,0	3,5	3,0
	1	20,0	15,2	12,6	11,5
	2	23,5	18,0	17,5	16,6
	3	21,5	16,5	14,0	13,5
154	Blank	9,5	6,5	5,5	5,0
	1	21,4	17,5	14,4	13,0
	2	24,0	23,0	22,6	21,5
	3	22,2	19,0	18,2	17,0
178	Blank	10,0	7,5	6,8	6,0
	1	20,0	17,0	16,5	16,2
	2	24,5	23,5	22,8	22,5
	3	19,0	17,5	15,5	15,0

The distillation data above results in different distillation volumes of each type of time and nutrient concentration. So in the measurement stage of ethanol content obtained, used the same volume ratio by looking at the highest distillation volume of 22.5 mL. The purpose of this volume equivalence is to graph the percentage of ethanol content obtained can be seen clearly decrease and increase in each treatment.

3.2 Measurement of Ethanol Levels

Determination of ethanol content resulting from distillation results that have equalized volume, was done by using heavy density analysis method and gas chromatography analysis at Biochemistry Laboratory of Faculty of Mathematics and Natural Sciences Mulawarman University, to obtain the result as in Table 2.

Table 2
Ethanol content from analysis of method of gas type and gas chromatography

Fermentation Time (hour)	Concentration of Nutrition (%)	Volume (mL)	Retention Time	Area	Concentration of Ethanol
130	Blank	22,5	4,925	1899511	11,650
	1	22,5	5,006	7178448	44,027
	2	22,5	5,034	10318498	63,286
	3	22,5	5,021	8770186	53,790
154	Blank	22,5	4,950	2754924	16,897
	1	22,5	5,014	8090663	49,622
	2	22,5	5,055	12912526	79,195
	3	22,5	5,035	1019903	63,908
178	Blank	22,5	4,965	3759356	23,057
	1	22,5	5,027	9562151	58,647
	2	22,5	5,061	13769394	84,451
	3	22,5	5,039	10869595	66,666
Absolute Ethanol			5,081	161275503	98,914

Based on Table 2, from the measurement of ethanol content of the two methods, it was found that the optimum addition of the dregs meal in each fermentation time was 2% and the highest ethanol content was obtained at the time of fermentation to 178 hours. In Table 2, the time of fermentation that produces the highest ethanol of 84.451% can not be said as the optimum time, because it does not close the possibility at the time of fermentation more than 178 hours an increase in the amount and content of ethanol produced. So if the graph data made with the relationship of ethanol content with fermentation time on each addition of nutrients obtained graphs such as Figure 1.

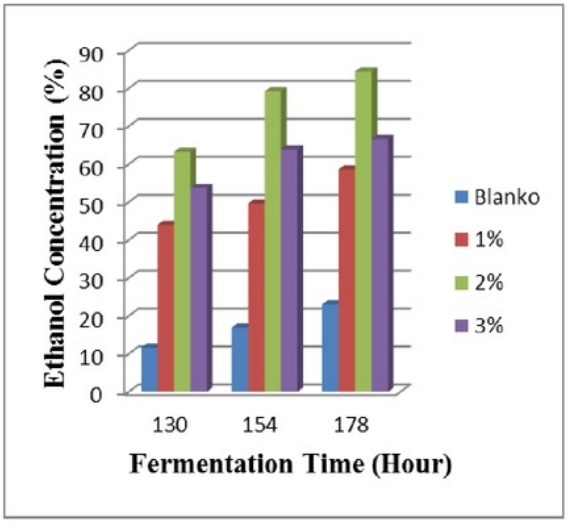

Fig.1 : Graphic relationship of ethanol content with fermentation time

Based on Figure 1, the ethanol content obtained continues to increase from the fermentation time to 130 hours up to 178 hours. So no optimum time has been found to ferment the ganyong tuber flour mixture with the pulp of tofu flour. While the role of the addition of tofu dregs flour know every fermentation time greatly affect the results obtained, where the results on the blank increase slowly and at the last time content of ethanol about 20%. This indicates that *Saccharomyces cerevisiae* needs additional nutrients for its survival, because the nutrients in the blank are insufficient for growth. So clearly visible when the addition of 1% flour dregs of tofu, the resulting ethanol content increased by about 30%. However, in the addition of 2% dregs know that the ethanol content obtained was higher, while in addition to the tofu dregs, 3% of the ethanol content obtained decreased.

This incident indicates that the optimum addition of tofu dregs flour as a source of nutrition as much as 2%, while the addition of 3% flour ganyong tubers ethanol content obtained decreased, because the amount of nutrients that have exceeded the limit of nutritional needs. Excess nutrients will adversely affect the survival of *Saccharomyces cerevisiae*, since the protein in nutrients is commonly used as a source of nitrogen for the formation of nucleic acids and amino acids in the growth of *Saccharomyces cerevisiae* cells. The high protein added in the fermentation process will form more and more amino acids. Amino acids that have been released, about 75% reused and excessive nitrogen will form urea and ammonia. Ammonia resulting from the decomposition of these amino acids may inhibit the synthesis of secondary metabolites [6].

3.3 Gas Chromatography Chromatogram results

The following chromatogram results of gas chromatographic measurements on ethanol absolute ethanol and fermentation results at any time of fermentation with the dregs knowing 2% concentration.

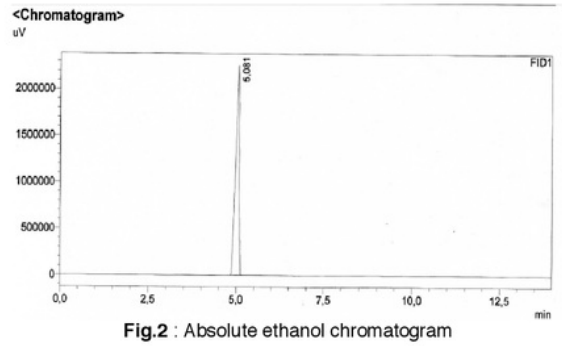

Fig.2 : Absolute ethanol chromatogram

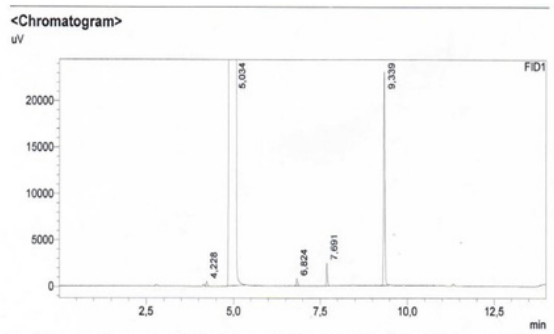

Fig.3 : Chromatogram at the time of fermentation to 130 hours with the addition of 2% nutrition.

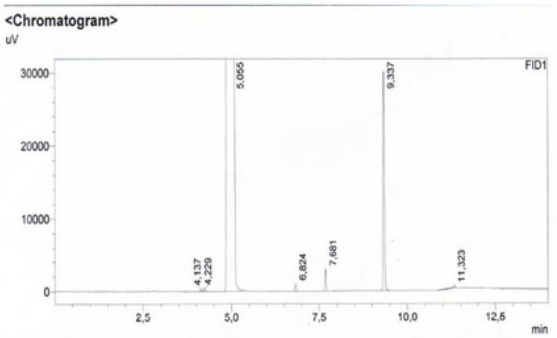

Fig.4 : Chromatogram at 154 hours fermentation with 2% addition of nutrients

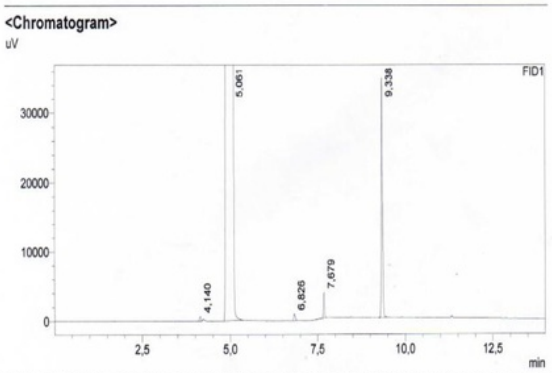

Fig.5 : Chromatogram at the time of fermentation to 178 hours with the addition of 2% nutrition.

Based on Figures 3 to 5 above, it can be stated that the results are still a lot of impurities, this is obvious when compared with absolute ethanol chromatgram (Figure 2). In general, readable chromatogram peaks other than ethanol also contain water, carboxylic acids and alcohol derivatives (such as methanol, 1-propanol, ethyl acetate and isobutanol) which are by-products of ethanol fermentation. The compounds are generally formed due to the longer fermentation time [6].

4.CONCLUSION

To get the optimum level of ethanol 84,451%, add 2% dregs of fermented flour with fermentation time for 178 hours.

5.CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest regarding the publication of this article.

6.ACKNOWLEDGMENT

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REFERENCES

[1]. F. Talebnia, D. Karakashev and I. Angelidaki, *Production Of Bioethanol From Wheat Straw: An Overview On Pretreatment, Hydrolysis and Fermentation,* Bioresource Technology, pp. 4744, 2010, doi :10.1016/j.biorte[illegible].2009.11.080

[2] D. Irawan and Z. Arifin, *Glucose Synthesis Of Organic Waste With Ultrasonic Technology Using Chloride Acid Catalyst Of Hydrolysis Process*, Rasayan Journal, pp. 1302, 2017, doi : 10.7324/RJC.2017.1041945

[3] M. J. Waites, N. L. Morgan, J. S. Rockey and G. Higton, *Industrial Microbiology*, School of Applied Science, South Bank University, London, pp. 86, 2001.

[4] A. Nur Aliyah, E. Deassy Edelweiss, M. Sahlan and A. Wijanarko, *Solid State Fermentation Using Agroindustrial Wastes To Produce Aspergillus Niger Lipase As A Biocatalyst Immobilized By An Adsorption-Crosslinking Method For Biodiesel Synthesis,* International Journal of Technology, pp. 1393, 2016, doi : 10.14716/ijtech.v7i8.6988

[5] T. Soenardi and S. Wulan, *Hidangan Nikmat Bergizi dari Bumi Indonesia Aneka Sajian Mi dan Olahan Lai,* PT. Gramedia Pustaka Utama, Jakarta, pp. 14, 2009.

[6] A. Adi Prabawa, E. Hanityo Utomo and Abdullah, *Produksi Enzim Invertase Oleh Saccharomyces cerevisiae Menggunakan Subtrat Gula Dengan Sistem Fermentasi Cair*, Jurnal Teknologi Kimia Industri UNDIP, pp. 144, 2012. http://ejournal-s1.undip.ac.id/index.php/jkti

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