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# Making Bioetanol from Glucose Off Grade With Fermentation Process Using Fermiol

*By Ni Ketut Sari*

# Making Bioethanol from Glucose Off Grade With Fermentation Process Using Fermiol

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**Abstract**—Fermentation can be defined as a process of anaerobic oxidation of carbohydrate which produces alcohol and carbon dioxide. Batch fermentation is fermentation system closed, where there is no addition of new media, there are no additions (O<sub>2</sub>), antifoam, acid/alkaline pH control is done in a way. the fermentation process using *Saccharomyces cerevisiae* producing optimum conditions on the addition of the *Saccharomyces cerevisiae* of 7% (v/v) and the time of fermentation produces ethanol for 10 days of 20.88% and a residual sugar of 2.44%. Use of microbial Nopkor Mz. held on the addition of the sugar with the levels of 15% (b/v) and time for 8 days of fermentation produces alcohol levels of 9.3%. From the research that has been done, the process of fermentation can take place using Microbe *Saccharomyces Cerevisiae*, *K. Marxianus*, Nopkor Mz, and *Zymomonas Mobilis*, Mz CP4. This research has been conducted on the analysis focusing on the purpose to find out the optimum time and glucose concentration, with a fermentation process for 7 days using glucose off grade and fermiol as well as urea and NPK nutrients as microorganisms. After dilution it is known that the content of glucose in glucose off grade raw materials amounting to 80%. From the results of this process of dilution is done according the levels of variables being run, then fermented for 7 days using fermiol. The best results on the fermentation of glucose levels at 14% and lasted for 6 days that generate levels of bioethanol of 11% (v/v) with the highest percentage yield of 61.63%.

**Keywords**—*bioethanol; glucose; fermiol*

## I. INTRODUCTION

Ethanol or ethyl alcohol is fermented product that can be manufactured from substrates containing carbohydrates. In the world of industrial ethanol is used as blends for beverages like sake or gin, pharmaceutical and cosmetic raw materials, fuel and a mixture of vehicles, fuel ethanol (gasohol) and as a source of oxygen for combustion cleaner substitute (methyl tertiary-butyl ether MTBE/). Because ethanol contains 35% oxygen, can increase the efficiency of combustion. Ethanol is also environmentally friendly because of the low levels of excess gas emissions of carbon monoxide, nitrogen oxides, and greenhouse gases that become pollutants so as not to

pollute the environment [1].

Bioethanol can be produced from a few raw materials, i.e. the sugared ingredients, starch, and lignocellulose. One of the materials that can be utilized to become sugared raw material in the manufacture of bioethanol is glucose off grade. Off grade glucose itself is not yet certified the glucose Standard National Indonesia (SNI). Glucose can be said to have fulfilled in a full grade LEVEL include, odorless, tasteless, colorless, sweet water content maximum 20% b/b, grey levels maximum 1% b/b, and reducing sugar levels at least 30% b/b. previous studies on the production of glucose from sago starch stated that one of the indicators of causes not yet certified sugar is a reddish yellow on the glucose that is caused by the presence of reducing sugars and protein in starch which led to occurrence of brownish, in addition total dissolved solids (TPT) expressed in ° Brix in the liquid sugar is still low i.e. by 60 ° Brix so still there is moisture content in large numbers, and also typical flavored sugar glucose [2].

In the year 2010 needs glucose in Indonesia reached 21360 tons every year and has increased in the year 2011 to reach 29331 tons annually. This indicates that the need of glucose in Indonesia has increased every year [3]. Food and beverage industry currently has a tendency to use glucose syrup as raw sugar substitute. But most of the industry prefers to use glucose syrup which has been SNI standard as the raw material manufacture its products than on using glucose off grade thus causing a decline in selling prices of glucose off grade on the market [2]. It need to be made off of glucose have grade to grade off glucose selling power can be increased. One solution is to change the grade off glucose into products bioethanol using the fermentation process.

Fermentation can be defined as a process of anaerobic oxidation of carbohydrate which produces alcohol and carbon dioxide. Batch fermentation is fermentation system closed, where there is no addition of new media, there are no additions, antifoam, acid/alkaline pH control is done in a way. Batch fermentation are widely used in industry to produce ethanol because of the ease of sterilization process and control tools [4]. But there are some disadvantages of

batch fermentation, i.e., barriers due to the high sugar levels, the concentration of the ethanol yield limited (12%) and productivity levels because the ethanol produced is low, due to the accumulation of ethanol, formed by the fermenters will poison the microorganism who was instrumental in the formation of ethanol itself, so that the growth and production of microorganism can be stopped [5]. Despite this batch fermenting still an option because the yield obtained is higher than with other methods [4].

Previous research for the optimization of fermentation process using *Saccharomyces cerevisiae* producing optimum conditions on the addition of the *Saccharomyces cerevisiae* of 7% (v/v) and the time of fermentation produces ethanol for 10 days of 20.88 % and the rest of the sugar of 2.44%. Optimum conditions of making wine from apples rejects using microbial Nopkor Mz. held on the addition of the sugar with the levels of 15% (b/v) and time for 8 days of fermentation produces alcohol levels of 9.3% [1]. Other studies on the production of ethanol from molasses optimum conditions in *Borassus flabelifer* fermentation using *Saccharomyces cerevisiae* bacteria i.e. at pH 4.8, the concentration of sugar is 110 g/L, a concentration inoculum 12,740,970 cells/ml and obtained yield ethanol 23.68% (y/y) [4]. Ethanol can also be retrieved from jackfruit seeds fermented using *Saccharomyces cerevisiae* grams 0.03, 0.1 grams of urea 0.05 grams NPK for 6 days generate levels of 13% ethanol % (v/v) [6]. According to [7] in the production of tuber bioethanol Ube using *Saccharomyces cerevisiae*, optimum conditions are held on with the addition of 1.5 % of the yeast and nutrients from 0.1 grams/100 ml of urea and NPK 0.1 g/100 ml and the time of fermentation for 12 days 9.97 bioethanol levels obtained% (v/v).

Optimum conditions for fermentation of ethanol from 30°C temperatures occur in the mangrove, the stirring speed 360 rpm and fermentation lasted 89 minutes produce ethanol with a yield maximum 69.58% [8]. Other studies mention that the optimum fermentation conditions of sugar cane by using *Zymomonas mobilis* CP4 obtained at concentrations of inoculum urea concentration of 15%, 0.3%, long fermentation 45 hours and the resulting optimum 1.257 ethanol % (v/v) [9]. Conditions optimum for the fermentation of ethanol from mango upgrade using microbe K. Marxianus is at a concentration of glucose 20 °Brix, pH 4.6 for 12 days, gained a 5% ethanol (b/v) [10].

From the research that has been done, the process of fermentation can take place using Microbe *Saccharomyces cerevisiae*, K. Marxianus, Nopkor and *Zymomonas mobilis*, Mz CP4. On the research we do focused knowing the concentration of glucose and the optimum time, with fermentation for 7 days using glucose off grade and Fermiol as well as urea and NPK nutrients as microorganisms. On the fermentation of glucose off grade be bioethanol for 7 th day use fermiol 0.15 g/L urea and NPK, as much as 0.1 g/100 ml as the nutrient levels of the highest bioethanol, allegedly resulting in glucose levels of 14% on the sixth day.

## II. THE PROCESS BIOETHANOL

### A. Glucose Off Grade

One of the materials that can be utilized to become sugared raw material in the manufacture of bioethanol is glucose off grade. This grade is off glucose has not certified the Standard National Indonesia (SNI) so it has a low price, in contrast to the price of the glucose has standard SNI which is still expensive in the market. In fact the need for glucose syrup is still high but this high requirement has yet to be fulfilled so do imports of sugar as an alternative. Glucose syrup is usually used as an additional ingredient in the manufacture of soy sauce, syrup, candy, and others [2].

TABLE I. THE QUALITY OF THE MOLTEN SUGAR BEST TREATMENT

Parameters	Unit	SNI
The smell of	-	Odorless
A sense of	-	Sweet
Color	-	Colorless
Water	%b/b	A maximum of 20
Abu	%b/b	A maximum of 1
Reducing sugar calculated as D-glucose	%b/b	At least 30
metal Impurities:		
Lead (Pb)	ppm	Maximum 1
Copper (Cu)	ppm	Maximum 10
Zinc (Zn)	ppm	Maximum 25
Arsenic (As)	ppm	Maximum 0.5
Microbial Impurities:		
Plate Number	colony/g	Maximum 5x10 <sup>2</sup>
Total Mold	colony /g	Maximum 50
Yeasts	colony /g	Maximum 50

(Source: [2])

### B. Fermentation process

The process of converting glucose generally require anaerobic conditions for maximizing the manufacture of ethanol [11]. Yeast used in the fermentation of alcohol which results primarily ethanol [12]. Anaerobic conditions, microbes that can be used is a *Saccharomyces Cerevisiae* [13]. *Saccharomyces cerevisiae* is a yeast which is important in the manufacture of alcoholic beverages like beer, wine, and is also used in making bread dough [12]. *Saccharomyces Cerevisiae* is a Microbe that is best for the fermentation of ethanol because it is relatively more efficiently convert the sugar into ethanol and more tolerant of ethanol [13]. In addition to *Saccharomyces Cerevisiae* and *Zymomonas Mobilis* is also very potential. However, these bacteria need to be developed further, due to a low against the toleration of salt in the media and in need of a sterile medium, so that makes it difficult for industrial-scale application [14].

Microorganism *Saccharomyces Cerevisiae* can be obtained from fermiol, red star, Baker's yeast, all tech, and gert strand. Based on the research results with the test conditions are some of the highest temperatures are obtained by successive ethanol yield is red star, fermiol, gert strand, Baker's yeast, and all tech [15].

The advantages the use of fermiol Strain No. 7221 DY: Reduce the addition of yeast, generate little or no foam, reduce glycerol, can the fermentation takes place at higher temperatures, without the risk of stuck fermentation, result of higher alcohols in the absence of a barrier [17], short adaptation Phase, low acid production [16].

**C. Bioethanol Product**

The ethanol used in the drink obtained from the brewing of carbohydrates catalyst enzymes. One type of enzyme changing carbohydrates into glucose, then into ethanol, the type of the other produce vinegar acid with ethanol as an intermediate. Source of carbohydrates for brewing relies on the availability of the purpose of the use of alcohol. Brewing of fruits, vegetables or whole grains stops when the alcohol levels had reached 14-16%. If a higher rate is desired, the mixture had to be distilled. Distillate (distilled) azeotrope is a mixture of 95% alcohol 5%. An azeotrope is a mixture boils at a constant boiling point, as if it's a pure compound). This distillate can be blended back into the mix of brewing to increase the levels of ethanol, or can be added to water to get the desired levels (Fessenden, 1982). Bioethanol has a higher oxygen content which is around 35% which helps more perfect combustion and environment-friendly because it contains gas emissions lower CO [11].

TABLE II. EXPERIMENTAL RESULTS OF VARIOUS ETHANOL-PRODUCING YEAST

	Residual Sugar	Total Sugar % v/v	Ethanol % v/v	Yield Ethanol
Temperature 37.5 °C				
Fermiol	9.33	37.84	13.73	62.16
Alltech	11.19	45.36	11.78	54.64
Red Star	8.44	34.23	14.34	65.77
Gert Strand	8.75	35.47	14.11	64.53
Baker's Yeast	11.38	46.13	11.88	53.87
Pada suhu 35°C				
Fermiol	5.93	24.05	15.85	75.95
Alltech	9.54	38.68	13.48	61.32
Red Star	5.37	21.78	16.06	78.22
Gert Strand	6.88	27.89	15.08	72.11
Baker's Yeast	9.21	37.35	13.79	62.65
Temperature 32°C				
Fermiol	4.85	19.65	17.03	80.35
Alltech	9.61	38.95	13.93	61.05
Red Star	4.23	17.15	17.10	82.85
Gert Strand	5.25	21.27	16.27	78.73
Baker's Yeast	8.99	36.46	14.27	63.54

(Source: [15])

Bioethanol is ethanol made from biomass containing cellulose or starch component. Raw materials bioethanol can be obtained from various plants which produce sugars (such as sugar cane and molasses), flour/starch such as corn, sago, and tubers such as cassava [7].

**III. METHODOLOGY RESEARCH**

Based on the results of the initial material analysis (glucose off grade) obtained the following data: level 80 %.

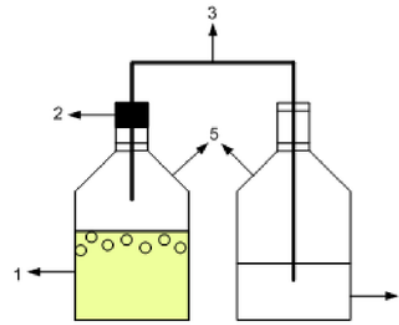


Image captions:

- (1) Off grade of glucose; (2) Bottle Cover; (3) The connecting Hose; (4) Aquadest; (5) The bottle

Fig. 1. Making off grade glucose setting

**Research procedure:**

- a. Prepare tools and materials as well as a series of fermenters were used.
- b. Glucose off grade that has been analyzed then dissolved with aquadest up to 800 ml with the appropriate concentration of the independent run.
- c. Stir in the glucose solution into each bottle, and then sterile with autoclave for 15 minutes, chill ± 30°C.
- d. Then enter fermiol into the bottle containing a solution of glucose.
- e. Add urea and NPK nutrients as microorganisms.
- f. Close the bottle meetings lest there is the air into the bottle. Connect the hose to the presence of CO<sub>2</sub> indicators 1 and 2 for the sampling interval.
- g. Once the bottle tightly closed and all hoses are already set, the fermentation process is done accompanied by stirring speed 100 rpm for 1 hour every day.
- h. Analysis of the filtrate is fermented according the independent variable (3, 4, 5, 6, 7) days.

**IV. RESULTS AND DISCUSSION**

Based on chart Figure 1 can note that on day 3 to day 5 each increase the levels of alcohol except at 10% glucose levels which increase the levels of alcohol on the 4th day, constant on day 5 and decline alcohol levels on day 6. On the 7th day of the samples with glucose levels by 10% and 18% experienced a decrease in the levels of alcohol. This condition indicates that the initial glucose levels that are too low and too high can affect the levels of alcohol produced. Where the levels of alcohol in the initial glucose levels that are too high and too low can cause the resulting alcohol levels will be low.

Fermentation lasted 7 days with variable levels of glucose 10%; 12%; 14%; 16%; and 18% and obtained the following data:

TABLE III. THE LEVELS OF ETHANOL

The Initial Glucose Levels (%)	Ethanol Levels (%)				
	The Fermentation Time (Day)				
	3	4	5	6	7
10	2	4	4	3	2
12	5	6	7	8	8
14	6	8	10	11	11
16	6	7	8	10	10
18	3	5	6	6	5

After the fermentation process takes place in accordance with the variables being run, then obtained the best conditions to obtain the highest levels of ethanol. The best conditions in glucose levels 14% with the best fermentation time on the 6th day. To see the levels of glucose which can conversion then do analysis of glucose levels before and after fermentation the fermentation. As for the results of the analysis of the levels of glucose are as in table IV:

TABLE IV. THE LEVELS OF GLUCOSE

The Initial Glucose Levels (%)	The Remaining Glucose Levels (%)				
	The Fermentation Time (Day)				
	3	4	5	6	7
10	7.8	6.8	6.4	6.2	5.8
12	7.4	6.6	5.4	4.6	4
14	8.2	6.8	4.6	3.6	3.2
16	11.4	10.2	8.8	6.6	6
18	16.4	15.4	14	13.2	12.2

Previous research for the fermentation process using Ube bulbs *Saccharomyces cerevisiae* producing optimum conditions on the addition of the *Saccharomyces cerevisiae* 1.5% (b/v) as well as the nutrients from 0.1 g/100 ml of urea and NPK 0.1 g/100 ml for 12 days 9.97 bioethanol levels obtained% (v/v) [7]. Optimum conditions of making wine from apples rejects using microbial Nopkor Mz. held on the addition of the sugar with the levels of 15% (b/v) and time for 8 days of fermentation produces alcohol levels of 9.3% [1]. If compared with our study, bioethanol from glucose off grade have higher levels of ethanol that is 10% on the initial glucose levels 16%, using fermiol 0.15 g/litre, the addition of urea and NPK 0.1 gr/100 ml, and long fermentation of 6 days. This is due to the use of fermiol that can produce higher alcohol levels of use of *Saccharomyces Cerevisiae*.

According to [1] to *Saccharomyces Cerevisiae* used in fermenting wine, the alcohol-making using low initial sugar

levels cause growth of yeast that short time resulting in low alcohol levels. This is because the number of microbes that form more when compared with the amount of food available at the beginning of fermentation.

TABLE V. YIELD AT OPTIMUM LEVELS OF ETHANOL

Glucose Levels (%b/b)	Mas of Glucose (grams)	Levels Ethanol (%v/v)	$\rho$ Ethanol (g/ml)	Volume Ethanol (ml)	The Mass Ethanol (grams)	Yield (%)
10	100	4	0.9910	32	31.7129	31.71
12	120	8	0.9848	64	63.0259	52.52
14	140	11	0.9805	88	86.2814	61.63
16	160	10	0.9819	80	78.5496	49.09
18	180	5	0.9894	40	39.5752	21.99

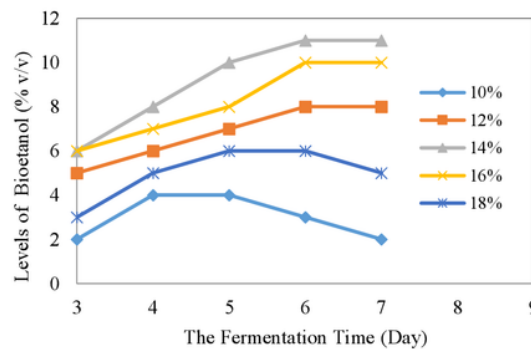


Fig. 2. The relationship between levels of bioethanol fermented with fermentation time

According to [14] initial substrate levels that are too high can reduce the amount of dissolved oxygen before the fermentation process takes place. Although in very small amounts, fixed oxygen needed by *Saccharomyces cerevisiae* for microbial metabolic processes.

After the fermentation runs and obtained the best time to reach the highest levels of alcohol, alcohol that is produced will be stable for some time and then declined. This is because in the fermentation process of bacterial stationary phase and then experiencing a phase of death, but the time to reach the stationary phase is different on each treatment. On treatment with glucose 10% stationary phase occurred from day 4 to day 5. On treatment with glucose 18% stationary phase occurred from day 5 to day 6. This shows that using the initial glucose concentration is too high and too low can accelerate the time to reach the stationary phase. In contrast to the treatment that uses the initial glucose levels 12%; 14%; and 16% to reach the stationary phase on day 6 to 7. Phase of death occurs on day 5 to day 7 for the initial 10% glucose levels, and on day 6 to day-7 initial glucose levels for 18%. In the phase of death not all of the bacteria die. Some bacteria

living oxidation alcohols so most alcohol formed is oxidized into pillars of compounds and carboxylic acids. So there happen levels decrease alcohol should do so the bacteria die warming.

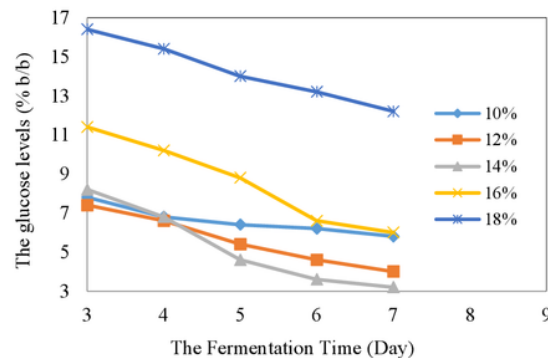


Fig. 3. The Relationship Between Levels Of Glucose Fermentation Time Remaining

Based on Figure 3 can note that the highest residual glucose levels found in the treatment using glucose levels beginning 18% and the lowest at the treatment by using the initial glucose levels 12%. On treatment with initial glucose levels that are too high may cause glucose levels remaining high because it cannot be broken down completely by microbes. According to [1] on the influence of the addition of sugar in the manufacture of wine of Apple rejects, the greater the ratio of glucose at the start of the fermentation process caused a collision between molecules of the reactants with the microbes increases, so the intrusion of molecules microbes into the substrate, occur more frequently and cause glucose has not broken down completely. On treatment with glucose levels too low can also cause glucose remaining high enough, this is because the amount of food a little microbes that cause early reproductive cells of microbes that bit anyway so that glucose has not unraveled the perfect by microbes.

On treatment with the use of an initial 12% glucose can give a fairly high levels of ethanol and glucose levels remaining low due to satisfy food needs early so that microbes can reproduce well and glucose can be fermented into alcohol. Based on the overall data, residual levels of glucose results obtained at the end of fermentation can be aware that the remaining glucose levels obtained in accordance with the results of glucose that does not conversion to alcohols.

The best conditions of fermentation of glucose off grade by using fermiol the initial glucose levels take place at 16% and time best fermentation lasted 6 days produce ethanol levels of 10% (v/v). This limits the use of *Saccharomyces cerevisiae* because tolerance with the trade name fermiol which can produce alcohol with levels of more than 10%

(v/v) than ordinary *Saccharomyces Cerevisiae* will die when the resulting alcohol levels touching 10% (v/v). Because fermiol is *Saccharomyces Cerevisiae* modified by the factory so that it has the capability of generating levels of alcohol more than 10% (v/v). However, the optimum levels of alcohol are produced in this research cannot be more than 10% (v/v) due to fermiol being used has been saved long ago so that their effectiveness slightly reduced so that it can lower the levels of alcohol produced.

#### V. CONCLUSION

- After dilution it is known that the content of glucose in glucose off grade raw materials amounting to 80%, from the results of this process of dilution is done according the levels of variables being run, then fermented for 7 days using fermiol.
- The best results on the fermentation of glucose levels at 14 (%v/v) and lasted for 6 days that generate levels of bioethanol of 11 (%v/v) with the highest percentage yield of 61.63%.
- The fermentation process takes place, obtained the smallest residual glucose levels 3.2% in treatment with the use of the initial glucose levels 14%, while the largest remaining glucose levels 12.2% in treatment with the use of the initial glucose levels 18%.

#### 4 ACKNOWLEDGMENT

The authors would like to acknowledge the financial support of the Directorate Research and Public Service. The Directorate of Research and Development Strengthening. The Ministry of Research, Technology, and Higher Education of the Republic of Indonesia with the Research-Based Competence Grant. Contract Number: 083/SP2H/LT/DRPM/2018.

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