NUSANTARA BIOSCIENCE
Vol. 2, No. 1, pp. 7-13
March 2010

ISSN: 2087-3948
E-ISSN: 2087-3956
DOI: 10.13057/nusbiosci/n020102

Comparasion of iles-iles and cassava tubers as a *Saccharomyces cerevisiae* substrate fermentation for bioethanol production

KUSMIYATI♥
Study Center for Alternative Energy, Department of Chemical Engineering, Faculty of Engineering, University of Muhammadiyah Surakarta. Jl. Ahmad Yani Tromol Pos 1 Pabelan, Kartosuro, Sukoharjo 57102, Central Java, Indonesia. Tel./Fax.: 271-717417 ext 442/ 271-715448. ♥email: rahmadini2009@yahoo.com

Manuscript received: 8 February 2010. Revision accepted: 16 March 2010.

Abstract. *Kusmiyati. 2010. Comparison of iles-iles and cassava tubers as a Saccharomyces cerevisiae substrate fermentation for bioethanol production. Nusantara Bioscience 2: 7-13.* The production of bioethanol increases rapidly because it is renewable energy that can be used to solve energy crisis caused by the depleting of fossil oil. The large scale production bioethanol in industry generally uses feedstock such as sugarcane, corn, and cassava that are also required as food resources. Therefore, many studies on the bioethanol process concerned with the use of raw materials that were not competing with food supply. One of the alternative feedstock able to utilize for bioethanol production is the starchy material that available locally namely iles-iles (*Amorphophallus muelleri* Blum). The content of carbohydrate in the iles-iles tubers is around 71.12 % which is slightly lower as compared to cassava tuber (83,47%). The effect of various starting material, starch concentration, pH, fermentation time were studied. The conversion of starchy material to ethanol has three steps, liquefaction and saccharification were conducted using α-amylase and amyloglucosidase then fermentation by yeast *S.cerevisiaie*. The highest bioethanol was obtained at following variables starch:water ratio=1:4 ;liquefaction with 0.40 mL α-amylase (4h); saccharification with 0.40 mL amyloglucosidase (40h); fermentation with 10 mL *S.cerevisiae* (72h) producing bioethanol 69,81 g/L from cassava while 53,49 g/L from iles-iles tuber. At the optimum condition, total sugar produced was 33,431 g/L from cassava while 16,175 g/L from iles-iles tuber. The effect of pH revealed that the best ethanol produced was obtained at pH 5.5 during fermentation occurred for both cassava and iles-iles tubers. From the results studied shows that iles-iles tuber is promising feedstock because it is producing bioethanol almost similarly compared to cassava.

Keywords: alternative energy, cassava, iles-iles, bioethanol.

Abatrak. *Kusmiyati. 2010. Perbandingan umbi iles-iles dan singkong sebagai substrat fermentasi Saccharomyces cerevisiae dalam produksi bioetanol. Nusantara Bioscience 2: 7-13.* Produksi bioetanol meningkat dengan cepat karena merupakan energi terbarukan untuk mengatasi krisis energi yang disebabkan oleh habisnya minyak fosil. Produksi bioetanol skala besar di industri umumnya menggunakan bahan baku seperti tebu, jagung, dan ubi kayu yang juga diperlukan sebagai sumber makanan. Oleh karena itu, banyak studi pada proses bioetanol terkait dengan penggunaan bahan baku yang tidak bersaing dengan pasokan makanan. Salah satu alternatif bahan baku dapat dimanfaatkan untuk produksi bioetanol adalah bahan berpati yang tersedia secara lokal yaitu iles-iles (*Amorphophallus mueller* Blum). Kandungan karbohidrat umbi iles-iles sekitar 71,12% yang sedikit lebih rendah dibandingkan dengan umbi singkong (83,47%). Pengaruh berbagai bahan awal, konsentrasi pati, pH, waktu fermentasi dipelajari. Konversi dari bahan berpati menjadi etanol memiliki tiga langkah, pencairan dan sakarifikasi dilakukan dengan α-amilase dan amyloglucosidase kemudian difermentasi dengan ragi *S.cerevisiaie*. Bioetanol tertinggi diperoleh pada variabel berikut rasio pati: air = 1:4; likuifaksi dengan 0,40 mL α-amilase (4h); sakarifikasi dengan amyloglucosidase 0,40 mL (40h); fermentasi dengan 10 mL S.cerevisiae (72h) memproduksi bioetanol 69,81 g/L dari singkong sementara 53,49 g/L dari umbi iles-iles. Pada kondisi optimum, gula total dihasilkan 33.431 g/L dari ubi kayu sementara 16.175 g/L dari umbi iles-iles. Pengaruh pH menunjukkan bahwa etanol yang dihasilkan terbaik diperoleh pada pH fermentasi 5,5 baik untuk ubi kayu maupun umbi iles-iles. Hasil studi menunjukkan bahwa umbi iles-iles menjanjikan sebagai bahan baku bioetanol karena menghasilkan bioetanol hampir sama dengan ubi kayu.

Key words: singkong, iles-iles, etanol, energi alternatif.

INTRODUCTION

Ethanol is an alternative fuel that is important in reducing the negative impacts of fossil fuel consumption (Cardona and Sanchez 2007). Fossil fuel consumption in the world reached 80%(Gozan et al. 2007). Fuel demand in Indonesia reached 5.6% per year, this has caused Indonesia to be the only OPEC member country that has to import crude oil by 487 thousand barrels/day since the end of 2004. Therefore, the use of biofuel such as bioethanol is one of the alternatives to overcome fuel crisis. Bioethanol is colorless liquid and environmentally friendly which results in the form of combustion gases, and air pollutants such as CO Nx are very small. Many researchers concluded that ethanol does not cause the greenhouse effect of fossil fuels because hazardous gases such as CO_2 which is reduced by 22% (Milan 2005). Bioethanol can be used to substitute premium and kerosene. According to Kusmiyati

Figure 1. a. 4-month-old cassava plants, b. 5 months old iles-iles plants, c. Cassava tubers after harvest, d. Iles-iles tuber that has been harvested

and Haryoto (2007), the use of bioethanol in Batik Industry has a higher efficiency than kerosene because the flame is stable, not too high and not easy to handle and use. It shows that 60% of bioethanol has better characterization and better efficiency compared to other ethanol in the concentration below 40% and above 90%).

Bioethanol can be produced from raw materials containing sugar, starch or cellulose. One type of natural resource which has potential to make bioethanol is the tubers. Tubers are agricultural products which contain carbohydrates or starch, and it is known that bioethanol can be made from raw materials containing carbohydrates. One kind of bulbs which is often used to make bioethanol is cassava. Cassava is an agricultural commodity grown in Indonesia and is the second highest source of carbohydrates after rice, the carbohydrate of which is 98.4% (Osunsami et al. 1988). Cassava can grow at of 2,000 m above sea level or in sub-tropical temperature of 16 º C. These plants flowers will bloom and produce bulbs properly when growing at altitude of 800 m above sea level, while at altitude of 300 m above sea level cassava can not bloom the flower, but they can only produce tubers. In 2005 cassava crop reached 19.5 tons with total area of 1.24 million hectares (Prihandana et al. 2007). Cassava can be harvested at the age of 9-12 months when the lower leaves growth begins to decrease. The color of the leaves begin to turn yellow and fall off a lot (www.warintek.ristek.go.id 2000). The product of cassava tubers are used to be processed into cassava flour, tapai, tiwul and others, while the cassava starch is used as raw materials of crackers, meatballs, and pempek, and this shows that cassava has an economic value. The use of cassava for bioethanol production could affect food supply, therefore it is necessary to find the diversification of raw materials such as tubers iles-iles (*Amorphophallus muelleri* Blume).

Iles-iles grows wild in Sumatra, Java, Flores and Timor (Jansen et al. 1996), as well as Bali and Lombok (Kurniawan et al. 2010, in press). Iles-iles belongs to the Araceae monocot plant family with compound flower of "cob" type which is covered by its leaves (spatha) (Jansen et al. 1996), it has dark brown tubers with rough rash that contain relatively high carbohydrate, i.e. 70-85% (Department of Agriculture 2009; Kusmiyati 2009). Iles-iles has the highest glucomannan among any other types of *Amorphophallus* in Indonesia, which ranged between 44-46% (Sumarwoto 2005). Glucomannan is a polysaccharide consisting of monomer β-1, 4 α-mannose and α-glucose (Widyotomo et al. 2000).

The availability iles-iles in Central Java is relatively abundant where forest land which is used reaches 640,000 hectares, while the productivity level is 30-40 tons/ha (Department of Agriculture 2009). However, this abundant amount of iles-iles has not been highly used; therefore iles-iles does not have high economic value. Because the nature of the sap causes itch, iles-iles tubers are not used as a food ingredient. According to Imelda et al. (2008), iles-iles is easy to be cultivated, either generative by using its seed or vegetative by using its bulbs, bulbils and leaf cuttings. Iles-iles naturally grows as secondary vegetation, on the outskirts of teak forests at an altitude of 700-900m above sea level, with rainfall level at 1000-1500 mm (Sumarwoto and Widodo 2008)

Iles-iles tuber is less fully utilized by society; it will be very beneficial when it is used as raw material for bioethanol production. This study aimed to compare the cassava tubers and iles-iles tubers as raw material for bioethanol production and to study the effect of variable concentrations of substrate and pH on levels of ethanol produced.

MATERIALS AND METHODS

Materials and equipment

These research materials are the cassava tubers found in traditional markets, while iles-iles tubers obtained from the

garden as a secondary plant in Wonogiri. The enzyme α-amylase, β-glucoamylase obtained from Daniso (Genericor, USA). Saccharomyces obtained from the Biology laboratory.

Sample preparation

Iles-iles tubers are peeled, washed, and cut into small pieces and dried in the sun up to 3 days so that maximum water content is 10. After that, tubers are mashed and sifted (approximately 40 meshes) so that the obtained particle size more uniform. The iles-iles flour is stored in a dry place and used in a long time. The same process is also carried on the manufacture of cassava tuber flour. The process of iles iles tubers flour making can be seen in Figure 2.

Stock culture of *S. cerevisiae*

Pure culture of *S. cerevisiae* is breed on for oblique (PGY medium) which has been sterilized before at a temperature of 121^0C and at pressure of 1 atm for 15 minutes. PGY medium (Peptone Yeast glucose) made by mixing 0.3 g of yeast extract, 0.3 g pantone, 0.4 g of malt and 20 g of agar which is dissolved in 300 aquadest. Stock cultures were incubated for 2-3 days at 28° C.

The process of enzyme production

Stock cultures of *S. cerevisiae* 200 is inoculated into liquid medium containing 5 g (NH) 2HPO4, 5 g KH2PO4, 1 g MgSO4.7H2O, 1 g of yeast extract. After that, it is incubated with a *rotary shaker* at 150 rpm speed and 30^0C for 24 hours. The same process for breeding pre-culture is done to breed the main culture; only with more liquid medium as much as 500 m. Enzyme that is formed by this process is used in the fermentation process.

The process of bioethanol production

The initial stage is liquification. First, dissolve 1 kg of cassava flour into water with a ratio (1:3,5, 1:4, 1:4,5, 1:5), and then add α-amylase enzyme of 0:48 mL/kg. This process is carried out by stirring the bulb flour at the speed of 250 rpm for 4 hours until it becomes mush at a temperature of 100 ° C. The hydrolysis process follows the process by using the enzyme β-glucoamylase with 0:48 concentration/kg, pH 4 for 40 hours. Glucose that is produced in the process of hydrolysis is analyzed with Nelson-Somogy method (Sudarmaji et al. 1984). The same process applies for manufacturing bioethanol from iles-iles tubers. Glucose that has been generated from the saccharification then is fermented by using the yeast S with a concentration of 10% (v/v) then DAP, urea, and NaOH are added to get the pH value at 6. This process lasts for 72 hours, levels of ethanol that is produced can be known from the GC where the sample can be taken at hour 2, 8, 12, 24 and 72.

Determining the water content

Petri dish was dried in an oven (105^0C) for ± 1 hour, then cooled down in a desiccator and weighed (A). Tubers samples (iles-iles and cassava) were weighed as much as 3 g (B). After that, the dish containing the sample was dried in an oven at a temperature of 105^0C for 2 hours, then cooled in a desiccator and weighed to obtain permanent weight (C). The water content can be calculated by formula (AOAC 1984).

$$Watercontent = \frac{(A+B)}{C} - C\ x100\%$$

Determining the starch content

Dissolve 5 g iles-iles tubers in 50, add HCl into it, close it, heat it above the heater water until boiling for 2.5 hours. When it is cool, neutralize it with NaOH solution and dilute it until 500. The sample is titrated with Fehling solution (Sudarmaji et al. 1984). Cassava tuber starch content is measured by the same method.

Crude fiber analysis

Mash and then sift dry bulbs of iles-iles and cassava. Weigh as much as 2 g and then extract the fat from it by using soxhlet. Move all materials into 600 mL of Erlenmeyer and add 3 drops of anti-foaming agent. After that add 200 mL of boiling solution of H_2SO_4 (1.25 g concentrated H_2SO_4) and cover it with coolant behind. Boil it for 30 minutes and shake it a few moments. Filter the suspension with filter paper, and then wash the filter paper with boiling distilled water until no longer acidic (acidity can be tested with litmus paper). Move the residue in the filter paper into Erlenmeyer by using a spatula and then wash the rest with 200 mL of boiling NaOH (1.25 g NaOH/100 mL = 0.313 N NaOH), until all the residue gets into the Erlenmeyer. Then boil it with cooler behind while shake it for 30 minutes. Next filter it using filter paper of known weight, while washing it with a solution of K_2SO_4 10%. Wash the residue again with boiling distilled water and then with 15 mL alcohol 95% (Sudarmaji et al. 1984).

a **b** **c** **d** **e**

Figure 2. The process of making flour iles-iles tuber, a. Tuber crops, b. Peeled tuber, c. Tuber is cut, d. Bulbs that have been dried in the sun, e. Tuber flour iles-iles.

Analysis of sugar reduction

Sugar reduction was done by using the Nelson-Somogy method. First, make the standard solution of 0.1 M natrium thiosulfate was prepared by dissolving $Na_2S_2O_3$ into and simmer for 5 minutes. Preparation of a solution of copper reagent was made by mixing some of Na_2SO_4 and KI solution, a solution of Na_2CO_3, $KNaC_4H_4O6.4H_2O$ solution, NaOH solution, a solution $CuSO_4.5H_2O$ and KIO_3 solution, then this copper reagent was stored in dark bottles. The Standardization of the copper reagent with the main liquor 0.005 M sodium thiosulfate was carried out with the main liquor thiosulfate. To analyze the levels of glucose in the sample, it was added with 1 sample into the reagent solution of copper and then it was simmer at a temperature of 95°C for 30 minutes. Then add H_2SO_4. Next, it was titrated the sample by using a solution of $Na_2S_2O_3$ with starch indicator, and TAT point is reached when the blue color turns clear. Each glucose levels is done in duplicate samples (Sudarmaji et al. 1984).

Determining ethanol by using GC

To analyze the levels of ethanol, centrifuge the fluid of fermentation sample with speed 6000 rpm for 30 minutes to separate supernatant and pellet. Take as much as 1μL supernatant samples and inject it into the chromatography gas column (6890 N, Agilent Technologies Inc., USA), then equip it with a column HP-Innowax. Set the column temperature at 200°C and set carrier gas using N_2 (40/min). Set the speed of gas flow rate for H_2 at 40/min and for O_2 at 500/min. Each sample is analyzed in duplicate.

RESULTS AND DISCUSSION

The content of raw materials

The material content percentage of iles-iles tubers is different from cassava. From the analysis, we find that total sugar content of iles-iles tubers is 73.43% and cassava is 86.42%. Comparison of the content of other materials contained in cassava and iles-iles can be seen in Table 1.

Table 1. Comparison of content of material found in the cassava and iles-iles.

Ingredients	Percentage (%)		
	Iles-iles		Cassava
	Wet	Dry	
Cellulose	1.67*	8.54*	-
Hemicelluloses	10.5*	43.3*	-
Lignin	0.597*	5.85*	-
Sucrose	1.35*	-	
Water	82.82*	-	62.50
Total sugar	-	73.43	86.42
Starch	-	71.25	83.47

* The Center Laboratory of Food and Nutrition Studies, Gadjah Mada University.

Table 1 shows that dry iles iles contains cellulose, hemicellulose, and lignin respectively, are 8.54%, 43.3%, and 5.85%. However, in wet conditions, the contents of cellulose, hemicellulose, and lignin on iles-iles becomes lower, that is 1.67%, 10.5% and 0.597%. The content of starch in the iles-iles is 71.25%, while cassava is 83.47%. Starch is a polysaccharide compound which consists of amylose and amylopectin (Campbell et al. 2000). Starch in the iles-iles is so high that the bulb can be converted into ethanol by using the enzyme amylase that will break the monosaccharide monomers on starch into glucose. Besides we can also use yeast *S. cerevisiae* to break down glucose into ethanol.

Glucose levels during the process of hydrolysis In general, bioethanol production from biomass consists of two main processes, i.e. hydrolysis and fermentation. Hydrolysis is a chemical process that uses H_2O as a breaker of a compound (Kuswurj 2008). The reaction between water and starch goes so slowly that it needs assistance to increase the reactivity of water catalyst. Acid solution is often used in the process to accelerate the process, but in this experiment, we use biological agent by using enzymes. According to Kolusheva and Marinova (2007), enzyme hydrolysis has more advantage compared to chemical hydrolysis. Chemical hydrolysis requires high temperatures (150-230° C), acid pH (1-2) and high pressure (1-4). This is different from the enzyme hydrolysis because it does not require a high temperature, medium pH of 6-8 and normal pressure. Enzymes are proteins that are catalysts, so often called biocatalysts. Enzymes have the ability to activate other specific compounds and to increase the accelerate of chemical reactions that will last longer if not using enzymes (Sun and Cheng 2002). Enzymes that are used in this study is the enzyme α-amylase and β-glucoamylase. The α-amylase enzyme plays important role in hydrolyzing α-1 ,4-glucoside 19 specifically. This enzyme works at pH 5.7 and temperature 95°C. Enzyme amylase cannot break down starch bond perfectly so that the process will produce dextrin with 6-10 chains units long (Schoonees 2004). The results of liquification process are then forwarded by the β-glucoamylase which can hydrolyze the bond of α-1,4-glucoside and α-1 0.6-glucoside with a temperature of 60°C and pH 4.2. Addition of β-glucoamylase in this experiment is aimed at producing more glucose because β-glucoamylase on starch can cut the starch bond that has not been cut by the addition of α-amylase, by producing glucose which has β-configuration in contrast to the results of hydrolysis by α-amylase, so that glucose generated will multiply or abundant. According to Kolusheva and Marinova (2007) hydrolysis that uses enzyme will produce higher reduction sugar if compared with the acid hydrolysis. Reduction of sugar concentration during the hydrolysis process is calculated using the Nelson-Somogy method. The process lasts for 40 hours with a temperature of 60°C. The comparison of reducing sugar in cassava and iles-iles can be seen in Figure 3.

Result shows that cassava has higher glucose content than iles-iles. Measurement of glucose is conducted by using Nelson-Somogy method. Iles-iles and cassava hydrolysis that has a ratio of tub: water 1:4 shows that glucose levels are influenced by the length of time. The largest concentration of glucose is formed at the time hydrolysis for 40 hours, which are 33.431 g/L for cassava

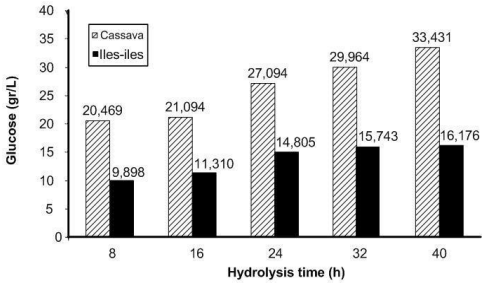

Figure 3. Sugar content in iles-iles and cassava.

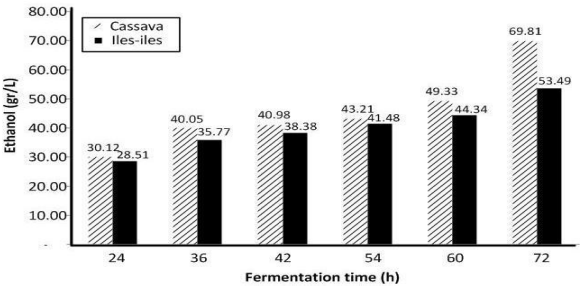

Figure 4. Ethanol content in the tuber iles-iles and cassava tubers.

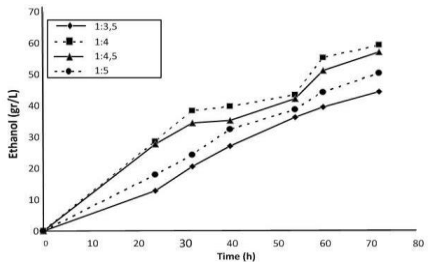

Figure 5. Effect of iles-iles substrate concentration and water concentrations toward ethanol, with yeast concentration of 10% (v/v) pH 5.5.

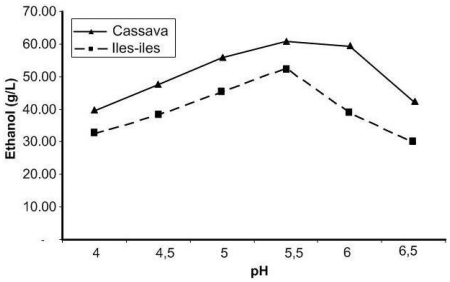

Figure 8. Effect of pH on ethanol production.

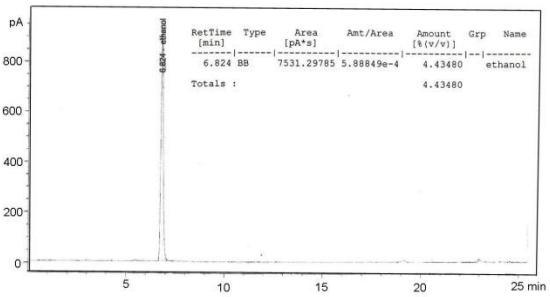

Figure 6. GC chromatogram resulted from fermented iles-iles for 60 hours at pH 4.5.

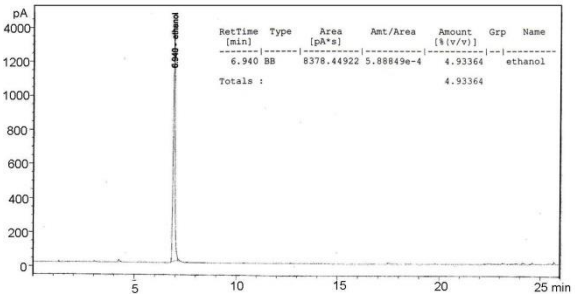

Figure 7. GC chromatogram of fermented cassava tuber for 60 hours pH 4.5.

and 16.175 g/L for iles-iles. This happens because the starch content in cassava is higher than in iles-iles, which is 83.47%, so that there is more glucose which can be converted. It is expected that the greater the hydrolysis of starch into glucose, the greater the ethanol produced in the fermentation process.

Temperature is a factor that affects the α-amylase hydrolysis process; hence in this study, we include the temperature variation in the process of α-amylase hydrolysis. Effect of temperature on variation of glucose levels can be seen in Table 2. From Table 2 we can conclude that the optimum temperature for the two tubers is 95°C, where the reduction sugar obtained from cassava and iles-iles respectively are 16. 176 g/L and 33. 431 g/L. This is the same stated by Kolusheva and Marinova (2007) where the research was performed under various temperatures (30, 60, 90 and 100ºC) and found that the optimum temperature was 90 and 100ºC where the process of hydrolysis runs faster so that result of reducing sugar is high.

Table 2. Effect of temperature variation on the hydrolysis process of the glucose levels, with concentrations of α-amylase 0:48/kg, time 40 hours, tubers and water concentration ratio (1:4).

Raw materials	Glucose level (g/L)				
	Temperature (°C)				
	90	**95**	**100**	**105**	**110**
Iles-iles tubers	14.57	16.176	16.041	15.221	13.245
Cassava tubers	29.145	33.431	30.451	28.451	27.219

Ethanol content during the fermentation process

After the hydrolysis process, the glucose which has been obtained will be converted into ethanol through a fermentation process. The basic principle is to activate the activity of microbial fermentation with the aim of changing the nature of raw materials to yield a product. The fermentation process of this study uses *S* because these organisms can ferment glucose, mannose, fructose, and galactose in anaerobe and low pH conditions. Besides that *S* is resistant to high alcohol content and high sugar levels (Kartika et al. 1992; Shen et al. 2008). The process of

fermentation by *S. cerevisiae* is done under anaerobic conditions, and if during the process the air enters then the ethanol formation process will be hampered. Therefore we must put small hose on the jar that serves to release CO2 gas. The purpose is to prevent a temperature rise inside the tube because *S. cerevisiae* is active at a temperature 4-32ºC (Chin et al. 2010). Ethanol is fermented substrate due to the activity of *S. cerevisiae.* Comparison of ethanol content of fermented iles-iles and cassava are shown in Figure 4.

Result analysis indicates that cassava yield higher ethanol than iles-iles. A 72-hour fermentation using the *S. cerevisiae* will produce highest ethanol either from iles-iles and cassava respectively 53.49 g/L and 69.81 g/L. The formation of ethanol is influenced by time, where the longer the time of fermentation the higher the level of ethanol will be. On the 24th-hour ethanol content of each tuber tends to be small, i.e. 30.12 g/L for cassava and 28.51 g/L for iles-iles. But the longer the fermentation time, the production of ethanol increases because the time that is used for converting glucose by *S. cerevisiae* is longer, resulting in higher ethanol. This is seen on the fermentation time 54 hours, where ethanol that comes from cassava and iles-iles are consecutively 43.21 g/L and 41.48 g/L.

Effect of substrate concentration and pH on ethanol production

Substrate concentration affects very much the production of ethanol, so to determine the effect of iles-iles substrate concentration toward the ethanol production we must perform variations of the adding water (1:3,5, 1:4, 1:4,5, 1:5 .) The level of ethanol content is then analyzed by using chromatography gas. The effect of water variation and iles-iels raw materials toward the ethanol is presented in Figure 5. From the result, it is known that the highest ethanol content obtained from the ratio of raw materials: water 1:4 with ethanol content of 59.36 g/L. The proper combination of raw materials and water will make the hydrolysis reaction run fast, because if the water is too little then the course of the reaction. According to Nowak (2000), if the substrate concentration is too high (a little water), the amount of oxygen will be too small; in fact, the oxygen is needed by *S. cerevisiae* to maintain life during the fermentation process.

Levels of ethanol fermentation results were analyzed by using Chromatography Gas. Figure 6 shows the chromatogram profile of iles-iles ethanol which was fermented for 60 hours. Result of the analysis shows that iles-iles with GC will produce ethanol at retention time of 6.929 minutes, whereas cassava at retention time of 6.940 minutes. Chromatogram from GC analysis on cassava is shown in Figure 7.

From the analysis using the GC we can see that the ethanol content of iles-iles tuber with early fermentation glucose concentration of 10% ethanol is formed by 44.4 g/L while the cassava 49.3 g/L. The degree of ethanol is determined by the activity of yeast with the sugar substrate which is fermented. According to Fessenden and Fessenden (1997), one molecule of glucose will form two molecules of ethanol and carbon dioxide. Too high concentration of glucose will obstruct yeast growth, which makes the ethanol content low. Ethanol is formed by the activities of microorganisms in the substrate complex changes. Yeast growth was greatly influenced by pH, because if the pH is not appropriate yeast cannot grow to a maximally, causing the death which lowers the ethanol. Effect of pH on ethanol is shown in Figure 8.

Bioethanol production is influenced by acid-base conditions. According to Liu and Shen (2008), optimum conditions of acid base can improve bioethanol production in the fermentation process because the acid-base conditions are closely related to the interaction of enzymes and raw materials. The degree of acid will affect the speed of fermentation. This is consistent with the results of research in which the process of cassava fermentation at pH of 5.5 produces highest amount of bioethanol, which is 60.85 g/L. While pH 4 produces the lowest ethanol concentration of 39.57g/L. Meanwhile, iles-iles can produce maximum ethanol at pH 5.5 where the ethanol content obtained is 52.61 g/L. The results obtained prove that the acid conditions increase enzyme *S. cerevisiae* work. This is consistent with the study presented by Wilkins et al. (2007) where the results of ethanol will increase at pH 5 and 5.5 and will decline at pH 4, and 4.5. The optimum conditions are from pH 5 to 5.2. This result is consistent with Liu and Shen study (2008), that acid pH is an important parameter that can increase production of ethanol in fermentation processes with enzyme *S. cerevisiae.*

Test the efficiency of bioethanol fuel

Bioethanol which is obtained, then used as fuel for batik stoves. Batik stove is made by the researchers from stainless steel with tank capacity 500 mL. This experiment aims to determine the efficiency of bioethanol in melting the wax. In this experiment, the researcher used some variation of ethanol degree as much as 100 mL to melt 20 g of wax.

Table 3. Variations of bioethanol degree toward its use in melting wax.

Parameter	Concentration of bioethanol fuel (%)					
	40	**50**	**60**	**70**	**80**	**90**
Time to boil (min)	22	22	19	18	17	11
The time required to evaporate out (hours)	2,7	2,5	2,4	1,65	1,6	1,4
Fire conditions when evaporation	Red	Red	Blue	Blue	Blue	Blue
The condition of wax the container	Soot	Soot	-	-	-	-

From table, it can be concluded 90% bioethanol grade takes the shortest time in boiling wax, with evaporation time of 1.4 hours for each 100mL. This is because the high levels of bioethanol (fuel: water = 90:10) is easier to vaporize. This is different for 40% and 50% bioethanol where the flame is red and soot appeared, but the time needed for ethanol to evaporate is longer than other levels. This is due to the water content that is high enough so that it took longer time to evaporate. However, high water

content also produces carbon and soot during combustion. The greater the water content in ethanol is the longer it takes for the flame to run out. From the efficiency, 90% bioethanol have a very high volatile nature that makes it less appropriate for use in combustion, because it tends to be wasteful and quickly burnt. While 40% and 50% bioethanol burns longer but it produces red flame and soot. This results in less energy than blue flame. Blue fire condition has more powerful energy for melting candle burning instead of changing the ethanol into carbon/soot. Based on efficiency 60% bioethanol then is more superior to other grades of ethanol. Water content that is not too high causes combustion produce blue and clean flame and clean of soot, and in turn, it will burn longer than any other levels of ethanol.

CONCLUSION

For 40 hours iles-iles tuber hydrolysis (*Amorphophallus muelleri*) has glucose content of 16.175 g/L and cassava 33.431 g/L. Degree of ethanol from iles-iles fermentation for 60 hours is 44.4 g/L, while cassava is 49.3 g/L. Temperatures difference will affect the speed of α-amylase hydrolysis in converting glucose. The result showed that the optimum temperature for hydrolysis is 95°C where the concentration of glucose obtained on cassava and cassava iles-iles are respectively 16 176 g/L and 33 431 g/L. The substrate concentration and acidity will affect the speed of fermentation. In this study, optimum ethanol degree was found in the ratio 1:4 and pH 5.5. The result shows that iles-iles tubers have the potential to be developed as a raw material for bioethanol production.

ACKNOWLEDGEMENTS

The author would like to thank DP2M Higher Education Research Grant Fund for the assistance of the fiscal year 2010 (Contract No. 089/SP2H/PP/DP2M/III/2010, dated March 1, 2010). The author would like to thank Agnes H, Agus Nur Arifin, Hesthi Chandra P, Diani Mentari, and Ina Istiqomah in conducting this research.

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