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The bioconversion of vegetable waste extract from Osowilangun Central Market Surabaya into bioethanol using *Saccharomyces cerevisiae*

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Abstract. This research was conducted to determine the effect of vegetable waste extract concentration, fermentation time, and the effect of its combination on ethanol content using *Saccharomyces cerevisiae*. This research used a complete randomized design with a factorial test consisting of two factors. The first factor is the vegetable waste extract concentration (100, 75, 50, and 25%). The second factor is the fermentation time (3, 6, 9, and 12 days). There were 16 treatments, each of which was performed 3 times. The dependent variable is ethanol content, while the control variables are pH, temperature, and *Saccharomyces cerevisiae* concentration of 10%. The measurement of reducing sugar used the luff method and the measurement of ethanol content used the pycnometry method. The data obtained were analyzed using two-way ANOVA, followed by the Duncan test on the difference between the treatments of waste extract concentrations and fermentation times. The results indicate that the concentration of vegetable waste extract, fermentation time, and its combination affect bioethanol production. The highest ethanol content was obtained at 100% vegetable waste extract concentration. The optimal time to produce ethanol is 9 days. The most optimal combination is at 100% concentration and 9 days of fermentation time with 4.40%.

1. Introduction

Bioconversion is the conversion of a less useful material into a useful one using microbes, resulting in a high economic value [1]. Energy needs in Indonesia are increasing every year. Conversion of biomass into energy is one solution to meet energy needs in Indonesia. The conversion of biomass into bioethanol can be used as a renewable fuel and as a substitute fuel for fossil fuels [2].

Bioethanol is an ethanol derived from biological sources, which can be used as an alternative fuel because it is environmentally friendly and it can be considered as future fuel which has the potential to replace fossil fuels [3]. Vegetable waste is used as a raw material for bioethanol because it contains cellulose and hemicellulose, which can be converted into sugar by the hydrolysis process, which through fermentation process will produce bioethanol [4]. Various microorganisms carry out fermentation process such as yeast, fungi, and bacteria [5]. Based on various physiological characteristic for bioethanol production at industrial level, *Saccharomyces cerevisiae* is preferable than the other yeasts. It can tolerate wide range of acidic pH as optimum, which protects contamination. It can also tolerate ethanol better than other ethanol producers [5]. The utilization of vegetable waste into bioethanol has advantages, namely being easily obtainable and being always available every day. Vegetable waste was obtained from the Surabaya Ososwilangun Central Market (PIOS). The size of PIOS waste was 46,325 m³/ day [6]. Research on bioconversion of vegetable waste extract from PIOS



to bioethanol using *Saccharomyces cerevisiae* was done by producing vegetable waste extract and fermentation time. This research was conducted to determine the effect of vegetable waste extract concentration, fermentation time, and combination of both on ethanol content. Ethanol has enormous benefits in various industries. Therefore, a research needs to be done on bioconversion of vegetable waste extract from PIOS to bioethanol.

2. Research Methods

This research was conducted by sampling vegetable waste at Surabaya's Osowilangon Central Market in the rain season. The research and analysis of test parameters were conducted at the Microbiology Laboratory and Environmental Laboratory, Department of Biology, Faculty of Science and Technology, Universitas Airlangga, Surabaya. The materials used in this research were microbial isolates of *Saccharomyces cerevisiae*, vegetable waste, Potato Dextrose Agar (PDA) 39 g / 1.1% yeast extract medium, 1% glucose media, demineralized water, luff solution, KI 20%, H₂SO₄, 1% amyllum indicator, Na₂S₂O₃ 0.1 N. The instrument used in this research were reaction tube, reaction tube shelf, petri dish, inoculation loop, 250 ml fermentation bottle, glass stirrer, thermometer, pH meter, Bunsen burner, Beaker glass, juicer, gauze, Erlenmeyer with glass cap, volume pipette, autoclave, shaker, incubator, burette, stative, water bath, analytical balance, distillation tube, 250ml volume pipette, distilling flask, and pycnometer.

The method used in this research consists of several stages. The first stage is to prepare raw materials and produce vegetable waste extract. The components of vegetable waste were analyzed in percent units. Vegetable waste was washed thoroughly, then extracted using a juicer. Furthermore, the researcher gave a sample treatment with extract concentration variations of 100%, 75%, 50% and 25% with water as the diluent. The second stage is the sterilization of tools and materials. The glassware was dry-heat sterilized using an oven with a temperature of 150°C for 2 hours, while the research materials were moist-heat sterilized using autoclave tool with a temperature of 121°C and the value of 1 atm for ten minutes [7]. The third stage was the production of culture stock. The culture stock was prepared by multiplying pure *Saccharomyces cerevisiae* culture inoculated into PDA media using aseptic scratch method, then incubated for 48 hours [7]. The fourth stage is the production of starter. The preparation of starter was done by inoculating *Saccharomyces cerevisiae* inoculated from the PDA agar slant into 600ml liquid medium yeast extract 1% and glucose 1%. The cultivated culture was homogenized using shakers for 4 hours and then stored in an incubator. After that, the *Saccharomyces cerevisiae* culture was incubated at room temperature for 3 days. Prior to being incorporated into the fermentation medium, the quantity of *Saccharomyces cerevisiae* in the starter was calculated using turbidimetric method and plate count (TPC) [7]. The fifth stage is the bio-ethanol fermentation process. Each bottle of fermenter corresponding to the concentration variations of vegetable waste extract (100%, 75%, 50%, and 25%) were added by 10% *Saccharomyces cerevisiae*. Fermentation was done using varying fermentation times of 3, 6, 9, and 12 days. The sixth stage is the measurement of reducing sugar using the luff method and the measurement of ethanol content using the pycnometry method. The final step is to perform statistical data analysis using Two-Way ANOVA to determine the relationship between treatments and Duncan test to determine the real difference between the effect of vegetable waste extract concentration variations and the length of fermentation time.

3. Result and Discussion

The composition of vegetable waste used in the study was 2.2 kg of carrots, 2.4 kg of potatoes, 3 kg of pak choi, 2 kg of cabbage, 0.5 kg of broccoli, 0.5 kg of long beans, and 0.5 kg of eggplant. The total mass of vegetable waste used was 11.6 kg. The vegetable waste composition was fermented using the *Saccharomyces cerevisiae* starter.

3.1. Effect of variation concentration of vegetable waste extract on ethanol content

This research used varying vegetable waste extract concentrations (100%, 75%, 50%, and 25%) and fermentation times (3, 6, 9, and 12 days). Kolmogorov Smirnov test results showed normal data. Two-Way ANOVA test results showed a significance value of vegetable extract concentration of 0.000 or less than (α) 0.05. Therefore, H₀₁ is rejected and H_{a1} is accepted, stating that there is an influence of vegetable extract concentration to ethanol content. Levene test results showed homogeneous data, and

Duncan test results showed a significant difference between the effect of concentration variation with ethanol content.

Vegetable waste extract in this research contained 4.58% sugar. The sugar will then be converted to bioethanol using *Saccharomyces cerevisiae*. *Saccharomyces cerevisiae* has invertase and zimase enzymes. If the sugar available in the substrate is disaccharide sugar, then the invertase enzyme will hydrolyze the disaccharides into a monosaccharide. Furthermore, zimase enzymes will convert monosaccharides to alcohol and CO₂ [8].

Ethanol content tends to decrease with the decrease of vegetable waste extract concentration. This also happens to reducing sugar content, which can be seen in Table 1. The more reducing sugars utilized by *Saccharomyces cerevisiae*, the higher the ethanol concentration that can be produced. This is in accordance with the opinion of Winarti [9] that the higher the concentration of substrate or reducing sugar that can be broken down into yeast cells into ethanol, the higher the concentration of ethanol produced. The concentration variation significantly affected the ethanol content from the 3rd day until the 12th day. Variations of vegetable waste extracts produce different ethanol levels. Ethanol content decreased with increasing dilution of vegetable waste extract. The thicker the substrate concentration, the higher the sugar content, so higher ethanol content can be produced. In this research, the researchers obtained the maximum ethanol content at 100% vegetable waste extract concentration.

Table 1. The averages of reducing sugar content

Vegetable waste extract concentration (%)	Fermentation Time (days)			
	3	6	9	12
25	0.82%	0.09%	0.06%	0.02%
50	1.10%	0.31%	0.10%	0.04%
75	1.25%	0.90%	0.50%	0.06%
100	1.88%	1.12%	0.66%	0.08%

3.2. The effect of fermentation time variation on ethanol content

Kolmogorov Smirnov test results showed homogeneous data. Two-Way ANOVA test results obtained a significance value of fermentation time variation of 0.000 or less than (α) 0.05. Therefore, H₀₂ is rejected and H_{a2} is accepted, which states that there is an influence fermentation time variation on ethanol content. Levene test results showed homogeneous data, and Duncan test results showed a significant difference between the effect of concentration variation with ethanol content. Based on research results, it is known that ethanol production has increased along with the increase of fermentation time. In all variations in the concentration of vegetable extracts, ethanol production increased from day 3 to day 9, and decreased on day 12. On day 3, ethanol production was still low because yeast was still in the adaptation phase. Ethanol production in the study increased until day 9 because the yeast was in the exponential phase and the time was most optimal for decomposing glucose into bioethanol. The exponential yeast growth indicates that there are still many nutrients, vitamins, and other elements that are still available in the fermentation medium. On the 12th day, the amount of ethanol decreases because the longer the fermentation time, the less nutrients there are in the fermenter, resulting in more and more cell numbers that lead to competition and the emergence of the death phase.

Based on the overall data, it can be seen that a pH value in the interval of 4.35- 4.5 is the optimum pH value for the growth of *Saccharomyces cerevisiae*. This is because to the substrate concentration contained in the fermenter is different. The pH of water is neutral, so the more substrate concentrations are diluted, the more substrate will be soluble in the water and the higher the pH will be. Yeast prefers acidic conditions at pH 4- 4.5 and has a pH tolerance of 2.2 - 8.0 [10]. Temperature measurement was performed to determine the environmental conditions in the fermenter according to the optimum conditions of *Saccharomyces cerevisiae*. The lowest temperature was 28.3°C, while the highest temperature was 28.6°C. Yeast can grow well at 20°C - 30°C [10].

3.3. The effect of combination between vegetable waste extract concentration and length of fermentation time on ethanol levels

The Kolmogorov Smirnov test results showed normal data ($\text{sig} > 0.05$). The Two-Way-ANOVA test obtained a significance value of 0.000 ($\text{sig} > 0.05$). This shows that H_{03} is rejected and H_{a3} is adopted, which states that the combination of vegetable extract concentration and vegetable waste extract concentration and fermentation time have significant effect on ethanol content.

Table 2. The average of ethanol content in the combination of vegetable extract concentration and fermentation time.

Incubation Time (day)	Average Ethanol Content (%)			
	Vegetable waste extract concentration (%)			
	25 %	50%	75%	100%
3	0.66 ± 0.06	1.20 ± 0.07	2.10 ± 0.10	2.34 ± 0.07
6	1.33 ± 0.07	2.07 ± 0.07	2.55 ± 0.07	3.39 ± 0.24
9	1.46 ± 0.07	2.69 ± 0.07	3.58 ± 0.07	4.40 ± 0.16
12	1.39 ± 0.07	2.48 ± 0.07	3.52 ± 0.09	4.31 ± 0.21

Table 2 shows the maximum ethanol content obtained in combination of 100% vegetable waste extract concentration and 9 days of fermentation time. The minimum ethanol content was obtained in combination of 25% extract concentration and 3 days of fermentation time. Based on the two-way ANOVA statistical analysis on the Levene test, the influence of fermentation time at 100% concentration is declared to be significant, while Duncan test showed that ethanol concentration at 100% concentration on day 9 and day 12 was not significantly different. However, fermentation with longer times is considered ineffective both in terms of cost and time. Therefore, the most optimal combination for the fermentation of vegetable waste extract into ethanol is at 100% concentration and 9 days, producing 4.40% of ethanol. Efforts to increase ethanol levels in vegetable waste extract should be performed, considering the abundance of vegetable waste in Indonesia. The initial reducing sugar contained in the vegetable waste extract is small, so it is advisable to add supplements or glucose into the fluid for further research on glucose levels to be added to the liquid so that the amount of ethanol produced is at maximum.

4. Conclusion

The conclusions of this research are as follows:

1. There is an influence of vegetable extract concentration variation on ethanol content from fermentation result using *Saccharomyces cerevisiae*. The highest ethanol content was obtained at 100% vegetable waste extract concentration.
2. There is an influence of the fermentation time variation on the ethanol content of the fermentation product using *Saccharomyces cerevisiae*. The most optimal fermentation time in this research is 9 days.
3. There is an influence of combination between vegetable waste extract concentration and fermentation time to ethanol content from the fermentation result using *Saccharomyces cerevisiae*. The most optimal combination is at 100% concentration and 9 days, which produces 4.40% ethanol.

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