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Effect of Time, pH, and Yeast Concentration on Bioethanol Levels in the *Ulva* sp. Fermentation Process

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Abstract. Bioethanol is a form of renewable energy that is used to reduce dependence on the use of fossil fuels which cause various negative impacts on the environment. *Ulva* sp. contains high carbohydrates so it has the potential as a raw material for bioethanol production. This study aims to determine the optimum conditions of the fermentation process with the variables used time, pH, and yeast concentration. This study used the results of hydrolysis of *Ulva* sp. with optimum operating conditions of 0.1 N HCl concentration, 80 mesh particle size, and 450 watt microwave power. Measurement of bioethanol levels was carried out using an alcoholmeter. The results showed that the optimal conditions for fermentation were 7 days of fermentation, pH 5.5, and yeast concentration of 1.5% which resulted in a bioethanol content of 7.55%.

Keywords: *Bioethanol, fermentation, yeast, Ulva sp.*

1. Introduction

Fuel is the energy that is needed by all countries in the world at this time and will increase over time. Meanwhile, oil and natural gas depend on fossil resources whose rate of formation is inversely proportional to the level of consumption. These fossil resources are included in non-renewable resources (non-renewable) if they are taken continuously one day their availability will run out [1]. Oil production in the last six months up to June 2022 shows a figure of 616.6 thousand barrels per day according to the Special Task Force for Upstream Oil and Gas Business Activities (SKK Migas). The use of motorized vehicles continues to increase every year, this is what makes energy scarce. Based on the Indonesian Statistics Center (BPS) in 2021 the use of motorized vehicles in Indonesia will reach around 270,688,529 vehicles. Alternative fuels are needed to be a solution to these problems, namely fuels that can

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be renewed so that they can be used optimally. Bioethanol is one of the potential that can be used as an alternative fuel [2]. Indonesia is a tropical country that has abundant biodiversity, agricultural products, and plantation products, so it has great opportunities for the bioethanol development process [3].

Ethanol has a high octane number, high laminar speed, and high heat of vaporization, so ethanol can be used as a transportation fuel [4]. Ethanol can be used as a substitute for transportation fuel, either directly or mixed with gasoline. Gasoline mixed with ethanol up to a maximum of 15% can be burned in a transportation combustion engine [5]. The level of carbon and hydrocarbon emissions in a mixture of gasoline and ethanol is lower than premium and pertamax because ethanol contains 35% oxygen which can increase combustion efficiency in vehicles [6]. Bioethanol with the chemical formula C_2H_5O is a single-chain alcohol with an octane number of 108, is difficult to evaporate, has a low calorie value, and is flammable [7]. Bioethanol production for the first generation generally still uses food as a raw material [8]. Sugar derived from carbohydrates (starch) is fermented with the help of microorganisms to produce bioethanol [9]. These microorganisms are *Saccharomyces cerevisiae*, *Zymomonas mobilis*, and *Escherichia coli* because they can convert simple sugars into ethanol [10]. Bioethanol is obtained by converting carbohydrates to form glucose using several processes such as acid or enzymatic hydrolysis [11]. Bioethanol can be produced from a variety of natural products, therefore it can be referred to as bioethanol [12]. Residue biomass of aquatic plants can be used as a raw material for making bioethanol, as is the case with the use of Algae *Ulva* sp.

Ulva sp. is a macroalga belonging to the class Chlorophyta. This is because *Ulva lactuca* contains quite a lot of chlorophyll cells that give the algae a green color. Green algae generally store starch as a food reserve [13]. Ulva sp. is a type of macroalgae that is widespread in the sea and Indonesian fresh waters [14]. It generally inhabits rocks on dead coral fragments and varies in shape and size with changes in environmental factors [13]. The content contained in *Ulva* sp. per 100 grams of net weight, namely water 18.7%, protein 15.26%, fat 0.1-0.7%, carbohydrates 46-51%, fiber 2-5%, and ash content 16-23%, and contains vitamins B1, B2, B12, vitamin C, and vitamin E [15]. *Ulva* sp. They range up to 100 cm in length and are bright apple green in color, and have the shape of a folded sword with smooth but wavy edges. The middle of each strand is pale and dark if it gets to the edges [16]. Ulva sp. can survive in the temperature range of 28-31°C [15]. *Ulva* sp. is one of the species of the *Ulvaceae tribe*, systematically classified as *Ulva* sp. can be seen in table 1 as follows [17]

Table 1. Classification of *Ulva lactuca*

Kingdom	***Plantae***
Division	*Chlorophyta*
Class	*Ulvophyceae*
Order	*Ulvales*
Family	*Ulvaceae*
genus	*Ulva*
Species	*Ulva lactuca*

The manufacture of bioethanol goes through several stages, the first of which is hydrolysis which converts polysaccharides into simple sugars and then converts them into ethanol [18]. The hydrolysis process generally uses an enzymatic chemical method with an acid catalyst [19]. The hydrolysis process can use a catalyst in the form of an acid or an enzyme [20]. The acid used is like a strong acid, namely hydrochloric acid (HCl). The effectiveness of the HCl catalyst type was higher in producing glucose at the same temperature, concentration, and time compared to H_2SO_4. This is because the nature of HCl is stronger with higher reactivity compared to H_2SO_4 [21]. The process of hydrolysis of cellulose with acid using the microwave method can convert starch into simple sugars in a relatively short period. Based on this, it can produce higher bioethanol production with lower acid concentrations so that it becomes environmentally friendly, saves costs, and shortens production time [22]. Hydrolysis is a reactant process with water capable of breaking down a compound to form its constituents [23]. The hydrolysis process can use a catalyst in the form of an acid or an enzyme [20]. One of them is hydrochloric acid (HCl) which can be used in the starch hydrolysis process because HCl is very easy to obtain, but hydrochloric acid must go through proper handling because this liquid is corrosive [24]. Although this compound is acidic, it contains chloride ions which are non-toxic and non-reactive. Hydrochloric acid with an intermediate concentration of 30% to 34% is stable enough to be stored and continues to maintain its concentration [25]. Therefore, the hydrolysis process is important in the manufacture of bioethanol, because this process determines the amount of glucose obtained, then fermented into bioethanol. Termination of starch polymer chains to form dextrose or monosaccharide units, namely glucose, is the principle of starch hydrolysis [26]. The following reaction equation for the hydrolysis process is shown in equation 1[27].

$$(C_6H_{10}O_5)n_{(s)} + nH_2O \xrightarrow[HCl_{(l)}]{} nC_6H_{12}O_{6(l)} \quad (1)$$

The second stage is the fermentation process, at this stage, it functions to convert glucose into ethanol and carbon dioxide (CO_2). The fermentation process is carried out by

adding yeast to work at optimal temperatures [18]. Factors that influence the fermentation method are temperature, nutrition, pH, and fermentation time. The optimal temperature for the fermentation process ranges from 27-30ºC and the optimal pH ranges from 4 – 7 [28]. Fermentation using ingredients that have ingredients such as glucose, starch, and fiber can produce liquid bioethanol [29]. The fermentation process is one of the ways to produce alcohol by precipitating a carbohydrate-containing substance in an anaerobic state [30]. Fermentation is a process in which chemical changes occur in an organic substrate from the activity of enzymes produced by microorganisms [31]. Fermentation usually uses microorganisms such as yeast or mold, but it can be done with bacteria and a mixture of various microorganisms [32]. The microorganism commonly used in bioethanol production is *Saccharomyces cerevisiae* because it can easily produce alcohol in large quantities and has a fairly high response to alcohol content [33]. *Saccharomyces cerevisiae* yeast is used to increase the yield of the bioethanol production process because the process does not require sunlight. The alcohol produced from the fermentation process can contain up to 8-10% alcohol content [34]. In the fermentation process, yeast has the role to convert glucose into ethanol and carbon dioxide gas [35]. The fermentation reaction for the formation of alcohol is shown in equation 2 [36]

$$C_6H_{12}O_{6\,(l)} + \textit{Saccharomyces cerevisiae} \longrightarrow C_2H_5OH_{\,(l)} + 2CO_{2\,(g)} \qquad (2)$$

The next process is the distillation process, this stage functions to obtain purer ethanol with the help of a distillation apparatus. Then do a test on the determination of ethanol levels [11]. Measurement of ethanol levels can be done using an alcoholmeter.

In this study, *Ulva* sp. hydrolyzed using hydrochloric acid to form simpler molecules. Then the hydrolysis results are carried out by the ethanol fermentation process with yeast. In several previous studies with various kinds of raw materials, it has been shown that the fermentation time and the concentration of *Saccharomyces cerevisiae* affect the bioethanol fermentation process. The longer the fermentation time, the higher the bioethanol content produced. However, bioethanol levels decreased when they reached the optimal point because the productivity of *Saccharomyces cerevisiae* decreased and nutrients were running out. High levels of bioethanol are also obtained from favorable environmental conditions, one of which is the influence of pH. This study aims to determine the optimum conditions of the fermentation process on *Ulva* sp. with the variables used, namely time, pH, and yeast concentration. References for this study were obtained from several journals which are summarized in Table 2.

Table 2. Research on Bioethanol Production

Raw Materials	Methods and Results	Ref
Ulva reticulata	Fermentation of *Saccharomyces cerevisiae* at pH 4.5 and temperature 30°C for 6 days. The results of the analysis of ethanol content have a purity of 5.02%	[37]
Microalgae *Nannochloropsis sp.*	*Saccharomyces cerevisiae* fermentation at pH 4.5. The best fermentation time to produce ethanol is 72 hours, which is 8.9%	[27]
Tetraselmis chuii Microalgae	*Saccharomyces cerevisiae* fermentation at 30°C for 5 days. The resulting ethanol content of 1%	[38]
Codium geppiorum Algae	Ferment at 27-30°C. The highest ethanol content was in fermentation with 20% yeast concentration for 7 days, namely 3.03%.	[39]
Green Algae *Spirogyra sp.*	*Saccharomyces cerevisiae* fermentation at pH 4.5 and temperature 30°C. The highest yield of ethanol was 0.0613 in 5 days of fermentation with 1% fermipan content	[40]
Elephant Grass (*Pennisetum purpureum* Schumach)	*Saccharomyces cerevisiae* fermentation at pH 4. The highest bioethanol yield was 17.30% with 11% starter content for 6 days	[36]
Microalgae *Chlorella pyrenoidosa*	Fermentation *Saccharomyces cerevisiae* at pH 5 was fermented for 3 days. The highest bioethanol concentration produced was 0.280% with 25% yeast concentration	[41]
Sargassum crassifolium	*Saccharomyces cerevisiae* fermentation at 30°C. The highest yield of bioethanol was obtained at pH 7 which was fermented for 72 hours, namely 67 ml	[1]
Powder Agar *Gracilaria verrucosa*	Fermentation of *Saccharomyces cerevisiae* at pH 7. Optimum ethanol content was achieved at 120 hours of incubation with 0.1M H2SO4 concentration of 0.77%	[42]
Nira Aren	*Saccharomyces cerevisiae* fermentation. The highest ethanol content was 45.70% in 6 days of fermentation using 7.5 ml of starter	[43]

2. Research Methods

2.1 Materials

The materials used in this study included hydrolysis of *Ulva* sp., yeast (*Saccharomyces cerevisiae*), distilled water, HCl, NaOH, and urea.

2.2 Material preparation

Algae *Ulva* sp. washed thoroughly to remove sand and other impurities. Furthermore, the material is dried using an oven, after which the material is mashed with a blender and sieved using an 80 mesh sieve to obtain *Ulva* sp. powder.

2.3 Hydrolysis Process

The hydrolysis process used 0.1N HCl, *Ulva* sp. powder. weighed and added 0.1 N HCl. Furthermore, the hydrolysis process was carried out using a microwave with a power of 450 watts. After the hydrolysis process is complete, the hydrolysate results are cooled in the beaker

glass until it reaches room temperature. The hydrolysate is then filtered using filter paper to separate the filtrate from the residue. The filtrate obtained was then put into a vial for further testing of reducing sugar levels.

2.4 Fermentation Process

The fermentation process uses the help of *Saccharomyces cerevisiae* with varying concentrations of 0.5%; 1%; 1.5% of the total hydrolysate volume, [40] and added 2M NaOH until the pH becomes 4; 5.5; 7 for variations in pH. After that, 1% of the total hydrolysate volume was added as a nutrient for the culture into the fermentation vessel. Fermentation is carried out anaerobically. Tightly cover the fermentation container, then the container is perforated and given a hose which is flowed into a container filled with water and fermented with variations of 3, 5, and 7 days [39] at room temperature (30°C).

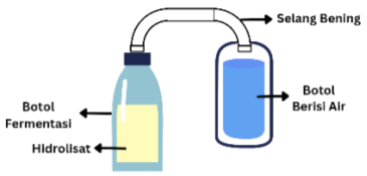

Figure 1. Series of fermentation equipment

2.5 Distillation Process

The results of the fermentation are then filtered so that the filtrate is separated from the residue. After that, the liquid is put into the distillation flask for the distillation process. Then heated at a temperature of 78˚C according to the boiling point of ethanol.

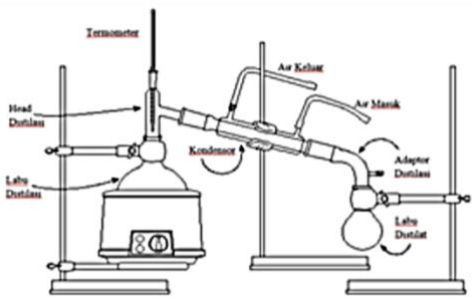

Figure 2. A series of distillation equipment [44]

2.6 Sample Analysis

This study tested the levels of ethanol produced from the process of making bioethanol. After carrying out the distillation process, cool the pure ethanol produced into a beaker glass and then test the levels by inserting an alcoholmeter into the beaker glass. Then let stand for 5-10 minutes and see the scale read on the alcoholmeter.

2.7 Data Analysis

Data analysis used Design Expert software version 13, adapted to the Response Surface Methodology (RSM) approach and the 17 running Box Behnken Design (BBD) model according to Table 3.

Table 3. Variation of *Ulva* sp. fermentation data.

No.	Fermentation time (days)	pH	Yeast Concentration (%)
1	7	4	1
2	3	5.5	1.5
3	3	4	1
4	5	7	1,5
5	7	7	1
6	5	5.5	1
7	5	5.5	1
8	3	5.5	0.5
9	7	5.5	1.5
10	5	5.5	1
11	5	4	0.5
12	3	7	1
13	5	5.5	1
14	5	5.5	1
15	5	4	1.5
16	5	7	0.5
17	7	5.5	0.5

3. Results and Discussion

3.1 Analysis of Bioethanol Content Results

Based on the bioethanol content test measured using an alcoholmeter, the highest bioethanol content was 7.55% with parameters of operating conditions namely 7 days fermentation time, pH 5.5, and yeast concentration 1.5%. While the lowest bioethanol content was 1.50% with parameters of operating conditions namely 3 days fermentation time, pH 4, and 1% yeast concentration.

Table 4. Results of Reducing Sugar Levels

No.	Fermentation time (days)	pH	Yeast Concentration (%)	Bioethanol Content (%)
1.	7	4	1	3.55
2.	3	5.5	1.5	1.55
3.	3	4	1	1.50
4.	5	7	1.5	3.10
5.	7	7	1	5.35
6.	5	5.5	1	4.60
7.	5	5.5	1	4.50
8.	3	5.5	0.5	3.85
9.	7	5.5	1.5	7.55
10.	5	5.5	1	4.10
11.	5	4	0.5	2.20
12.	3	7	1	1.75
13.	5	5.5	1	4.30
14.	5	5.5	1	3.95
15.	5	4	1.5	2.40
16.	5	7	0.5	2.55
17.	7	5.5	0.5	3.70

3.2 *Analysis of Variance* (ANOVA)

Analysis of Variance (ANOVA) is a form of statistical hypothesis testing by drawing conclusions based on inferential statistical data or groups. Significant results can be seen from the p-value (probability value) <0.05. The results of the ANOVA can be seen in table 4, and the results obtained are p-value <0.05. This shows that the variables used in this study affect the levels of bioethanol. Lack of Fit is a deviation from the model. The p-value for Lack of Fit was >0.05 and showed insignificant results, in this study the Lack of Fit value was 0.5177. This shows the suitability of the model. A significant relationship between the variables and the yield of bioethanol content can be seen from the R^2 value. Table 5 shows that the R2 value is 0.9872, which indicates that there is a significant relationship between fermentation time, pH, and yeast concentration on bioethanol levels. In addition, there is a difference between the Predicted R^2 value and the Adjusted R^2 value <0.2 which indicates that the data is reasonable.

Table 5. Results of Analysis of Variance (ANOVA)

Source	*Sum of Squares*	*df*	*Mean square*	*F-value*	*p-value*	
Model	37.87	9	4.21	60.40	< 0.0001	*significant*
A- Fermentation time	16.68	1	16.68	239.38	< 0.0001	
B-pH	1.16	1	1.16	16.69	0.0047	
C- Yeast Concentration	0.6612	1	0.6612	9.49	0.0178	
AB	0.6400	1	0.6400	9.19	0.0191	

Source	*Sum of Squares*	*df*	*Mean square*	*F-value*	*p-value*	
AC	9.46	1	9.46	135.74	< 0.0001	
BC	0.0306	1	0.0306	0.4396	0.5285	
A^2	0.1181	1	0.1181	1.70	0.2340	
B^2	8.64	1	8.64	124.03	< 0.0001	
C^2	0.3664	1	0.3664	5.26	0.0555	
Residual	0.4876	7	0.0697			
Lack of Fit	0.1956	3	0.0652	0.8933	0.5177	*not significant*
Pure Error	0.2920	4	0.0730			
Cor Total	38.35	16				

The suitability between the experimental data and the model can be seen based on the parity plot graph in Figure 3. The straight lines on the graph are the predicted data, while the actual data from each run is shown as dots on the graph. The experimental data values spread around the line indicating that there is a match between the model and the experimental data so that the model is significant.

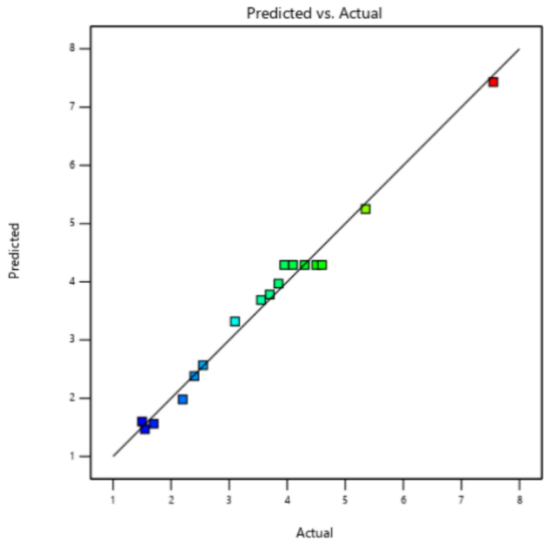

Figure 3. Graph of comparison of model data with experimental data on ANOVA

3.3 Effect of Parameters on Bioethanol Levels

3.3.1 Effect of Fermentation Time and pH on Bioethanol Levels

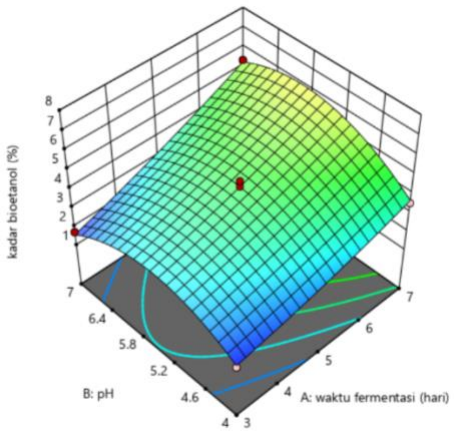

Figure 4. Effect of bioethanol content on fermentation time (days) and pH

Figure 4 is a graph of the effect of the variable fermentation time and pH on the levels of bioethanol produced. The effect of fermentation time on significant bioethanol levels is shown in table 5 that p-value = <0.0001. The effect of pH on bioethanol levels is also significant as shown in Table 5 that p-value = 0.0047. The interaction between fermentation time and pH for bioethanol is significant because p-value = 0.0191 <0.005.

The graph in Figure 4 shows that the longer the fermentation time and the higher the pH, the higher the bioethanol content. High levels of bioethanol can be influenced by favorable environmental conditions, one of which is pH. However, the optimal pH condition is pH 5.5 because *Saccharomyces cerevisiae* has an optimal pH for the growth process, namely pH 4-5 [45]. Enzyme performance in yeast is affected by pH, if the pH is too acidic or alkaline it will disrupt enzyme activity. High pH conditions can cause the value of bioethanol levels to decrease because when the fermentation media conditions lead to a neutral pH *Saccharomyces cerevisiae* enters a stationary phase or is no longer working and is experiencing growth again. During the fermentation process, the pH can decrease in the presence of organic acids produced by microorganisms [46]. This is what causes the condition of pH 4, yeast can not work optimally so the level of bioethanol produced is lower. The length of time of fermentation also affects the levels of bioethanol, because the yeast will continue to reproduce over time in a fermenting solution medium that is capable of converting glucose into bioethanol [36]. It can be seen in Figure 4 that for 3 days, yeast concentration of 1.5% and pH 5.5 produced a bioethanol content of 1.55%. Meanwhile, 7 days of fermentation, yeast concentration of 1.5% and pH 5.5 produced a bioethanol content of 7.55%.

3.3.2 Effect of Fermentation Time and Yeast Concentration on Bioethanol Levels

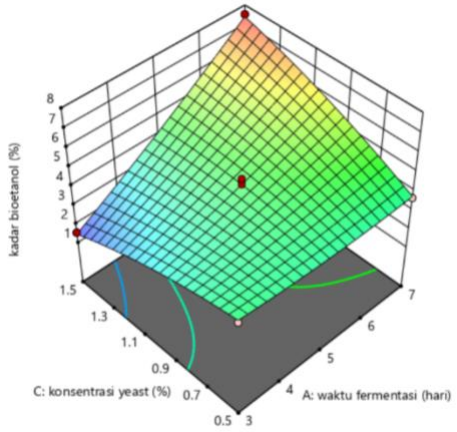

Figure 5. Effect of bioethanol content on fermentation time (days) and yeast concentration (%)

Figure 5 is a graph of the effect of the variable fermentation time and yeast concentration on the levels of bioethanol produced. The effect of fermentation time on significant bioethanol levels is shown in table 5 that p-value = <0.0001. The effect of yeast concentration on bioethanol levels is also significant as shown in table 5 that p-value = 0.0178. The interaction between fermentation time and pH towards bioethanol is significant because p-value = <0.0001.

The bioethanol content with the operating conditions of 7 days fermentation time, pH 5.5 and 1.5% yeast concentration is 7.55%, whereas, in the operating conditions of 3 days fermentation time, pH 5.5 and 0.5% yeast concentration produces bioethanol 3.85%. Figure 5 shows that the bioethanol content increases with the length of time of fermentation and the high concentration of yeast. Yeast can develop and grow in fermented solution media so that it can convert glucose into bioethanol. Thus a high concentration of yeast will increase the level of bioethanol formed in the fermentation process. However, the fermentation time has a maximum limit of 7 days, but the fermentation time that exceeds the maximum number does not affect the increase in bioethanol levels because the yeast undergoes a death phase so that the activity of the yeast in converting glucose to bioethanol decreases [47].

3.3.3 Effect of pH and Yeast Concentration on Bioethanol Levels

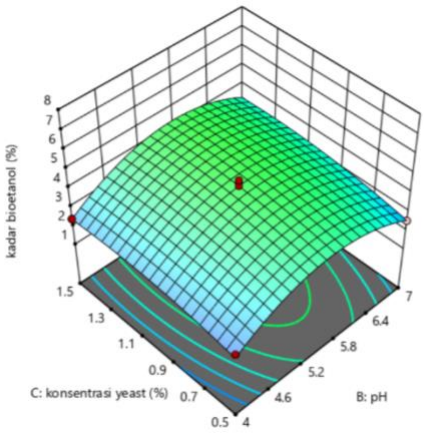

Figure 6. Effect of bioethanol content on pH and yeast concentration (%)

Figure 6 is a graph of the effect of the variable pH and concentration of yeast on the levels of bioethanol produced. The effect of pH on significant bioethanol levels is shown in Table 5 that p-value = 0.0047. The effect of yeast concentration on bioethanol levels is also significant as shown in table 5 that p-value = 0.0178. However, the interaction between fermentation time and pH for bioethanol was not significant because of the p-value = 0.5285 > 0.05.

The bioethanol content at 5 days, pH 5.5, and 1% yeast concentration was 4.6%, while at 5 days, pH 4 and 0.5% yeast concentration was 2.2%. The high concentration of yeast accelerates the occurrence of fermentation so that microorganisms can decompose glucose into ethanol maximally. This is because the higher concentration of yeast will increase the population of microorganisms that work in it so that the level of bioethanol produced is greater [41]. The pH variable in the fermentation process is very important for yeast growth because yeast can only grow at certain pH conditions. Optimal growth of *Saccharomyces cerevisiae* takes place in media with a pH of 4-5. However, the speed of the bioethanol fermentation process will decrease if the pH is below 3. Therefore, the level of bioethanol produced will be low if the pH is below 4 [45].

Table 6. Optimization of Maximum Bioethanol Content Expert Design

Fermentation time (days)	**pH**	**Yeast Concentration (%)**	**Bioethanol Content (%)**	***Desirability***
7	5,5	1,5	7,283	1,000

Table 6 shows the optimal results in response to bioethanol content of 7.283% when the operating conditions were 7 days of fermentation, pH 5.5 and yeast concentration of 1.5%, and the desirability value reached 1.000. The suitability of the model for the optimization value is obtained when the desirability value is close to one.

Table 7. Comparison of bioethanol levels with previous studies

No.	Raw material	Operating Conditions	Bioethanol Content Results	Ref
1.	*Ulva reticulata*	Fermentation time 6 days, pH 4.5	5.02%	[37]
2.	Mikroalga *Chlorella pyrenoidosa*	Fermentation time 3 days, pH 5	0.280%	[27]
3.	Alga Hijau *Spyrogyra* sp	Fermentation time 5 days, pH 4.5	0.281%	[40]
4.	Alga Merah *Gracilaria verrucosa*	Fermentation time 5 days, pH 7	0.77%	[39]
5.	*Ulva* sp.	Fermentation time 7 days, pH 5.5	7.55%	In this research

Table 7 shows the results from previous studies using different operating conditions. From the table above, the lowest bioethanol content resulted from operating conditions, namely 3 days of fermentation with a pH of 0.280%. Meanwhile, the highest bioethanol content resulted from the operating conditions of 6 days of fermentation with a pH of 4.5 of 5.02%. However, judging from the operating conditions in Table 7, it is known that previous studies produced lower levels of bioethanol compared to this study. This can be due to differences in operating conditions used in the fermentation process.

4. Conclusion

This research uses variables that include fermentation time (days), pH, and yeast concentration (%). This variable has a significant effect on the bioethanol content because it has a p-value <0.05. The results of the bioethanol content test are supported by the Analysis of Variance (ANOVA). The results show that the value of R2 is 0.9872. In this study, the bioethanol content was obtained at 7.55% under operating conditions of 7 days of fermentation, pH of 5.5, and yeast concentration of 1.5%.

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REFERENCES

[1] M. Taslim, M. Mailoa, Dan M. Rijal, "Pengaruh Ph, Dan Lama Fermentasi Terhadap Produksi *Ethanol* Dari *Sargassum Crassifolium*," 2017.

[2] B. F. S. Negara, N. Nursalim, N. E. Herliany, P. P. Renta, D. Purnama, Dan M. A. F. Utami, "*Bioethanol Production From Waste Paper Using Separate Hydrolysis Dan Fermentation*," 2019.

[3] L. Arlianti, "Bioetanol Sebagai Sumber Green Energi Alternatif Yang Potensial Di Indonesia *A Review Article*," 2018.

[4] H. Zabed, J. N. Sahu, A. Suely, A. N. Boyce, Dan G. Faruq, "*Bioethanol Production From Renewable Sources: Current Perspectives Dan Technological Progress*," *Renewable Dan Sustainable Energy Reviews*, Vol. 71. Elsevier Ltd, Pp. 475–501, 2017.

[5] A. Prisca Dan A. Kurniawan, "Pembuatan Bioetanol Dengan Teknik Imobilisasi Sel *Saccharomyces cerevisiae* Dari Limbah Kertas Hvs," *Pembuatan Bioetanol Dengan ...)* Jurnal Teknik Kimia Vokasional, Vol. 1, No. 2, Pp. 45–50, 2021.

[6] I. Winarni Dan T. B. Bardant, "Pembuatan Bioetanol Dari Limbah Kayu Sengon (Falcataria Moluccana (Miq.) Barneby & J.W. Grimes) Dengan Metode Substrat Konsentrasi Tinggi (*Bioethanol Production Of Sengon Wood (Falcataria Moluccana (Miq.)* Barneby & J.W. Grimes) Waste Using High Loading Substrate Method)," *Pusat Penelitian Dan Perngembangan Hasil Hutan*, 2017.

[7] T. H. Nufus *Et Al.*, "Efek *Flame Temperature* Dan Magnetisasi Bahan Bakar Campuran Bensin-Bioetanol Terhadap Emisi Gas Buang Pada Engine Satu Silinder 4 Langkah 125 Cc," Vol. 6, No. 1, 2022.

[8] Ansar, Nazaruddin, A. D. Azis, Dan A. Fudholi, "*Enhancement Of Bioethanol Production From Palm Sap (Arenga Pinnata (Wurmb) Merr) Through Optimization Of Saccharomyces cerevisiae As An Inoculum*," *Journal Of Materials Research Dan Technology*, Vol. 14, Pp. 548–554, Sep. 2021.

[9] N. Hasanah, S. Zaenab, Dan A. Rofieq, "Peran Biologi Dan Pendidikan Biologi Dalam Menyiapkan Generasi Unggul Dan Berdaya Saing Global," 2015.

[10] I. Mishbach, N. S. Permatasari, M. Zainuri, H. P. Kusumaningrum, Dan D. Hastuti, "Potensi Mikroalga Anabaena Sp. Sebagai Bahan Utama Bioetanol *Potential Of The Microalgae Anabaena Sp. As Bioetanol Feedstock*," Jurnal Penilitian Biologi, Botani, Zoologi Dan Mikrobiologi, Vol. 07, No. 1, Pp. 69–76, 2022.

[11] S. F. Pradigdo, F. Arifan, W. Broto, Dan S. N. Noviana, "Pemanfaatan Kulit Kentang Dalam Pembuatan Bioetanol Dengan Metode Hidrolisa Asam Di Desa Sikunang," Vol. 02, No. 1, Pp. 12–20, 2021.

[12] N. Ketut Dan E. Dira, Teori Dan Aplikasi Pembuatan Bioetanol Dari *Selulose* (Bambu). 2017.

[13] F. Azizah, "Uji Aktivitas Antibakteri Ekstrak Etanol Alga Hijau (*Ulva Lactuca*) Dengan Variasi Waktu Sonikasi," 2021.

[14] L. Ktari, "*Pharmacological Potential Of Ulva Species: A Valuable Resource*," *J Anal Pharm Res*, Vol. 6, No. 1, Sep. 2017.

[15] E. Dewi Nurcahaya, "*Ulva Lactuca*," 2018.

[16] Y. Natawijaya Albert, "*Use Of Bromelain Enzyme To Extract Glutamic Acid At Seaweed Ulva* Sp.," 2019.

[17] Tri Hdanayani, "Karakteristik Dan Aspek Biologi *Ulva* Sp. (*Chlorophyta, Ulvaceae*)," *Oseana*, Vol. Xli, Pp. 1–8, 2016.

[18] N. Hendrasarie Dan E. Mahendra, "Pemanfaatan Sampah Sayur Dari Pasar Tradisional Untuk Produksi Bioetanol," *Serambi Engineering*, Vol. V, No. 3, Pp. 1115–1122, 2020.

[19] I. Febriana, I. Purnamasari, J. Teknik Kimia, Dan P. Negeri Sriwijaya Jl Srijaya Negara Bukit Besar, "Pengaruh Temperatur Inkubasi Dan Jenis Ragi Dalam Pembuatan Bioetanol Dari Limbah Kulit Pisang (*Musa Paradisiaca*)," 2017.

[20] A. Dewi N.K Dan A. Hartiati, "Pengaruh Suhu Dan Jenis Asam Pada Hidrolisis Pati Ubi Talas (*Colocasia Esculenta* L. *Schott*) Terhadap Karakteristik Glukosa," *Jurnal Rekayasa Dan Manajemen Agroindustri*, Vol. 6, Pp. 307–315, 2018.

[21] A. Monika, "Uji Hidrolisis Pati Dengan Asam *Hydrolysis Test Of Starch With Acid*," 2021.

[22] S. M. D. Kolo Dan E. Edi, "Hidrolisis Ampas Biji Sorgum Dengan Microwave Untuk Produksi Gula Pereduksi Sebagai Bahan Baku Bioetanol," *Jurnal Saintek Lahan Kering*, Vol. 1, No. 2, Pp. 22–23, Dec. 2018.

[23] R. T. Putri, L. Hardjito, Dan J. Santoso, "Optimasi Hidrolisis Mikrobiologi Serta Bioaktivitas Antibakteri, Antioksidan, Dan Antikoagulan Hidrolisat *Ulva Lactuca*," *Jurnal Pascapanen Dan Bioteknologi Kelautan Dan Perikanan*, Vol. 15, No. 2, P. 123, Dec. 2020.

[24] M. Saiful Fajri *Et Al.*, "Produksi Gula Cair Dengan Proses Hidrolisis Asam Dengan Bahan Pati Singkong," 2022.

[25] S. Bahri, A. Aji, Dan F. Yani, "Pembuatan Bioetanol Darikulit Pisang Kepok Dengan Cara Fermentasi Menggunakan Ragi Roti," *Jurnal Teknologi Kimia Unimal*, Vol. 7, No. 2, Pp. 85–100, 2018.

[26] M. Gustina Dan S. Bahri, "Pengaruh Lama Waktu Fermentasi Terhadap Kadar Bioetanol Dari Pati Ubi Jalar Ungu (Ipomea Batata L)," 2022.

[27] Y. A. Erlangga, C. Nugroho, Dan S. Miskah, "Pembuatan Bioetanol Dari Mikroalga Dengan Variasi Konsentrasi Asam, Waktu Hidrolisis, Dan Fermentasi," 2017.

[28] A. I. Nuraini Dan N. Ratni, "Pengaruh Waktu Dan Nutrien Pada Proses Fermentasi Sampah Organik Menjadi Bioetanol Dengan Metode Ssf," 2021.

[29] T. Widyaningrum Dan M. Parahadi, "*Bioethanol Levels Of Dragon Fruit (Hylocereus Polyrhizus) Peel With The Addition Of Blend Crude Cellulase Enzyme From*

Trichoderma reesei Dan Aspergillus Niger," *J Trop Biodivers Biotechnol*, Vol. 5, No. 1, Apr. 2020.

[30] A. Khoiri *Et Al.*, "Review Teknologi Fermentasi Bioetanol Dari Berbagai Bahan Organik," Vol. 5, No. 2, Pp. 272–276, 2020.

[31] K. Zhang, G. Zheng, K. Saul, Y. Jiao, Z. Xin, Dan D. Wang, "*Evaluation Of The Multi-Seeded (Msd) Mutant Of Sorghum For Ethanol Production*," *Ind Crops Prod*, Vol. 97, Pp. 345–353, Mar. 2017.

[32] Y. Suryani, I. Hernaman, Dan D. Ningsih, "*The Effect Of Urea Dan Sulfur Addition In Solid Waste Bioethanol Fermented By Em-4 On Contents Of Crude Protein Dan Fiber*," 2017.

[33] I. Febriana, A. Zikri, S. Hatina, J. Teknik Kimia, Dan P. Negeri Sriwijaya Jl Srijaya Negara Bukit Besar, "Pengaruh Konsentrasi Ragi Roti (*Saccharomyces Cereviseae*) Dan Lama Fermentasi Dalam Pembuatan Bioetanol Menggunakan Kulit Pisang," 2018.

[34] M. M. Maharani Dan M. Bakrie, "Pengaruh Jenis Ragi, Massa Ragi Dan Waktu Fermentasi Pada Pembuatan Bioetanol Dari Limbah Biji Durian," Muhammad Bakrie, 2021.

[35] S. Schläfle, T. Senn, P. Gschwind, Dan R. Kohlus, "*Feasibility Dan Energetic Evaluation Of Air Stripping For Bioethanol Production*," *Bioresour Technol*, Vol. 231, Pp. 109–115, 2017.

[36] N. Herawati, U. R. Defo, Dan Atikah, "Pengaruh Jenis Katalis Asam Dan Waktu Fermentasi Terhadap % *Yield* Bioetenol Dari Rumput Gajah (*Pennistum Purpureum Schumach*)," 2019.

[37] S. M. D. Kolo, J. Presson, Dan P. Amfotis, "Produksi Bioetanol Sebagai Energi Terbarukan Dari Rumput Laut *Ulva Reticulata* Asal Pulau Timor," *Alchemy Jurnal Penelitian Kimia*, Vol. 17, No. 2, P. 159, Sep. 2021.

[38] B. F. S. Negara, N. Nursalim, N. E. Herliany, P. P. Renta, D. Purnama, Dan M. A. F. Utami, "Peranan Dan Pemanfaatan Mikroalga *Tetraselmis Chuii* Sebagai Bioetanol," *Jurnal Enggano*, Vol. 4, No. 2, Pp. 136–147, Sep. 2019.

[39] W. Karta, N. Made Puspawati, Dan Y. Ciawi, "Pembuatan Bioetanol Dari Alga *Codium Geppiorum* Dan Pemanfaatan Batu Kapur Nusa Penida Teraktivasi Untuk Meningkatkan Kualitas Bioetanol," 2015.

[40] D. Jaya, R. Setiyaningtyas, Dan S. Prasetyo, "Pembuatan Bioetanol Dari Alga Hijau *Spirogyra* Sp," 2018.

[41] S. J. Sari *Et Al.*, "Pembuatan Bioetanol Dari Mikroalga Limbah Cair Kelapa Sawit Dengan Variasi Konsentrasi Ragi Menggunakan *Saccharomyces Cerevisiae*," 2018.

[42] W. A. Saputra, A. Susanto, Dan R. Pramesti, "Produksi Bioetanol Dari Tepung Agar G*racilaria Verrucosa (Hudson) Papenfuss* Yang Dihidrolisis Dengan Menggunakan Larutan Asam Sulfat," 2013.

[43] A. Ansar, G. M. Dwi Putra, S. H. Abdullah, Dan M. S. Siahaya, "Pengaruh Variasi Konsentrasi Starter Dan Npk Terhadap Kadar Etanol Hasil Fermentasi Dan Destilasi Nira Aren," *Jurnal Teknotan*, Vol. 13, No. 2, P. 35, Dec. 2019.

[44] N. Ihda, F. Nisa, Dan A. Aminudin, "Pengaruh Waktu Distilasi Etanol-Air Terhadap Konsentrasi Overhead Product Dan Bottom Product," *Cheesa*, Vol. 2, No. 1, 2019.

[45] I. Gede Yogi Wikrama Yuda *Et Al.*, "Studi Pengaruh Ph Awal Media Dan Konsentrasi Substrat Pada Proses Fermentasi Produksi Bioetanol Dari Hidrolisat Tepung Biji Kluwih (*Actinocarpus Communis*) Dengan Menggunakan *Saccharomyces Cerevisiae*," 2018.

[46] U. Fadilah, I. Made Mahaputra Wijaya, N. Semadi Antara, M. Jurusan Teknologi Industri Pertanian, F. Teknologi Pertanian Unud, Dan D. Teknologi Industri Pertanian, "Studi Pengaruh pH Awal Media Dan Lama Fermentasi Pada Proses Produksi Etanol Dari Hidrolisat Tepung Biji Nangka Dengan Menggunakan *Saccharomycess Cerevisiae*," 2018.

[47] F. H. Moede, S. T. Gonggo, Dan Ratman, "Pengaruh Lama Waktu Fermentasi Terhadap Kadar Bioetanol Dari Pati Ubi Jalar Kuning (Ipomea Batata L)," 2017.