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# Microwave Assisted Hydrolysis Ulva sp. Using HCl for the Production of Bioethanol Raw Materials

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**Abstract.** The challenges faced by the Indonesian state are increasing greenhouse gases, climate change, and depleting fossil fuel reserves. This requires the exploration of alternative energy that is environmentally friendly and sustainable. Algae biomass, especially *Ulva* sp. is one of the resources that have the potential for bioethanol production as an alternative energy producer. The purpose of this study is to determine the potential of *Ulva* sp. as raw material for bioethanol and to determine the effect of particle size, solvent concentration, and power on the hydrolysis process. This study used *Ulva* sp. as raw material. hydrolyzed with HCl solvent using the microwave assisted hydrolysis method. Hydrolysis with HCl concentrations of 0.1 N, 1 N, 2 N, variations of microwave power 150 watts, 300 watts, 450 watts, and particle sizes of 60 mesh, 80 mesh, and 100 mesh. Measurement of reducing sugar levels was carried out using the dinitro salicylic acid (DNS) method. The results showed that the best conditions for hydrolysis were when the HCl concentration was 0.1 N, the microwave power was 450 watts, and the particle size was 80 mesh which resulted in a reducing sugar content of 20.751 mg/mL.

**Keywords:** *Bioethanol, reducing sugar, hydrolysis, microwave assisted hydrolysis, Ulva sp.*

## 1. Introduction

Energy needs increase every year along with the increasing population and economy of a country, including Indonesia. This also has an impact on increasing the use of transportation and industrial activities which results in the use of fuel oil (BBM) increasing. The world's energy needs still rely on fossil energy [1]. Meanwhile, non-renewable energy supplies, such as coal and oil, are running low. Therefore the need for alternative energy that can replace the availability of non-renewable energy that is environmentally friendly and renewable. The National Energy Policy issued by the government in Presidential Decree No. 5 of 2006 states

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that the target for the use of biofuels in 2010 is 2% and in 2025 it is 5% [2]. Bioethanol is an alternative that can replace fuel oil derived from natural resources. Bioethanol is obtained from cellulose, starch, and simple sugars [3]. Bioethanol is more widely used as an alternative energy due to its environmentally friendly use [4], [5].

Bioethanol with the molecular formula $C_2H_5OH$ can be produced using biomass containing starch, cellulose, and sugar [6]. Generally, the main processes in the conversion of biomass to ethanol consist of two processes, namely hydrolysis, and fermentation. Hydrolysis aims to break down polysaccharides into monosaccharides [7]. Polysaccharides become ethanol through chemical and biological processes. One of the potential biomass that can be utilized as bioethanol is seaweed. The development of marine resources owned by Indonesia has great and promising potential. The development of Indonesia's marine cultivation land is 80,929 ha with a beach area of 46,734,300 tons/year [8]. However, only about a quarter of seaweed cultivation can be used as industrial raw materials and processed products. Seaweed cultivation is influenced by chemical factors, namely the quality of the water at the location of the aquaculture waters [9]. Seaweed contains essential amino acids, crude fiber, unsaturated fats (omega 3 and omega 6), polysaccharides (non-starch), minerals, and vitamins [10], [11]. Seaweed cultivation as an alternative energy is the latest innovation and must be supported. Data from the Inha University of Korea states that 58700 L of biodiesel can be produced in one hectare of seaweed with an estimated oil content of 30% [12]. The content of macroalgae consists of 60% carbohydrates, 10-47% protein, 1-3% lipids, and 7-38% other minerals [13]–[16]. This causes macroalgae to be considered as a future method to become an alternative to sustainable biomass production. The high carbohydrate fraction includes a wide variety of soluble polysaccharides, such as laminarian and alginate in the brown type, starch and sulfate gelectan in the red type, and *Ulva* in the green type [17].

*Ulva sp.* is a type of green macroalgae that has the potential as a raw material for biomass [18]. This is due to its fast growth rate and adaptability to a variety of habitats with different abiotic conditions. Carbohydrates in *Ulva* sp. consists of C5 and C6 monosaccharides, iduronic acid, and glucuronic acid [19]. Ulva sp. carbohydrates. mostly in the form of complex hydrocolloid *Ulva* and with cellulose which is a structural component of the cell wall and starch as a place of intracellular energy storage [20], [21]. Acid hydrolysis is often used to hydrolyze cellulose [22], [23]. Acid hydrolysis is divided into two types, namely hydrolysis of dilute acids and concentrated acids. The temperature used for the hydrolysis of concentrated acids is lower than that of dilute acids. This can minimize the degradation of sugar. Acid functions as a catalyst

which can accelerate the rate of the hydrolysis process [24], [25]. The resulting sugar conversion will be high when using concentrated acids up to 90% [26]. The use of acid in the hydrolysis process can produce a greater yield compared to hydrolysis with enzymes [27]. Acid hydrolysis has advantages, including the yield of more ethanol compared to using enzymatic hydrolysis. The hydrolysis process is also faster and more on the random breaking of glycosidic bonds [28]. The effectiveness of acid hydrolysis can be increased by using microwave irradiation [29], [30].

Alkaline and acid treatment at 100 –120ºC can only digest hemicellulose, so cellulose must be hydrolyzed further with the addition of acid so that it can be converted into fermentable sugar. However, it cannot effectively hydrolyze some types of algae due to different cell wall compositions [31]. A study conducted by Rabelo, et al [32] showed that the maximum release of glucose reached 68.2% after being hydrolyzed using Chlorococcum humicola with 20 mg of cellulose at 40ºC for 72 hours. Enzymatic hydrolysis shows a suitable potential in microalgae biomass. However, the disadvantages of enzymatic hydrolysis such as long processing time and high costs indicate the need for more processing and treatment to produce optimal bioethanol [33]. Lignin binding compounds that protect cellulose make cellulose difficult to hydrolyze [34]. Pre-treatment aims to break down and reduce the amount of lignin and hemicellulose, increase the porosity of the material, and destroy the crystal structure contained in cellulose. Several methods in the pre-treatment process, including chemically by adding acids or bases and enzymatically [35]. Enzymatic pretreatment has higher complexity and is more expensive than acid pretreatment [36]. Hydrolysis which is commonly used on the type of seaweed *Ulva* sp. for bioethanol is enzymatic polysaccharide hydrolysis and fermentation with the help of microorganisms (*Saccharomyces cerevisiae*) [37].

**Table 1.** Hydrolysis of Ulva that has been carried out

| Material | Standard Method and Results | Ref |
|---|---|---|
| *Ulva reticulata* | Acid hydrolysis without delignification using $H_2SO_4$ 2% (v/v) at 75-150°C for 30 minutes (microwave), obtained a reducing sugar content of 23.7 g/L | [27] |
| *Ulva reticulata* | Acid hydrolysis by delignification using $H_2SO_4$ 2% (v/v) at 75-150°C for 30 minutes (microwave), obtained a reducing sugar content of 27.3 g/L | [27] |
| *Ulva lactuca* | Hydrothermal hydrolysis at 121°C for 30 minutes using an autoclave, obtained a reducing sugar content of 2.24% | [38] |
| *Ulva reticulata* | Acid hydrolysis using 2% $H_2SO_4$ for 30-50 minutes (microwave irradiation), obtained reducing sugar levels of 16.41-27.97 g/L | [39] |
| *Ulva reticulata* | Acid hydrolysis using 2% $H_2SO_4$ with a temperature variation of 75-150°C (microwave irradiation), obtained reducing sugar content of 5.80-27.30 g/L | [39] |

| Material | Standard Method and Results | Ref |
|---|---|---|
| *Ulva lactuca* | Hydrothermal hydrolysis at 180°C for 60 minutes, obtained a reducing sugar content of 7.83 mg/g | [40] |
| *Ulva prolifera* | Thermal acid hydrolysis with 0.9M $H_2SO_4$ using a temperature of 121°C for 50 minutes, obtained a reducing sugar content of 11.07% | [41] |
| *Ulva lactuca* | Acid hydrolysis with 1N $H_2SO_4$ using a temperature of 121°C for 30 minutes, obtained a reducing sugar content of 158 mg/g | [42] |
| *Ulva lactuca* | Acid hydrolysis with 1% HCL using a temperature of 121°C for 60 minutes (autoclave), obtained a reducing sugar content of 13.17% | [43] |
| *Ulva sp.* | Hydrolysis using polyoxometalate (POM) with microwave irradiation for 4-10 minutes at 140°C, obtained reducing sugar levels of 349-435 mg/g | [44] |

This research was conducted to determine the potency of *Ulva* sp. as raw material for bioethanol and to determine the effect of particle size, solvent concentration, and power on the hydrolysis process. The use of hydrochloric acid in the process of making bioethanol because hydrochloric acid produces glucose higher than sulfuric acid. The variables used in this study were particle size, solvent concentration, and microwave power.

## 2. Materials and Methods

### 2.1 Materials

The materials in this study were *Ulva* sp., hydrochloric acid (HCl), aquadest, glucose, 2M NaOH, potassium sodium tartrate, and dinitro salicylic acid (DNS).

### 2.2 Material preparation

*Ulva sp.* washed to remove sand and other impurities. Then the material is dried using an oven with a temperature of 70°C for 3 hours. Physical pretreatment was carried out by pulverizing the material using a blender, which was then sieved using 60 mesh, 80 mesh, and 100 mesh sieves to produce *Ulva* sp. powder. Then an analysis of the water content was carried out. The purpose of the water content analysis is to determine the water content in the sample. This is because water is very influential on metabolic activity both enzymatic, microbial, and chemical activity. The principle of water content analysis is heating and weighing using an oven at a temperature of 105°C until a constant weight is obtained. The temperature used is above the boiling point of water 100°C for maximum evaporation of water [45]. This research resulted in a water content of 8.1%.

### 2.3 Hydrolysis Process

*Ulva sp* powder. Weigh 3 grams using an analytical balance. Then it was put into an erlenmeyer plus 100 mL HCl with various concentrations, namely 0.1 N, 1 N, and 2 N. The hydrolysis process took place with several microwave powers of 150 watts, 300 watts, and

450 watts. The optimum time for the hydrolysis process is 15 minutes [46]. Furthermore, the cooling process in the hydrolyzed solution until it reaches room temperature. The resulting hydrolysis solution is filtered using filter paper to separate the filtrate from the residue. Then the next process is the analysis of reducing sugars.

### 2.4 Sample Analysis

Reducing sugar levels can be analyzed using the dinitro salicylic acid or 3,5-dinitro salicylic acid (DNS) method using a spectrophotometer [47]. DNS reagent was prepared by dissolving 1 gram of DNS powder, 20 mL of 2M NaOH, and 30 grams of Ka-Na tartrate until the volume reached 100 mL. Ka-Na tartrate was dissolved separately using sufficient distilled water, then 1 gram of DNS powder was dissolved using 20 mL of 2 M NaOH. After the powder dissolves, the two solutions are homogenized using a stirrer. The solution is stored in the refrigerator solution.

Preparation of the glucose standard curve begins with the preparation of a 1000 ppm glucose mother liquor made by dissolving 100 mg of anhydrous glucose in 100 mL of distilled water. The standard glucose solutions were made with concentrations of 50 ppm, 200 ppm, 400 ppm, 600 ppm, 800 ppm, and 1000 ppm. Each standard glucose solution was taken 1 mL and filled in each test tube. Then 1 mL of Reagent DNS and 1 mL of distilled water were added. The solution was then homogenized and heated with a water bath for 5 minutes. After that, the solution was cooled to room temperature and added distilled water to a final volume of 5 mL and homogenized again. The adsorption value was measured using a spectrophotometer with a wavelength of 540 nm. The standard curve obtained will be used to determine the concentration of the sample.

Reducing sugar was tested by taking 1 mL of the sample and putting it into a test tube, then adding 1 mL of DNS reagent and 2 mL of distilled water. Additions were made to each test tube using a pipette. The filled test tube was heated using a water bath for 5 minutes. The purpose of heating is for a reaction to occur between glucose and DNS. After that, the solution was cooled to room temperature, then added distilled water to a final volume of 5 mL and homogenized again. Next, the absorbance of each solution was measured at a wavelength of 540 nm. With a wavelength of 540 nm, the dinitro salicylic acid compound is able to absorb these waves strongly [48]. The measured values are then plotted on a standard curve. The reduced sugar content is obtained from the standard curve equation where y is the absorbance value and x is the reduced sugar content [49].

### 2.5 Data Analysis

Data analysis was used using Design Expert version 13 software with the Box-Behnken Design (BBD) Surface Response Method (BBD) 17 times as shown in table 2. The choice of method was based on the variables used, namely three variables, which were considered more effective compared to other methods [50 ], [51].

**Table 2.** *Hydrolysis Run Order*

| No. | Particle Size (mesh) | Concentration of HCl (N) | Microwave Power (*watt*) |
|---|---|---|---|
| 1. | 80 | 0.1 | 450 |
| 2. | 100 | 2 | 300 |
| 3. | 60 | 2 | 300 |
| 4. | 60 | 1 | 450 |
| 5. | 100 | 0.1 | 300 |
| 6. | 100 | 1 | 450 |
| 7. | 80 | 2 | 150 |
| 8. | 60 | 1 | 150 |
| 9. | 80 | 1 | 300 |
| 10. | 80 | 2 | 450 |
| 11. | 60 | 0.1 | 300 |
| 12. | 80 | 1 | 300 |
| 13. | 80 | 0.1 | 150 |
| 14. | 100 | 1 | 150 |
| 15. | 80 | 1 | 300 |
| 16 | 80 | 1 | 300 |
| 17. | 80 | 1 | 300 |

## 3. Results and Discussion

### 3.1 Standard Glucose Curve

The glucose standard curve gives results as shown in Figure 1.

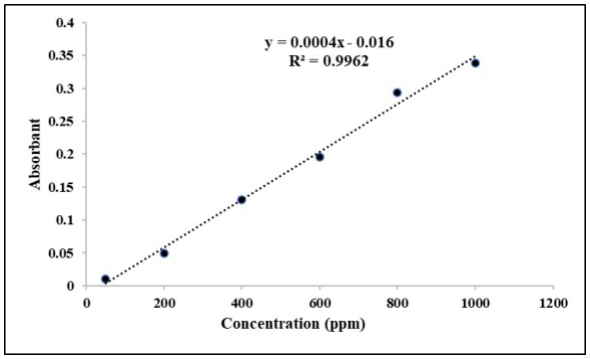



**Figure 1.** Glucose Standard Curve

Glucose standard curve results with $R^2 = 0.9962$ and linear equations $y = 0.0004x – 0.016$ (1) for determining the concentration of glucose as the determination of reducing sugars.

### 3.2 Quantitative Analysis of Reducing Sugar Content

Based on the reducing sugar test on the *Ulva* sp. measured using a UV-Vis spectrophotometer, the highest reducing sugar content was 20.751 mg/mL with operating conditions parameters, namely particle size of 80 mesh, HCl concentration of 0.1 N, and microwave power of 450 watts. The results of a study conducted by Dave, et al [41] yielded a reducing sugar content of 1.61 mg/mL when hydrolyzed using $H_2SO_4$ at 121°C where these results were lower than this study. Differences in results may occur due to differences in operating conditions.

**Table 3.** Results of Reducing Sugar Levels

| No. | Particle Size (mesh) | Concentration of HCl (N) | Microwave Power (watt) | Reducing Sugar Levels (mg/mL) |
|---|---|---|---|---|
| 1. | 80 | 0.1 | 450 | 20.751 |
| 2. | 100 | 2 | 300 | 11.083 |
| 3. | 60 | 2 | 300 | 5.672 |
| 4. | 60 | 1 | 450 | 7.584 |
| 5. | 100 | 0.1 | 300 | 16.172 |
| 6. | 100 | 1 | 450 | 11.253 |
| 7. | 80 | 2 | 150 | 7.252 |
| 8. | 60 | 1 | 150 | 3.021 |
| 9. | 80 | 1 | 300 | 5.674 |
| 10. | 80 | 2 | 450 | 8.333 |
| 11. | 60 | 0.1 | 300 | 14.751 |
| 12. | 80 | 1 | 300 | 6.012 |
| 13. | 80 | 0.1 | 150 | 9.924 |
| 14. | 100 | 1 | 150 | 4.331 |
| 15. | 80 | 1 | 300 | 6.252 |
| 16. | 80 | 1 | 300 | 6.083 |
| 17. | 80 | 1 | 300 | 5.834 |

### 3.1 *Analysis of Variance* (ANOVA)

*Analysis of Variance* (ANOVA) is a form of statistical hypothesis testing in which conclusions are 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. In the table, the p-value obtained is <0.0001. This shows that the variables used in this study affect the levels of reducing sugars. Lack of Fit is a deviation from the model. The p-value and lack of fit which were > 0.05 showed insignificant results and in this study the lack of fit value was 0.1230 where the results indicated suitability for the model. A significant relationship between the variable and the yield of reducing sugar can be seen from the $R^2$ value obtained of 0.9979. In table 4 you can see the difference between the predicted $R^2$ value and the adjusted $R^2$ value <0.2 which shows reasonable data.

**Table 4.** Results of Analysis of Variance (ANOVA)

| *Source* | *Sum of Squares* | *df* | *Mean square* | *F-value* | *p-value* | |
|---|---|---|---|---|---|---|
| **Model** | 352.11 | 9 | 39.12 | 366.94 | < 0.0001 | *significant* |
| A- Particle Size | 17.49 | 1 | 17.49 | 164.07 | < 0.0001 | |
| B- Concentration of HCl | 107.02 | 1 | 107.02 | 1003.73 | < 0.0001 | |
| C- Microwave Power | 68.50 | 1 | 68.50 | 642.50 | < 0.0001 | |
| AB | 3.98 | 1 | 3.98 | 37.33 | 0.0005 | |
| AC | 1.37 | 1 | 1.37 | 12.84 | 0.0089 | |
| BC | 23.77 | 1 | 23.77 | 222.90 | < 0.0001 | |
| $A^2$ | 0.9085 | 1 | 0.9085 | 8.52 | 0.0224 | |
| $B^2$ | 126.77 | 1 | 126.77 | 1188.95 | < 0.0001 | |
| $C^2$ | 0.0505 | 1 | 0.0505 | 0.4735 | 0.5135 | |
| **Residual** | 0.7463 | 7 | 0.1066 | | | |
| *Lack of Fit* | 0.5454 | 3 | 0.1818 | 3.65 | 0.1230 | *not significant* |
| Pure Error | 0.2009 | 4 | 0.0502 | | | |
| **Cor Total** | 352.86 | 16 | | | | |

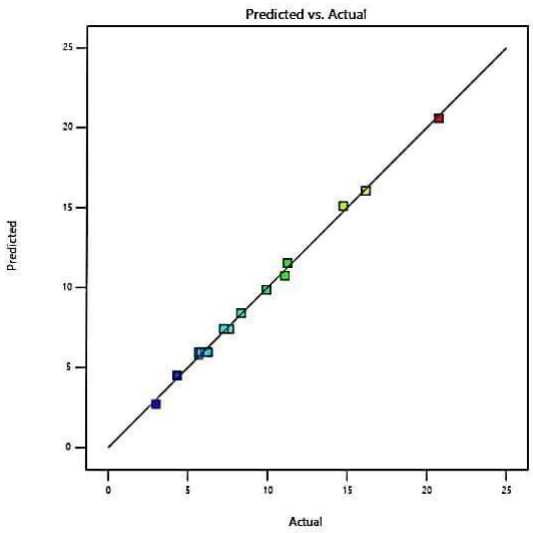



**Figure 2.** Graph of comparison of model data with experimental data

The fit between the experimental data and the model can be seen based on the porosity plot graph in Figure 2. The response of reducing sugar levels will increase in direct proportion to particle size, microwave power, the interaction between particle size and HCl

concentration, and the interaction between particle size and microwave power. This is indicated by a positive constant value. The response of reducing sugar levels will decrease along with decreasing HCl concentration, the interaction between HCl concentration and microwave power. This is indicated by a negative constant value

$$Y = 5.97 + 1.48A - 3.66B + 2.93C + 0.9975AB + 0.5850AC - 2.44BC + 0.46456A^2 + 5.49B^2 + 0.1095C^2 \quad (1)$$

where,

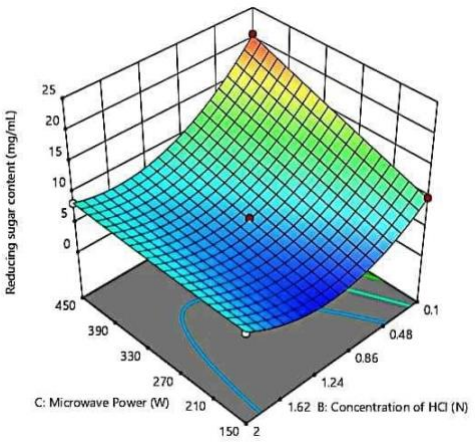
Y = Reducing sugar content (mg/mL)

A = Particle Size (mesh)

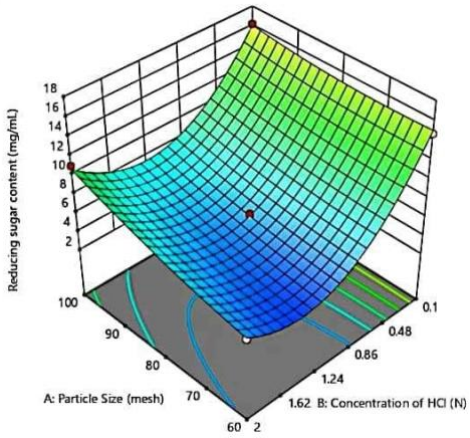
B = Concentration of HCl (N)

C = Microwave Power (W)

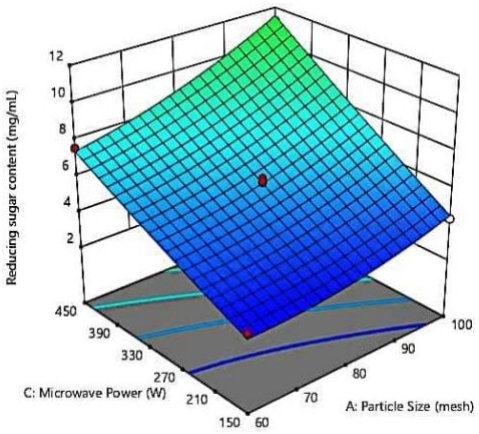
3.2 Effect of Parameters on Reducing Sugar Levels




(a)




(b)




(c)

**Figure 3.** Variable relationship to reducing sugar content between (a) microwave power (watts) and HCl concentration (N); (b) particle size (mesh) and concentration of HCl (N); (c) particle size (mesh) and microwave power (watts)

Figure 3 is a graph of each variable (microwave power, concentration, and particle size) on the resulting reducing sugar content. The reducing sugar content is affected by the HCl concentration as shown in Figure 3(a) and Figure 3(b) which shows that at a concentration of 0.1 N, 450 watt microwave power, and 80 mesh particle size, a reducing sugar content of 20.75 mg/mL. Meanwhile, at a concentration of 2 N, microwave power of 450 watts, and a particle size of 80 mesh, a reducing sugar content of 8.33 mg/mL was obtained. This is because the use of acids with low concentrations can reduce the decomposition of glucose by acids, while the use of acids with high concentrations can accelerate hydrolysis, but the resulting reducing sugars will be less [28].

Figure 3(a) shows that a concentration of 0.1 N can produce the maximum reducing sugar from Ulva sp. N, and 2 N which showed an increase in reducing sugar levels as the concentration of HCl decreased. This decrease is due to an increase in acid concentration in the hydrolysis process so that glucose is degraded into other compounds [28].

Figures 3(a) and 3(c) show that the higher the microwave power used, the higher the reduced sugar content obtained. The effect of power on the process of hydrolyzing carbohydrates is that the higher the conversion obtained will be higher, but if the power is too high the conversion obtained will decrease [52]. The microwave power used affects the reducing sugar content where there is a significant release of organic matter in the range of 360 watts to 630 watts as research has been conducted by Kumar, et al [46].

Figures 3(b) and 3(c) show that at a particle size of 100 mesh, a concentration of 0.1, and a microwave power of 300 watts, a reducing sugar content of 16.17 mg/mL was obtained.

Meanwhile, at a particle size of 60 mesh, a concentration of 0.1, and a microwave power of 300 watts, a reducing sugar content of 14.75 mg/mL was obtained. The finer the particle size of the raw material, the greater the ethanol content produced, which is also directly proportional to the reduced sugar content produced [53]. This is also because the smaller the particle size, the greater the surface area in contact with the HCl solution so the greater the reducing sugar produced [54].

Table 5 shows the optimal results for a reducing sugar content response of 20.584 mg/mL when the particle size is 80 mesh, the HCl concentration is 0.1 N, the microwave power is 450 watts and the desirability value is 1,000. The suitability of the model for the optimization value is obtained when the desirability value is close to one [55].

**Table 5.** Optimization of Maximum Reducing Sugar Content Expert Design

| Particle Size (mesh) | Concentration of HCl (N) | Microwave Power (watt) | Reducing Sugar Yield (mg/mL) | *Desirability* |
|---|---|---|---|---|
| 80.00 | 0.100 | 450.00 | 20.584 | 1.000 |

## 3.3 Comparison of Reducing Sugar Levels with Previous Research

**Table 6.** Comparison of Reducing Sugar Levels with Previous Research

| No. | Research Title | Method | Reducing Sugar Yield | Ref |
|---|---|---|---|---|
| 1. | *Ulva reticula* | Microwave irradiation | 23.7 mg/mL | [27] |
| 2. | *Ulva* sp. | Autoclave, $H_2SO_4$ | 12 mg/mL | [57] |
| 3. | *Ulva prolifera* | Enzymatic | 420 mg/mL | [58] |
| 4. | *Ulva* sp. | Microwave, HCl | 20.75 mg/mL | |

Table 6 shows the results from previous *Ulva* studies using different methods. From the table above the lowest reducing sugar content resulted from the autoclave method of 12 mg/mL. Meanwhile, the highest reducing sugar content resulted from the enzymatic method of 420 mg/mL. However, the enzymatic method has several drawbacks including being more expensive, the process taking longer, and being complicated.

## 4. Conclusion

Research variables which include particle size (mesh), HCl concentration (N), and microwave power (watts) have a significant effect on reducing sugar levels. Testing for reducing sugar content was supported by the Analysis of Variance (ANOVA) test which obtained an $R^2$ value of 0.9979. This research produced the highest reducing sugar content, namely 20.751 mg/mL with the conditional parameters of 80 mesh particle size, 0.1 N HCl concentration, and 450 watt microwave power.

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