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Research Article

Optimization of *Ulva* sp. Decomposition using H_2SO_4 with Microwave-Assisted Hydrolysis Method as Feedstock of Bioethanol

Optimasi Dekomposisi Ulva sp. Menggunakan H_2SO_4 dengan Metode Microwave-Assisted Hydrolysis Sebagai Bahan Baku Pembuatan Bioetanol

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Abstract

Bioethanol is a renewable energy used to reduce dependence on fossil fuels, which have negative impacts on the environment. Furthermore, *Ulva sp.* contains high levels of carbohydrates, making it potentially suitable as a raw material for bioethanol production. Therefore, this study aims to determine the optimal decomposition process using the microwave-assisted hydrolysis method with an acid solvent (H_2SO_4), by examining the effects of acid concentration, hydrolysis time, and microwave power. Optimization was carried out using several parameters such as hydrolysis time, microwave power, and the ratio of raw materials to solvents. The ANOVA test results showed that the hydrolysis variable parameter had a significant effect on the reducing sugar content obtained, evidenced by the R^2 value of 0.9892. The highest reducing sugar content of 19.71 mg/mL was produced under the operating conditions of 15 min hydrolysis time, 450 W microwave power, and 0.065 g/mL ratio of raw material to solvents.

Keywords: bioethanol; hydrolysis; microwave-assisted hydrolysis; reducing sugar; Ulva sp.

Abstrak

Bioetanol merupakan bentuk energi terbarukan yang digunakan dalam mengurangi ketergantungan terhadap penggunaan bahan bakar fosil yang menimbulkan berbagai dampak negatif terhadap lingkungan. Ulva sp. mengandung karbohidrat yang tinggi sehingga berpotensi sebagai bahan baku produksi bioetanol. Penelitian ini bertujuan untuk mengidentifikasi konsentrasi asam, waktu hidrolisis, dan daya microwave untuk mendapatkan proses dekomposisi yang optimal menggunakan metode microwave-assisted hydrolysis dengan pelarut asam (H_2SO_4). Optimasi dilakukan dengan menggunakan beberapa parameter seperti waktu hidrolisis, daya microwave, dan rasio massa bahan baku terhadap volume pelarut. Hasil uji ANOVA menunjukkan bahwa parameter waktu, daya, serta rasio bahan dan pelarut pada proses hidrolisis berpengaruh signifikan terhadap kadar gula pereduksi yang diperoleh, yang didukung oleh nilai R^2 sebesar 0,9892. Kadar gula pereduksi tertinggi pada penelitian ini yaitu sebesar 19,71 mg/mL pada parameter kondisi operasi berupa waktu hidrolisis selama 15 menit, daya microwave 450 W, dan rasio massa bahan baku terhadap volume pelarut 0,065 g/mL.
***Kata kunci**: bioetanol; gula pereduksi; hidrolisis; microwave-assisted hydrolysis; Ulva sp.*

1. Introduction

As Indonesia continues to witness advancements and progress in multiple areas, including population growth, transportation sector expansion, and technological developments, the demand for fuel energy in the country has experienced a consistent rise year after year [1,2]. According to the World Energy Agency, the world's demand for energy will increase by up to 45% or approximately 1.6% annually by 2030. However, the primary energy supply is still dominated by the use of fossil fuel energy which contributes to increased carbon dioxide gas emissions and significant changes to climate instability [3-5]. Carbon emissions originating from the burning of carbon compounds such as carbon dioxide (CO_2), diesel, and other fuels are the biggest contributors to climate change, leading to a rise in the earth's temperature [6]. To reduce dependence on fossil fuels, efforts must be made to develop biomass conversion into bioethanol [7-9].

Bioethanol is a form of renewable energy that has various advantages, such as a high octane rating, easy-to-obtain raw materials, and environmental friendliness [10, 11]. Meanwhile, macroalgae have been considered the 3rd generation of biomass for the production of bioethanol and other forms of biofuels due to their high carbohydrate content of up to 60% [12-14]. This innovation certainly supports government programs related to accelerating the development of new renewable energy with targets of fulfilling 23% and 31% of the total national energy demand in 2025 and 2050 respectively [15].

Ulva sp. commonly known as sea lettuce is a type of macroalgae commonly found in Indonesian waters [7, 16]. It has a morphology consisting of small cells which are colonies in the form of branched filaments and its vegetative cell division occurs in more than one area. Furthermore, *Ulva sp*. belongs to the Chlorophyta division because it contains high chlorophyll content, evidenced by its green leaves [17,18]. It also contains carbohydrates, protein, lipids, and ash of up to 50.34%, 25.69%, 2.31%, and 21.66% respectively [19]. The high carbohydrate content has the potential to be used as a raw material for bioethanol through the role of bacterial and yeast microorganisms [20-22]. Several species of *Ulva* sp. with a high carbohydrate content include *Ulva fasciata* (43.0%), *Ulva lactuca* (54.3%), *Ulva pertusa* (52.3%), and *Ulva rigrida* (53%) [23]. Bioethanol yields based on various biomass sources are presented in Table 1 [24].

Table 1. Bioethanol yield from various biomass sources

Biomass	***Yield* (L/ha)**
Sorghum	3.050 - 4.070
Corn	3.460 - 4.020
Beet sugar	5.010 - 6.680
Cassava	3.310
Corncob	3.460 - 4.020
Algae	46.760 - 140.290

One of the main steps in the conversion of macroalgal biomass into chemicals and biofuels is the deconstruction of complex carbohydrates into sugars [25, 26]. Reducing sugars include monosaccharides such as fructose, glucose, and galactose, as well as disaccharides namely maltose and lactose. However, they do not include polysaccharides such as starch and sucrose [27]. Hydrolysis is a step used to break down the algae cell wall and convert complex carbohydrates into simple sugars, which will then be fermented to produce

ethanol [28]. Conventional hydrolysis processes generally use concentrated strong acids [29, 30]. These strong acids, such as hydrochloric acid (HCl) or sulfuric acid (H_2SO_4), have several drawbacks, including negative impacts on the environment, long reflux times, and low yields due to very fast decomposition. [29, 31].

The relatively slow hydrolysis process can be overcome by using microwave assistance [32, 33]. The use of microwave irradiation can increase the rate of carbohydrate hydrolysis into glucose by 50-80% [34, 35]. Furthermore, microwave heating shortens the hydrolysis time and reduces energy consumption, making it effective in preventing the formation of other unwanted products [11, 31, 36]. Pretreatment of algae biomass using a microwave at a power of 360 W for a relatively short time of 5-10 min exhibited optimal conditions for the release of organic compounds [37, 38]. Various previous studies regarding the hydrolysis of *Ulva* sp. are shown in Table 2.

Based on the background in Table 2, further study is needed on the production of biomass-based bioethanol raw material in the form of *Ulva* sp. commonly found in Indonesian waters. Therefore, this study aims to determine the optimal decomposition process of *Ulva* sp. with the Microwave-Assisted Hydrolysis (MAH) method using an acid solvent (H_2SO_4). Optimization was performed with parameters of hydrolysis time, microwave power, as well as the ratio of materials to solvents.

Table 2. Studies on Hydrolysis of *Ulva* sp.

Raw Materials	Methods and Results	Ref
Ulva reticulata	Acid hydrolysis with 2% H_2SO_4 (150°C, 50 min) yielded a reducing sugar content of 27.30 g/L	[39]
Ulva reticulata	Acid hydrolysis with H_2SO_4 2% v/v (75−150°C, 30 min) yielded a reducing sugar content of 2.3−23.7 g/L	[21]
Ulva lactuca	Hydrothermal hydrolysis by autoclaving (121°C, 30 min) yielded a reducing sugar content of 2.9%	[40]
Ulva lactuca	Acid hydrolysis using H_2SO_4 (with concentrations of 2, 3,5, and 5%; for 60, 90, and 120 min, at 121°C) yielded a reducing sugar content of 29,050 mg/L	[41]
Ulva rigrida	Acid hydrolysis using H_2SO_4 and HCl (0-10% v/v, autoclaved for 30, 45, and 60 min) yielded a reducing sugar content of 342 mg/g	[12]
Ulva fasciata	Enzymatic hydrolysis with cellulase 2% v/v in a volume of 20 mL of sodium acetate buffer (36 h incubation, 45°C, 150 rpm) yielded a reducing sugar yield of 206.82 ± 14.96 mg/g	[42]
Ulva lactuca	Acid hydrolysis with 4% H_2SO_4 (80°C, 120 min) yielded a sugar yield of 155 mg/g	[43]
Ulva sp.	Acid hydrolysis with 2% H_2SO_4 (autoclaved 121°C, 30 min) yielded a sugar yield of 225 mg/g	[26]
Ulva sp.	Acid hydrolysis with 4% H_2SO_4 (121°C, 20 min) yielded a reducing sugar content of 21.1%	[19]
Ulva sp.	Hydrolysis with the addition of polyoxometalate (POM) in the microwave (4-10 min, 140°C) yielded a sugar content of 349-435 mg/g	[44]
Ulva prolifera	Enzymatic hydrolysis using *S. cerevisiae* at pH 4.8, temperature 50°C, 48 h, yielded reducing a sugar content of 0.42 g/g	[45]
Ulva fasciata	Enzymatic hydrolysis with cellulase (sodium acetate buffer pH 4.8, 45°C, 36 h) yielded a reducing sugar content of 20.6 g/L	[46]

2. Research Methods

2.1 Tools and Materials

The tools used in this study were a blender (Philips-HR2115), 100 mesh sieve (CBN), container, analytical balance (OHAUS-PX224), measuring cup (Pyrex), Erlenmeyer (Pyrex), round bottom flask (DURAN), condenser, microwave (Electrolux-EMM2308X), model 752AP UV-Vis spectrophotometer, metal spatula, dropping pipette, measuring pipette, vials, micropipette and tip, test tube and rack, cuvette. Meanwhile, the materials used were *Ulva* sp. (from the Cemara Indah Beach area, Situbondo, East Java), aquadest, sulfuric acid (H_2SO_4) 98% p.a (Smartlab), 3,5-dinitrosalicylic acid p.a 98% (Sigma Aldrich), anhydrous glucose p.a (Merck), potassium sodium tartrate (Kna-Tartrate) p.a (Pudak), and technical 2M NaOH solution (ROFA).

2.2 Methods

The procedures followed included the pretreatment, the hydrolysis, and the analysis stage for reducing sugar levels, as well as statistical analysis using the Response Surface Methodology (RSM) as shown in Figure 1.

Ulva sp. pretreatment stage began with a washing process to remove sand and other impurities. Subsequently, it was conventionally dried in direct sunlight for 2-3 days, until the mass reached a constant value and obtained a texture that could be easily crushed [47]. The water content used was 10%, then *Ulva sp.* was reduced in size by grinding using a blender. The sample was filtered using a 100 mesh sieve [41], yielding a powder with a uniform size of 0.147 mm.

Furthermore, the hydrolysis process was conducted using sulfuric acid (H_2SO_4). The series of tools used during the process included a microwave as shown in Figure 2. *Ulva* sp. powder was weighed with variations in the ratio of the mass to the volume of solvent between 0.03-0.1 [19, 21]. An analytical balance was used to measure the powder, and 100 mL of 2% (v/v) sulfuric acid solution (H_2SO_4) was added [39][21]. The hydrolysis process was carried out using a microwave with a power of 150-450 W [37] over a period of 5-15 min [38]. Afterward, the hydrolyzate results were cooled in the beaker glass until room temperature was reached. The hydrolyzate was then filtered using filter paper to separate the filtrate from the residue. The filtrate obtained was placed into a vial for further testing of reducing sugar levels.

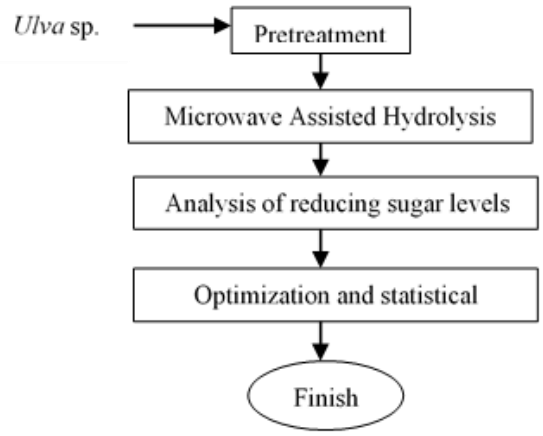


Figure 1. Study procedure

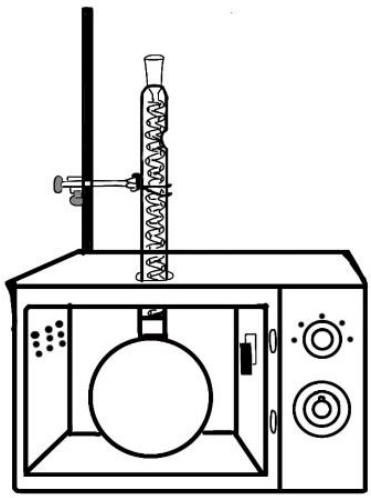


Figure 2. MAH process toolset

2.3 Analysis of Reducing Sugar Levels

The analysis of reducing sugar levels was carried out using a reagent in the form of 3,5-dinitrosalicylic acid (DNSA) and a spectrophotometer. Absorbance as a quantitative analysis was assessed based on the Lambert-Beer Law [48] which states that "The amount of visible light radiation (ultraviolet, infrared, etc.) absorbed or transmitted by a solution is an exponential function of the concentration of the substance and the thickness of the solution" [49]. The absorbance obtained showed the ratio of the absorbed light to the incident light intensity. The resulting absorbance value was directly proportional to the concentration of the substance contained in the sample [50].

DNSA is an aromatic compound that reacts with reducing sugar to form 3-amino 5-nitrosalicylic acid characterized by a reddish-orange color change [31]. The testing stages included sample preparation, preparing DNSA reagents, creation of glucose standard solutions and curves, as well as determining reducing sugar levels. The hydrolyzed sample was filtered using filter paper to separate it from the residue.

To prepare the DNSA reagent, 1 g of DNSA was added to 20 mL of 2M NaOH and then homogenized. Separately, 30 g of KNa Tartrate was dissolved in distilled water and the two solutions were homogenized using a hotplate with a magnetic stirrer. The solution was placed in a 100 mL volumetric flask and added with distilled water to the limit and further homogenized. The homogeneous solution was then placed in a 100 mL reagent bottle [51].

Standard glucose solutions were made in concentrations of 200, 300, 400, 500, 600, and 1000 ppm. A total of 0.5 mL of glucose solution was added to 0.5 mL of DNSA reagent and 1 mL of distilled water in a test tube. The glucose solution was homogenized and heated in a water bath (boiling water) for 5 min. It was then cooled to room temperature, added with distilled water to a final volume of 5 mL, and homogenized. Absorbance values were measured using UV-Vis spectrophotometry at a wavelength (λ) of 540 nm. Afterward, a standard curve was made to determine the linear regression equation which was used in determining the sample concentration [51].

The reducing sugar levels were tested by taking 3 mL of the sample solution into a test tube and adding 0.5 mL of DNSA reagent along with 1 mL of distilled water. The mixture was homogenized and heated in a water bath (boiling water) for 5 min. It was then cooled at room temperature, added with distilled water to a final volume of 5 mL, and then homogenized. Absorbance measurements were carried out using UV-Vis spectrophotometry at a wavelength (λ) of 540 nm [39, 52].

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

No.	A (mins)	B (W)	C (g/mL)
1	10	150	0.1
2	5	150	0.065
3	10	450	0.1
4	15	150	0.065
5	5	300	0.03
6	15	450	0.065
7	10	300	0.065
8	10	300	0.065
9	10	150	0.03
10	5	300	0.1
11	15	300	0.1
12	15	300	0.03
13	10	300	0.065
14	10	450	0.03
15	5	450	0.065
16	10	300	0.065
17	10	300	0.065

2.4 Data analysis

Data analysis used the Design Expert software version 13, adapted to the Response Surface Methodology (RSM) approach and the Box-Behnken Design (BBD) model [53] for 17 runs. Table 3 shows that A is the hydrolysis time (min), B is the microwave power (W), and C is the ratio of the mass of the raw material to the volume of solvent (g/mL).

3. Results and Discussion

3.1 Results of Reducing Sugar Levels

To determine the reducing sugar levels, the standard glucose curve was initially derived to ascertain the most suitable linear regression equation. This curve was obtained by measuring the absorbance of standard solutions at various concentrations of 200, 300, 400, 500, 600, and 1000 ppm. Based on the curve in Figure 3, a linear regression equation was obtained for y = 0.0004x - 0.0117, with an R^2 value of 0.9937.

The results of reducing sugar levels obtained from the hydrolysis of *Ulva* sp. using the MAH method are presented in Table 4. As shown in the table A, B, and C represent the hydrolysis time, the microwave power, and the ratio of the mass of the raw material to the volume of the solvent. The highest reducing sugar content of 19.71 mg/mL was obtained at operating conditions of 15 min hydrolysis time, 450 W microwave power, and 0.065 g/mL ratio of raw material mass to solvent volume. Meanwhile, the lowest value of 1.67 mg/mL was obtained at similar operating conditions of 5 min, 300 W, and 0.1 g/mL respectively. Kolo et al. [21] produced a lower reducing sugar content compared to this study, with a value of 2.3 g/L or equivalent to 2.3 mg/mL when hydrolyzed using H_2SO_4 at 75°C. This variation was probably due to differences in operating conditions used in the hydrolysis process.

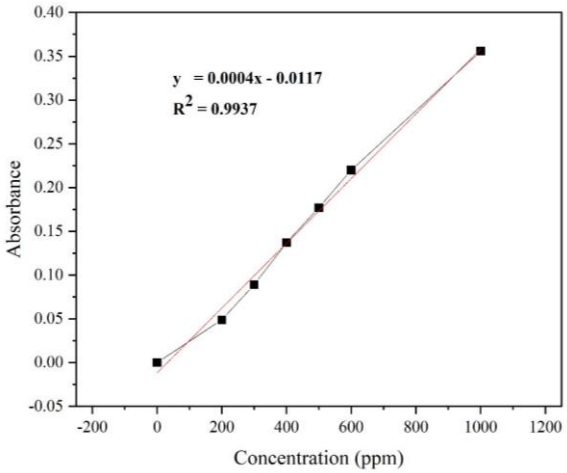


Figure 3. Glucose standard solution curve

Table 4. Reducing sugar levels in the filtrate hydrolyzed by *Ulva* sp.

No.	A (min)	B (W)	C (g/mL)	Reducing sugar levels (mg/mL)
1	10	150	0.100	3.31
2	5	150	0.065	4.37
3	10	450	0.100	5.04
4	15	150	0.065	7.3
5	5	300	0.030	4.73
6	15	450	0.065	19.71
7	10	300	0.065	5.24
8	10	300	0.065	5.37
9	10	150	0.030	3.68
10	5	300	0.100	1.67
11	15	300	0.100	7.74
12	15	300	0.030	18.81
13	10	300	0.065	5.24
14	10	450	0.030	19.23
15	5	450	0.065	4.16
16	10	300	0.065	6.89
17	10	300	0.065	6.22

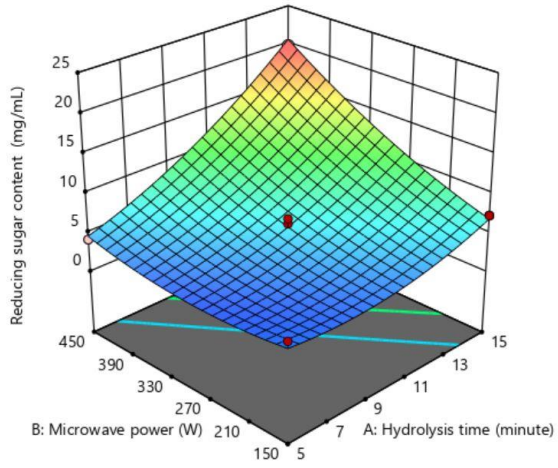
3.2 The Effect of Parameters on Reducing Sugar Levels

Figure 4 is a graph showing the effect of each variable used in the hydrolysis process of *Ulva* sp. including hydrolysis time, microwave power, and mass ratio of raw materials to solvent volume on the reducing sugar content.

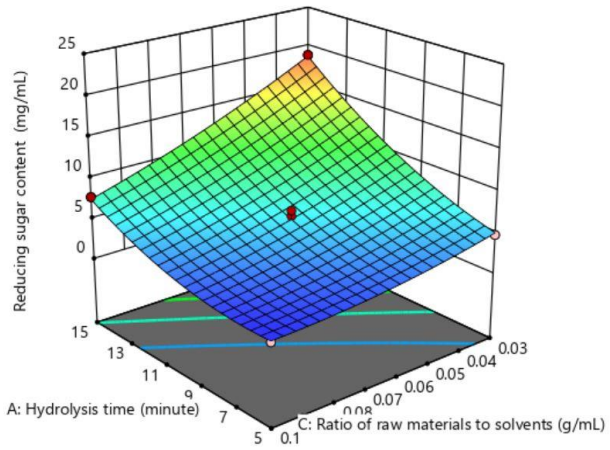
Based on the results, the concentration of reducing sugars was strongly influenced by hydrolysis time, as shown in Figures 4(a) and 4(b). There was an increase in the reducing sugar from 4.37 mg/mL to 7.3 mg/mL along with an extended hydrolysis time from 5 to 15 min, at similar microwave power and the ratio of material to solvent conditions.

Figure 4(a) shows that the highest reducing sugar yield was obtained after 15 min of hydrolysis time. This was consistent with Ngamput & Herrani [54] which used variations of hydrolysis time namely 15, 30, 45, and 60 min. The results obtained showed an increase in ethanol content. This was because the longer the hydrolysis process, the more carbohydrates were degraded into reducing sugars (glucose) [41].

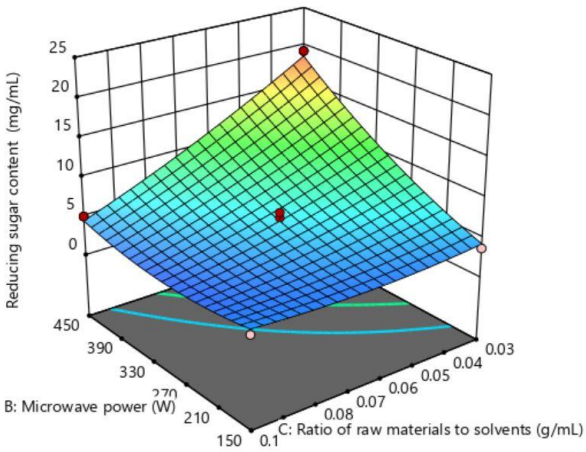
Figures 4(a) and 4(c) also show that the higher the microwave power, the greater the reducing sugar content produced. This was in accordance with the studies conducted by Kumar et al. [37] and Kavitha et al. [55] with varying microwave power of 90−630 W. The results showed that 2 phases of organic release occurred, namely in the range of 90-270 W (minor phase) and 360-630 W (major phase). In the major phase, there was a significant increase in the release of organic matter [37, 55].



(a)



(b)



(c)

Figure 4. Variable relationship to reducing sugar content between (a) hydrolysis time (min) and microwave power (W); (b) raw material mass ratio to solvent volume (g/mL) and hydrolysis time (min); (c) raw material mass ratio to solvent volume (g/mL) and microwave power (W)

Based on Figures 4(b) and 4(c), the ratio of the raw material in the form of *Ulva* sp. to solvent (H_2SO_4) indicated that the higher the ratio, the lower the reducing sugar content produced. Hydrolysis treatment with a ratio of 0.03 g/mL (3 g *Ulva* sp. in 100 mL H_2SO_4) for 5 min yielded a reducing sugar content of 4.73 mg/mL. Meanwhile, in the treatment with a ratio of 0.1 g/mL solvent (10 g *Ulva* sp. in 100 mL H_2SO_4), the reducing sugar level was reduced to 1.67 mg/mL. This was attributed to the high ratio of ingredients which reduced the homogeneity of *Ulva* sp. powder in the MAH process. The insufficient amount of liquid and high viscosity caused by the high ratio of ingredients posed a challenge to the decomposition reaction, leading to a decrease in the yield of reducing sugars. These results were in line with Dave et al. [56] stating that a higher dose of macroalgae reduced its homogeneity with the solvent, thereby prolonging the hydrolysis time [57].

3.3 Analysis of Variance (ANOVA)

Analysis of variance was used to determine the effect of a combination of process variables on the resulting response [58]. The results obtained from the analysis are shown in Table 4. The parameters were considered significant when the p-value (probability) was <0.05 or α=5% according to a predetermined level of significance [58]. In this study, the p-value for the model was <0.001 indicating that the variable hydrolysis time, microwave power, and the ratio of raw material mass to solvent volume had an effect on the reducing sugar content obtained. Furthermore, the lack of fit demonstrated the level of deviation or inaccuracy of the model. The p-value for the lack of fit was> 0.05 indicating not significant results [59]. The p-value obtained for the lack of fit was 0.2483 (not significant) implying the suitability of the response to the model.

Table 5. ANOVA test results

Source	Sum of Squares	df	Mean square	F-value	p-value	
Model	526.41	9	58.49	70.91	< 0.0001	significant
A- Hydrolysis time	186.53	1	186.53	226.15	< 0.0001	
B- Microwave power	108.63	1	108.63	131.70	< 0.0001	
C-Ratio of raw materials to solvents	102.89	1	102.89	124.74	< 0.0001	
AB	39.82	1	39.82	48.27	0.0002	
AC	16.04	1	16.04	19.45	0.0031	
BC	47.75	1	47.75	57.89	0.0001	
A^2	13.01	1	13.01	15.77	0.0054	
B^2	7.51	1	7.51	9.10	0.0195	
C^2	1.99	1	1.99	2.41	0.1642	
Residual	5.77	7	0.8248			
Lack of Fit	3.60	3	1.20	2.20	0.2301	not significant
Pure Error	2.18	4	0.5441			
Cor Total	532.18	16				

A = hydrolysis time; B = microwave power; C = raw material mass ratio to solvent volume

The R^2 value obtained was greater or closer to 1 indicating a better model [59]. The results of the ANOVA analysis presented in Table 5 show that R^2 was 0.9892, implying a significant relationship between hydrolysis time, microwave power, and the ratio of the mass of the raw material to the volume of solvent with the reducing sugar content obtained. The Adjusted R^2 and Predicted R^2 values were considered reasonable because the difference was <0.2.

Table 6. Fit Statistic

R^2	Adjusted R^2	Predicted R^2	Adeq precision
0.9892	0.9752	0.8855	26.8875

Mathematically, the equation model for determining reducing sugar content is shown in Equation (1). A positive parameter coefficient indicated an increase in the value of the reducing sugar content, and vice versa. Based on the results, the ratio of the mass of the raw material to the volume of solvent did not significantly affect the reducing sugar content, as indicated by the negative equation coefficient.

The suitability between the experimental data and the model is demonstrated based on the porosity plot graph in Figure 5.

$$Y = 5.79 + 4.83A + 3.69B - 3.59C + 3.15AB - 2.00AC - 3.46BC + 1.76A^2 + 1.34B^2 + 0.6878C^2 \quad \text{.....(1)}$$

where,

Y = reducing sugar content (mg/mL)

A = hydrolysis time (min)

B = microwave power (W)

C = raw material mass ratio to solvent volume (g/mL)

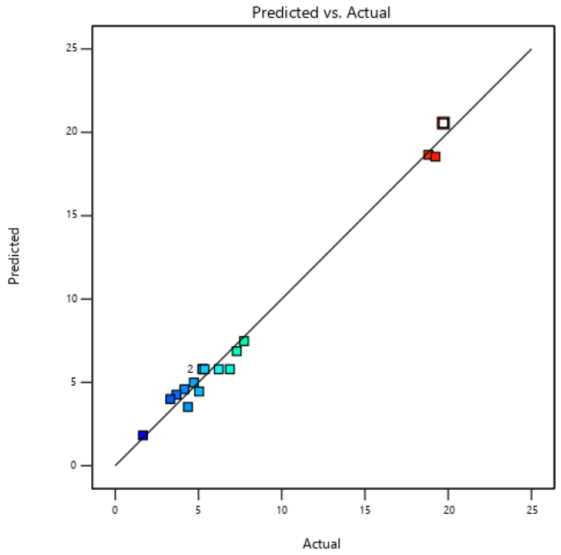


Figure 5. Comparison of model data with experimental data

4. Conclusion

In conclusion, the optimization of the hydrolysis process of *Ulva* sp. using the MAH method, with the Response Surface Methodology (RSM) model of the Box-Behnken Design (BBD), has demonstrated the significant impact of hydrolysis time (min), microwave power (W), and the ratio of the mass of the raw material to the volume of solvent (g/mL) on reducing sugar levels. This conclusion is further supported by the ANOVA test, which yielded an R^2 value of 0.9892. The highest reducing sugar content of 19.71 mg/mL was achieved by employing a hydrolysis time of 15 min, microwave power of 450 W, and a ratio of raw material mass to solvent volume of 0.065 g/mL.

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