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Study of the Delignification Process of Green Algae *(Chlorophyceae)* for α-Cellulose Production

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A B S T R A C K

Green algae (*Chlorophyceae*) are a lignocellulosic biomass with considerable potential as a source of α-cellulose due to their relatively high cellulose content. This study aimed to produce α-cellulose via alkaline delignification and to investigate the effects of NaOH concentration and delignification time on the resulting product's characteristics. The delignification process was carried out using NaOH concentrations ranging from 6% to 14% and delignification times of 30–90 min at 60°C. The resulting product were analyzed using the Chesson-Datta method and further characterized by FTIR and SEM analyses, while process optimization was performed using Response Surface Methodology (RSM). The results showed that the optimum conditions were achieved at an NaOH concentration of 8% and a delignification time of 45 min, yielding an α-cellulose content of 51.2%, a hemicellulose content of 16.9%, and a lignin content of 5.4%. FTIR analysis confirmed the presence of functional groups characteristic of α-cellulose, whereas SEM observations revealed morphological changes in the fiber surface, which became rougher and more porous due to the removal of lignin and hemicellulose. RSM optimization predicted optimum conditions yielding 51.234% α-cellulose, 12.990% hemicellulose, and 4.963% lignin.

Contribution to Sustainable Development Goals (SDGs):
SDG 9: Industry, Innovation, and Infrastructure
SDG 12: Responsible Consumption and Production
SDG 13: Climate Action
SDG 14: Life Below Water
SDG 15: Life on Land

1. INTRODUCTION

1.1. Research Background

Green algae (*Chlorophyceae*) are members of the division *Chlorophyta* that contain chlorophyll *a* and chlorophyll *b* as the primary pigments involved in the photosynthetic process [1]. These organisms exhibit a wide range of morphological forms, including unicellular, colonial, and multicellular structures. The cell walls of green algae are generally composed of cellulose, which serves as a structural support component of the cell [2]. One of the most extensively studied genera of green algae is *Cladophora*, which has been reported to contain up to 51% cellulose based on its total biomass. This high cellulose content indicates that *Cladophora* has significant potential as a lignocellulosic feedstock for various industrial applications [3]. Furthermore, the substantial cellulose content of green algae makes them a promising alternative source of cellulose in addition to terrestrial plant biomass [4].

Cellulose in green algae serves as the primary structural component of the cell wall and is composed of glucose units linked by β-1,4-glycosidic bonds, forming long polymeric chains [5]. Based on their solubility in strong alkaline solutions, cellulose fractions can be classified into α-cellulose, β-cellulose, and γ-cellulose. Among these fractions, α-cellulose exhibits the highest degree of purity and stability due to its long polymer chains with a degree of polymerization ranging from

approximately 600 to 1500 and its insolubility in 17.5% NaOH solution [6]. These characteristics make α-cellulose a widely utilized raw material for the production of various cellulose-based products. Therefore, the content and purity of α-cellulose are important parameters that determine the quality of the final product, necessitating an effective purification process to obtain α-cellulose with a high level of purity [7].

Although the lignin content of green algae is relatively lower than that of terrestrial plant biomass, its presence remains a significant obstacle in the cellulose isolation process. Lignin is associated with hemicellulose, forming a complex lignocellulosic matrix that is resistant to natural degradation [8]. To overcome this challenge, a delignification process is required to disrupt the lignin structure, thereby facilitating the separation of cellulose [9]. One of the most commonly employed delignification methods is alkaline treatment using sodium hydroxide (NaOH) solution, as it is capable of dissolving a portion of the lignin and hemicellulose present in the lignocellulosic matrix. The effectiveness of this process is influenced by several factors, particularly the NaOH concentration and the duration of the treatment [10,11].

Numerous studies have reported cellulose extraction from green algae through alkaline delignification. Wahlström et al. demonstrated the successful isolation and characterization of cellulose from green algae and highlighted its potential as an alternative source of cellulose-based materials [12]. In addition, Prasetia et al. investigated alkaline delignification of Cladophora sp. and reported that NaOH treatment significantly affected cellulose purity and the removal of non-cellulosic components [13]. These findings confirm that alkaline treatment is an effective method for improving cellulose purity through the removal of lignin and hemicellulose.

However, previous studies have mainly focused on cellulose isolation, characterization, or the influence of a single process variable, while information regarding the combined effects of NaOH concentration and delignification time on α-cellulose production from Cladophora glomerata remains limited. Furthermore, the optimum operating conditions required to maximize α-cellulose content while minimizing lignin residue have not been comprehensively established for this biomass. Therefore, this study evaluates the interaction between NaOH concentration (6–14%) and delignification time (30–90 min) using Response Surface Methodology (RSM) to determine the optimum delignification conditions for α-cellulose production. The novelty of this work lies in the systematic optimization of alkaline delignification parameters for Cladophora glomerata and in establishing the relationship between NaOH concentration, delignification time, and α-cellulose content to obtain optimum process conditions for producing high-purity α-cellulose.

1.2. Literature Review

Cladophora glomerata is a green alga containing lignocellulosic components such as cellulose, hemicellulose, and lignin, and has considerable potential as a raw material for α-cellulose production due to its relatively high polysaccharide content. However, the presence of hemicellulose and lignin within the cell wall matrix limits access to cellulose, thereby necessitating a pretreatment step prior to the isolation process. α-Cellulose is a highly purified cellulose fraction obtained after the removal of most hemicellulose and lignin components. This material is widely utilized in the biomaterials, paper, and biopolymer industries due to its stable structure and the presence of hydroxyl (–OH) functional groups, which contribute to its reactivity and hydrogen-bonding capabilities. The isolation of α-cellulose is commonly carried out through alkaline delignification using sodium hydroxide (NaOH), which functions by dissolving lignin and a portion of the hemicellulose through the cleavage of lignin–carbohydrate linkages, thereby creating a more accessible and open fiber structure.

I.2.1 Cellulose

Cellulose is a naturally occurring polysaccharide with the chemical formula $(C_6H_{10}O_5)_n$, composed of glucose units linked through β-1,4-glycosidic bonds. It is the primary structural component of plant cell walls and is recognized as the most abundant organic polymer in nature. Cellulose provides mechanical strength and rigidity to the cell wall, enabling it to maintain cellular structure and withstand external environmental stresses [7]. Based on its solubility characteristics in alkaline solutions, cellulose can be classified into three fractions: α-cellulose, β-cellulose, and γ-cellulose.

a. α-Cellulose

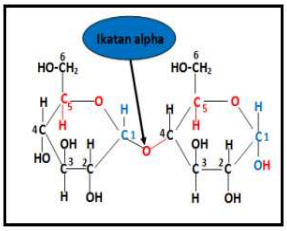

Figure 1. Chemical Structure of α-Cellulose

α-Cellulose is a cellulose fraction with a degree of polymerization (DP) ranging from 600 to 1500 and is insoluble in a 17.5% sodium hydroxide (NaOH) solution. This fraction contains a higher proportion of pure cellulose than β-cellulose and γ-cellulose, making it a key indicator for assessing cellulose purity [14].

b. β-Cellulose

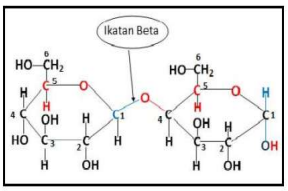

Figure 2. Chemical Structure of β-Cellulose

β-Cellulose is a cellulose fraction that is soluble in 17.5% sodium hydroxide (NaOH) solution as well as other strong alkaline solutions and has a degree of polymerization (DP) ranging from approximately 15 to 90. This fraction consists of shorter polymer chains than α-cellulose and is characterized by its ability to reprecipitate upon neutralization of the alkaline solution.

c. γ- Cellulose

γ-Cellulose (Gamma Cellulose) is a cellulose fraction that is soluble in 17.5% sodium hydroxide (NaOH) solution as well as other strong alkaline solutions and has a degree of polymerization (DP) of less than 15. This fraction is composed of shorter polymer

chains than both α-cellulose and β-cellulose and is predominantly associated with hemicellulosic components [15].

I.2.2 Lignocellulosic Composition of Green Algae

Green algae (*Chlorophyceae*) generally contain lignocellulosic materials composed of three major cell wall components, namely cellulose, hemicellulose, and lignin. The proportion of these components may vary among species depending on environmental growth conditions, habitat location, and the analytical methods employed. Table presents the lignocellulosic composition of several green algae species.

Table 1. Lignocellulosic Composition of Green Algae

Component	Content (%)
Cellulose	33,43%
Hemicellulose	24,55%
Lignin	15,48%

The compositional data indicate that green algae possess a relatively high cellulose content, highlighting their potential as a raw material for α-cellulose production. However, the presence of lignin and hemicellulose, although in lower amounts, can still hinder the separation and purification of cellulose. Therefore, a delignification step is required to reduce lignin content and partially remove hemicellulose, thereby obtaining α-cellulose of higher purity. Furthermore, the variation in lignocellulosic composition among different species of green algae emphasizes the importance of raw material characterization to determine the actual composition of the biomass being utilized [16].

1.3. Research Objective

This study aimed to produce α-cellulose from green algae (*Chlorophyceae*) using an alkaline delignification method with sodium hydroxide (NaOH) solution. It also sought to investigate the effects of NaOH concentration and delignification time on the α-cellulose content obtained. Furthermore, the resulting products were characterized using the Chesson–Datta method, Fourier Transform Infrared Spectroscopy (FTIR), and Scanning Electron Microscopy (SEM). Process optimization was carried out using Response Surface Methodology (RSM) to determine the optimum operating conditions for α-cellulose production.

2. MATERIALS AND METHODS

The green algae used in this study were tentatively identified as Cladophora glomerata based on their morphological characteristics, including filamentous thalli, branching patterns, and green coloration, and were collected from Mojokerto, East Java, Indonesia. The collected biomass was thoroughly washed with tap water to remove adhering impurities such as sand, soil, and other debris. Subsequently, the biomass was dried, milled, and stored in sealed containers prior to use. Cladophora glomerata was selected as the raw material due to its lignocellulosic composition, including cellulose, hemicellulose, and lignin, which makes it a potential source of α-cellulose. The delignification process was carried out in a 500 mL beaker serving as the reaction vessel. The beaker was equipped with a magnetic stirrer and heating system to provide simultaneous agitation and temperature control throughout the process. During delignification, the biomass was mixed with sodium hydroxide (NaOH) solution at predetermined concentrations. The mixture was heated to the desired operating temperature and continuously stirred for the specified reaction time to facilitate the removal of lignin and hemicellulose from the biomass structure. The experimental setup used for the delignification process is shown in Figure 3.

Figure 3. Experimental Setup for the Delignification of Green Algae

A total of 1000 g of green algal biomass was washed with distilled water to remove impurities and residual salts, then sun-dried until a constant dry weight was achieved. The dried algae were then ground using a blender to obtain a fine powder. Delignification was carried out by reacting 40 g of green algae powder with NaOH solutions at concentrations of 6%, 8%, 10%, 12%, and 14% in a 500 mL beaker. The mixture was heated at 60°C and stirred using a magnetic stirrer at 150 rpm for 30, 45, 60, 75, and 90 min. After the delignification process, the mixture was filtered, and the resulting residue was dried in an oven at 100°C for 4 h, then ground into a powder. The α-cellulose purification step was performed by immersing the delignified material in a 17.5% NaOH solution contained in a beaker and maintaining the mixture at 60°C for 30 min while stirring at 150 rpm using a magnetic stirrer. The mixture was then filtered to separate the residue from the filtrate. The recovered residue was dried again in an oven at 100°C for 4 h and ground into α-cellulose powder. The resulting product was analyzed using the Chesson–Datta method to determine the contents of α-cellulose, hemicellulose, and lignin. Furthermore, the samples were characterized using Fourier Transform Infrared Spectroscopy (FTIR) and Scanning Electron Microscopy (SEM) to identify their functional groups and surface morphology. The calculations of hemicellulose, α-cellulose, and lignin contents based on the Chesson–Datta method were performed using the following equations:

$$\text{Hemiselulosa (\%)} = \frac{b - c}{a} \times 100\%$$

$$\alpha - \text{selulosa (\%)} = \frac{c - d}{a} \times 100\%$$

$$\text{Lignin (\%)} = \frac{d - e}{a} \times 100\%$$

Optimization of α-cellulose production was carried out using Response Surface Methodology (RSM) with a Central Composite Design (CCD) generated by Design-Expert software. Two independent variables were evaluated, namely NaOH concentration (6–14%) and delignification time (30–90 min). The experimental design consisted of 13 runs covering factorial and axial points. The experimental data were fitted to a second-order polynomial model to describe the relationship between the independent variables and α-cellulose content. Analysis of variance (ANOVA) was employed to evaluate the significance of the model and its terms. Model adequacy was assessed using the

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coefficient of determination (R^2), adjusted R^2, predicted R^2, and lack-of-fit analysis.

3. RESULT AND DISCUSSION

3.1. Analisis Chesson - Datta

To determine the composition of cellulose, hemicellulose, and lignin in the samples, an analysis was conducted using the Chesson–Datta method. This analysis was intended to quantitatively determine the content of each component and to evaluate changes in composition following the applied treatments. The method involves a series of extraction and fractionation steps to remove non-cellulosic components, thereby enabling the accurate determination of cellulose, hemicellulose, and lignin contents. The results obtained from the Chesson–Datta analysis were subsequently used to evaluate the effectiveness of the delignification process and served as the basis for data interpretation, as presented in Table 2.

Table 2. Experimental Results of Lignocellulosic Components at Various NaOH Concentrations and Delignification Times

No.	NaOH Consentration (%)	Delignification Time (Min)	Hemicellulose Content (%)	α-Cellulose Content (%)	Lignin Content (%)
1.	6	30	18.2	46.5	6.2
2.	6	45	17.5	47.8	6
3.	6	60	17	48.9	5.8
4.	8	30	17.6	48.8	5.9
5.	8	45	16.9	51.2	5.4
6.	8	90	15.5	45.8	5
7.	10	45	16	48.2	6.9
8.	10	60	15.3	48.5	6.3
9.	10	90	14.5	42.5	6
10.	12	30	15.9	46.2	7.9
11.	12	45	15.1	45.8	8.1
12.	14	75	13.3	41.2	8.4
13.	14	90	13	40	8.5

Based on Table 2, the results of the lignocellulosic component analysis using the Chesson-Datta method at 60°C indicate that variations in NaOH concentration and delignification time significantly affected the contents of hemicellulose, α-cellulose, and lignin. Increasing the NaOH concentration from 6% to 8% increased the α-cellulose content to an optimum of 51.2% at a delignification time of 45 min, while also reducing lignin content compared to other treatment conditions. However, at higher NaOH concentrations (10–14%), the α-cellulose content tended to decrease, whereas the lignin content increased, suggesting that excessive alkali treatment may adversely affect the cellulose structure. In contrast, the hemicellulose content decreased continuously as NaOH concentration and delignification time increased, owing to its greater susceptibility to degradation under alkaline conditions. Based on these results, the optimal delignification conditions were achieved at an NaOH concentration of 8% and a treatment time of 45 min.

Table 3. Predicted Optimum Conditions and Lignocellulosic Component Contents Obtained from the RSM Model

No.	NaOH Consentration (%)	Delignification Time (Min)	Hemicellulose Content (%)	α-Cellulose Content (%)	Lignin Content (%)
1.	6	30	18.2	46.5	6.2
2.	6	45	17.5	47.8	6
3.	6	60	17	48.9	5.8
4.	6	75	16.4	47.1	5.7
5.	6	90	16	44.6	5.5
6.	8	30	17.6	48.8	5.9
7.	8	45	16.9	51.2	5.4
8.	8	60	16.2	49.1	5.3
9.	8	75	15.7	47.7	5.1
10.	8	90	15.5	45.8	5
11.	10	30	16.7	47.8	6.8
12.	10	45	16	48.2	6.9
13.	10	60	15.3	48.5	6.3
14.	10	75	14.8	46.9	6.1
15.	10	90	14.5	42.5	6
16.	12	30	15.9	46.2	7.9
17.	12	45	15.1	45.8	8.1
18.	12	60	14.6	46.3	7
19.	12	75	14.1	44.8	6.9
20.	12	90	13.8	42.1	6.7
21.	14	30	15.1	42.5	8.9
22.	14	45	14.4	43.2	9
23.	14	60	13.8	42.8	8.7
24.	14	75	13.3	41.2	8.4
25.	14	90	13	40	8.5

Based on Table 3, the optimum conditions predicted by the Response Surface Methodology (RSM) model using Design-Expert software indicated that variations in NaOH concentration and delignification time significantly affected the contents of α-cellulose, hemicellulose, and lignin at 60°C. The model predicted that the highest α-cellulose content (51.2%) could be achieved at an NaOH concentration of 8% and a delignification time of 45 min. Under these optimum conditions, the lignin content was relatively low (5.4%), indicating effective delignification. However, further increases in NaOH concentration and delignification time were associated with a decrease in α-cellulose content. These findings are consistent with the study reported in [17], which demonstrated that alkaline treatment effectively removes lignin and hemicellulose, whereas excessive treatment may negatively affect cellulose quality.

The α-cellulose content obtained in this study (51.2%) is comparable to or higher than values reported in previous studies on green algae cellulose extraction. Wahlström et al. reported cellulose isolation from Ulva lactuca with cellulose yields in the range of 30–45%, while Prasetia et al. reported that alkaline delignification improved cellulose purity from Cladophora sp. but did not establish optimum operating conditions through multivariable optimization. Therefore, the present study contributes additional information by providing statistically

optimized delignification conditions for maximizing α-cellulose content from Cladophora glomerata.

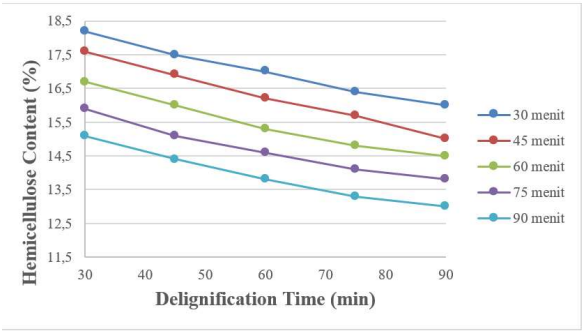

Figure 4. Effect of Delignification Time on Hemicellulose Content

Based on Figure 4, the hemicellulose content decreased as delignification time increased at 60°C. The reduction in hemicellulose content occurred gradually over the treatment period from 30 to 90 minutes, indicating that the duration of delignification significantly influenced the removal of hemicellulose from the green algae structure. As the treatment time increased, more hemicellulose was dissolved and removed, resulting in a lower residual hemicellulose content in the material. These findings suggest that delignification time is an important factor in enhancing the effectiveness of hemicellulose removal. The results are consistent with those reported by [18] and [19], who demonstrated that extending the duration of alkaline pretreatment promotes greater solubilization and removal of hemicellulose from lignocellulosic structures.

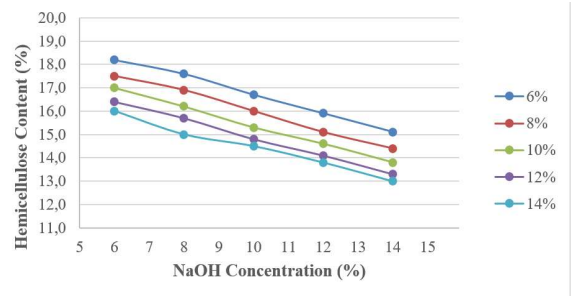

Figure 5. Effect of NaOH Concentration on Hemicellulose Content

Based on Figure 5, the hemicellulose content decreased with increasing NaOH concentration across all delignification time variations. At a NaOH concentration of 6%, the hemicellulose content remained relatively high, but it gradually declined as the concentration increased to 14%. This reduction indicates that higher NaOH concentrations were more effective in dissolving and removing hemicellulose from the lignocellulosic structure. All curves exhibited a similar trend despite differences in delignification time, suggesting that NaOH concentration had a greater influence on hemicellulose removal than the duration of the process. These findings are consistent with the results reported by [20] and [21], which demonstrated that increasing NaOH concentration enhances hemicellulose solubilization and reduces the residual hemicellulose content in lignocellulosic materials.

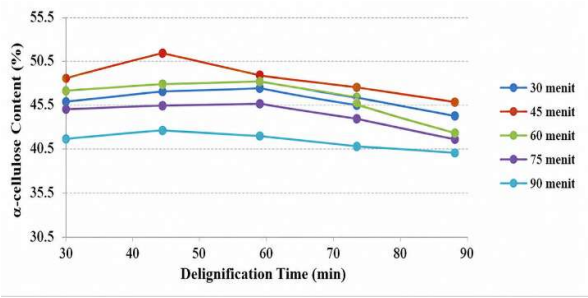

Figure 6. Effect of Delignification Time on α-Cellulose Content

Based on Figure 6, delignification time had a significant effect on α-cellulose content. At all treatment temperatures, α-cellulose content tended to increase during the initial delignification period (30–45 minutes), reaching its highest value of 51.2% at 60 °C and 45 minutes. Subsequently, α-cellulose content decreased gradually as the delignification time was extended to 60, 75, and 90 minutes. At 90 minutes, the α-cellulose content ranged from approximately 39.8% to 45.8%, depending on the treatment temperature. This behavior indicates that moderate delignification times effectively removed lignin and hemicellulose, thereby increasing cellulose purity. In contrast, prolonged alkaline treatment may cause cellulose depolymerization and degradation, leading to a reduction in α-cellulose content. Similar observations have been reported by [22], who found that alkaline delignification improves cellulose purity but excessive treatment can damage the cellulose structure.

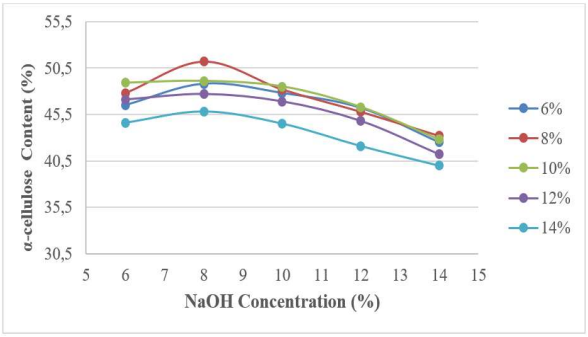

Figure 7. Effect of NaOH Concentration on α-Cellulose Content

Based on Figure 7, NaOH concentration had a significant effect on the α-cellulose content obtained after the delignification process. In general, α-cellulose content increased as the NaOH concentration increased from 6% to 8%, reaching the highest value of 51.2% at 8% NaOH. This result indicates that delignification was most effective at this concentration, where lignin and hemicellulose removal occurred without causing significant damage to the cellulose structure. However, further increases in NaOH concentration to 10%, 12%, and 14% resulted in a gradual decline in α-cellulose content. At the highest concentration tested (14%), α-cellulose content decreased to approximately 39.8–42.0%, depending on the delignification time. The decrease in α-cellulose content at higher NaOH concentrations suggests that excessive alkaline treatment promoted cellulose depolymerization and degradation, thereby reducing cellulose purity. These findings are consistent with those reported by [23], who demonstrated that NaOH pretreatment effectively enhances cellulose content through the removal of lignin and hemicellulose, whereas excessive alkali concentrations may damage the fiber structure and lower α-cellulose content.

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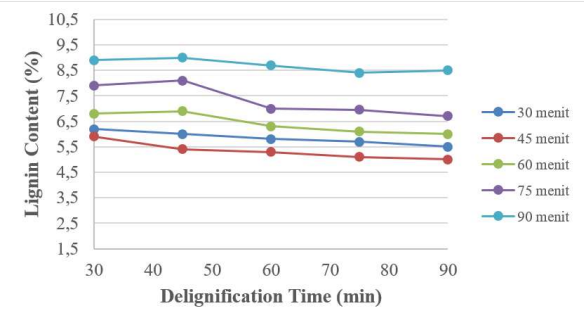

Figure 8. Effect of Delignification Time on Lignin Content

Based on Figure 8, the lignin content generally decreased during the delignification process, indicating that lignin was progressively dissolved and removed by the NaOH solution. The lowest lignin content obtained was approximately 5.0%, demonstrating the effectiveness of alkaline treatment in breaking down lignin strucaractures within the lignocellulosic matrix. However, under certain treatment conditions, the measured lignin content appeared to increase to as much as 9.0%. This apparent increase does not necessarily indicate an actual increase in lignin content. Instead, it may be attributed to the greater degradation and dissolution of cellulose and hemicellulose fractions during the delignification process. As a result, the proportion of lignin relative to the remaining biomass becomes higher, leading to an increase in the measured lignin percentage. Therefore, the observed fluctuations in lignin content are likely associated with changes in the relative composition of the lignocellulosic components rather than the formation of additional lignin. These findings are consistent with the study reported in [24], which demonstrated that changes in lignin content during alkaline treatment may be influenced by the different degradation rates of lignocellulosic components.

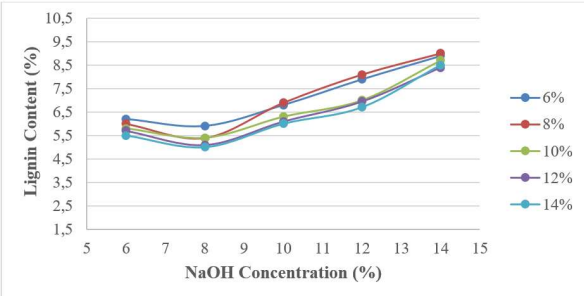

Figure 9. Effect of NaOH Concentration on Lignin Content

Based on Figure 9, NaOH concentration significantly influenced the lignin content obtained after the delignification process. The lignin content generally decreased as the NaOH concentration increased from 6% to 8%, reaching its lowest value at 8% NaOH. However, further increases in NaOH concentration up to 14% resulted in a rise in lignin content, reaching approximately 9%. These findings indicate that the use of NaOH beyond the optimum concentration does not enhance lignin removal efficiency. This result is consistent with the findings reported by [25], who demonstrated that alkaline treatment at an optimum concentration promotes more effective delignification, whereas excessive alkali concentrations may reduce process efficiency. Therefore, the application of NaOH at appropriate concentrations is essential to maximize lignin removal while maintaining the effectiveness of the delignification process.

3.2 FTIR Analysis

Fourier Transform Infrared Spectroscopy (FTIR) analysis was conducted to identify the functional groups present in the α-cellulose extracted from green algae. This method provides information regarding the chemical structure of a material through the absorption of infrared radiation at specific wavelengths. The FTIR spectrum obtained shows characteristic absorption bands corresponding to the functional groups of cellulose. Therefore, FTIR analysis was used to confirm the presence of α-cellulose in the purified sample.

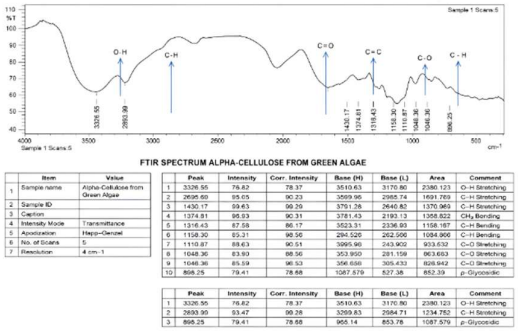

Figure 10. FTIR Spectrum of α-Cellulose

Based on Figure 10, the FTIR spectrum exhibits characteristic absorption bands corresponding to α-cellulose. The broad absorption band at 3326.55 cm^{-1} is attributed to O–H stretching vibrations, while the peak at 2893.99 cm^{-1} corresponds to C–H stretching vibrations in the polysaccharide backbone. Absorption bands observed at 1430.17 cm^{-1}, 1374.81 cm^{-1}, and 1316.43 cm^{-1} are associated with CH_2 bending, C–H bending, and O–H bending vibrations, respectively. In addition, the peak at 1158.30 cm^{-1} is assigned to C–O–C asymmetric stretching, whereas the bands at 1110.87 cm^{-1} and 1046.36 cm^{-1} correspond to C–O stretching vibrations. The absorption band at 896.25 cm^{-1} is attributed to the β-(1→4)-glycosidic linkage, which is a characteristic feature of cellulose. A weak absorption peak at 1736.43 cm^{-1} corresponding to C=O stretching vibrations may indicate the presence of trace amounts of residual non-cellulosic compounds. Furthermore, no prominent absorption bands commonly associated with aromatic lignin structures were observed in the region of 1500–1600 cm^{-1}. Overall, the FTIR results confirm the presence of characteristic functional groups of α-cellulose in the purified sample [26].

3.3 SEM Analysis

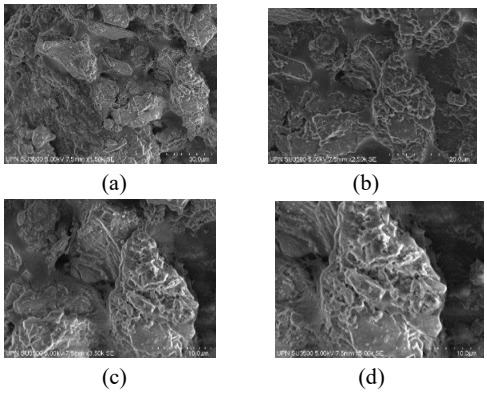

Figure 11. SEM Micrographs Illustrating the Porous Surface Morphology at Magnifications of (a) 1500×, (b) 2500×, (c) 3500×, and (d) 5000×.

Based on Figure 11, SEM observations of the sample treated with 8% NaOH for 45 min revealed an irregular and heterogeneous surface morphology. At a magnification of 1500× (Figure 11a), the surface appeared relatively compact and agglomerated. Increasing the magnification to 2500× and 3500× (Figures b and c) revealed cracks and pore-like structures distributed across the surface. At 5000× magnification (Figure d), these features became more clearly visible, revealing a rougher surface with more pores and fragmented structures. The observed morphology suggests that alkaline treatment modified the biomass structure and increased surface heterogeneity. Similar morphological characteristics have been reported for alkali-treated lignocellulosic materials, in which the partial removal of non-cellulosic components resulted in rougher, more porous surfaces [27]. Although no untreated control sample or quantitative pore-size measurements were included in this study, the SEM observations provide qualitative evidence of structural modification. Furthermore, the observed morphological changes are consistent with the compositional analysis, which showed reduced lignin and hemicellulose contents and increased α-cellulose content under the optimum treatment conditions.

3.3 Optimization of α-Cellulose Content in the Delignification Process Using RSM

As an initial step in the optimization process, the effects of NaOH concentration and delignification time as independent variables on the resulting α-cellulose content were evaluated. This assessment was based on α-cellulose content measurements obtained from 13 experimental runs designed according to the selected experimental design. The complete results of all experiments are presented in Table 4. The experimental data obtained from the 13 runs were analyzed using Design-Expert software to develop a predictive model for α-cellulose production. A quadratic polynomial model was selected based on the statistical evaluation of model performance. The significance of the model and the contribution of each independent variable were assessed using analysis of variance (ANOVA). The developed model was subsequently used to generate response surface plots and determine the optimum operating conditions for maximizing α-cellulose content.

Table 4. Experimental Design Matrix of 13 Runs for RSM Modeling

Run	Factor 1 NaOH Consentration (%)	Factor 2 Delignification Time (Min)	Response α-Selulosa Content (%)
1	6	30	46.5
2	6	45	47.8
3	6	60	48.9
4	8	30	48.8
5	8	45	51.2
6	8	90	45.8
7	10	45	48.2
8	10	60	48.5
9	10	90	42.5
10	12	30	46.2
11	12	45	45.8
12	14	75	41.2
13	14	90	40

Based on the results presented in Table 4, variations in NaOH concentration and delignification time influenced the α-cellulose content. At a NaOH concentration of 6%, the α-cellulose content ranged from 46.5% to 48.9%. An increase in α-cellulose content was observed at a concentration of 8%, reaching the highest value of 51.2% at a delignification time of 45 minutes. At a concentration of 10%, the α-cellulose content remained relatively stable but gradually decreased to 42.5% after 90 minutes of treatment. Furthermore, at NaOH concentrations of 12–14%, a more pronounced decline was observed, with α-cellulose content decreasing to approximately 40.0–41.2%. Overall, the optimal conditions were achieved at a NaOH concentration of 8% and a delignification time of 45 minutes, yielding the highest α-cellulose content.

Table 5. Comparision of RSM Models

Source	Sequential p-value	Adjusted R^2	Predicted R^2	
Linear	0.0077	0.5466	0.4244	
2FI	0.1020	0.6318	0.5046	
Quadratic	0.0735	0.7755	0.6602	Suggested
Cubic				**Allowed**

Based on the results presented in Table 5, the quadratic model was selected as the most appropriate for describing the relationship among NaOH concentration, delignification time, and α-cellulose content. This selection was based on its Adjusted R^2 value of 0.7755 and Predicted R^2 value of 0.6602, indicating that the model possesses a satisfactory ability to explain the variability of the response and to predict experimental data. These values were higher than those obtained for the linear and two-factor interaction (2FI) models. Although the cubic model exhibited a higher Adjusted R^2 value (0.8620), it was not selected because its negative Predicted R^2 value (−0.0155) indicated poor predictive capability. Furthermore, the quartic model was found to be aliased and therefore could not be considered for further analysis. Consequently, the quadratic model was chosen as the basis for developing the mathematical equation and optimizing α-cellulose content.

Table 6. Analysis of Variance (ANOVA) for Quadratic Model

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	180.05	5	36.01	9.29	0.0054	significant
A-NaOH concentration	55.75	1	55.75	14.38	0.0068	
B-Delignification time	22.93	1	22.93	5.91	0.0453	
AB	0.0626	1	0.0626	0.0161	0.9025	
A^2	16.61	1	16.61	4.28	0.0773	
B^2	19.26	1	19.26	4.91	0.0611	
Residual	27.14	7	3.88			
Cor Total	207.19	12				

Based on the ANOVA results in Table 6, the quadratic model was statistically significant ($p = 0.0054 < 0.05$), indicating

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that it adequately describes the relationship among NaOH concentration, delignification time, and α-cellulose content. Among the model terms, NaOH concentration (A) and delignification time (B) significantly affected α-cellulose production, with p-values of 0.0068 and 0.0453, respectively. In contrast, the interaction term (AB) was not significant ($p > 0.05$), suggesting that the interaction effect between the two variables was relatively weak within the studied range. These results confirm that both NaOH concentration and delignification time are important factors influencing α-cellulose content.

The relationship between NaOH concentration and delignification time on α-cellulose content was visualized using contour and three-dimensional (3D) surface plots, as presented below:

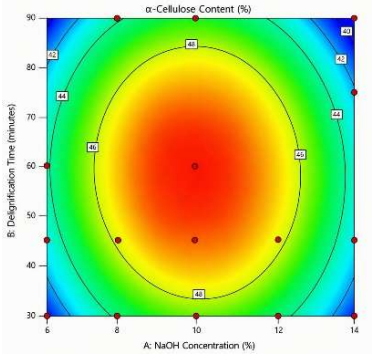

Figure 14. Contour Plot Illustrating the Effects of NaOH Concentration (%) and Delignification Time (min) on α-Cellulose Content (%)

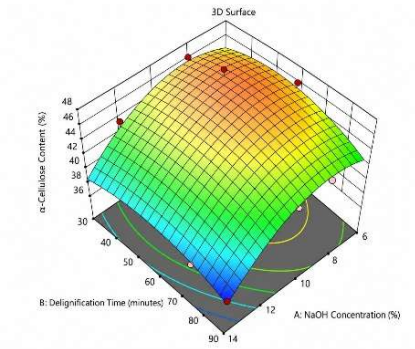

Figure 15. Three-Dimensional Surface Plot Illustrating the Combined Effects of NaOH Concentration (%) and Delignification Time (min) on α-Cellulose Content (%)

Based on the contour plot presented in Figure 14, the optimum region was observed at a NaOH concentration of 8–9% and a delignification time of 45–55 minutes, as indicated by the yellow zone representing the highest α-cellulose content. Under these conditions, the delignification process proceeded efficiently, allowing lignin and a portion of hemicellulose to be removed without causing significant damage to the cellulose structure. However, increasing the NaOH concentration to 12–14% and extending the delignification time to 75–90 minutes resulted in a decline in α-cellulose content, as reflected by the color transition from yellow to green–blue. A similar trend was observed in the three-dimensional surface plot shown in Figure 15, where the response surface exhibited a dome-shaped profile, indicating an optimal point at a specific combination of process variables. The α-cellulose content increased progressively to a maximum, then decreased under more severe treatment conditions. These findings suggest that an effective delignification process requires a balance between lignin removal and the preservation of cellulose integrity to prevent degradation. The reduction in α-cellulose content at excessive NaOH concentrations and prolonged treatment times can be attributed to cellulose degradation under strong alkaline conditions and extended exposure, which cleaves cellulose chains. These results are consistent with the findings reported by [28] and [29], which demonstrated that although NaOH is effective in removing lignin, excessive alkali concentration and treatment duration may adversely affect cellulose quality and reduce the resulting α-cellulose content.

4. CONCLUSION

The initial characterization of green algae using the Chesson–Datta method revealed cellulose, hemicellulose, and lignin contents of 31.8%, 22.4%, and 11.7%, respectively, indicating its potential as a raw material for α-cellulose production. Variations in NaOH concentration and delignification time significantly influenced the composition of α-cellulose, hemicellulose, and lignin. The optimum delignification condition was achieved at a NaOH concentration of 8% and a delignification time of 45 min, resulting in an α-cellulose content of 51.2%, a hemicellulose content of 16.9%, and a lignin content of 5.4%. FTIR characterization confirmed the presence of O–H, C–H, C–O, and β-(1→4)-glycosidic functional groups, which are characteristic features of α-cellulose. Furthermore, SEM analysis revealed significant morphological changes in the surface structure, characterized by a rougher and more porous appearance. These structural modifications were attributed to the removal of lignin and hemicellulose during delignification, thereby increasing exposure of the cellulose matrix.

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