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Isolation and Characterization of Microcrystalline Cellulose from Alginate Residue of Brown Seaweed *Sargassum Polycystum*

Ilham Wahyu Pamungkas, Amir Husni*, Siti Ari Budhiyanti

Department of Fisheries, Faculty of Agriculture, Universitas Gadjah Mada, Yogyakarta, Indonesia

Abstract: Seaweed has great potential in the pharmaceutical field, developing into microcrystalline cellulose (MCC). MCC is a pharmaceutical solid dosage form as an excipient and is very suitable for manufacturing direct-printed tablets that function as binders and fillers. The purpose of this study was to isolate and characterize MCC from brown seaweed *Sargassum polycystum* and its alginate extraction residues through acid and enzyme hydrolysis methods. *S. polycystum* was prepared, and then alginate was extracted. The residue obtained from the alginate extraction and seaweed powder was then isolated to obtain α-cellulose and then hydrolyzed using 2.5 N HCl and cellulase. The characteristics of MCC tested were yield, solubility, water content, ash content, pH, Fourier Transform-Infra Red (FTIR) analysis, and X-Ray Diffraction. The results showed that MCC produced from different types of samples and isolated treatment of microcrystalline cellulose did not meet the requirements of the British Pharmacopeia. However, the results showed that MCC from alginate extraction residue with enzyme hydrolysis treatment was closer to the standard (Avicel PH 101) with a yield of 35.16 ± 9.02%, pH 6.60 ± 0.36, water content 2.50 ± 1.34%, ash content 5.55 ± 0.09%, and solubility 35.23 ± 10.57%. The results of the FTIR analysis test also showed typical peaks of cellulose, namely 3444.5 cm^{-1} for O-H and 2907.61 cm^{-1} for C-H, with a crystallinity degree of 74.73%.

Keywords: microcrystalline cellulose, alginate extraction residue, *Sargassum polycystum*, acid hydrolysis method, enzyme hydrolysis method.

從褐海藻馬尾藻多囊藻酸鹽殘留物中分離和表徵微晶纖維素

摘要： 海藻在製藥領域具有巨大潛力，可發展成微晶纖維素。微晶纖維素是一種作為賦形劑的藥物固體劑型，非常適合製造用作粘合劑和填充劑的直接印刷片劑。本研究的目的是通過酸和酶水解方法從褐海藻馬尾藻多囊及其藻酸鹽提取殘留物中分離和表徵微晶纖維素。製備多囊馬尾藻，然後提取海藻酸鹽。然後分離從藻酸鹽提取物和海藻粉中獲得的殘餘物以獲得β-纖維素，然後使用 2.5N 鹽酸和纖維素酶水解。測試的微晶纖維素的特性是產量、溶解度、水含量、灰分含量、酸鹼度、傅立葉變換紅外分析和 X 射線衍射。結果表明，不同類型樣品產生的微晶纖維素和微晶纖維素的分離處理不符合英國藥典的要求。然而，結果表明酶水解處理海藻酸鹽提取渣中的微晶纖維素更接近標準（微晶纖維素 PH 101），產率為 35.16±9.02%，酸鹼度 6.60±0.36，含水量 2.50±1.34%，灰分 5.55± 0.09%，溶解度 35.23±10.57%。傅里葉變換-紅外分析測試的結果還顯示出纖維素的典型峰，即氧-氫的 3444.5 厘米-1 和碳-氫的 2907.61 厘米-1，結晶度為 74.73%。

关键词：微晶纖維素、海藻酸鹽提取渣、海藻多囊、酸解法、酶解法。

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About the authors: Ilham Wahyu Pamungkas, Amir Husni, Siti Ari Budhiyanti, Department of Fisheries, Faculty of Agriculture, Universitas Gadjah Mada, Yogyakarta, Indonesia
Corresponding author Amir Husni, a-husni@ugm.ac.id

1. Introduction

Indonesia is one of the world's main seaweed producers with a contribution of 21% of the world's total seaweed production and is the 2nd seaweed producer after China [1]. One of the most common species of seaweed found in Indonesia is *Sargassum polysetum* [2]. According to Manteu et al. [3], *S. polycystum* has a chemical composition including water content, protein, fat, carbohydrates, crude fiber, and bioactive compounds including flavonoid compounds, saponins, steroids, alkaloids, phenol hydroquinone, and triterpenoids. According to Vijay et al. [4], carbohydrates in brown seaweed generally consist of polysaccharides such as fucoidan, laminaran, cellulose, and alginate.

Sargassum is one of the alginate-producing seaweeds. In alginate extraction, one by-product is solids in the form of alginate residues [5]. The alginate residue of S. polycystum still contains cellulose. Cellulose in the alginate extraction residue can be isolated into microcrystalline cellulose (MCC) through hydrolysis [6]. MCC is α-cellulose which is partially depolymerized and purified until it is white, odorless, tasteless, and has easy-flowing properties, and is a material that can act as a filler-binder disintegrant. MCC is widely used in the pharmaceutical industry as the best excipient compound in manufacturing direct-printed tablets that function as binders and fillers [7].

So far, the pharmaceutical industry uses microcrystalline cellulose from synthetic materials, which are relatively expensive [8]. Therefore, it is necessary to search for other cheaper alternative materials in isolating microcrystalline cellulose as a pharmaceutical preparation material, one of which is from seaweed. Alternative materials used in must-have conditions include a fairly high cellulose content and abundant availability [9]. Several studies on the isolation of microcrystalline cellulose from seaweed include the red seaweed *Eucheuma cotonii* [7], the green seaweed *Cladophora sp*. [10], and *Posidonia oceanica* [11], as well as some fishery waste produced such as agar waste [12]. However, some of the MCC produced by these raw materials do not follow commercial MCC's standard characteristics from the British Pharmacopoeia [13].

Microcrystalline cellulose can be isolated through hydrolysis [7] and enzymatic reactions [14]. Edison et al. [7] reported that the 2-2.5 N HCl concentration gave the best results in MCC's physical and organoleptic characteristics from seaweed *E. cottonii*. Cellulase has been used to isolate α-cellulose from water hyacinth into MCC, and its quality was equivalent to standard MCC [14]. Until now, the authors have not found any research on the isolation of MCC from brown seaweed *S. polycetum* and its alginate residues. Therefore, this study aimed to isolate and characterize MCC from brown seaweed *S. polycystum* and its alginate extraction residues through acid hydrolysis and enzymatic methods.

2. Material and Methods

2.1. Materials

The main material used in this study was *S. polycystum* taken from Drini Beach, Gunungkidul, Yogyakarta, Indonesia, in September 2020. The chemicals used in the process of making alginate extraction residues and isolation of MCC were HCl, Na_2CO_3, NaOH (Merck KGaA, Germany), NaOCl (Caledon Laboratory, Canada), Avicel PH 101 (Sigma-Aldrich, USA), cellulase (Sigma-Aldrich C2605, USA), Acetate buffer and KOH (Merck KGaA Germany).

2.2. Sample Preparation

Sample preparation refers to the method of Husni et al. [15] with some modifications. Two samples are used to isolate microcrystalline cellulose, namely from dried seaweed and alginate extraction residue. In the seaweed samples, S. polycystum was washed thoroughly in running water to remove impurities in the subsequent seaweed. The grass is cut to speed up the drying process and then dried at 100°C to dry. The dried seaweed was then mashed using a miller machine, and S. polycystum powder was obtained. The alginate extraction residue sample was prepared by washing the seaweed thoroughly, soaking it in 0.1% KOH for 1 hour, and then washing it with running water to remove the residual alkali. Furthermore, the seaweed was treated the same as making *S. polycystum* seaweed powder to obtain *S.polycystum* seaweed powder, which would extract alginate residues.

2.3. Making Alginate Residue

The residue obtained was obtained from the extraction of alginate by separating the residue and the filtrate. Alginate extraction method using acid pathway extraction method refers to Husni et al. [15] with some modifications. The first step was to weigh 100 grams of the dried powdered seaweed. Next, it was soaked in 1% HCl solution for 1 hour, washed with clean running water until neutral pH, and then extracted with 2% Na_2CO_3 in a ratio of 1: 30 with a temperature of 70°C for 2 hours. The extraction process was stirred periodically every 15 minutes within 2 hours of extraction. Then the extract was filtered to separate the filtrate, and the residue was rinsed until neutral pH using a calico cloth.

2.4. Isolation of α-Cellulose

The α-cellulose isolation method used refers to the research of Edison et al. [7] with some modifications. Each 100 g of seaweed samples and alginate extraction residue were extracted with 20% NaOH at 70-80°C for

2 hours. The extracted sample was filtered and washed until the pH was neutral. Then bleaching was done with 3.5% NaOCl and water (1:1). The bleached sample was then rinsed with distilled water until the pH was neutral.

2.5. Isolation of Microcrystalline Cellulose by Acid Method

Isolation of microcrystalline cellulose using the acid method refers to the research of Edison et al. [7] with some modifications. The cellulose samples obtained were then hydrolyzed with 2.5N HCl in a ratio of 1: 20, boiled at 105°C for 15 minutes. The hydrolyzed sample was then rinsed using distilled water until the pH was neutral and then dried in an oven at a temperature of 60-70°C for 1 hour.

2.6. Isolation of Microcrystalline Cellulose by Enzime Method

The process of isolation of microcrystalline cellulose with the enzyme method refers to the research of Suryadi et al. [14] with some modifications. The sample was weighed 10 g and added 100 ml of 0.05 M acetate buffer and 2 ml of cellulase. Then the sample was stirred at a speed of 160 rpm with a magnetic stirrer for 1 hour at room temperature. Then the sample was centrifuged at 30,000 rpm for 30 minutes. The sample in the form of residue was then separated with the supernatant. Then washed and dried using an oven at a temperature of 60-70°C for 1 hour to obtain MCC.

2.7. Yield of MCC

The yield of MCC was calculated as a percentage comparison between the weight of MCC resulting from hydrolysis and the weight of the material used in the form of α-cellulose, where the calculation of the yield of MCC was based on the dry weight of the material [7]. The calculation formula is:

$$\text{Yield} = \frac{\text{Weight of MCC}}{\text{Weight } \alpha\text{-cellulose}} \text{ x } 100\%$$

2.8. Solubility Test

Solubility was tested by the method described by Gusrianto et al. [16] with some modifications. A sample of 0.2 g was stirred with 10 mL of distilled water for 10 minutes, filtered under vacuum through filter paper weighed beforehand. The filtrate was transferred into a beaker that had been weighed, then evaporated to dryness at a temperature of 105°C for 1 hour, cooled in a desiccator, and weighed. The calculation formula is as follows:

$$\text{Solubility (\%)} = \frac{\text{Initial wight - Final weight}}{\text{Initial weight}} \text{ x } 100\ \%$$

2.9. pH Test

The pH test was carried out based on the British Pharmacopoeia [13] using a pH meter. A total of 0.2 g of sample was added with 10 mL of distilled water, stirred for 5 minutes, and the pH of the supernatant was measured with a pH meter.

2.10. Water Content Analysis

The water content test was carried out to determine the water content using the OHAUS MB35 moisture analyzer. A total of 0.5 grams of MCC samples were put into a pan moisture analyzer whose weight was known. Then the moisture analyzer was closed then the drying process at a temperature of 110°C will start until the drying process was completed automatically and the results of the moisture content (%).

2.11. Ash Content Analysis

Ash content was tested based on the AOAC method [17]. A sample of 3 g was placed in a porcelain dish with known constant weight and then heated over a Bunsen burner until it no longer smoked. The sample was put into a furnace at 500°C for 5 hours. After constant weight, the sample was weighed again, and the ash content was calculated.

2.12. Functional Group Analysis

Functional group testing with Fourier Transform Infrared (FTIR) was carried out as described by Sunardi et al. [18]. A sample of 5 mg was mixed with 0.1 g of KBr, then crushed and made into pellets, then heated in an oven for 24 hours. FTIR analysis was carried out in the wavenumber region around 500-4000 cm^{-1}. The resulting spectra will show a peak at a certain wavenumber.

2.13. X-Ray Diffraction (XRD) Analysis

XRD analysis was performed to determine the crystallinity index of MCC. XRD analysis was carried out as described by Sunardi et al. [18]. A sample of 0.3 g was placed on a plastic plate and carried out in transmission mode using Cu-Kα radiation, operated at 40 kV and 35 mA, then scanned through a diffraction angle (2θ) = 5-80° with a step size of 0.02θ. The crystallinity index (CrI) was determined by the formula:

$$Crl\,\% = \frac{I002 - Iam}{I002} \times 100\%$$

where:

$I002$ - the peak intensity of microcrystalline cellulose at 2θ =22.5°;

Iam - amorphous intensity at 2θ = 18.7.

2.14. Statistical Analysis

All data are expressed by the mean standard deviation (n = 3). Data were processed using Excel 2019 and Statistical Package for Social Sciences (SPSS) Version 25 for Windows (Microsoft Windows, Inc) to test for normality using the Kolmogorov-Smirnov test. If the data is normally distributed, the parametric test is continued by using a further test

using Duncan's test.

3. Results and Discussion

3.1. Yield of MCC

The yield of MCC from the powder of seaweed and residues of alginate extraction by acid and enzyme methods can be seen in Table 1. MCC from seaweed powder and alginate residues hydrolyzed using acid had yields of 5.83 ± 0.51 and 2.28 ± 0.42%, respectively. Meanwhile, the MCC from seaweed powder and alginate residue hydrolyzed using enzymes were 23.93 ± 2.41 and 35.16 ± 9.02%, respectively. The yield of MCC with the acid hydrolysis method was lower than the enzyme hydrolysis method caused by the different stages of each treatment, especially the manufacture of α-cellulose.

Putri & Suryadi [19] reported that MCC from water hyacinth with cellulase enzymes and beta-glucosidase extract produced lower yields (63%) than expected (70%) that can be caused by the stages of the delignification and washing process on the alpha-cellulose samples to reduce the yield of MCC produced. Other factors such as the influence of the temperature used, hydrolysis time, and the concentration used or the solvent used can affect the difference in yield [20].

3.2. Water Content

The results of the water analysis of MCC from *S. polycsetum* can be seen in Table 1. MCC from seaweed powder and alginate residue hydrolyzed by the acid method had a water content of 12.81±2.90 and 9.38±0.76%, respectively. Meanwhile, the MCC of seaweed powder and alginate residue hydrolyzed by the enzyme method obtained water content of 7.08 ± 1.04 and 2.5 ± 1.34%, respectively. The Avicel PH101 as a standard has a moisture content of 2.44 ± 0.46%.

The water content of MCC, which was hydrolyzed by the acid method, was higher than that of MCC hydrolyzed by the enzyme method because the MCC with the acid method is still not completely hydrolyzed, and part of the cellulose structure is still amorphous. Amorphous properties are easier to absorb and store water than crystalline properties, so that the water holding capacity is too high in the structure. Too low a concentration of acid can cause an imperfect hydrolysis process. [7]. This fact is evidenced in the research of Edison et al. [7], where the MCC produced from the red seaweed *E. cottonii* experienced an increase in the water content at the 2 N HCl concentration of 9.6% compared to the 3 N HCl concentration of 4%. In contrast, in the enzyme hydrolysis method, the value of the water content in MCC was low because the enzyme was cellulase. In a study conducted by Suryadi et al. [14], cellulase can produce water hyacinth MCC with a water content of 3.68%. Cellulase is a type of endoglucanase enzyme that can hydrolyze amorphous regions of cellulose. The amorphous region that has been hydrolyzed leaves a crystalline part, resulting in a structure that cannot store water [12]. Based on the results obtained, the residual MCC of alginate extraction by hydrolysis with the enzyme method met the standard requirements for water content in microcrystalline cellulose [13].

3.3. Ash Content

The results of the analysis of MCC ash content from *S. polycsytum* are shown in Table 1. MCC from seaweed powder and alginate residue hydrolyzed by the acid method had an ash content of 15.38 ± 0.11 and 4.36 ± 0.29%, respectively. Meanwhile, MCC from seaweed powder and alginate residue hydrolyzed by the enzyme method obtained ash content of 15.24 ± 0.08 and 5.55 ± 0.09%, respectively. The Avicel PH101, as a comparison, has an ash content of 0.14 ± 0.02%. MCC *S. polycystum* produced from seaweed powder and alginate residue by acid hydrolysis method had significantly different ash content. The MCC ash content of seaweed powder was much higher than that of alginate residue due to differences in the treatment of the MCC of the alginate residue at the pre-treatment time and the process of making alginate residue.

In the pre-treatment process, seaweed was soaked in 1% KOH solution; while making alginate residue; alginate was extracted using Na_2CO_3. According to Nurkhanifah & Husni [21], the provision of KOH at the time of soaking seaweed affects the amount of ash content because mineral salts, impurities, and organic substances contained in seaweed can dissolve in KOH solution. In contrast, the alginate extraction using Na_2CO_3 could extract Na contained in the alginate extraction residue, resulting in a large amount of Na content in the alginate filtrate. The immersion in KOH solution and the removal of alginate with Na_2CO_3 at the time of the alginate residue sample reduced the ash content compared to samples that were not soaked. The ash content of MCC produced by the alginate residue with the acid hydrolysis method was lower than the enzyme method. It was suspected that demineralization occurred in addition to the hydrolysis process due to the influence of hydrochloric acid.

The demineralization process is the process of removing minerals from materials using an acid solution [22]. When the hydrolysis process occurs, the minerals still left by the pre-treatment process on the cellulose are also dissolved by hydrochloric acid. This process results in a decrease in the ash content of MCC produced by acid hydrolysis. Meanwhile, the MCC produced by the enzyme hydrolysis method did not occur, so that the ash content was still left in the MCC sample. The MCC ash content of the research results was higher than the MCC ash content of *E. cottonii* (1.42%) [7] and water hyacinth (0.20%) [14], so it did

not meet British Pharmacopia standard is a maximum of 0.10% [13] probably because there is still a high mineral content in *S. polycystum* seaweed samples. According to Edison et al. [7], seaweed generally has a high mineral content because it was obtained from the sea rich in minerals. Seaweed with high enough minerals can produce high MCC ash content after the hydrolysis process.

3.4. pH Analysis

The pH test results of MCC *S. polycsetum* isolated by the acid and enzyme methods can be seen in Table 1. MCC from seaweed powder and alginate residue hydrolyzed by the acid method had a pH of 5.00 ± 0.79 and 4.60 ± 0.35, respectively. Meanwhile, the MCC of seaweed powder and alginate residue hydrolyzed by the enzyme method obtained pH of 7.40 ± 0.59 and 6.60 ± 0.36, respectively. The Avicel PH101, as a comparison, has a pH of 6.10 ± 0.21.

MCC from seaweed powder and alginate residues with acid hydrolysis method had a not significantly different pH. In contrast, with the enzyme method, it was significantly different caused by the alkaline mineral content in seaweed. The mineral content is in the form of nutrients with a high pH consisting of sodium, calcium, magnesium, and potassium derived from seawater [23]. The MCC produced by the alginate residue has a much lower pH due to the addition of Na_2CO_3. Na_2CO_3 is known to reduce alkaline mineral levels in seaweed when swelling occurs in the cellulose structure so that the resulting pH decreases [21]. In addition, it also influences the delignification process using NaOH solution and the bleaching process using NaOCl solution, which creates an alkaline atmosphere during the isolation of α-cellulose [24].

The difference in the hydrolysis method had a significant effect on the pH of the MCC. MCC from the enzyme hydrolysis method has a high pH compared to the acid hydrolysis method because the cellulase enzyme requires a pH of 4.5-6.0 for the hydrolysis process optimally. The pH that is too low or acidic can reduce enzyme activity in the hydrolysis process to the extreme, and if the pH is too high or alkaline, it can cause damage to the active site in the cellulose structure. Optimal pH at the hydrolysis of α-cellulose gave MCC results with a neutral pH [14]. While the MCC produced from the acid hydrolysis method has a low pH because the hydrolyzing agent is hydrochloric acid, a strong acid compound, and the resulting MCC has a lower pH than the use of enzymes [24]. Previous research, isolation of MCC using red seaweed *E. cottonii*, which was hydrolyzed using 2.5 N HCl, and water hyacinth, which was hydrolyzed with cellulase, resulted in a pH of 6.49 [7] and 7 [14], respectively. Thus the pH of the MCC in this study was lower than in the two studies. The low pH value of MCC in this study could be caused during the MCC neutralization process that did not run perfectly so that the MCC produced still had a lower pH [25]. Based on pH, MCC from *S. polycystum* powder using acid and enzymes hydrolysis has met the MCC standard [13].

3.5. Solubility of MCC

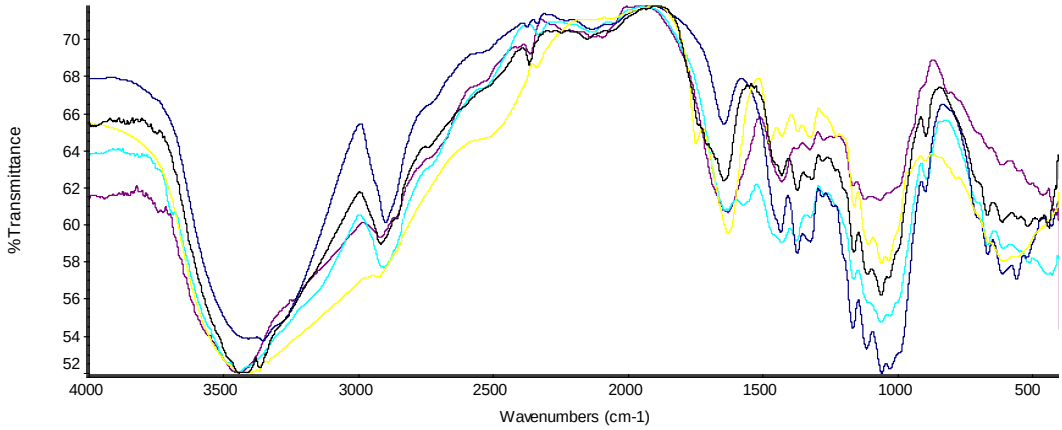
The MCC *S. polycsytum* solubility test results using the acid and enzyme isolation method can be seen in Table 1. MCC from seaweed powder and alginate residue hydrolyzed by the acid method had a solubility of 56.93 ± 10.11 and 27.07 ± 7.27%, respectively. Meanwhile, the MCC of seaweed powder and alginate residue hydrolyzed by the enzyme method had a solubility of 24.02 ± 6.16 and 35.23 ± 10.57%, respectively.

Table 1 Effect of treatment on yield, moisture content, ash content, pH, and solubility of microcrystalline cellulose (MCC) from *Sargassum polycystum*

Treatments	Yield (%)	Water content (%)	Ash content (%)	pH	Solubility (%)
Acid hydrolysis of seaweed powder	5.83 ± 0.51^{a}	12.81 ± 2.90^{b}	15.38 ± 0.11^{d}	5.0 ± 0.79^{a}	56.93 ± 10.11^{b}
Acid hydrolysis of alginic residue	2.28 ± 0.42^{a}	9.38 ± 0.76^{a}	4.36 ± 0.29^{b}	4.6 ± 0.35^{a}	27.07 ± 7.27^{a}
Enzyme hydrolysis of seaweed powder	23.93 ± 2.41^{b}	7.08 ± 1.04^{c}	15.24 ± 0.08^{d}	7.4 ± 0.59^{c}	$24{,}02 \pm 6.16^{a}$
Enzyme hydrolysis of alginate residue	35.16 ± 9.02^{c}	2.50 ± 1.34^{b}	5.55 ± 0.09^{c}	6.6 ± 0.36^{bc}	35.23 ± 10.57^{a}
Avicel PH101	-	2.44 ± 0.46^{a}	0.14 ± 0.02^{a}	6.1 ± 0.21^{b}	22.95 ± 5.75^{a}

The solubility of MCC produced based on different samples, namely seaweed powder and alginate residue in the enzyme hydrolysis method, showed no significant difference. In contrast, the acid hydrolysis method was significantly different. MCC of *S. polycystum* powder by acid method had higher solubility than alginate residue due to the high levels of lignin in MCC from seaweed powder. According to Amalia [26], lignin has a complex, irregular, random structure, and its main constituents are aromatic compounds, which add to the elasticity of the cellulose and hemicellulose matrix. As a result of this complexity, cellulose is not completely hydrolyzed into MCC, causing high solubility. The MCC produced from alginate residues was quite low due to the low concentration of HCl immersion treatment during making alginate residues capable of reducing lignin levels in lignocellulosic due to the Cl^- playing a role in disrupting the stability of bonds in the network. The hydrogen bonds in the lignocellulosic structure will be broken, resulting in the decay of lignin and hemicellulose compounds in the cell wall, resulting in

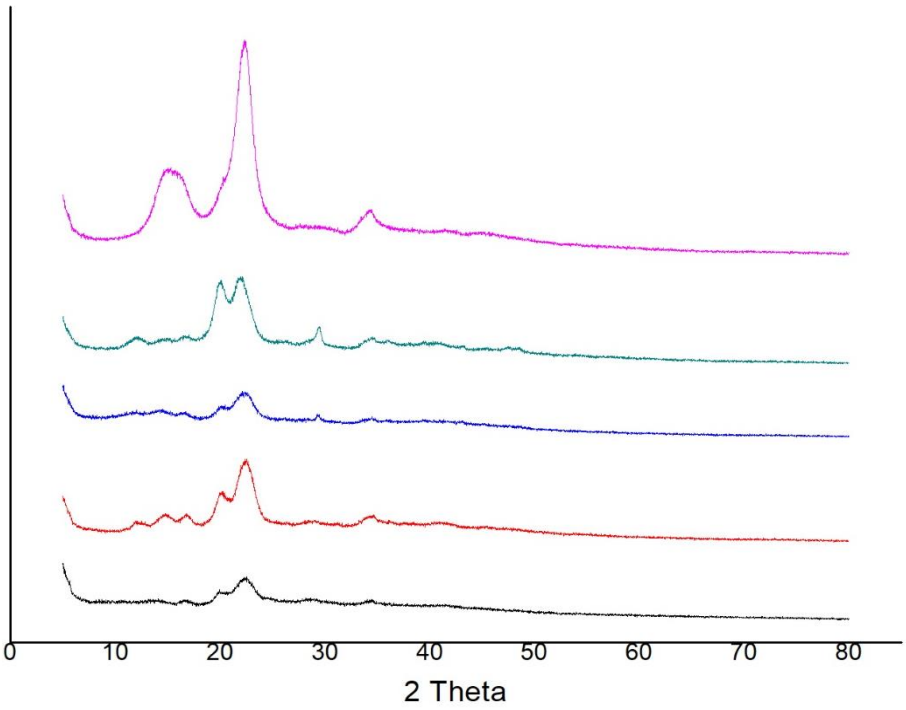
low lignin and hemicellulose levels [27], also the lower the lignin content, the lower the solubility [26].



Notes:

- Avicel Ph101
- Acid hydrolysis of seaweed powder
- Acid hydrolysis of alginic residue
- Enzyme hydrolysis of seaweed powder
- Enzyme hydrolysis of alginate residue

Fig. 1 FT-IR spectra of microcrystalline cellulose from *Sargassum polycystum*



Notes:

- Avicel Ph101
- Acid hydrolysis of seaweed powder
- Acid hydrolysis of alginic residue
- Enzyme hydrolysis of seaweed powder
- Enzyme hydrolysis of alginate residue

Fig. 2 X-ray diffraction of microcrystalline cellulose from *Sargassum polycys*

3.6. Fourier Transform Infrared (FT-IR) Analysis

The analysis was used to determine the functional groups contained in MCC produced from various hydrolysis methods and types of samples using FT-IR. The working principle of FT-IR is the interaction between energy and matter. Infrared passes through the slit to the sample, where the slit controls the amount of energy delivered to the sample. Then some infrared is absorbed by the sample, and others are transmitted through the sample's surface so that infrared rays pass to the detector. The measured signal is then sent to a computer and recorded in the form of peaks to distinguish the compounds [31]. Based on the results of infrared spectra in Fig. 1, hydrolysis treatment using acid method on MCC of seaweed powder showed wave peaks at 3411.75 cm^{-1}, 1747.47 cm^{-1}, 1627.81 cm^{-1},

1473.23 cm^{-1}, 1323.32 cm^{-1}, 1161.32 cm^{-1}, 1060.83 cm^{-1} and 608.87 cm^{-1}. For MCC from alginate extraction residue, it shows infrared spectra at the peak of 3441.96 cm^{-1}, 2961.3 cm^{-1}, 2366.8 cm^{-1}, 1644.17 cm^{-1}, 1429.5 cm^{-1}, 1372.82 cm^{-1}, 1162.62 cm^{-1}, 1061.28 cm^{-1}, 895.44 cm^{-1}, 667.94 cm^{-1}, and 437.95 cm^{-1}. While the results of infrared spectra with the hydrolysis treatment of the enzyme method on MCC from seaweed powder showed wave peaks at 3447.14 cm^{-1}, 2918.34 cm^{-1}, 2095.07 cm^{-1}, 1629.6 cm^{-1}, 1429.91 cm^{-1}, 1066.87 cm^{-1}, and 809.57 cm^{-1}. For MCC of the alginate extraction residue showed a wave peak of 3445.5 cm^{-1}, 2907.1 cm^{-1}, 1640.78 cm^{-1}, 1429.55 cm^{-1}, 1061.87 cm^{-1} and 894.22 cm^{-1}. While the Avicel PH 101 showed a wave peak of 3355.10 cm^{-1}, 2899.58 cm^{-1}, 2134.63 cm^{-1}, 2134.63 cm^{-1}, 1644.23 cm^{-1}, 1433.39 cm^{-1}, 1372.86 cm^{-1}, 1166.35 cm^{-1}, 1115.54 cm^{-1}, 1058.98 cm^{-1} and 666.19 cm^{-1}.

According According to Yugatama et al. [32], the infrared spectrum in pure cellulose has a peak between 3400-3500 cm^{-1} which indicates the presence of O-H groups, 2800-2900 cm^{-1} C-H groups, 1035-1060 cm^{-1} C-O groups. The peaks of 1374 cm^{-1} and 1373 cm^{-1} indicate the presence of a C-H group. The peak of about 3400-3500 cm^{-1} is the wavenumber for the O-H strain in cellulose which shows inter and intra bonds and a decrease in the O-H group, which indicates the increasing number of crystalline regions in MCC. The peak at the wavenumber around 2800-2900 cm^{-1} is the wavenumber for the C-H strain in cellulose. There is a shift in wavenumber at this peak, wherein α-cellulose peak at a wavenumber of about 2900 cm^{-1}, where at MCC from seaweed powder, and the residue of alginate extraction decreases. The decreasing amount of C-H was since the chains between the cellulose in MCC became denser and denser, thus preventing the movement of the C-H groups [33]. While at the peak of about 1600 cm^{-1}, there is a strong interaction between cellulose and water [33]. The peak at a wavenumber of around 1429 cm^{-1} indicates the presence of C-O-C groups in cellulose [34]. The peak at a wavenumber of around 1318 cm^{-1} indicates an O-H bend [33]. The peak at a wavenumber of about 1059 cm^{-1} indicates the presence of C-O-C vibrations from the pyranose ring framework [34]. The peak at a wavenumber of about 894.97 cm^{-1} indicates the presence of α-glycosidic bonds in cellulose [33].

3.7. X-Ray Diffraction (XRD) Analysis

XRD analysis identifies the crystalline phase in the material by determining the lattice structure parameters and obtaining the particle size. The working principle of XRD is that X-rays are diffracted, successively formed by crystal atoms of the material [35]. With the emergence of various angles that arise, the diffraction pattern formed expresses the characterization of the sample. XRD characterization in this study was conducted to determine the effect of differences in MCC crystallinity on the samples used, namely seaweed powder and alginate extraction residue with acid and enzyme methods used for hydrolysis.

The results of the MCC diffractogram (Fig. 2) show the wave crests formed due to different treatments each occur at an angle of 2θ 22.45º for Avicel PH101; 22.49º for seaweed powder by acid hydrolysis; 22.78 for seaweed powder with enzyme hydrolysis; 22.33 for residues of alginate extraction by acid hydrolysis and 21.93 for residues of alginate extraction by enzyme hydrolysis. According to Sunardi et al. (2019)[18], the angle around 2θ = 12° is the angle for the amorphous region, while the angle around 2θ = 20°-22° is the angle for the crystalline region, where at the angle of about 2θ = 20° is a type of cellulose, while at about 2θ = 22° is a type of cellulose I. The crystallinity index of MCC *S. polycystum* is presented in Table 2.

Table 2 Crystallinity index of microcrystalline cellulose from Sargassum polycystum

Sample	Crystallinity Index (%)
Avicel PH101	59.88
Acid hydrolysis of seaweed powder	80.16
Acid hydrolysis of alginic residue	65.61
Enzyme hydrolysis of seaweed powder	66.99
Enzyme hydrolysis of alginate residue	74.73

MCC from seaweed powder and alginate residue hydrolyzed by acid method had crystallinity index of 80.16% and 65.61%, respectively, while by enzyme method 66.99% and 74.73%, respectively. The Avicel PH101, as a comparison, has a crystallinity index of 59.88%. The highest crystallinity index was found in the MCC from seaweed powder using the acid method, and the lowest residue was found in the acid method of alginate extraction. In previous studies, MCC produced from water hyacinth with the enzyme method and waste agar produced a crystallinity index of 70 [14] and 62.03[12]. The high and low degree of crystallinity of material is seen in the higher intensity formed. Ansharrullah et al.[29] stated that the high crystallinity index was caused by a large number of cuts in the amorphous regions of the cellulose during hydrolysis. The low degree of crystallinity is caused by the high hemicellulose content resulting in a more amorphous structure.

4. Conclusion

Brown seaweed *Sargassum polycystum* contains cellulose, which has the potential as raw material for MCC. In general, the alginate residue sample type has better characteristics than seaweed powder. Also, the enzyme hydrolysis produces better characteristics than the acid method. As for overall results of the parameters test conducted, the best results were MCC from enzyme hydrolyzed alginate residue with a yield, water content, ash content, pH, solubility, and

crystallinity of 35.16 ± 9.02%, 2.50 ± 1.34%, 5.55 ± 0.09%, 6.60 ± 0.36, 35.23 ± 10.57%, and 74.73%, respectively. Producing microcrystalline cellulose from *S. polycystum* is strongly influenced by the isolation process, including the type of raw materials and isolation methods. Therefore, it is necessary to optimize the optimum condition to get the best quality and efficiency for producing MCC in the future.

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