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# Effect of Isolation Method on Characterization of Microcrystalline Cellulose from Brown Seaweed *Sargassum vulgare*

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**ABSTRACT**

Microcrystalline cellulose (MCC) has been reported to be purely derived from depolymerized cellulose which forms crystals. Therefore, this study aims to determine the characteristics of MCC samples which were in form of powder and alginate residues of *Sargassum vulgare,* hydrolyzed with acid and enzymes. The observed characteristics included yield, water content, ash content, pH, solubility, functional groups, and crystallinity index. Furthermore, the results showed that the MCC from acid-hydrolyzed seaweed powder and alginate residue, as well as enzymatically hydrolyzed seaweed powder and alginate residue each, had a yield of 3.83±2.74%, 5.66±1.52%, 10.03±2.58%, and 17.17±3.50%, crystallinity index of 91.37%, 80.26%, 84.67%, and 81.90%, water content of 4.92±1.85%, 4.92±1.11%, 3.88±2.01%, and 3.58±0.40%, ash content of 13.88±0.12%, 3.49±0.13%, 9.86±0.17%, and 7.43±0.09%, pH of 5.13 ± 0.38, 5.00±0.10; 5.70±0.17, and 5.97±0.06, and solubility of 22.82±1.20%, 23.73±1.09%, 19.12±3.55%, and 21.10±1.48%, respectively. The functional group analysis showed that there were similarities with the standard Avicel PH101. However, the results of enzymatic hydrolysis were better than acid hydrolysis, and samples from alginate residues had better results than seaweed powder. Considering these results, the best MCC was obtained from enzymatically hydrolyzed alginate residue with a yield, water content, ash content, pH, solubility, and crystallinity index of 17.17±3.50%, 3.58±0.40%, 7.43±0.09%, 5.97±0.06, 21.10±1.48, and 81.90%.

**Keywords:** Brown seaweed, characterization, isolation, microcrystalline cellulose, *Sargassum vulgare.*

## INTRODUCTION

Indonesia is one of the largest producers of seaweed in the world, and the second after China (FAO, 2021), and a common type of seaweed in Indonesian waters is *Sargassum vulgare* (Pakidi and Suwoyo, 2017) which had potent radical scavenging activities, and contains high carbohydrate, ash and crude fiber content (Arguelles *et al*., 2019). Carbohydrates in brown seaweed generally consist of polysaccharides such as fucoidan, laminarin, cellulose, and alginate (Vijay *et al*., 2017 ). Furthermore, the extraction of alginate produces by-products, namely solids in the form of residues (Diharningrum and Husni, 2018). The cellulose contained in this residue is isolated through the process of hydrolysis into microcrystalline cellulose (MCC) which is widely used in the pharmaceutical industry as the best excipient compound in the manufacture of tablets (Edison *et al*., 2019). The pharmaceutical industry uses MCC from relatively expensive synthetic materials (Rahmi *et al*., 2020). Therefore, it is necessary to study other cheaper alternatives, one of which is obtained from seaweed. Several studies on the isolation of MCC from seaweed have been carried out, namely the isolation of MCC from red seaweed *Eucheuma cotonii* (Edison *et al*., 2019) and green seaweed, including *Cladophora sp*. (Prasetia *et al*., 2018) and *Posidonia oceanica* (Tarchoun et al., 2019). Meanwhile, no study was being conducted on the isolation of MCC from *S. vulgare*, both from alginate extraction residue and seaweed powder.

In general, MCC extraction is performed chemically through acid and enzymatic hydrolysis methods (Suryadi *et al*., 2017), whereby the best results of MCC from *E. cottonii* seaweed were obtained using 2-2.5 N HCl (Edison *et al*., 2019).

The cellulase enzymes have been used to isolate cellulose from water hyacinth into MCC of equivalent standard quality (Suryadi *et al*., 2017). However, little information is available on the isolation of MCC from *S. vulgare* seaweed and its alginate residues. Therefore, this study aims to isolate and characterize MCC from *S. vulgare* seaweed and its alginate extraction residues through acid and enzymatic hydrolysis methods.

## MATERIAL AND METHODS

The main ingredient used was *Sargassum* v*ulgare* seaweed obtained from the Tabanan Bali, Indonesia, and the chemicals used in the production of alginate residue extraction residues and isolation of microcrystalline cellulosewere KOH, HCl, $Na_2CO_3$, NaOCl (Sigma Aldrich, Germany), NaOH (Merck, Germany), cellulase (Sigma Aldrich, USA), and acetate buffer (Sigma Aldrich, Germany).

### Sample preparation.

*S. vulgare* seaweed samples were collected from Atap Beach, Selemadeg, Tabanan Regency, Bali on 5-6 September 2020. Seaweed samples were collected by cutting the lower thallus using scissors. The samples were then cleaned and divided for morphological identification. Other samples were further deposited within a cool box and stored in the refrigerator, to be delivered to the laboratory. Some of the samples were identified at the Plant Systematics Laboratory, Faculty of Biology, Universitas Gadjah Mada (Letter number: 014890/S.Tb/IX/2020), and were divided into two according to the treatment that was to be performed, namely samples from seaweed powder that did not go through the extraction stage and those of alginate residue obtained through the alginate extraction stage. The samples for alginate residues were pretreated by soaking in KOH and then dried in an oven at 60$^o$C for 1-2 days, while those for seaweed powder were directly dried in the oven without firstly soaking them in KOH. The dried samples were then powdered using a miller machine (FOMACFCT-Z300 PT. Toksindo, Indonesia) and ready for extraction.

### Alginate residue making

A total of 100 g of seaweed powder samples that had been pretreated with KOH were soaked in 1% HCl for 1 hour with a ratio of 1:30 (w/v). Afterward, they were washed with running water until the pH was neutral, and were extracted with 2% $Na_2CO_3$ at a temperature of 60-70°C for 1 hour with a ratio of 1:30 (w/v). Therefore, the filtrate was separated from the residue to obtain alginate residue (Husni *et al*., 2012).

### α-Cellulose isolation

A total of 100 g of seaweed powder samples or alginate residue were heated in 20% NaOH solution at 80°C for 2 h to degrade the lignin polymer. Then the sample was filtered and washed until the pH was neutral. Furthermore, it was bleached with 3.5% NaOCl and water in a ratio of 1:1 while heated to remove residual lignin in the sample and to obtain a brighter appearance of the sample. After which the sample was filtered and neutralized to obtain α-cellulose in the form of pulp (Edison *et al*., 2019).

### Isolation of MCC by acid hydrolysis method

The acid hydrolysis method refers to Edison *et al*. (2019) with some modifications, whereby the α-cellulose pulp samples obtained were hydrolyzed using 2.5N HCl at 105°C for 15 min at a ratio of 1:20 (w/v) to extract the MCC content. Afterward, the sample was rinsed with distilled water and dried in an oven at 40°C for 2 days until it dries and was grounded to obtain MCC.

### Isolation of MCC by enzyme hydrolysis method

The enzymatic hydrolysis method refers to Suryadi *et al*. (2017) with some modifications, whereby the cellulose pulp sample obtained was mixed with 0.05 M acetate buffer and cellulose enzyme in a ratio (2:20:1) which was aimed at hydrolyzing the sample enzymatically. Furthermore, the mixture was stirred at 160 rpm at 50°C for 1 hour, and the hydrolyzed sample was centrifuged at 3.000 rpm for 30 min to separate the residue and supernatant. Afterward, the samples were rinsed with distilled water and dried in an oven at 40°C for 2 days until it dries and was grounded to obtain MCC.

### Yield and physical characteristics

The obtained MCC was tested for its characteristics and compared with Avicel PH101 as a standard which is shown in its appearance or shape, color, and smell. MCC is a powder, white or bright in color and odorless (Rowe *et al*., 2009), and it was obtained from the ratio between the weight of MCC and that of α-cellulose used and expressed in percent (%). Therefore, the following formula is used for calculating the yield of MCC as stated by Edison *et al*. (2019).

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

**Water content analysis**

The water content in MCC was determined using a moisture analyzer (Ohaus, USA) whereby a total of 0.5 g of MCC samples were put into a weighed pan (moisture analyzer), after which it is closed to begin the drying process at a temperature of 110°C until completed automatically and the results of the moisture content (%) are displayed.

**Analysis of ash content**

The quantity of ash content contained in MCC was determined based on the AOAC method (2005). A total of 5 grams of the sample was placed in a weighed porcelain dish which was then heated on a bunsen. Afterward, it was placed in a muffle furnace at a temperature of 500°C for 5 hours and then weighed to a constant weight. The following formula is used to calculate the ash content.

$$\text{Ash content (\%)} = \frac{\text{Initial weight} - \text{Final weight}}{\text{Weight of sample}} X\ 100$$

**pH analysis**

The pH analysis was determined according to the method of Sunardi *et al*. (2019) with slight modifications. A total of 0.2 g of MCC powder was dissolved into 10 mL of distilled water and was stirred for 5 min after which the pH was measured using a pH meter.

**Solubility analysis**

The solubility of MCC was determined according to the method of Gusrianto *et al*. (2011) with some modifications. A total of 0.2 g of the sample was dissolved into 10 mL of distilled water and stirred for 10 min. Afterward, it was filtered with a weighed filter paper which was then heated in an oven at 105°C for 1 h and weighed. The following formula is used for calculating the solubility test.

$$\text{Solubility (\%)} = \frac{\text{S} - (\text{K2} - \text{K1})}{\text{S}} X\ 100$$

Note:
S = Weight of sample before heating (gram)
$K_2$ = Weight of filter paper and sample after heating (gram)
$K_1$ = Weight of filter paper before heating (gram)

**Fourier Transform Infrared (FT-IR) analysis**

FT-IR analysis was performed according to the method described by Sunardi *et al*. (2019). Furthermore, it was conducted to determine the functional groups contained in MCC. In this process, the sample was mixed with potassium bromide and grounded until homogeneous. Afterward, the sample was made into a thin tablet and the infrared spectrum was recorded at a wavelength of 500-4000 $cm^{-1}$.

**X-ray diffraction (XRD) analysis**

XRD analysis was performed based on the method described by Sunardi *et al*. (2019). Furthermore, it was conducted to determine the crystallinity index of MCC, whereby the sample was placed on a plastic plate and operated at 40 kV and 35 mA, after which it was scanned through a diffraction angle (2)=5-80°C to obtain the crystallinity index.

**Data analysis**

The method used was a Completely Randomized Design (CRD) with 2 samples, namely seaweed powder with and without alginate extraction (alginate residue). Each sample received 2 treatments, namely acid and enzymatic hydrolysis methods with 3 replications, however, the data obtained were analyzed using Analysis of Variants (ANOVA) to determine the effect of the extraction method on the characteristics of MCC, when there is a significant difference in the results, further tests are conducted using Duncan (DMRT) with a significance level of 95%.

## RESULT AND DISCUSSION

**MCC yield and appearance**

The yield of MCC (Table I) by acid hydrolysis method of seaweed powder and alginate residue samples were 3.83±2.74% and 5.66±1.52%, respectively, while that of enzymatic hydrolysis method was 10.03±2.58% and 17.17±3.50%, respectively. Therefore, examining the yield of both types of samples, it is observed that the alginate residue was higher than that of the seaweed powder samples, and this is because it contains higher α-cellulose. The alginate residue obtained from its extraction process undergoes separation from cellulose by $Na_2CO_3$ to produce pure and higher MCC yields, while in seaweed powder samples there is alginate contamination that inhibits the hydrolysis process in MCC extraction. Examining the yield data of the two methods, it is observed that the enzyme hydrolysis resulted in higher yields than the acid method from both alginate residue and seaweed powder samples. This shows that the enzyme hydrolysis is better in producing MCC than the acid method. This is because enzymes do not degrade sugar (Suryadi *et al*., 2017), while strong acids which are non-specific

catalysts degrade sugar into monomers to easily dissolve in washing, therefore resulting in low MCC yields (Edison *et al*., 2019).

Table I. Effect of treatment on yield of microcrystalline cellulose (MCC) *Sargassum vulgare*

| Treatments | Yield (%) |
|---|---|
| Acid hydrolysis of seaweed powder | 3.83±2.74[a] |
| Acid hydrolysis of alginic residue | 5.66±1.52[ab] |
| Enzyme hydrolysis of seaweed powder | 10.03±2.58[b] |
| Enzyme hydrolysis of alginate residue | 17.17±3.50[c] |

The same letter in the same column shows no significant difference (P>0.05)

Nawangsari (2019) reported that the MCC yield from bagasse using the acid hydrolysis method was 26.03%, while that from *P. oceanica* seaweed using the acid hydrolysis method was 74.23% (Tarchoun *et al*. 2017), which is higher than MCC *S. vulgare*. Likewise, in the study of Suryadi *et al*. (2019) which was conducted using the enzyme hydrolysis method on water hyacinth, the yield was very high, reaching 95%, this was because the purification stage was performed in such a way that the yield was higher. The MCC yield from *Saccharina japonica* seaweed using the acid hydrolysis method and ball-milling treatment was 9.9% (He *et al*., 2018). Therefore, the difference in yield results is influenced by the sample used.

Avicel PH101 standard MCC has a fine powder form, white in color and odorless, while that which is hydrolyzed by acid and enzyme has a coarse powder form with a different appearance of color and odor. The MCC which is hydrolyzed using acid has a darker color than compared to enzyme hydrolysis, and this is because the use of concentrated acid solutions destroys MCC into carbon which is marked with a brown color (Suryadi *et al*., 2017). Examining the type of sample, MCC from seaweed powder has a slightly darker color compared to alginate residues. This is because the alginate residue sample has gone through the extraction stage, whereby the NaOCl solution is easily absorbed into the alginate residue during the bleaching process compared to seaweed samples (Figure 1). Acid-hydrolyzed MCC has a slightly acidic odor, while that which is hydrolyzed using enzyme has no odor. This is because the concentrated acid is still attached, thereby causing the smell to be slightly sour. However, the two samples of seaweed and alginate residues were not different.

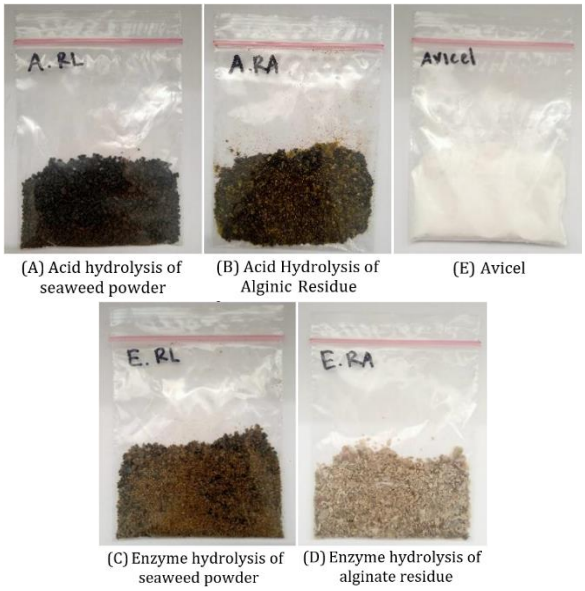


Figure 1. The appearance of MCC results from Acid hydrolysis of seaweed powder (A) and alginic residue (B), Enzyme hydrolysis of seaweed powder (C), and alginate residue (D), and Avicel (E).

MCC is pure cellulose derived from α-cellulose which is depolymerized and purified to form crystals (Schuh *et al*., 2013). Furthermore, it is made from a-cellulose that undergoes a purification process. Cellulose as raw material for MCC produced from seaweed powder or alginate residue which is a solid waste by-product of seaweed processing that contains cellulose and mineral salts. This is because in the alginate extraction process there are steps that function to separate it from cellulose so that the cellulose that is left behind as a filtrate and alginate residue (Anwar *et al*., 2013). The alginate extraction process is conducted by soaking seaweed powder using 1% HCl for 1 h which aims to remove mineral salts that are still attached and to break down the cell walls (Kamisyah *et al*., 2020). Also, it was performed using 2% $Na_2CO_3$ at a temperature of 600-700°C for 1 h which aims to separate the alginate from its residue containing cellulose (Diharningrum and Husni, 2018), hence resulting dregs or filtrate is an alginate residue containing cellulose.

Table II. Effect of treatment on water and ash content of microcrystalline cellulose (MCC) *Sargassum vulgare*

| Treatments | Water content (%) | Ash content (%) |
|---|---|---|
| Acid hydrolysis of seaweed powder | 4.92±1.85[a] | 13.88±0.12[e] |
| Acid hydrolysis of alginic residue | 4.92±1.11[a] | 3.49±0.13[b] |
| Enzyme hydrolysis of seaweed powder | 3.88±2.01[a] | 9.86±0.17[d] |
| Enzyme hydrolysis of alginate residue | 3.58±0.40[a] | 7.43±0.09[c] |
| Avicel PH101 | 2.44±0.46[a] | 0.14±0.03[a] |

The same letter in the same column shows no significant difference (P>0.05)

**Water content**

The results of the MCC water content test (Table II) showed that the MCC moisture content ranges from 3.58±0.40 to 4.92±1.85%. It is observed that the type of sample and the hydrolysis method did not affect the water content. However, there is the low water content of MCC in this study compared to MCC from *E.cottonii* (8.9%) hydrolyzed by HCl at a concentration of 2.5N (Edison *et al*. 2019) and MCC from *P.oseanica* (5.8%) (Tarchoun *et al*., 2019), but comparable to the MCC moisture content of bagasse (4.96%) hydrolyzed by 2.5 N HCl (Nawangsari, 2019). Higher water content weakens the mechanical properties of polymer composites (Jumaidin *et al*., 2017). Therefore, the moisture content of the four samples corresponds to the standard quality according to the Handbook of Pharmaceutical Excipients, which was less than 5% (Rowe *et al*., 2009).

**Ash content**

The quality standard of ash content according to the Handbook of Pharmaceutical Excipients is not more than 0.1% (Rowe *et al*., 2009). The results of the MCC ash content test (Table II), whereby the seaweed powder and acid-hydrolyzed alginate residues had an ash content of 13.88±0.12% and 3.49±0.13%, respectively, while those which were hydrolyzed enzymatically are 9.86±0.17% and 7.43±0.09%, respectively. Statistically, the type of sample and the hydrolysis method had a significant effect (p<0.05) on the ash content of MCC which was higher in the seaweed powder sample than that of the alginate residue. This is because the seaweed sample contains high contamination, while the alginate residue sample has gone through the extraction stage which causes the sample to be more pure and contain less contamination. The ash content of the four samples was higher than the MCC ash content of *E.cottonii* which was 0.94 - 4.90% (Edison *et al*., 2019), *P.oceanica which* was 0.75% (Tarchoun *et al*., 2019), and the standard Avicel PH101 (0.14±0.03%). According to Suryadi *et al*. (2017), the high ash content is influenced by the material used, namely, seaweed which is rich in mineral content is removed during the hydrolysis process.

**pH analysis**

The results of the MCC pH analysis (Table III), where the four samples had a pH between 5.0±0.1 to 5.97±0.06 which is statistically not significantly different (p>0.05). Furthermore, the MCC of the four samples corresponds to the pH quality standard, which was in the range of 5.0-7.5 (Rowe *et al*., 2009). However, the MCC *S. vulgare*, in general, has the same pH as studied by Edison *et al*. (2019) which is 5.73-6.82, and Nawangsari (2019) which is 6.90.

**Solubility of MCC**

The results of the MCC solubility test of *S. vulgare* (Table III), where seaweed powder and acid-hydrolyzed alginate residues have a solubility of 22.82±1.20% and 23.73±1.09%, respectively, while those which hydrolyzed enzymatically were 19.12±3.55% and 21.10±1.48%. Furthermore, MCC from seaweed powder samples had lower solubility than alginate residue, but not statistically significant. The acid method has a higher level of solubility than that of an enzyme, although statistically, it is also not significantly different. These results show that the sample with enzyme hydrolysis has better quality than that of acid. This is because the enzyme can hydrolyze cellulose and produce MCC with a stronger structure and lower solubility than the acid method. Strong acids degrade sugar into monomers, therefore having high solubility in water (Edison *et al*., 2019). The lower the MCC solubility, the better the quality, which is caused by the presence of strong hydrogen bonds between hydroxyl groups in the bond chain adjacent to the crystalline structure (Nawangsari *et al*., 2018).

Table III. Effect of treatment on pH and solubility of microcrystalline cellulose (MCC) *Sargassum vulgare*

| Treatments | pH | Solubility (%) |
|---|---|---|
| Acid hydrolysis of seaweed powder | 5.13±0.38[a] | 22.82±1.20[a] |
| Acid hydrolysis of alginic residue | 5.00±0.10[a] | 23.73±1.09[a] |
| Enzyme hydrolysis of seaweed powder | 5.70±0.17[b] | 19.12±3.55[a] |
| Enzyme hydrolysis of alginate residue | 5.97±0.06[b] | 21.10±1.48[a] |
| Avicel PH101 | 6.10±0.21[b] | 22.95±5.74[a] |

The same letter in the same column shows no significant difference (P>0.05)

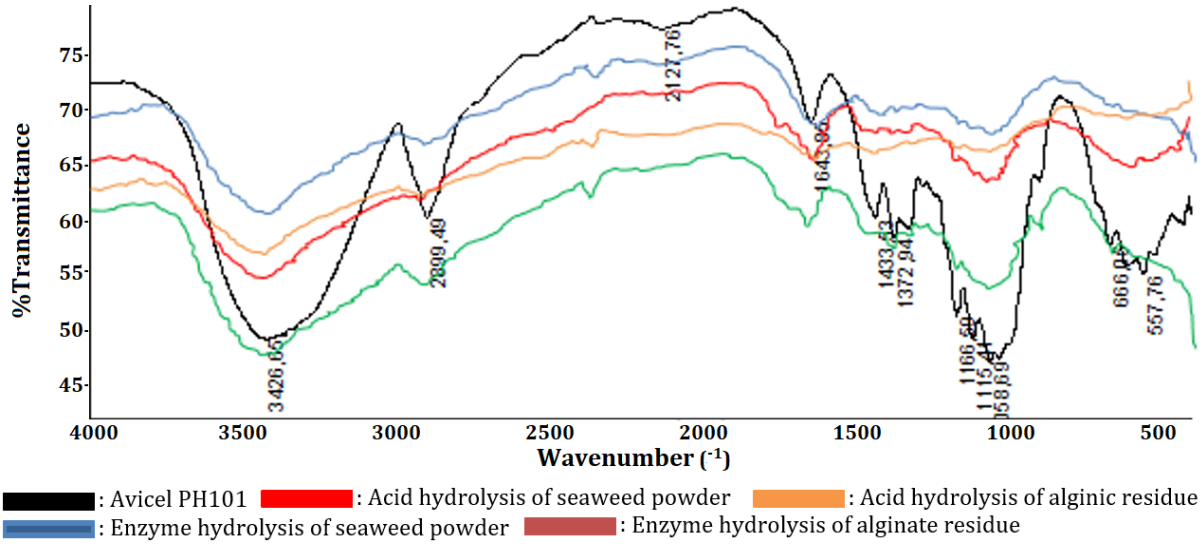


Figure 2. FT-IR spectra of microcrystalline cellulose *Sargassum Vulgare*

Based on the research of Gusrianto *et al*. (2011) the extraction of MCC from sawdust waste has a very low solubility of 0.12%, while *S. vulgare* has a higher solubility level which shows that there are still amorphous parts that have not yet formed crystals. In addition, it is suspected that during the hydrolysis process glucose monomers are formed which are easily soluble in water (Edison *et al*., 2019). When compared with the standard MCC Avicel PH101 which has a solubility of 22.95 ± 5.74%, the MCC sample hydrolysis of alginic acid residues has higher solubility, while that of seaweed acid and enzyme hydrolysis, as well as alginate residue enzyme hydrolysis, have higher levels. However, both the Avicel standard and the four MCC samples do not meet the requirements for water solubility which was less than 0.25% according to the Handbook of Pharmaceutical Excipients (Rowe *et al*., 2009).

**Fourier Transform Infrared (FT-IR) analysis**

FT-IR analysis was conducted to determine the functional groups contained in MCC. Furthermore, its principle was to recognize a functional group of compounds based on infrared absorbance, hence distinguishing compounds (Sankari *et al*., 2010). Based on the results of the FT-IR test, MCC *S.vulgare* from acid and enzymes hydrolysis, both from seaweed powder and alginate residues samples have similar functional groups to standard microcrystalline Avicel PH101 (Figure 2). The sequence of MCC functional groups which is close to that of standard Avicel PH101 are acid hydrolyzed alginate residue, enzymatically hydrolyzed alginate residue, enzymatically hydrolyzed seaweed powder, and acid hydrolyzed seaweed powder.

Based on the FT-IR spectra graph, it suggested that the Avicel PH101 standard has several wavelengths that show the functional groups possessed by MCC including the wavelength of 1058.69 $cm^{-1}$ which shows the COC functional group of the pyranose ring, and 1372.94 $cm^{-1}$ shows the bending of the group. CH in cellulose (Liu *et al*., 2018), 1115.41 $cm^{-1}$ and 1166.59 $cm^{-1}$ show a $CH_2$ group (Effendi *et al*., 2018), 1433.53 $cm^{-1}$

Table IV. Crystallinity index of microcrystalline cellulose *Sargassum vulgare*

| Sample | Crystallinity Index (%) |
|---|---|
| Avicel PH101 | 59.88 |
| Acid hydrolysis of seaweed powder | 91.37 |
| Acid hydrolysis of alginic residue | 80.26 |
| Enzyme hydrolysis of seaweed powder | 84.67 |
| Enzyme hydrolysis of alginate residue | 81.90 |

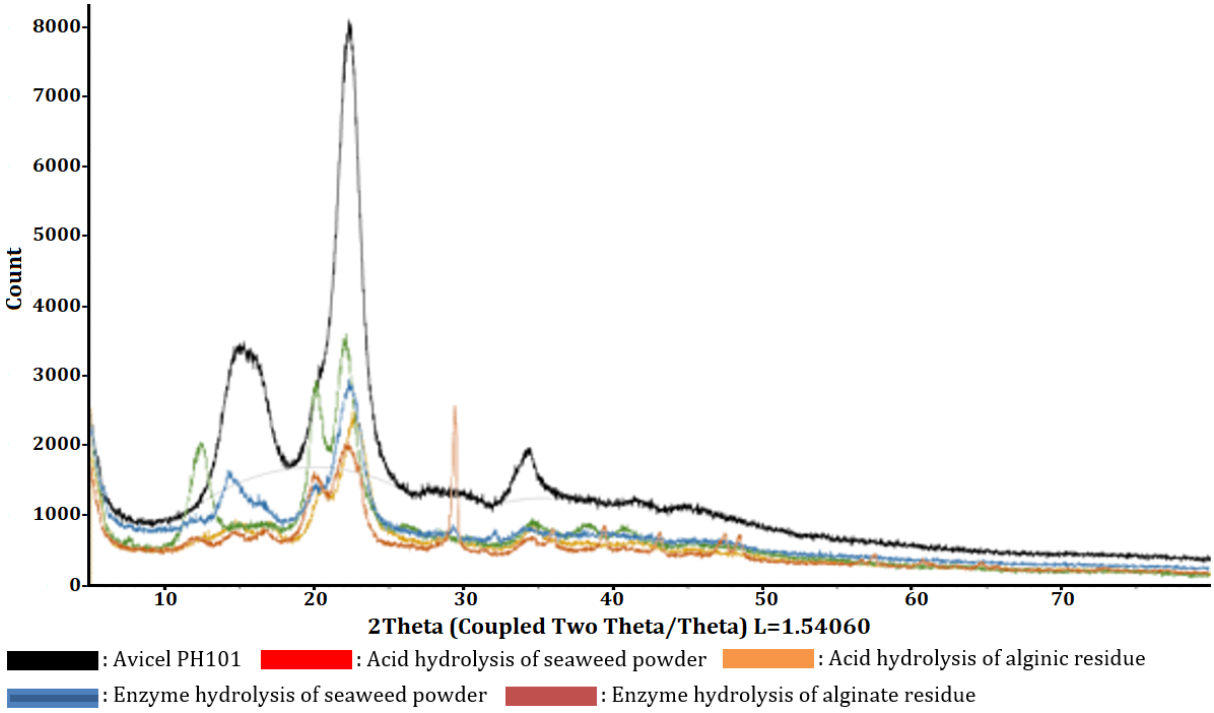


Figure 3. X-ray diffraction of microcrystalline cellulose *Sargassum Vulgare*

a symmetrical bending of the $CH_2$ group (Purwanti and Dampang, 2017), 3426.65 $cm^{-1}$ is the OH functional group in cellulose (Liu *et al*., 2018), 2899.49 $cm^{-1}$ shows the strain of the CH group (Purwanti and Dampang, 2017), and 1643 $cm^{-1}$ shows a carbonyl group (C=O) (Effendi *et al*., 2018). The MCC from acid hydrolyzed seaweed powder was similar to Avicel standard at the wavelengths of 3436.45 $cm^{-1}$, 1062.29 $cm^{-1}$, 1634.64 $cm^{-1}$, and 1374.49 $cm^{-1}$, while that from alginate residues which were hydrolyzed by acid had similarities with Avicel as a standard, including 3444.11 $cm^{-1}$, 1059.75 $cm^{-1}$, 1644.09 $cm^{-1}$, 1374.57 $cm^{-1}$, and 2912.63 $cm^{-1}$. However, there are also wavelengths discovered in MCC from acid hydrolyzed alginate residues but not in the Avicel, namely the wavelength of 892.6 $cm^{-1}$ which shows glycosidic bonds in cellulose (Effendi *et al*., 2018). The MCC of acid hydrolyzed seaweed powder had the same wavelength as the Avicel standard, including 3434.79 $cm^{-1}$, 1062.86 $cm^{-1}$, 1632.03 $cm^{-1}$, 1431.15 $cm^{-1}$, and 2919.53 $cm^{-1}$. Likewise, that from acid hydrolyzed alginate residues had similar wavelengths to Avicel standards including 3432.24 $cm^{-1}$, 1062.79 $cm^{-1}$, 1633.96 $cm^{-1}$, 1439.64 $cm^{-1}$, and 2919.21 $cm^{-1}$. Therefore, the results of the FT-IR test shows that the functional groups contained in the four MCC samples have similarities with the Avicel PH101 standard which already represents the MCC functional groups.

**X-ray diffraction (XRD)**

XRD analysis was performed to determine the crystallinity index of the MCC samples, and this is a property that shows the bonds between molecular chains that are arranged in an orderly manner. The higher the crystallinity index (Table IV), the more amorphous areas are hydrolyzed by

acids or enzymes into the crystalline phase (Sunardi *et al*., 2019).

The MCC crystallinity index of *S. vulgare* samples ranged from 80.26-91.37%, where that of the alginate residue was lower than the seaweed powder. Furthermore, the acid method had a higher crystallinity index than the enzyme in the seaweed powder sample, while in the alginate residue the enzyme method had a higher crystallinity index than the acid. Thus, the methods does not affect the crystallinity index of MCC.

The XRD test results showed that the MCC crystallinity index of S. Vulgare was higher than the standard Avicel PH101 (59.88%) and the MCC of *P. oseanica* which was 74.23%. (Tarchoun *et al*., 2019), is comparable to that of *Neolitsea latifolia* at 82.17% (Sunardi *et al*., 2019) and *Saccharina japonica* at 88.60% (He *et al*., 2018). The high and low crystallinity index is because MCC still contains amorphous which is more dominant than crystals (Suryadi *et al*., 2017), and this is influenced by the concentration of the solvent used (Sunardi *et al*., 2019). MCC samples that were hydrolyzed by acid or enzymes had a high crystallinity index because the process breaks the glycosidic bonds (Sunardi *et al*., 2019). Therefore, this aims to remove the amorphous phase of cellulose into a crystalline or rigid phase to increase crystallinity (Suryadi *et al*., 2017). In the acid hydrolysis method, hydronium ions from HCl break the glycosidic bonds in the amorphous region (Kale *et al*., 2018), while In the enzyme hydrolysis method, cellulase enzymes from the bacteria *Aspergillus sp*. acts as a catalyst in the MCC hydrolysis process, thereby selectively break the amorphous part. The formation of the crystalline phase is shown on the peaks that appear on the X-ray diffraction graph (Figure 3).

## CONCLUSION

Brown seaweed *Sargassum vulgare* contains cellulose, which has the potential as raw material for MCC. In general, the alginate residue sample type has better characteristic results than seaweed powder. Also, the enzyme hydrolysis produces better characteristics than the acid method. Considering the overall results of the parameters test conducted, the best results were MCC from enzyme hydrolyzed alginate residue.

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## REFERENCES

Anwar, F., Djunaedi, A., & Santosa, G. W. (2013). The Effect of different KOH Concentration Toward the Quality of Alginate from Brown Alga *Sargassum duplicatum* J.G. Agardh. *Journal of Marine Research,* 2(1), 7-14. https://doi.org/10.14710/jmr.v2i1.2049

Arguelles, E. D., Monsalud, R. G., & Sapin, A. B. (2019). Chemical Composition and In Vitro Antioxidant and Antibacgterial Activities of *Sargassum vulgare* C. Agard from Lobo, Batangas, Philippines. *Journal of the International Society for Southeast Asian Agricultural Sciences*, 25(1), 112-122.

Association of Official Analytical Chemistry (AOAC). (2005). Official Method of Analysis of the Association of Official Analytical Chemistry. Washington DS (US): AOAC International. Retrieved August 24, 2021, from https://www.aoac.org/official-methods-of-analysis-21st-edition-2019/

Diharningrum, I. M., & Husni, A. (2018). *Acid and calcium alginate extraction method affected the quality of alginate from brown seaweed Sargasum hystrix J. Agardh. Jurnal Pengolahan Hasil Perikanan Indonesia*. *21*(3), 532–542. https:/doi.org/10.17844/jphpi.v21i3.2473/7

Edison, Diharmi, A., & Sari, E. D. (2019). Characteristic of microcrystaline cellulose from Red Algae *Eucheuma cottonii* | *Jurnal Pengolahan Hasil Perikanan Indonesia*. *22*(3), 483–489. https://doi.org/10.17844/jphpi.v22i3.28946

Effendi, F., Elvia, R., & Amir, H. (2018). Preparation and Characterization of Microcrystaline Cellulose Derived from Baku Tanda Kosong Kelapa Sawit. *Jurnal Pendidikan dan Ilmu Kimia,*2(1),52-57. https://doi.org/10.33369/atp.v2i1.4672

FAO. (2021). Global seaweeds and microalgae production, 1950–2019. World Aquaculture Performance Indicators (WAPI) factsheet. 172 pp.

Gusrianto, P., Zulharmita, Rivai, H. (2011). Preparation and Characterization of Microcrystalline Cellulose from Waste of Sawdust. *Jurnal Sains dan Teknologi Farmasi,* 16(2), 180-188.

He, Q., Wang, Q., Zhou, H., Ren, D., He, Y., Cong, H., & Wu, L. (2018). Highly Crystalline Cellulose from Brown Seaweed *Saccharina japonica*:

Isolation, Characterization and Microcrystallization.*Cellulose*, *25*(10), 5523–5533.https://doi.org/10.1007/s10570-018-1966-1

Husni, A., Subaryono, Pranoto, Y. , Tazwir, Ustadi (2012). The Development of Alginat Extraction Method from Seeweds *Sargassum sp. AS Thijkening Agent*. Agritech 32(1):1-8. https://doi.org/10.22146/agritech.9649

Jumaidin, R., Sapuan, S. M., Jawaid, M., Ishak, M. R., & Sahari, J. (2017). Thermal, Mechanical, and Physical Properties of Seaweed/Sugar Palm Fibre Reinforced Thermoplastic Sugar Palm Starch/Agar Hybrid Composites. *International Journal of Biological Macromolecules*,*97*,606–615. https://doi.org/10.1016/j.ijbiomac.2017.01.079

Kale, R. D., Bansal, P. S., & Gorade, V. G. (2018). Extraction of Microcrystalline Cellulose from Cotton Sliver and Its Comparison with Commercial Microcrystalline Cellulose. *Journal of Polymers and the Environment*, *26*(1),355–364. https://doi.org/10.1007/s10924-017-0936-2

Kamisyah, S., Sapar, A., Brilliantoro, R., & Sayekti, E. (2020). Isolation and Characterization of Alginate from seeweed (*Sargassum polycystum*) isolated from Singkawang West Kalimantan. *Jurnal Kimia Khatulistiwa,* 8(3), 62-71. https://jurnal.untan.ac.id/index.php/jkkmipa/article/view/42395

Liu, Y., Liu, A., Ibrahim, S. A., Yang, H., & Huang, W. (2018). Isolation and characterization of microcrystalline cellulose from pomelo peel. *International Journal of Biological Macromolecules*,*111*,717–721. https://doi.org/10.1016/j.ijbiomac.2018.01.098

Nawangsari, D., Chaerunisaa, A. Y., Abdassah, M., Sriwidodo, S., Rusdiana, T., & Apriyanti, L. (2018). Isolation and Physicochemical Characterization of Microcristalline Cellulose from Ramie (*Boehmeria nivea* L. Gaud) Based on Pharmaceutical Grade Quality. *Indonesian Journal of Pharmaceutical Science and Technology,* 5(2), 55-61. DOI:10.24198/ijpst.v5i2.15040

Nawangsari, D. (2019). Isolation and Characterization of Microcrystaline Cellulose from *Saccharum officinarum* L. waste. *Pharmacon: Jurnal Farmasi Indonesia*, *16*(2), 67–72. https://doi.org/10.23917/pharmacon.v16i2.9150

Pakidi, C. S., & Suwoyo, H. S. (2017). The Use of Active ingredient in Brown Alga *Sargassum sp. Jurnal Ilmu Perikanan,* 6(1), 551-562. https://doi.org/10.26618/octopus.v6i1.708

Prasetia, I. G. N. J. A., Deviana, S., Damayanti, T., Cahyadi, A., & Wirasuta, I. M. A. G. (2018). The Effect of NaOH Concentration in Delignification Process on Microcrystalline Cellulose from Green Algae (*Cladophora* sp.) as the Renewable Marine Product. *Jurnal Farmasi Sains Dan Komunitas (Journal of Pharmaceutical Sciences and Community)*, *15*(2), 68–71. DOI:10.24071/jpsc.1521026

Purwanti, E., & Dampang, S. (2017). The Effect of Different Hydrolysis Condition toward The Yield of Nanocrystaline Cellulose from Zea Mae Waste. *Indonesian Journal of Chemical Research*, 5(1), 12-16. https://doi.org/10.30598//ijcr.2017.5-end

Rahmi, D., Marpaung, M. T., Aulia, R. D., Putri, S. E., Aidha, N. N., & Widjajanti, R. (2020). Extraction and Characterization of Microcellulose from Brown SeaWeed *Sargassum sp* as an an Ingredient for Bioplastic Film. *Jurnal Kimia Dan Kemasan*, *42*(2), 57–65. https://doi.org/10.24817/jkk.v42i2.6401

Rowe, R. C., Sheskey, P. J., Owen, S. C. (2009). Handbook of Pharmaceutical Excipients Sixth Edition. Pharmaceutical Press. London.

Sankari, G., Kriahnamoorhty, E., Jayakumaran, S., Gunaeakaran, S., Priya, V. V., Subramanlam, S., & Mohan, S.K. (2010). Analysis of Serum Immonuglobulins Using Fourier Transform Infrared Spectral Measurements. *Biology and Medicine*, 2(3), 42-48. https://doi.org/10.4172/0974-8369.1000066

Schuh, V., Allard, K., Herrmann, K., Gibis, M., Kohlus, R., & Weiss, J. (2013). Impact of Carboxymethyl Cellulose (CMC) and Microcrystalline Cellulose (MCC) on Functional Characteristics of Emulsified Sausages. *Meat Science*, 93(2), 240–247. https://doi.org/10.1016/j.meatsci.2012.08.025

Sunardi, Lestari, A., Junaidi, A.B., Istikowati, W.T. (2019). Isolation of Microcrystalline Cellulose from Medang Wood *(Neolitsea*

*latifolia)*. *Konversi,* 8(2), 7-14. https://doi.org/10.20527/k.v8i2.6881

Suryadi, H., Sari, H. R., & Rosikhoh, D. (2017). Preparation of Microcrystalline Ccellulose from Water Hyacinth Powder by Enzymatic Hydrolysis Using Cellulase of Local Isolate. *Journal of Young Pharmacists*, *9*(1), S19–S23. https://doi.org/10.5530/jyp.2017.1s.6

Tarchoun, A. F., Trache, D., & Klapötke, T. M. (2019). Microcrystalline Cellulose from *Posidonia oceanica* Brown Algae: Extraction and Characterization. *International Journal of Biological Macromolecules*, *138*, 837–845. https://doi.org/10.1016/j.ijbiomac.2019.07.176

Vijay, K., Balasundari, S., Jeyashakila, R., Velayathum, P., Masilan, K., & Reshma, R. (2017). Proximate and Mineral Composition of Brown Seaweed from Gulf of Mannar. *International Journal of Fisheries and Aquatic Studies*. 5(5), 106-112.