

Organic and Inorganic Fillers' Role on the Amelioration of *Kappaphycus* spp.-based Biopolymer Films' Performance

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The potential of organic and inorganic fillers at different loadings (ranging from 1% to 10%) was evaluated as a means to enhance the performance of *Kappaphycus* spp.-derived biopolymer films. The morphological properties of organic and inorganic fillers were characterized by scanning electron microscopy (SEM). The organic fillers displayed an irregular rod shape with sizes from 60 μm to 80 μm , while the inorganic fillers displayed spherical shapes with a much smaller size range (0.08 μm to 0.25 μm). The contact angle (CA) and mechanical properties of the fabricated biopolymer films were remarkably enhanced compared to the neat biopolymer films due to the incorporation of organic and inorganic fillers. The highest tensile strength (TS), CA, and water barrier properties were attained by biopolymer films that incorporated 5% organic and 1% inorganic fillers. Up to now, there has been no such report on the role of organic and inorganic fillers on tuning the performance of the *Kappaphycus* spp.-based biopolymer films. This work demonstrated that the biopolymer films containing inorganic fillers promoted better physical-mechanical performance compared to the biopolymer films with organic fillers, achieving desirable properties for various packaging applications.

Keywords: Biopolymer films; *Kappaphycus* spp.; Polymer composite films; Microcrystalline cellulose; Calcium carbonate; Packaging material

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INTRODUCTION

Marine resources have attracted research interest in recent years due to their advantages in reducing land use, pesticides, and fertilizers compared to terrestrial plants, which require a long process before the land can be used again for the next plantation (Jumaidin *et al.* 2017). Seaweed, a wild, primitive plant that has been considered to be nothing more than weeds in the ocean in the past decades is now becoming one of the most attractive sources of biopolymer. It has been used extensively in South Asian countries for food, animal feeds, and fertilizer (Diyana *et al.* 2015). Research works have sought to exploit seaweed and have discovered its intrinsic values based on its growth, yield, chemical, and physical properties.

Seaweed is a fast-growing plant that can be cultivated either at a small scale or a large scale. It yields production over a shorter time duration compared to terrestrial plants. It contains good nutritional content, such as carbohydrates, fibers, minerals, vitamins, and even antioxidant properties, which makes it one of the best candidates to replace conventional plastic (Diyana *et al.* 2015).

In Malaysia, especially in Sabah, *Kappaphycus alvarezii* is one of the most popular red seaweeds that has been cultivated at a large scale for food, health beverages, cosmetics, and pharmaceutical applications (Phang 2006; Hussin and Khoso 2017). It has been commercialized worldwide mainly due to its hydrocolloid carrageenan, as a thickener and gelling agent (Jumaidin *et al.* 2017). Moreover, carrageenan has been used widely as the base-matrix in biofilms due to its excellent gel-forming properties, which are attributed to the presence of D-galactose-4-sulphate and 3,6-anhydro-D-galactose (Abdul Khalil *et al.* 2018). Instead of using carrageenan as the base matrix, raw seaweed can be used to fabricate film to shorten the time of film fabrication by reducing the process of extracting seaweed hydrocolloid (Siah *et al.* 2015; Abdul Khalil *et al.* 2018).

Because raw seaweed polymer could be an alternative resource to glass fibre and petroleum-based plastic in the future, studies have used raw seaweed as a base matrix for various applications such as packaging, plasticulture, *etc.* However, the main concern for either raw seaweed-based film or carrageenan-based film is still its hydrophilic characteristic, which reduces the mechanical and barrier properties of the films and consequently reduces its potential applications (Abdul Khalil *et al.* 2018). For this reason, the incorporation of fillers or modification is necessary to reduce its hydrophilicity while enhancing its mechanical and barrier properties (Hasan *et al.* 2019).

The incorporation of fillers is one of the widely used approaches to enhance film properties, especially the physical and mechanical properties, due to its feasibility and cost effectiveness (Abdul Khalil *et al.* 2019). Previous studies have reported on the possibility in reinforcing polysaccharide-based matrices by incorporating with either organic or inorganic fillers. Common organic fillers, including microcrystalline cellulose (MCC), nanocrystalline cellulose (NCC), nanofibrillated cellulose (NCF), and inorganic fillers including calcium carbonate (CaCO₃), nanosilver particles (AgNPs), zinc oxide (ZnO), and montmorillonite (MMT), have been used to improve polysaccharide-based film properties (Abdul Khalil *et al.* 2019). Each type of filler has its own unique feature and characteristic that contribute to the composite system to enhance film properties.

This study highlighted the effect of organic fillers (bamboo microcrystalline cellulose, B-MCC) obtained from a fast-growing bamboo species, *Semantan* bamboo, and inorganic fillers microbially induced calcium carbonate (M-CaCO₃) obtained from microbially-induced calcite precipitation (MICP) technique, on the physical-mechanical performance of the seaweed-based biopolymer films. Previous studies have focused on the effect of an individual type of filler, either organic or inorganic, on the properties of polysaccharide-based films (Zarina and Ahmad 2015; Swain *et al.* 2017). However, research that compares the effect of organic and inorganic fillers on the properties of polysaccharide-based film is still lacking. Therefore, the current study intended to compare the physical (contact angle, water vapour permeability), mechanical (tensile strength and elongation at break), and morphological performances of seaweed-based biopolymer films incorporated with organic fillers and inorganic fillers for potential packaging material applications.

EXPERIMENTAL

Materials

Edible red seaweed (*Kappaphycus alvarezii*) was used for film fabrication was purchased from Green Leaf Synergy Sdn. Bhd. in Tawau, Sabah, Malaysia. Organic fillers derived from *Semantan* bamboo (*Gigantochloa scortechinii*) and inorganic fillers induced by the bacteria agent *Bacillus sphaericus* LMG 22557 (Belgian Coordinated Collection of Microorganisms, Ghent, Belgium) were used as fillers in seaweed-based biopolymer films. Analytical grade glycerol was used as the plasticizer (Ajax Chemicals Pty. Ltd., Sydney, Australia).

Methods

B-MCC extraction

The B-MCC was extracted from *Semantan* bamboo *via* pulping, bleaching, isolation of cellulose, and acid hydrolysis techniques. Approximately 500 g of bamboo chips with the size of 3 cm × 2 cm were prepared, followed by a pulping process in which 23% of sodium hydroxide (NaOH) was used as pulping liquor and 0.1% of anthraquinone was used as the catalyst at the ratio of 1:7. The bamboo chips were pulped at 160 °C for 2 h (Hasan *et al.* 2019). After that, bamboo pulps were bleached according to the method used by Suvachitanont and Ratanapan (2011). Initially, 3g of sodium chlorite and 10% of acetic acid were added to the bamboo pulps and cooked in a water bath for 2 hours at 85 °C with constant agitation (140 rpm). Later, the bleached white pulp was further treated with 17.5% NaOH for 1 h based on the method used by Pachuau *et al.* (2014) to obtain high purity cellulose. To obtain the B-MCC, bamboo cellulose underwent an acid hydrolysis process using 2.5 N of hydrochloric acid (HCl) at 100 °C for 30 min with constant agitation. The liquor ratio was 1:25 according to the method described by Chuayjuljit *et al.* (2010).

Microbially-induced calcium carbonate precipitation

Bacillus sphaericus LMG 22557 was the bacterial agent used to produce calcium carbonate. Inoculum preparation, fermentation of synthetic urea, and precipitation were the main three steps of obtaining calcium carbonate precipitates. Inoculums were prepared according to the method described by Azmi *et al.* (2019). Bacteria were sub-cultured into a new growth medium of 10% inoculum and incubated for 24 h at 30 °C and 100 rpm. Fermentation started when 720 mL of urea growth medium was inoculated with 80 mL of *B. sphaericus* inoculums in a 2-L shaker flask and incubated at 30 °C and 100 rpm. Fermentation stopped when stationary growth phase was achieved. After that, the cells were harvested and removed by centrifugation (Kubota, Tokyo, Japan) at 6000 rpm for 10 min. Precipitation of calcium carbonate took place when calcium salt, calcium nitrate, and Ca(NO₃)₂·4H₂O (Bendosen Laboratory Chemicals, Bendosen, Norway) was added according to the same concentration of bicarbonate acids. The solution was then mixed and allowed to settle before being filtered with medium-flow filter paper (Whatman, Buckinghamshire, England). The precipitates were then washed with distilled water and oven-dried at 70 °C for 24 h according to the method described by Azmi *et al.* (2019).

Films fabrication

The solution casting technique was employed to fabricate seaweed (SW) composite films. An amount of 4 g of clean raw seaweed was blended together with 2 g of glycerol.

Different organic and inorganic filler loadings were added at 1%, 5%, and 10%, respectively, based on the dry weight of the seaweed (wt%). The solution was then heated to 90 °C with constant agitation for 1 h to obtain a homogenous solution. After that, the solution was poured into a casting tray and placed in a ventilated oven at 40 °C to dry overnight. Dried films were peeled off and further conditioned at 50% relative humidity prior to film testing to maintain the integrity of film for further analysis.

Film thickness

Film thickness was measured to the nearest 0.001 mm using a precision digital micrometer (Mitutoyo, Kanagawa, Japan) at 10 random locations on every single film, and the corresponding average values were used in the water vapour permeability and tensile tests. The average thickness of the films for this study ranged from 0.068 mm to 0.080 mm.

Contact angle (CA)

The CA was determined using a contact angle analyser (KSV CAM 101; KSV Instruments Ltd., Helsinki, Finland) under ambient temperature. Approximately 6 µL of water drop was released on the surfaces of the films using a syringe. The CA between the baseline and the tangent at the drop boundary was measured and averaged. At least three measurements were taken to determine the average.

Water vapour permeability (WVP)

The WVP of the films was determined and calculated according to the wet cup method of the standard ASTM E-96 (1996). The test cup was filled with distilled water before the circular film samples were sealed over the mouth of the test cup. The diameter of the cup was 7.40 cm. The initial weight was measured before the cup was placed in a humidity chamber. The controlled humidity chamber was subjected to 25 °C and 50% relative humidity for 6 h. The change in the weight of the cup was recorded every hour.

Mechanical properties of seaweed composite films

The tensile strength (TS) and percentage of elongation at break (% EAB) were determined according to the ASTM D882-02 (2002) standard with slight modification. The films were cut into strips of 10 mm × 150 mm width with three replicates for each film. The films were tested using the Dia-Stron Miniature Tensile Tester (Dia-Stron Instruments, Hampshire, UK) equipped with a 5 kg load cell. The initial grip separation was at 100 mm and crosshead speed was fixed at 50 mm/min. The TS (MPa) and % EAB were calculated using Eqs. 1 and 2, where F is the maximum force (N) at rupture, A is the film cross-section (thickness × width, mm), D is the elongation at break (%), and I is the initial grip separation (mm):

$$\text{Tensile strength (MPa)} = \frac{F}{A} \quad (1)$$

$$\text{Elongation at break (\%)} = \frac{D}{I} \times 100 \quad (2)$$

Fourier transform infrared (FTIR) analysis of seaweed composite films

Seaweed+ B-MCC and seaweed+ M-CaCO₃ composite films were characterized using FTIR (Perkin-Elmer, PC1600, USA) within the wavelength range 400 to 4000 cm⁻¹. Prior to the FTIR analysis, film samples were cut into square shape (3 x 3 cm) and dried in the oven at 60 °C for 24 h.

Morphological properties of seaweed composite films and fillers

Prior to observing the surface morphology, the films were cut into 1 cm × 1 cm and oven-dried at 60 °C overnight. The dried films were then mounted on double-sided Scotch tape and further coated with thin layer of gold (Au) (Sputter coater SCD 005; Baltec Inc., Oberkochen, Germany). The surface morphology of the films was observed under a scanning electron microscope (SEM) (EVO MA 10; Carl-ZEISS SMT, Oberkochen, Germany). The surface morphology of organic and inorganic fillers was also determined using SEM and their length sizes were measured using Image J software (LOCI, J1.51k, Madison, WI, USA).

RESULTS AND DISCUSSION

Characterization of B-MCC and M-CaCO₃ Particles

The yield of MCC (B-MCC) obtained from *Gigantochloa scortechinii* bamboo was 82.58% ± 1.03%. The yield was lower compared to the MCC obtained from *Melocanna baccifera* (92.5%) and *Dendrocalamus longispatus* (92%) bamboo as reported by Pachuau *et al.* (2013, 2014) but higher than the reported yields for *Luffa cylindrica* (60.69%), jute (48%), orange mesocarp (25.3%), *Bambusa vulgaris* bamboo (25%), and *Cochlospermum planchonii* (67%) (Ohwoavworhwa and Adelakun 2005; Jahan *et al.* 2011; Umeh *et al.* 2014; Macuja *et al.* 2015). The differences in MCC yield were attributed to the different source of raw materials and their method of MCC production.

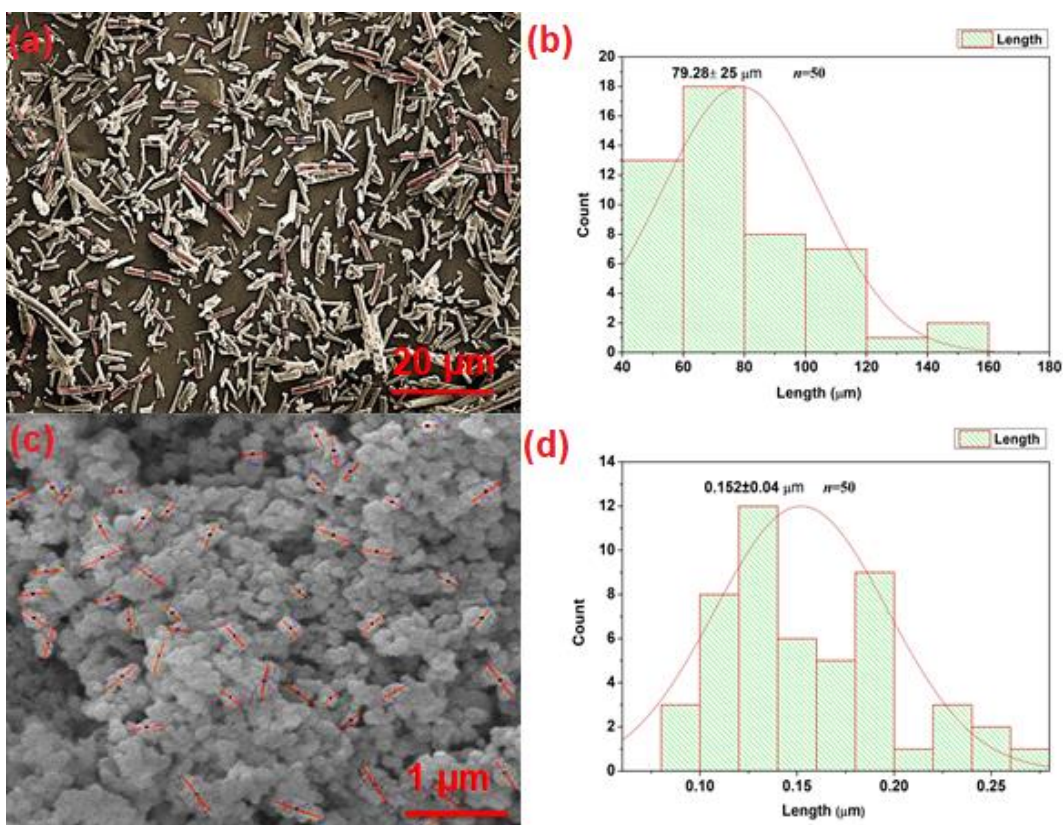


Fig. 1. (a) and (b): Morphological properties and sizes of B-MCC, and (c) and (d): morphological properties and sizes of M-CaCO₃

Meanwhile, the microbially induced calcium carbonate (M-CaCO₃) was precipitated by the *Bacillus sphaericus* LMG 22557 bacteria strain, and its specific yield productivity was 0.28 mol/g cell/h. The crystallinity of B-MCC and M-CaCO₃ was 75.0% and 82.0%, respectively. The higher crystallinity of M-CaCO₃ than B-MCC likely promoted better improvement in the mechanical properties of seaweed films.

The morphology of B-MCC and M-CaCO₃ are shown in Fig. 1(a), (b), (c), and (d). The B-MCC displayed a morphological pattern of irregular rod shapes with rough surfaces. Figure 1(b) shows the histogram of B-MCC length counts, using Image J software. The graph shows that most of the B-MCC ranged from 60 μm to 80 μm, with a mean value of 79.28 ± 2.5 μm. The lowest amount of B-MCC size distribution was between 120 μm to 140 μm. Nevertheless, M-CaCO₃ was spherical in shape and smaller in size compared to B-MCC. Figure 1(d) shows that the size distribution of M-CaCO₃ ranged from 0.080 μm to 0.250 μm, with a mean value of 0.152 ± 0.04 μm. However, the results were slightly different compared to previous studies that found a range of 0.066 μm to 0.109 μm (Abdul Khalil *et al.* 2018). Overall, B-MCC was larger in size than M-CaCO₃ due to the differences of raw materials and method of production.

Morphological Properties of Seaweed Composite Films

The morphological properties of seaweed + B-MCC and seaweed + M-CaCO₃ composite films were investigated using SEM. Figure 2(g) shows the surface of neat seaweed films (NE-SW), which had a smooth and homogeneous surface, indicating that the seaweed solution was well dispersed in the aqueous media. However, with the addition of B-MCC from 1% to 10%, the seaweed (SW) film surface became rough and uneven. As shown in Fig. 2(a), (c), and (e), B-MCC were clearly embedded in the seaweed matrix, as filler loading increased from 1% to 10% as denoted by the circle and arrow. At the highest loading, B-MCC was observed to agglomerate in the SW films. Similar morphological patterns were also reported by Shankar and Rhim (2016) with the addition of MCC particles into the agar film.

The morphological surfaces of SW + M-CaCO₃ composite films were different compared to the B-MCC + SW composite films. The films remained smooth and compact even with the addition of M-CaCO₃ particles up to 10%. There were no morphological changes observed in the SW films reinforced with 1%, 5%, and 10% M-CaCO₃ particles. This could probably have been due to the small size of M-CaCO₃ particles (0.080 μm to 0.250 μm) as shown in Fig. 1(c) and Fig. 1(d). However, tiny M-CaCO₃ particles that were embedded in the SW matrix could still be denoted by the circle and arrow as shown in Fig. 2(b), (d), and (f). Overall, B-MCC and M-CaCO₃ displayed good compatibility with the SW matrix without the formation of cracks and voids due to homogenous dispersion within the films.

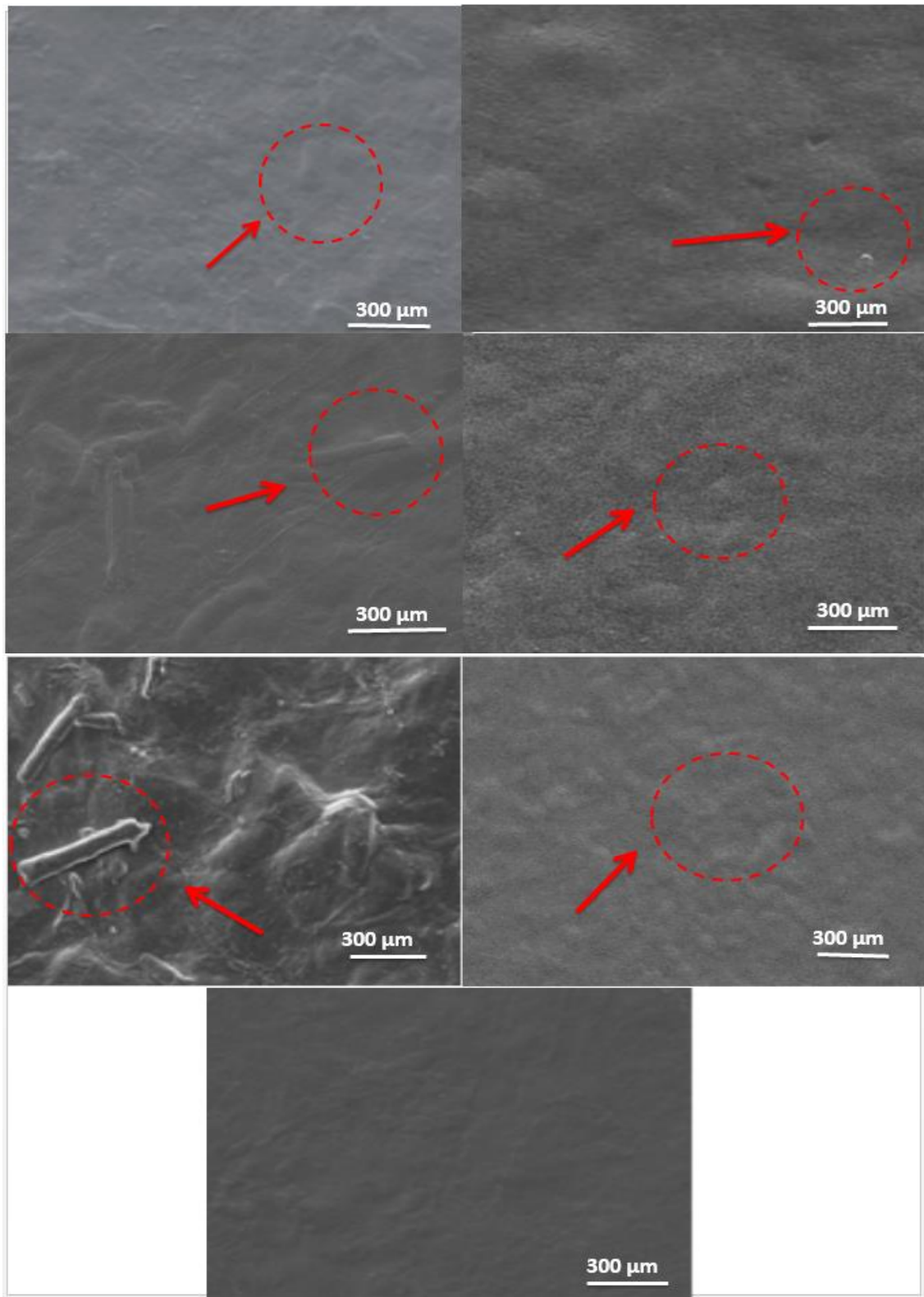


Fig. 2. SEM images of (a) SW + 1% B-MCC, (b) SW + 1% M-CaCO₃, (c) SW + 5% B-MCC, (d) SW + 5% M-CaCO₃, (e) SW + 10% B-MCC, (f) SW + 10% M-CaCO₃, and (g) neat seaweed films (NE-SW)

Mechanical Properties of Seaweed Composite Films

Mechanical properties are an important factor in determining film performance, especially in packaging. The effect of B-MCC and M-CaCO₃ particles on the TS and EAB of SW composite films at different filler loadings are shown in Figs. 3(a) and (b). The TS and EAB of NE-SW films were 20.0 MPa and 18.4%, respectively. The TS of SW films reinforced with 1% B-MCC, 5% B-MCC, and 10% B-MCC were 25.8, 41.9, and 30.2 MPa, respectively, with the highest TS observed at 5% B-MCC loading.

The enhancement of TS of SW composite films upon addition of B-MCC particles was likely due to the good compatibility between the B-MCC and SW matrix and the good dispersion of B-MCC particles in SW due to the strong intermolecular hydrogen bonding between B-MCC and seaweed (Hasan *et al.* 2019; Hermawan *et al.* 2019). The good compatibility and homogeneous dispersion of the filler and matrix resulted in the efficient load transfer from the SW to the B-MCC particles network.

In contrast, the EAB of SW films reinforced with 1% and 5% B-MCC particles showed a decreasing trend with values of 17.2% and 13.4%, respectively. The lowest EAB value was observed at 5% B-MCC loading. Meanwhile, the EAB of SW composite film was 16.6% upon the addition of 10% B-MCC particles. The incorporation of high crystallinity B-MCC particles (75.0%) could have restricted the flexibility of the SW polymer, causing the slight reduction of EAB values. The EAB of SW+ 10% B-MCC composite film was higher compared to SW+5% B-MCC composite film. This was probably due to the agglomeration of B-MCC particles in seaweed matrix as shown in Fig. 2(e). Similar findings have been reported by Zarina and Ahmad (2015) with the incorporation of cellulose nanocrystals (CNC) into the commercial kappa-carrageenan films.

For M-CaCO₃, incorporation of 1% M-CaCO₃ caused TS enhancements to seaweed composite film (43.8 MPa), which was 23.8% higher than NE-SW films (20.0 MPa) and 18.0% higher than SW + B-MCC composite films at the same loading.

The M-CaCO₃ particles showed a higher mechanical reinforcing effect on the SW composite films compared to B-MCC particles, probably due to the high crystallinity index of M-CaCO₃ particles (82%). According to Nasution *et al.* (2017), higher crystallinity of the filler is more efficient in achieving a higher reinforcement effect for composite materials. However, at 5% and 10% M-CaCO₃ loading, the TS of SW composite films tended to decrease to 40.7 MPa and 36.8 MPa, respectively. This could have been due to the agglomeration of M-CaCO₃ in SW films due to its high surface area (Fig. 1c). Similar results were reported by Sun *et al.* (2014) with the addition of CaCO₃ into the starch-based films. They revealed that the TS of starch films was significantly improved due to the homogeneous dispersion of CaCO₃ particles in the starch matrix.

In contrast, the EAB of seaweed composite films was reduced to 17.2% with the addition of 1% M-CaCO₃ particles. The rigidity and high crystallinity of M-CaCO₃ particles could have been the factor that restricted the flexibility of the seaweed polymer chain. These results slightly contradicted the results reported by Sun *et al.* (2014), who reported that the EAB of starch films increased significantly instead of decreasing upon the addition of 0.02% to 0.50% of CaCO₃. The differences could have been due to the particle sizes of their CaCO₃ particles ($0.035 \pm 2\mu\text{m}$) compared to the M-CaCO₃ particles (approximately 0.080 μm to 0.250 μm) used in this work. The differences could also have been due to the different material used for the base matrix and the method of film fabrication applied in both studies.

In this work, as filler loading increased from 5% to 10%, the EAB of the SW composite films increased up to 18.4% and 19.1%, corresponding to the decrease of their TS. The agglomeration of M-CaCO₃ at 5% to 10% loadings could have been the main cause that interrupted the bonding of seaweed and M-CaCO₃. The poor filler-matrix adhesion at the interfaces due to the agglomeration of M-CaCO₃ particles might seriously weaken the seaweed composite films because M-CaCO₃ particles no longer act as reinforcement but as defects in the matrix. Thus, the EAB of SW+5% and SW+10% M-CaCO₃ composite films tend to increase and their TS decreased, as shown in Figs. 3(a) and (b). As presented in Fig. 3(a) and (b), the optimum loading was attained by SW + 1% M-CaCO₃ and SW + 5%. The B-MCC composite composite films with the highest TS were achieved at 43.76 MPa and 41.87 MPa.

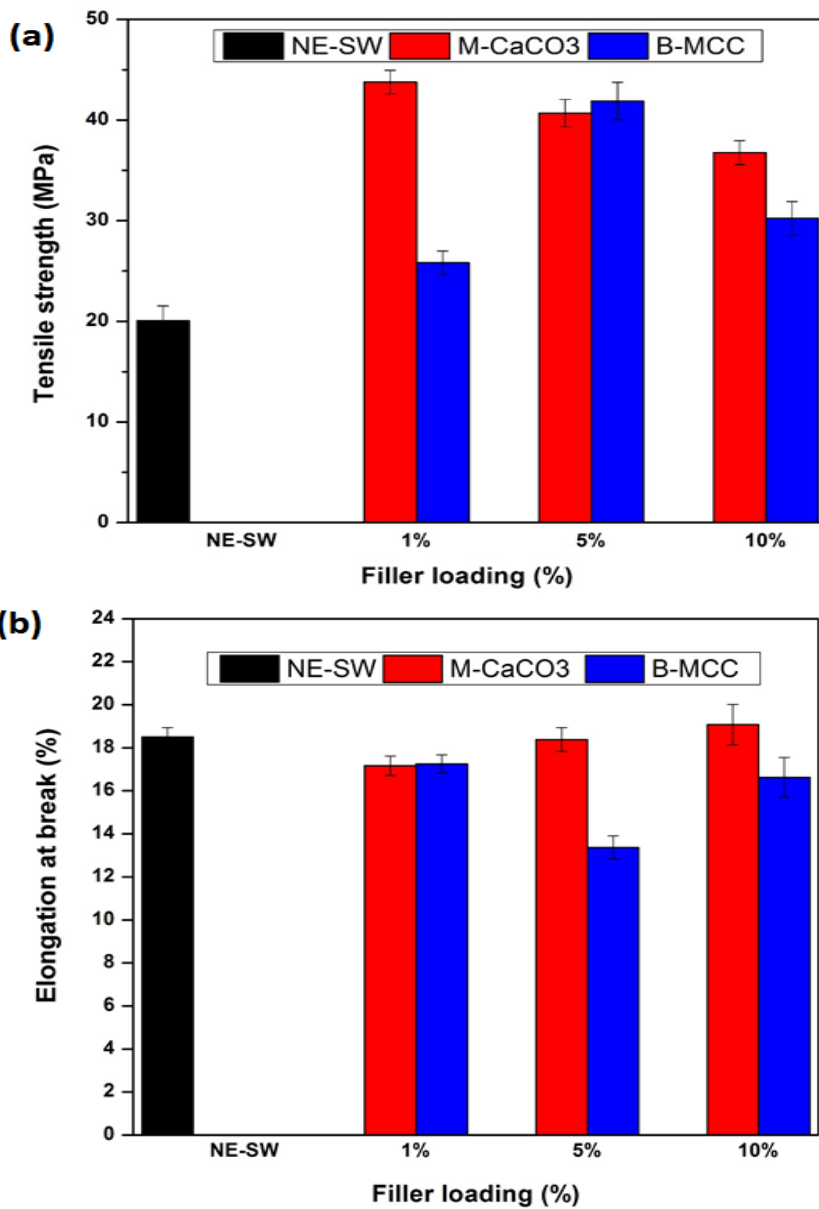


Fig. 3. (a) TS of SW + B-MCC and SW + M-CaCO₃ composite film (b) EAB of SW + B-MCC and SW + M-CaCO₃ composite film

FTIR Characterization of Seaweed Composite Films

Figure 4 presents the FTIR spectra of seaweed+B-MCC and seaweed+CaCO₃ composite films. Overall, the neat seaweed (NE-SW) spectra, seaweed+B-MCC and seaweed+CaCO₃ showed a relatively similar pattern of bands. This indicated that there were good miscibility between organic and inorganic particles with the seaweed matrices. However, there were slight changes in the shifting of wavenumber (cm⁻¹) for certain functional groups (-OH, C-O, S=O). The peak that appeared at 3323 cm⁻¹ for NE-SW could be attributed to the presence of hydroxyl group (-OH). However, the peak shifted to higher wavenumber 3331 cm⁻¹ after incorporated with B-MCC particles, indicating good interactions between hydroxyl group of seaweed and B-MCC particles. On the other hand, the wavenumber of seaweed+ M-CaCO₃ composite films shifted to 3327 cm⁻¹ with addition of M-CaCO₃ particles. According to Swain *et al.* (2017), this was due to the occurrence of intermolecular hydrogen bonding of the hydroxyl (-OH) group in seaweed and M-CaCO₃ particles. In this study, all seaweed composite films exhibited peaks at wavenumber 1217 to 1219 cm⁻¹, which was probably due to stretching of the sulphate ester group (S=O) of kappa carrageenan in seaweed. Another strong peak appearing at wavenumber 1031 to 1032 cm⁻¹, as shown in Fig. 4, was assigned to the glycosidic linkage (C-O) of 3,6-anhydro-D-galactose, which typically refers to the presence of carrageenan in seaweed composite films. Finally, the peak at wavenumber 845 cm⁻¹ was correspond to D-galactose-4-sulphate due to the stretching vibration of C-O-C found in seaweed.

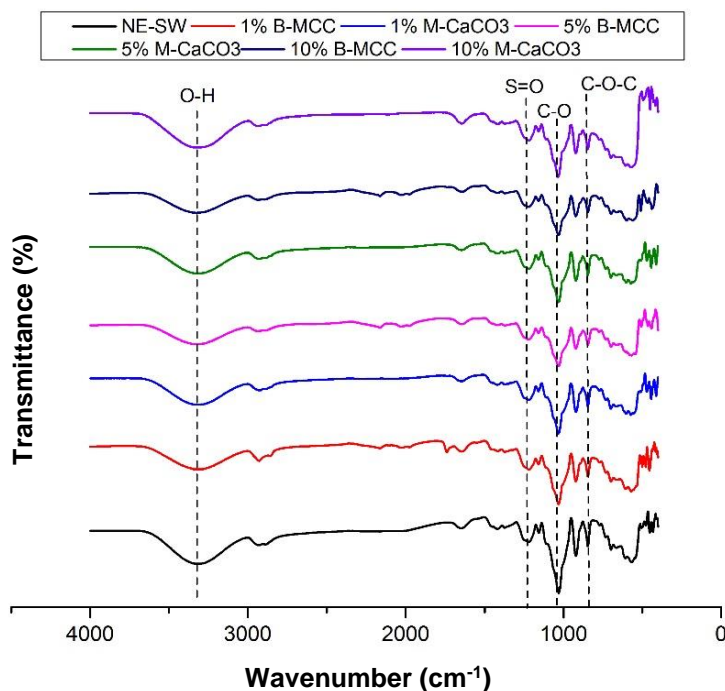


Fig. 4. FTIR spectra of seaweed+B-MCC and seaweed+M-CaCO₃ composite films

CA of Seaweed Composite Films

In this study, the CA showed the wettability of the water on a solid surface of the thin film. The contact angles (°) of the SW film incorporated with different types of fillers, including B-MCC and M-CaCO₃, at different filler loadings are presented in Fig. 5.

It was noticed that the highest CA of 75.9° was achieved by the SW film incorporated with M-CaCO₃ at 1% loading. A film with a contact angle higher than 90° is normally considered hydrophobic (Yuan and Lee 2013). In this case, the results showed that all of the films were still considered hydrophilic. However, the hydrophilicity was successfully reduced as the SW film was incorporated with either organic or inorganic fillers (B-MCC or M-CaCO₃). The CA was also observed to increase with the increase of filler loading up to an optimum loading, and thereafter it decreased after the optimum loading. The optimum loading for B-MCC and M-CaCO₃ was noted to be at 5% and 1%, respectively. For seaweed films incorporated with B-MCC, the increase of CA up to the optimum loading could have been due to the interaction between the hydroxyl groups of B-MCC and hydroxyl groups of carrageenan in seaweed, which hinders the binding of the available hydroxyl groups with the moisture. While for seaweed films incorporated with M-CaCO₃, the increase of CA was due to the hydrogen bonds that formed between the carboxyl groups of M-CaCO₃ and the hydroxyl groups of carrageenan from the seaweed. Similar cases were found from previous studies when excessive filler content was loaded (Suvachitanont and Ratanapan 2011; Shankar *et al.* 2016). This result was also in agreement with the WVP of the films shown in Fig. 6.

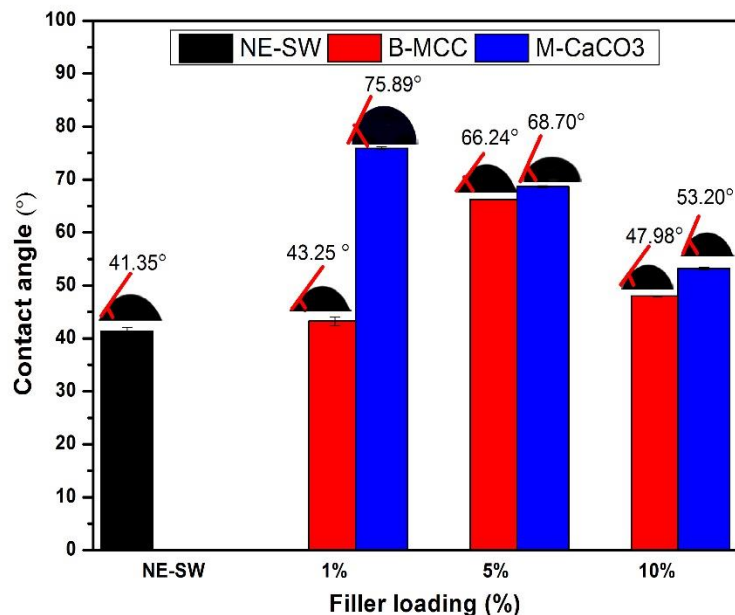


Fig. 5. Comparison of CA of seaweed-based film incorporated with B-MCC and M-CaCO₃ at different filler loadings

WVP of Seaweed Composite Films

The WVP was dependent on the diffusibility of water vapour across the film. Lower WVP values corresponded with better water barriers in the films. From Fig. 6, the WVP values of SW films incorporated with B-MCC and M-CaCO₃ particles were lower than the NE-SW film.

The results shown in Fig. 6 verified that the incorporation of fillers successfully enhanced the water barrier properties of the SW films. Abdul Khalil *et al.* (2019) reviewed

that the incorporation of organic and inorganic fillers, such as MCC, NCC, MMT, and AgNPs, has the ability to enhance film water barriers due to the formation of a tortuous pathway, which may vary between different composite systems. In this study, the lowest WVP was attained by SW films incorporated with 5% B-MCC and 1% M-CaCO₃. As the filler loading increased, WVP values decreased up to the optimum loading of 5% for SW films incorporated with B-MCC and 1% for SW films incorporated with M-CaCO₃.

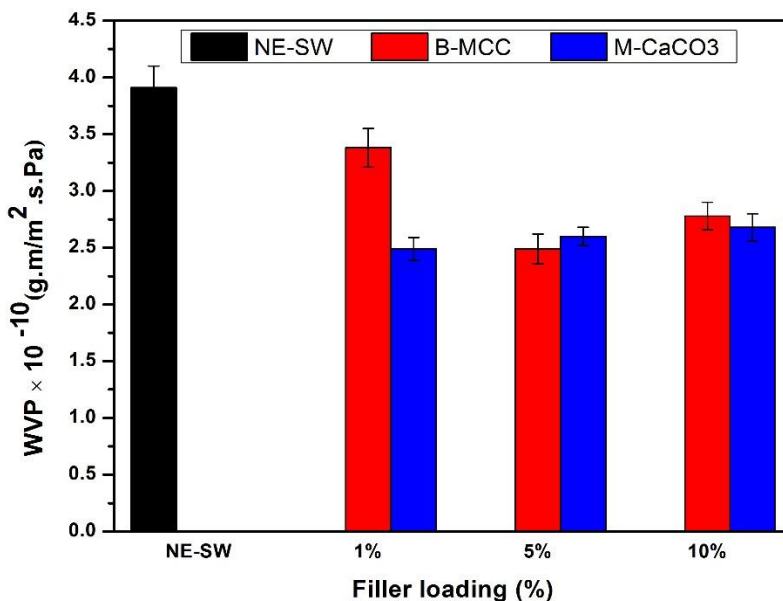


Fig. 6. Water vapour permeability of seaweed-based films incorporated with B-MCC and M-CaCO₃ at different loadings

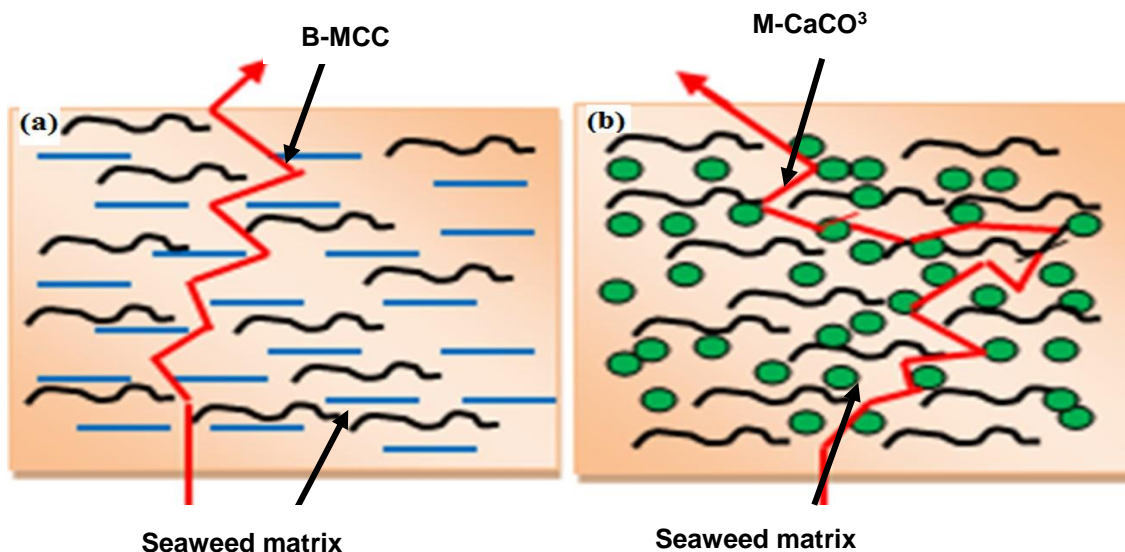


Fig. 7. Schematic diagram of tortuous pathway formed by seaweed-based film incorporated with a) B-MCC and b) M-CaCO₃

As the filler loading increased from 1% to the optimum loading, the water barrier improved as more tortuous pathways were formed. However, WVP increased as fillers were loaded more than the optimum loading. This behaviour could have been due to the agglomeration of fillers within the matrix that caused more voids for the moisture to pass through the pathway. Figure 6 also shows that SW films incorporated with B-MCC had higher values of WVP compared to those incorporated with M-CaCO₃. This indicated that SW films incorporated with B-MCC promoted lower water barrier properties compared to SW films incorporated with M-CaCO₃. This reduction could have been due to the larger size of B-MCC compared to M-CaCO₃ particles, which resulted in the reduction of lesser tortuous pathways, allowing more frequent penetration of water vapour across the films. Besides the size, the different shape of B-MCC and M-CaCO₃ may also affect the penetration of water vapour. The spherical shape of M-CaCO₃ particles within the SW matrix could have created more tortuous pathways, creating longer distances for water vapour to migrate across the films from the environment, compared to the rod-like B-MCC particles as illustrated in the schematic diagram in Fig. 7(a) and (b).

CONCLUSIONS

1. Microcrystalline cellulose produced from *Gigantochloa scortechinii* bamboo and CaCO₃ produced from the MICP method using *Bacillus sphaericus* were successfully reinforced into SW-based films at different loading concentrations (0%, 1%, 5%, and 10%) via the solvent casting technique.
2. A satisfactory enhancement in mechanical properties and water barrier properties of SW films was obtained mainly due to the good dispersion of B-MCC and M-CaCO₃ particles within the SW matrix.
3. The incorporation of B-MCC and M-CaCO₃ particles into the SW matrix resulted in the enhancement of CA, especially at 5% B-MCC and 1% M-CaCO₃, which were

66.24° and 75.89°, respectively. This indicated that the incorporation of B-MCC and M-CaCO₃ particles was able to reduce the hydrophilicity of the SW film to some extent.

4. The WVP of the SW composite film was reduced due to the homogenous dispersion and the formation of a tortuous path by B-MCC and M-CaCO₃ particles, which hindered the permeability of water vapor *via* the films. The mechanical properties of SW composite films were enhanced with the incorporation of B-MCC and M-CaCO₃ particles.
5. The optimum TS was attained by SW films incorporated with 5% B-MCC and 1% M-CaCO₃ particles, which was 41.9 and 43.8 MPa, respectively. The M-CaCO₃ demonstrated a higher mechanical reinforcement effect in SW + M-CaCO₃ films compared to SW + B-MCC composite films. It was noted that the crystallinity index of M-CaCO₃ was 82%, compared to 75.0% in the case of B-MCC.
6. Overall, the incorporation of B-MCC and M-CaCO₃ demonstrated remarkable improvements in the mechanical and physical properties on SW composite films. Thus, SW-based composite films incorporated with B-MCC and M-CaCO₃ have the potential to be used as bio-based packaging material for food and agricultural applications.

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