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The Novelty Nethod for Biofilm Made from the Gel of *Kappaphycus Alvarezii* in Vietnam

Ha-Anh Thi Huynh, Thanh Thi Ngoc Lan Mai*

Thu Dau Mot University, 6 Tran Van On, Phu Loi District, Ho Chi Minh City
thanhmtnl@tdmu.edu.vn

With the growing awareness of plastic pollution, the need for sustainable alternatives has been more urgent. Bioplastics and biodegradable plastics present promising solutions that can help us reduce our environmental impact. Embracing these innovative materials is a vital step toward a cleaner, greener future. Vietnam is a country that uses packaging such as nylon bags and plastic products in large quantities. The use of plastic packaging puts a lot of pressure on environmental protection. Therefore, packaging made from natural and biodegradable materials is the current trend. Biodegradable materials used to develop bio-packaging are cellulose, pectin, chitosan, starch and protein extracted from plant and animal sources. Among them, seaweed is one of the potential biopolymers in biofilm applications. Gelling agents found in seaweed are mostly carrageenan, ager, alginate, these substances have the ability to form good biodegradable films and have properties that help them to be commercially applied as additives used in the food industry, as gelling agents, emulsifiers, stabilizers and thickeners, but they are gradually recognized as valuable materials, so it is necessary to strengthen basic research to discover new functions, make the most of its potential, especially in the production of biodegradable films. Biofilm made from gelling agent is one of the potential applications for creating biofilm with antibacterial properties to help preserve food, while also helping to reduce waste polluting the environment with biodegradability. The gelling agent is isolated from *Kappaphycus alvarezii* through the green method, which is the preprocessing by sonication and gelling agent is isolation by the temperature, these results were reported in the National Conference in Vietnam. In this study, biofilm made from gelling agent, which was isolated from *Kappaphycus alvarezii*, is reported first time. The biofilm show that the ability applied packaging and biodegradable materials used to develop bio-packaging.

1. Introduction

Microplastic pollution poses an enormous challenge, so bioplastics and biodegradable plastics have emerged as promising alternatives. Natural materials comprise the biofilm, offering significant advantages in the packaging industry, such as odour reduction and improved food safety. *Kappaphycus alvarezii* is a natural material, has the commercial name Cottonii. The seaweed is green to reddish brown in color, grows upright, 20 – 60 cm high, the main stem and branches are about 1 – 2 cm in diameter. The branches are sparse, the distance between 2 branches is from 4 – 10 cm. Curved, wide, tapering and elongated branches are typical of this species. When growing in areas with good water flow, the seaweed can grow longer than 2 m (Trono JR and Gavino C, 1992). The chemical composition of *Kappaphycus alvarezii* always changes depending on the physiological state, growth time and living conditions (radiation intensity, chemical composition of the environment). In *Kappaphycus alvarezii*, the water content accounts for 77 % -91 %, the remaining few percent is dry matter. Dry matter mainly contains: Glucide, protein, minerals, lipids, pigments, enzymes. Glucides include monosaccharides, disaccharides and polysaccharides. Monosaccharides are usually in the form of galactose combined with glyceric acid to form unstable compounds that can be extracted with high-proof alcohol. Disaccharides are usually in the form of mannose combined with glyceric acid and sodium to form disaccharide compounds accounting for 15 %. Polysaccharide is carrageenan. A complex mixture of at least five polymers: Carrageenan is composed of D-galactose and 3,6- anhydro D-galactose units. These units are linked together by alternating 1,4 and 1,3 bonds. D-galactose residues are highly sulfated. Carrageenans differ in the degree

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of sulfation. The polysaccharide chain of carrageenan has a double helix structure. Each turn of the helix is made up of 3 disaccharide units. The common polysaccharides of carrageenan are kappa, iota, lambra (Amarnath Praphakar R. et al., 2017). Biofilm is a thin layer that ranges in thickness from 0.050 to 0.250 mm and adherent layer created from a bioactive polymer matrix. Biofilms play a crucial role in preserving food quality, and avoidance of flavour compounds are evaporated, act as a barrier against microorganisms, and enhance color or shine. This makes the food more appealing to consumers (Jooyandeh H, 2011). Biofilms are classified based on the origin of the film-forming materials, including: Protein membrane; Synthetic membrane. Carrageenan, a natural polysaccharide derived from red algae, are widely utilized in the creation of biofilms and edible coatings. The effectiveness of these seaweed biofilms can be enhanced by synergy with compounds purified from plant extracts (Nair et al., 2020). Crude extracts from seaweed in the future have been trending to develop edible coating materials. Ramani et al. (2020) prepared seaweed extracts from *Sargassum tenerrimum (*a type of brown algae) and *Kappaphycus alvarezii* (a type of red algae) were used to coat tomatoes. The results determined the antifungal and antibacterial activities of *K. alvarezii*. Tomatoes coated with biofilm exhibited better quality characteristics than both uncoated tomatoes, over a 30-day storage period (Ramani et al., 2020). Biofilm created from the combination of chitosan and starch. Starch films are brittle, the addition of chitosan helps increase the tensile strength and elongation at break, while reducing the water vapor transmission rate of the film. XRD analysis shows that gelatinized starch reduces the crystallinity of chitosan films. FTIR spectrum shows a shift in the absorption band of the amino group in chitosan, demonstrating the interaction and homogeneity at the molecular level between chitosan and starch (Xu et al., 2005). Many methods and materials for film formation have been studied in the world, and seaweed extract has also been applied in agricultural product preservation. The gelling compound of *Kappaphycus alvarezii* were isolated by ultrasonic pretreatment, a green extraction method, and the film formation process from the gel-forming substance have not been previously reported. Based on the chemical composition characteristics of *Kappaphycus alvarezii*, the study "The novelty method for biofilm made from the gel of *Kappaphycus alvarezii* in Vietnam" proposed to proceed.

2. Material and methods

Kappaphycus alvarezii was supplied from Lam Hong Phat Agricultural Products Company in Dong Nai. The gelling agent was separated from *Kappaphycus alvarezii*, which is reported in the before research (Anh et Mai, 2024) and was supplied from Thu Dau Mot University laboratory.

2.1 Investigation of gel forming agent mass on biofilm formation process

The mass of gelling agent was investigated for its ability to affect the biofilm formation process, respectively 0.5; 1.0; 1.5; 2.0; 2.5; 3.0 g then add 150 mL of 20 % glycerol and heat at 70 °C. After the mixture boils, collect the supernatant. Pour the heated mixture into a dish with a diameter of 8.7 cm. The experiments were dried at 60 °C for 24 h, and the film was obtained.

2.2 Testing the load-bearing capacity of biofilms

The biofilm was successfully created, the membrane was fixed in a ring with a diameter of 4 cm, the diameter of the membranes tested for load bearing capacity had an average diameter of 8.7 cm. Two analytical balance calibration blocks with standard weights of 200 g and 300 g, respectively were used to test the load bearing capacity of the membrane. The two balance calibration blocks were placed on the biofilm fixed on the ring and the time the membrane was able to withstand the weight of the two blocks until the membrane broke was recorded.

2.3 Biofilm solubility testing

The membrane was cut into 3 x 3 cm squares and dried to constant mass at 60 °C in a vacuum dryer, weighing the initial mass (wt_0). Then, the membrane was placed in a beaker containing 20 mL of distilled water, shaken well for 24 h at 25 °C. The membrane was then dried under the same conditions, weighing the mass (wt_1) (Guenter Kieckbusch et al., 2012). The solubility of the membrane was calculated according to Eq(1):

$$H\ (\%) = \frac{\mathrm{Wt0} - \mathrm{Wt1}}{\mathrm{Wt0}} . 100\ \% \qquad (1)$$

H (%): Percentage of membrane solubility; wt_0: Initial mass of the film after drying; wt_1: Mass of dried membrane after shaking

2.4 Testing the grape preservation ability of biofilm compared with commercial plastic

The grape preservation ability of biofilms was evaluated based on the change in fruit color. Record the color change of stored grapes at 0 and 7 days.

2.5 Testing the dye removal ability of biofilm

The experiment to determine the dye separation efficiency was conducted by vacuum pump. The pressure-stabilized biofilm of gelling agent from carrageenan determined the dye separation efficiency. The dye separation efficiency is determined by Eq(2):

$$R = \frac{(Cf - Cp)}{Cf} . 100\ \% \qquad (2)$$

C_f: initial dye concentration; C_p: concentration of dye in solution after filtration (Đặng Thị Tố Nữ và cs., 2022).

2.6 Scanning electron microscopy (SEM)

A biofilm created from the gelling agent *Kappaphycus alvarezii* was adhered to a carbon board and placed in a platinum (Pt) coating sprayer. For imaging purposes, a field emission scanning electron microscope (FESEM) S4800 was used to capture a photo of the *Kappaphycus alvarezii* biofilm in the imaging chamber (Hitachi, Japan) (Mai, 2024).
The assay was practised repeatedly three times. The statistical analysis of the results was conducted using STATGRAPHICS Centurion XV (Statgraphics Centurion, 2006).

3. Results and discussion

3.1 Investigation of gel forming agent mass on biofilm formation process

The results of the experiment investigating the effect of gelling agent mass on the biofilm formation process are presented in Figure 1.

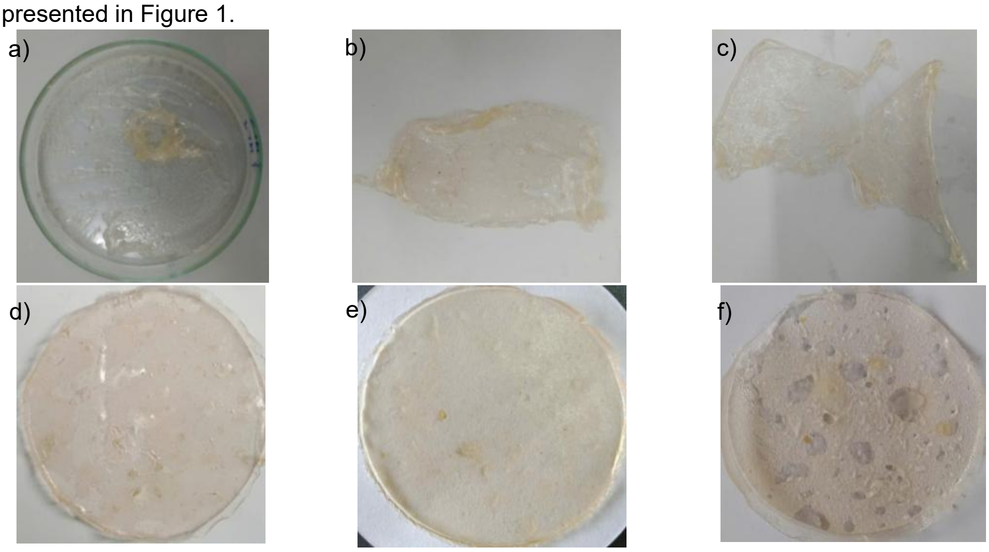


Figure 1: The biofilms were made of (a) 0.5 g of gelling agent; (b) 1.0 g of gelling agent; (c) 1.5 g of gelling agent; (d) 2.0 g of gelling agent; (e) 2.5 g of gelling agent; (f) 3.0 g of gelling agent.

The results of determining the gelling agent mass were 2.0 g; 2.5 g; 3.0 g, respectively, and the biofilm was successfully formed. Continously, a membrane tensile test was designed to determine the optimum gelling agent mass for biofilm formation. The results are presented in Table 1. The results determined that the gelling agent mass of 2.5 g affected the structure formation process of the biofilm, so the biofilm formed had better load-bearing capacity than the two biofilms formed from gelling agent mass of 2.0 g and 3.0 g. Specifically, the biofilm formed from 2.5 g of gelling agent had the ability to withstand weights of 200 g and 300 g at times of 11.0567±0.0801 min and 10.0333±0.1008 min, respectively. In contrast, the biofilms made from gelling agents weighing 2 g and 3 g, the two films have almost the same load-bearing capacity of 3 min on a 200-gram calibrated balance. This result may be due to the fact that the biofilms made from 2.5 g of gelling agents have a tightly bound polymer network that creates the appropriate elasticity for the film to withstand large loads, while the biofilms made from 2.0 g of gelling agents have a flexible structure due to flexible but loose polymer bonds, so the film is easy to break, while the biofilms made from 3 g of gelling agents have a higher concentration of polymer, so the hard film does not have elasticity.

Table 1: Biofilm weight bearing results of balance calibration

balance calibration mass	Gelling agent mass (g)		
	2.0	2.5	3.0
200 g	3.8500^{b}±0.0356	11.0567^{a}±0.0801	3.8100^{b}±0.0829
300 g	X	10.0333±0.1008	X

Letters corresponding to different values are statistically significant; values are expressed as mean ± SD (n=3)
Compared with the study of Siti Hajar Othman et al. (2019), in this study, the cassava starch film combined with cellulose was successfully created with a tensile strength of 2.35 MPa equivalent to 359 g of force (Siti Hajar Othman et al., 2019), the biofilm from the gelling agent had a tensile strength almost equivalent to the cassava starch film reinforced with cellulose, which proves that the process of creating biofilm from the gelling agent obtained from *Kappaphycus alvarezii* has optimised the mass of the gelling agent to create biofilm, which is reported for the first time. This trend indicates that an increase in the concentration of the gelling agent during the biofilm process enhances molecular bonding and strengthens interactions among the polysaccharides in the biofilm. However, if the mass of the gelling agent exceeds 2.5 grams, it may cause changes in the molecular structure, which could lead to a reduction in tensile strength.

3.2 Biofilm solubility testing

The biofilm with the best load-bearing structure was evaluated for its solubility in aqueous solvent, presented in Table 2. The results of determining the biofilm with an average percentage of solubility of 22.4711 ± 0.5243 % with a standard deviation of 0.5243 (Sx<1) were within the allowable range, statistically significant. Compared with the study of Siah Watt Moey et al. (2014), in the study the author used *Kappaphycus alvarezii* as raw material and the molding method with solvent, the biofilm formed had a solubility determined at 66.08 % (Moey, S. W et al., 2014), this indicates that the biofilm formed by the research group of the author Siah Watt Moey et al. (2014) has an unstable structure and is more soluble than the biofilm successfully formed in this study. The solubility of the biofilm formed in this study was 22.4711 %, which was 2.94 times lower than the solubility of the biofilm of Siah Watt Moey et al. (2014). Therefore, the stable structure of the biofilm formed from the gelling agent of *Kappaphycus alvarezii* in this study is applied in food packaging and in wastewater filtration for environmental treatment.

Table 2: Percentage of membrane solubility

	Mean ± SD (%)
Biofilm solubility	22.4711 ± 0.5243

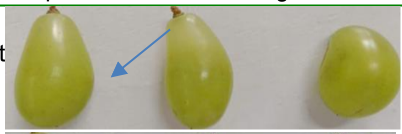
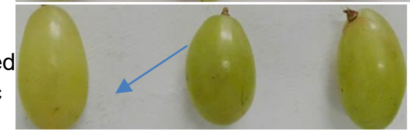
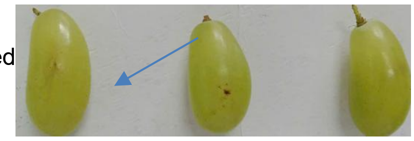
Letters following the same values are not statistically different, values are expressed as mean ± SD (n=3)

3.3 Testing the grape preservation ability of biofilm compared with commercial plastic

The results of determining the color change of grapes are presented in Table 3. After 7 days of storage, grapes not covered with biofilm, grapes covered with commercial plastic film, and grapes covered with biofilm were observed and recorded for color change at room temperature. The results determined after 7 days of storage, grapes not preserved with biofilm showed signs of damage, the skin turned yellow-brown, grapes preserved with biofilm had no change in color on the skin compared to the original color of the grapes before storage. As a result, grapes were not preserved by biological membranes, the fruits reached physiological maturity and entered the aging stage of the fruit. Storing grapes at room temperature exposes the fruit to air, increasing the respiration rate of the ripe fruit, thus promoting physiological processes in the fruit that decompose the components in the fruit. Storing grapes at room temperature also exposes the fruit to microorganisms in the surrounding environment that can cause the fruit to spoil. Grapes preserved in plastic have turned from green to brown. The main reason is that covering the grapes with plastic causes the grapes to switch to anaerobic respiration. The plastic prevents the grapes from being exposed to air. Covering the grapes tightly causes the temperature inside the grapes to increase, so the grapes switch from aerobic respiration to anaerobic respiration. In anaerobic respiration, the components present in grapes such as sugar are converted into ethanol and water, and go into fermentation which causes the sour smell in the fruit. Grapes preserved with biofilm after 7 days still retain the original color of the grapes, this can explain why biofilm can provide a layer of separation between grapes and the surrounding air, but biofilm can have small air holes to help air in the environment circulate properly so it does not cause anaerobic respiration in the fruit, only limits it to a minimum and prevents physical and chemical damage and prevents the growth of pathogenic microorganisms. The results in this study are similar to the study of Kundian Che et al. (2025) in preserving grapes using a synthetic coating of 3 % sodium alginate (SA) extracted from brown algae combined with quercetin/copper nanoparticles (QC NPs). After

9 days of storage, grapes coated with 3 % sodium alginate (SA) extracted from brown algae combined with quercetin/copper nanoparticles (QC NP) showed no signs of damage to the grapes. In the study of Kundian Che et al. (2025), grapes were covered with biofilm with suitable thickness and gas barrier ability, maintaining suitable fruit temperature without causing anaerobic respiration or rotting in the fruit as well as prolonging the storage time of the fruit.

Table 3: Color change of grapes after preservation with nylon and biofilm

Sample	Grape color before coating	Grape color after 7 days of storage
Control (grapes without biofilm coating)		
Grapes are preserved with commercial plastic		
Grapes are preserved with biofilm		

3.3 Testing the dye removal ability of biofilm

The test of the ability to separate the color of the red dye was carried out, the purpose was to evaluate the ability of the color to filter and to demonstrate that a biofilm with air holes is meaningful in the preservation of grapes. The results are presented in Table 4 and Figure 2.

Table 4: Biofilm weight bearing results of balance calibration

	Dye concentration before passing through the membrane *(mg/L)*	
	500	62.5
Dye separation efficiency (%)	0.363^{b}±0.096	86.831^{a}±0.034

The separation efficiency of Congo Red dye of the biofilm was determined to be 86.831 ± 0.034 % when the dye concentration before passing through the membrane was 62.5 mg/mL, compared to the separation efficiency of Congo Red dye of the membrane which was only 0.363 ± 0.096 % when the dye concentration before passing through the membrane was 500 mg/L, this indicates that the biofilm has air holes, and the appropriate density is not too much to be able to hold the dye at a concentration higher than 62.5 mg/mL, and the biofilm also has a suitable air hole density for grape preservation. In the study of Dang Thi To Nhu et el. (2022), cellulose acetate (CA) membrane and NF/CA-PDA composite membrane when separating Congo red dye gave a separation efficiency of 99.3% (Nu et al., 2022), the dye separation results of the biofilm were similar, the results in the test also aimed to prove the ability of the biofilm to preserve grapes.

(a) (b)

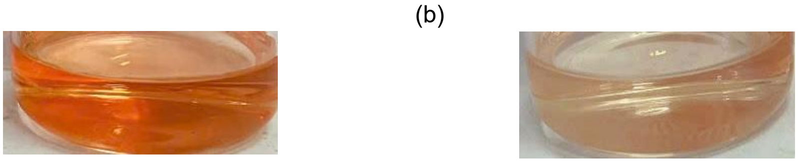


Figure 2: The separation efficiency of the Congo red dye of the biofilm a) before filtration and b) after filtration

3.4 Scanning electron microscopy (SEM)

To prove the above arguments, SEM images of the biofilm surface were taken. The results are presented in Figure 3. The biofilm surface is uniform, with many small holes on the membrane surface with a size of less than 50 μm. The biofilm has a uniform structure that helps increase adhesion, the appearance of a density of small air holes helps prevent excessive diffusion of oxygen and water vapor. The continuous surface without large air holes helps the biofilm create an effective protective layer against the invasion of harmful microorganisms and reduce anaerobic respiration in agricultural products. The membrane's load-bearing capacity may be due to the uniform structure of the biofilm, so the membrane is able to withstand the weight of a 300-gram calibration ball.

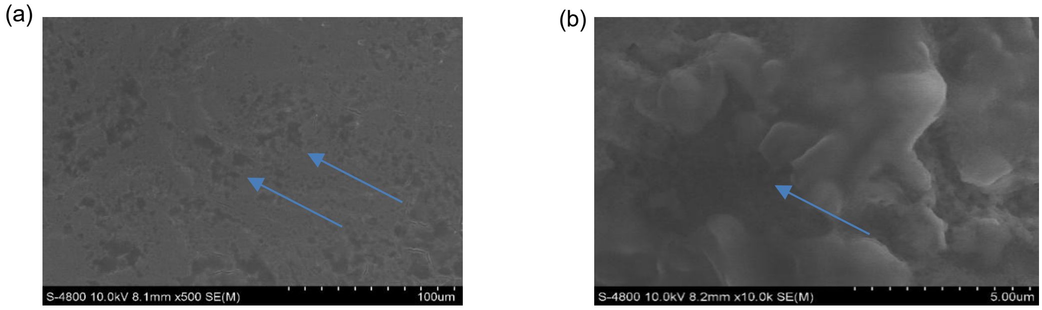


Figure 3 Biofilm structure image by scanning electron microscopy (SEM)for a) Membrance structure at 500X magnification and b) Membrane structure at 10,000 X magnification

4. Conclusions

The study successfully created a biofilm from gelling agent extracted from *Kappaphycus alvarezii.* The biofilm was applied in grape preservation, the results proved that the biofilm had a homogeneous structure, had air holes and air hole density suitable for grape preservation. Testing the ability to separate Congo Red dye of the biofilm, the result was that the film was able to separate the dye with a separation efficiency of 86.831 %. The SEM images determined the biofilm surface is uniform and features many small holes, each measuring less than 50 μm in size. This consistent structure enhances adhesion, while the presence of small air pockets helps limit the excessive diffusion of oxygen and water vapour, which is beneficial for food preservation and packaging.

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