

Research Article

Synthesis and Characterization of a Biodegradable and Robust Film Using *Gracilarialichenoides* and *Sargassumhorneri* for Packaging Applications

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In this study, a biodegradable and mechanically robust film was synthesized using seaweeds (*Gracilarialichenoides* and *Sargassumhorneri*), and its physicochemical and mechanical properties were characterized. In addition, its applicability in the packaging area was studied. A synthetic process including cleaning, drying, softening, pulping, and forming was developed. The microscopic morphology of the developed biodegradable film was similar to wood-based paper. In order to characterize its chemical composition and structure, FE-SEM, FT-IR, GC-MS, and TGA analyses have been performed. In addition, an aerobic biodegradability test was performed in accordance with ISO 14855-1:2012. From those experimental studies, it was shown that the major ingredient of the developed material is cellulose and other bio-originated organic substances. The developed seaweed-based film has excellent biodegradability which is 17 times higher than biodegradable polybutylene succinate (PBSe, hereafter) film, and seven times higher than other seaweed-based film in literature. The tensile strength of the film was enhanced by more than 40% compared with other seaweed-based film of previous studies. A hazardous material test was conducted in accordance with IEC62321, and the result indicated that all of the concerned toxic material was estimated to be under the method detection limit (MDL). Based on the aforementioned results, a prototype egg carton was successfully developed in accordance with ISO 534.

1. Introduction

Polymers are widely used for industrial and personal purposes due to their favorable properties, such as high mechanical strength per weight, chemical stability, and low production cost [1, 2]. Polymers are regarded as very important materials due to their widespread usage in various fields, such as food packaging, clothes, shelter, communication, transportation, construction, health care, and the leisure industries. However, the increased use of polymer-based products focused the view on the environmental impact of the polymers [3]. Around 300 million tons of nonbiodegradable polymeric waste is reportedly generated every year in the United States and Western Europe [3]. Among the annual polymer products (“plastic” hereafter), up to 10%, namely, approximately 30 million tons of plastics

are estimated to end up in the oceans in the form of “microplastics,” [4] which is thought to constitute 60–80% of the overall marine litter [5]. In this regard, the accumulation of the microplastic is emerging as a serious environmental problem [6]. Once it enters the ocean, the microplastic is hardly biodegraded but accumulated due to its intrinsic chemical stability [2], whereas what happens to microplastics in the end over time is still not disclosed. The more important technological concern lies within the ecological harm caused by the microplastics and how to develop an eco-friendly material that can effectively substitute the polymers.

Many researchers attempted to develop innovative biodegradable and eco-friendly materials of which the functional properties are comparable to those of commercially available plastics. Practically, Polylactic acid (PLA), which is invented in 1989, is the most widely known eco-

friendly polymer. It is produced from renewable sources such as corn, cassava, or supercane [7]. It has been further developed into poly D,L-lactic acid (PDLA) and poly L-lactic acid (PLLA). However, the PLA are still not a commodity polymer because its application is hindered by poor mechanical properties and low processibility [7]. In the 1990s, Polyhydroxybutyrate (PHB) was synthesized from *Arabidopsis thaliana* and elephant grass [8]. It received significant industrial attention since it is biocompatible and biodegradable and has good mechanical strength than that of polypropylene (PP). However, it was not commercialized due to its high cost and some unsatisfactory properties, such as low glass transition temperature.

More recently, attempts have been made focusing on biomass-originated plastics such as starch-based plastics [9], cellulose-based plastics [10], and protein-based plastics [11]. Di Filippo et al. [12] developed a new packaging material made of commercial cellulose derivatives mixed with snail mucus extracted from *Helix Aspersa* Muller. The developed material is biodegradable and even has antimicrobial properties. Shih et al. [13] developed a pineapple leaf/recycled disposable chopstick hybrid fiber-reinforced biodegradable composites. Jeong et al. [14] developed a novel eco-friendly starch paper for flexible, transparent, and disposable organic electronics and shed light on biodegradable green electronics. Some studies utilized seaweeds for developing biodegradable materials [15, 16]. In addition to being environment friendly, seaweed has the advantage of being very cheap compared to petroleum- or wood-based materials, because it is abundant in the ocean. They developed seaweed-based films and characterized their physicochemical properties. However, materials derived from those studies generally showed insufficient mechanical properties, and their biodegradability was not quantitatively demonstrated. In this study, a biodegradable and mechanically robust film is synthesized and characterized using seaweeds: *Gracilarialichenoides* and *Sargassumhorneri* and its applicability is studied, particularly aiming at packaging areas. The microscopic appearance, physicochemical properties, biodegradability, and mechanical properties of the developed film are experimentally studied. From those studies, the practical applicability of the seaweed-based film is discussed.

2. Experiment

2.1. Material Preparation and Synthesis. In this study, *Gracilarialichenoides* and *Sargassumhorneri* were used as the raw materials. The *Gracilarialichenoides* and *Sargassumhorneri* used were harvested during April–June 2021 when the ocean temperature was 15–18°C in Jeju, Korea. Based on raw materials, the seaweed-based biodegradable material is made by the following procedure: cleaning, drying, softening, pulping, second cleaning, drying, and stacking (forming). The overall process is shown in Figure 1. The *Gracilarialichenoides* and *Sargassumhorneri* first go through a cleaning/drying process (Figure 2, left). The input ratio of *Gracilarialichenoides* and *Sargassumhorneri* is 1:1. Subsequently, the mixture goes through a softening process, where the mixture was agitated in a Na₂SO₃ aqua solution. Then, the

mixture turned into fiber-like raw material with white color (Figure 2, right). The resulting material was then cleaned with purified water and concentrated. The concentrated softened seaweeds, pulps, and purified water were supplied into a pulper. The mixture of the raw material was then pumped into a dump chest and is mixed there using a stirrer. In the dump chest, the pH was maintained above 5, and the temperature was maintained at 60°C.

The mixed material was then transferred to a pulp cleaning process and subsequently moved into a storage chest. The treated material was again mixed with water and moved to a molding machine with moving conveyor trays. The conveyor trays proceed through an internal channel of a hot drying oven. The dried material was stacked to form a film or pellets. The films were fabricated by applying roll-to-roll processes to the stacked material, and the pellets were fabricated by chopping seaweeds-based fibers, which were made by applying extrusion processes to the stacked material.

Storage and the processing of samples are all conducted at room temperature. Prior to the film synthesis, raw material went through toxicity and compatibility tests. Heavy metals and hazardous chemicals detection tests were performed in Korea Testing and Research Institute (KTR) in accordance with the IEC 62321 test standard. Additionally, the elution test was performed using ICP-OES after acid-grinding of the sample. The pH of the seaweed-mixed pulp was also measured according to ISO 6588-1 [17], which is a test method specially designed for paper, board, and pulp wherein the quantity of extractable ionic material is extremely low. In this method, the pH value is defined by the electrolytes extractable by cold water from the sample [18]. The resulting pH value was shown to be 7.9. The moisture content, which is the percentage equivalent to the ratio of the weight of water to the weight of the dry matter, of the seaweed-mixed pulp was measured in accordance with ISO 287:2009. This is an oven-drying method for the determination of the moisture content of papers and boards [19]. The moisture content value was estimated to be 6.0%. The aforementioned test was conducted at room temperature (23.0 ± 1°C) with a moderate humidity condition (50 ± 2% RH).

2.2. Physicochemical Characterization

2.2.1. FE-SEM Visualization. Field-Emission Scanning Electron Microscopy (FE-SEM) was used to investigate the microscopic appearance of the synthesized film. Both the raw material (*Gracilarialichenoides* and *Sargassumhorneri*) and synthesized film were analyzed by FESEM. Before the microscopy, a platinum coating was applied on the surface of the samples. The voltage was set as 10 kV.

2.2.2. Thermogravimetric Analysis. A thermogravimetric analysis (TGA) was performed to determine the thermal stability and fraction of volatile components of the synthesized sample. In this test, the weight change of a sample is monitored while the atmospheric temperature around the sample is elevated at a constant rate using TGA Q500 (TA Instrument, Inc.). The temperature elevation rate was set as

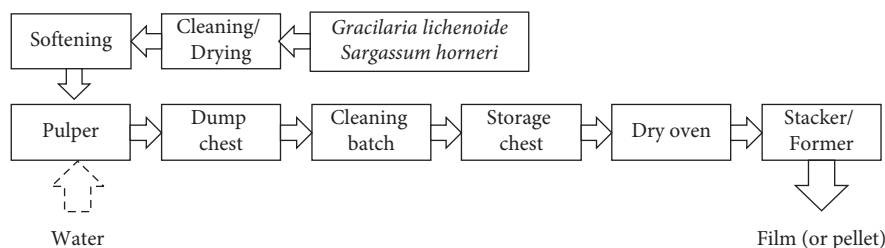


FIGURE 1: Synthesis process of the present biodegradable material.



FIGURE 2: Appearance of raw materials (*Gracilarialichenoides* and *Sargassumhorneri*).

20°C/min and was steadily increased up to 800°C to investigate the organic composition of synthesized materials. The air was used as an atmospheric gas.

2.2.3. FT-IR Analysis. A Fourier Transform Infrared (FT-IR) analysis was conducted to verify the structure of the sample. Each material has a corresponding intrinsic infrared spectrum, which acts as a finger print. Based on this principle, FTIR provides an effective way for structural analysis, material analysis, and qualitative analysis of the organic matter. FT-IR facility from Mettler-Toledo Inc. was used in this study.

2.2.4. Pyrolysis GC-MS. A pyrolysis gas chromatography—mass spectrometry (pyrolysis GC/MS) was used to analyze the chemical composition of the prepared sample. The pyrolysis GC/MS is a method of chemical analysis in which the sample is heated to decomposition to produce smaller molecules that are separated by gas chromatography and detected using mass spectrometry [20, 21]. As the equipment, Agilent 7890B, 5977B MSD system was used.

The pyrolysis condition was set as 600°C through a multi-shot pyrolyzer (EGA/PY-3030D). UA5 (Frontier Laboratories, Inc.) was used as the column. The helium was used as the carrier gas, and the flow rate was set as 1.0 mL/min.

2.3. Biodegradability Test. The aerobic biodegradability test was performed in accordance with ISO 14855-1:2012; AYHA20-06963 method provided by SGS CSTC Standards Technical Services (Shanghai) Co., Ltd. This test method determines the ultimate biodegradability and degree of disintegration of the test items under conditions simulating an intensive aerobic composting process. The used inoculum consists of stabilized, mature compost derived from composting the organic fraction of solid municipal waste. The test item is mixed with the inoculum and introduced into a static composting vessel where it is intensively composted under optimum oxygen, temperature, and moisture conditions for a test period not exceeding six months. During the aerobic biodegradation of the test item, carbon dioxide, water, mineral salts, and new microbial cellular constituents

(biomass) are the ultimate biodegradation products. The carbon dioxide produced is continuously monitored or measured at regular intervals, in test and blank vessels to determine the cumulative carbon dioxide production. The percentage of biodegradation is given by the ratio of the carbon dioxide produced from the test item to the maximum theoretical amount of carbon dioxide that can be produced from the test item. The maximum theoretical amount of producible carbon dioxide is calculated from the measured total organic carbon (TOC) content. The percentage biodegradation does not include the amount of carbon converted to new cell biomass, which is not metabolized into carbon dioxide during the course of the test.

The experimental apparatus consists of reaction equipment (reaction vessels), a pressurized-air system, a gas absorption device, a gas rotameter, a humidity controller, a gas detector, and a thermostat water bath cauldron. A total 360 g inoculum (dry mass) and 60 g test item (dry mass) were added to the reaction vessel for testing. A more detailed experimental procedure was described in Supplementary Information (SI) section of this paper. In the test, TLC (thin-layer chromatography) grade cellulose microcrystalline (Molecular formula $(C_6H_{10}O_5)_n$, molecular weight $(162.14)_n$, CAS No 9004-34-6) is used as a reference item. The aerobic compost used as the inoculum was treated so that the coarse particles and inert matters can be removed, and it is sieved on a screen of 0.5 mm mesh element size. The parameters such as pH, moisture content, total dry solids, volatile solids, total nitrogen, and total organic carbon were measured for quality control of the compost. The results were listed below: pH 7.57, moisture content 42.3%, total dry solids 57.7%, volatile solids 45.0% (of dry solid), total nitrogen 1.73%, and total organic carbon 26.35%. The portion of the added water to adjust the amounts of the total dry solids to the content of the wet solids was measured to be 50.4% before the compost is used. The total organic carbon and moisture content were measured to be 38.44% and 5.1%, respectively. Those reference items were determined as 43.16% and 4.0%, respectively. The ratio of the dry mass of the inoculum to the dry mass of the test material was set as 6 : 1.

2.4. Test for Mechanical Properties. In order to demonstrate the applicability of the seaweed-based biodegradable material, its mechanical properties were measured. Four kinds of mechanical properties were tested: tensile strength, elongation, tearing strength, and bursting strength. The test standards employed for the mechanical property characterization in this study are ISO 1924-2 [22] for tensile strength and elongation; ISO 1974 [23] for tearing strength; and ISO 2758 [24] for bursting strength. The tensile strength of a material is the maximum amount of tensile stress that it can take before failure (yield or fracture) [24]. The tensile stress denotes the magnitude of force applied along the axial direction of an elastic object subject to a tension, which is divided by the cross-sectional area of the object in a direction perpendicular to the applied tension force. The elongation is

the maximum allowable deformation of an elastic object subject to a tension before it is fractured. The elongation is sometimes called as the maximum extension ratio and is defined as follows:

$$\varepsilon = \frac{\Delta L_{\max}}{L_0}, \quad (1)$$

where ε is the elongation, ΔL_{\max} is the final length of the object subtracted by the initial length of the object, and L_0 is the initial object length. It should be noted that the tensile strength and the elongation are simultaneously determined through ISO-1924-2. In this test, a test piece of given dimensions is strained to break at a constant rate of elongation using a testing machine that records both the tensile force and the elongation. If the tensile force and elongation are continuously recorded, the strain at break, the tensile energy absorption per unit area (J/m^2 or N/m), and the elongation (%) can be determined. The tearing strength is defined as the force required to start or to continue to tear an object (generally defined for thin objects such as papers or fabrics) in either weft or warp direction, under torsion or warping conditions. The bursting strength is the capacity of thin packaging materials such as papers or textiles to maintain in continuity when subject to pressure broadly. In the bursting strength test, the material is formed as a balloon-like shape, and air pressure is applied inside the balloon through the hole. The pressure is gradually increased until the balloon bursts. The pressure value when the balloon bursts is recorded as the bursting strength.

3. Results and Discussion

3.1. Physical Properties. Figure 3 illustrates the change of microscale morphology of raw material during the synthesis process. After cleaning, marine micro-organisms and a portion of galactose (Figure 3(a)) are washed out, as shown in Figure 3(b). After pulping, mixing, and second cleaning, the morphology of the material turns into a cellulose-like (bundles of fibers) configuration. After film forming, fibers were pressed flat as shown in Figure 3(c). Figures 4(a) and 4(b) show the appearance of the fabricated film. Figure 4(b) compares FE-SEM images of seaweed-based film with commercially available wood-pulp-based papers. This figure indicates that the microscale morphology is similar to wood papers, having an even denser and sturdier structure. Since seaweed is a much more common and inexpensive sustainable resource than wood, this result suggests that the film developed in this study has the potential to replace paper-based films.

3.2. Chemical Composition. Figure 5 shows the TGA result of the synthesized film. As the temperature increases, the weight of the sample slowly decreases. When the temperature reaches 100°C , the weight loss becomes 5.9%, which is attributable to the small amount of wax, fixative, degassing agent, and volatile components attached to seaweed. The weight loss then hardly changes with the temperature elevation, and the weight loss at 250°C , was measured to be only

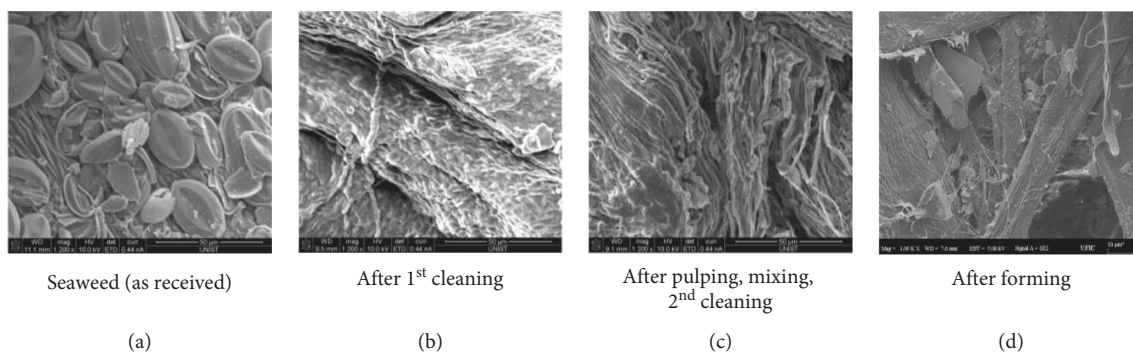


FIGURE 3: FE-SEM image of raw material (a seaweed: *Gracilarialichenoides*): (a) as-received; (b) 1st cleaned; (c) pulped, mixed, 2nd cleaned; (d) formed into a sheet. The magnification rate is 1,000 for all images.

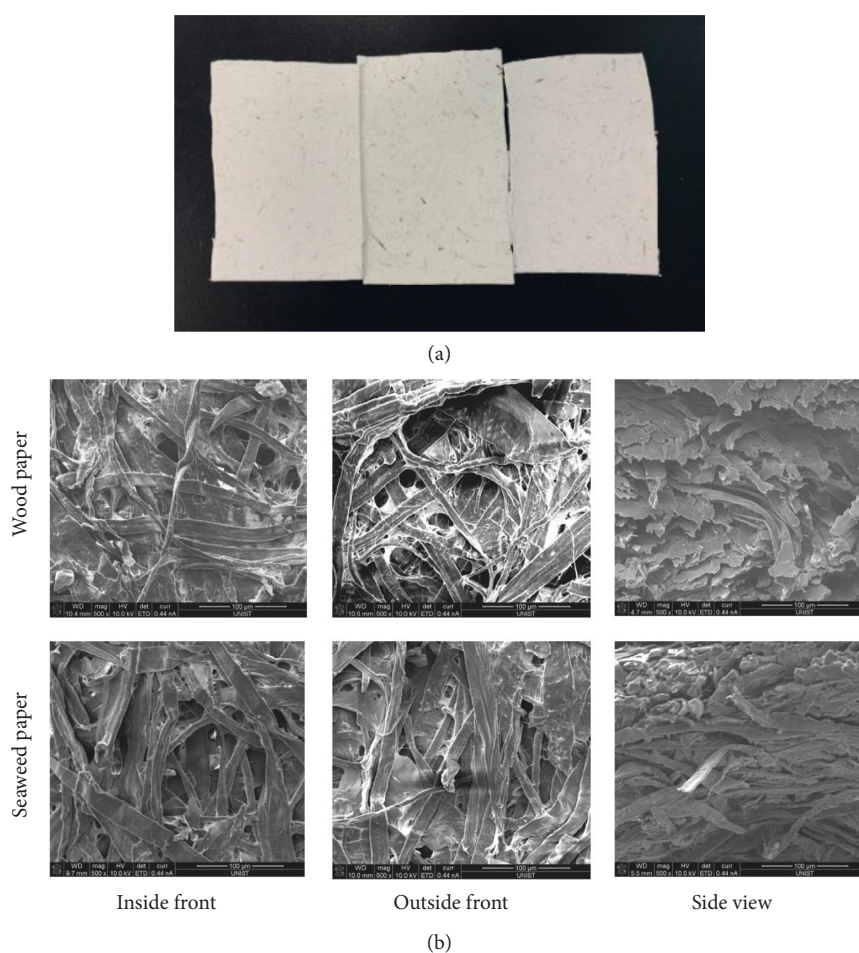


FIGURE 4: Appearance of synthesized film. (a) Synthesized seaweed-based biodegradable film, (b) FE-SEM image of the synthesized film: (i) inside front; (ii) outside front; (iii) side view. The magnification rate is 500 for all images.

7.2%. However, the weight loss starts to abruptly increased when the temperature increased approximately beyond 300 d-C, and the weight loss is researched 86.9% and 92.8% when the temperature is 400 and 800°C, respectively. From the TGA results, it is postulated that the synthesized film is mainly composed of cellulose, considering that the cellulose has thermal stability up to 350°C.C [10].

Figure 6 shows the FT-IR results for the fabricated film. A peak around $3,332\text{ cm}^{-1}$ represents typical cellulose I peak, which is related to the valence vibration of hydrogen bonded -OH groups [25]. Peak around $2,896\text{ cm}^{-1}$ is attributed to C-H symmetrical stretching vibrations of all hydrocarbon constituents in polysaccharides [26–28]. Typical bands assigned to cellulose were observed in the region of

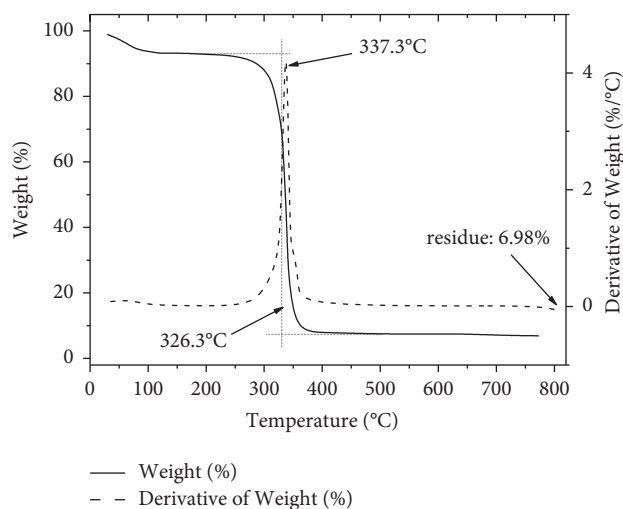


FIGURE 5: TGA result of the synthesized film (The raw data are available in the supplementary file (available (here))).

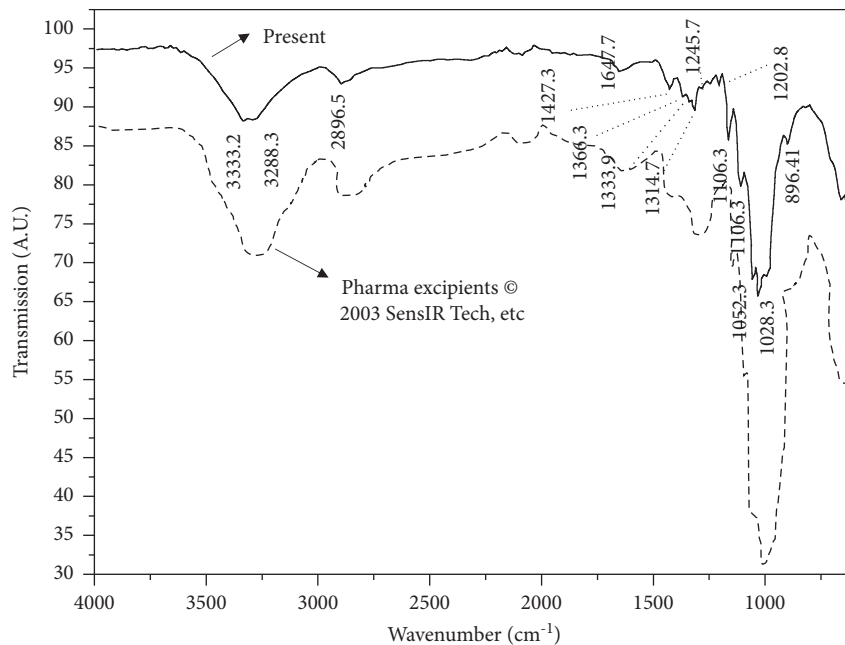


FIGURE 6: FT-IR result of the synthesized film: solid line represents measured data; its y -value is in accordance with the y -axis; the dashed line represents a reference data (a commercially available cellulose powder/fiber) while its y -value is not to scale (The raw data are available in the supplementary file (available (here))).

1,630–900 cm^{-1} . The absorption bands at 1,427 cm^{-1} belong to HCH and OCH in-plane bending vibration, which is associated with the amount of the crystalline structure of the cellulose. The dominant peak at 1366 cm^{-1} signifies C-H deformation. Peak around 1,028 cm^{-1} is related to C-C, C-OH, C-H ring, and side group vibrations [29–31]. The band at 896 cm^{-1} is attributed to the aromatic C-H vibration of cellulose, which is assigned to the amorphous region in cellulose. From the comparative analysis of the FT-IR spectrum and the library, the sample was shown to be a cellulose-like material (Pharmaexcipients © 2003SensIR-Tech, SigmaAldrich, S.T. JapanCellulosepowder).

From the pyrolysis GC-MS test, as shown in Figure 7, the 21 compounds identified in the programs are given in Table 1. Compounds have been identified using a combination of a mass spectral database and retention data for standard components. As shown in this table, furans, phenols, aldehydes, ketones, carboxylic acid, hydrocarbons, esters, and nitrogen-containing compounds are contained in the tested specimen. They all correspond to organic substances involved in biological metabolism and are substances commonly found in seaweed [32–34]. The most prominent compound is shown to be β -d-Glucopyranose, which is a beta isoform of d-glucopyranose, a simple synthetic

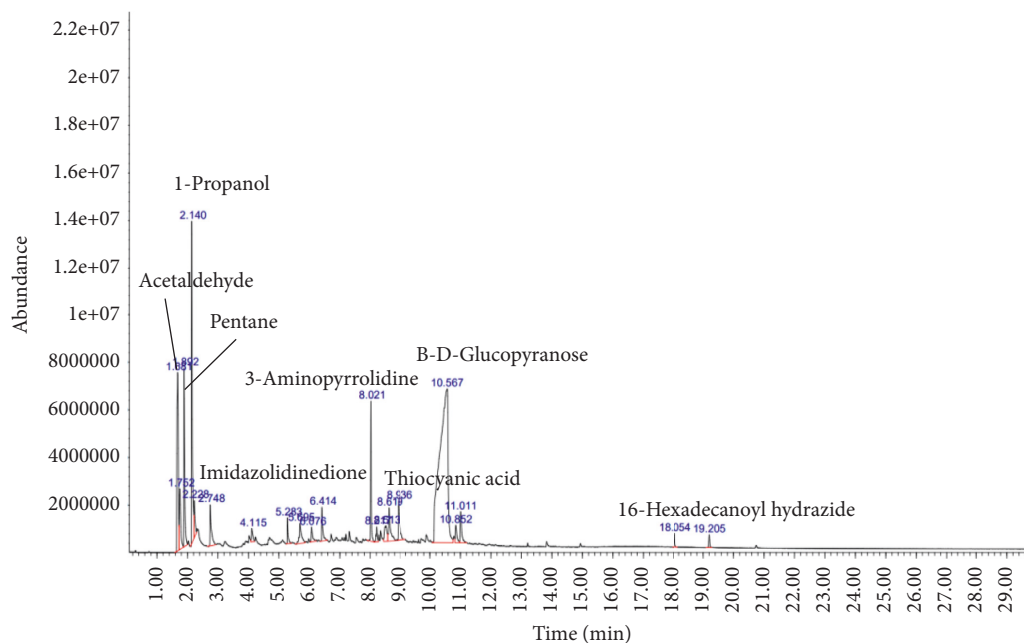


FIGURE 7: Results of pyrolysis GC-MS (600°C); it shows phytochemical compounds (The raw data is available in the supplementary file (available (here))).

TABLE 1: Compounds identified in pyrograms from Py-GC/MS.

No.	Retention time	Content (area%)	Name of compound	Molecular formula	Molecular weight (g/mol)
1	1.681	8.97	Acetaldehyde	C ₂ H ₄ O	44.05
2	1.752	3.05	Ethylene oxide	C ₂ H ₄ O	44.05
3	1.892	5.53	Pentane	C ₅ H ₁₂	72.15
4	2.140	10.58	1-Propanol	C ₃ H ₈ O	60.10
5	2.228	1.50	Acetaldehyde, hydroxy-	C ₂ H ₄ O ₂	44.05
6	2.748	2.20	2-Propanone, 1-hydroxy-	C ₃ H ₆ O	58.08
7	4.115	0.69	n-Methyl-n'-nitroguanidine	C ₂ H ₆ N ₄ O ₂	118.10
8	5.283	0.86	1,3,5,7-Cyclooctatetraene	C ₈ H ₈	104.15
9	5.695	1.60	2-hydroxy-2-cyclopenten-1-one	C ₅ H ₆ O ₂	98.10
10	6.076	0.71	Hydrazine, (2-methyl-1-propenyl)-	C ₄ H ₁₀ N ₂	86.136
11	6.414	1.38	2,4-imidazolidinedione, 3-methyl-	C ₄ H ₆ N ₂ O ₂	114.10
12	8.021	3.30	3-Aminopyrrolidine	C ₄ H ₁₀ N ₂	86.136
13	8.217	0.63	Valeric acid hydrazide	C ₅ H ₁₂ N ₂ O	116.16
14	8.513	1.44	Cyclohexanone, 3-hydroxy-	C ₆ H ₁₀ O ₂	114.14
15	8.619	2.29	Butanoic acid, 2-oxo-	C ₅ H ₈ O ₃	116.12
16	8.936	2.69	Thiocyanic acid, ethyl ester	C ₃ H ₅ NS	87.143
17	10.567	49.06	β-D-Glucopyranose	C ₆ H ₁₂ O ₆	180.15
18	10.852	1.03	Guanosine	C ₁₀ H ₁₃ N ₅ O ₅	283.241
19	11.011	1.69	Phloroglucinol	C ₆ H ₆ O ₃	126.11
20	18.054	0.36	16-hexadecanoyl hydrazide	C ₁₆ H ₃₄ N ₂ O	270.454
21	19.205	0.44	18-pentatriacontanone	C ₃₅ H ₇₀ O	506.9

monosaccharide as an energy source. D-glucopyranose is known to be oxidized in various tissues either under aerobic or anaerobic conditions through glycolysis, and the oxidation reaction produces carbon dioxide, water, and ATP [32]. The Py GC-MS results demonstrate that the synthesized film is bio-friendly.

3.3. Biodegradability. The materials tested in the present biodegradation study are given in Table 2. Compost,

reference material, and test material were used. For each kind of material, three samples were prepared. For all samples, the same amount of compost inoculum is applied. For samples 1–3, only compost is tested for comparison. For samples 4–6, thin layer chromatography-grade cellulose microcrystalline was used. For samples 7–9, the biodegradable material synthesized in this study was used. For all samples, the dry content (ratio between dry solid and wet solid) of the inoculum was 57.7%. The total dry solid (M_{TOT}) of the tested samples was set as 60 g with an accuracy of three

TABLE 2: Calculation of the theoretical amount of carbon dioxide ($m\text{CO}_2$): $m_{w,i}$ is wet solid mass of inoculum; $m_{d,i}$ is total dry solids of inoculum; $m_{w,s}$ is a wet solids of material.

No.	Compost			Reference material			Test material		
	1	2	3	4	5	6	7	8	9
$m_{w,i}$ (g)	623.93	623.94	623.93	623.94	623.94	623.95	623.94	623.93	623.95
$m_{d,i}$ (g)	360.1	360.1	360.1	360.1	360.1	360.1	360.1	360.1	360.1
$m_{w,s}$		—		62.49	62.5	62.5	63.22	63.22	63.22
M_{TOT} (g)		—		60	60	60	60	60	60
TOC (%)		26.35			43.16			38.44	
C_{TOT} (g/g)		0.2635			0.4316			0.3844	
$m\text{CO}_2$, g/vessel		—		94.9	94.9	94.9	84.6	84.6	84.6

significant figures. The dry content of the tested sample was around 95% for all samples. The total organic carbon ratio (C_{TOT}) was measured to be 0.2635, 0.4316, and 0.3844 for the compost, reference material, and test material, respectively. As a result, the theoretical CO_2 per vessel was estimated to be 94.9% and 84.6% for the reference material and the test material, respectively. Figure 8 shows the change in the appearance of the sample. As shown in this figure, the white-colored sample was biodegraded with time and gradually turned into black powder.

Figure 9 quantitatively illustrates the biodegradation of the synthesized film during the test. This figure shows the accumulated CO_2 production over time for all of the tested samples. This figure shows that the CO_2 production rate of the synthesized seaweed-based film is significantly higher than that of compost itself, representing an excellent biodegradability of the synthesized material. From the CO_2 production rate, the degree of biodegradation in Figure 10 can be estimated using the equation (Available (here)) of the Supplementary Information of this paper. As shown in this figure, the degree of biodegradation is slightly lower than the reference material (a TLC grade cellulose). However, as time passes beyond 30 days, the gradient of the tested film abruptly increases and eventually chases over the curve of the reference material. This result, albeit small difference in biodegradation rate, represents that the seaweed-based material synthesized in this study has at least comparable biodegradation properties with well-refined organic chemical (here, TLC grade microcrystalline), which is expensive. The degree of biodegradation of the tested material is shown to reach a plateau after approximately 20 days. It should be noted that the aforementioned biodegradation rate is significantly higher than conventional biodegradable polymers: a PBSe film [35] by 17 times, a bamboo-based cellulose film [15] by 25%, and a seaweed-based film [36] by seven times, when based on their data at 20 days after the start of the experiment. In addition, considering that it takes hundreds of days for commercially available packaging paper boards to be biodegraded under a composting condition, it can be stated that the developed seaweed-based biodegradable film has an excellent biodegradability compared with conventional packaging materials. Nevertheless, in the atmospheric condition, the synthesized film was not biodegraded in atmospheric condition at least for three months, suggesting that the film can be used as packaging materials reliably.

The materials used in the experiment are mainly composed of natural polymers such as cellulose and chitin. In the biodegradation process, it is estimated that cellulose is decomposed into glucose through hydrolysis and then finally decomposed into carbon dioxide [37]. In the case of chitin, it is postulated that it is decomposed into monomers such as glucosamine and finally converted into carbon dioxide as the monomer is oxidized [38].

3.4. Mechanical Properties. The experimental methods and results are given in Table 3. The tensile strength in [kN/m] in accordance with ISO1924-2 can be converted into that in [MPa] by simply dividing the thickness [mm] of the tested specimen. As a result, the tensile strengths are 7.5 MPa for 10% C_{SW} , and 6.66 MPa for 30% C_{SW} , respectively. As shown, the tensile strength was measured to be ~ 7 kN/m. Both tensile strength and elongation are shown to decrease with increase in the C_{SW} (the seaweeds content out of the test material on the weight basis), but the dependence was not significant. It should be noted that the measured tensile strength is higher than that of seaweed-based films in literature: Bacteriocin from *P. acidilactici*—2.85 MPa; *E. faecium*—5.24 MPa.; Crystalline nanocellulose—3.3 MPa [13]. However, the measured tensile strength is slightly lower than commercially available packaging board (~ 10 MPa) [39] or biodegradable polymers that are not bio-originated such as Polydioxanone, PLA, PLLA, whose value typically amounts to 10–30 MPa [40, 41]. The elongation was measured to be $\sim 2\%$, which was lower than literature values typically amount to 5–20% [14, 39–41]. The tearing strength and the burst strength were measured to be $\sim 5,000$ mN and ~ 5 kPa, respectively, suggesting that the robustness of the developed material with respect to the tearing and bursting forces are comparable to or slightly better than commercially available packaging paperboards [39]. The effect of the seaweeds content on the tearing and bursting strengths was shown to be insignificant.

3.5. Environmental Toxicity Evaluation and Practical Application. In order to reconfirm that the seaweed-based film is bio-friendly and not hazardous to the environment and people, the toxicity evaluation was performed for the tested film material, considering heavy metals are sometimes detected in seaweed. In addition, organic substances such as PBBs and PBDEs are used in polymers for the purpose of

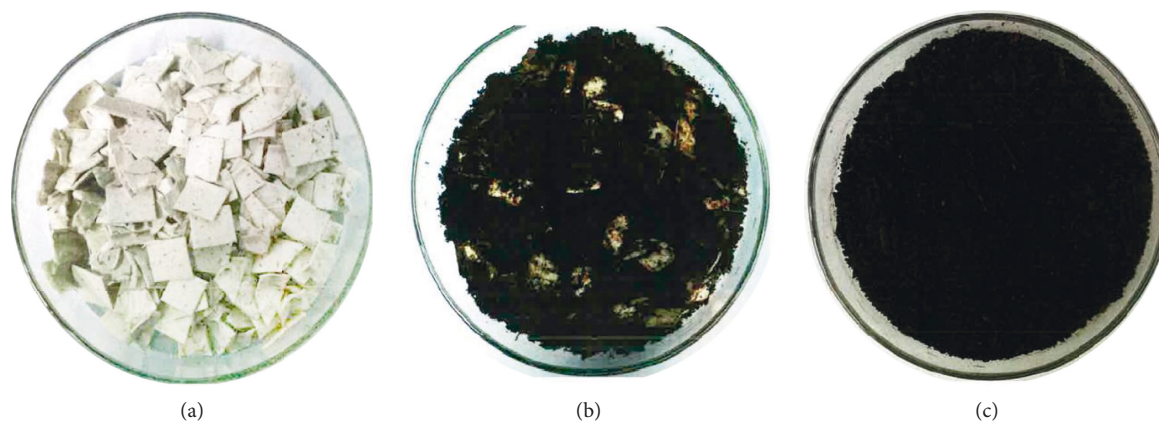


FIGURE 8: Change of sample appearance with time ((a): sample before the test; (b): sample and composts before the test; (c): sample and compost after 45 days test).

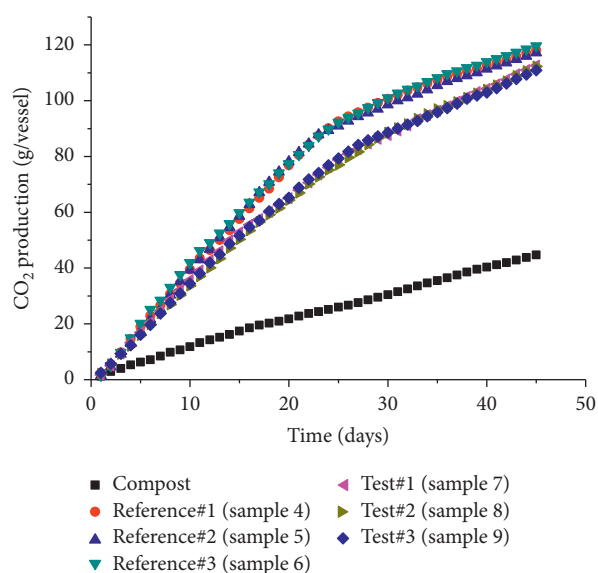


FIGURE 9: Accumulated CO_2 production in the “blank” vessel with only compost, and vessels of test materials.

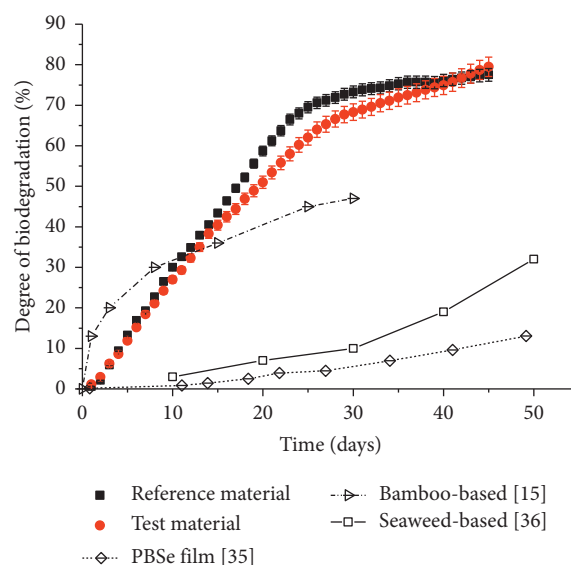


FIGURE 10: Biodegradation estimation: comparative plot of biodegradation rate with respect to reference material (averaged over three samples each) and literature [15, 35, 36].

TABLE 3: Test methods and results for the mechanical property characterization.

Test item	Unit	Test method	Test results	Remark
Tensile strength	kN/m	ISO 1924-2 [22]	7.35 ($C_{sw} = 10\%$)	
			6.53 ($C_{sw} = 30\%$)	
Elongation	%	ISO 1974 [23]	2.40 ($C_{sw} = 10\%$)	$23.0 \pm 1^\circ\text{C}$ $50 \pm 2\%$ R.H.
			1.60 ($C_{sw} = 30\%$)	
Tearing strength	mN	ISO 2758 [24]	4,990 ($C_{sw} = 10\%$)	
			4,480 ($C_{sw} = 30\%$)	
Bursting strength	kPa		537 ($C_{sw} = 10\%$)	
			560 ($C_{sw} = 30\%$)	

flame retardancy and are also detected in commercial plastics. From the IEC62321 hazardous material test for the film specimen, lead, cadmium, Mercury, and hexavalent chromium were not detected to be above the method detection limit (MDL). Their MDL values are 5 mg/kg, 1 mg/kg,

1 mg/kg, and 1 mg/kg, respectively. From the elution test, also, arsenic, lead, optical brightener (fluorescent whitening agent), and formaldehyde were not detected to be above the MDL. Their MDL values are all 0.05 mg/L, respectively. The existence of hazardous organic materials such as

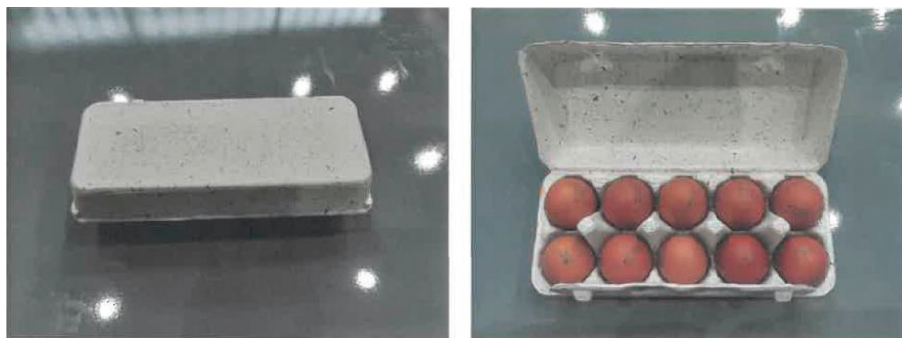


FIGURE 11: An image of the fabricated egg carton, based on the biodegradable film considered in this study.

bromobiphenyls (PBBs, from mono- to deca-) and bromodiphenyl ethers (PBDEs, from mono- to deca-) were also not detected, whereas the MDL is 5 mg/kg.

3.6. Practical Applicability. In order to demonstrate the practical applicability of the synthesized film, we fabricated an egg carton, as shown in Figure 11. The thickness and the density of the seaweed-based biodegradable plate used to make the egg carton were measured in accordance with a test standard: ISO 534 [42] and were measured to be 1.56 mm and 0.43 g/cm³, respectively. The weight per unit area was estimated by ISO 536 [43]. The estimated value was 677 g/m². The aforementioned tests were conducted at room temperature (23.0 ± 1°C) with a moderate humidity condition (50 ± 2% RH). Those values, combined with toxicity test, composition analysis, biodegradability, and mechanical properties, suggest the seaweed-based film developed in this study has a high potential for replacing currently used packaging materials based on petroleum polymers, and wood papers, which are subject to depletion risk, relatively low sustainability, and high cost.

4. Conclusion

In this study, a biodegradable and mechanically robust film was developed and characterized using seaweeds: *Gracilarialichenoides* and *Sargassumhorneri* and its applicability was studied, particularly aiming at packaging areas. From the study, the following conclusions were derived:

- (1) From the developed synthetic process, wood-paper-like biofilm was successfully developed.
- (2) From the SEM, FT-IR, GC-MS, and TGA analyses, it was concluded that the major component of the developed material is cellulose. There are also various types of nontoxic bio-originated organic substances, as shown in Figure 7.
- (3) The developed seaweed-based film has excellent biodegradability amounts to 17 times higher value of biodegradable PBSe film, and even seven times higher value of other seaweed-based films in literature. The tensile strength of the film also was enhanced by more than 40% compared with other seaweed-based film in literature. Based on the excellent biodegradability and

mechanical properties, it can be stated that the developed biodegradable film has a high potential for application as a substitute for plastic packaging materials.

- (4) A hazardous material test was also conducted in accordance with IEC62321, and the result indicated that all of the concerned toxic material was estimated to be under the method detection limit (MDL). Based on the aforementioned results, a prototype egg carton was successfully developed in accordance with ISO 534.

Data Availability

The FT-IR, Pyrolysis-GCMS, and TGA data used to support the findings of this study are included within the supplementary information files.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Supplementary Materials

The test vessel is maintained at 58 °C ± 2 °C, with CO₂-free, water-saturated air through the test mixture at a flow rate of 0.05 L/min. The oxygen concentration in the reaction vessel is measured twice daily in the first week of testing. Afterward, the measurement frequency can be reduced. The amount of carbon dioxide absorption is measured once a day during the first 45 days of the test. (1) CO₂ determination is performed using a sodium hydroxide absorption method [44]. In this method, the CO₂ was collected by an absorption bottle (containing sodium hydroxide solution) which was connected with a backflow preventer in the reaction vessel. When the absorption bottle with CO₂ was taken away, the next absorption (containing sodium hydroxide solution) in the series was connected with the backflow preventer while a new absorption bottle (containing sodium hydroxide solution) was added in the series. The absorption bottle with CO₂ was titrated with HCl standard solution. (2) The air tightness of the test device was checked every day. (3) The composting vessels were shaken and mixed well once weekly. Mixture pH was determined and the parameters such as smell of the exhaust air, humidity of the compost, color, fungal development, structure change, and disintegration of the test item were determined. (4) The theoretical

amount of carbon dioxide (ThCO_2) in grams per vessel is estimated as follows: $m_{\text{CO}_2} = M_{\text{TOT}} \times C_{\text{TOT}} \times 44/12$ where M_{TOT} is the total dry solid (in gram unit) in the test material introduced into the composting vessels at the start of the test; C_{TOT} is the proportion of total organic carbon in the total dry solids in the test material, in grams per gram. (5) The portion of the biodegradation (D_t) of the test material is estimated from the cumulative amount of carbon dioxide released, as the following: $D_t = ((\text{CO}_2)_T - (\text{CO}_2)_B)/m_{\text{CO}_2} \times 100$ where $(\text{CO}_2)_T$ is the cumulative amount of carbon dioxide evolved in each composting vessel containing the test material, in grams per vessel; $(\text{CO}_2)_B$ is the mean cumulative amount of carbon dioxide evolved in the blank vessels. Each test is conducted three times. When the difference between the individual results is less than 20%, the average value is recorded; if not, the result is used separately. The degree of biodegradation is maintained to be larger than 70% after 45 days of testing, to manage the reliability of the data. For all the tests, the inoculum in the blank test was set to produce more than 50 mg but less than 150 mg of carbon dioxide per gram of volatile solids after ten days of testing, for justification of the reliable test. (*Supplementary Materials*)

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