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Biodegradable Plastics from Cellulose Extracted from Caulerpa Lentilifera and Sugarcane Baggasse for a Sustainable Environment

Syed Taufiq Akmal Syed Nasiruddin[1], Muhammad Firdaus Abdul Hadi[2], Sharir Aizat Kamaruddin[2,], Rozilah Rajmi[2]*

[1]Department of Applied Sciences, Universiti Teknologi MARA, Cawangan Pulau Pinang, Kampus Permatang Pauh, 13500 Permatang Pauh, Penang, Malaysia
[2]Faculty of Applied Sciences, Universiti Teknologi MARA Perlis Branch, Arau Campus, 02600 Arau, Perlis, Malaysia

[*]Corresponding Author's E-mail: shariraizat@uitm.edu.my

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ABSTRACT

The primary objective of this investigation is to explore the fabrication of bioplastic films utilizing corn starch as the foundational component, along with cellulose extracted from Caulerpa lentilifera, a form of marine algae and sugarcane bagasse (SCB). The extraction process incorporates the application of innovative techniques, notably alkali sonicator extraction (UAE), in alignment with contemporary developments in algae biotechnology. These bioplastic films are systematically produced with varying concentrations of cellulose originated from Caulerpa lentilifera and SCB, specifically at levels of 10%, 15%, and 20% (w/w), with a control sample devoid of cellulose extract. Interestingly, all cellulose-reinforced bioplastic films exhibited lower tensile strength (TS) and elongation at break (EAB) compared to the control sample. In Caulerpa lentillifera-based films, increasing cellulose concentration led to slight improvements in TS and EAB, whereas SCB-based films showed minimal changes across concentrations. These results suggest that the addition of extracted cellulose, despite increasing film thickness, did not enhance mechanical performance as expected, indicating that factors such as cellulose dispersion or matrix interaction may play a more dominant role. Evaluation through bacterial susceptibility tests reveals a conspicuous absence of inhibition zones, signifying the absence of antibacterial properties in the cellulose extracts

from both seaweed and SCB. Finally, the biodegradation process for the biofilms showed rapid biodegradation, completing within two weeks under soil burial conditions. While the incorporation of cellulose did not enhance mechanical performance relative to the control, the use of marine algae and agricultural waste demonstrates a promising step toward the development of biodegradable materials from renewable sources.

Keywords: Algae; Bioplastics; Caulerpa lentillifera; Marine; Sustainability; Seaweed

INTRODUCTION

The global demand for plastic products has increased, which puts pressure on waste management systems. In Malaysia, sales of petroleum-based products have risen significantly, indicating the importance of plastics manufacturing in the economy [1]. To address the environmental impact of petroleum-based plastics, there is a shift towards biodegradable alternatives sourced from natural materials like agricultural waste, aquatic resources, and microbes. These efforts align with global goals to promote bioplastics and biopolymers [2-3], contributing to sustainability and environmentally conscious plastic use.

Bioplastics and biopolymers are often used interchangeably, but they represent distinct concepts. Bioplastics are plastic materials that are bio-based, biodegradable, or both, whereas biopolymers specifically refer to polymers synthesized by living organisms or derived from renewable biological sources use [4]. In this study, “bioplastic” refers to biodegradable films made from natural biopolymers such as starch and cellulose.

In recent years, there has been a growing interest in finding renewable resources for bioplastics and biodegradable materials. Seaweed polysaccharides, found in various green algae, have become a focus of research. These underutilized resources offer potential for creating bioplastic films and biodegradable products. This study utilizes cellulose extracted from *Caulerpa lentilifera*, a green algae found along Sabah's coast, to make bioplastics when combined with a plasticizer. *Caulerpa lentilifera* was selected due to its fast growth rate and natural abundance along the Sabah

coastline, making it a highly renewable marine biomass [5-6].

Furthermore, the use of agricultural biomass to address environmental concerns is gaining traction. In Europe, biomass is widely used for sustainable energy, like biofuel and biodiesel [7], utilizing abundant agricultural waste. Malaysia, an agricultural nation repurposes farming waste such as corn silage for livestock [8] and coconut husk coco peat for gardening [9]. Sugarcane bagasse, a byproduct of sugarcane, is commonly used to improve soil quality[10]. A prior study in 2015 explored sugarcane bagasse for bioplastics [11]. This study reevaluates this research, focusing on cellulose extraction from sugarcane bagasse for bioplastic creation. It also compares sugarcane bagasse-derived bioplastics, a terrestrial source, with those from marine seaweed Caulerpa lentilifera, providing insights into sugarcane bagasse's potential as a bioplastic raw material. The research evaluates the mechanical, antibacterial, and biodegradation properties of the resulting films, aiming to assess their viability for bioplastic applications. By doing so, this study contributes to the development of environmentally friendly alternatives to conventional petroleum-based plastics through the utilization of both land-based and marine biomass.

EXPERIMENTAL METHODOLOGY

The process begins with washing, drying, and finely grinding the raw materials. Cellulose was then extracted using ultrasonic-assisted alkali treatment. The extracted cellulose was subsequently incorporated into a corn starch-based matrix, which uses glycerol as a plasticizer and vinegar as a pH modifier, following the formulation details outlined in Table 1 and Table 2.

Table 1: The composition of cellulose film from seaweed extract.

Material	Composition			
	Control	BPCL-1	BPCL-2	BPCL-3
Corn starch (g)	10.0	10.0	10.0	10.0
Glycerol (ml)	10.0	10.0	10.0	10.0
Acetic acid (ml)	7.0	7.0	7.0	7.0
Cellulose (Caulerpa lentilifera) (g)	0.0	1.0	1.5	2.0

Table 2: The composition of cellulose film from sugarcane bagasse extract.

Material	Composition			
	Control	BPSCB-1	BPSCB-2	BPSCB-3
Corn starch (g)	10.0	10.0	10.0	10.0
Glycerol (ml)	10.0	10.0	10.0	10.0
Acetic acid (g)	7.0	7.0	7.0	7.0
Cellulose (sugarcane bagasse) (g)	0.0	1.0	1.5	2.0

In this bioplastic formulation, corn starch functions as the base polymer forming the structural matrix, glycerol acts as a plasticizer to enhance flexibility and reduce brittleness, acetic acid (vinegar) serves as a pH modifier and blending aid to facilitate uniform mixing, and the extracted cellulose from Caulerpa lentillifera or sugarcane bagasse was incorporated as a reinforcing agent to improve the mechanical strength of the resulting films. This mixture was then homogenized using a hot plate and magnetic stirrer, heated to 100 °C, and cast into film-shaped molds. The bioplastic mixture was poured onto a 31 cm × 26 cm silicone sheet, which served as a mold. These filled molds were then dried in an oven at 40 °C for at least 24 hours, or until the films were fully dried. Once dried, each resulting bioplastic film was then cut into smaller, uniform test specimens according to the requirements of each characterization method. For mechanical and antibacterial testing, three replicate specimens were taken from the same film and tested to ensure consistency and reliability of the results. A flowchart representing the methodology of the study was shown in Figure 1.

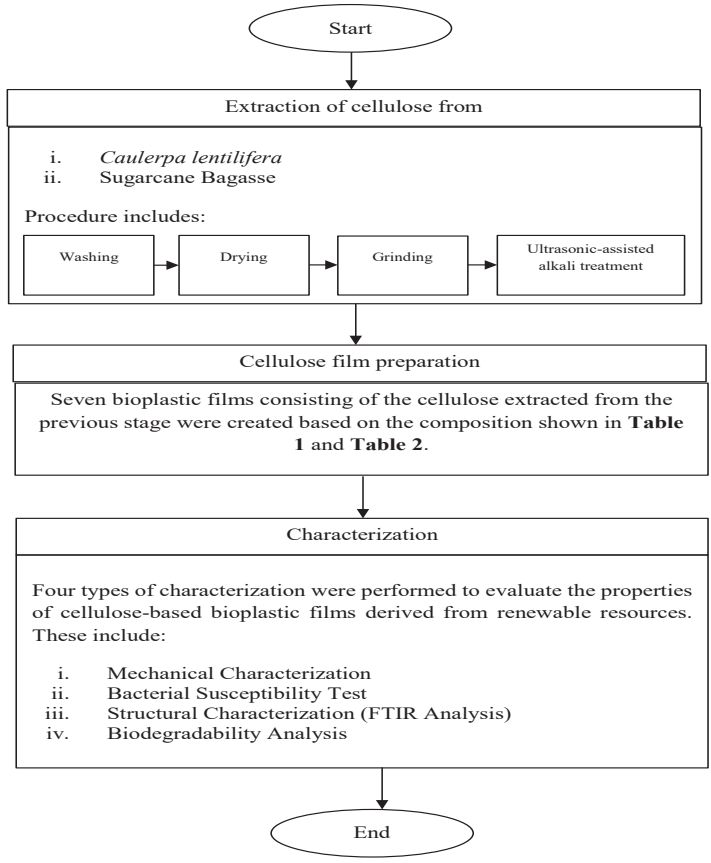

Figure 1: Flowchart of the methodology.

The resulting films undergo comprehensive characterization using various methods. Tensile testing assesses their mechanical properties, providing insights into strength and flexibility. Fourier Transform Infrared spectroscopy (FTIR) analyzes the films' chemical composition and structural properties. Antibacterial susceptibility testing evaluates their effectiveness against bacterial growth, indicating potential antimicrobial properties. Biodegradation properties are also examined to gauge the films' natural breakdown over time, contributing to environmental sustainability. These characterization methods offer a holistic understanding of the films' physical, chemical, antimicrobial, and biodegradation characteristics.

Extraction of Cellulose from Caulerpa lentilifera and Sugarcane Bagasse

Cellulose extraction followed a methodology akin to that employed for the isolation of cellulose from sugarcane bagasse, as documented by Phuang[12]. The process commenced by introducing 10 g of finely ground sample into a Scott bottle containing 100 ml of 1.00 M KOH solution, recognized for its optimal efficacy in disrupting the covalent bonds within the lignocellulosic components of green seaweed. This step facilitated the hydrolysis of hemicellulose, lignin, and pectin, compounds that might otherwise interfere with the extraction process.

Subsequently, ultrasonic-assisted alkali treatment was implemented to extract cellulose from the green seaweed. Under the influence of ultrasonic energy, at a frequency of 40 kHz and a temperature of 80 °C, for a duration of 30 minutes, the cellulose experienced breakdown and dissolution, thereby enhancing extraction efficiency. Post-extraction, rigorous washing with distilled water and filtration were undertaken to eliminate any residual potassium hydroxide and soluble by-products.

Following this purification process, the cellulose residues were subjected to drying in an oven, set initially at 50 °C for a period of 24 hours. In cases where complete drying remained unattained, adjustments were made, with the temperature raised to 60 °C, and drying time extended by an additional six hours, conditional on the sample's moisture content. The procedure yielded purified, dry cellulose from *Caulerpa lentilifera*, now prepared for integration into corn starch-based biofilm matrices. Successful drying culminated in the production of a white and free-flowing powder [13]. The extraction process was repeated for the sugarcane bagasse.

Cellulose Film Preparation

Film bioplastics were made via solvent casting albeit with slight modifications to suit this study's objectives [14]. A total of six distinct bioplastic film formulations were prepared, denoted as follows: control, BPCL-1, BPCL-2, BPCL-3, BPSCB-1, BPSCB-2, and BPSCB-3. These formulations, as detailed in Tables 1 and Table 2, encompassed a combination of corn starch, glycerol, acetic acid, and cellulose extracted

from the respective samples.

The creating process initiated with the dissolution of 10 g of corn starch in 50 ml of distilled water, complemented by the addition of 10 ml of glycerol plasticizer and 7 ml of white vinegar (acetic acid). Subsequently, cellulose extracts, varying in composition, were incorporated into the mixture. In the ensuing steps, the mixture was poured into a beaker, stirred, and subjected to heating at 100 °C until achieving a thickened consistency. Finally, the viscous blend was evenly spread within a mold, followed by drying in an oven set at 40 °C for a duration of 24 hours.

Tensile Strength and Elongation at Break

The tensile strength of the samples, measuring 6 cm x 1 cm, was assessed by securing them onto a tensile strength and elongation tester. The analysis adhered to the ASTM D882-97 method, employing a laboratory Instron Testing machine. This machine systematically subjected the sample to a pulling force at a rate of 100 mm per minute until the sample fractured. Subsequently, the computer displayed the maximum tensile strength value following the sample's rupture, which was then calculated for further evaluation.

Bacterial Susceptibility Test

In this study, two growth media, Mueller-Hinton agar (MHA) and tryptone soy broth (TSB), were prepared to support *E. coli* growth [15]. MHA, solid at room temperature, was used to observe bacterial inhibition zones, while TSB facilitated bacterial cultivation. MHA was prepared by dissolving 40 g of agar powder in a liter of heated distilled water, followed by autoclaving at 121 °C for 15 minutes. For TSB, 30 g of dehydrated tryptone soy broth were dissolved in a liter of distilled water, autoclaved, and the broth was incubated with *E. coli* at 37 °C for 18 hours [16]. After incubation, bacterial concentration was standardized to 0.5 McFarland standard using UV spectrophotometer, and MHA plates were inoculated with E. coli. Bioplastic film samples were cut into 5 mm x 5 mm dimensions, placed on MHA plates with control disks, and incubated. The resulting zones of inhibition were measured with a ruler, to measure its antibacterial activity.

Functional Group Properties

The chemical structure of the formed film incorporated with cellulose extracted from Caulerpa lentilifera and sugarcane bagasse were determined using FTIR analysis [17]. The ATR-FTIR Frontier 100842 was set to run at wavenumber range of 4000 - 400 cm^{-1} with 16 scans per analysis.

Biodegradation Test

To assess biodegradation, a soil burial test was conducted [14]. Bioplastic film samples with varying cellulose extract concentrations were cut into 2 cm x 2 cm pieces, weighed, and buried in pots filled with local environmental soil near UiTM Arau. These pots were left uncovered indoors in the laboratory setting for two weeks or until the sample had completely deteriorated. Any remaining larger film debris was collected and weighed. Environmental conditions such as temperature and humidity were not artificially controlled, but average daily temperatures during the test period ranged between 28 °C and 32 °C, based on local environmental conditions. At the end of the test, any remaining visible film residues were retrieved, dried, and reweighed. However, most samples showed complete degradation, preventing accurate calculation of intermediate weight loss.

RESULTS AND DISCUSSION

Percentage Yield of Cellulose from *Caulerpa lentilifera* and Sugarcane Bagasse

The initial and dried weight of *Caulerpa lentilifera* was 2817.0705 g and 133.3200 g respectively. Thus, the calculated moisture content is 95.267%, leaving 4.73% as its dried weight in relation to the initial weight. This finding is consistent with previous research on various green seaweed species, which reported dried weight ranges between 1.6% and 34%[18-19].

Additionally, an evaluation of cellulose content in the dried green seaweed revealed that extracted cellulose constituted approximately 32.13% of the sample. Extrapolating from this, it can be inferred that around 1.52% of the *Caulerpa lentilifera* seaweed's total weight consists of

cellulose. In contrast, dried sugarcane bagasse (SCB) had an initial weight of 32.1172 g, and after pre-treatment, which involved lignin, pectin, and hemicellulose removal, its dried weight decreased to 24.6416 g, resulting in a pre-treatment yield of 76.72%. Subsequent ultrasonic-assisted alkali treatment successfully extracted 12.3716 g of pure cellulose, indicating an overall cellulose content of 38.52% in the dried SCB sample.

Thickness, Tensile Strength, and Elongation at Break

Tensile strength (TS) and elongation at break (EAB) of corn starch biofilm are influenced by thickness and cellulose extract concentration [20]. Increased cellulose concentration adds reinforcing fibers, enhancing TS, and preventing rupture. Thicker biofilms generally exhibit higher TS due to greater material and load-bearing capacity [21]. However, there might be a limit to proportional strength increase with thickness. EAB, indicating stretchability before fracturing is influenced by cellulose concentration and biofilm thickness. Higher cellulose levels limit EAB due to increased stiffness, while thicker biofilms are less flexible and have lower EAB [22]. Understanding the interplay between cellulose concentration and biofilm thickness is crucial for optimizing corn starch biofilm mechanical properties. However, the results in this section differed from expectations and previous references.

The control sample, with a minimal thickness of 0.51 mm, shows the highest tensile strength at 0.19 MPa and elongation at break at 11.69%. Interestingly, this outperforms sample BPCL-3, which has the highest thickness of 1.08 mm, with the second-highest tensile strength at 0.162 MPa and elongation at break at 9.69%. These results reveal a non-linear relationship between bioplastic thickness and mechanical characteristics. The control sample's exceptional performance hints that factors beyond thickness and the absence of cellulose significantly contribute to its superior TS and EAB. Further analysis is needed to understand these mechanisms fully and their role in bioplastics' mechanical behavior. Reducing cellulose concentration to less than 10 w/w and modifying biofilm preparation methods may affect TS and EAB. Table 3 displays various samples of corn starch bioplastics with different thicknesses, tensile strengths, and EABs when incorporating cellulose from seaweed and SCB.

Table 3: Mechanical analysis of the samples.

Sample	Thickness, mm	Tensile strength, MPa	Elongation at break, %
Control	0.51 ± 0.05	0.19 ± 0.06	11.69 ± 3.79
BPCL-1	0.58 ± 0.02	0.11 ± 0.01	6.88 ± 0.87
BPCL-2	0.79 ± 0.01	0.13 ± 0.02	7.61 ± 1.35
BPCL-3	1.08 ± 0.05	0.16 ±0.05	9.69 ± 3.00
BPSCB-1	0.63 ± 0.04	0.09 ± 0.05	5.14 ± 0.29
BPSCB-2	0.87 ± 0.08	0.09 ± 0.01	5.57 ± 0.52
BPSCB-3	0.83 ± 0.04	0.09 ±0.01	5.22 ± 0.55

The study observed an unexpected trend in the impact of cellulose extract concentration from *Caulerpa lentilifera* on bioplastic properties. Contrary to expectations, increasing cellulose concentration from 10% to 20% (w/w) led to significant improvements in both tensile strength (TS) and elongation at break (EAB). Specifically, TS increased from 0.11 MPa to 0.62 MPa, and EAB improved from 6.88% to 9.69%. This differs from the usual expectation that higher cellulose concentration would correlate with higher TS and lower EAB. Notably, even though increasing cellulose concentration also increased bioplastic thickness, none of the cellulose-extracted samples matched the TS and EAB of the control sample, which remained the thinnest.

Variance analysis highlighted the significant influence of thickness on both TS and EAB in cellulose extracted from *Caulerpa lentilifera*. This suggests that variations in thickness can notably affect bioplastic mechanical properties. The increased thickness wasssociated with improved TS and EAB, possibly due to undisclosed additives or substances in *Caulerpa lentilifer*a that interact with cellulose, enhancing bioplastic characteristics. Further research is required to investigate these additives and their impact on TS and EAB.

For cellulose extracted from sugarcane bagasse (SCB), thickness remained consistent across different cellulose concentrations. TS consistently measured around 0.09 MPa, the lowest among tested samples, and EAB remained at 5%, the lowest observed value. It is important to note that the control sample consistently exhibited superior mechanical

properties. Among various cellulose concentrations, the BPCL-3 sample emerged as the most optimal choice for biofilm modification.

Functional Group Properties

All six spectra showed absorption bands at approximately 3300-3600, ~2900, ~1630, and 1000-1100 cm^{-1}, indicating the presence of OH, C-H, C-O-C, and C-O functional groups in all corn starch matrix samples. The FTIR spectra of the samples are presented in Figure 2. The absorption peak detected at 3600-3300 cm^{-1} for all biofilm indicated the structure of the hydrogen-bonded hydroxy group (O-H) of the corn starch and the glycerol functional group. The result is consistent with corn starch film [17], [23] and shows a similar range of stretching hydroxyl group at 3448 cm^{-1} and 3300 cm^{-1} respectively. Furthermore, the presence of a band at 2932 cm^{-1} in the biofilms can be attributed to the stretching vibration of the C-H bonds in the carbon methyl group of the corn starch film [24].

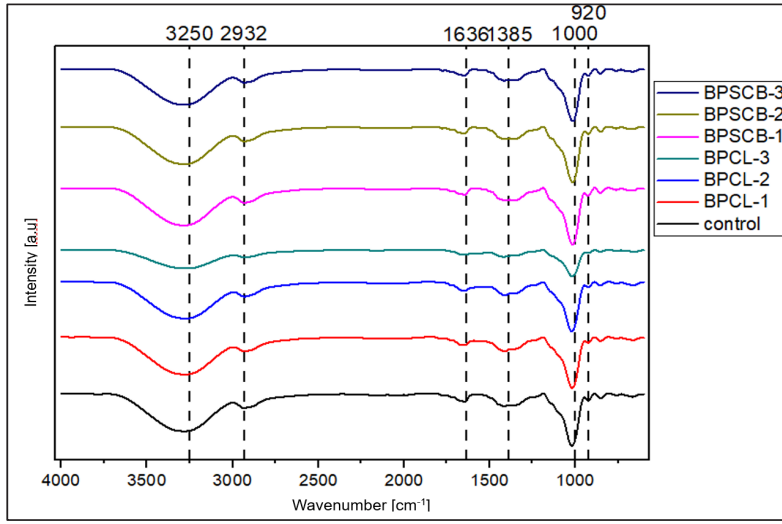

Figure 2: FTIR spectra of samples

The medium absorption peak at 1636 cm^{-1} in all biofilm samples indicates the presence of C-O bending related to the O-H group, signifying the involvement of glycerol, cellulose, and corn starch in interacting with water molecules [25-26]. The IR vibration band at 1385 cm^{-1} in all biofilms indicates the bending vibration of the C-H bond in the methylene ($-CH_2-$) groups. This band confirms the presence of methylene groups in the glycerol and corn starch molecules. Such stretching and bending vibrations of the C-H bond in methylene groups are typical in organic compounds containing carbon and hydrogen atoms [27].

The observed band peak in the range of 1019 cm^{-1} to 1014.57 cm^{-1} corresponds to the C-O stretching in alcohols and ethers present in the corn starch matrix, cellulose, and glycerol. This contribution is likely due to the C-O stretching vibration of D-glucopyranose units, linked together by glycosidic bonds to form polysaccharide chains, which are a key component of corn starch [28]. The characteristic C-O-C ring vibration in corn starch, associated with the absorbance peak at around 920 cm^{-1} corresponds to the stretching of the cellulosic β-(1→4)-glycosidic linkages within glucose molecules [17]. Therefore, the presence of cellulose extract further supports the existence of C-O-C stretching vibrations [29].

Additionally, it is important to note that no observable shift in the FTIR spectra occurred across the samples, even with increasing cellulose concentration. This indicates that the interaction between the cellulose and the corn starch matrix was mainly physical in nature such as hydrogen bonding and matrix entanglement rather than the formation of new chemical bonds. The consistent spectral profiles across all formulations support the conclusion that the cellulose was successfully incorporated without altering the chemical structure of the film matrix.

Bacterial Susceptibility Analysis

None of the bioplastic samples demonstrated antimicrobial properties or exhibited inhibition zones against *E. coli* as depicted in Figure 3.

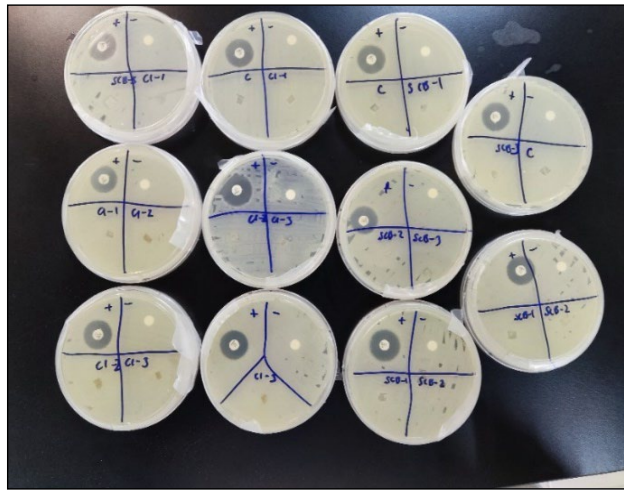

Figure 3: Result of the bacterial susceptibility analysis.

This outcome aligns with expectations, as the primary component of the extract cellulose is not inherently antimicrobial. *E. coli* and many other bacterial strains are known to produce cellulase enzymes that can degrade cellulose-based materials [30]. Thus, it is plausible that the bacteria did not encounter inhibitory conditions, but rather a potential carbon source.

Additionally, although seaweed and lignocellulosic materials can contain bioactive compounds, any such components in the extracted cellulose were likely present in concentrations too low to exert antimicrobial effects. The dense and hydrophilic nature of the corn starch matrix may also have limited the diffusion of any minor antimicrobial compounds to the surrounding media. In contrast, the positive control demonstrated a clear inhibition zone (average 19 mm), confirming the validity of the test.

Overall, the absence of antibacterial activity suggests that cellulose derived from *Caulerpa lentillifera* and SCB in the tested formulations does not inhibit *E. coli* growth. Future studies could explore whether surface-functionalizing the films or concentrating specific bioactive fractions from seaweed may enhance antimicrobial performance.

Biodegradation Analysis

The biodegradability analysis unveiled that the biodegradable films, upon burial in soil, underwent complete degradation within a remarkably brief span of two weeks, consistent with findings reported by Hidayati et al. [14], despite five-week duration of the study. The rapid degradation phenomenon can be attributed to the presence of cellulose and corn starch, natural polysaccharides highly amenable to biodegradation by soil microorganisms [31]. While the study initially intended to monitor the weight loss progression, the first measurement point was taken too late by which time most of the samples had already degraded. As a result, accurate intermediate weight loss data could not be captured due to the late timing of the initial measurement, by which point significant degradation had already occurred. Nevertheless, the total disappearance of samples confirms their high biodegradability in natural soil conditions. The prior bacterial susceptibility investigation confirmed the absence of antimicrobial activity in any of the samples against *E. coli* bacteria. Notably, *E. coli* is renowned for its cellulase enzyme production, capable of breaking down cellulosic materials, including pulp and paper, into fermentable sugars for biofuel production [30]. The availability of these carbon sources within the cellulose-infused corn starch films fosters the proliferation of microorganisms proficient in cellulose utilization as an energy substrate. Consequently, the corn starch films augmented with cellulose experienced an accelerated biodegradation process within the soil environment, culminating in the complete dissolution of all samples within a mere two-week timeframe. The absence of residual biofilm samples at the experiment's conclusion underscores their pronounced susceptibility to biological degradation in the soil milieu. Hence, the incorporation of cellulose extracts from both SCB and Caulerpa lentilifera into corn starch biofilms renders them eminently biodegradable through soil-based biological processes.

Despite the promising biodegradation performance observed, there are certain limitations to this study. First, intermediate weight loss data could not be collected, as the first measurement occurred after significant degradation had already taken place. This prevented a detailed analysis of degradation kinetics over time. Additionally, environmental parameters such as soil moisture and relative humidity were not quantitatively measured or controlled. The soil burial test was conducted under natural ambient

conditions, with local daily temperatures ranging between 28 °C and 32 °C, and the soil was manually watered to maintain moisture. While this approach reflects realistic environmental exposure, the lack of precise environmental data may limit reproducibility. Future studies are recommended to adopt standardized biodegradation protocols with controlled parameters to provide a more comprehensive understanding of degradation behavior.

CONCLUSION

In conclusion, the mechanical properties of cellulose extracted from Caulerpa lentilifera surpass those from sugarcane bagasse (SCB). *Caulerpa lentilifera's* highest cellulose concentration exhibits superior tensile strength (0.16 MPa) and elongation at break (9.69%) compared to SCB's 20 w/w concentration, which records 0.09 MPa and 5.22% for the same properties. However, the cost-effectiveness aspect is pivotal. *Caulerpa lentilifera* yields cellulose at a rate 25 times lower than SCB, making it economically less favorable for bioplastic applications. Bacterial susceptibility tests reveal no inhibition zone, indicating ineffective inhibition of bacterial growth by cellulose extracts. Moreover, high cellulose content accelerates biodegradation, with complete degradation in just two weeks. These findings suggest that while *Caulerpa lentilifera* cellulose shows promising mechanical properties, factors like cost-effectiveness and antibacterial characteristics must be considered in cellulose source selection for bioplastics. It is worth noting that despite promising results, the control sample outperforms others in mechanical properties, antibacterial activity, biodegradability, and cost-effectiveness. In FTIR analysis, functional groups (OH, C-H, C-O-C, C-O) match those reported in the literature. A recommendation for further investigation involves refining results regarding cellulose extract concentration, specifically comparing it to the control sample. Currently, none of the cellulose extracts match the control sample's performance in terms of tensile strength (TS) and elongation at break (EAB). Additionally, the expected relationship, where higher cellulose concentration yields higher TS but lower EAB, did not hold true. Therefore, it is suggested to reduce *Caulerpa lentilifera* cellulose concentration to below 10 w/w to assess how near lower concentrations can approach the control sample's high performance, which lacks cellulose. This refined study will offer insights into the impact of cellulose concentration on biofilm mechanical properties.

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