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Shaping Sustainable Bioplastics: Illuminating *Chlorella* sp. Growth with Light Variations and NPK Levels

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ABSTRACT: This study delved into the exploration of a biodegradable alternative to synthetic plastics through the production of biodegradable bioplastics derived from microalgae. The investigation suggests that utilizing *Chlorella* sp. as a viable source for sustainable biomass in bioplastic creation holds promise due to the convenience of microalgae cultivation and its rapid growth rate. However, the large-scale cultivation of microalgae requires a considerable amount of nutrients, posing challenges to its economic viability. To address this hurdle, it becomes imperative to prioritize the enhancement of growth parameters for microalgae development. This study endeavors to identify the optimal NPK (nitrogen, phosphorus, and potassium) concentration and light spectrum for *Chlorella* sp. using a cost-effective NPK fertilizer medium. Various combinations of light spectra and NPK levels were examined to optimize growth conditions. Cultures of *Chlorella* sp. were subjected to red (660 nm), blue (460 nm), and white light (380 ~ 760 nm) which acted as experimental control over a span of 10 days. Among these, blue light yielded the highest optical density at 0.687, while red light exhibited the lowest optical density at 0.349. The findings underscore that the quantity of NPK fertilizer employed as a growth medium correlate directly with the observed cellular growth in *Chlorella* sp. cultures. The study also encompassed tensile strength and biodegradability assessments to characterize the resulting bioplastics. Tensile tests disclosed that bioplastics synthesized with sorbitol displayed a lower tensile strength of 0.106 MPa, in contrast to bioplastics containing both sorbitol and chitosan, which demonstrated a tensile strength of 0.167 MPa. In conclusion, both the appropriate light wavelengths and NPK nutrients emerge as pivotal factors influencing photosynthesis and the growth of photoautotrophic microalgae. The overarching objective of this research was achieved by successfully producing bioplastics using microalgae biomass residue cultivated under optimized parameters.

KEY WORDS: bioplastics, *Chlorella* sp., light wavelength, NPK nutrient, biodegradability

INTRODUCTION

The worldwide demand for plastics has grown gradually alongside industrial advancements. Despite their adaptability and widespread availability, plastics have imposed

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a substantial toll on the environment through pollution. Since the discovery of polymers and the rise of mass production, the annual requirement for plastic has steadily risen due to its flexibility, durability, and cost-effectiveness, making it applicable across various industries. The major contributor to plastic pollution arises from inadeuqate management of macroplastics, as well as the breakdown of plastics leading to the buildup of microplastics within ecosystems (Zolotova et al., 2022). Microplastics are small fragments of artificially synthesized polymers with a diameter less than 5 mm. Due to its small size, microplastics can easily penetrate through water filter systems and enter the ocean, eventually posing a threat to aquatic wildlife and the ecosystems. Cverenkárová et al. (2021) found that microplastic particles with sizes between 1.01 and 4.75 mm have a negative impact on marine ecosystems, particularly the food chain. According to Lebreton (2018), the current area occupied by microplastics in the Pacific Ocean is only about 1% (1.6 mln km^2/165 mln km^2). Despite its easy processing and commercial feasibility, dealing with plastic waste has long been a contentious issue. The implementation of plastic waste recycling programs alone is insufficient in order to reduce plastic pollution. To alleviate the consumption of nonrenewable resources, biodegradable plastics are gaining acceptance as a viable alternative to fossil-based polymers on the market.

Recently, microalgae have garnered attention in the bioplastic sector for their ability to produce bioplastic components efficiently and affordably. Microalgae is the primary source of raw materials for various high-value bioproducts include vitamins, lipids, chlorophyll, and carotenoids. Niccolai et al. (2019) emphasised that *Chlorella* sp. has biotechnological potential for application in several industrial sectors because of its biochemical composition (protein 56.8%; lipids 16.9% and carbohydrates 5.9%), and pigments such as carotenoids and chlorophylls that can reach 1%–2% dry weight. Research by Arora et al. (2023) underscored the urgent need for sustainable alternatives to traditional plastics and highlighted microalgae as a promising resource for bioplastic production. These researchers have emphasized the potential of microalgae-based bioplastics in reducing environmental impacts while meeting the escalating demand for plastics in various industries.

Microalgae can produce a substantial biomass in a relatively small area. They can be cultivated in various environments, including open ponds, photobioreactors, or even wastewater streams, making them versatile and adaptable. It is worth noting that different algae species have their own specific growth requirements. Selection of suitable microalgae and cultivation methods is necessary in order to enhance the economic feasibility in bioplastic production. The type of microbe and its strain also have a significant impact on the quality and quantity of the bioplastic generated. Therefore, proper adjustment in the growth parameters will be made primarily depending on the microalgae of interest and the cultivation purposes such as to obtain maximum biomass productivities or optimal metabolite production. Despite the advantageous properties of microalgae, their entry into the market is hindered by the complexity of their production methods in comparison to petroleum-based alternatives. The initial setup and ongoing maintenance expenses associated with microalgae cultivation systems can be substantial, particularly in the case of

advanced photobioreactors and controlled environmental systems. The operation and upkeep of essential growth parameters such as lighting, temperature control, and aeration systems can result in a significant energy expenditure, thereby contributing to elevated operational costs. High operational costs accompanied with scale-up issues make microalgae cultivation less profitable and sustainable. One strategy to overcome the limitations of using microalgae in the bioplastic industry involves accelerating their growth rate to compensate for their lower cell density and intricate extraction processes. Achieving higher microalgae production in a shorter timeframe necessitates significant efforts.

As a response to these challenges, this study embarks on an exploration of the growth parameters of *Chlorella* sp. with the aim of generating biodegradable bioplastics. Referring to ~~a~~ research conducted by Khalili et al. (2015), which explored innovative strategies for enhancing microalgae growth rates, the present research delves into the influence of different nitrogen sources, LED light wavelengths, and light intensities on the biomass production of *Chlorella* sp. This investigation aligns closely with the objectives of this study. This study focuses on investigating the optimal growth of *Chlorella* sp. in an open pond system by manipulating the different LED light wavelengths and NPK concentrations. These factors significantly impact photosynthesis efficiency, biomass production, and bioplastic synthesis. Hence, this study seeks to fine-tune these growth parameters as it became crucial in augmenting microalgae productivity and achieving economically sustainable large-scale production of biodegradable bioplastics.

MATERIALS AND METHODS

Microalgae collection and identification

This research experiment used 1.5 L plastic bottles to obtain microalgae samples. The starter culture algae samples were collected from ponds with visible microalgal population, at Jeli Hot Spring (5.66316° N, 101.71661° E). Water samples were examined under a microscope for identification of microalgae.

Preparation of agar plate

2% agar (w/v) was mixed into the BBM solution. The mixture was then poured onto agar plates. The agar plates were sent or autoclaved at 126 °C for 15 min. Next, the plates were allowed to cool down before streaking.

Isolation of microalgae

Using a micropipette, small water samples were transferred to an agar plate. The Streaking method will be used to isolate the microalgae of interest. The single colony was then picked up using a loop. The isolated microalgae was then inoculated in Erlenmeyer flasks with a working volume of 0.8 L BBM that was autoclaved for 15 min at 121 °C before used for inoculation. The microalgae inoculated were incubated at 20±4 °C. Throughout the isolation process, strict aseptic techniques were used to minimize the risk of contamination. Practices included working in a clean environment (laminar flow hood), wearing sterile gloves, and using sterile tools. The cultures were periodically tested for

contamination by checking for the presence of other microorganisms (fungi). Contaminated cultures were eliminated.

Cultivation of microalgae

The inoculated microalgae were then transferred to the plant aquarium. The microalgae were cultivated for 14 days, and the temperature was controlled at 25±1 °C at pH 7.5–8.0, with NPK as culture medium, and light intensity 80 μmol·m^2/sec. Microalgal culture samples were monitored every 2 days for cellular growth rates. The growth rate and biomass productivity of *Chlorella* sp. was analyzed in optical density (OD680) using UV-spectrophotometer.

Study on the effect of different wavelengths of LED lights on growth of *Chlorella* sp.

The most optimal light spectrum for microalgae growth was determined by exposing the algae tanks to various light wavelengths: red, blue, and natural white.

Study on effect of nutrient on biomass production of *Chlorella* sp.

The effect of different nitrogen concentrations on growth of microalgae is analyzed by manipulating the amount of NPK added into the culture medium: 7.5; 8.0; 8.5 and 9.0 g/L (Mtaki et al., 2021)

Extraction of microalgae

Centrifugation is conducted to separate the microalgae from water. Microalgae were centrifuged for 15 min at 18−20 °C at a speed of 5500 rpm in order to obtain the microalgae residuals (Wong, Roma, 2021).

Preparation of bioplastic

1 g of sorbitol was added into 50 mL of distilled water in a beaker and stirred for 1 min, at a temperature of 28 °C. Then, 1 g of microalgae was combined with the mixture, stirring for 30 min, at a temperature of 88 °C before it was casted onto a petri dish. Following this, the mixture was heated in an oven at 50 °C for 3 hours. The bioplastic film was allowed to cool at room temperature before being removed from the molding. These steps were repeated with the addition of 1 g of chitosan into the bioplastic mixture (Wong, Roma, 2021)

Tensile strength test

The goal of tensile testing is to determine the characteristics of mechanical properties of the synthesized bioplastic. To test the tensile strength of the synthesized bioplastic, the bioplastics were cut down into strips with dimensions of; 7 cm in length, 3 cm in width and with varied thickness.

Biodegradability test

To determine the biodegradability of the bioplastics, the pH and moisture of soil will be measured first. 150 g of soil was added into a 250 mL beaker. The 4 × 4 cm samples of the plastics were buried aerobically in soil for 14 days. Initial weight and ultimate weight were recorded for each sample (Nissa et al., 2019).

RESULTS AND DISCUSSION

Identification of microalgae

Under a light microscope with 40x magnification, *Chlorella* sp. cells were detected with the appearance of small, green, single-celled spheres with the absence of flagella, containing a single cup-shaped chloroplast, which is responsible for photosynthesis. *Chlorella* sp. is a genus of green microalgae characterized by its unicellular and spherical morphology. These microscopic organisms typically range in size from 2 to 10 µm in diameter (Chowdury et al., 2020). The cell wall of *Chlorella* sp. is relatively thin and consists of cellulose, giving the cells a soft and flexible appearance. *Chlorella* sp. is also a type of microalgae that is capable of forming colonies. As can be seen in Fig. 1, *Chlorella* sp. formed small clusters in such a way that they can produce an environment that is conducive to their growth.

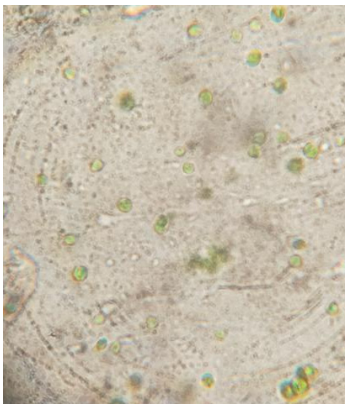


FIG. 1: Photographic view of the microalgae *Chlorella* sp. used in the present study under a light microscope with 40× magnification

Optical density of *Chlorella* sp. in different light wavelengths

The monitoring of water quality was one of the most essential aspects of *Chlorella* sp. culture management. The temperature and the pH were the two most critical factors that were measured during the course of this experiment. Microalgae colony populations are extremely sensitive to changes in the culture conditions, which can include light intensity, temperature, pH, and other factors. For the water quality monitoring, water parameters were measured every two days, as referred to in the literature (Baidya et al., 2021).

The parameters of temperature and pH were analyzed throughout the experiment (Table 1). The temperature of the water was measured every other day, and the results demonstrate that there is no significant difference in temperature between the tanks or the days. During the course of the experiment, there were no discernible variations in any of these parameters between the various treatments.

During the process of growing *Chlorella* sp., the temperature and pH varied from a range of 23.80 to 24.75 °C and from 6.0 to 8.1, respectively. In terms of temperature, the highest value of 25.60 °C was measured on the eighth day of culture under the white light treatment, whereas the initial day under the red-light treatment produced the lowest value of 23.80 °C.

TABLE 1: Physico- chemical parameters of *Chlorella* sp. medium under different wavelengths of LEDs light treatment

Treatment	Conditions	Sampling time, day					
		Initial	**2**	**4**	**6**	**8**	**10**
White	Temperature, °C	23.85 ± 0.05	24.12 ± 0.01	24.72 ± 0.01	24.78 ± 0.03	25.60 ± 0.03	24.35 ± 0.20
	pH	6.9	7.2	7.5	7.5	7.8	8.1
Red	Temperature, °C	23.80 ± 0.02	24.22 ± 0.01	24.35 ± 0.03	24.63 ± 0.05	25.19 ± 0.02	24.15 ± 0.05
	pH	6.8	7.2	7.4	7.5	7.8	7.9
Blue	Temperature, °C	23.82 ± 0.05	24.21 ± 0.05	24.73± 0.02	24.89 ± 0.05	25.12 ± 0.01	24.75 ± 0.08
	pH	6.9	7.3	7.5	7.7	7.9	8.1

TABLE 2: Optical density of *Chlorella* sp. culture under different light wavelengths treatment

Light treatment	Sampling time, day					
	Initial	**2**	**4**	**6**	**8**	**10**
White	0.166	0.193	0.288	0.381	0.394	0.531
Red	0.159	0.184	0.247	0.265	0.314	0.349
Blue	0.161	0.197	0.381	0.592	0.658	0.687

The results of the optical density of *Chlorella* sp. under white light, red light, and blue light using a spectrophotometer (OD680) are presented in Table 2. The initial optical density under white light, red light, and blue light were OD 0.166; 0.159, and 0.161 respectively.

Based on Fig. 2, on the second day, the increase in growth rate is insignificant, as the growth is still at a lag phase. On the fourth day, an insignificant increase in growth can be detected, as the optical density of blue light increased from 0.197 to 0.381 (from 2nd to 4th day). The highest optical density obtained was DO 0.687 from culture under blue light treatment, whereas the lowest was DO 0.349 from culture under red light treatment. The results of the analysis show that the different light wavelengths have significant effects on microalgae growth.

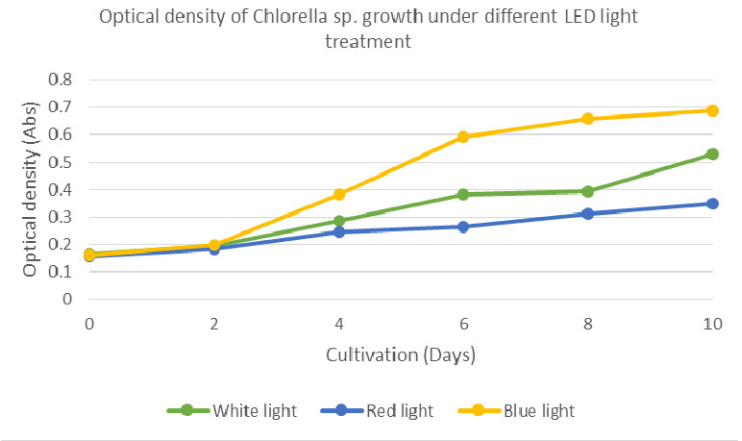
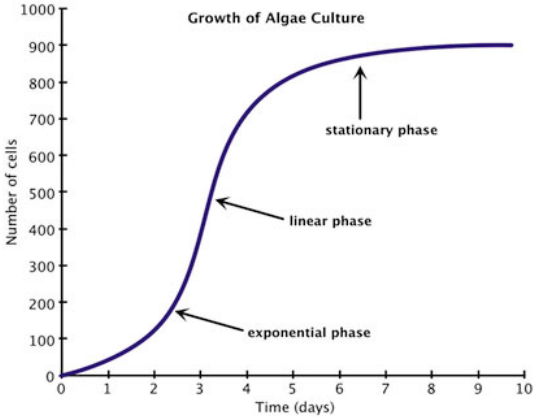


FIG. 2: *Chlorella* sp. Growth curve under different LED lights treatment

FIG. 3: Standard growth curves of algae culture



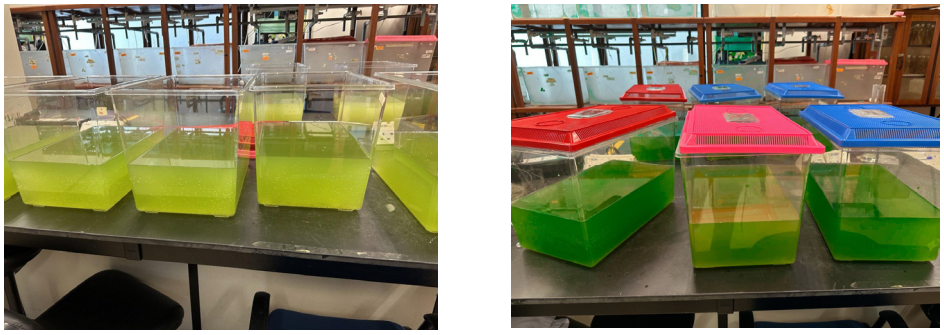
The growth curves of all three different light treatments displayed a similar growth pattern to the general growth displayed in Fig. 3.

The *Chlorella* sp. that was cultivated under blue light with a wavelength of 460 nm had the highest optical density value of 0.687 on the 10th day. On the other hand, the *Chlorella* sp. that was cultivated under white light with a wavelength range of 380–760 nm had a lower optical density value of OD 0.531, while the red light yielded the lowest optical density value of OD 0.349. Microalgae cultivation involves four growth stages: lag phase, logarithmic phase, stationary phase, and death phase. The logarithmic phase experiences exponential growth rate based on favorable conditions like medium, temperature, light intensity, pH, and dissolved oxygen. Based on the results of experimental data, blue light is the most beneficial for chlorella growth, while red light is the least beneficial. After the 10th day onwards, the transition to the death phase occurred due to a reduction in the number of cell divisions caused by factors such as extreme pH, abundance of dissolved carbon dioxide

and oxygen, intense light, and fungal contamination. A study by Hancheng G. and Fang Z. (2020) shows that blue light is the most favorable condition for *Chlorella pyrenoidosa* growth, while the present study shows relatively lower results due to different light intensity. Chlorophyll plays a vital role in photosynthesis, and its concentration and composition in the alga determine the amount of light absorbed and converted into chemical energy through photosynthesis.

Effectof different amounts of NPK on *Chlorella* sp. growth

Fig. 4 presents the color of the culture medium as it transitions from pale light green to dark green from the initial day to day 10. The transition in color is most likely attributed to an accumulation in the chlorophyll pigments which may reflect a change in their physiological status, transiting from exponential to stationary growth phase.



Initial day **Day 10**

FIG. 4: Photographic view of the batch cultivation for *Chlorella* sp. with time period

The growth parameters analyzed were optical density to determine the growth productivity of *Chlorella* sp. culture. Fig. 5 shows the *Chlorella* sp. Growth patterns display the general characteristics of growth curves (lag, exponential and stationary phases).

Microalgae require nutrients to sustain their growth. In order to achieve the optimal growth for an industrial scale, their cultivation in the lab requires a medium that is economically sustainable and rich in both major and minor nutrients. According to Fig. 5 and Table 3, the NPK nutrient at a concentration of 9.0 g/L has the greatest optical density value of 1.351. The lowest optical density measurement recorded with 7.5 g/L NPK is OD 0.52. In the meantime, NPK media containing 8.0 g/L and 8.5 g/L yielded optical densities of 0.89 and 1.078, respectively. The highest optical density value of OD 1.351 was obtained with 9.0 g/L of NPK nutrient, which was over 582.32% higher than the initial measurement. The lowest optical density was OD 0.525 with 7.5 g/L of NPK. Mediums with 8.0 g/L and 8.5 g/L of NPK showed a 340.82% and 401.40% increase in growth performance, respectively, compared to the initial cultivation. The production of cells for the microalgae with increasing amounts of NPK is observed.

TABLE 3: Optical density of cell culture with different amount of NPK fertilizer at 680 nm

Treatments with different amount of NPK, g/L	Sampling time, day					
	Initial	**2**	**4**	**6**	**8**	**10**
7.5	0.198	0.297	0.363	0.441	0.472	0.526
8.0	0.196	0.343	0.471	0.589	0.741	0.894
8.5	0.215	0.347	0.501	0.664	0.825	1.076
9.0	0.198	0.331	0.526	0.678	0.975	1.351

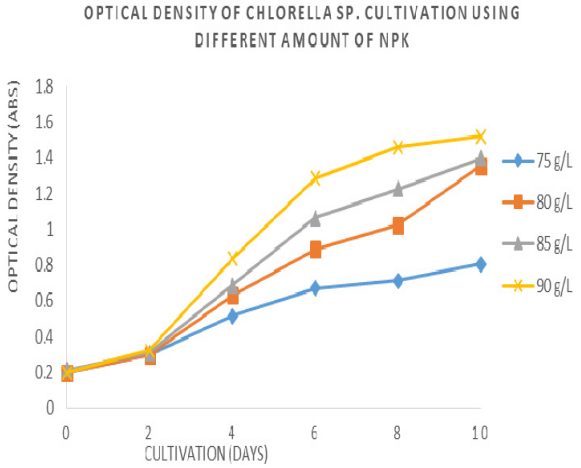
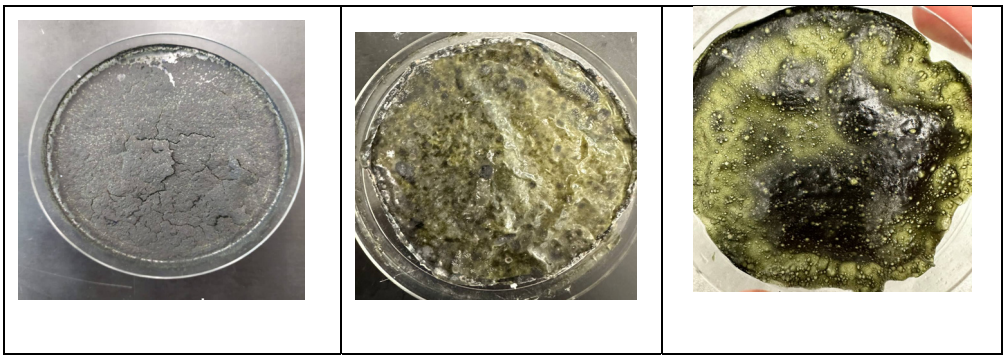


FIG. 5: Optical density of *Chlorella* sp. cell culture with different amount of NPK fertilizer at 680 nm

Moderate optimization is important as an excess of NPK can cause overgrowth of microalgae, which induces a eutrophication process. Compared to previous studies, death phases are expected on the 14th day, however, the cultures showed visible signs of fungal contamination on the 12th day. Due to this issue, analysis was only conducted until the 10th day.

Therefore, the use of moderate amounts of NPK (8 g/L and 8.5 g/L) as a medium formicroalgae cultivation on a wide scale is recommended.

A prior study done by Azrina et al. (2021) showed that a low-cost fertilizer medium with 2 g/L NPK and Jourdan modified medium yielded the most biomass (OD 0.68), which was corroborated by the present study. However, the values obtained in this study were much higher, possibly due to differences in media composition and concentration used.



A B C

FIG. 6: Photographic image of sample *A* (synthesized with no plasticizer); *B* (synthesized bioplastic with sorbitol), and *C* (synthesized bioplastic with sorbitol)

The synthesis of *Chlorella* based bioplastic

Table 4 and Fig. 6 describe the visual evaluation and physical properties of the synthesized bioplastic films. Sample A is a control sample, synthesized using *Chlorella* powder and cassava starch only. The bioplastic (sample A) that was created without the addition of a plasticizer was hard, brittle, and fragile. They shatter into pieces, making it more difficult to peel and handle them. This is due to the strong intramolecular hydrogen bonds, which grant the macromolecular chains less mobility. Consequently, the further testing of sample A was not conducted due to the difficulty of handling fragile cracked films. The formation of bioplastic film using this formulation was not successful, as the film cracked, due to its fragile, and brittle characteristics.

TABLE 4: Physical properties of synthesized bioplastics with different formulation

Sample	Type of plasticizer(s) used	Appearance	Thickness, mm	Weight, g
A	None	Cracked, fragile, brittle	-	-
B	Sorbitol	Harder to peel, bendable, dull surface	0.28	0.1566
C	Sorbitol and chitosan	Easy to peel, bendable, glossier surface	0.34	0.1864

Sample B is a bioplastic formulated with sorbitol. The bioplastic film can be successfully formed, although the film is hard to peel. It was able to bend and had a dull surface. The bioplastic had a weight of 0.28 g and thickness of 0.1566 mm. This demonstrates that the inclusion of sorbitol improves the mobility of the macromolecular

segments, which ultimately results in the bioplastic films exhibiting a much-increased degree of flexibility.

The bioplastic formulated with sorbitol and chitosan (sample C) was able to form a bendable and glossy film with a thickness of 0.34 mm and weight of 0.1865 g. The appearance of sample C appears to be glossier and more bendable.

Mechanical property of synthesized bioplastic

Based on Table 5, sample B obtained the tensile strength of 0.106 mPa. Sample C, on the other hand, obtained a higher value of 0.167 mPa tensile strength.

TABLE 5: Tensile strength of synthesized bioplastics

Sample	Tensile strength, mPa
B	0.106
C	0.167

To determine the tensile strength of the synthesized plastics, a tensile examination of several plasticized films was performed. Bioplastics formed without sorbitol or chitosan cracked, making it impossible to conduct testing. The bioplastic film with sorbitol was found to have a tensile strength of 0.116 mPa, while the bioplastic containing sorbitol and chitosan exhibited a tensile strength of 0.147 mPa. These results were similar to the study conducted by Dianursanti and Noviasari (2018), which revealed that starch talas bioplastic film had a higher tensile strength than other plasticizers. The addition of chitosan to starch strengthened the intermolecular hydrogen bond between the NH_3^+ of the chitosan backbone and OH^-. This resulted in a stronger molecule link which necessitated a greater amount of energy to be used in order to rupture the film. This result also supports the study conducted by Utami (2021) as his mung bean derived starch based bioplastics with 40% chitosan showed the highest tensile strength as compared to the bioplastics without the addition of chitosan. The addition of chitosan to bioplastics is beneficial as it has several beneficial properties, such as biodegradability, biocompatibility, and antibacterial activity. When chitosan is added to a bioplastic, it can improve the material's mechanical strength, making them more sustainable and environmentally friendly alternatives to traditional plastics.

Biodegradability test of the synthesized bioplastics

The results of the biodegradability test using the aerobic soil burial method are shown in Table 6. The outcomes of the biodegradability test conducted using the SBT method are depicted in Table 6. It is clear that the synthesized bioplastic experienced 100% biodegradability, as both of the bioplastic films totally disintegrated over the course of the burial. A natural polymer that had a hydroxyl group (-OH) is the most important component of a naturally biodegradable; as a result, this group was able to be easily degraded by microorganisms (Chamas et al., 2020). The temperature, oxygen content, relative humidity, and microbiological conditions of polymeric materials have a significant

impact on both the rate and the process of biodegradation of plastic materials. These results are consistent with those of Barragán et al. (2016), who reported that they only recovered >10% of the PHB film after 180 days in loam soil at room temperature.

TABLE 6: Biodegradability of the synthesized bioplastics

Sample	Initial weight	Final weight	Biodegradability
B	0.1566	0.00	100%
C	0.1864	0.00	100%

CONCLUSIONS

Both the appropriate wavelength of light and the presence of NPK nutrients are significant factors influencing the processes of photosynthesis and the development of photoautotrophic microalgae, which are also harnessed for energy production and the generation of chemical energy. The findings of the current study indicate that blue light with a wavelength of 460 nm is the optimal light spectrum for cultivating *Chlorella* sp. Furthermore, the intensity and patterns of light also directly impact the growth and photosynthesis mechanisms of microalgae. The empirical data demonstrated a direct correlation between the amount of NPK utilized in the culture medium and the growth of microalgae. However, excessive NPK quantities might lead to rapid overgrowth of microalgae, negatively affecting the quality of the culture medium.

The central aim of this research is to successfully synthesize bioplastics using microalgae biomass residue cultivated under optimized parameters. The incorporation of chitosan as a plasticizer into bioplastics has been proven to enhance the tensile strength properties. Additionally, biodegradability testing of the *Chlorella*-based bioplastics formulated with sorbitol and chitosan revealed their rapid degradation compared to petroleum-derived plastics, emphasizing the importance of optimal light intensity, color, and wavelengths for fostering a more sustainable microalgal production process.

However, the research is limited in terms of microalgae harvesting. The centrifugation method employed for harvesting microalgae is time-consuming, especially when dealing with high culture volumes and lacking access to large-scale centrifuges. Overcoming this challenge requires the adoption of flocculation methods for harvesting large-scale microalgae suspensions. One potential approach involves introducing a flocculant into the growth medium during microalgae cultivation and then triggering flocculation externally. According to Eyley (2015), pH adjustments can modify the surface charge of flocculants, facilitating flocculation management. Alkaline flocculation, for instance, allows for pH-induced reversible flocculation and deflocculation of algal biomass. Employing a fluctuation-based method could potentially reduce the time required for harvesting.

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