

## Synthesis and Characterization of Bioplastic Derived from *Chlorella* sp. Residue Biomass

Wong Y.C. & Chan M.Y.\*

Bioproduct and Bioprocessing Technology Research Group, Faculty of Bioengineering and Technology, University Malaysia Kelantan, Jeli Campus, Jeli 17600, Kelantan, Malaysia

\* yeeching@umk.edu.my

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**ABSTRACT:** The accumulation of plastic waste is a significant global pollution issue in the age of globalization. Despite their irreplaceable properties and multiple applications, conventional fossil-based plastics cannot decompose naturally in the environment, resulting in exponential accumulation over time. To address this problem, biodegradable and biobased plastics have been introduced as alternatives to fossil-based plastics. This study aimed to characterize bioplastics formulated from *Chlorella* sp. microalgae biomass. Various formulations of biodegradable plastics were developed using microalgae *Chlorella* sp. and tapioca starch, with varying levels of plasticizer glycerol or sorbitol (25 wt%, 50 wt%, or 75 wt% based on the weight of starch) and the addition of chitosan as a filler. The physical, mechanical, and chemical properties of bioplastics were characterized. The study found that the *Chlorella* bioplastics were ductile and had low elasticity, despite the addition of glycerol or sorbitol. However, the bioplastic exhibited an exceptional rate of biodegradability, with 100% weight loss when buried in loamy soil for 7 days. Furthermore, the *Chlorella* bioplastic with 75 wt% of glycerol, without the addition of chitosan, exhibited the highest solubility at 62.35%. The results indicated that increasing the amount of plasticizer tended to increase solubility, with glycerol having a more significant effect than sorbitol. Meanwhile, chitosan loading reduced bioplastic solubility.

**KEY WORDS:** bioplastic, *Chlorella* sp., glycerol, sorbitol, chitosan

### INTRODUCTION

Plastic is a synthetic or semi-synthetic substance made of polymers, with the majority of plastics produced today being made from fossil fuels. Plastics are versatile in shape and protective properties, allowing for the mass production of plastic packaging for food and beverages, such as bottles and containers. However, single-use plastics waste, including eating utensils, containers, and plastic bags, has significantly increased after the COVID-19 pandemic (Lai et al., 2022), posing a threat to the environment and human health due to non-biodegradable properties and the emission of greenhouse gases.

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To achieve a sustainable biobased economy, the feasibility of algae as a biomass feedstock was analyzed. Algae biomass possesses advantages over first- and second-generation biomass, such as low cost, high renewable energy outcome, high surface productivity, and the ability to grow on non-agricultural land and waste resources. In this study, the microalgae were utilized as the residue biomass in bioplastic synthesis instead of macroalgae. This is because microalgae contain high levels of starch, cellulose, polyhydroxyalkanoates (PHA), and polyhydroxybutyrate (PHB) content that can be utilized in the production of bioplastic (Chia et al., 2020). Microalgae biomass can be used directly for bioplastic production by blending it with other polymers or cultivating it under stress conditions to produce PHA, a thermoplastic or elastomeric material (Cinar et al., 2020). Meanwhile, the *Chlorella* can be a sustainable feedstock in bioplastic production due to its complex heteropolymer protein (Devades et al., 2021). The amino acids in heteropolymer are connected to each other by peptide bonds, thus showing better mechanical properties compared to polysaccharides.

According to Carissimi et al. (2018), the bioplastic with microalgae *Heterochlorella luteoviridis* J. Neustupa, Y. Nemcova, M. Eliás & P. Skaloud or *Dunaliella tertiolecta* Butcher extracts tend to enhance the antioxidant properties, which make it a potential alternative as food packaging. Meanwhile, the findings of Dianursanti and Noviasari (2018) discovered *Spirulina platensis* Turp. as a biopolymer material with high protein content. It was found that the bioplastic had higher tensile strength, but lower elongation compared to the conventional plastic bag.

This study synthesized microalgae bioplastics from microalgae *Chlorella* residue with varying amounts (25 wt%, 50 wt%, and 75 wt%) and types of plasticizers (glycerol and sorbitol), as well as the addition of chitosan as a filler. The synthesized bioplastics were characterized through tensile tests, water solubility tests, and biodegradability tests.

## MATERIALS AND METHODS

### Identification of microalgae species

One liter of water samples were collected from Jeli Hot Spring (5.66316° N, 101.71661° E) by using a plastic bottle. The samples were then observed under microscope for morphological screening process and the microalgae species were determined by comparing with Algae Identification lab guide (Nancy, Mai-Linh, 2011).

### Isolation of microalgae

Agar plates were prepared by dissolving 2% agar (*w/v*) in BBM. The composition and formulation of BBM is shown in Table 1. The plates were autoclaved at 126 °C for 15 min. The agar plates were allowed to cool for at least 72 hours before using and kept in an

inverted position to avoid the accumulation of condensation on the agar. For the isolation of microalgae, 10 L water samples were transferred in the agar plate by using a micropipette. The suspension was spread over the plate by using a streaking method with aseptic technique applied. After that, the agar plates were incubated in an inverted position for 10 days at 25 °C. The isolated microalgae were inoculated in Erlenmeyer flasks with a working volume of 0.8 L medium.

**TABLE 1:** Composition of Bold’s Basal medium (BBM) per litre (Canadian Phycological Culture Centre)

Basal medium	Components	Stock solution	Amount used in final medium
Chemicals	KH <sub>2</sub> PO <sub>4</sub>	8.75 g/500 mL	10.0 mL/L
	CaCl <sub>2</sub> · 2H <sub>2</sub> O	12.5 g/500 mL	1.0 mL/L
	MgSO <sub>4</sub> · 7H <sub>2</sub> O	37.5 g/500 mL	1.0 mL/L
	NaNO <sub>3</sub>	125.0 g/500 mL	1.0 mL/L
	K <sub>2</sub> HPO <sub>4</sub>	37.5 g/500 mL	1.0 mL/L
	NaCl	12.5 g/500 mL	1.0 mL/L
	Na <sub>2</sub> EDTA · 2H <sub>2</sub> O	10.0 g/L	1.0 mL/L
	FeSO <sub>4</sub> · 7H <sub>2</sub> O	4.98 g/L	1.0 mL/L
	H <sub>3</sub> BO <sub>3</sub>	5.75 g/500 mL	0.7 mL/L
	KOH	6.2 g/L	–
	H <sub>2</sub> SO <sub>4</sub>	1.0 mL/L	–
Trace metal solution	H <sub>3</sub> BO <sub>3</sub>	–	2.86 g/L
	MnCl <sub>2</sub> · 4H <sub>2</sub> O	–	1.81 g/L
	ZnSO <sub>4</sub> · 7H <sub>2</sub> O	–	0.222 g/L
	Na <sub>2</sub> MoO <sub>4</sub> · 2H <sub>2</sub> O	–	0.390 g/L
	CuSO <sub>4</sub> · 5H <sub>2</sub> O	–	0.079 g/L
	Co(NO <sub>3</sub> ) <sub>2</sub> · 6H <sub>2</sub> O	–	0.0494 g/L

### Cultivation of microalgae

After that, the microalgae were then transferred in a clear plastic aquarium with NPK as culture medium for cultivation. The microalgae cultivation was conducted with following specifications: temperature at  $25 \pm 1$  °C, pH 7.5–8.0, under light intensity  $80 \text{ mol/m}^2/\text{sec}$  for 14 days.

### Harvesting of microalgae biomass

After cultivation, the microalgae were harvested from the water medium and centrifuged at 6000 rpm for 15 min. The supernatant was discarded and the microalgae residues were dried in the oven overnight at 60 °C.

### Synthesis of Bioplastic

First, 1% chitosan solution was prepared by dissolving 1 g of chitosan in a 100 mL solution of 5% acetic acid at 50 °C while stirring. The microalgae residue and tapioca starch were mixed with distilled water with microalgae residue: starch: distilled water ratio of 1.9 : 3.8 : 94.3. The final volume of the mixture was maintained at 100 mL. The solution was heated on a hot plate at 80 °C and stirred with a glass rod. During heating, 1 mL chitosan solution and glycerol with varied amounts (25 wt%, 50 wt%, 75 wt% toward starch weight) were then added into the mixture. The mixture was continuously stirred until a gel-like structure was formed. 20 mL of the viscous suspension was poured into a petri dish for moulding. The procedures were repeated by replacing the glycerol with sorbitol of different formulations (Table 2). The suspension was dried in an oven at 50 °C for 3 hours and peeled off from the petri dish after drying.

TABLE 2: The formulation of bioplastic samples

Sample	Amount				
	Microalgae residue, g	(Tapioca) starch, g	Distilled water, mL	Plasticizer, g	Filler (chitosan), mL
Control	1.9	3.8	94.3	–	–
G1	1.9	3.8	94.3	G 0.5	0
G2	1.9	3.8	94.3	G 0.5	1.0
G3	1.9	3.8	94.3	G 1.0	0
G4	1.9	3.8	94.3	G 1.0	1.0
G5	1.9	3.8	94.3	G 1.5	0
G6	1.9	3.8	94.3	G 1.5	1.0

S1	1.9	3.8	94.3	S 0.5	0
S2	1.9	3.8	94.3	S 0.5	1.0
S3	1.9	3.8	94.3	S 1.0	0
S4	1.9	3.8	94.3	S 1.0	1.0
S5	1.9	3.8	94.3	S 1.5	0
S6	1.9	3.8	94.3	S 1.5	1.0

\*G – represents glycerol; S – represents sorbitol.

### **Mechanical analysis**

The mechanical properties were analysed by measuring the tensile strength and elongation rate of bioplastic by using a universal tensile testing machine according to the ASTM D638 and ASTM D882 standard. The bioplastic was cut into a  $7 \times 2$  cm strip and the thickness of the film was measured and recorded by using a digital vernier calliper. The thickness was measured by holding the film between the external jaws and reading the value directly. The strip of film was held on the hydraulic grip and the tensile testing was performed with 1.5 mm/min crosshead speed until the breaking point was reached. The tensile strength and elongation at peak were recorded and tabulated.

### **Biodegradability test**

The biodegradable properties of bioplastic were tested via aerobic soil burial degradation test according to the ASTM D5338 standard. The bioplastic was first cut into  $4 \times 2$  cm. The strips of bioplastic were buried in the garden soil at 6 cm depth and left inside the room for 7 days. The soil was collected to study the moisture and pH of the soil. The characteristics of the soil were recorded and tabulated. After that, the initial weight and final weight after the biodegradation test were recorded and tabulated. The biodegradability of the sample was calculated by using the formula in Equation 1.

$$\text{Weight loss (\%)} = (W_i - W_f) / W_i \times 100 \quad (\text{eq. 1})$$

### **Water solubility test**

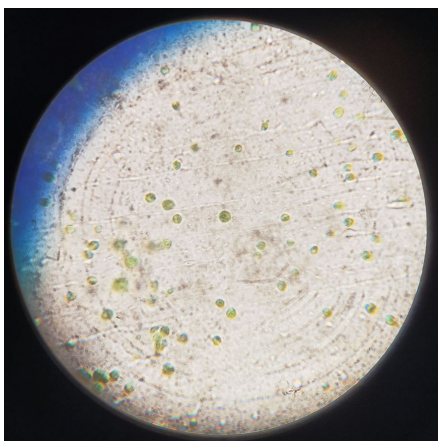
The bioplastic was first cut into  $4 \times 2$  cm and immersed in 50 mL of distilled water for 24 h. After that, the solution was filtered, and the residue was dried in the oven at  $60^\circ\text{C}$  until a constant weight was achieved. The initial weight and final weight after drying were recorded and tabulated. The solubility of the bioplastic was calculated by using equation 2.

$$\text{Solubility (\%)} = (W_f - W_i) / W_i \times 100 \quad (\text{eq. 2})$$

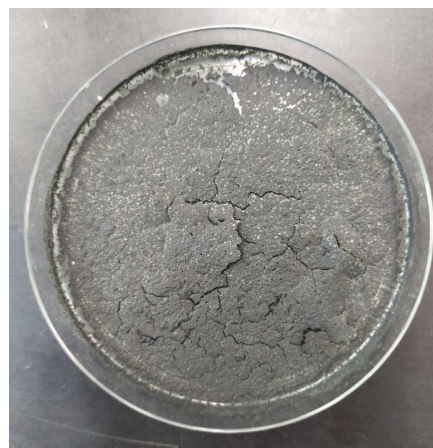
## RESULTS AND DISCUSSION

### Identification of microalgae species

The morphology of microalgae was identified under microscope at 40x magnification. The culture of *Chlorella* sp. was represented by small spherical green cells without flagella and often solitary (Nancy, Mai-Linh, 2011).



**FIG. 1:** Cells under microscope at 40x magnification



**FIG. 2:** Control sample without addition of plasticizer and filler

### Synthesis of bioplastic

The control sample formulated without plasticizer and filler was fragile and brittle, causing it to be difficult to peel off completely (Figs 1 and 2). Additionally, the bioplastics without chitosan showed a higher level of saturated green color, whereas those with chitosan had a darker green appearance. The drying rate of the films varied depending on the type of plasticizer used, with films containing glycerol requiring a longer drying time than those containing sorbitol. As a result, bioplastics with a higher concentration of glycerol were soft and highly flexible, while those with sorbitol were harder, stronger, and more flexible, making them easier to peel off after drying.

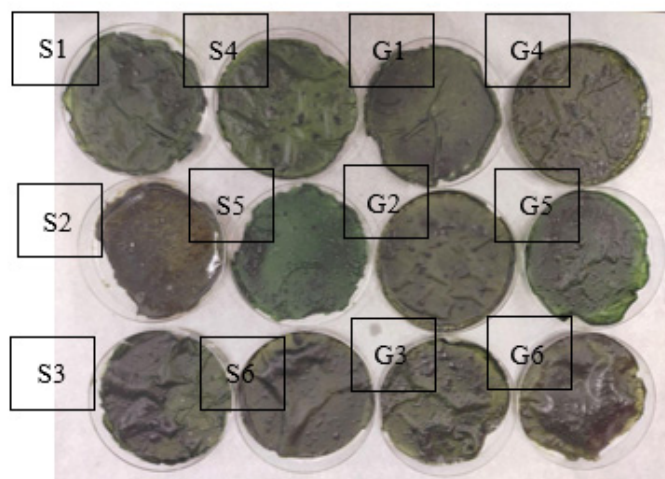
Microalgae biomass can be used as the main ingredient or an additive for bioplastic synthesis due to its high carbohydrate and lipid content. Carissimi et al. (2018) found that adding microalgae biomass from *Heterochlorella luteoviridis* and *Dunaliella tertiolecta* enhances biodegradability, solubility, opacity, elongation, and rupture of bioplastic while reducing its tensile strength. In this study, tapioca starch was selected as the biopolymer to produce blended bioplastics with microalgae to improve plastic properties. Tapioca starch contains approximately 16.7% amylose and 83.3% amylopectin, which is responsible for the gelatinization during bioplastic formation, and its high viscosity makes it an excellent binder.

However, films made from pure starch and microalgae biomass were brittle and lacked plastic properties. Therefore, *Chlorella*-based bioplastics were formulated with plasticizers ranging from 25 wt% to 75 wt% of the weight of starch to increase flexibility and extensibility by dissolving in each polymer chain. Bioplastics made without plasticizer and filler were brittle and rigid, unable to detach from the petri dish due to a lack of flexibility and plasticity. Meanwhile, excessive plasticizer caused the plastic to be greasy and difficult to dry completely, leading to very flexible, soft, and sticky films. Moisture content is also critical to avoid microbial or fungal growth, as low moisture content reduces the potential for growth. However, an adequate moisture level is necessary to manifest the plastic properties of bioplastic, and overdrying or extensive low moisture content can result in brittle properties.

### **Mechanical analysis**

The mechanical properties of bioplastic samples were measured by referring to the ASTM D638 standard which a tensile force is applied to a sample and analyzing various properties of the sample under stress. The measures taken in this test included tensile strength, elongation at break and Young's modulus (Table 3).

Figure 3 suggests that materials that are brittle can tolerate more stress but exhibit limited elasticity. Conversely, ductile materials are more elastic and display a linear stress-strain relationship. However, once the ductile materials have reached their elastic limit, they cannot return to their original shape. Nevertheless, they may reach their ultimate tensile strength at the second peak before breaking. In contrast, plastic materials often exhibit the greatest tensile strain with low stress applied.



**FIG. 3:** *Chlorella*-based bioplastics with twelve different formulations of plasticizer and filler

**TABLE 3:** The mechanical properties of bioplastic are based on its thickness, tensile strength, elongation at break and Young's Modulus

Sample	Thickness, mm	Tensile strength, MPa	Elongation at break, mm	Young's modulus, N/mm <sup>2</sup>
G1	0.75	0.147	4.519	1.240
G2	0.33	0.152	3.009	19.675
G3	0.34	0.147	7.908	5.082
G4	0.31	0.161	7.352	8.229
G%	0.32	–	–	–
G6	0.29	–	–	–
S1	0.13	–	–	–
S2	0.43	0.135	2.814	–
S3	0.21	0.116	9.443	4.995
S4	0.34	0.147	5.716	4.227
S5	0.23	0.217	7.872	–
S6	0.37	0.278	6.457	21.245

In this experiment, the mechanical properties of *Chlorella*-based bioplastics were found to have exhibited low elasticity and ductility. The tensile strength of these bioplastics was much lower than that found in other studies, such as Lusiana et al. (2019) who obtained a tensile strength of 16.12 MPa for sago-PVA starch film with sorbitol loading, and Tan et al. (2022b) who obtained a tensile strength of 5.19 MPa with the additives 40 wt% glycerol and 20 wt% chitosan. However, Marichelvam et al. (2019) found a maximum tensile strength of 12.5 MPa for starch-based bioplastics with the combination of rice starch and cornstarch with glycerol loading. In our study, the effects of glycerol and sorbitol loading on the mechanical properties of *Chlorella*-based bioplastics were not significant. We observed that chitosan loading tended to increase the tensile strength while reducing the elongation at break of the bioplastics. The amylose content of starch polymer plays an important role in enhancing the tensile properties of bioplastics, as proposed by Marichelvam et al. (2019). They suggested the formulation of starch-based bioplastics with a total starch to water ratio of 1:10, while we used a ratio of 1.9 microalgae: 3.8 starch: 94.3



water. Therefore, the rigid and ductile properties of bioplastics can be attributed to the formulation of bioplastics with a low interstitial volume of the polymeric matrix, which reduces the polymer-polymer interactions in bioplastics. Despite the low plastic properties of the bioplastics formed, the films were found to be more flexible and softer than bioplastic without any additives. According to Tan et al. (2022a), the plasticizer interrupts the internal hydrogen bonds of the polymer chain between amylose and amylopectin, which increases the molecular spacing, enhancing the elasticity and flexibility of bioplastics.

Apart from that, the results shown that the mechanical properties of the formulated bioplastics were inconsistent while some could not be analysed. This may be caused by the uneven surface of bioplastic and defective sample used in mechanical test (Wong et al., 2021). Therefore, the defective sample will break outside the gauge length, causing inaccurate strain readings and stress measurements.

#### **Biodegradability test**

In order to examine the biodegradability of *Chlorella*-based bioplastic with different formulations, the weight loss of films is calculated. The weight loss of each film during biodegradability tests were tabulated and recorded in Table 4.

**TABLE 4:** The biodegradability of each bioplastic based on the formulation

Sample	Initial weight, g	Final weight, g	Weight loss, %
G1	0.1368	0	100
G2	0.1301	0	100
G3	0.1412	0	100
G4	0.3652	0	100
G5	0.2366	0	100
G6	0.2819	0	100
S1	0.2133	0	100
S2	0.1243	0	100
S3	0.1566	0	100
S4	0.1864	0	100
S5	0.2040	0	100
S6	0.2279	0	100

The production of biodegradable *Chlorella*-based bioplastics is an important step towards reducing the amount of plastic waste generated annually and lowering carbon emissions and greenhouse gases. These bioplastics are made from biobased materials, including microalgae and starch, which enable them to be broken down by microorganisms in composting processes into water, carbon dioxide, and biomass. When buried in soil, the films undergo biodeterioration as microorganisms attach to the surface layer, weaken the mechanical structure, and facilitate the biofragmentation process that hydrolyzes organic materials into short polymers (Table 5).

**TABLE 5:** The physicochemical properties of soil

Properties	Conditions
Type of soil	Loamy soil
Moisture (%)	44.8–49.2
pH	5.2
Acidic or alkaline	Acidic

Enzymatic reactions then break down long-chain polymers into monomers and oligomers to obtain nutrients and energy. Biodegradation rates are affected by several factors, such as moisture level, pH, oxygen, temperature, and light.

Table 3 shows that all the bioplastics had high biodegradability rates in soil, with a weight loss of 100% after seven days of burial. This biodegradation rate is higher than previous research, such as Ahimbisibwe et al. (2019), which took five to ten weeks to achieve 98.3% decomposition of cassava starch bioplastics, and Lusiana et al. (2019), which found that sago-PVA starch bioplastics with glycerol had a biodegradability of 82.38% at best.

In this experiment, the ex-situ method was used to test biodegradability in loamy soil with growing plants. Loamy soil is a mixture of sandy soil, silt soil, clay soil, and humus, commonly used in agricultural activities due to its high ability to retain moisture and nutrients. The high biodegradability of bioplastics may be due to the moisture of the soil, which allows for more water uptake by the bioplastics. Ahimbisibwe et al. (2019) found that water uptake by bioplastics creates optimal conditions for the enzymatic activity of microorganisms, thereby accelerating the biodegradation process. Bioplastics disintegrate due to soil moisture and are digested by microorganisms in the soil until they are completely degraded. Additionally, the presence of plant residue in the loamy soil enhances

the biodegradation process due to the higher level of nutrients for soil microorganisms. Finally, the biodegradability rate is affected by environmental temperature, with the test carried out in the range of 22 °C to 31 °C, which is suitable for the growth of mesophilic microorganisms.

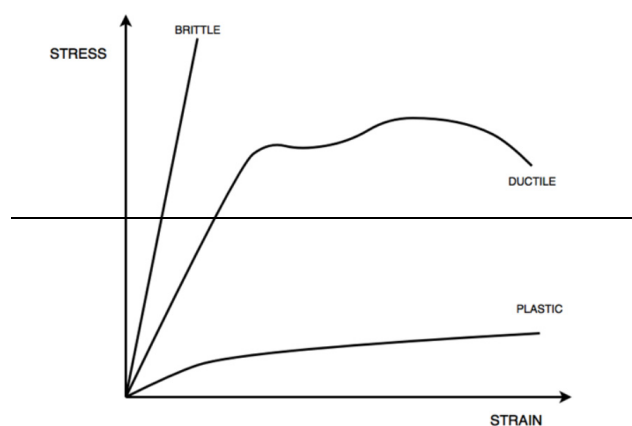
#### **Solubility test**

The solubility test was conducted by immersing the bioplastic in distilled water overnight with environmental temperature at a range of 20 °C to 25 °C and recording the initial and final weight of bioplastics. The tendency of bioplastic molecules to dissolve in the water is referred to as solubility. Therefore, the solubility of the bioplastic is calculated and tabulated in Table 6.

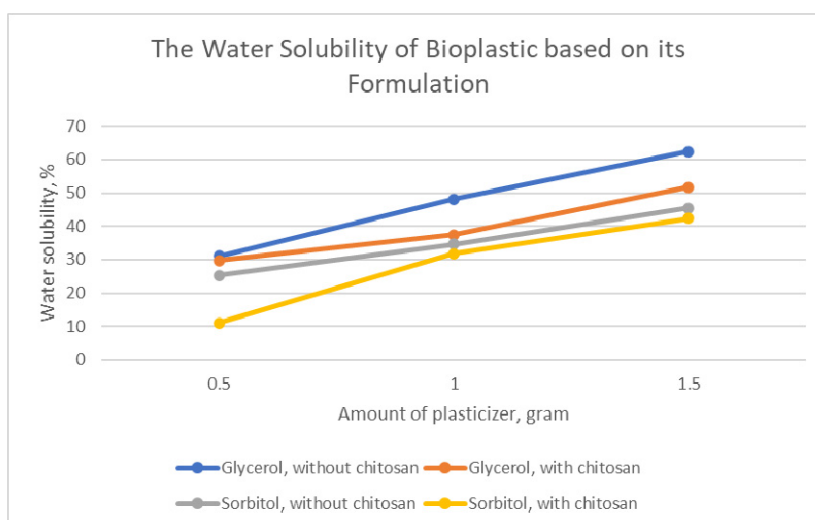
**TABLE 6:** The water solubility of each bioplastic based on the formulation

<b>Sample</b>	<b>Initial weight, g</b>	<b>Final weight, g</b>	<b>Solubility, %</b>
G1	0.1363	0.0939	31.11
G2	0.1057	0.0743	29.71
G3	0.1649	0.0855	48.15
G4	0.1668	0.1043	37.47
G5	0.2064	0.0777	62.35
G6	0.2916	0.1407	51.75
S1	0.1619	0.1201	25.26
S2	0.1132	0.1007	11.04
S3	0.1671	0.1091	34.71
S4	0.1861	0.1269	31.81
S5	0.2374	0.1296	45.41
S6	0.2348	0.1353	42.38

Figure 4 shows that bioplastics G<sub>5</sub> with 1.5 mL glycerol as a plasticizer, without the addition of chitosan solution, had the highest solubility (62.35%) among all the formulations. The average solubility of glycerol-plasticized bioplastic was 11.66% higher than that of sorbitol-plasticized bioplastic. Shafqat et al. (2021) also found that the solubility of bioplastics with glycerol was higher than that of bioplastics with sorbitol.



**FIG. 4:** Characteristics of materials based on stress-strain plot (Source: University of Birmingham)



**FIG. 5:** The water solubility of bioplastic based on the formulation

This can be attributed to glycerol's stronger affinity to water than sorbitol, which tends to bind to water by forming strong hydrogen bonds. Glycerol's lighter molecular weight also facilitates water molecules penetrating the polymer chain. Additionally, the water solubility of bioplastics rose with an increase in the amount of plasticizer used, even when chitosan solution was added. For example, the solubility of glycerol-bioplastic increased from 29.71% to 62.35%, while sorbitol-bioplastic showed an increase from 11.04% to 45.41%.

The water solubility of bioplastics is a key factor in determining their applicability and functionality in our daily lives (Fig. 5). Highly water-soluble bioplastics are considered environmentally friendly as they can help reduce the carbon footprint of plastic. These

bioplastics, made from edible materials, can be used as packaging and preservation coatings for some food products. Based on Oduro (2021), the edible coatings or films provide protection against microorganisms, gases, and moisture for fresh food products, and are both consumable and eco-friendly. However, some packaging materials may require low water solubility and absorption to increase their resistance to water or water vapor. Low solubility films can hold their shape and remain unaffected by water under specific conditions, making them suitable for use in wet environments or underwater, as well as for storing liquid products. On the other hand, plastic films with high solubility are less suitable for use in high moisture conditions despite being considered environmentally friendly.

However, adding chitosan solution to the bioplastic formulation tended to reduce the solubility of bioplastics in both glycerol-bioplastic and sorbitol-bioplastic, regardless of the type of plasticizer used. The average solubility of glycerol-bioplastic without the addition of chitosan (47.20%) was 7.56% higher than that with 1.0 mL chitosan solution (39.64%). Similarly, the sorbitol-bioplastic showed a 6.72% difference with the presence of chitosan. This is because chitosan is insoluble in water and most organic solvents due to the strong intermolecular and intramolecular hydrogen bonds between chitosan molecules. Additionally, the  $\text{NH}_3^+$  groups of chitosan bind with the  $\text{OH}^-$  groups of starch, forming intermolecular hydrogen bonds during bioplastic formation, which results in lower water affinity as water is unable to bind with either group.

## **CONCLUSIONS**

To summarize, the addition of glycerol or sorbitol can enhance the plastic properties of bioplastic made from microalgae *Chlorella* sp. and tapioca starch. All the bioplastics exhibited high biodegradability with complete weight loss after being buried in soil for 7 days. However, the highest solubility value of 62.35% was achieved with 75 wt% glycerol without chitosan. Adding chitosan solution to the bioplastic formulation reduces its solubility in water due to strong hydrogen bonds between chitosan molecules. While the biodegradability and solubility properties are favorable for the environment, they limit the use of bioplastics in various applications due to poor moisture and gas barriers. Based on the mechanical properties, which are crucial for application of bioplastics, the current formulations are not a viable alternative to conventional fossil-based plastics. Therefore, further research is recommended to improve the plastic properties of bioplastic based on microalgae biomass for suitable application.

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