

## Potential of the Biodegradability and Characteristics of Bioplastic From Microalgae Residues\*

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**ABSTRACT:** Petroleum-based plastic has been widely used in many industries. However, it takes hundreds of years to degrade and causes widespread pollution to our environment. These problems led to the invention of bioplastics, which were comprised of natural biopolymers made from starch. The production of bioplastics from food-based starches such as tapioca and corn created competition between food and bioplastic production industries. Hence, this research study focuses on producing bioplastic from microalgae residue, which is a non-food based raw material that uses four different types of plasticizers: glycerol, sorbitol, glutaraldehyde and polyethylene glycol (PEG). Microalgae species for identification were obtained from the fish pond at the University Malaysia of Kelantan, before cultivating the species for 14 days. The microalgae residues were extracted through the centrifugation process. Three species were identified under the light microscope, *Chlorella* sp., *Scenedesmus* sp. and *Monoraphidium* sp. The production of bioplastic involved a manual stirring method using a hotplate magnetic stirrer, followed by drying the bioplastic in an oven at 60 °C. Results obtained showed that sorbitol and glycerol from microalgae are suitable to be used as a plasticizer for the production of bioplastic, however glutaraldehyde and PEG are not suitable. Bioplastics that used PEG and glutaraldehyde became cracked and brittle after the drying process. The characterization of bioplastics includes universal tensile testing machines, Fourier-transform infrared analysis and biodegradability tests being processed/undertaken on glycerol-based and sorbitol based bioplastic. Characterization of bioplastics proved that both glycerol and sorbitol have high potential for applications in daily human life. Bioplastics which used sorbitol as a plasticizer could be used in can be applied the production of plastic goods such as toys and household items due to its good resistance toward stress and minimal flexibility. Meanwhile bioplastics which used glycerol as a plasticizer could be applied to the production of plastic bags and plastic food wrap due to its elastic and flexible nature.

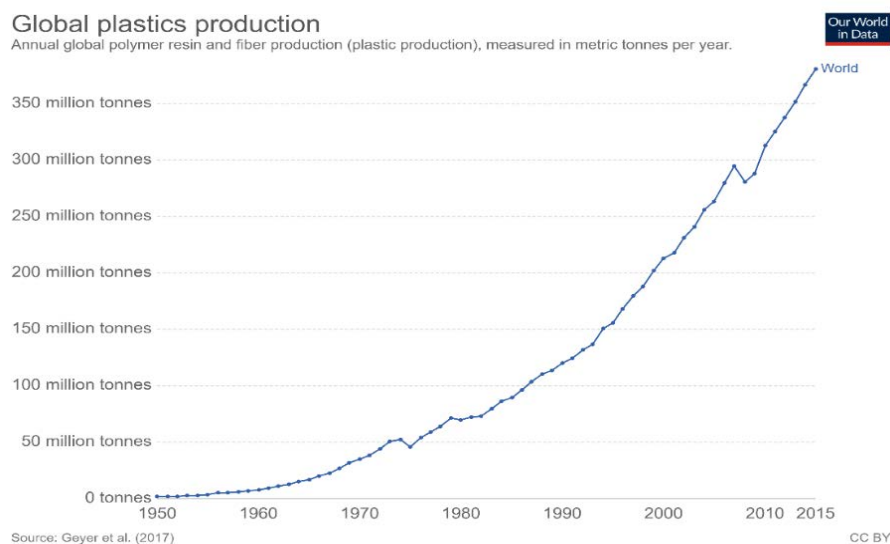
**KEY WORDS:** bioplastic, glycerol, sorbitol, microalgae, algal cultivation

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## INTRODUCTION

Plastic has come to constitute an important component of many materials which are used in everyday life within modern society. Plastic is widely used as the main material in many household items such as: carrier bags, food packaging, and utensils. The global plastics industry began in 1907 when Bakelite, the first synthetic plastic, was produced. The production of plastics began to increase in the 1950s, when global plastic production started to produce 2 million tons of plastic per year. Over the next 65 years, global plastic production increased ~200 fold, approaching 381 million tons globally in 2015 (Geyer et al., 2017; Fig. 1). This sharp increase in plastic production is worrying due to the consequences which are being caused by an over abundance of non-degradable fossil-fuel based plastic pollution in the world.



**FIG. 1:** Global plastics production from the 1950s until 2015 (Geyer et al., 2017)

The depletion of fossil fuel sources and the harmful effects of fossil fuel-based plastic on the environment, encourages the plastic production industry to produce plastic derived from bio-based materials, known as bioplastic. Bioplastics have been produced since 1941, and since then have gone through considerable innovation and development (Rajendran et al., 2012). In this research, extracted microalgae residues were used as the main source of biopolymers for the production of bioplastic. Microalgae are a renewable source; therefore a continuous supply of microalgae for the production of bioplastic can be guaranteed.

Microalgae is a non-feed stock source; the production of bioplastic from microalgae will not impact the sustainability of global food supply, nor the carbon footprint. The recent

rapid developments in the bioplastics industry have mainly been driven by using feed-stock as biopolymer sources, such as: potato starch, corn starch, wheat starch, and barley (Table 1). This is quite worrying as while it meant that bioplastics could contribute to the reduction of fossil fuel usage it threatens food availability at the same time. This research will focus on producing bioplastics from non-feedstock sources: microalgae residues.

**TABLE 1:** History of the development of bioplastic (Rajendran et al., 2012)

Year	Development
1941	Henry Ford experimented with plastics made from soya beans and produced a plastic car. World War II played an important role in the development of bioplastics
1992	Metabolix, a bioscience company provided solutions for the worlds needs for plastics, chemicals and energy
2000	Metabolix initiated research programs for the development of engineered industrial crops for the production of bioplastics
2005	Toyota began a pilot plant at Hirose plant in Toyota City, Japan to test the ease of producing bioplastics
2006	London-NEC corporation and Unitika Ltd developed a bioplastic material reinforced with fibre from the Kenaf plant to reduce the environmental impact of mobile phones
2010	Cardia bioplastics Malaysia manufacturing was developed to manufacture bioplastics products

Glycerol, sorbitol, glutaraldehyde and PEG are being used as a plasticizer for the production of bioplastics. A comparison is also made between the physical properties and morphology of the bioplastic produced. Comparison of the plasticizers is determined using the universal tensile testing machine, Fourier transform infrared spectroscopy (FTIR) analysis and biodegradable tests based on method ISO 11266 (Wang, 2014).

## MATERIALS AND METHODS

### Identification of microalgae species

10 mL of water samples were extracted from the aquarium and kept inside sample bottle for identification purpose. Microalgae species were identified using a microscope.

### Cultivation of microalgae

9 L of water sample that contained microalgae from UMK's fish pond were collected and mixed with 4 L of distilled water. The microalgae were cultivated inside a clear plastic aquarium for 14 days between the temperature of 27–30 °C, pH of 6–8, and a supply of light intensity between 4000–6000 lx, with the presence of NPK fertilizer as nutrient supply.

### **Centrifugation of microalgae**

Centrifugation was undertaken to separate the microalgae from water. Microalgae were centrifuged for 15 min at 18–20 °C with the speed of 5500 rpm.

### **Preparation of bioplastic**

Microalgae residue of 4 g, 1 g of starch, 1 mL of glycerol and 44 mL of distilled water were mixed inside a 250 mL beaker. The solution was stirred on a hot plate by using a magnetic stirrer. The solution was heated until 90 °C and stirred until it coagulated. Once the solution coagulated or became thick, 30 mL of the solution was poured into a petri dish. The petri dish was transferred into the oven for 45 min to 1 hour under the temperature of 60 °C in order to let the bioplastic to dry. The same procedures were repeated using different concentrations of microalgae residues and plasticizer, using sorbitol to replace glycerol.

### **Characterization of bioplastic. Universal tensile testing machine**

Samples were cut into 7 × 2 cm sections. The upper and lower part of the samples were pasted with 1 cm of double-sided tape to facilitate the installation of samples on to the hydraulic grip of the universal tensile testing machine. A digital calliper was used to measure the thickness of samples. The samples were pulled at a speed of 3 mm/min until breaking point. The value of elongation at break (mm), Young & Modulus (N/mm<sup>2</sup>) and stress at peak (N/mm<sup>2</sup>) were obtained and tabulated.

### **Fourier-transform infrared spectroscopy (FTIR) analysis**

Samples were cut into 3 × 2 cm and placed on the detector of FTIR. The range of wavelengths used for the analysis fell between 400 and 4000 cm<sup>-1</sup>. The results obtained were displayed graphically.

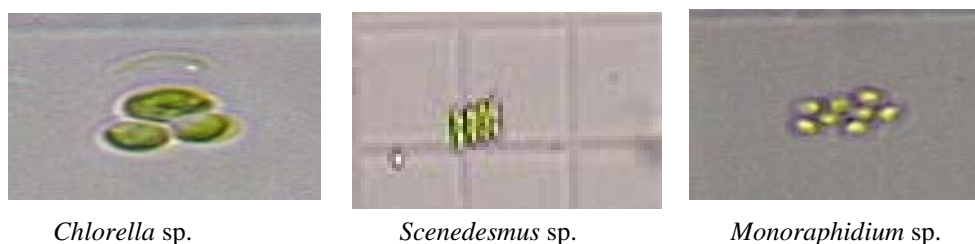
### **Biodegradable test**

Samples were cut into 5 × 3 cm and buried in the soil for 14 days. Initial weight and final weight of the samples were recorded. The pH of the soil was also checked and recorded.

## **RESULTS AND DISCUSSION**

### **Identification of microalgae species from water samples**

In this study, a mixed culture of microalgae was obtained from UMK's fish pond as the starter culture for the cultivation of microalgae. The identification of microalgae species was undertaken to observe the type of microalgae species which may be present in the water. The microalgae species present the water samples taken from the fish pond at the University Malaysia of Kelantan, Jeli Campus were: *Chlorella* sp., *Scenedesmus* sp. and *Monoraphidium* sp. (Photo).



**PHOTO:** Microalgae species discovered

The identification of the microalgae species in water samples was based on the morphological characteristics of microalgae. *Chlorella* sp. is a green microalgae with a spherical or oval shape. *Chlorella* sp., are unicellular microorganisms that are found abundantly in the fish pond, as it obtains nutrients for growth from fish's feedstock, as well as the waste that is expelled by the fish (Ramaraj et al., 2016).

*Scenedesmus* sp. is a green microalgae that grew in small chains. This species is usually found in colonies of small chains that comprises four cells in each colony, and grow on the surface of the water. This species floated on the surface of the water instead of becoming embedded at the bottom of the pond or on rocks. This species is more often found floating in the creek water and not attached to rocks on the bottom. During the sampling process, the green layer was observed on the surface of pond water hence, supporting the presence of *Scenedesmus* sp. (Unpaprom et al., 2017).

The third species identified in water samples was *Monoraphidium* sp. This green microalgae has a curved, coccoid shape, and is found in a colony of several cells (Lin et al., 2019). A fish pond can be considered as a good environment for the growth of *Monoraphidium* sp. due to an abundance of nutrients such as nitrogen, phosphorus, and iron being readily available inside the fish pond, possibly from fertilizer runoff from agricultural activities around the fish pond.

#### **Cultivation of microalgae**

The cultivation of microalgae was undertaken in the aquarium under an extreme light source. NPK fertilizer was used as a source of nutrients. NPK fertilizer is widely used in the agriculture industry for the growth of crops such as maize and coffee. The growth rate of microalgae was extensive due to the rapid diffusion of NPK fertilizer into the cells of microalgae, since the microalgae were being cultivated underwater. The rate of absorption of NPK fertilizer is higher in water compared to in soil (Ibiremo, Akanbi, 2016).

Additionally, NPK fertilizer contains 15% nitrogen, 15% phosphorus and 15% potassium, which contributes to the massive growth of microalgae (Fig. 2). This nutrient supply is enough for the growth of microalgae. The growth of microalgae underwent 4

phases: a lag phase, exponential growth phase, stationary phase, and a declining phase can be observed. This life cycle proved that the microalgae grew well with the presence of just the NPK fertilizer (Mahmood et al., 2017).

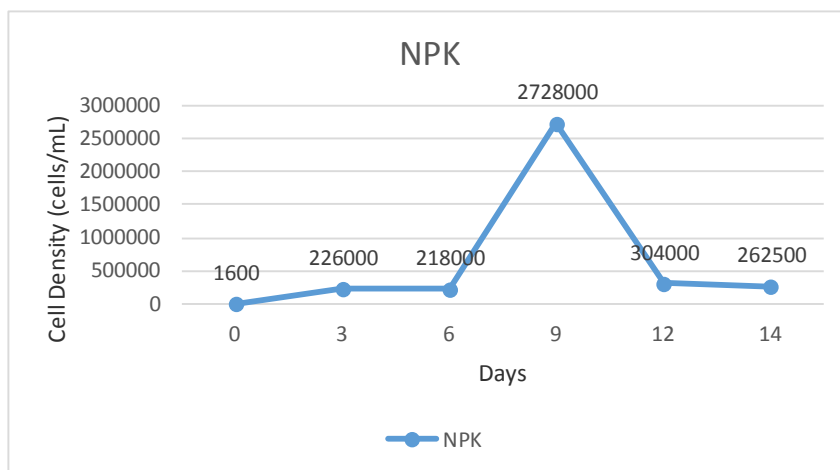


FIG. 2: Growth of microalgae species for 14 days based on cell density

#### Preparation of bioplastic thin film

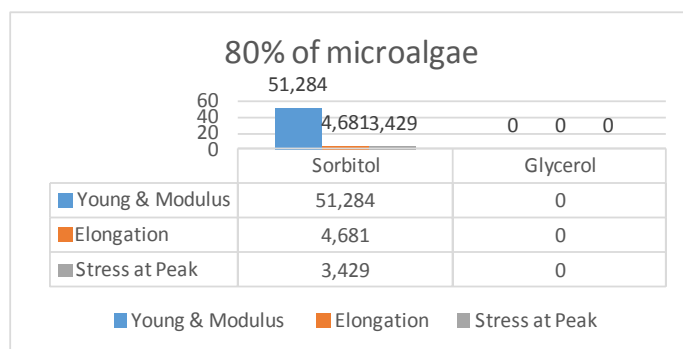
Bioplastics were prepared using four different types of plasticizer with a different ratio of microalgae residues. Based on the results obtained, a bioplastic that used glycerol and sorbitol turned out well, whereas a bioplastic which used glutaraldehyde and PEG were not viable. This is because glycerol and sorbitol consist of hydroxyl groups that can combine with the natural biopolymer in microalgae such as polyhydroxy butyrate (PHB) in order to form a bioplastic. The concentration of PHB inside microalgae is influenced by the sources of the nutrients obtained by microalgae during the cultivation process. Nitrogen and phosphorus are two main nutrients needed by microalgae in order to produce a high PHB content (Martins et al., 2017). Hence, microalgae which are to be used as raw materials to produce bioplastic need to be cultivated with a sufficient amount of phosphorus and nitrogen. However, there are other useful natural biopolymers present in microalgae, such as proteins and polysaccharides. Polysaccharides can be found either in storage or cell wall (Bernaerts et al., 2019).

Solutions for bioplastic production that used glutaraldehyde as a plasticizer turned out to be dry and brittle after being dried inside the oven. However, a solution with a higher percentage of microalgae residues did not become as brittle as a solution that had a lower percentage of microalgae residues. This brittleness is thought to be caused by the presence of aldehyde groups in glutaraldehyde which combine with the hydroxyl groups to form water. Water evaporated during the drying process and the only substances left were

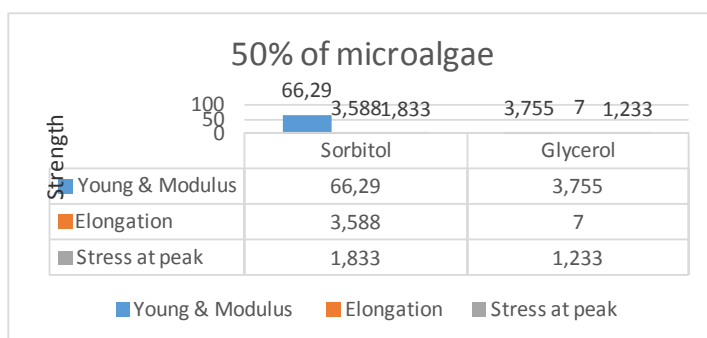
microalgae residues. The right selection of plasticizers for the production of bioplastic is very important as it affects the plasticity, structural integrity and energy required for molecular motion of the bioplastic (Vieira et al., 2011).

**Characterization of bioplastic. Mechanical properties of bioplastic using universal tensile testing machine**

Tensile strength was affected by the concentration of microalgae and types of plasticizers being used. Bioplastic made with sorbitol has stronger tensile strength compared to bioplastic using glycerol as a plasticizer. The difference in tensile strength in the formulation of 80% microalgae and 50% microalgae (for sorbitol) was significant at 1.596, thus proving that different concentrations of microalgae in preparation of bioplastic affected the tensile strength (Figs 3, 4).



**FIG. 3:** Comparing-the potential of bioplastics using different types of plasticizer but with the same ratio of microalgae residues (80%)



**FIG. 4:** Comparing the potential of bioplastics using different types of plasticizer but with the same ratio of microalgae residues (50%)

However, a bioplastic made with glycerol has a weaker tensile strength when compared to sorbitol with a significant difference in tensile strength (0.6). Sorbitol is a type of polyol that has a high molecular weight. High molecular weight increases tensile strength, Young & Modulus and also creating a bioplastic with better thermal stability (Li et al., 2011). This explains why sorbitol has better tensile strength compared to glycerol.

Elongation at break refers to the ability of a bioplastic to return to its original shape before it breaks. Bioplastic made from 50% microalgae and mixed with glycerol performed better in regard to elongation compared to bioplastics which used sorbitol as a plasticizer, which elongated two fold less. This is because glycerol contains OH bonds that can reduce the intermolecular bonds between matrixes of polymers and replace it with hydrogen bonds. Resulting in lowering the rigidity and increasing the flexibility of bioplastics (Muscat et al., 2012). Glycerol is classified under humectant-plasticizer, as it reacts with water to increase the softness and flexibility of bioplastics (Jantrawut et al., 2017). This could be a plausible reason that contributes to the failure in detecting the tensile strength, Young & Modulus and elongation of bioplastics made up from 80% of microalgae residues; due to microalgae residue in paste form being used, where it contains a high amount of water. Once glycerol is mixed with microalgae paste, it produces a very soft and fragile bioplastic (Tian et al., 2017).

Young & Modulus indicate the stiffness and strength of a bioplastic. The lower the value of Young & Modulus the more flexible a plastic is. In terms of stiffness, bioplastics mixed with sorbitol are stiffer (51.284 and 66.29) for both concentrations of microalgae, compared to bioplastics that used glycerol (6.639) as a plasticizer. Addition of sorbitol into a bioplastic affected the hydrogen bonds between polymers of matrixes. Hydrogen bonds become stronger and at the same time, reduce the original hydrogen bonds of the matrixes (Tian et al., 2017), thus resulting in producing stiff and brittle bioplastics.

#### **Fourier-transform infrared spectroscopy (FTIR) analysis**

Fresh microalgae residues underwent FTIR analysis. The band between  $3402.66\text{ cm}^{-1}$  and  $3281.37\text{ cm}^{-1}$  showed the presence of an O–H stretch bond while the band at  $2918.90\text{ cm}^{-1}$  showed the presence of a C–H bending bond (Table 2).

Microalgae contain a polysaccharide that comprises of 3 different elements which are C, O, and H (Johnsson, Steuer, 2018). Hence, the presence of both O–H and C=C bond is expected as it relates to the presence of polysaccharides inside the microalgae residues. This clearly showed that microalgae residues are an organic compound that consist of hydrocarbons. Bending bonds between  $1702.18\text{ cm}^{-1}$  until  $1229.29\text{ cm}^{-1}$  showed the presence of other bonds such as C=O and C–O.

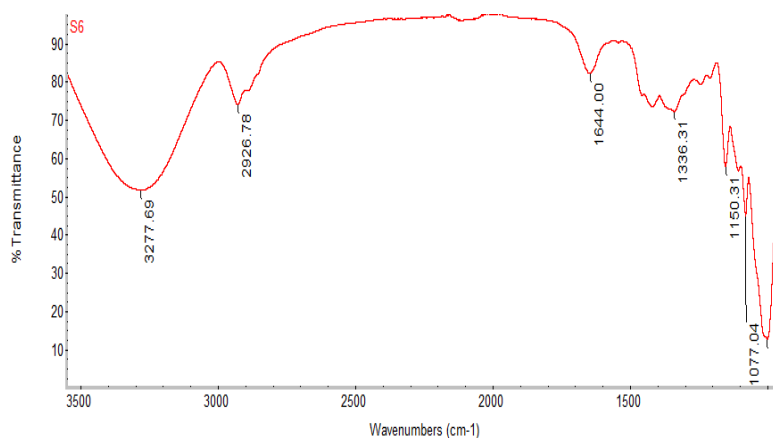
The formulation of bioplastic consists of 4 main substances which are microalgae residues, starch, plasticizer (glycerol or sorbitol) and distilled water (to mix the substances).



**TABLE 2:** Classification of bond type and functional groups

Wavenumber, $\text{cm}^{-1}$	Bond type	Functional group
3550–3200	O–H	Alcohol
3000–2840	C–H	Aliphatic
1740–1720	C=O	Carbonyl
1662–1626	C=C	Alkenes
1650–1566	C=C	Alkenes
1420–1330	O–H	Alcohol
1275–1200	C–O	Ether
1075–1020	C–O	Ether

All the substances involved are organic compounds comprised of elements such as carbon, hydrogen, and oxygen. The chemical formula for glycerol is  $\text{C}_3\text{H}_8\text{O}_3$  while the chemical formula for sorbitol is  $\text{C}_6\text{H}_{14}\text{O}_6$ . Hence, from the FTIR analysis obtained, the presence of the O–H bond in bioplastics is at  $3277.69 \text{ cm}^{-1}$  and  $1336.31 \text{ cm}^{-1}$ . This showed the presence of a free hydroxyl group that allows bioplastic to absorb water and contributes to their high biodegradability properties (Lebreton et al., 2018). There are also other bonds present in bioplastics such as C–H bond at  $2926.78 \text{ cm}^{-1}$ , C=C at  $1644 \text{ cm}^{-1}$  and C–O bond at  $1150.31 \text{ cm}^{-1}$  and  $1077.04 \text{ cm}^{-1}$ . Based on the bonds found in FTIR analysis, it is proven that both glycerol and sorbitol reacted with microalgae residues in other to form the bonds and resulted in the production of bioplastic film (Figs 5–7).



**FIG. 5:** FTIR analysis of fresh microalgae residues

### Biodegradable test

Test method ISO 11266 was used in this experiment to determine the biodegradation of organic chemicals (bioplastics) (Briassoulis et al., 2014). Samples of bioplastic being were placed under aerobic soil conditions to allow the process of biodegradation to occur. Biodegradable testing of 6 different samples of bioplastic was undertaken for two weeks to analyse the biodegradability of each bioplastic (Table 3). Observation and changes in weight of samples was recorded on the first, seventh and fourteenth day.

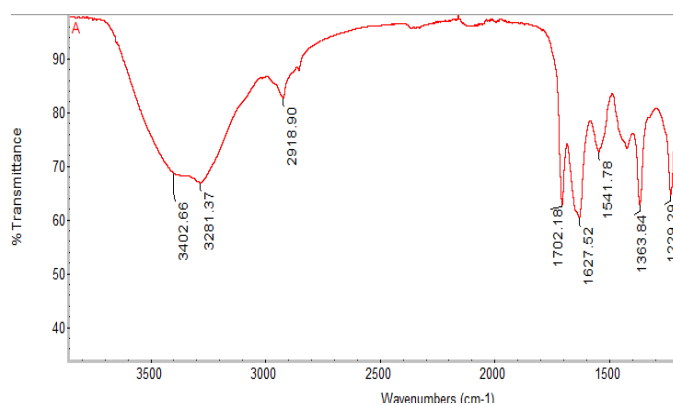


FIG. 6: FTIR analysis of glycerol-based bioplastic

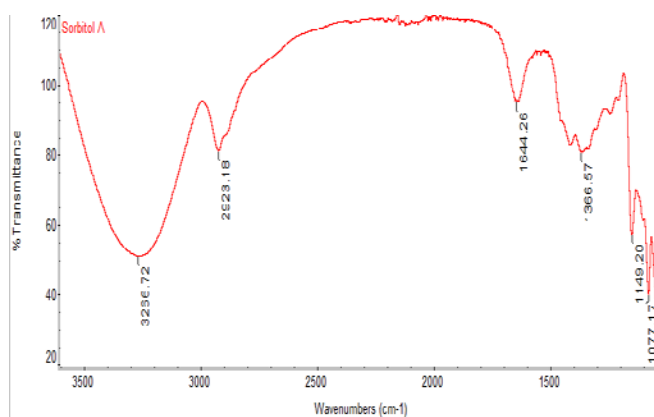


FIG. 7: FTIR analysis of sorbitol-based bioplastic

From the results obtained, as shown in the Table 3 below, the weight of each sample reduced when weighed on the seventh day. The reduction in weight of samples indicated that bioplastics are degraded. However, on the last day of testing (14<sup>th</sup> day), no observation could be made as no samples could be found inside the soil where the samples were being buried.

**TABLE 3:** Lists of standards for biodegradable test sources (Briassoulis et al., 2014)

Standards	Title
American society for testing and materials international (ASTM)	
ASTM D 5988-12	Standard test method for determining aerobic biodegradation of plastic materials in soil
International standards	
ISO 17556-2012	Plastics – determination of the ultimate aerobic biodegradability of plastic materials in soil by measuring the oxygen demand in a respirometer or the amount of carbon dioxide evolved
ISO 11266-1994	Soil quality – guidance on laboratory testing for biodegradation of organic chemicals in soil under aerobic conditions
French and Italian Normalisation Organisations (AFNOR, UNI)	
NF U52-001 February 2005	Biodegradable materials for use in agriculture and horticulture – Mulching products – requirements and test methods
UNI 11462:2012	Plastic materials biodegradable in soil – types, requirements and test methods
OECD guidelines	
304A	Inherent biodegradability in soil
307	aerobic and anaerobic transformation in soil

The percentage rate of biodegradation of bioplastics for both plasticizers increased as the percentage of microalgae residues increased in the samples. This showed that the presence of microalgae residues helps to increase the rate of biodegradability of a bioplastic (Table 4). Microalgae are known to have a high content of PHB, a type of polymer that classifies under the polyester class, which enhances the biodegradability of a plastics (Abdou et al., 2019). Hence, a high amount of microalgae in bioplastics increase the rate of biodegradability of the bioplastic.

Based on the results obtained, bioplastics from microalgae that used glycerol as plasticizer have higher rate of biodegradability when buried under natural soil conditions,

compared to using sorbitol as a plasticizer. This is because glycerol has better hygroscopic properties which make glycerol plasticized bioplastic have a higher rate of water absorption compared to sorbitol (Ooi et al., 2012). Therefore, the changes in natural weather such as rain contribute to the absorption of water into the bioplastic and hasten the duration of degradation of the bioplastic. The other effects that might contribute to the degradation of bioplastics are pH or the concentration of bacterial biomass in soil (Adhikari, Hartemink, 2016).

**TABLE 4:** Percentage rate of biodegradability of bioplastics

Samples	Initial weight, g	Weight (7 <sup>th</sup> day), g	Difference in weight, g	Biodegradable rate, %
Glycerol (0% microalgae)	0.216	0.135	0.081	37.5
Glycerol (50% microalgae)	0.209	0.118	0.091	43.54
Glycerol (80% microalgae)	0.376	0.120	0.256	68.09
Sorbitol (0% microalgae)	0.194	0.112	0.082	42.27
Sorbitol (50% microalgae)	0.182	0.106	0.076	41.76
Sorbitol (80% microalgae)	0.257	0.118	0.139	54.09

## CONCLUSIONS

In conclusion, both glycerol and sorbitol are suitable for use as plasticizers in the preparation of bioplastic from microalgae. However, the applications of the bioplastic made up from glycerol and sorbitol are different. Glycerol-based bioplastic is suitable to be used as a plastic bag or food wrapper, as it is more flexible compared to sorbitol-based bioplastic. Although sorbitol-based bioplastic is less flexible, it can resist high force better than glycerol-based bioplastic. The addition of different ratios of microalgae residues in the formulation of bioplastic, increases the percentage of biodegradability of bioplastic. This due to the presence of additional natural biopolymers such as polysaccharides in microalgae residues. The biodegradability of bioplastic is also being influenced by the hygroscopic properties of both plasticizers that contribute to the solubility of bioplastic when mixed with water.

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