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The influence of polypropylene- and polyethylene microplastics on the quality of *Spirulina* sp. harvests

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Abstract

Microplastics are polymer-based materials that require a variety of organic and inorganic chemical additives during the manufacturing process. These chemicals have an impact on aquatic organisms. This study aimed to quantify the impact of microplastics on the growth and quality of microalgae *Spirulina* sp. We studied the interaction between *Spirulina* sp. and 500 mg of polyethylene (PE) and polypropylene (PP) microplastics. Three glass bioreactors containing 2 L of *Spirulina* sp. were observed for 30 days. The first bioreactor was untreated and acted as a control. The second was treated with PE microplastic of 1 mm in size, and the third was treated with similarly sized PP microplastics. Each day, the optical density (OD) was measured to determine the rate of growth of the *Spirulina* sp. After harvesting, the *Spirulina* sp. biomass was dried in an oven at 30-35°C for 24 hrs and subsequently analyzed using Fourier transform infrared spectroscopy (FTIR). With PE treatment, the results showed a change in the organic structure on *Spirulina* sp., as well as a decline of polysaccharides and the loss of one peak at wave number 875.45 cm^{-1}. Meanwhile, in *Spirulina* sp. with PP treatment, two peaks that showed polysaccharides at wavelengths of 875.45 cm^{-1} and 1,245.67 cm^{-1} were lost. The results also indicated that microplastics had a significant impact on the growth and the quality of *Spirulina* sp., especially in decreased polysaccharide content.

1. Introduction

Microplastics are small (< 5 mm) particles formed from organic polymers. They have a profound influence on freshwater and marine ecosystems as they get ingested and do not get caught in planktonic nets (Setälä *et al*., 2014). Microplastics also influence higher trophic levels (Farrell and Nelson, 2013; Van Cauwenberghe and Janssen, 2014; Besseling *et al*., 2015) and show toxicity and increased mortality in marine plankton (Bergami *et al.*, 2017). Several studies on the toxicity of microplastics of various sizes and characteristics show how aquatic ecosystems, including algae, ciliate, invertebrates, crustaceans, and fish (Bergami *et al.*, 2017).

Saturated polymers, or polyolefins, have a broad range of applications, packaging being one of them. Several broad classes of plastics that are used in packaging are PE, PP, polystyrene, Polyethylene terephthalate, and Polyvinyl chloride (Andrady, 2011). However, PE and PP (also known as CnH2n), are most commonly used. They share similar polymer characteristics, namely in polyolefin groups with linear carbon chains. PE and PP are also the most widely used linear hydrocarbon polymers (Arutchelvi *et al.*, 2008). As a thermoplastic polymer resin with a semi-crystalline structure, PP is used in the production of packaging for mineral water, bottle caps, drinking straws, yogurt containers, and more. PE, on the other hand, is made into a soft, transparent, and flexible film that has good impact and tearing resistance and is therefore commonly used as plastic bags. They are both high molecular weight elements however that cannot be biodegraded (Lagarde *et al.*, 2016). More specifically, plasticizer content such as phthalate, nonylphenol, and bisphenol A found in microplastics can harm aquatic microorganisms (Campanale *et al.*, 2020). One such microorganism is the microalga *Spirulina* sp., often used in the food, cosmetics, and medicine industry.

Spirulina sp. can produce exopolymer substances

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(EPS), viscous gel-like structures that are species-specific. These are long-chain polysaccharides that are composed of repeating units of sugar derivatives with a structural diversity arisen from a broad range of non-carbohydrate substituents and linkage types (Bazaka *et al.*, 2011). Production of EPS from microalgae such as *Spirulina* sp. can be encouraged by providing adequate nutrients, substrates, and oxygen during cultivation. On *Spirulina* sp., EPS act antibacterial, anticoagulant, anti-oxidative, anticancer, and anti-inflammatory (Chentir *et al.*, 2017). As a photosynthetic microorganism, the *Spirulina* sp. utilizes several carbon sources. It uses CO2 as a carbon source to form biomass (Khoironi *et al.*, 2019). Additionally, Gu (2003) explained that EPS from microalgae are able to break polymer bonds to produce shorter rings by utilizing microplastics to become carbon sources. Finally, Rummel *et al*. (2017) reported similarly by stating that plastic can be used by microalgae as a carbon source, and the release of additives in plastics can increase the growth of microorganisms by acting as carbon sources. EPS also plays a role in the biodegradation process, where it is used by microbes as a growth medium and forms heteroaggregation involving plastics, microbes and detritus (Long *et al.*, 2015; Khoironi *et al.*, 2019). Sjollema *et al*. (2016) also reported on the impact microplastics can have on the growth of microalgae and showed that microalgae absorbed microplastics so as to inhibit their own growth.

Spirulina sp. has organic functional groups in the form of polysaccharides, amides, lipids, proteins, carboxyl groups, alkyl and other useful compounds such as antioxidant (Costa *et al.*, 2019). These are important components of *Spirulina* sp. These organic functional groups contained in *Spirulina* sp. can be an indicator of the quality of the biomass of *Spirulina* sp. Dmytryk *et al*. (2014) did an FTIR analysis to identify organic functional groups in *Spirulina* sp. They reported that peaks for polysaccharides appeared at wavelengths of 861.48 cm^{-1}; for γCC/CO, γOH at 1,049.44 cm^{-1}; for ether polysaccharides and polysaccharides at 1,150.86 cm^{-1}; for amides, polysaccharides, and esters at 1,241.96 cm^{-1}; for the carboxyl group at 1,400.08 cm^{-1}; for alkyl rings at 1,454.64 cm^{-1}; for the second amide at 1,541.50 cm^{-1}; for the first amide protein at 1,654.16 cm^{-1}; for alkyl chopped at 2,874.17 cm^{-1}, 2,917.79 cm^{-1}, and 2,960.11 cm^{-1}; and for amines at 3,302.49 cm^{-1}. This study aimed to determine the impact PE and PP microplastics have on the growth of *Spirulina* sp. and the quality of *Spirulina* sp. biomass.

2. Materials and methods

Spirulina sp. was cultivated, treated with microplastics and analyzed. During the cultivation of *Spirulina* sp., Each day, the content's OD was measured to determine the rate of growth of the *Spirulina* sp. The following variables were used: *Spirulina* sp. without microplastic treatment (control) (variable 1), *Spirulina* sp. with PE treatment (variable 2), and *Spirulina* sp. with PP treatment (variable 3).

2.1 Preparation of Spirulina sp.

Microalgae *Spirulina* sp. seeds were obtained from Neoalgae, Sukoharjo, Central Java. Microalgae cultivation, testing, and result analysis were carried out at UPT C-BIORE Laboratory, Diponegoro University, Semarang. Three glass bioreactors for all three variables were filled with 2 L of *Spirulina* sp., each glass equipped with an aerator for oxygen supply and each illuminated by conventional LEDs. The OD was then measured at 0.42 for all three variables. For 30 days, the pH values were maintained at 7-8 and the temperatures at 24-26^{o}C. Nutrition was given every 5 days in the form of a mixture of TSP 15 ppm, Urea 70 ppm, and $NaHCO_3$ 1 g/L, in order to maintain the growth of *Spirulina* sp. The OD was measured using a spectrophotometer (OPTIMA SP-300) to determine cell concentrations in the *Spirulina* sp. For 30 days wavelengths at 680 nm were used to observe the presence of chlorophyll-α. Increasing chlorophyll-α levels indicated that the *Spirulina* sp. was growing.

2.2 Preparation of microplastics

Microplastics used in this study were PE obtained from unused white plastic bags, and PP was provided by AQUA and designed as the packaging of mineral water. PE and PP were cut to around 1 mm^2 in size. Afterwards, the microplastics were washed with ethanol and dried at room temperature for 24 hrs. They were then weighed carefully to 500 mg and mixed into the bioreactor glasses already containing 2 L of *Spirulina* sp. culture. Aerators on the glass jars were used for the stirring process.

2.3 Harvesting of Spirulina sp.

To separate biomass and filtrate, a stainless steel screen wire mesh with a size of 40 microns was used. The microplastics were previously filtered out with a Whatman filter in order to avoid the mixing of *Spirulina* sp. and wet biomass. Wet biomass *Spirulina* sp. was subsequently dried in an oven at 30-35^{o}C.

2.4 FTIR analysis

An FTIR analysis was done to evaluate the effect of microplastic on organic elements with changes in organic functional groups. Measurements of chemical structures of the biomass were made at a wavelength range of 400-4,000 cm^{-1}.

3. Results and discussion

3.1 The influence of microplastics on the growth of Spirulina sp.

The results of the OD measurements are depicted in Figure 1. All three variables have experienced an increase in OD. On day 15, the OD in variable 1 saw a spike up to 0.772. Meanwhile, variable 2 had a similar spike on day 21 up to 0.762. Lastly, variable 3 experienced a spike on day 22 up to 0.779. On the last day (day 30), variable 1 had reached OD of 0.994. The OD in variable 2, however, remained lower and reached 0.912 on the last day, followed by variable 3 which reached an OD of 0.886. This is in line with research conducted by Cunha *et al*. (2019), who demonstrated that microalgae contaminated with microplastic showed a decrease of up to 42% in the number of cells. From Figure 1, the growth rate of *Spirulina* sp. can be calculated with the following formula:

$$\mu = \frac{lnOD_2 - lnOD_1}{t_2 - t_1}$$

Where μ = growth rate (day^{-1}), OD = Optical density at 680 nm and t = time (day)

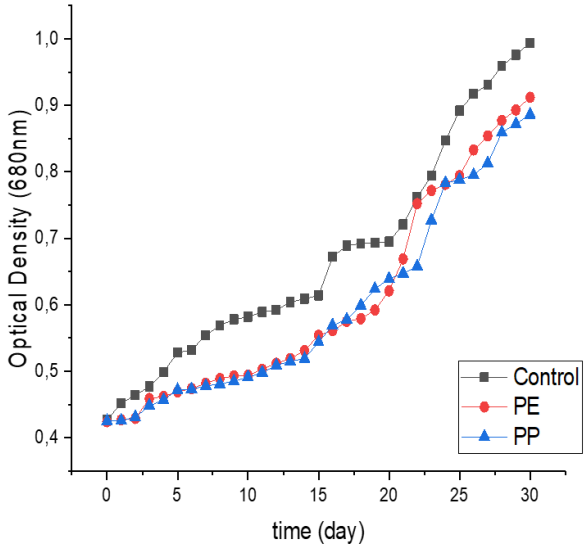

Figure 1. Microalgae growth of *Spirulina* sp for all three variables

Table 1 shows the growth rate of *Spirulina* sp. during the 30 days study of the three variables. The growth rate of variable 1 (control) was higher than with variables 2 and 3. This is due to microplastics inhibiting the distribution of light and air and thus the process of photosynthesis on the surface of the cultivation area in *Spirulina* sp. (Hadiyanto *et al.*, 2012; Sjollema *et al.*, 2016). This is in line with Bhattacharya *et al*. (2010), who added microplastics 20 nm in size to the cultures of *Chlorella* and *Scenedesmus* and showed that there too, algae inhibited the process of photosynthesis due to the blockage of light and air.

Table 1. The growth rates of *Spirulina* sp for all three variables

	System	Grow rate (μ)
Fresh water	Variable 1	0.229/day
	Variable 2	0.208/day
	Variable 3	0.189/day

On the other hand, the presence of PE and PP microplastics also had positive effects. We saw a higher growth rate in *Spirulina* sp. that received PE and PP microplastics treatment than in *Spirulina* sp. without treatment. This seems to support the assessment that *Spirulina* sp. is able to utilize microplastics as a carbon source.

3.2 FTIR analysis and the changes in polysaccharide functional groups in Spirulina sp.

Negi *et al*. (2011) showed that polymers consisting of double bonds (such as PE) and triple bonds (such as PP) can be identified at wavelengths of 330 to 2,150 cm^{-1}. The results of the FTIR analysis on variables 1, 2 and 3 are depicted in Figure 2. The FTIR analysis showed that variables 2 had a loss of polysaccharides at wavelengths 875.45 cm^{-1}. This is due to PE having double bonds and degrading as much as twice as fast as PP. On the other hand, PP has a longer triplicate in aggregation, which causes an interaction between PP microplastic and *Spirulina* sp. to increase. This results in increased EPS production by *Spirulina* sp., but excessive EPS production can have a toxic effect. Sheng *et al*. (2010) explained that EPS from microalgae can absorb metals and organic compounds such as proteins and polysaccharides. The loss of two peaks indicates the presence of polysaccharides at wavelengths 1,245.67 cm^{-}

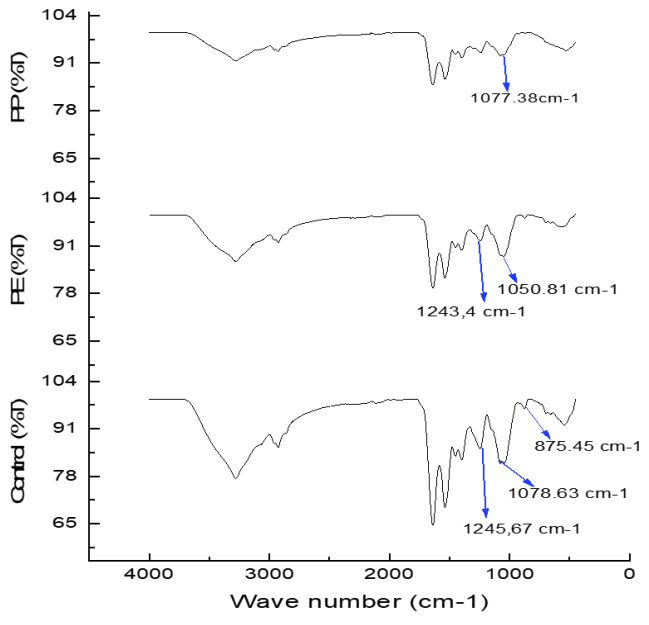

Figure 2. FTIR analysis results of *Spirulina* sp for all three variables

Table 2. Results of FTIR analysis results on *Spirulina* sp for all three variables, showing the presence of polysaccharides

Variable 1		Variable 2		Variable 3	
polysaccharides	%T	polysaccharides	%T	polysaccharides	%T
1,245.67	85.58	1,243.40	92.37	n.d	n.d
1,078.63	81.48	1,050.81	88.19	1,077.38	93
875.45	96.52	n.d	n.d	n.d	n.d

[1] and 875.45 cm^{-1}.

The increase of % T can be seen in Table 2, showing that % T PP > % T PE > % T. The greater the value of % T, the lower the productivity of the resulting polysaccharides. The results of this study indicate that the presence of microplastics has a significant impact on the growth and quality of *Spirulina* sp. produced, especially in polysaccharide content.

4. Conclusion

The presence of PE and PP plastics showed a strong influence on the growth of *Spirulina* sp. The increased growth rate of *Spirulina* sp. due to added microplastics is not proportional to the increased quality of the produced biomass. Organic functional groups contained in biomass are indicators of the quality of *Spirulina* sp. The longer the interaction time between *Spirulina* sp. and plastic, the further the PE and PP plastics were degraded which caused the loss of some functional groups in the dry biomass of *Spirulina* sp. This is most notable in the polysaccharide group, an important component in *Spirulina* sp. The interaction between plastic and microalgae pro-vides phenomena that need to be further studied to devise a solution for handling the abundance of plastic waste in aquatic systems and the protection of the health of aquatic organisms.

Conflict of interest

The authors declare no conflict of interest.

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