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Effects of Polystyrene Microparticles on Growth and Physiological Metabolism of Microalgae *Scendesmus obliquus*

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Abstract: Plastic products are widely used due to their lightness, durability, low cost, and malleability, and their threat to the environment is becoming increasingly apparent. However, the detailed mechanism of such an effect is unclear. Due to improper treatment, microplastic contamination is very much studied in the areas around sewage outfalls or some plastic manufacturers, and its content is high. To investigate the ecological impact of microplastics in this particular area, we evaluated the toxic effect of polystyrene microplastics (PS-MPs) on the microalgae *Scenedesmus obliquus* (*S. obliquus*). In particular, the cell growth, photosynthesis, antioxidant enzyme activity, membrane permeability, and morphology of the microalgae in the presence of different PS-MP concentrations were monitored. These new data revealed an increase in microalgae cell growth as an early response to low-dose PS-MP exposure with a maximum inhabitation rate of −3.33%. In contrast, a higher concentration of PS-MP solution leads to a significant inhibition within the test concentration, with a maximum inhabitation rate of 43.62%. Meanwhile, a decreased photosynthesis activity of *S. obliquus* was observed during the exposure. Moreover, PS-MPs induced oxidative damage to the algae cells, exhibiting decreased antioxidant activities and enhanced lipid peroxidation reactions, as evidenced by the reduction in the superoxide dismutase (SOD) level and increased malondialdehyde (MDA) content, as well as damaged cell membrane and soluble protein. Collectively, our study systematically investigates the toxicity of microplastics on microalgae, providing a deep insight into the possible influences of plastic pollution on the whole food web in the aquatic ecosystem.

Keywords: microplastic; microalgae; toxicity; antioxidant activity; growth; cell membrane damage

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1. Introduction

During the COVID-19 pandemic, there was a significant increase in the use and disposal of personal protective equipment (PPE), such as masks, body protectors, and so on. As most (PPE) are made of synthetic polymers, they may undergo chemical and physical degradation after entering the environment, leading to microplastic (MP) pollution [1]. MPs refer to plastic particles with particle sizes of less than 5 mm, and they are produced directly by industry or by large-scale plastic degradation. According to the classification of forming method, MPs are generally divided into primary microplastics and secondary microplastics [2,3]. Two of the most produced and detected MPs in aquatic environments and treated waters are polymethyl methacrylate (PMMA) and polystyrene (PS). PS is commonly used in industry, owing to its corrosion resistance and economic feasibility [4]. Given the fact that the existence of these MPs leads to serious consequences on the functioning of the aquatic ecosystem [5,6], investigating the underlying mechanisms has been a long-term goal of many research groups.

Most studies related to MPs have been conducted in the seawater environment. However, there is evidence showing that MPs are ubiquitously present in the freshwater ecosystem [7–10]. In Poyang Lake, high abundances of MPs were detected in all surface water and sediment samples, with a range from 5 to 34 items/L and 54 to 106 items/kg dw, respectively [11]. A recent report on the abundance of MPs in Cisadane River showed that

PS, polyethylene (PE), and polypropylene (PP) dominated the chemical composition, and the abundance ranged between 13.33 and 113.33 particles/m^3 in surface water [12]. Some studies also indicated that remote areas have fewer MPs compared to urban areas. As the main collection and treatment of urban sewage, wastewater treatment plants (WWTP) are also an important point source for microplastics to enter the environment. The emission is 1.24×10^{10} items daily, and they are discharged into the Black Sea. Meanwhile, the abundance of MPs is also very high in areas around sewage outfalls [13]. MPs were widespread in aquatic systems, from surface water to benthic sediment, and their specific locations depended on the shapes and densities of polymers [8,14]. MPs were easily absorbed and accumulated in aquatic organisms at different trophic levels [15,16], causing severe mechanical injuries and inflammatory responses during digestion [17–19]. Humans, as nature's highest consumers, have been shown that they could ingest MPs on the food chain, thus posing a potential threat to health [20–22]. We noticed that feces, meconium, placental and lung tissues, and breast milk were detected MPs [23]. Behavioral, hematological, and biochemical changes, histopathological damage, embryonic toxicity, and neurotoxicity are the main ways in which MPs affect organisms [14].

Microalgae, a fundamental component at the base of aquatic food, serving as primary producers essential to oxygen production, are closely associated with the presence of microplastics [24–27]. Therefore, it is of great importance to investigate the influence of the presence of microplastics on microalgae. As described in Table 1, extensive research demonstrated that MPs have a negative effect on microalgae, and the specific performance was growth inhibition and cell morphological damage [14]. For example, the decrease in the biomass of *Phaeodactylum tricornutum* was observed after long-term exposure to MPs [4]. It was also found that PVC and PP would greatly inhibit photosynthetic activity when algal cells completed self-regulation [24]. In a study on *Chlamydomonas reinhardtii*, the increasing toxicity of cells was related to more carbonyl groups and the zeta potential of UV-aged PVC [25]. On the other hand, PS affects lipid metabolism in algae, which was important for photosynthesis [26]. Although many studies showed that there were harmful effects of MP on microalgae, it is not unclear about the toxicity mechanisms.

The microalgae *S. obliquus*, an ideal candidate that is commonly used in eco-toxicological studies, was employed as the test species. Their advantages are associated with a short growth period and sensitivity to external stimuli [18]. In this present study, we investigated the effects of 5 μm polystyrene microplastics (PS-MPs) that may be exerted on microalgae. The effect of PS-MPs on the photosynthesis of microalgae was studied by measuring Chlorophyll content. The measurements of the antioxidant enzymes and soluble protein contents of microalgae were used to evaluate the oxidative damage of PS-MPs on microalgae. In combination with the characterization of the ultrastructure of *S. obliquus*, the adsorption of PS-MPs on the algal cell was investigated, as well as the influences on the toxic effects of the agglomeration process on microalgae. Collectively, these studies revealed important details regarding the potent influence of microplastics on microalgae in freshwater environments.

Table 1. Ecotoxicological effects of microplastics on microalgae.

Test Species	Microplastics			Exposed Time	Effect Mechanism	Refs
	Polymer	Size/μm	Concentration (mg/L)			
Phaeodactylum tricornutum	PS; PMMA	70–80 for PS and 40–60 for PMMA	0.25, 0.5, 25, 50	27 d	Pigments, carbohydrates, and Biomass production severely decreased	[4]
Chlorella pyrenoidosa and *Microcystis flos-aquae*	PP PVC	D_{90}: 216 D_{10}: 236	5, 10, 50, 100, 250, 500	11 d 7 d	High concentrations of microplastics inhibited photosynthesis of algae	[24]
C. reinhardtii	v-PVC and a-PVC	50 to 100	10, 20, 50, 100, and 200	96 h	Reduction in chlorophyll-a level; oxidative damage	[25]
Chlorella sorokiniana	PS	<70	60	28 d	PS disrupted lipid composition: reduction in two essential fatty acids, linoleic and linolinic.	[26]
Chlorella vulgaris	PE, PA, PLA, PBS	77.75; 59.88; 57.41; 53.33	10, 100, and 1000	11 d	Inhibitory effects on microalgal growth; promote the accumulation of photosynthetic pigments and trigger the evolution of antioxidative substances; heterogeneous aggregation of MPs and microalgae	[27]
Chlorella pyrenoidosa	PS	1 and 5	2, 10, 50	10 d	Significant inhibition of growth and photosynthesis, decrease in photosynthetic pigment content, induction of oxidative stress, and changes in transcript levels of genes related to photosynthesis and energy metabolism	[28]
Microcystis aeruginosa	PS	1 and 5	0.01, 0.1, 0.5, 1, 2, 5, and 10	12 d	Significantly increase ROS content and SOD activity; high concentrations of PS-MPs can alter genes transcription; promote MC-LR synthesis and transport	[29]
Rhodomonas lens	HDPE + CPF + Hg	10–15	1	96 h	Affect cell viability, population growth, and pigment content	[30]
Microcystis aeruginos	PS	0.1 and 1.0	1, 10, and 100	17 d	Alleviate photosynthetic efficiency, triggered EPS release, and changed composition of specific EPS fractions	[31]
Thalassiosira pseudonana, *Skeletonema grethae*, *Phaeodactylum tricornutum* and *Dunaliella tertiolecta*	PS	0.055, 1 and 6	0.0001, 0.001, 0.01, 0.1, 1, 10, 50, 250	48 h	Inhibition of growth; lower DNA values when exposed to high concentrations of nano-polystyrene particles (55 nm); induced higher P/C ratios of EPS	[32]
Microcystis aeruginosa	PS	0.02, 0.10 and 1.00	100	120 h	EPS compositions changed; inhibition on the algae grew	[33]

Table 1. *Cont.*

Test Species	Microplastics			Exposed Time	Effect Mechanism	Refs
	Polymer	Size/μm	Concentration (mg/L)			
Chlamydomonas reinhardtii.	PS + sulfadiazine	1 and 5	5, 10, 20, 50, 100, and 200	7 d	Increased antioxidant enzyme activity; resulted in an increase in extracellular secretory proteins and a decrease in chlorophyll content.	[34]
Microcystis aeruginosa	PE and PVC	0.5	5, 10, and 50	16 d	Hormesis effect on microalgal growth at intital period; increasingly inhibited growth as MPs dosed increased; antioxidant and morphology analyses revealed unevident; MCs production/release ability increased	[35]
P. tricornutum	PS and PMMA	0.08–0.1	0.01, 0.1, 1, and 10	240 h	ROS levels and growth inhibition; increased membrane permeability and inhibition of chlorophyll biosynthesis; most metabolism and genes expression were changed and disturbed	[36]

Note: D10 represents the particle diameter, corresponding to 10% cumulative (from 0 to 100%) undersize particle size distribution; v-PVC represents virgin PVC, and a-PVC represents aged PVC; PE represents polyethylene; PA represents polyamide; PLA represents polylactic acid; PBS represents polybutylene succinate; HDPE represents high density polyethylene; PMMA represents polymethyl methacrylate; CPF represents chlorpyrifos; PS represents polystyrene; Hg represents hydrargyrum.

2. Materials and Methods

2.1. Materials and Reagents

PS-MPs with green fluorescence were purchased from Tianjin Base Line Chrom Tech Research Centre (Tianjin, China), with an initial concentration of 10 mg/mL, and stored as dispersions at 4 °C in darkness (2.5% *w*/*v*, 10 mL). PS-MPs disperse evenly in water, and do not settle or accumulate during the culturing. The excitation and emission wavelengths of PS-MPs were at 488 nm and 518 nm, respectively. This series of fluorescent microspheres possessed excellent characteristics, including good optical properties, highly uniform particle size, less leakage of fluorescent dye, good biocompatibility, and surface functionalization. The dye used for fluorescence labeling was contained inside the microbeads rather than being attached to the surface. Therefore, the potential chemical effect of dye on the experiment was generally perceived to be negligible [37]. The reagents used in the experiment were of analytical grade. All the medium and conical flasks were sterilized.

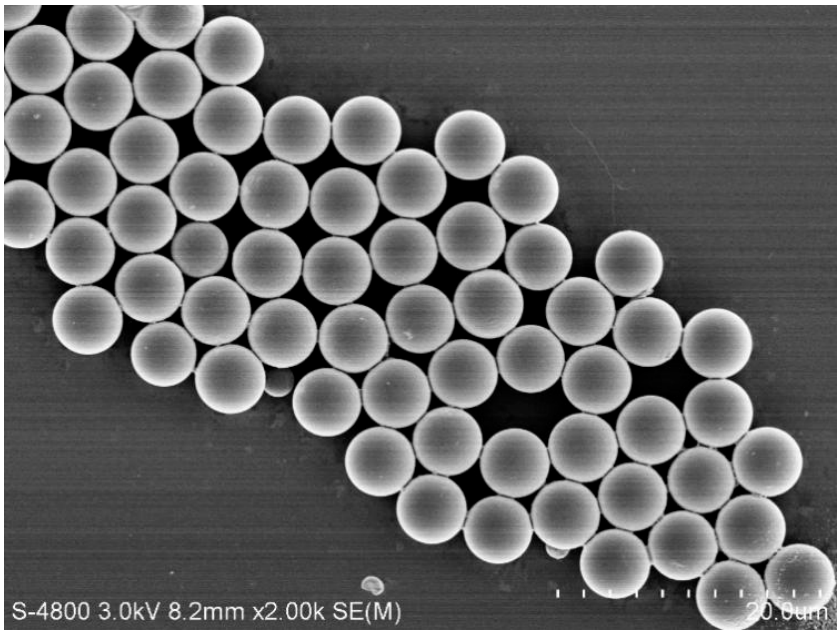
To further exclude effects from other matter in PS-MPs, we determined the physicochemical properties, including Scanning electron microscopy (SEM, Hitachi S4800, Tokyo, Japan) analysis and Fourier transform infrared spectroscopy (FT-IR) analysis. The physicochemical characterizations of PS-MPs are depicted in Figure 1. The PS-MPs with 5 µm particle size exhibited a uniform and smooth microsphere shape. Moreover, we also observed that the MPs particles adsorbed and accumulated with each other, primarily driven by van der Waals forces [38]. The FTIR spectra displayed characteristic peaks of a functional group, indicating that the particles were composed of PS, ensuring a high-level purity and minimizing contamination during exposure experiments. In the previous study, two types of PS (with fluorescence or not) were used for comparative experiments. The results obtained from both treatments were generally consistent, so the effects of the dyes that were contained in the PS-MPs on the algal cells could be disregarded [28].

2.2. Microalgae Cultivation

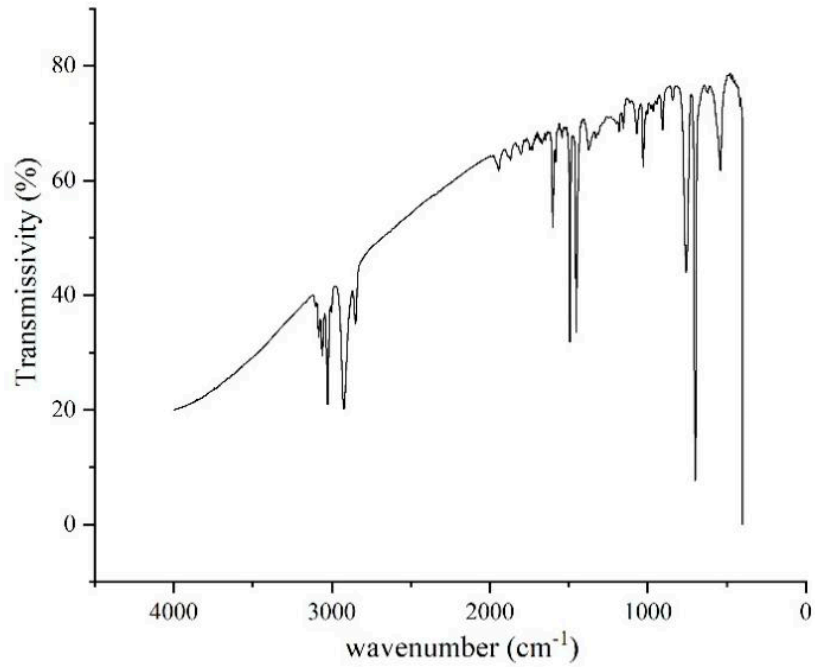
The experimental microalgae *Scenedesmus obliquus* (*S. obliquus*) (FACHB-12) was obtained from the Institute of Hydrobiology of the Chinese Academy of Sciences in Wuhan, China. The cultivation and enrichment of algae were conducted using BG-11 medium, and the receipt of BG-11 medium was described in Tables S1 and S2. To obtain the algae with stable characteristics and high activity, the algae strain was inoculated. The sterilized culture containing the algae strains was inoculated in an illumination incubator and grown under 20 ± 1 °C with a light intensity of 4800 lux of a 12:12 h photoperiod. The cultures were shaken at least three times during a day, as a way to prevent the adhesion and sedimentation of algae. The incubation period lasted for 7 days, during which most of cells exhibited logarithmic phase growth [39]. All the experiments were conducted under a sterilized environment, and cultivation equipment used for cultivation was pre-autoclaved at 121 °C for 30 min.

2.3. Microalgal Exposure to Microplastics

The initial algal density in 200 mL BG-11 medium was 8.5×10^5 cells/mL, and it was transferred to sterilized Erlenmeyer flasks. Before the toxicity assay, the PS-MPs suspension was well dispersed through the sonication for 1 h at 50 W using an ultrasound device. At t = 0 h, PS-MPs were added into algae solution with exponentially growing algae to reach the final gradient concentrations of 10, 25, 50, 75, and 100 mg/L. These samples were gently mixed and incubated in an illumination incubator (temperature: 20 ± 1 °C; light/dark cycle: 12 h/12 h; the light intensity of 4800 lux). Algal cultures without PS addition were used as experimental controls. Each treatment was repeated in triplicate.



(**A**)



(**B**)

Figure 1. The SEM image (**A**) and FT-IR spectra (**B**) of PS-MPs.

2.4. Algal Growth Assay

Cell density of each algal culture was sampled every 24 h for cell density determination. As that the cell density of *S. obliquus* is positively related to optical density $(OD)_{628nm}$, OD_{682nm} was measured using a spectrophotometer (UV-8000S, Inesa Instrument, Shanghai, China). The number of algal cells was determined via a calibration curve (Figure S1). The growth inhibition ratios (*IR*) of microalgae *S. obliquus* were calculated according to the equation below: $IR(\%) = \left(1 - \frac{T}{C}\right) \times 100\%$, where *T* and *C* represent cell density the in test group and control group, respectively.

2.5. Chlorophyll Content Determination

In order to quantify the amount of chlorophyll in the microalgae, the chlorophyll was extracted using the traditional organic solvent method [40]. Algal cultures exposed to PS-MPs of different concentrations every 24 h were collected for the measurement. Precisely, 20 mL of each culture was centrifuged at 5000 rpm for 10 min at 4 °C, and the supernatant was discarded. The algal were then resuspended in 5 mL of 90% acetone for 24 h under the condition of 4 °C in the darkness. Afterward, the algal in acetone was treated by centrifugation at 7000 rpm for 15 min, and its chlorophyll composition was collected. OD values were measured at two wavelengths (645 nm and 663 nm, respectively) to calculate the total content of chlorophyll by using the following formula (T_{chl}): $T_{chl} = 20.2 \times A_{645\text{nm}} + 8.02 \times A_{663\text{nm}}$.

2.6. Measurement of Super Oxide Dismutase (SOD), Malondialdehydes (MDA), and Soluble Proteins

Algal cultures exposed to PS-MPs of different concentrations every 24 h were collected for the measurement of SOD, MDA and soluble proteins. Assay kits (Shinuoda Biological Technology Co., Ltd., Chuzhou, China) were utilized to measure these biomarkers. Following to the instructions provided with the kits, the algae cells were harvested by centrifuging the culture at 5000 rpm for 10 min, and the precipitation was resuspended in 2 mL Phosphate buffer (PBS, pH = 7.2) and then further broken down by sonication in ice bath (5 min, ultrasonic rest cycle: 4–4 s). The resultant homogenate was centrifuged again at 5000 rpm for 10 min at 4 °C, and the supernatant of cell lysate was collected to detect the contents of MDA, SOD, and soluble protein, respectively.

2.7. Cell Membrane Permeability

The permeability of algae membrane was investigated via the derivate fluorescein diacetate (FDA) cytometric assay [41]. Algal cells exposed to PS-MPs were collected for measurement every 24 h. A 5 mL algae solution was mixed with 40 µL of 0.01 mg/mL FDA solution for 15 min at room temperature in the darkness. The fluorescent product retained in the cells was examined by fluorescence spectroscopy, with excitation and emission wavelengths of 488 nm and 530 nm, respectively. Cell membrane permeability was expressed according to the ratio of the fluorescence value of each treatment group to the fluorescence value of the control group. The ratio indirectly reflected the degree of damage to algal cells caused by PS-MPs.

2.8. Morphologic Properties

At the end of the exposure test, algal cells exposed to PS-MPs were collected for morphological property measurements. The algae solution was harvested by centrifugation at 5000 rpm for 10 min. The obtained algae cells were resuspended in PBS (pH = 7.4) and fixed by 2.5% glutaraldehyde at 4 °C overnight. Afterward, the samples were washed with 0.01 mol/L PBS three times and dehydrated with gradient alcohol (30%, 50%, 70%, 80%, 90%, and 100%) for 30 min. Finally, samples were freeze-dried with a lyophilizer for 24 h. The completely dried samples were stabilized on the conductive adhesive and coated with colloidal gold for observation.

2.9. Data Analysis

Statistical analysis was performed using IBM SPSS Statistics software 19.0. Data were processed by Origin Pro 2021 and Excel 2010 software. Results were expressed as means ± standard deviation (SD). Analysis of variance was performed by one-way ANOVA method, following Duncan multiple comparison analysis. A value of $p < 0.05$ was used to reveal a significant difference.

3. Results

3.1. Effect of PS with Different Concentrations on the Growth of S. obliquus

The effects of PS-MPs of different concentrations on the growth of *S. obliquus* are presented in Figure 2. Various treatment concentrations exerted slightly different effects on the growth of the logarithmic phase of *S. obliquus*, as evidenced by the growth inhibition of PS-MPs on microalgae (Figure 2B). During the first 24 h, the treatment groups with PS concentrations lower than 50 mg/L showed growth stimulation phenomena, and algal cell reproduction was promoted, with ratios of 2.42%, 3.33%, and 2.52%, respectively. In contrast, higher concentration treatments, such as 75 mg/L and 100 mg/L, exhibited significant inhibitions of algal cell growth. Notably, the 48th h inhibition ratio at 25 mg/L was negative, indicating a growth stimulation, whereas the IR at other concentrations showed an evident growth inhibition at the 48th h. The IR value after 48 h was gradually increased along with the PS concentration addition, showing growth inhibition effects, indicating a dose–effect relationship. The most significant inhibition of microalgae growth occurred at 96 h of PS exposure; the growth IR values were 15.55%, 19.98%, 31.08%, 39.08%. and 43.62%, under the PS concentrations of 10, 25, 50, 75, and 100 mg/L, respectively.

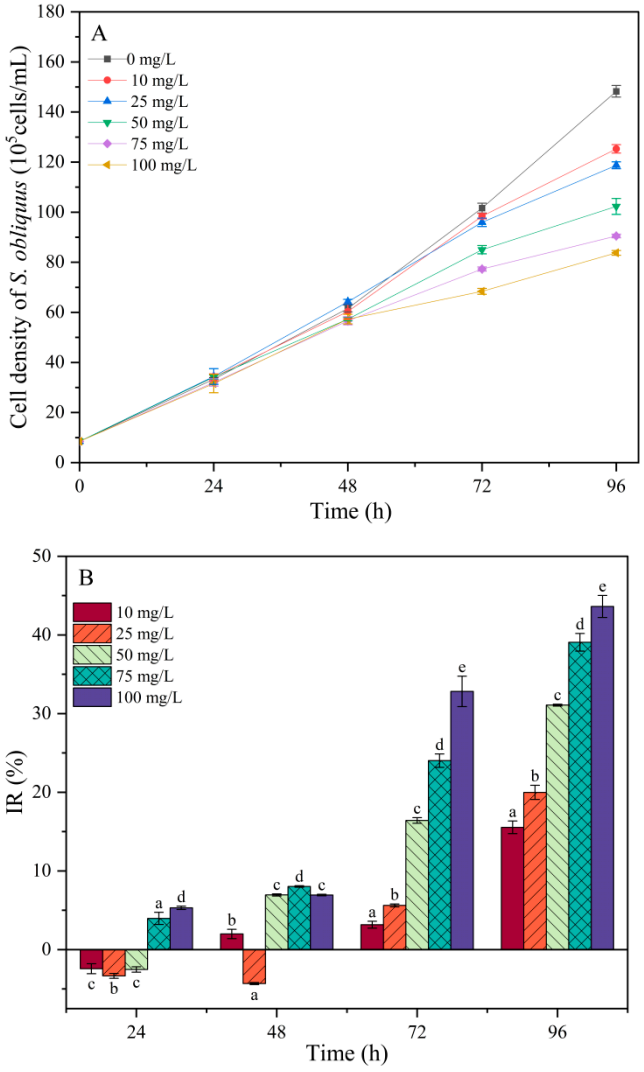


Figure 2. The effects on the growth of *S. obliquus* in response to exposure time at different PS-MP concentrations. (**A**) Microalgae cell density; (**B**) Inhibitory rate (IR). The error bars represent standard error (n = 3). Means not sharing the same letter are significantly different from each other ($p < 0.05$).

3.2. Effect of PS with Different Concentrations on Chlorophyll Content of S. obliquus

The chlorophyll content was measured to monitor the algae's photosynthetic efficiency upon microplastic exposure. As illustrated in Figure 3, it was observed that the total chlorophyll content increased with the exposure time. However, the chlorophyll productivity was inhibited as PS concentration grew, with the strongest inhibition observed at the 96th h. This indicated that there was a dose–effect relationship between total chlorophyll content and PS concentration. Notably, after 72 h exposure, the 5 μm microplastics with the concentrations of 10 and 25 mg/L had strong inhibitory effects on the photosynthetic efficiency of *S. obliquus*. In contrast, the inhibition effect was comparable when the exposure concentration was greater than 50 mg/L at all time periods. Importantly, significant differences were observed between each treatment group compared with the control group at 96 h; the total chlorophyll contents significantly decreased by 17.57%, 31.81%, 33.00%, 35.01%, and 40.00% in each treatment group, respectively, indicating a substantial reduction in the algae photosynthetic activity with the exposure time.

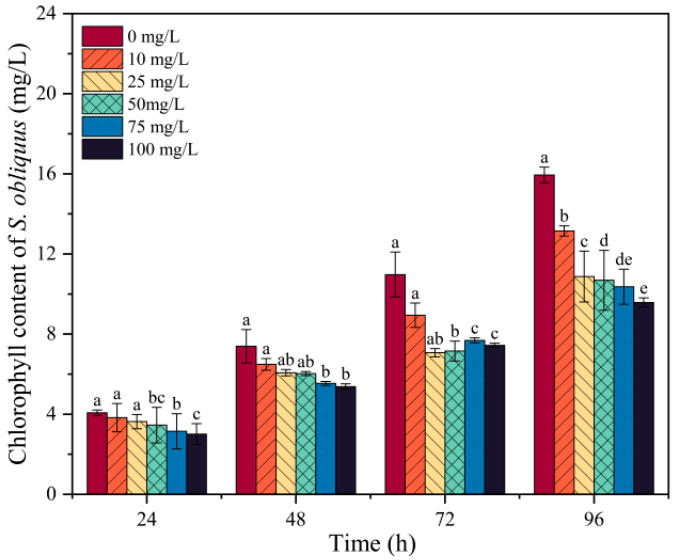


Figure 3. Effects of PS-MPs of different concentrations on photosynthesis of microalgae with different treatment time. The error bars represent standard error (n = 3). Means not sharing the same letter are significantly different from each other ($p < 0.05$).

3.3. Effect of PS with Different Concentration on SOD Activity of S. obliquus

Figure 4 illustrates the changes in SOD activity in the algae upon the upon the microplastic exposure for 96 h. As depicted in the figure, the index of SOD in each concentration's treatment group were distinct with the control, showing a trend in initially increasing and then gradually decreasing SOD activity over time. During the initial 24 h, the SOD activity was significantly increased under the PS concentrations of 50 mg/L, 75 mg/L, and 100 mg/L; the increases were 18.14%, 18.80%, and 24.54%, respectively, compared with the control group. Treatment groups with concentrations lower than 50 mg/L failed to significantly differ from the control group; meanwhile, they showed relatively similar results, indicating that low concentrations of microplastics had less effect on SOD activity. After 24 h, SOD activity was significantly inhibited upon the increased PS exposure concentration in all treatment groups. As for the extent of inhibition, the inhibition of the treatment groups with concentrations of 25 mg/L and 50 mg/L were similar, whereas the inhibition of the treatment groups with concentrations greater than 50 mg/L was significantly stronger than the former, and the difference was greater with the increase in exposure time, which might have been a certain "dose-dependent" relationship. At 96 h, the inhibition of SOD activity was the strongest in each treatment group, with inhibition rates of 31.09%, 43.59%, 46.59%, 62.95%, and 69.81%, respectively.

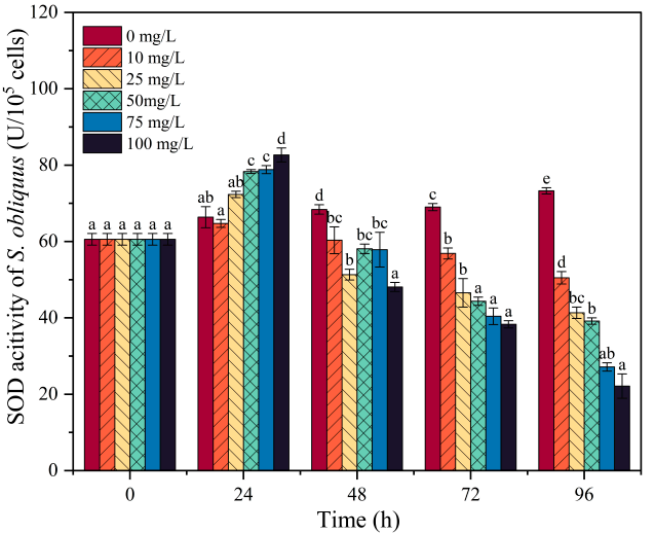


Figure 4. Variations in SOD activity of *S. obliquus* in response to exposure time at different PS-MP concentrations. The error bars represent standard error (n = 3). Means not sharing the same letter are significantly different from each other ($p < 0.05$).

3.4. Effect of PS with Different Concentration on MDA Content of S. obliquus

The changes in the MDA content in response to exposure time at different PS-MP concentrations are shown in Figure 5. The MDA content of algal cells in each treatment group exhibited positive responses to the PS's exposure time. Initially, the increase in MDA content in the control group was smaller and significantly lower than in other treatment groups. Following 48 h of exposure, the content of MDA increased significantly when the microplastic concentration was higher than 10 mg/L and gradually increased with the increase in the exposure time, which was mainly ascribed to the reduced electron transport rate and reduced photosynthesis. It is noteworthy that the MDA content in algal cells exhibited the largest changes at 96 h, which were 1.79, 1.93, 1.93, 2.12, and 2.45 times higher than that of the control group, respectively. In addition, it is important to address that the MDA content is positively correlated with the microplastic concentration, indicating an amplified oxidative stress intensity.

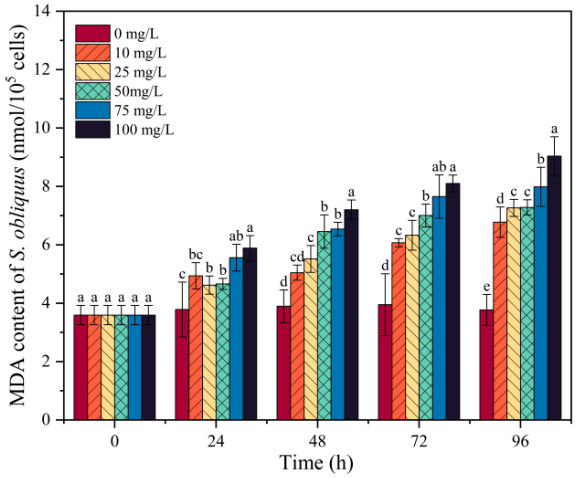


Figure 5. Variations in MDA content of *S. obliquus* in response to exposure time at different PS-MP concentrations. The error bars represent standard error (n = 3). Means not sharing the same letter are significantly different from each other ($p < 0.05$).

3.5. Effect of PS-MPs with Different Concentrations on Soluble Proteins of S. obliquus

Figure 6 exhibits the variations in soluble protein content in *S. obliquus* in response to exposure time at different PS-MP concentrations. The soluble protein content increased across all treatment groups, indicating that PS-MPs had a facilitative effect on soluble protein production in algal cells. Specifically, the soluble protein content showed an "A" pattern at 24 h, 48 h, and 72 h, i.e., the soluble protein content of algal cells increased at the lower dose and then decreased with the microplastic concentration, the maximum value of 67.90 mg/L was observed under the 10 mg/L treatment at 48 h exposure. However, the higher concentration groups (75 mg/L and 100 mg/L) always showed significant inhibition in the exposure test, with the maximum inhibition occurring at 96 h of exposure, corresponding to inhibition rates of 16.34% and 19.19%, respectively. Furthermore, the remaining treatment groups also demonstrated significant differences from the control group at 96 h, and there was a dose–effect relationship between microplastics and soluble protein content in algal cells at this point.

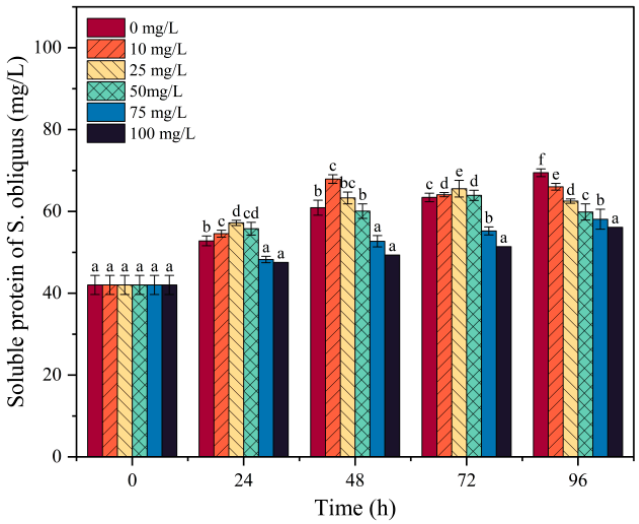


Figure 6. Variations in soluble proteins content of *S. obliquus* in response to exposure time at different PS-MP concentrations. The error bars represent standard error ($n = 3$). Means not sharing the same letter are significantly different from each other ($p < 0.05$).

3.6. Effect of PS-MPs with Different Concentrations on Membrane Permeability of S. obliquus

Figure 7 presents the changes in cell membrane permeability of *S. obliquus* under different concentrations of PS-MPs exposure. The cell membrane permeability of each treatment appeared to increase with the growth of treatment time at a certain exposure concentration. In detail, the ratio of cell membrane permeability in the algae at the corresponding time was gradually increased in each treatment group compared to that of the control group. The high-concentration groups (75 mg/L and 100 mg/L) showed significant dose–effect relationships, whereas the low-concentration treatment groups (10 mg/L and 25 mg/L) had negligible effects on cell membrane permeability. At the 96th h, the treatment groups above 25 mg/L exhibited the maximum degree of difference, and it was detected that the cell membrane permeability of each treatment group performed 1.61, 1.77, and 2.36 times higher compared to the control group, respectively.

The SEM images illustrated the changes in *S. obliquus* morphology under the microplastics exposure. As shown in Figure 8A, the algal cells aggregate with each other to form homogeneous aggregates in the presence of microplastic. On the other side, the algal cells could attach to the surface of microplastic clearly, which serves as a good growth substrate, leading to the formation of heterogeneous aggregates between the microalgae and PS-MPs (Figure 8B,C). In addition, the algal cells could secrete the extracellular polymers that could either exist in the interface of microalgae or attach to the surface of plastic,

thereby facilitating the generation of the homo-aggregation and hetero-aggregation. It is clearly demonstrated that the growth space of algal cells is greatly limited, and it is possible to observe the cellular deformation and significant damage (Figure 8D) caused by microplastics that can be observed in algal cells.

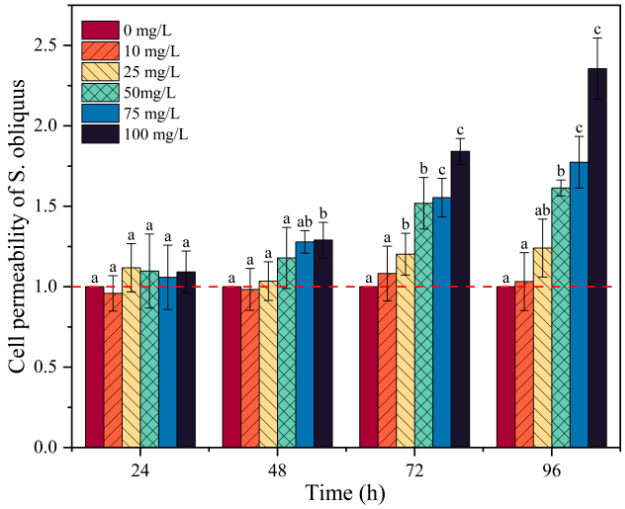


Figure 7. Variations in cell permeability of *S. obliquus* in response to exposure time at different PS-MP concentrations. The error bars represent standard error (n = 3). Means not sharing the same letter are significantly different from each other ($p < 0.05$).

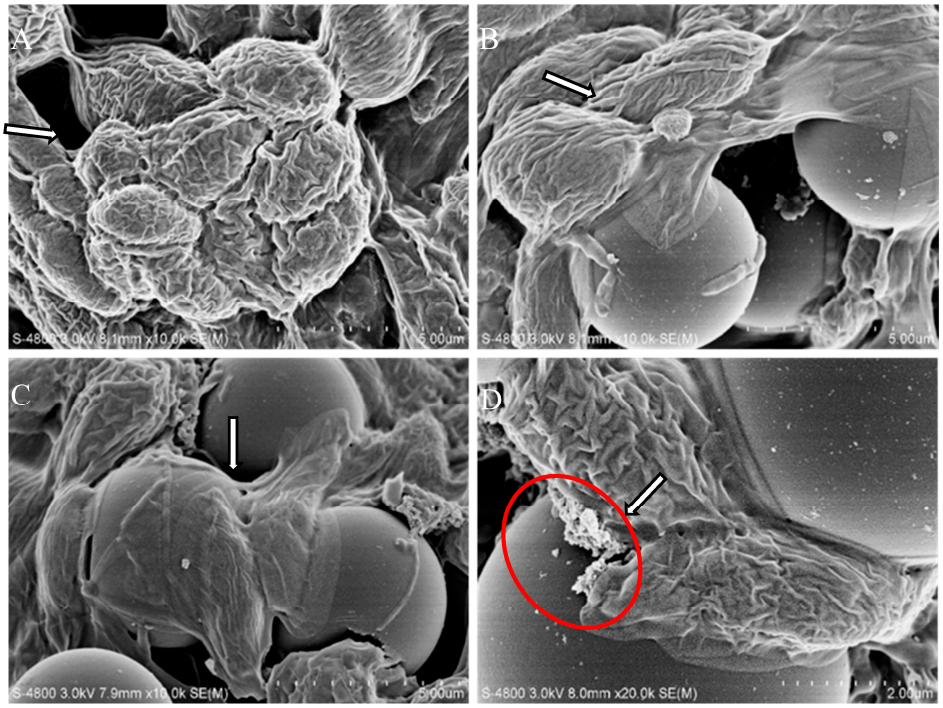


Figure 8. The Scanning Electron Microscope (SEM) images of *S. obliquus* homo-aggregated with others (**A**), PS-MPs attached to *S. obliquus* forming hetero-aggregation (**B**,**C**), the rupture of *S. obliquus* (**D**).

4. Discussion

PS-MPs are considered more toxic than other types of MPs (e.g., PP and PVC) [29]. The ecological benefits of microplastics, in areas with high concentrations, such as those near landfills and plastic commodity production plants, are unknown now [42]. Thus, to investigate the response mechanisms of widely distributed algal species to microplastics

in areas with high plastic pollution, PS-MPs of micro-scales (5 μm) were chosen for toxicity tests on *S. obliquus* at a high concentration (0–100 mg/L), which exceeded existing maximum environmental concentration (from 0 to 100 mg/L) [43]. We found that low concentrations of PS-MPs promoted the growth of *S. obliquus*; however, the phenomenon disappeared by the third day, indicating a toxic excitatory effect of the microalgae in low concentrations of PS-MPs, which gradually faded over time [44]. This effect lent credit to the idea that some microalgae species have the ability to use MPs as substrates and develop biofilms on them [30]. The concentration of freely dispersed PS-MPs in the solution was reduced due to hetero-aggregation between microalgae and PS-MPs. Moreover, the PS-MPs hetero-aggregation would tend to sink downward, creating a better environment without microplastic wrapping. Thus, microalgae that were not adsorbed by the microplastics remained capable of self-reproduction in the liquid cultures [31], facilitating the growth of algal cells to a certain extent. In addition, the SME images revealed that homogeneous aggregates were formed between the algal cells, which reduced the contact between the algal cells and PS-MPs. As the exposure time and concentration of the microplastics increased, the growth rate of algal cells in each treatment decreased significantly, indicating that the high concentration of microplastics still had an inhibitory effect on *S. obliquus*. A pattern of population changes that were similar to those described in this study had been previously found in other marine and freshwater species when they were exposed to MPs. These organisms are known to release exopolymeric substances (EPS) due to the cellular stress caused by the interaction with plastic particles of high concentrations.

We observed the heterogeneous aggregation formed by microplastic and algal cells, which would accelerate the settlement of aggregations. These substances were attached to the surfaces of algal cells, and enhanced the interaction between algal cells, extracellular polymers, and microplastics. Thus, this resulted in a reduction in light availability and substance exchange in microalgae [28,32,33,45]. In addition, a significant growth inhibition allowed us to hypothesize that contamination with MPs could induce a shading effect, hindering the microalgal exposure to light and negatively affecting microalgal growth and photosynthetic efficiency [4]. To cite an example, the light required for the normal survival of algal cells is blocked; thus, the uptake of substances and interfering with gas exchange with the outside world is negatively affected [46]. Adhesion is recommended to be the primary method by which PS-MPs affect the growth of *S. obliquus*. Furthermore, the high concentration of microplastics may cause mechanical damage to the algal cells by destroying the cells' structures, as shown in Figure 8 where the algal cells are fractured.

The content of chlorophyll (CHL) is one of the essential indicators for confirming whether the growth of algal cells is normal, reflecting the ability of algal cells to utilize light. According to the ability, we have strong evidence to judge the strength of the photosynthetic ability of algal cells, and this parameter indirectly responds to the degree of stress of external substances on algal cells [27]. The results of this study manifested that PS-MPs did not evidently affect photosynthetic ability during initial period. However, with the extension of exposure time, the variability compared with the control group increased, which indicated that the ability of algal cells to synthesize chlorophyll was significantly inhibited. Considering the changes in the activities of the enzymes related to the antioxidant system, the decrease in chlorophyll content could be attributed to the accumulation of ROS in the algal cells, resulting from strong oxidative stress on the cells. Furthermore, microplastics had a "shading" effect on the algal cells, so the algal cells lacked the ability to receive the light required for normal growth, and the efficiency of light energy conversion was reduced [4]. Ultimately, the mechanical damage to algal cells and the increase in cell membrane permeability (Figure 8) resulted in cytoplasmic leakage and a significant decrease in photosynthetic activity.

Superoxide dismutase (SOD) is a protective enzyme used in the antioxidant enzyme system to regulate intracellular reactive oxygen radicals (ROS). SOD is widely distributed in various organisms, and its activity level can reflect the degree of oxidative stress on cells [46]. In the absence of external influences, the antioxidant system of algal cells maintains a

dynamic equilibrium. When faced with external stress, SOD is rapidly activated to mitigate the ROS as ROS accumulation. Low levels of ROS can regulate multiple physiological and biochemical reactions in cells; however, ROS is evidently toxic to organisms at high concentrations [34,46]. In this experiment, the SOD activity of microplastic-treated algal cells exhibited a pattern of initial increase followed by a subsequent decrease. Moreover, there was a dose–effect relationship between the concentration of microplastics and SOD activity during the later stage of the experiment, indicating a significant inhibitory effect. This implies that highly sensitive algal cells in the logarithmic phase of growth rapidly activated SOD activity within a short period of exposure, in response to the explosion of ROS also as a way to maintain a normal state [35]. However, as time progresses, and the degree of external stress gradually increases, ROS accumulation reaches the threshold of clearing by the algal cells themselves. At this juncture, the intracellular antioxidant system was imbalanced, the antioxidant system of the organism was damaged, and the SOD enzyme activity was significantly inhibited, indicating that the cells were seriously damaged [28].

Turning our attention to another crucial marker of oxidative stress, malondialdehyde (MDA) serves as one of the end products of cellular lipid peroxidation. It is commonly utilized to confirm whether lipid peroxidation occurs in cellular membranes under environmental stress, and its content can be employed as an indicator to assess the extent of oxidative damage in cells. When lipid peroxidation occurs in cells, the structure of the cell membrane is also affected, resulting in cell deformation and increased permeability of the cell membrane. Therefore, the normal energy exchange between algal cells and external substances is also impacted [46]. In this study, the exposure of microplastics caused a significant increase in MDA content in algal cells, indicating that lipid peroxidation occurred in the algal cells. Previous studies have identified that the content of MDA increased by many times with the exposure under MPs as compared with that of the control group [27]. It revealed that MPs caused superfluous ROS to algal cells and anoxidative bursts. Several studies studied exposure of MPs significantly increased the intracellular ROS content of *Euglena gracilis* and inhibited the production of pigment [47]. When the intake and accumulation of MPs exceeded the threshold, the antioxidant enzyme systems of microalgae cells fail to maintain a dynamic balance of ROS production and elimination, leading to the inhibition of enzyme activity and serious oxidative damage to the antioxidant enzyme system, triggering apoptosis [37].

As key nutrients, soluble proteins play an important role in the osmoregulation of algal cells, facilitating water retention and protecting cell membranes and intracellular materials [48]. Under stress, algal cells produce a stress response to disturb the internal cellular equilibrium, causing changes in various metabolic activities. The results of this study showed that the trend in soluble protein in algal cells treated with less than 75 mg/L initially showed an increase, followed by a slight decrease. This may be traced back to the self-repair and defense mechanism of *S. obliquus* following the adsorption of PS-MPs to produce physical damage for a short period of time [39,48]. However, as the exposure time increased, the toxic effects exceeded the self-regulation capacity of the algal cells. Meanwhile, a decrease in the synthesis of raw materials was observed, as reflected by the decreased chlorophyll content (Figure 3), which pointed to the fact that the high-concentration treatment group showed significant inhibition of protein synthesis throughout the exposure.

The cell membrane is the first barrier that provides defense and isolates the cell from the outside world [33]. The cell membrane can indirectly confirm whether the cell wall/plasma membrane of algal cells has been damaged [36]. In this study, the authors examined the membrane permeability of algal cells in each treatment group. Our results showed that PS-MPs disrupted the membrane integrity of algal cells, and the cell permeability was evidently improved by 2.36 times. In general, membrane permeability is closely related to membrane damage and metabolic activity. Cell ruptures in SEM images and reductions in chlorophyll contents in our study also offered compelling evidence for

this result. In addition, the change in permeability was closely associated with the risk of some unfavorable substances entering the algal cells and accumulating in the cells, which eventually produce toxicity and thus inhibit the growth of algal cells [34]. The results of this experiment unveiled that the cell membrane permeabilities of algal cells positively responded to the high concentrations of PS-MPs over long periods of time. Conversely, none of the treatment groups exhibited differential changes over a short period of time. This suggests that the exposure time contributes to a considerable share to the effect of PS on cell membranes, probably as a collective outcome of the imbalance of various metabolic activities to a certain level and the time required for toxic accumulation. Concurrently, we used SEM to observe algal cells subjected to microplastic stress, and the algal cells underwent significant deformations and even fractures, such that cell damage was denatured.

5. Conclusions

In this paper, the biological effects of PS-MPs on *S. obliquus* were investigated by measuring the growth curve, chlorophyll content, SOD activity, MDA content, soluble protein content, cell membrane permeability, and cell morphology. The results showed that PS-MPs had a significant inhibitory effect on the growth of *S. obliquus*, and the intrinsic mechanism was that PS significantly promoted the photosynthesis and metabolic activities of *S. obliquus* and induced serious oxidative damage. Furthermore, the mutual contact between PS-MPs and *S. obliquus* led to rupture of algal cells. The results of this study are helpful to understand the interaction between PS-MPs and microalgae and have a certain reference value for assessing the impact of PS on aquatic ecosystems.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/su151411223/s1, Figure S1: The relationships between optical density and Cell Density of microalgae; Table S1: The recipe of BG-11 (Blue Green Medium); Table S2: The recipe of Mixture of trace metals; Table S3: The instruments and chemicals employed in the experiment.

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