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Article

Effects of Suspended Particles on Exopolysaccharide Secretion of Two Microalgae in Jinjiang Estuary (Fujian, China)

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Abstract: The effects of suspended particles (SP) of different concentrations and sizes on the secretion of exopolysaccharide (EPS) by *Chlorella pyrenoidosa* (*C. pyrenoidosa*) and *Microcystis flos-aquae* (*M. flos-aquae*) in Jinjiang Estuary, Fujian Province, China were studied in co-cultures of microalgae and SP. The results show that the SP concentration has an "inhibit–promote–inhibit" effect on the secretion of EPS by *C. pyrenoidosa*, whereby there is an optimal concentration of SP corresponding to the largest amount of EPS secreted by *C. pyrenoidosa*. Low concentrations had no significant effect on the secretion of EPS from *M. flos-aquae* ($p > 0.05$), whereas higher concentrations had an inhibitory effect. The *C. pyrenoidosa* EPS content was found to be significantly decreased in response to SP of small particle sizes (0–75 and 75–120 μm) and significantly increased for SP of large particle sizes (120–150 and 150–500 μm). Small particle sizes (0–75 and 75–120 μm) were found to be beneficial to the secretion of EPS from *M. flos-aquae*, and the promotion of EPS secretion gradually decreased with the increase in SP particle size. However, when the particle size was too large (120–150 and 150–500 μm), SP had no significant effect on EPS secretion. This study is helpful for understanding the microalgae EPS secretion response to SP and provides a scientific basis for studying the mechanism of EPS secretion by algae and the effect of SP on eutrophication of the estuary.

Keywords: suspended particles; exopolysaccharide; *Chlorella pyrenoidosa*; *Microcystis flos-aquae*

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1. Introduction

Eutrophication and its resulting red tides have become one of the significant environmental problems in estuaries and coastal waters worldwide [1–3]. Some studies have shown that blooms are caused by a large number of cyanobacteria and green algae [4–6]. Yerli et al. [7] found that cyanobacteria and green algae dominate the microalgae community of Lake Mogan (Ankara, Turkey). Exopolysaccharide (EPS) is a bioactive polysaccharide secreted by algal cells during growth and reproduction. The synthesis and release of EPS are considered a result of the coordination between organisms and their growth environment [8,9]. The bloom of microalgae may lead to an increase in EPS production.

An estuary is a zone where there is mixing of marine and fresh water resulting in creation of a gradient of brackish water, to which the regional ecosystem is susceptible. At the same time, frequent human activity in the estuarine area affects the ecosystem and has led to an imbalance [10]. As an important part of water environment, suspended particles (SP) mainly affect algae by blocking light from entering the water, releasing or absorbing nutrients, and acting as algae growth carriers [11]. At the same time, SP may also cause algae cells to lyse, resulting in death of the microalgae [12]. In addition, EPS can combine with SP to form transparent exopolymeric particles (TEP), which increase the weight of bound particles and affect the settling ability of particles [13]. There are a wide range of SP concentrations and particle sizes in different regions of estuary, such as in the Belgian nearshore area (southern North Sea), where SP particle size range between 30–180 μm [14].

The SP concentration and particle size of the San Francisco Bay (San Francisco, CA, USA) are 21–230 mg/L and 24–639 μm, respectively [15]. The average concentration of SP in Jinjiang River (Fujian, China) ranges from 10.74 to 136.76 mg/L [16].

Microalgae are the main primary producers of the aquatic ecosystem and the basis of the food chain. Therefore, all changes to microalgae will eventually affect the stability of the structure and function of the aquatic ecosystem [4,17]. *Chlorella pyrenoidosa* (*C. pyrenoidosa*) and *Microcystis flos-aquae* (*M. flos-aquae*) are the most widely distributed and common types of algae in freshwater environments and are often used in toxicology [18]. In aquatic ecosystems, the effects of antibiotics, heavy metals and organic matter on EPS secretion of algae have been studied. For example, increased EPS content plays a defensive role in protecting *Dictyosphaerium* sp. against antibiotic stress, and there is a strong positive correlation between EPS content and antibiotic concentration [19]. Wang et al. [20] found that EPS plays an important role in the ability of symbiotic systems to cope with the stress of heavy metals and resist their toxicity. Lv et al. [21] showed that particulate organic matter significantly promotes EPS production in microalgae via cell lysis and the stress induced by carbon starvation. Based on these observations, we hypothesize that the existence of SP is important to the secretion of EPS from microalgae.

Specifically, SP is known to control biological secretions, and the effect of SP in the estuarine environment on the EPS secretion of algae is seldom reported. Therefore, *C. pyrenoidosa* and *M. flos-aquae* were selected as the study objects for the mixed culture of microalgae and SP to investigate the effects of different concentrations and particle sizes of SP in the Jinjiang Estuary on the secretion of EPS by algal cells. The results provide a scientific basis for studying the mechanism of the effects of SP on EPS secretion by algae and on eutrophication of the estuary.

2. Materials and Methods

2.1. Experimental Materials

SP in water was collected from Jinjiang Estuary in April 2019. *C. pyrenoidosa* (FACHB-9) and *M. flos-aquae* (FACHB-1028) were purchased from the Freshwater Algae Culture Collection at the Institute of Hydrobiology (FACHB), Wuhan, China, and cultured in BG11 medium. The composition of the culture medium is shown in Supplementary Table S1. The main apparatus is shown in Supplementary Table S2. Chemicals used were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China).

2.2. Experimental Methods

2.2.1. Determination Parameters of SP

The sediment particle size was determined by using a laser particle size analyzer (Mastersizer, 2000) [22]. Particulate organic matter was measured using the "$K_2Cr_2O_7$ volumetric method" stipulated in the China Agricultural Standard (NY/T1121.6E2006). The total nitrogen (TN) in particulates was determined based on $K_2S_2O_8$ oxidation [23]. The total phosphorus (TP) of particulate matter was determined as per Lu et al. [24]. The particles were visualized by scanning electron microscopy (SEM) (TESCAN MIRA4).

2.2.2. SP Stock

To better simulate the effect of SP on microalgae in the natural state, particle samples from Jinjiang Estuary in their untreated natural state were naturally air-dried, ground with an agate mortar and pestle, and sieved with stainless steel mesh into 200 mesh (75 μm), 120 mesh (120 μm), 100 mesh (150 μm) and 32 mesh (500 μm), and then stored in self-sealing bags with corresponding labels and stored under dry conditions until use. The particles were added to distilled water one day in advance and stirred for 24 h to ensure that the SP stock was as evenly dispersed as possible.

2.2.3. Microalgae Cultivation

The microalgae were cultured in a 250 mL conical flask containing 100 mL BG11 medium, with a light intensity of 3000–4000 lx, temperature of 25 °C, and light to dark ratio of 14 h:10 h. They were shaken slowly in a circular motion by hand three times a day, randomly changing the position of the conical flask. The absorbance (OD_{680}) of the culture was measured every day, and the growth stage of the culture was determined by drawing the growth curve. All of the above operations were carried out under aseptic conditions. When the microalgae were in the logarithmic growth phase, they were used for the following experimental treatments.

2.2.4. Preliminary Experiment

In this experiment, a range of SP concentrations and particle sizes similar to those of real water were used to explore the influence of SP concentration and particle size close to the environmental level on microalgae. The concentration and particle size selections were based on Hou et al. [25] but with slight differences. Prepared according to the above culture method, SP of different concentrations and particle size gradients were added to the microalgae culture flasks with a logarithmic growth period to culture *C. pyrenoidosa* and *M. flos-aquae*. The aim was to determine the concentration and particle size gradient of SP in formal experiments through the response of algae cells to different treatments.

2.2.5. Experimental Design

The microalgae in the logarithmic growth phase were mixed and the same volume was added to each conical bottle. The reserve SP solution was added to the conical bottle with microalgae according to the different concentrations, and the particle sizes are shown in Table 1. There were three replicates in each group, with 60 culture flasks in total. Culture was carried out under the same conditions as earlier. *C. pyrenoidosa* and *M. flos-aquae* were treated with the SP for 10 and 7 days [26,27], respectively, then the content of EPS was determined. CK represents the control check, in which no SP has been added.

Table 1. Experimental design of the effect of SP on EPS secretion of microalgae.

SP Addition	Concentration (mg/L) & Particle Size (μm)				
C. pyrenoidosa	CK & 75–120	30 & 75–120	50 & 75–120	70 & 75–120	90 & 75–120
M. flos-aquae	CK & 75–120	100 & 75–120	150 & 75–120	200 & 75–120	250 & 75–120
C. pyrenoidosa	30 & CK	30 & 0–75	30 & 75–120	30 & 120–150	30 & 150–500
M. flos-aquae	100 & CK	100 & 0–75	100 & 75–120	100 & 120–150	100 &150–500

2.2.6. Determination of Glucose Standard Curve

The content of EPS was mostly determined using the phenol—sulfuric acid method proposed by Dubois et al. [28]. The standard glucose solution of 150 mg/L was prepared with distilled water. Glucose standard solutions (150 mg/L) of 0.0, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, and 1.0 mL were added to the test tube and supplemented with distilled water to a volume of 2.0 mL, to give different concentration gradients. Then, 1.0 mL 6% phenol solution was added to each gradient and mixed well, and then 5.0 mL concentrated sulfuric acid was added. The solutions were then placed in a boiling water bath for 10 min, followed by running water to cool to room temperature. The standard curve was drawn with the glucose concentration as the abscissa and the absorbance OD_{490} as the ordinate.

2.2.7. Determination of EPS Content

After the microalgae in the culture medium were treated with SP, the algae EPS was extracted by centrifugal filtration. The culture medium with microalgae was centrifuged at 10,000× *g* (15 min), and the supernatant was subjected to a 0.45 μm cellulose acetate ultrafiltration membrane for filtration. The filtrate contained EPS. The content of EPS was determined using the phenol sulfuric acid method [28]. Although the color reaction of

glucose with other monosaccharides is similar, there are some differences, so the standard curve drawn with glucose as the standard was corrected with a correction coefficient of 0.9 [29]. The calculation of EPS content referred to the following formula:

$$\text{EPS content (mg/L)} = C \times V \times 0.9 \quad (1)$$

where C is the standard glucose solution concentration, V is the volume of standard glucose solution concentration corresponding to the measured absorbance, and 0.9 is the correction coefficient of glucose.

2.3. Data Analysis

All the data presented were expressed as mean ± standard deviation. Excel 2016 (Microsoft, Redmond, WA, USA) was used for statistical processing, Origin 2018 (OriginLab, Northampton, MA, USA) was used for plotting, and the SPSS 25.0 software package (International Business Machines Corporation, Armonk, NY, USA) was used for significant difference analysis. The difference from the control group was considered significant at $p < 0.05$ or very important at $p < 0.01$. The vertical error bars indicate standard deviations. The asterisks highlight significant differences from control (* $p < 0.05$; ** $p < 0.01$). The different lowercase letters indicate significant differences ($p < 0.05$) between comparisons and the same lowercase letters indicate insignificant differences ($p > 0.05$).

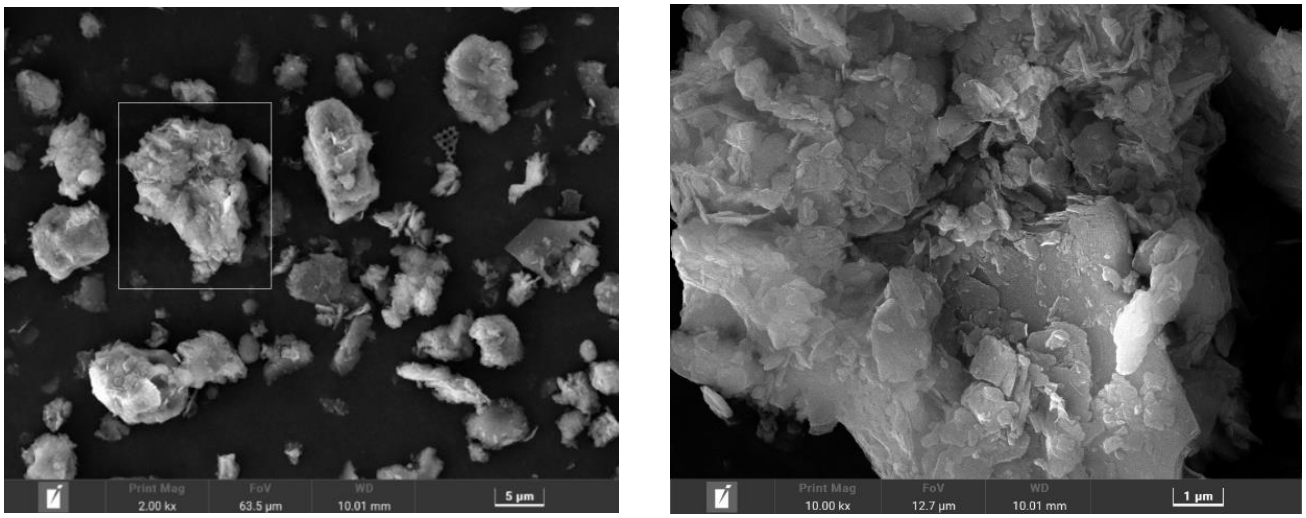
3. Results

3.1. SP Parameters

The measured values of physical and chemical parameters of SP are shown in Table 2 [30]. Most SP were classified as silt (2–20 μm), about 78.37%, with relatively fewer SP being classified as clay (<2 μm) and sand (>20 μm). SEM images of the SP samples are shown in Figure 1.

Table 2. Physicochemical parameters of SP.

Clay (<2 μm)	Silt (2–20 μm)	Sand (>20 μm)	Organic Matter ($mg \cdot kg^{-1}$)	TN ($mg \cdot kg^{-1}$)	TP ($mg \cdot kg^{-1}$)
4.34% ± 1.63%	78.37% ± 1.14%	17.26% ± 2.69%	21.29 ± 7.9	388.49 ± 59.14	549.70 ± 8.43



(**a**) (**b**)

Figure 1. SEM images of SP in the present study. (**a**) SEM images of SP; (**b**) SEM images of SP in detail of the box of (**a**).

3.2. Influence of SP of Different Concentrations on EPS Content of C. pyrenoidosa in Jinjiang Estuary

The effects of different concentrations of SP on EPS from *C. pyrenoidosa* in Jinjiang Estuary are shown in Figure 2. The EPS content of *C. pyrenoidosa* decreased firstly, then increased, and then decreased with the increase of SP concentration. The EPS content from *C. pyrenoidosa* in the 30 mg/L particle experimental group was significantly lower than that in the control group ($p < 0.01$), and its EPS content reached the lowest value (0.03550 mg/mL). There was no significant difference in EPS content between the 50 mg/L experimental group and the control group ($p > 0.05$). The EPS content in the 70 mg/L experimental group was significantly higher than that in other groups ($p < 0.01$) and reached a maximum value of 0.05537 mg/mL.

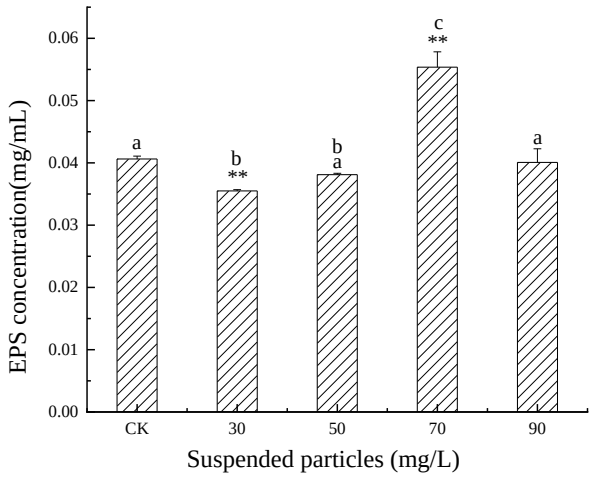


Figure 2. Influence of SP of different concentrations on the EPS content of *C. pyrenoidosa* in Jinjiang Estuary.

3.3. Influence of SP of Different Concentrations on the EPS Content of M. flos-aquae in Jinjiang Estuary

The effects of SP of different concentrations on the EPS content of *M. flos-aquae* in Jinjiang Estuary are shown in Figure 3. The EPS content of *M. flos-aquae* in the 150 mg/L particle concentration group was significantly lower than that in the 100 and 250 mg/L groups ($p < 0.05$). When the concentration of SP was 150 mg/L, the EPS content reached the minimum value (0.01992 mg/mL). There was no significant difference between the 200 and 250 mg/L particle concentration groups ($p > 0.05$). The EPS content in all experimental groups was lower than in the control group. There was no significant difference between the 100 mg/L group and control groups ($p > 0.05$). The other concentration groups were significantly lower than the control group ($p < 0.01$).

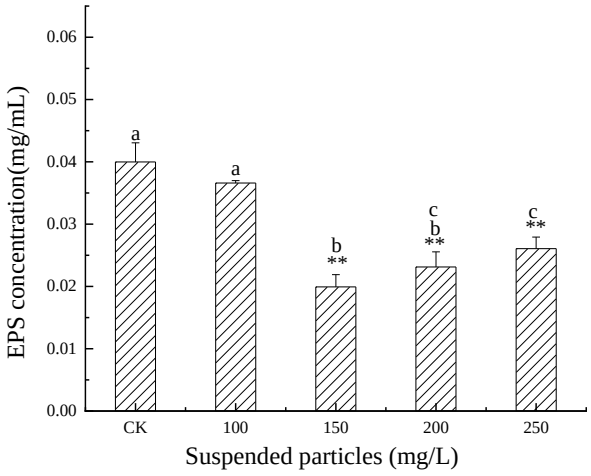


Figure 3. Influence of SP of different concentrations on the EPS content of *M. flos-aquae* in Jinjiang Estuary.

3.4. Influence of SP of Different Particle Sizes on EPS Content of C. pyrenoidosa in Jinjiang Estuary

Figure 4 shows the effects of SP of different particle sizes on the EPS content of *C. pyrenoidosa* in the Jinjiang Estuary. As can be seen in Figure 4, the EPS content of the 0–75 μm particle size of *C. pyrenoidosa* was significantly lower than that of the control group ($p < 0.05$), the content of EPS in the 75–120 μm particle size group was significantly lower than that in the control group ($p < 0.01$) and was the lowest overall (0.02467 mg/mL). The EPS content in the 120–150 and 150–500 μm particle size groups was significantly higher than that in the control group ($p < 0.01$), and the EPS content of the 120–150 μm group was the highest overall (0.04975 mg/mL) and 1.58 times that of the control group.

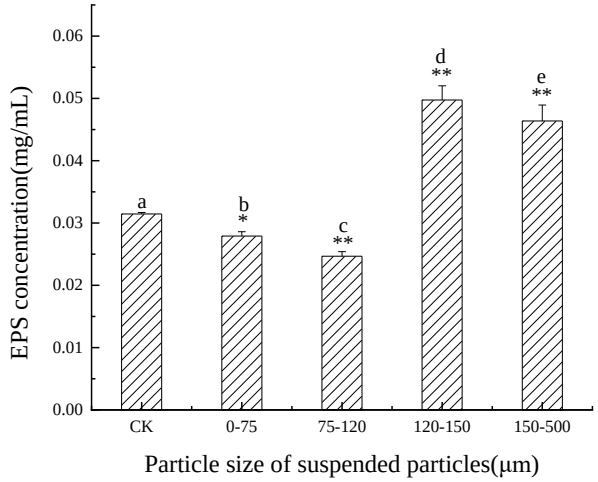


Figure 4. Influence of SP of different particle sizes on the EPS content of *C. pyrenoidosa* in Jinjiang Estuary.

3.5. Influence of SP of Different Particle Sizes on the EPS Content of M. flos-aquae in the Jinjiang Estuary

Figure 5 shows the influence of SP of different particle sizes on the EPS content of *M. flos-aquae* in the Jinjiang Estuary. With the increase in particle size, the content of EPS of *M. flos-aquae* first increased and then decreased. When the particle size was 0–75 μm, the EPS content was significantly higher than that of the control group ($p < 0.01$), and the EPS content was 0.05021 mg/mL, which was 1.81 times that of the control group. The content of EPS in the 75–120 μm group was significantly higher than that in the control group ($p < 0.05$) (1.28 times as much as the control group) and there was no significant difference in EPS in the 120–150 and 150–500 μm particle size groups compared with the control group ($p > 0.05$).

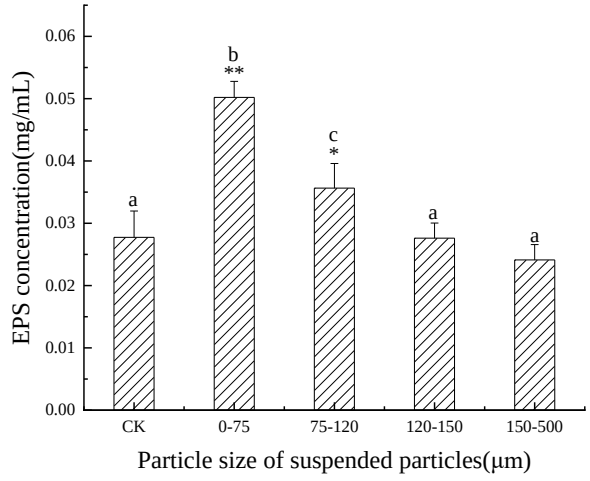


Figure 5. Influence of SP of different particle sizes on the EPS content of *M. flos-aquae* in the Jinjiang Estuary.

3.6. The Light Attenuation Coefficient(k_d) by SP of Different Concentrations and EPS per Unit Chlorophyll A of C. pyrenoidosa and M. flos-aquae

Tables 3 and 4 shows the light attenuation coefficient (k_d) by SP of different concentrations and EPS per unit chlorophyll a of *C. pyrenoidosa* and *M. flos-aquae*. As shown in Table 3, with the increase of SP concentration, the coefficient of k_d increases continuously. In the 70 mg/L experimental group, the EPS per unit chlorophyll a of *C. pyrenoidosa* was higher. In the experimental group of 150–250 mg/L, the EPS secretion per unit chlorophyll a by *M. flos-aquae* was reduced. The EPS content of the 250 mg/L experimental group is higher than that of the 150 mg/L experimental groups. As shown in Table 4, under 120–500 μm particle size of *C. pyrenoidosa*, the EPS secretion per unit biomass increased. In Table 4, the unit EPS content of *M. flos-aquae* of small particle size groups (0–75 and 75–120 μm) is 1.91 and 1.39 times that of the control group, respectively.

Table 3. K_d of SP and EPS per unit chlorophyll a of *C. pyrenoidosa* and *M. flos-aquae*.

C. pyrenoidosa	CK	30 mg/L	50 mg/L	70 mg/L	90 mg/L
K_d	0.00	1.94	2.92	3.82	4.67
EPS per unit Chlorophyll a $\times 10^{-6}$ (mg/mg)	4.16	4.22	5.19	7.76	5.50
M. flos-aquae	CK	100 mg/L	150 mg/L	200 mg/L	250 mg/L
K_d	0.00	5.08	7.03	8.85	10.58
EPS per unit Chlorophyll a $\times 10^{-5}$ (mg/mg)	13.90	12.99	7.33	8.85	9.78

$K_d = 0.127\ SSC^{0.801}$ ($R^2 = 0.88$, $p < 0.001$), where SSC is the concentration of SP in water (mg/L) [31].

Table 4. EPS per unit chlorophyll a of *C. pyrenoidosa* and *M. flos-aquae*.

EPS per Unit Chlorophyll A (mg/mg)	CK	0–75 μm	75–120 μm	120–150 μm	150–500 μm
C. pyrenoidosa ($\times 10^{-6}$)	5.84	5.56	5.14	10.52	10.09
M. flos-aquae ($\times 10^{-5}$)	8.69	16.59	12.11	9.01	8.02

4. Discussion

4.1. Effects of SP of Different Concentrations on EPS Secretion of C. pyrenoidosa and M. flos-aquae

Studies have shown that when pyrenoidosa faces external environmental stress, it will promote the secretion of cellular polysaccharides, especially EPS, to form a protective layer and prevent the invasion of cells by external adverse factors [32,33]. Li et al. [34] showed that the EPS secretion was higher when *Aphanothece halophytica* growth was eugonic. In this experiment, the effect of SP at different concentrations on the EPS of *C. pyrenoidosa* was "inhibit-promote-inhibit", which means there was an optimal concentration of SP on the secretion of EPS by *C. pyrenoidosa*. With the extension of culture time, the nutrients in the culture medium gradually decreased. On the one hand, SP can be used as a nutrient source to provide nutrients for the growth of microalgae; on the other hand, it may also place microalgae in a stressful environment, resulting in increased EPS secretion. In addition, the existence of SP will limit the absorption of light, thus affecting photosynthesis. As a product of photosynthesis, the yield of EPS will also be limited. The EPS content of *C. pyrenoidosa* in the 30 mg/L experimental groups was significantly lower than that in the control group ($p < 0.01$). It may be that a lower concentration of SP cannot provide enough nutrition to promote the growth of *C. pyrenoidosa*, nor can it provide enough stress to increase EPS secretion. The light attenuation coefficient in water is often expressed by K_d. Through study of the water of Jinjiang River, Li [31] found that there was a correlation between K_d and the concentration of suspended solids. As shown in Table 3, the existence of SP will inevitably limit the absorption of light, even if the concentration of SP is low. So the yield of EPS will also be low. An et al. [35] also proposed that the effect of light intensity on EPS was higher than that of nitrogen and phosphorus. There was no significant difference in the content of EPS between the 50 mg/L experimental group and the control group

($p > 0.05$). The reason is that SP provided nutrition for microalgae, which compensated for the negative effect caused by light limitation. However, the EPS content in the 70 mg/L experimental group was significantly higher than that in the control group ($p < 0.01$). This may be because the SP at 70 mg/L could provide a large number of nutrients to algal cells. In addition, microalgae thrived on carriers with an excellent living environment, resulting in increased EPS secretion (Table 3). Thus, the EPS content was higher. However, there was no significant difference in EPS contents between the 90 mg/L high concentration group and the control group ($p > 0.05$). This may be because the high SP concentration provided sufficient nutrients for microalgae growth. Sufficient nutrients counteracted the adverse effect of algae cell growth inhibition due to light obstruction by SP. Similarly, Hou et al. [25] found that the concentration of particles within a specific range (30–50 mg/L) can promote the growth of *M. aeruginosa*. However, algae growth will still be inhibited if the concentration exceeds a certain level (60 mg/L). This concentration range is different from that in our experiment, indicating there are interspecific differences in the response of different microalgae to SP.

Wang [36] found that there were interspecific differences in the effects of natural nanoparticles on *C. pyrenoidosa* and *M. flos-aquae.* Some studies have shown that the sensitivity of green algae and cyanobacteria to antibiotics differs by several orders of magnitude [37,38]. In addition, this study also found that cyanobacteria and green algae have different sensitivities to SP. It may be due to the different mechanisms of action of SP on microalgae or the different tolerances of microalgae to SP. Therefore, the choice of SP with different concentrations and particle sizes were associated with responded of *C. pyrenoidosa* and *M. flos-aquae* in this study. In this experiment, the EPS content of *M. flos-aquae* in different concentration groups was lower than that in the control group. There was no significant difference between the 100 mg/L experimental group of *M. flos-aquae* and the control group ($p > 0.05$). This may be because the concentration of SP had not reached the level of significant effect on the growth of algal cells. That is, the nutrient release effect and extinction effect of particles on *M. flos-aquae* are similar, and the two are balanced. So, the low concentration of particles has no significant impact on algal cells. However, in the experimental group of 150–250 mg/L, the EPS secretion by *M. flos-aquae* was significantly lower than that of the control group ($p < 0.01$). This indicates that the presence of high-concentration particles seriously hindered the entry of light, thus inhibiting the photosynthesis of microalgae and reducing the EPS secretion per unit biomass (Table 3). He and Lu [39] confirmed that SP has dual ecological effects of providing nutrients to and facilitating extinction of the ecosystem. The extinction effect of SP is stronger than the release effect of nutrients in the turbidity zone, which results in significantly reduced phytoplankton biomass. The higher the SP concentration, the more pronounced the flocculation and sedimentation with microalgae. In this experiment, the EPS content of the 250 mg/L experimental group was significantly higher than that of the 150 mg/L experimental group ($p < 0.05$). This may be due to the faster settling speed of SP, which made microalgae stay in the light for a longer time, which is beneficial to the synthesis of EPS. As shown in Table 3, the EPS content of the 250 mg/L experimental group is higher than that of the 150 mg/L experimental groups.

4.2. Effects of SP of Different Particle Sizes on EPS Secretion of C. pyrenoidosa and M. flos-aquae

This study shows that different types of algal cells have different stress responses based on different particle sizes. SP of different particle sizes had significant effects on the secretion of *C. pyrenoidosa*. Small particle size SP was not beneficial to the secretion of EPS from *C. pyrenoidosa*. By contrast, large particle size was beneficial to the secretion of EPS from *C. pyrenoidosa*. The particle size of 120–150 μm had the maximum effect in promoting EPS secretion. The EPS content was significantly decreased under small particle sizes of 0–75 μm ($p < 0.05$) and 75–120 μm ($p < 0.01$). This may be distinct from the effect of different light intensities on the photosynthesis of *C. pyrenoidosa*. Under the same illumination conditions, SP with the same concentration and different particle sizes can have varied

blocking effects on light [40]. The distribution of SP with the same concentration and different particle sizes in water can also vary. It is a pity that changes in the size of total suspended solids is one of the reasons for the unresolved K_d variability [41]. Therefore, the K_d of SP of different particle sizes could not be calculated in this study. However, studies have shown that the smaller the particle size, the higher the number of SP particles. In addition, the larger the distribution density, the greater the ability to block light, and the lower the light intensity that can be used by algal cells [25,40]. Thus, photosynthesis is weakened, algal cell growth is inhibited, and the secretion of EPS is also reduced. The EPS content of *C. pyrenoidosa* in the 75–120 μm group was the lowest, which may be because its particle size was larger than that in the 0–75 μm group. The specific surface area of particles was smaller, and the nutrients adsorbed by particles were insufficient considering the supply capacity to algal cells, which is less favorable for the reproduction of algal cells. The EPS content of *C. pyrenoidosa* increased significantly under 120–150 μm particle size ($p < 0.01$). This may be because the extinction effect of large particle size was much smaller than that of small particle size. This particle size was the most suitable environment for the growth of algae cells in the experimental group. Microalgae photosynthesis enhanced, with an increase in EPS secretion per unit biomass (Table 4). However, a particle size of 150–500 μm might be too large, leading to more algae cells sinking. By comparison, we found that there was a difference in EPS content between the 30 mg/L group in Figure 2 and the 75–120 μm group in Figure 4 under the same conditions of SP concentration and particle size. In addition, it can be seen from Tables 3 and 4 that the EPS content per unit biomass of the two experimental groups are different. This may be due to the different attenuation abilities of light caused by the irregular shape of SP [41]. The specific reasons need to be further explored.

The results showed that small particle sizes had a more significant effect on EPS secretion than large particle sizes. Small particle sizes (0–75 and 75–120 μm) are beneficial to the secretion of EPS from *M. flos-aquae*. However, the promotion of EPS secretion was gradually decreased with the increase in particle size. When the particle size was excessively large (120–150 and 150–500 μm), SP had no significant effect ($p > 0.05$) on EPS secretion from *M. flos-aquae*. Some scholars have pointed out that the secretion of EPS of *Cyanobacteria* is essentially a reaction of organisms to adverse environmental factors [42]. Guo et al. [43] found that disturbance, especially persistent disturbance, could promote EPS secretion to a certain extent. Qin et al. [44] showed that due to the presence of EPS, moderate or small wind and wave disturbances lead to the aggregation of algal cells into larger groups and accelerates the formation of cyanobacteria blooms. As a result of the negative impact of small-size particles on the weakening of light intensity, the absorption of nutrients and the consumption of dissolved oxygen is greater than the positive impact of the release of nutrients and the growth carrier of the algae on the algae. Therefore, the EPS secretion is increased as a defense mechanism against the stress environment to maintain average algae growth. Chlorophyll a is an important index reflecting phytoplankton biomass [45]. As shown in Table 4, the unit EPS content of small particle size groups (0–75 and 75–120 μm) is 1.91 and 1.39 times that of the control group, respectively. It can be seen that the increase in EPS content in the small particle size group is due to increased secretion. With the increasing particle size, the adverse effects of particles gradually decreased. The stress response of algae was no longer strong, and EPS secretion slowly approached normalization. The difference in EPS content between algae and the control group diminished to the point of not being significantly different. Hou et al. [25] found in their study that particles with small sizes have a more substantial impact on algae growth than those with large particle sizes, which is consistent with our conclusion on *M. flos-aquae*.

5. Conclusions

This study shows that:

1. The effect of SP concentration on the EPS of *C. pyrenoidosa* was "inhibit-promote-inhibit", that is, there was an optimal concentration of SP on the EPS secretion of *C. pyrenoidosa*;
2. SP in a particular concentration range (0–100 mg/L) had no significant effect on the EPS content of *M. flos-aquae*. When the particles exceeded a specific critical concentration (150 mg/L), the EPS secretion of *M. flos-aquae* was inhibited;
3. A small particle size did not promote the secretion of EPS from *C. pyrenoidosa*. By contrast, large particle size promoted the secretion of EPS from *C. pyrenoidosa*, with the maximum effect observed for 120–150 μm particle size;
4. A small particle size promoted the secretion of EPS from *M. flos-aquae*, but the effect gradually weakened with the increase in particle size.

The above findings are helpful to understand the change patterns of EPS in the response process of microalgae under the action of SP and provide a scientific basis for the study of EPS secreted by algae and the eutrophication mechanism of estuarine SP.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/jmse10020277/s1, Table S1: The composition of BG11 medium; Table S2: The main apparatus of the experiment.

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