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Microplastics removal from water body by extracellular polymeric substances (EPS) extracted from microalge through surfactants pre-treatment

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Abstract

Microplastics (MPs) have recently been identified as an important global problem which affects marine organisms and even human being. The extracellular polymeric substances (EPS) of microalge showed a potential to form hetero-aggregates with MPs and remove them from water body. However, the EPS extraction from microalge is a challenging and costly process. In this research, MPs removal from water body by using EPS extracted form microalge through surfactants pre-treatment was studied. To do this, cationic surfactants of Cetyl trimethyl ammonium bromide (CTAB) and dodecyl trimethyl ammonium bromide (DTAB) were added to microalgae for cell disruption and EPS extraction. Chitosan has also used as control. The results revealed that CTAB and DTAB could efficiently extract EPS from microalge up to 75% and 47%, respectively. While, chitosan could only release 17% of EPS. Additionally, microalgae were tested against micro plastics, including Polyethylene (PE) and polypropylene (PP). A maximum removal efficiency of 86% was achieved for all MPs after treating microalge with CTAB. The main mechanism of the MPs removal was identified as hetero-aggregation. The results of this study showed and effective removal efficiency of multiple kinds of MPs by microalgae EPS.

Key words: Microalgae, Microplastic, Biological Wastewater Treatment, Extracellular polymeric substances.

1. Introduction

Plastic pollution has become a concern due to the increased plastic production every year. Their safe disposal has attracted more and more attention due to the increased production and disposal of plastic products and low biodegradation rates. Microplastics (MPs), with the diameter less than 5 mm, are ubiquitous in the aquatic environment [1]. In recent years, several technologies including flocculation [2], electrocoagulation [3], dynamic membranes [4] and photocatalytic [5] have been applied for microplastics removal from fixed emissions like wastewater before being discharged into natural environment. Nevertheless, existing microplastics removal techniques are still inefficient or expensive, in which both too ineffective and inflexible that might have a negative effect to the environment [6].

Microalgae are typical organisms present in all freshwater systems and at the lowest level of the trophic chain [7]. For the past years, the attention given to extracellular polymeric substances (EPS) of microalgae has increased due to their potential applications within different industries [8]. EPS are biodegradable and range from gels to fully dissolved states, displaying viscous gel-like structures [9]. Microalgae can excrete exopolymer substances (EPS) with a potential to form hetero-aggregates with microplastic particles [10]. In other words, the capability of microalgae to produce EPS with sticky properties makes them potential candidates for hetero-aggregation which can attach to microplastics [11].

Several studies have already reported that microplastics interacted with microalgae cells and this has impacts on their respective fates. Microplastic surfaces constitute suitable substrates for the formation of biofilms, and microalgae are an important constituent of colonizing biotic communities [12]. Several studies on microalgae have demonstrated significant interactions and the rapid formation of hetero aggregates when microalgae were exposed to 400–1000 μm diameter polypropylene and high-density polyethylene microplastics at a concentration of 1 g/L [13]. Recently, the interaction between microplastics (MPs) and microalgae had been investigated [14]. Sjollema et al. (2016) indicated that microplastics might reduce photosynthesis and growth in microalgae. However, the interaction mechanism between microplastics and microalgae highly depends on the type of plastic and species of algae. Therefore, it would be necessary to develop a new strategy for enhance microplastic removal from water body by microalgae EPS.

Surfactants have received great attention in the field of microalgae harvesting and cell disruption. Surfactants have solubilization property which allows them to disrupt the cell walls of microalgae and release EPS into an aqueous phase, which is a significant advantage over other methods [15]. In our previous work, different types of surfactants have demonstrated distinctive cell disruption ability in which higher EPS components have extracted, indicating a substantial microalgae cell lysis [16].

Regarding microplastics particles removal from aquatic ecosystems in an effective hetero-aggregates process and biodegradable approach, this work aimed to investigate: (i) the performance of different types of surfactants on microalgae cell disruption and EPS extraction to form strong tangled networks between microalgae and microplastic, (ii) the potential of *Chlorella sorokiniana* microalgae, focusing on its EPS production and hetero-aggregation ability in the presence of micro-plastics. The interaction between some typical microplastics of Polyethylene (PE) and polypropylene (PP) and microalgae will be analyzed. PP and PE are the most produced and used thermoplastics in the industrial sector. Plastic PE and PP represent up to 85% of the synthetic plastics produced and are mainly used for single-use packaging [17].

2. Materials and methods

2-1- Microalgae cultivation and chemicals

Chlorella sorokiniana was selected as sample microalgae [18]. The microalgae were cultivated in 8 L pilot-scale flat-panel photo bioreactor (HAFP-PBR) using standard BG-11 medium at 28 °C and exposed to fluorescent light of 2800 lx in a cycle of 12/12 h light/dark. The components concentration in the BG-11 is reported in a previous study [18]. The original pH of the medium was 7.5. The optical density at 680 nm (OD_{680}) and final dry weight of *C. sorokiniana* were 0.325 and 0.63 ± 0.05 g/L after incubation for two weeks, respectively.

In this study, three surfactants with different ionicity were selected. Cetyl trimethyl ammonium bromide (CTAB) and dodecyl trimethyl ammonium bromide (DTAB) and chitosan represent cationic surfactants. All chemicals purchased from Merck.

2-2- Microplastic preparation

The two types of microplastics including two types of microplastics of Polyethylene (PE) and polypropylene (PP) were obtained by fragmentation using a milling machine (230 V ~ 50 Hz, 120 W). After this, all microplastics were mesh sieved (AnalysensiebeRetsch), collected and separated according to their size. The microplastics were washed with dichloromethane, on a magnetic stirrer for 2 h, at room temperature. Then, the microplastics were filtered, oven-dried at 40 °C overnight and kept in a desiccator. Distinct stock solutions of microplastics were used and prepared for this study. The microplastic removal percentage was measured as [19]:

$$\text{MPs removal percentage (\%)} = (C_0 - C_f) / C_0 \times 100 \quad (1)$$

Where, C_0 and C_f are the initial and final microplastic concentrations before treatment (t_0) and after treatment (t_f), respectively.

2-3- Experimental design and procedures

The biomass concentration was determined by washing and drying the samples of microalgae cells in an oven at 70 °C for 24 h to measure the growth rate. After that, the performance of three different types of surfactants on microalgae EPS extraction will comprehensively study. Microalgae solution after being treated with different doses of surfactants, the microalgae suspensions will be stirred rapidly at 300 rpm followed by slow mixing at 50 rpm. To do this, first, 400 mL samples of microalgal suspension were filled into 500 mL beakers. Then, the three surfactants were added to the beakers to obtain a predetermined dose of 50, 100, 200, 300, 400, 500 and 1000 mg/L. After being treated with different doses of surfactants, the microalgal suspensions were stirred rapidly at 300 rpm for 5 min followed by slow mixing at 50 rpm for 20 min. Finally, the microalgal suspensions were allowed to settle for 180 min, and the samples were carefully withdrawn from the suspension at 2/3 from the bottom every 30 min.

2-4- Analytical methods and EPS determination

The biomass concentration was measured by using the optical density at 680 nm (OD₆₈₀) in a UV-VIS spectrophotometer (Unico 2800 UV-Visible, 4nm, 110 V, China). To measure the extracted EPS from microalgal cells, 40 mL of the harvested microalgae samples before and after treatment with surfactant were collected and centrifuged at 5000 rpm for 10 min. The samples were filtered throughout a 0.45 µm syringe membrane to achieve soluble EPS. The total bioproducts content was shown as percentage of cell dry weight (CDW). The protein content was determined based on the Bradford assay method using bovine serum albumin (BSA) standard curve. The microalgal samples were mixed with Bradford Dye reagent and then measured in OD₅₉₅ absorbance using a spectrophotometer (2800 UV/VIS UNICO, China). Anthronesulphuric acid method from the protocol of Deng et al. (2016) was used for the carbohydrate concentration, in which 1 mL of diluted microalgal supernatant was mixed with 1 mL of phenol (5% w/v) and 5 mL of 96% H₂SO₄ and then the absorbance of was measured at OD₄₉₀. For the standard and calibration curve, glucose (from 0.2 mg/mL to 1 mg/mL at an interval of 0.2 mg/mL) was used [12]. To obtain lipid content, the microalge samples were weighted before and after the flocculation process in a pre-weighed tube. For this purpose, the middle layer of supernatant containing lipids were taken and centrifuged and the volatile liquid was evaporated in a rotary evaporator (RV 10, IKA, Germany). Finally, the extracted lipids remained in the tube was dried in a vacuum oven (VacuCell, Germany) at 100 °C until a constant weight was achieved. Gompertz equation can be represented by Eq. (2):

$$C_m = C_{max} \exp\{-\exp[\mu e / C_{max} (\lambda - t) + 1]\} \quad (2)$$

where, C_m is biomass concentration (g/L) at time (t) predicted by Gompertz model, C_{max} is the maximum biomass concentration (g/L), μ is the maximum biomass growth rate (g/L/d) and λ is the lag phase duration (d).

3. Result and discussion

3-1- Microalgae growth rate (curve)

Figure 1 shows the experimental results and modeling of microalgae growth rate after 10 days. The maximum biomass concentrations were obtained as 1.8 g/L after 8 days of cultivation at temperatures of 30 °C at 3000 Lux. Similarly, Qu et al. (2019) obtained the optimum temperature of 30 °C for cultivations of *Parachlorella kessleri* QWY28, produced maximum biomass productivity of 646 mg/L/d [20]. In addition, the simulation of the growth behavior of the microalgae showed a perfect match between experimental data and predicted data by using Gompertz model (Eq. 2).

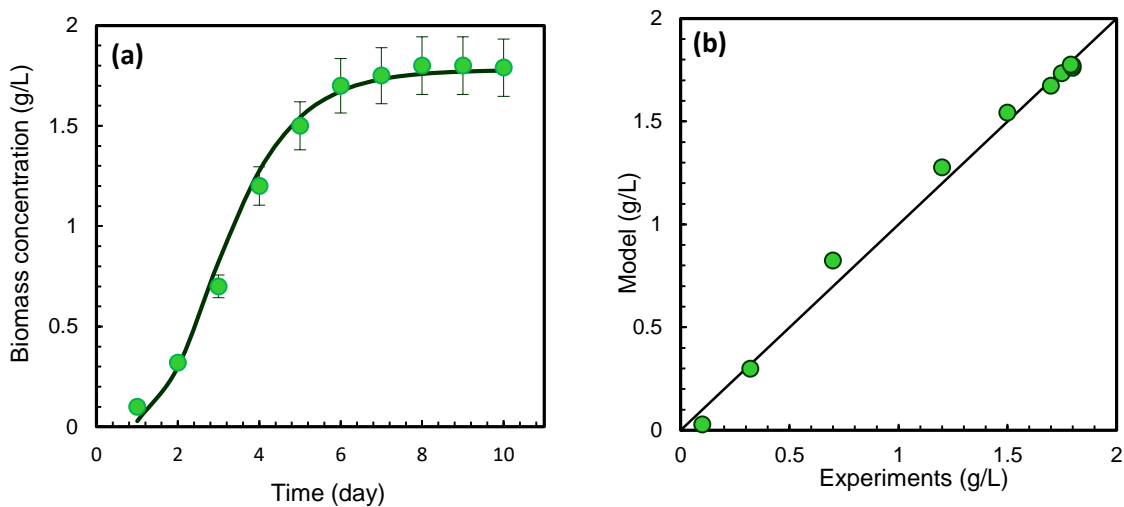


Figure 1: Microalgae growth rate: (a) biomass concentration vs. cultivating time and (b) Gompertz model evaluation

3-2- Effect of various surfactants on EPS extraction and microplastic removal

Extracellular polymer (EPS) is a polymer produced during the metabolism of microbial cells. The main components include carbohydrates such as polysaccharides, proteins (i.e. enzymes and structural proteins), and lipids [21]. Figure 2 demonstrates the concentration of the released EPS fractions from *Chlorella sorokiniana* microalgae after treatment with chitosan and the two surfactants. As can be seen from the Figure 2, the addition of cationic surfactants of CTAB and DTAB could increase the EPS generation of *Chlorella sorokiniana* up to 15 times of the control group. The protein, polysaccharide and lipid concentrations in EPS for untreated microalgae were very similar and about 1 mg/L. The final concentrations of protein and carbohydrate released by adding chitosan and the surfactants treatment were in the following order: for protein: CTAB (15 mg/L) > DTAB (13 mg/L) > Chitosan (10 mg/L), for Carbohydrate CTAB (40 mg/L) > DTAB (22 mg/L) > Chitosan (7 mg/L) and for lipids CTAB (20 mg/L) > DTAB (17 mg/L) > Chitosan (6 mg/L).

When surfactants are used as flocculent for microalgae harvesting is that, the linear hydrocarbon chains of surfactant can interact with the hydrophobic components of the microalgae cells (e.g. protein and nucleic matters) that accelerate EPS release from biomass. Alkyl-chain surfactant (CTAB) could achieve higher release of EPS compared to the shorter alkyl-chain surfactant (DTAB). It is hypothesized that the longer hydrocarbon chain of CTAB can insert into hydrophobic cell membrane and release EPS from microalgae more efficiently. Moreover, CTAB has a lower critical micelle concentration (CMC) (~328 mg/L) contributing to its higher surface activity and surface charge density [14].

Moreover, by adding surfactants of chitosan, DTAB and CTAB, the microplastic removal percentage increased. According to Figure 2 (the blue line), approximately 45%, 70%, and 86% of microplastic removal were obtained by adding chitosan, DTAB and CTAB as surfactant in microalgae harvesting, respectively. Microalgae shown the potential to colonize, adsorb and adhere the micro plastic particles, which was confirmed using scanning electron microscopies. Additionally, it has been shown that EPS are able to aggregate morphologically irregular microplastics within a wide spectrum of sizes. Cunha et al. (2019) observed that EPS secreted by microalgae could form hetero-aggregates with microplastic particles [13]. The results indicated that the microalgae have great potential to produce EPS to adsorb and aggregate microplastic from freshwater and marine environment.

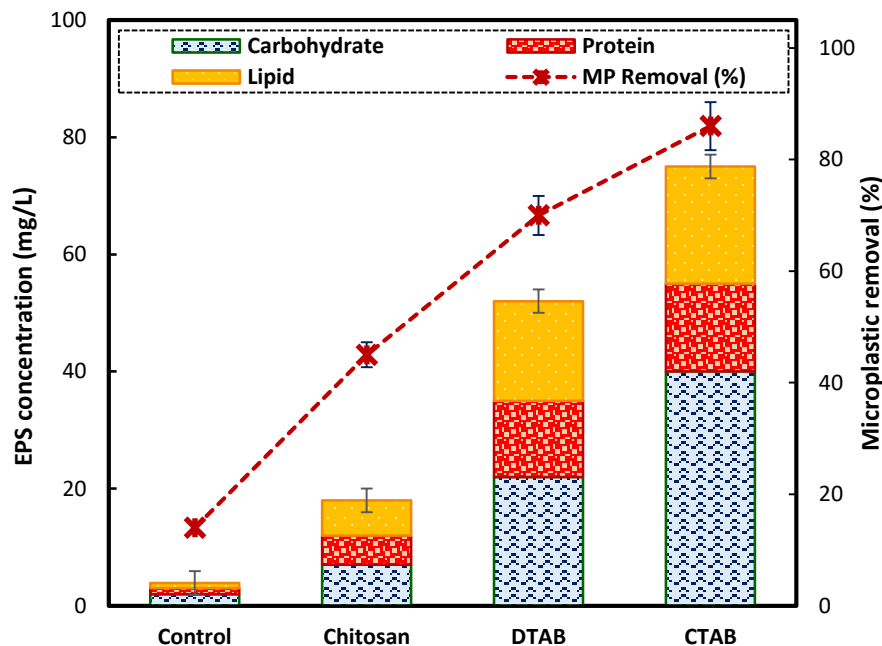


Figure 2: Effects of different surfactant on concentration of released EPS fractions (protein and polysaccharide) and Microplastic removal from *C. sorokiniana* induced by Chitosan, CTAB and DTAB

3-3- Detection of hetero-aggregates by Scanning electron microscope images

In order to further explore the interaction between microplastics and microalgae, SEM characterization was also carried out with a magnification of 1000X. Figure 3 shows the structural characterization of microplastics and algal cells. *Chlorella sorokiniana* cells had spherical shape with average 10 μm diameter. The SEM images shows the original surface morphological structure of microplastics, as well as its influence on the morphological and internal structure of *Chlorella sorokiniana*. After treatment, the SEM results of microplastic revealed many cracks in the form of crystals filled with a white substance. This white substance was EPS slime produced by *Spirulina* sp., which plays an important role in the biodegradation process by adhering to and damaging the surface of microplastics. When *Chlorella sorokiniana*. Produces EPS, it accumulates and forms a biofilm on the surface of any solid object in contact with *Chlorella sorokiniana* including microplastics in culture. This can be seen in the SEM results, where the surface of the plastic looks like a crack. This crack is an indicator that the degradation process occurs due to the presence of EPS, which forms a biofilm on microplastics surface. The aggregation of microspheres outside the algal cells can also be observed in Figure 3, and these small particles tend to aggregate into a whole to maintain a stable state, and then adsorb to the algal cell surface wholly or individually.

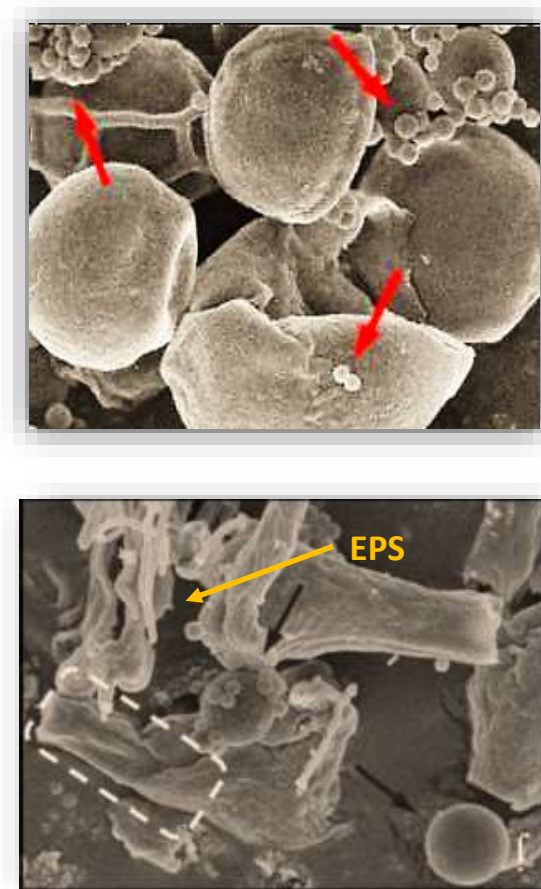


Figure 3: Scanning electron microscope image

3-4- mechanism between microalgae and microplastics

Figure 4 shows the schematic view of interaction between microplastic and microalgae cells. The interaction mechanism between microplastics and *Chlorella sorokiniana* is depended on two aspects. Firstly, the leaching of microplastic additives presented toxicity to microalgae and secondly removal of microplastic through EPS releasing. In the first case, toxicity resulted in the increase of reactive oxygen species (ROS). Excessive amounts of ROS might prompt membrane lipid peroxidation, which could be confirmed by the increase of MDA content. In that case, the intracellular membranes composed of unsaturated phospholipids were easily attacked by ROS. Additionally, microplastics caused slight cell deformation which indicated the damage of intracellular membranes. Secondly, microplastics could be moved to the surface of microalgae by means of EPS [11].

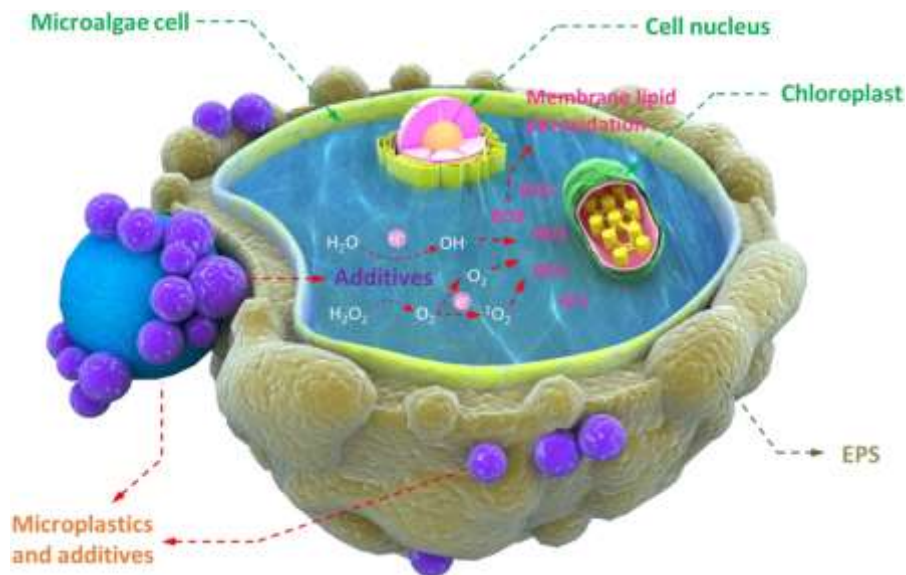


Figure 4: Interaction mechanism between microalgae and microplastics [14]

4. Conclusion

The interaction between microplastics and microalgae (*Chlorella sorokiniana sp.*) has been investigated in this work. The secretion of EPS could facilitate adsorption and aggregation between microplastics particles and microalgae cells. The SEM analysis further confirmed these processes. The result showed that adding CTAB, DTAB and chitosan as surfactants play an important role to release EPS up to 75%, 47% and 17%, respectively. Furthermore, maximum removal efficiency of 86% for microplastic was observed. As a result, microalgae presented potential to be a promising bio-solution for microplastics treatment in freshwater and marine environment. However, it should be noted that in order to adsorb or aggregate microplastics in an efficient pathway, more research related to the behavior of microplastics aggregation via microalgae is needed to be conducted.

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