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Review Article

Extracellular polymeric substances from *Spirulina* sp. for the bioremediation of fishing net–derived microplastics in seawater

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Abstract. The increasing accumulation of plastic waste from fishing nets is primarily driven by the large volume of nets introduced into aquatic systems. Fishing nets are predominantly manufactured from polyethylene, a polymer known for its high resistance to degradation, allowing it to persist in marine environments for hundreds of years. Consequently, discarded or degraded fishing nets represent an ongoing environmental challenge, which is further exacerbated by their fragmentation into microplastics. In response to this issue, this study aims to evaluate the effectiveness of extracellular polymeric substances (EPS) produced by Spirulina sp. for the bioremediation of polyethylene-derived microplastics from fishing nets in seawater. A quasi-experimental design was employed, consisting of control and treatment reactors. EPS was applied at concentrations of 20, 30, and 40 mg in the treatment reactors, while the control reactor received no EPS addition. The remediation process was conducted over treatment periods of 5, 9, and 13 days. Following the treatment, laboratory analyses were performed to assess changes in key water quality parameters, including salinity, dissolved oxygen (DO), pH, total dissolved solids (TDS), chemical oxygen demand (COD), and Spirulina growth rate. In addition, the residual microplastics that were not flocculated were quantified. The results indicated that the presence of microplastics influenced the growth dynamics of Spirulina. Nevertheless, EPS application resulted in a measurable reduction in microplastic mass, with decreases of 5.0 g, 10.1 mg, and 11.6 mg observed in the 20, 30, and 40 mg treatments, respectively. Overall, the findings demonstrate that bioremediation using Spirulina-derived EPS is effective in reducing polyethylene microplastics in seawater. Future studies are recommended to extend the treatment duration, utilize natural seawater under environmentally realistic conditions, and evaluate the broader ecological impacts of EPS-based bioremediation to support its practical application in marine environments.

Keywords: Microplastics, Spirulina, Polyethylene, Seawater, Salinity

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1. Introduction

Indonesia, as the world's largest archipelagic nation, possesses extensive marine and coastal resources that play a critical role in sustaining biodiversity and supporting coastal livelihoods (Darson & Prapto, 1999). The vastness of Indonesia's maritime territory confers substantial potential in the fisheries and marine sectors, resulting in a high dependence of coastal communities on fishing activities. Fishing operations rely heavily on gear such as nets and fishing lines; however, improper management and unsustainable practices have led to significant environmental consequences. Among these, abandoned, lost, or otherwise discarded fishing gear—commonly referred to as *ghost nets*—represents a persistent and underrecognized source of marine pollution with severe ecological implications (Harahap et al., 2021). Once lost, ghost nets remain uncontrolled in the marine environment, where they continue to entangle marine organisms and degrade habitats over extended periods. Ghost nets constitute a substantial fraction of plastic debris in marine ecosystems. Polyethylene is widely used in fishing nets due to its low cost, flexibility, resistance to low temperatures, low friction coefficient, and high chemical stability (Pampi & Jayashree, 2020). In the marine environment, polyethylene-based nets are highly persistent and gradually fragment into microplastics rather than undergoing complete degradation.

Microplastics, defined as plastic particles with diameters smaller than 5 mm, have emerged as contaminants of global concern due to their persistence, bioavailability, and potential risks to ecosystems and human health (Sun et al., 2023). Their small size limits the effectiveness of conventional water treatment processes. Filtration and sedimentation, when applied without appropriate primary treatment such as flocculation, are largely ineffective for removing microplastics, as these processes primarily target larger particulate matter. Without the development of effective mitigation strategies, microplastic contamination in marine waters is expected to intensify, accompanied by deteriorating water quality parameters, including pH, chemical oxygen demand (COD), dissolved oxygen (DO), and total dissolved solids (TDS). Bioremediation using extracellular polymeric substances (EPS) produced by microalgae has gained attention as an environmentally sustainable alternative. EPS, which may constitute 50–90% of the total organic content in

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microbial aggregates, acts as a natural bioflocculant capable of facilitating the aggregation and removal of microplastic particles from aquatic systems (Ajao et al., 2018). Through EPS-mediated interactions, microplastics can form larger flocs that enhance sedimentation efficiency (Matter et al., 2019).

This study aims to investigate the effectiveness of microalgae-derived EPS-based bioremediation in reducing microplastic contamination and improving key physicochemical parameters (pH, COD, DO, and TDS) in seawater contaminated by fishing lines and nets. The findings are expected to contribute to the development of sustainable remediation strategies for mitigating microplastic pollution in marine environments.

2. Material and method

2.1. Equipment and Materials

The equipment used in this study included glass reactor tubes, transparent tubing, straws, an aerator, LED lamps (6000 lux), graduated cylinders, pipettes, cuvettes, beakers, a stereo zoom microscope (NSZ 606), microscope slides and cover glasses, sieves, wire mesh, glass stirring rods, test tubes, glass sample bottles, scissors, a COD reactor, a 25-hole test tube heater (HANNA Instruments HI 839800), a pH meter, a dissolved oxygen (DO) meter, a digital salinity meter, a TDS/EC meter, a UV–Vis spectrophotometer (Thermo Scientific™ Genesis 20) operated at a wavelength of 680 nm, centrifuge tubes, and a DLAB clinical centrifuge. The materials used were *Spirulina* sp. microalgae, artificial seawater (ASW), polyethylene (PE) microplastics derived from fishing nets, Walne's nutrient medium, distilled water (aquades), and COD reagents.

2.2. Cultivation of Spirulina *sp. Microalgae*

Prior to the bioremediation experiments, *Spirulina* sp. microalgae were cultivated by transferring 500 mL of *Spirulina* sp. culture into a glass reactor. The reactor was covered with plastic wrap to prevent contamination and equipped with an aerator and LED lighting. Walne's nutrient medium was supplied throughout the cultivation period. Cultivation was continued until a sufficient biomass of *Spirulina* sp. was obtained for subsequent experiments.

2.3. Acclimatization of Spirulina *sp. Microalgae*

Acclimatization was performed prior to bioremediation to determine the optimal salinity conditions for *Spirulina* sp. growth. A volume of 30 mL of *Spirulina* sp. culture was added to artificial seawater adjusted to specific salinity levels using salt. Acclimatization was conducted for 7 days under two salinity conditions: low salinity (10–15 ppt) and moderate salinity (20–25 ppt). Changes in *Spirulina* sp. growth and condition were observed after the acclimatization period.

2.4. EPS (Extracellular Polymeric Substances) Presence Test

The presence of extracellular polymeric substances (EPS) was assessed prior to the bioremediation process, as EPS production is a key indicator of the microalgae's potential for bioremediation. The test was conducted by mixing *Spirulina* sp. microalgae with 98% ethanol that had been pre-chilled in a refrigerator for 24 hours. The mixture was then returned to the refrigerator for an additional 24 hours. EPS production was indicated by the formation of a slimy precipitate in the test tube.

2.5. Bioremediation Process

The bioremediation experiment was conducted using four 2-L glass reactors, consisting of one control reactor and three treatment reactors containing different concentrations of microplastics. Each reactor was initially filled with 50 mL of *Spirulina* sp. culture that had been previously adjusted with artificial seawater to achieve a salinity of 10–15 ppt. Polyethylene microplastics, cut into fragments smaller than 5 mm, were added to the treatment reactors at concentrations of 20 mg/500 mL, 30 mg/500 mL, and 40 mg/500 mL, respectively. Optical density was measured using a UV–Vis spectrophotometer, and cell morphology was observed under a microscope on days 5, 9, and 13. Nutrients were added every two days at a dosage of 2 mL per liter of culture. Floc formation was also examined microscopically for each microplastic concentration.

2.6. Measurement of pH, TDS, Salinity, and Dissolved Oxygen (DO)

Water quality parameters, including pH, total dissolved solids (TDS), salinity, and dissolved oxygen (DO), were measured prior to treatment and on days 5, 9, and 13 of the bioremediation process. pH was measured using pH paper, TDS using a TDS/EC meter, salinity using a digital salinity meter, and DO using a dissolved oxygen meter.

2.7. Chemical Oxygen Demand (COD) Analysis

Chemical oxygen demand (COD) analysis was performed on day 13 of the bioremediation experiment. Prior to reagent addition, the *Spirulina* sp. samples were placed in centrifuge tubes and centrifuged for 15 minutes using a DLAB clinical centrifuge to separate the microalgal cells from the supernatant. The COD reagent was then added to the supernatant, and analysis was conducted using a COD reactor and a 25-hole test tube heater (HANNA Instruments HI 839800).

2.8. Analysis of Remaining Microplastics Not Forming Flocs

Remaining microplastics that did not form flocs were collected from the top, middle, and bottom sections of each reactor. Microplastics at the surface were collected using tweezers, those in the middle layer were obtained by filtering the *Spirulina* sp. culture through wire mesh, and settled microplastics at the bottom were scraped from the reactor surface. The collected microplastics from each section were then dried and weighed.

2. Result and Discussion

3.1. Growth Medium for Spirulina sp.

The Figure 1 illustrates the stark morphological and physiological differences in *Spirulina sp.* when subjected to varying salinity levels. The culture at 10.7 ppt exhibits a rich, vibrant green color, indicating a high concentration of chlorophyll and a robust cell population. In contrast, the sample at 20.9 ppt shows a distinct shift toward a brownish-olive hue and increased transparency. This discoloration suggests that at higher salinities, the organism suffers from pigment degradation and a reduction in cell density. While *Spirulina* is known for its halotolerance, the visual evidence confirms that exceeding the 20 ppt threshold induces significant physiological stress, likely due to the metabolic burden of maintaining osmotic balance. This stress response has a direct impact on the organism's growth kinetics and photosynthetic efficiency. At the higher salinity of 20.9 ppt, *Spirulina* must redirect energy away from biomass production toward osmoregulation and the synthesis of protective compatible solutes. This metabolic shift typically results in the inhibition of Photosystem II (PSII) activity, which explains the decrease in chlorophyll concentration noted in the study. The observed reduction in green intensity at 20.9 ppt serves as a macroscopic indicator of a microscopic slowdown in carbon fixation and cellular division, reinforcing why the lower range of 10–15 ppt is superior for maintaining high-density cultures.

The implications of these findings are critical for the success of bioremediation applications. Because the effectiveness of *Spirulina* in removing pollutants—such as heavy metals or excess nutrients—is intrinsically linked to its metabolic rate and total surface area, the stunted growth at 20.9 ppt would result in diminished treatment efficiency. By optimizing the salinity to 10.7 ppt, the study maximizes the bioremediation potential, ensuring the cells remain in an active growth phase with high photosynthetic turnover. Consequently, maintaining a controlled salinity environment is not merely a matter of survival for the species, but a fundamental requirement for achieving the highest possible rate of water purification.

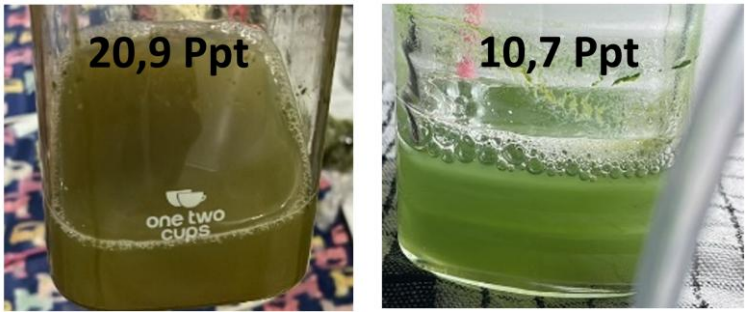

Figure 1. Growth medium for microalgae

3.2. Cultivation of Spirulina sp.

The cultivation of *Spirulina sp.* over a 60-day period exhibited a fluctuating growth pattern, as reflected by changes in optical density (OD). These fluctuations were primarily attributed to dilution effects applied during cultivation. In continuous culture systems, the dilution rate strongly influences biomass productivity; excessive dilution can reduce biomass concentration and lead to unstable OD values (Khannapho et al., n.d.). Therefore, maintaining an optimal dilution rate is essential to balance nutrient availability and biomass production, thereby ensuring more stable growth dynamics.

Figure 2 shows the reactor treated with 40 mg of microplastics showed a higher increase in OD compared to the control. This observation suggests that, at certain concentrations, microplastics may act as an alternative carbon source or be perceived as particulate nutrients by *Spirulina sp.*, potentially stimulating growth. In contrast, the reactor treated with 20 mg of microplastics exhibited an initial decline in OD on day 5, followed by recovery by day 13. This pattern likely reflects complex interactions between *Spirulina sp.* and microplastics, including an initial stress response followed by physiological adaptation.

Conversely, the reactor treated with 30 mg of microplastics showed a pronounced and sustained decrease in OD. At specific concentrations, microplastics may induce the production of reactive oxygen species (ROS), which are toxic to microalgae and can significantly inhibit growth. Environmental factors such as light intensity, nutrient availability, and dissolved oxygen further influence stress responses in microalgae and are difficult to control precisely. Previous studies have reported that microplastic exposure induces oxidative stress in *Spirulina sp.*, as evidenced by increased ROS production, cellular damage, and the release of volatile organic compounds associated with unpleasant odors (Yang et al., 2023).

In the 20 mg microplastic treatment, the observed decrease in OD on day 9 followed by recovery on day 13 is characteristic of bioremediation processes involving *Spirulina sp.* Similar adaptive responses have been reported in studies where *Spirulina* initially experienced biomass reduction upon exposure to pollutants but subsequently recovered as adaptation occurred. For instance, Adamia et al. (2018) reported a 38% initial biomass decrease despite *Spirulina* achieving up to 87% TNT removal. Comparable trends have been observed in other pollutant-removal studies involving *Spirulina* (Barone et al., 2024; Lee et al., 2015).

Daily OD measurements further indicated that microplastic treatments were generally associated with reduced growth rates, demonstrating a direct relationship between microplastic exposure and algal physiological stress (Dianratri et al., 2020). By the final day of cultivation, the reactor containing 30 mg of microplastics had entered the death phase, as indicated by discoloration of the culture, excessive foam formation, and the presence of strong odors, all of which are consistent with severe cellular degradation.

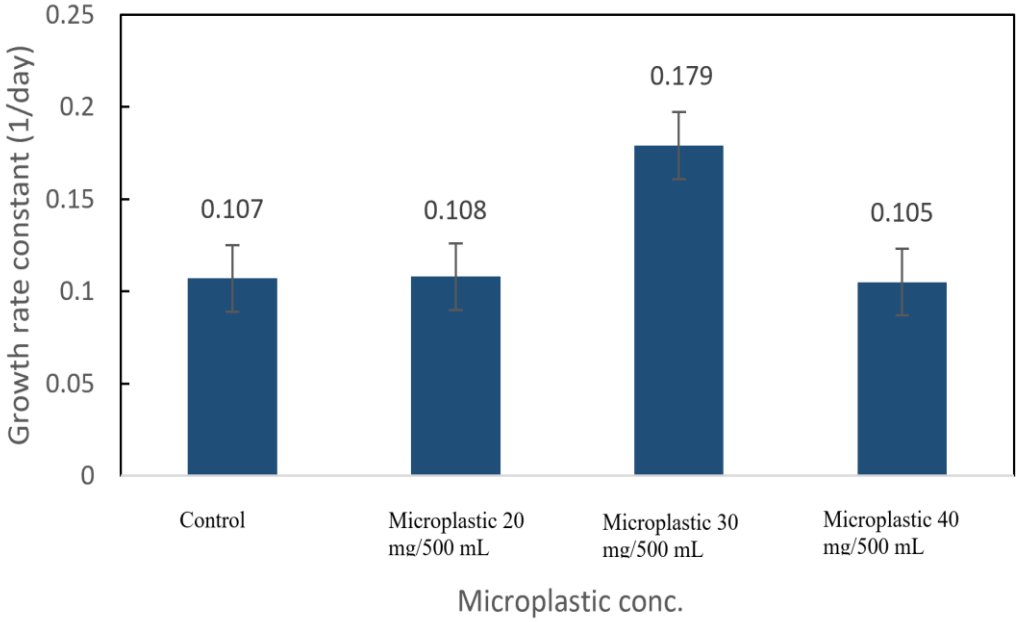

Figure 2. growth rate constant of microalgae in microplastic condition

3.3. EPS (Extracellular Polymeric Substances) Content

Figure 3 demonstrates the formation of extracellular polymeric substances (EPS), as indicated by the appearance of a slime-like material in the test tube after 24 hours of refrigeration. The visible increase in viscosity and gel-like consistency confirms the presence of EPS produced by *Spirulina sp.* during the experimental process.

The EPS content test revealed clear slime formation, which is a commonly used qualitative indicator of EPS presence. Under low-temperature conditions, EPS components—primarily polysaccharides, proteins, and other biopolymers—can undergo partial dehydration and aggregation, resulting in a gelatinous or mucous-like structure. This physical transformation is characteristic of EPS and distinguishes it from dissolved cellular components.

The formation of this gel-like EPS is particularly significant because EPS plays a crucial role in biofilm development, microbial protection, and aggregation processes. In aquatic systems, EPS functions as a natural binding agent that enhances cell-to-cell adhesion and promotes attachment to solid surfaces, including contaminants such as microplastics. By forming a protective matrix, EPS also helps shield microbial communities from environmental stressors and improves their survival and functional stability.

In the context of this study, the observed EPS formation supports the flocculation and heteroaggregation phenomena reported in reactors containing microplastics. The slime-like EPS matrix facilitates the entrapment and immobilization of microplastic particles, thereby enhancing the potential of *Spirulina sp.* for microplastic bioremediation through biofloc formation and contaminant sequestration.

Figure 3. Exo-polysacaharide from the experiment

3.4. Floc Formation via Heteroaggregation

Figure 7 illustrates the formation of flocs between microplastics and *Spirulina sp.* microalgae after 13 days of bioremediation. The control reactor shows a homogeneous distribution of *Spirulina* cells with no visible aggregation, indicating normal algal growth in the absence of microplastic contamination. In contrast, reactors containing microplastics (20, 30, and 40 mg) exhibit clear aggregate and floc structures, confirming strong interactions between the microalgae and microplastics. At a microplastic concentration of 20 mg, initial heteroaggregation is observed, characterized by partial attachment of *Spirulina* cells to microplastic surfaces. Increasing the concentration to 30 mg results in larger and denser flocs, suggesting enhanced collision frequency and stronger binding between microplastics and algal biomass. The 40 mg treatment shows the most pronounced floc formation, with compact aggregates in which microplastics are extensively embedded within algal biomass and extracellular polymeric substances (EPS).

The observed flocculation is primarily attributed to heteroaggregation mechanisms involving EPS secreted by *Spirulina sp.* EPS acts as a natural bioadhesive, promoting surface attachment, physical entrapment, and biofilm formation on microplastic particles. These processes reduce microplastic mobility and facilitate their incorporation into larger aggregates. As reported by Khoironi, Adian, and Anggoro Sutrisno (2019), the extent of aggregation is strongly influenced by microplastic concentration, as well as particle size and shape, which affect surface area availability and interaction efficiency. Overall, the results demonstrate that *Spirulina sp.* has significant potential for microplastic bioremediation through EPS-mediated floc formation, with higher microplastic concentrations enhancing heteroaggregation and aggregate stability.

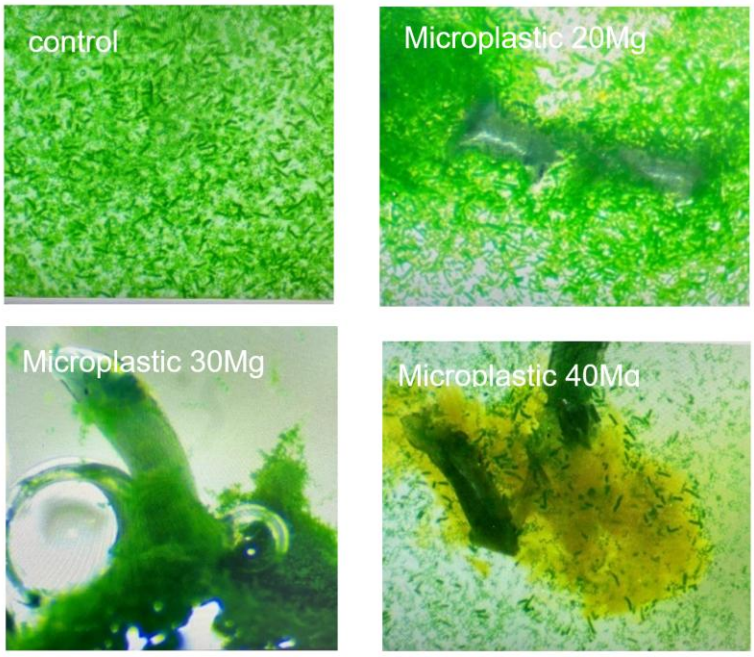

Figure 4. Floc formation during treatment

3.5. Effect of Bioremediation on Salinity

Salinity reduction in the control reactor was lower than that in microplastic-treated reactors due to better algal growth in the absence of stressors. Elevated salinity can inhibit photosynthesis and respiration in *Spirulina* sp., thereby affecting growth and overall bioremediation performance (Pramesdistian, 2019). However, *Spirulina* has demonstrated the ability to adapt to higher salinity levels in some cases, resulting in non-significant reductions in growth rate. Additionally, *Spirulina* extracts have been reported to confer protective effects against salinity stress in other organisms (Hamouda et al., 2022). The results demonstrate that microplastic concentration significantly influences changes in salinity within the bioremediation system. As shown in the figure, the control reactor exhibited only a minimal increase in salinity (0.012), indicating stable baseline conditions without microplastic interference. In contrast, the reactor containing 20 mg/500 mL microplastics showed the highest salinity increase (0.075), followed by a moderate increase at 30 mg/500 mL (0.043), while the lowest increase among treatments was observed at 40 mg/500 mL (0.022).

The initial rise in salinity at lower and intermediate microplastic concentrations can be attributed to physicochemical interactions between microplastics and dissolved ions in the aquatic medium. Polyethylene (PE) microplastics are known to adsorb salts, organic compounds, and nutrients on their surfaces, altering ion distribution and increasing apparent salinity and total dissolved solids (TDS) in the water column (Koelmans et al., 2016; Wang et al., 2021). In addition, microplastics can act as carriers for residual additives and adsorbed contaminants, which may further contribute to ionic release during immersion. The observed decline in salinity increase at the highest microplastic concentration (40 mg/500 mL) suggests a system-level adaptation driven by biological processes. At higher microplastic loads, enhanced production of extracellular polymeric substances (EPS) by *Spirulina* sp. likely promoted microplastic aggregation and floc formation. EPS-mediated flocculation can immobilize microplastics and associated ions,

reducing their dispersion and measurable contribution to salinity in the bulk medium (Sheng et al., 2010; Zhang et al., 2020). Similar findings have been reported where algal EPS reduced the mobility and reactivity of particulate pollutants through heteroaggregation.

Furthermore, microalgal uptake of dissolved ions for metabolic processes during growth may also contribute to salinity stabilization at higher microplastic concentrations. *Spirulina* sp. has been reported to regulate osmotic stress by adjusting intracellular ion balance, which can indirectly reduce fluctuations in external salinity under stress conditions (Vonshak & Richmond, 2008). Overall, these results indicate that microplastic concentration does not exert a linear effect on salinity. Instead, salinity changes are governed by a balance between microplastic–ion interactions and biological mitigation mechanisms, particularly EPS production and microalgal acclimation. This non-linear trend highlights the importance of incorporating biological responses when evaluating the impact of microplastics on aquatic physicochemical parameters and supports the role of *Spirulina* sp. as an effective agent in stabilizing water quality during microplastic bioremediation.

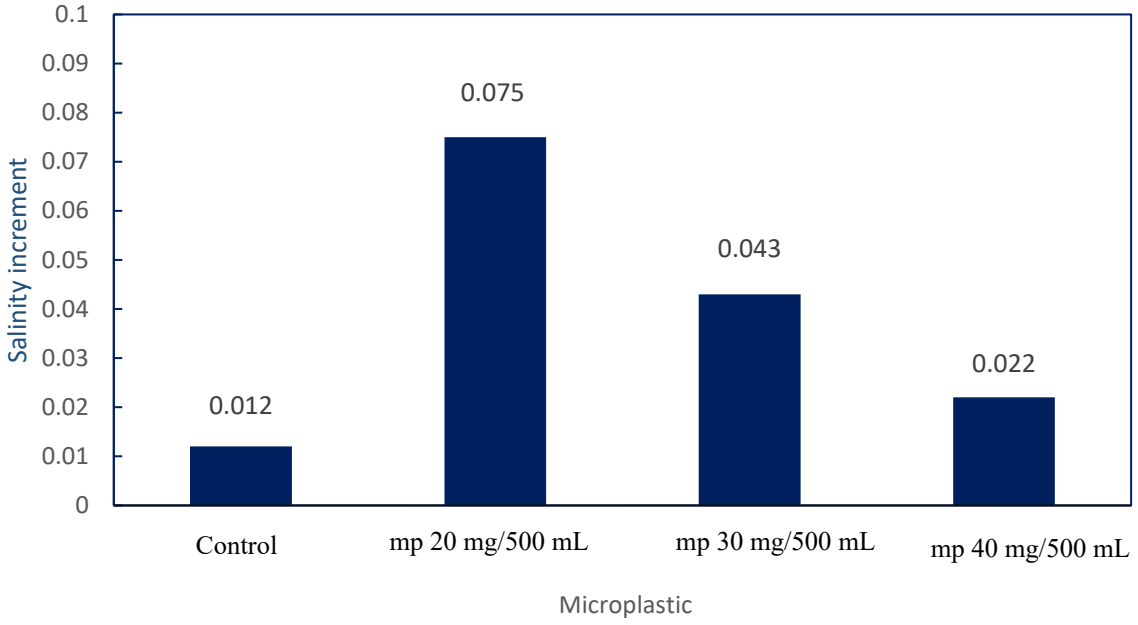

Figure 5. The increase of salinity after treatment

3.9. Effect of Bioremediation on Dissolved Oxygen (DO)

Dissolved oxygen (DO) is essential for oxidative processes involved in pollutant degradation in aquatic systems and plays a critical role in bioremediation. Typically, higher pollutant loads correspond to increased COD and decreased DO levels. However, in this study, both DO and COD values were high. This discrepancy can be attributed to differences in sampling times, with DO measured on day 9 and COD measured on day 13. By day 9, *Spirulina* activity and oxygen production had increased due to partial degradation of microplastics. The results indicate that dissolved oxygen (DO) concentrations varied with microplastic loading in the bioremediation reactors. The control reactor exhibited a relatively high DO value (~7.3 mg L^{-1}), reflecting favorable conditions for photosynthesis and gas exchange in the absence of microplastic stress. Similar DO levels were observed in the reactor treated with 40 mg/500 mL microplastics, whereas lower DO values (~7.2 mg L^{-1}) were recorded in the 20 mg/500 mL and 30 mg/500 mL treatments.

The decrease in DO at intermediate microplastic concentrations (20–30 mg/500 mL) can be attributed to physiological stress experienced by *Spirulina* sp. Microplastics are known to induce oxidative stress in microalgae, increasing metabolic oxygen consumption and suppressing photosynthetic efficiency, which can lead to reduced net oxygen production (Dianratri et al., 2020; Yang et al., 2023). In addition, microplastics may interfere with light penetration and gas exchange, further limiting oxygen generation in algal cultures. Interestingly, the recovery of DO levels at the highest microplastic concentration (40 mg/500 mL) suggests an adaptive response of *Spirulina* sp. over the experimental period. At this concentration, enhanced EPS production and floc formation were observed, which may have reduced the bioavailability and toxicity of microplastics by immobilizing them within aggregates. Such heteroaggregation can alleviate stress on microalgal cells, allowing photosynthetic activity—and consequently oxygen production—to recover (Matter et al., 2019). Similar adaptive behavior has been reported in studies where microalgae initially experience stress but subsequently restore metabolic activity following acclimation (Adamia et al., 2018; Lee et al., 2015).

From a bioremediation perspective, the relatively high DO values across all treatments indicate that the system remained aerobic, which is favorable for oxidative degradation processes. Dissolved oxygen plays a critical role in supporting microbial and algal metabolism during pollutant removal; thus, maintaining adequate DO levels is essential for effective bioremediation (Magro et al., 2012). The observed DO patterns further suggest that EPS-mediated interactions between *Spirulina* sp. and microplastics can mitigate oxygen depletion under higher pollutant loads. Overall, these findings demonstrate that while moderate microplastic concentrations may temporarily suppress oxygen availability due to algal stress, higher concentrations can trigger adaptive mechanisms that stabilize DO levels. This highlights the resilience of *Spirulina* sp. and supports its potential application in microplastic bioremediation under aerobic marine conditions.

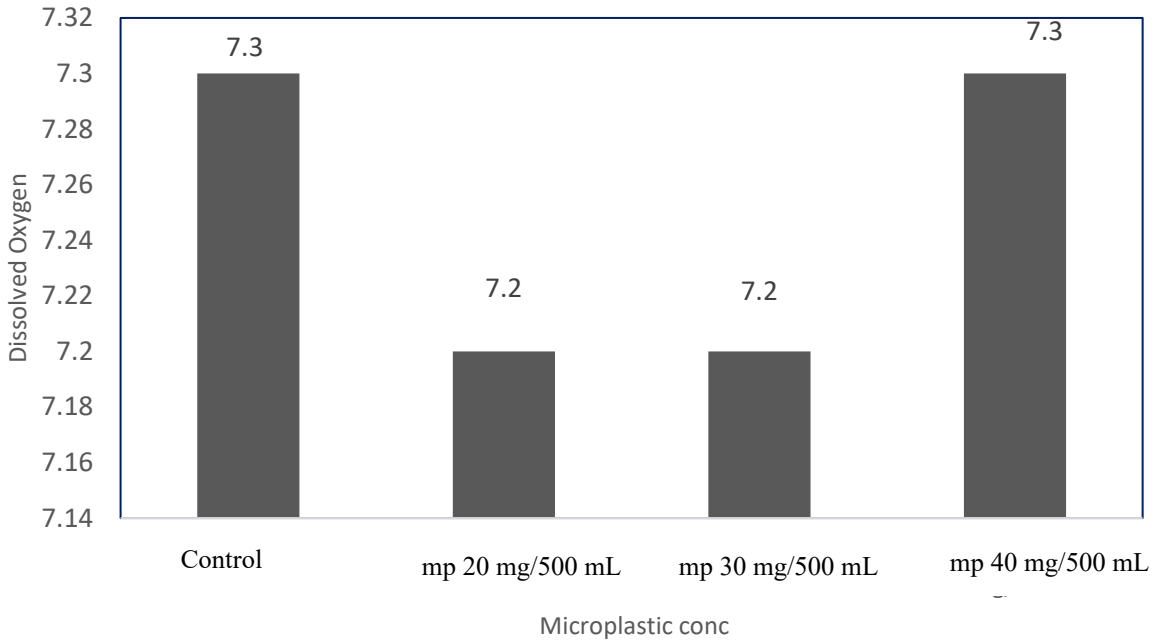

Figure 6. Dissolved oxygen after treatment

3.10.Effect of Bioremediation on Total Dissolved Solids (TDS)

The provided chart illustrates the temporal dynamics of Total Dissolved Solids (TDS) concentration in *Spirulina sp.* cultures across various microplastic treatments over a 13-day period. Across all experimental groups, including the control and the three microplastic concentrations (20 mg, 30 mg, and 40 mg per 500 mL), a consistent fluctuating trend is observed. Initial readings on day 5 show relatively high TDS levels, ranging from 6,891 to 7,007 ppm, which then undergo a uniform decline by day 9, reaching their lowest recorded values in the study. This initial reduction suggests that the microalgae were actively utilizing dissolved substances during their vigorous growth phase, effectively lowering the overall concentration of solids in the medium.

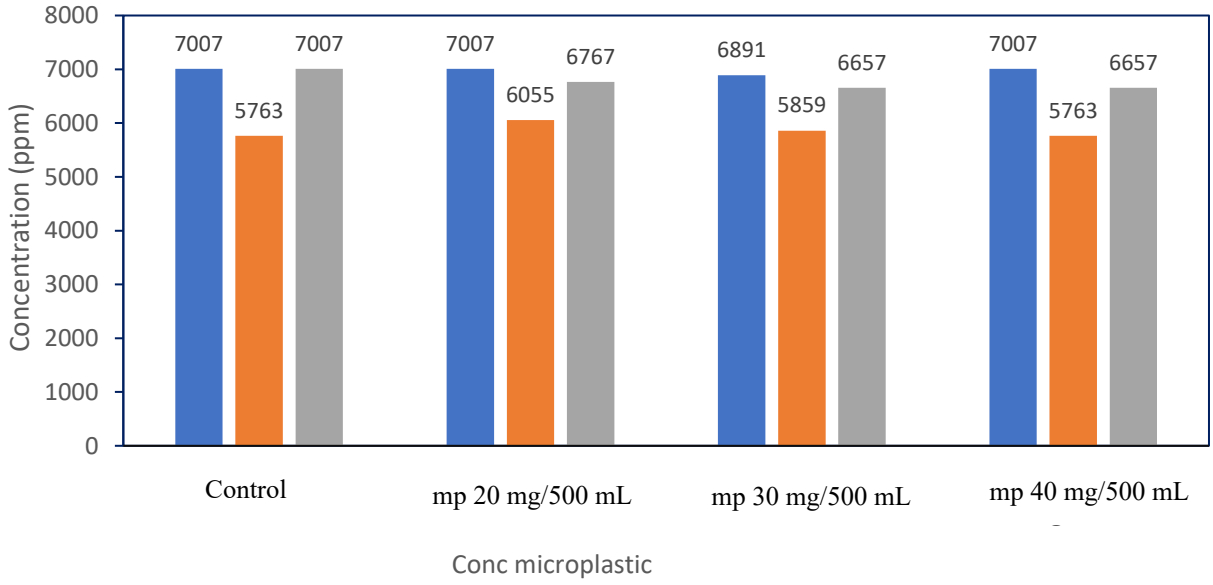

Figure 7. Total dissolved solid (TDS) after treatment

The decline in TDS observed on day 9 is attributed to the peak metabolic activity and stable growth rate of *Spirulina sp.* during this timeframe. At this stage of the experiment, the microalgae are able to efficiently absorb and process dissolved nutrients and organic matter, outperforming any potential accumulation of waste products. Interestingly, the chart shows that the treatment with

20 mg/500 mL of microplastics maintained the highest TDS concentration on day 9 (6,055 ppm) compared to the other groups, including the control (5,763 ppm), indicating that the presence of microplastics may subtly influence the rate of nutrient uptake or metabolic efficiency. By day 13, however, the trend reverses as TDS levels across all reactors show a significant increase, generally returning to concentrations between 6,657 and 7,007 ppm. This shift corresponds with a noticeable decline in the growth rate of *Spirulina sp.* compared to the early stages of treatment. As the growth rate slows, the ability of the microalgae to effectively reduce or sequester dissolved solids becomes limited. The resulting rise in TDS may be linked to the accumulation of metabolic byproducts or the release of organic compounds back into the medium as the culture enters a stationary or decline phase, ultimately reducing the overall effectiveness of the bioremediation process at later stages.

3.11.Effect of Bioremediation on Chemical Oxygen Demand (COD)

The experimental results reveal a clear correlation between microplastic concentration and the degradation of water quality parameters, specifically Chemical Oxygen Demand (COD) and Total Dissolved Solids (TDS). As shown in Figure 12, the COD values exhibited a significant upward trend as the microplastic dosage increased, with the control group recording the lowest value at 718 ppm and the 40 mg treatment reaching a peak of 1298 ppm. This substantial increase suggests that high concentrations of polyethylene microplastics introduce significant organic loading into the system, likely through the leaching of chemical additives or by inducing cellular stress in *Spirulina sp.*, which leads to the release of organic metabolites into the medium. While *Spirulina sp.* is traditionally effective at organic pollutant removal under nutrient-rich conditions, the presence of these stressors appears to overwhelm its metabolic capacity, resulting in lower removal efficiency compared to untreated environments.

The temporal fluctuations in TDS, as illustrated in Figure 11, further elucidate the physiological state of the microalgal culture during the bioremediation process. Across all treatments, a distinct decrease in TDS was observed by day 9, where levels dropped to their lowest point (e.g., 5763 ppm in the control and 40 mg reactors). This reduction coincides with the period when *Spirulina sp.* maintained a stable growth rate, allowing for the effective sequestration and utilization of dissolved solids for biomass production. The initial success in lowering TDS levels highlights the microalgae's potential for water purification when metabolic activity is high and the inhibitory effects of microplastics have not yet reached a critical threshold.

However, the subsequent rise in TDS levels observed by day 13 indicates a shift in the system's equilibrium. By this stage, the growth rate of *Spirulina sp.* had noticeably declined, which directly limited its ability to continue absorbing dissolved compounds from the aqueous medium. The rebound in TDS—returning to levels as high as 7007 ppm in the control—suggests that as the microalgae enter a stationary or decline phase, the rate of waste accumulation or cellular leakage exceeds the rate of nutrient uptake. These findings underscore the importance of optimizing the duration of the bioremediation cycle; extending the process beyond the point of peak microalgal vitality may lead to a reversal of the initial purification gains, particularly in the presence of high microplastic concentrations that accelerate the onset of culture senescence.

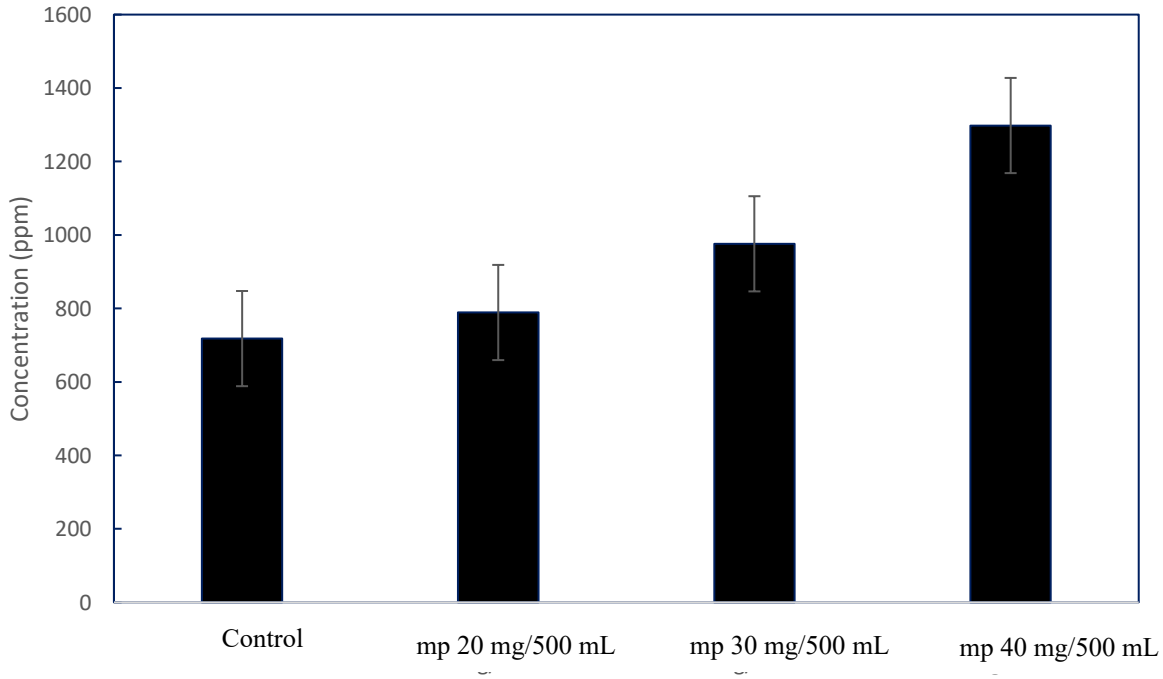

Figure 8. Chemical oxygen demand (COD) after treatment

3.12.Residual Microplastics after 13 Days of Bioremediation

The effectiveness of *Spirulina sp.* in remediating polyethylene microplastics is primarily driven by the secretion of extracellular polymeric substances (EPS), yet the presence of residual particles highlights the limitations of the flocculation process. EPS, composed of polysaccharides and proteins, acts as a natural bio-flocculant that facilitates hetero-aggregation, trapping microplastic particles within a sticky matrix (Costa et al., 2020). However, studies indicate that the removal efficiency is highly dependent on the

initial concentration and contact time; residual microplastics often persist in the supernatant when the EPS-to-microplastic ratio is insufficient to overcome the buoyant forces of polyethylene (Sendra et al., 2017). These residuals are frequently the smaller-sized fractions that escape the primary flocculation network, suggesting that while *Spirulina* significantly reduces the plastic load, it may require a secondary polishing step to achieve near-total removal (Tabagari et al., 2019).

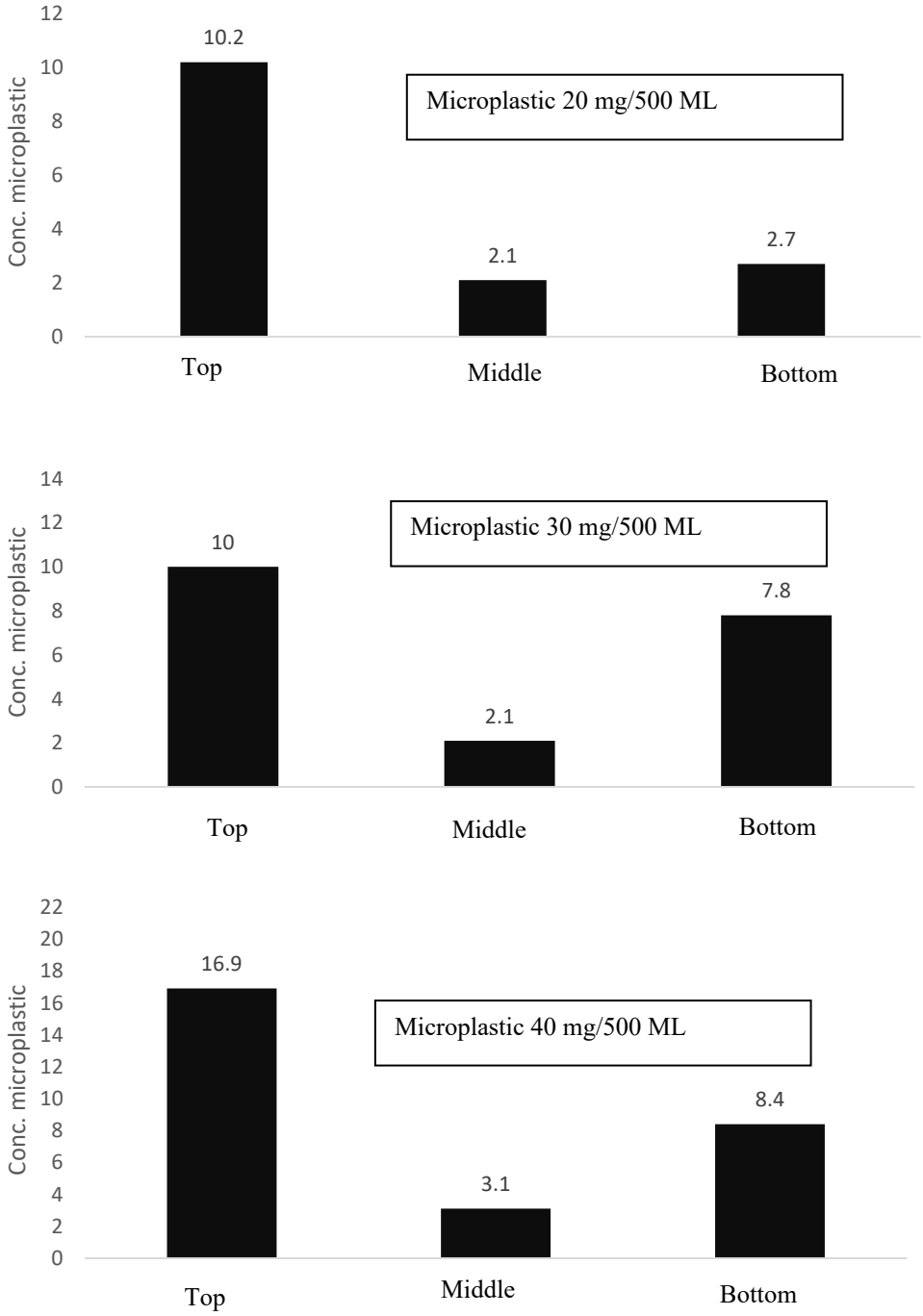

Figure 9. Residual microplastic after treatment

The characterization of residual microplastics post-treatment often reveals significant surface modifications and biophysical changes compared to their original state (Figure 9). Exposure to *Spirulina sp.* can lead to the formation of a "bio-corona," where proteins and lipids from the algae coat the plastic surface, altering its hydrophobicity and chemical reactivity (Lagarde et al., 2016). Research using FTIR analysis has shown that residuals can exhibit increased carbonyl and hydroxyl groups, indicating the onset of biodegradation or photo-oxidation facilitated by the alkaline conditions often created in microalgal cultures (Sari et al., 2022). These surface changes mean that the remaining particles are no longer inert; they are more likely to interact with heavy metals or other organic pollutants, potentially altering their environmental toxicity if they are not recovered from the system (Magro et al., 2012).

Finally, the distribution of residuals is influenced by the settling dynamics and the eventual senescence of the *Spirulina* culture. As shown in the experimental data, the decline in microalgal growth rates by day 13 corresponds with a reduced capacity to sustain

stable flocs, leading to a potential re-suspension of previously trapped microplastics. In high-salinity environments or under the stress of high microplastic loading, the stability of the EPS matrix can weaken, causing the release of residuals back into the water column (Pramesdistian, 2019). This highlights a critical challenge in bioremediation: the window for harvesting the flocs must be precisely timed to coincide with peak EPS production and cell vitality to minimize the volume of residual plastic remaining in the treated effluent (Lee et al., 2015).

4. Conclusion

The findings of this study establish that Spirulina sp. maintains an optimal growth profile within a salinity range of 10–15 ppt, which balances osmotic pressure and metabolic efficiency. While the bioremediation of polyethylene microplastics (PE-MPs) from fishing nets led to measurable increases in salinity, Total Dissolved Solids (TDS), and Chemical Oxygen Demand (COD), the aqueous medium's pH remained stable, indicating a robust buffering capacity within the culture system. The primary mechanism for microplastic removal was the secretion of extracellular polymeric substances (EPS) by the microalgae, which facilitated the formation of large, settleable flocs through physical and chemical interactions with the polyethylene particles.

To build upon these results, future investigations should incorporate a broader array of analytical parameters, such as FTIR spectroscopy to analyze surface functional group changes or SEM imaging to visualize the EPS-microplastic interface, thereby strengthening the dataset's scientific rigor. Additionally, extending the experimental timeframe is vital to observe the long-term stability of the formed flocs and the potential for microplastic degradation or desorption over time.

Finally, transitioning from synthetic media to natural seawater is highly recommended for subsequent studies. Utilizing natural seawater introduces a complex matrix of indigenous microorganisms and varying mineral concentrations, which would provide a more environmentally realistic representation of how *Spirulina sp.*-based bioremediation performs in situ. This approach is essential for scaling the technology from laboratory-controlled environments to practical applications in contaminated marine or brackish water ecosystems.

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