Biodegradation of oxidized high-density polyethylene and oxo-degradable plastic using microalgae *Dunaliella salina*

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Published online: 30 Sep 2022.

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ABSTRACT

This research aimed to evaluate the effectiveness of microalgae *Dunaliella salina* in the biodegradation process of oxidized oxium and HDPE plastics. Microalgae and microplastic interactions were evaluated in two 1 L glass bioreactors containing *D. salina* with oxium microplastics and oxidized HDPE at various concentrations (100 mg/500 mL, 200 mg/500 mL, and 300 mg/500 mL) for 15 d. The results showed a more significant decrease in alkene functional groups in oxium plastics than in HDPE. In addition, there was a change in the oxium functional group with the formation of carbonyl, ether, and primary alcohol. The growth rate of *D. salina* decreased significantly after interaction with oxidized HDPE microplastics compared to oxium interaction. We established that oxium plastics have a faster biodegradation ability owing to the addition of additives to the plastic. However, oxidation pre-treatment with H_2O_2 on HDPE plastic can also accelerate the plastic degradation process.

1. Introduction

Plastic pollution continues to impact the environment, especially in the ocean. Earth, dominated by almost 70% of the ocean area, seems to be covered by waste, especially plastic waste, such as plastic bags, bottles, and packaging products. Around 0.8 to 30 million metric tons of plastic waste are estimated to accumulate annually in the oceans [1]. Environmental pollution causes ozone layer depletion, climate change, and the greenhouse effect, resulting in decreased biodiversity and animal extinctions [2]. The growing industrial revolution and human urbanization play a crucial role in increasing the amount of plastic waste throughout the world's oceans. However, the inability of the government system to handle plastic waste, strengthened by the absence of restrictions on the production of plastic products in various plastic industries and the lack of education on environmental health, has prolonged the problem of plastic waste. Several studies have proven that plastic waste is harmful to the environment. Plastic waste has the potential to break down into microparticles that damage marine ecosystems, increase carbon dioxide emissions by 90% by 2050, and lead to the death of living organisms [3]. Due to low degradability of plastic, it takes hundreds of years to decompose into microplastics [4]. In addition, plastic additives such as bisphenol A, plasticizers, phthalates, and heavy metals increase the strength and resistance of plastics to abiotic and biotic degradation by microorganisms. However, these additives are toxic to the environment [5]. Plastic waste management continues to be a challenge and is being studied by scientists to find solutions to minimize plastic waste [6].

The growth of plastic waste in the last decade has pushed the government to address and provide solutions to plastic management problems. Several solutions to minimize plastic waste have been discussed, such as recycling used plastic and reducing plastic products, particularly packaging products in the form of plastic bags [6]. However, only a few types of plastics can be recycled; polymers with cross-links, such as thermoplastics and thermosets, form irreversible chemical bonds and cannot be melted back into new products [7]. In addition, the recycling process is considered uneconomical and inefficient because of the poor-quality end products [8]. Simultaneously, reducing plastic products is undoubtedly detrimental to the plastics industry. There is a trend toward developing several types of environmentally friendly plastics as a form of innovation. One of them is oxo-degradable plastics (oxium). According to CEN/TR 15351:2006, oxo-degradable plastics are polymers made from conventional polyolefins added with pro-oxidants as oxo compounds to accelerate the breaking of macromolecular chains in plastics. Rizzarelli et al. [9] reviewed the literature on several types of pro-oxidants added to

oxo-degradable plastics, including pro-oxidants derived from the transitions of heavy metal ions containing metal salts of carboxylic acids or dithiocarbamates Co^{2+}/Co^{3+}, Mn^{2+}/Mn^{3+}, Fe^{2+}/Fe^{3+}, and dan Ti^{4+} [10]. Other pro-oxidants come from more environmentally friendly additives, such as talc, starch, and organoclay, to accelerate the degradation process [9]. Adding these pro-oxidants facilitates photooxidation from UV heat radiation and formation of free radicals, which then react with atmospheric oxygen, causing fragmentation and the production of carbonyl groups (C = O) [10]. The main effects of pro-oxidants in oxo-degradable plastics are the reduction of the molecular weight of the matrix on the plastic, increase in the number of oxygenated groups, and decrease in the mechanical strength of the plastic in 2–5 years [9,11]. According to Chiellini et al. [12], after 12 weeks (84 d) at sea, oxo-degradable plastic bags decomposed by almost 70%, much faster than conventional plastic bags that showed no signs of disintegration. Da Luz et al. [13] observed the formation of fragments owing to the photooxidation of oxo-degradable plastics for 120 d. Abdelmoez et al. [14] reported that oxo-degradable plastics could undergo chain breaking by heat or light within 24 months, generating free radicals that facilitated quick formation of low-molecular-weight plastics. In addition to oxo-based plastics, microorganisms are also involved in manufacturing environmentally friendly plastics, such as microalgae-based bioplastics, which are considered environmentally friendly. Microalgae-based bioplastics are considered to be of high mechanical strength, with reduced carbon dioxide emissions, lower toxicity, and high degradability [15]. Vriend et al. [1] reported that microalgae have the potential to become raw materials for the production of bioplastics and to replace conventional plastics. According to Persistence Market Research, oxo plastics can be considered a solution to minimize plastic waste by creating environmentally friendly plastic products to produce oxo-degradable plastics. Their usage continues to increase, especially in the Middle East and other Asian countries such as India, Indonesia, and China [6]. Currently, several types of environmentally friendly plastics are being continuously produced. They are expected to reduce the use of non-degradable conventional plastics and lead to a safer and healthier environment.

However, knowledge about the benefits of oxo-degradable plastics, which should be able to suppress the increase in the production of polyethylene plastic bags, is still lacking among people. They need more education about the dangers of non-degradable polyethylene plastic waste. Chia et al. [15] reported that single-use polyethylene plastic bag waste dominated the environment, especially in the oceans. Polyethene is a polyolefin with powerful C-C and C-H bonding molecules, resulting in a high molecular weight and difficulty in decomposition [16,17]. Currently, environmentally friendly degradable plastics are produced by several industries worldwide [6]. Several studies have tested the level of degradability of this plastic, both abiotic and biotic. In this study, the authors observed the degradation ability of oxo-degradable plastic, particularly Oxium plastic. Oxium plastics contain pro-oxidants from natural minerals that are non-toxic and in low doses and that have been tested and passed ASTM 6954, ASTM D5208, and ASTM G21-09 (www. greenhope.co/oxium). In addition to being able to decompose abiotically, oxium is claimed to be an oxo-biodegradable plastic that microorganisms can metabolize. Abdelmoez et al. [14] reported that microorganisms could convert the oxidation products of pro-oxidants in oxium plastics into water, carbon dioxide, and biomass. Muthukumar et al. [18] also reported that the biodegradation process in oxium plastic produces low-molecular-weight hydrocarbons, such as carboxylic acid alcohols and ketones. Da Luz et al. [13] observed the formation of fragments due to the photo-oxidation of oxium plastic and biodegradation by microorganisms for 120 d. Abdelmoez et al. [14] reported that within 20 d, microorganisms biofouling on the surface of oxium plastic. This research focuses on exploiting microorganisms such as microalgae, which are considered capable of colonizing foreign substrates, such as plastic surfaces, to form biofilms and release ligninolytic enzymes and exopolysaccharides (EPS) [19]. EPS from microalgae released into the water media and EPS in the biofilm interact with macromolecules on the plastic surface and trigger plastic damage [20]. Kumar et al. [21] examined transverse damage to polyethene sheets after colonization by microalgae such as *Anabaena Spiroides*, which showed the highest percentage of polyethene degradation (8.18%), followed by the diatom *Navicula Pupula* (4.44%) and green algae *Scenedesmus dimorphus* (3.74%). In addition, using microalgae in the biodegradation process is considered safer than using other microorganisms, such as bacteria. According to Chia et al. [15], bacteria can also decompose plastics, but they produce endotoxins. Therefore, they are considered biological pollutants. Sarmah et al. [22] reported that the freshwater microalgae *Phormidium lucidum* and *Oscillatoria subbrevis* could colonize and decompose polyethene efficiently without the addition of pro-oxidants. Sarmah et al. [22] showed that in the absence of pro-oxidants, microalgae can colonize plastic, so adding pro-oxidants to oxyplastic will make it easier for microalgae to decompose plastic. In this study, we utilized the microalgae *D. salina*, an autotrophic-photo microalgae capable of biodegrading conventional and oxium plastics [23]. Parsy et al. [24] also observed the effect of plastic biodegradation on the growth of several microalgae, such as *D. salina*, *Nannochloropsis oceanica*, *Tetraselmis suecica*,

Picochlorum Costavermella, Coccomyxa simplex, Synechococcus rubescens, and *Chlorella vulgaris* in waste. They reported that microalgae that produce large amounts of biomass, including *D. salina*, are most resistant to waste containing hydrocarbons, heavy metals, and other additives. These compounds are also included in conventional plastics and plastic-labeled oxo-degradable.

Research on the success of microalgae in plastic biodegradation is still being conducted. New research continues to be conducted to further deepen the potential of microalgae in dealing with environmental problems. Microalgae are considered to have the renewable potential for waste biodegradation, waste treatment (bioremediation), and environmental monitoring [15]. To date, an increasing number of industrial companies have been involved in the bioremediation of waste using microalgae. Ahmad et al. [25] reported that various microalgae had been proven to carry out bioremediation by reducing pollutants and fixing CO_2 from wastewater, especially plastic waste, with the simultaneous production of biomass. As a result of bioremediation, microalgal biomass can be used as a raw material for producing environmentally friendly plastics and other by-products, such as oxo-biodegradable, bioplastic, and biofuel [25]. Asiandu et al. [26] summarized several microalgae proven to accelerate the rate of plastic degradation by reducing the weight of significant pollutants. Recently, in addition to adding pro-oxidants to oxium plastic, the initial pre-treatment of conventional plastics, such as artificial UV, ozone, and peroxide treatment, as the initial oxidation step, can also be carried out before biodegradation by microorganisms. Kim et al. [27] reported the success of various abiotic pre-treatments of conventional plastics. However, further studies are required to evaluate plastic biodegradation using microalgae and the effects of the resultant biodegraded product on microalgae.

Microalgae contain high-value compounds widely used for food products, so they must be free from pollutants, especially plastic waste [28]. Since oxo-degradable plastics are degraded by microorganisms and the environment, there is currently a lot of discussion about the impacts of oxo-degradable plastics on the environment. Nava et al. [29] reported a negative impact of conventional plastics on microorganisms. Based on previous research on the success of microalgae in the biodegradation process of oxium plastic and oxidized plastic, the impact of accumulated toxins from degradation products on microalgae has been discussed in this study. We aimed to assess the ability of microalgae to decompose conventional plastics and oxo-degradable plastics. Further, we evaluated the potential of microalgae, specifically *D. salina*, to biodegrade two different types of plastic, namely conventional HDPE plastic bags with pre-treatment and Oxium plastic bags. We also aimed to

understand the rate of plastic degradation after its interaction with *D. salina* and the potential toxicity of the two plastics on the growth of *D. salina*. This research intends to educate the public to be wiser about plastic issues and provide an overview for entrepreneurs in the plastics industry to increase increase environment-friendly plastic products.

2. Material and method

2.1. Ingredient

Oxo-degradable oxium plastic was standardized by SNI 7188.7:2016 (Indonesian National Standard), and conventional high-density polyethylene (HDPE) plastic was standardized by SNI 19–4370-2004 (Indonesian National Standard for Plastic Waste Bags). The photoautotrophic microalgae, *D. salina*, used in this study was obtained from the Jepara Brackish Water Cultivation Center, cultivated in the C-Biore laboratory, Diponegoro University.

2.2. Preparation of culture media D. salina

We observed the growth of *D. salina* in a 1 L Erlenmeyer flask and measured the maximum cell density (optical density) using a UV-Vis spectrophotometer at a wavelength of 680 nm [30]; we also measured pH and temperature. The pH was 8–9, while the temperature was 15–35°C [31]. *D. salina* was cultured until the cell density reached 1 ppm and then put into a bioreactor filled with salt water with controlled salinity in the range of 30–35 ppt. *Dunaliella salina* was nourished using Walne (1 ml/L *D. salina*) with a mixture of triple super phosphate (TSP) 36 ppm, urea 80 ppm, ammonium sulfate 40 ppm, ethylene diamine tetra acetic acid (EDTA) 5 ppm, and $FeCl_3$ 1 ppm administered once every four days. The optical density (OD) of *D. salina* was measured every day for one week to determine the increase in growth before the microplastic treatment.

2.3. Preparation of HDPE and oxium microplastic samples

HDPE and Oxium plastics were cut to a size of 2 mm^2 and a thickness of 1–2 μm, each as much as 2 g, and then soaked in distilled water for 24 h to ensure that the microplastics were clean of contaminants that might still stick to the HDPE and Oxium surfaces and dried. In this study, dried HDPE microplastics were subjected to an initial oxidation treatment using a Hydrogen Peroxide (H_2O_2) solution to obtain oxidized HDPE. HDPE pieces were immersed in a glass beaker containing 100 ml of H_2O_2 at a concentration of 15 M. The glass beaker was equipped with an aerator for stirring so that the HDPE plastic could be

distributed into the H_2O_2 solution. The HDPE immersion was performed at room temperature (25°C) for 72 h.

2.4. Preparation of culture media D. salina with microplastic

Seven reactors containing 500 mL of *D. salina* media, pretreated for one week, were prepared to be treated with microplastics. The seven reactors had aerators and LED lights (3000 lux). The reactors were divided into three groups. Group 1 was a reactor containing *D. salina* without microplastics, group 2 contained three reactors containing *D. salina* with oxidized HDPE microplastic, and group 3 contained *D. salina* with Oxium microplastic. Groups 2 and 3 were treated with microplastics at concentrations of 100, 200, and 300 mg, respectively. The reactor conditions were a temperature of 15–35°C, a salinity of 30–35 ppt, pH of 8–9, and nutrients once every four days. The optical density measurements in the seven reactors were carried out every day for 15 d to evaluate the potential effect of increasing the concentration of microplastics on the growth of *D. salina*.

2.5. Analysis preparation

The microplastics to be analyzed were HDPE microplastics oxidized by H_2O_2, Oxium control microplastics without treatment (0 d, to be used as a reference), oxidized HDPE microplastics treated with *D. salina*, and Oxium microplastics treated with *D. salina*. After 15 d of microplastic interaction with *D. salina*, the OD value for the growth of *D. salina* was obtained. Then, the microplastics in the *D. salina* culture were separated and rinsed using distilled water to remove the *D. salina* biomass that was still attached to the surface of the microplastics. The microplastics were then dried for 24 h at room temperature. The four dried microplastics were further analyzed. *D. salina* was then filtered using a 40 mesh stainless steel filter to separate the *D. salina* biomass from the filtrate. The experimental data obtained were the OD value of *D. salina*, the value of *D. salina*'s growth rate, and the data from the analysis of the four microplastics (Fourier transform infrared spectroscopy (FTIR), scanning electron microscopy (SEM), and X-ray diffraction (XRD) data).

2.6. Fourier-transform infrared spectroscopy (FTIR), scanning electron microscopy (SEM), and X-ray diffraction (XRD) analyses

The four dried microplastics were analyzed using FTIR, SEM, and XRD. FTIR is a common technique used to study macromolecules, such as HDPE polymers, which are recommended for plastic degradation investigations using UV exposure as mentioned in ISO 4582

and ISO 4892 and surface colonization of microorganisms as mentioned in ISO 846 and ISO 11266 [32]. FTIR analysis was performed using a Perkin Elmer Type Frontier instrument, producing a spectrum from 4000–500 cm^{-1} (SNI 19–4370-2004 method) and ASTM D6288-89. SEM analysis was used to determine changes in the surface morphology of the microplastics after treatment [32]. SEM analysis was performed at room temperature and standarized using an Au.A Jeol instrument (model JSM-6510 LA) at a magnification of 10000x. SEM and FTIR analyses were performed on the four microplastics with a size of 1 mm^2. XRD analysis of microplastics was carried out using a Siemens D500 X-ray diffractometer with Cu Kα radiation. XRD samples of microplastics was performed at the same thickness (0.3 mm). The thickness of the microplastics was measured using a screw micrometer with a Digital Micrometer Syntek brand, with an accuracy of 0.001 mm.

3. Results and discussion

During the interaction between *D. salina* microalgae and plastics in the water system, reducer microorganisms in the culture, in the form of bacteria or fungi that are heterotrophs or chemoautotrophs, also play a role in the plastic biodegradation process. Microalgae *D. salina* produces EPS, which accumulates and forms a biofilm on the plastic surface. Microalgae reducers use biofilms to grow and reproduce by utilizing the oxygen from microalgae. In the hetero-aggregation process between EPS, reducer microorganisms, and microplastics, these reducer microorganism colonies carry out the microplastic bio-decomposition process to produce inorganic carbon dioxide (CO_2), and microalgae reuse this inorganic CO_2 as an energy source [33].

Celente et al [34] reported that the *D. salina* did not utilize organic carbon in culture for its growth but instead used the inorganic CO_2 produced from the nutrient sodium bicarbonate ($NaHCO_3$). The CO_2 produced from the catalysis increased the density of *D. salina* cells. Thus, microplastics are not directly used by microalgae as a carbon source. *D. salina* did not experience an increase in growth in the presence of organic carbon from microplastics or other sources [34]. However, the biodegradation process of microplastics occurred because of the EPS produced from *D. salina*, and EPS provides organic carbon as a food source for reducing microorganisms that colonize the surface of microplastics. EPS itself is also able to accelerate the heteroaggregation process together with reducer microorganisms to produce the final products of biodegradation, namely in the process of assimilation, in the form of carbon dioxide (CO_2) + water (H_2O) (aerobic microorganisms) and CO_2 + H_2O + methane gas (CH_4) + hydrogen sulfide (H_2S) (anaerobic microorganisms), which contribute to providing inorganic

carbon as an energy source for *D. salina* to photosynthesis and generate new biomass ([34,35]). This new biomass is the result of the participation of plastic biodegradation products in the form of acetyl coenzyme A, which plays a role in the formation of proteins, carbohydrates, and lipids. Various studies have proven the ability of microalgae in the biodegradation of microplastics, and the ability of microplastics to inhibit the growth of microalgae [29,36] is discussed in this study.

3.1. Evaluation of the impact of microplastics on the growth rate of D. salina

The presence of microplastics in *D. salina* culture media affects the growth rate of microalgae. Various studies have proven the ability of microalgae cells to absorb microplastics and microplastic additives [29]. A study by Song et al [37] reported significant inhibition of microalgae by microplastics. Celente et al [34] reported that all microplastics can penetrate the microalgal cell wall through passive diffusion to enter the intracellular cell membrane. If the compound adsorbed by microalgae in the form of inorganic carbon accelerates the growth, the adsorption of additive pollutant compounds will stimulate microalgae metabolism to increase reactive oxygen species (ROS) such as O_2 and H_2O_2 in algal cells, which are toxic and able to inhibit their growth. The excessive increase in ROS indicates stress conditions in microalgae [38], resulting in intracellular damage to cells, especially the permeability of cell membranes and other molecular structures such as DNA, proteins, and lipids [39]. Cell damage is usually characterized by a significant decrease in the density and growth rate of microalgae [37,40].

The growth and density of *D. salina* (Figures 1 & 2) in oxium treatment increased with time at each concentration variation until the 12th day, whereas in the oxidized HDPE treatment, growth and density increased until the 10th day. The increase in growth at the beginning of this period is an adaptation stage for *D. salina* in the presence of microplastics. The presence of inorganic carbon sources from the assimilation process by reducer microorganisms is considered an energy source for *D. salina* to increase its growth [41]. Chae et al [42] observed an increase in the growth rate of microalgae after the first 6 d, and a decrease in growth rate the next day indicated that *D. salina* began to experience stress due to microplastics [38]. Research by [43] and [29] reported that the time of exposure to microplastics affects the increase in the concentration of additives leached by microplastics, which inhibit microalgae growth. Based on Figures 2 and 3, a comparison of the growth rate of [of *D. salina* after interacting with microplastics is provided in Table 1.

We compared the growth rate of *D. salina* in the treatment of oxidized oxium and HDPE microplastics with variations in the concentration of microplastics (Table 1). The growth rate of *D. salina* on both microplastics decreased with the addition of microplastics. This was due to the microplastic exerting a shadow effect on the surface of the *D. salina* cultivation area, which hindered the distribution of light that entered the *D. salina* culture. As a result, the photosynthetic efficiency decreased. Increasing the concentration of microplastics in the media causes a more significant shadow effect, thus resulting in a slower growth effect [44]. In addition, a greater concentration of microplastics also affects the production of excess EPS and is toxic [36]. Several studies have shown that microalgae utilize microplastic organic carbon. Microalgae can permanently capture and store inorganic carbon dioxide resulting from the bio-decomposition by reducing microorganisms to produce biomass. Consequently, carbon dioxide is not emitted into the environment. In addition to CO_2, various toxic contaminants are also adsorbed into microalgae cells, thereby reducing their growth rate as the concentration of microplastics increases and the length of exposure time increases [15].

However, an in-depth comparison (Table 1) showed a drastic decrease in the growth rate of *D. salina* after treatment with oxidized HDPE microplastic compared to growth rate after treatment with Oxium microplastic. The growth rate of *D. salina* with the Oxium microplastic treatment was better. This was due to the different conditions of the microplastics used in this study. Oxium microplastics contain several additional additives, such as pro-oxidants, which have greater degradation capabilities than conventional plastics [6]. added additives to plastics as the main indicator of the inhibition of microalgae growth, especially to oxium plastics, with the addition of pro-oxidants that trigger a higher toxicity level. However, oxidized HDPE microplastics derived from conventional HDPE were subjected to a pre-oxidation treatment using a peroxide solution for 72 h. This process aims to provide an early stage of damage to the HDPE surface so it can be more easily degraded biotically [45]. reported that the biodegradation process of microplastics begins with chemical oxidation, which can increase the susceptibility of plastics to damage [46]. stated that the hydrophobic and structural properties of the plastic surface could be drastically reduced by oxidation treatment with peroxide. The initial damage to the oxidized HDPE surface, which was then mixed into the *D. salina* culture, had a direct contaminant effect in the form of carbon and additives with a higher concentration on *D. salina*, thus providing a higher inhibitory effect. Contaminants released from HDPE are the dominant factors that cause toxicity, inhibit growth, damage cell membranes, and have the
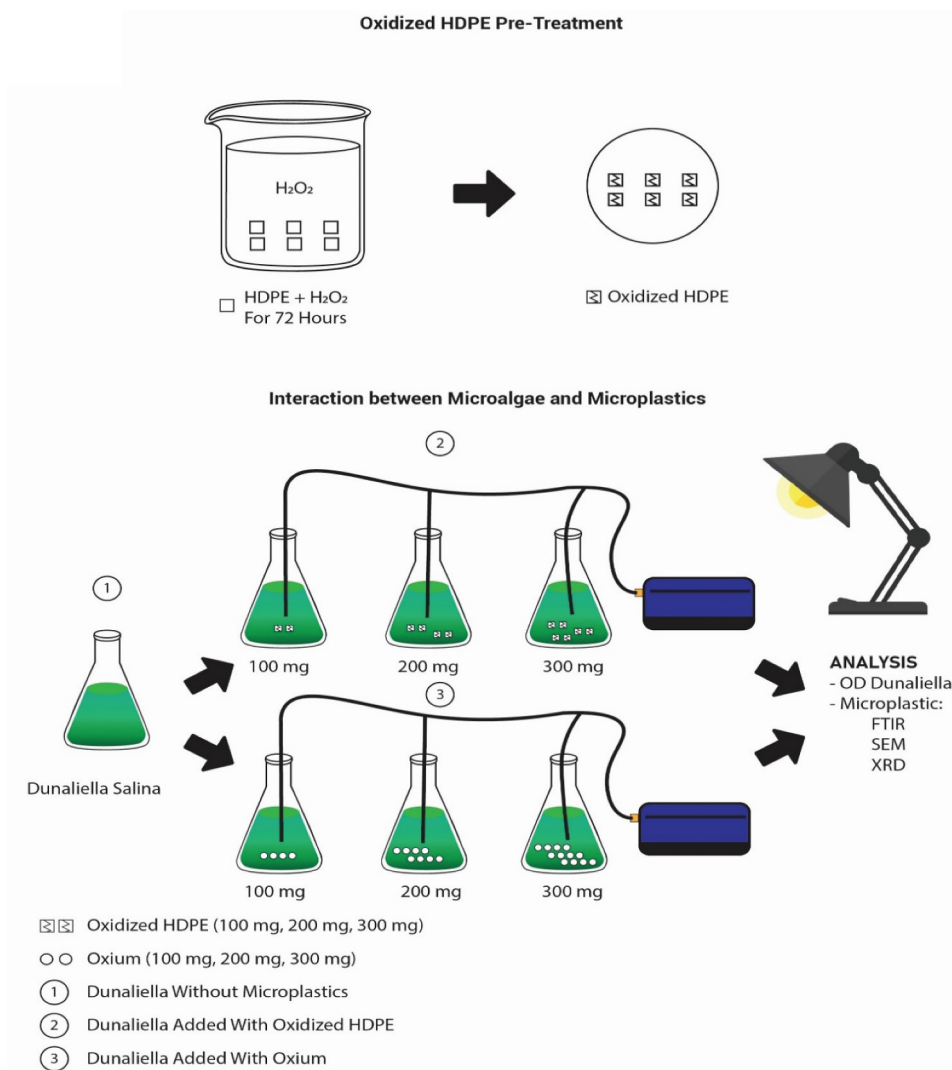
Figure 1. The series of biodegradation processes in this research.
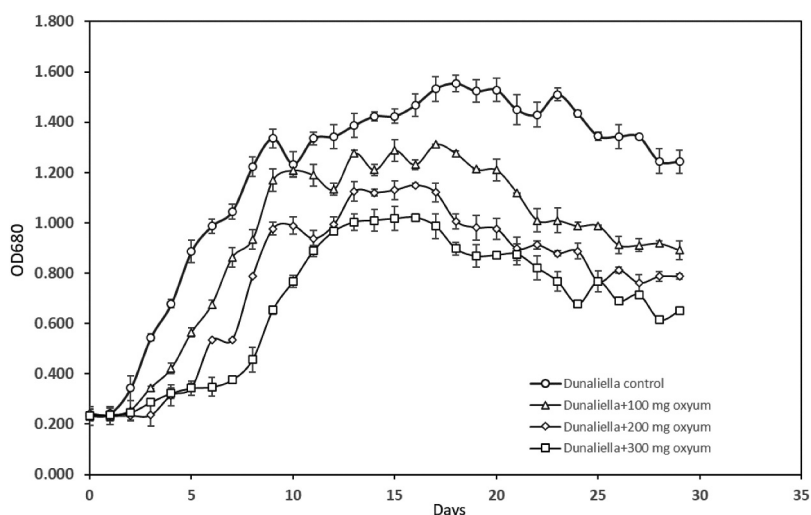

Figure 2. The growth of microalgae *D. salina* treated with oxium microplastic.

potential to accumulate in microalgae tissues [47]. Meanwhile, oxium plastic, which contains pro-oxidants, when put into *D. salina* culture, still takes time to undergo oxidation, so the inhibition rate is slower than that with oxidized HDPE. Esmaeili et al [48] reported that polymer chain breakage and scission triggered by pro-oxidants in oxium plastic occurred for at least one week in microorganism culture [49]. observed changes in the
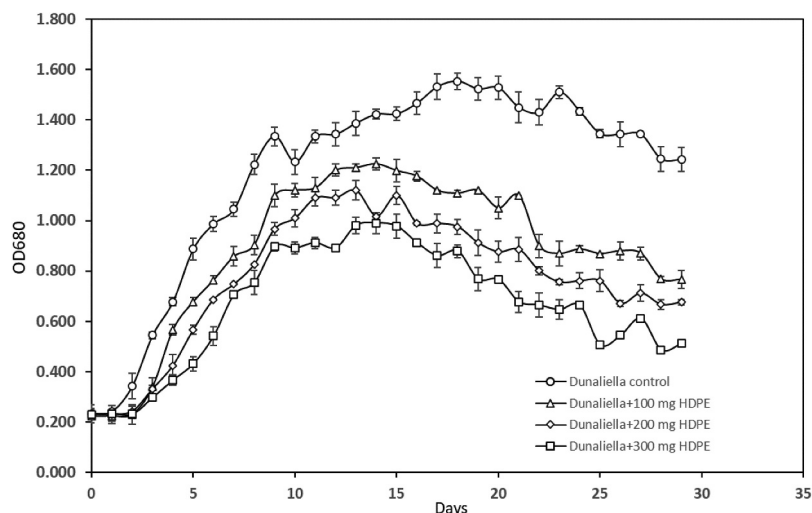
Figure 3. The growth of *D. salina* treated with oxidized HDPE microplastic.

Table 1. Comparison of growth rates of *D. salina* after interaction with oxium microplastics and oxidized HDPE for 15 d.

Microplastic (mg)/ 500 ml *D. Salina*	Oxium/day	Oxidized HDPE /day
Control (0 mg)	0.2195 ± 0.034	0.2195 ± 0.035
100 mg	0.1587 ± 0.052	0.0448 ± 0.076
200 mg	0.1343 ± 0.071	0.0411 ± 0.054
300 mg	0.1237 ± 0.062	0.0409 ± 0.045

surface of oxo-degradable plastics by microorganisms after 45 d of interaction [14]. reported that oxium-based PE plastics could be biofouled by microorganisms 20 d after interaction.

Furthermore, several studies have shown that plastic waste containing additives can reduce the quality of microalgae [29]. The release of additives from microplastics significantly affects the growth of microalgae. However, the ability of microplastic additives to enter the microalgal cell membrane is strongly influenced by their chemical properties. More hydrophobic contaminants will more easily undergo passive transportation (diffusion) through the lipid-filled cell membranes. At the same time, more hydrophilic pollutants have more potential to mix with the aquifer media. Adding a pro-oksidan to accelerate the degradation of oxium plastic causes an increase in the hydrophilic properties of oxium plastic [14]. The addition of a pro-oxidant to polyethylene to form Oxium causes breakage of the long polymer chain to form a carbonyl group [50], increasing the hydrophilicity of the polymer [10]. The results are presented in Table 1. The decrease in the growth rate of *D. salina* was more drastic with the addition of the oxium microplastics. This proves that the different concentrations of pro-oxidant additives in oxium plastic compared to conventional plastics can accelerate the degradation process in oxium plastic and the rate of growth inhibition is also greater. This is evident from the range of decreased growth rate of *D. salina* with oxium plastic, which was greater than that of *D. salina* with oxidized HDPE.

Conventional HDPE microplastics have better hydrophobicity than oxium. After HDPE is oxidized with peroxide, its hydrophobic properties decrease, and it has more potential to carry out the diffusion process through *D. salina* cell membranes [40]. This was evident from the slight decrease in the growth rate of *D. salina* (Table 1). However, the addition of microplastic concentration and oxidized HDPE treatment caused the growth rate of *D. salina* to decrease drastically compared to oxium treatment.

3.2. SEM analyzed changes in morphological characteristics

Microalgae with mucilaginous secretions of EPS play an essential role in the oxidation process in the environment, especially in aquatic systems. EPS is the primary colonizer of several materials, including plastics, that are physically impacted, as indicated by the destruction of the morphology of the plastic surface [51]. Changes in the surface morphology of plastics that are influenced by the presence of EPS in biofilms, which affect the corrosion process in microplastics, are an important assessment for plastic degradation. Several factors that play an important role in biofilm formation are electrostatic forces, surface charge, hydrophobicity of plastic surfaces, and the availability of cations [52].

An electronic magnification scan analysis was carried out to determine the morphological changes in the plastic surface after treating with *D. salina* . There were several instances of plastic surface damage due to microbial colonization (Figure 4). Figure 4 shows SEM micrographs of oxidized HDPE and oxium before and after 15 d of treatment with *D. salina*. Figure 4(a) depicts that after treatment with *D.salina*, the oxidized HDPE showed a softer surface with smooth erosion and defects caused by the HDPE oxidation process [53]. reported that the morphology of oxidized HDPE

after 30 d exhibited an uneven surface and formed cavities [46]. also reported that the oxidation of plastics using peroxide results in morphological changes on the plastic surface. However, oxidized HDPE showed a different picture after being treated by *D. salina*. Differences were observed between the surfaces. Figure 4(b) shows an uneven surface and brittleness, which is influenced by the formation of pits and the higher frequency of voids produced by the biofilm owing to the aggregation process on the surface of the film [49].

Interestingly, in Figure 4(c), the morphology of the oxium plastic before the threat shows that some erosion spreads over the entire plastic surface, which is similar to that in [49], where oxo-degradable plastic without treatment showed an uneven surface but no voids. Further, oxium plastic, which contains pro-oxidants, is ready to attach to microorganisms. After incubation with Oxium for 15 d, large wounds were observed, indicating greater biofilm formation. This biofilm functions as a growth medium for bacteria and fungi involved in the biodegradation process on plastic surfaces [54]. The formation of biofilms in oxium is triggered by pro-oxidants in oxium, which can break polymer chains so that microorganisms can work optimally after at least one week [48]. Research by da Luz et al [49] showed large cracks and holes in biodegradable oxo-plastic after 45 d of treatment with fungi. Abdelmoez et al [14] reported that oxium-based PE plastics could be biofouled by microorganisms 20 d after the interaction. When considering the damage to the two plastics after the *D. salina* treatment, the damage that occurred was almost the same. HDPE, which had been oxidized by peroxide first, showed surface damage, and the damage was even more significant after *D. salina* treatment. While oxium is more easily degraded, it showed tremendous damage even though it was not pre-treated, indicating that the pro-oxidant in oxium plastic works effectively to accelerate the plastic degradation process.

3.3. Organic evaluation of altered functional groups by FTIR

The ability of plastic polymers to degrade, resulting in changes in the polymer molecular structure, is strongly influenced by the characteristics of the plastic and its degradation process. Functional groups in plastic polymers play an important role in determining the characteristics of plastic and chain length, branched chains, cross rings, chain structure (RSC, Advancing the Chemical Sciences), and chemical additives. Furthermore, the degradation process also determines the resulting degradation products so that each degradation phase can be recognized [55,56]. During a photooxidation process, the plastic absorbs UV light, and with the help of oxygen from the surroundings, the polymer undergoes formation of free radicals that accelerate the termination of the polymer chain. The reaction results in the formation of a carbonyl group, which reduces the molecular weight and increases the hydrophilicity of the plastic. This concept
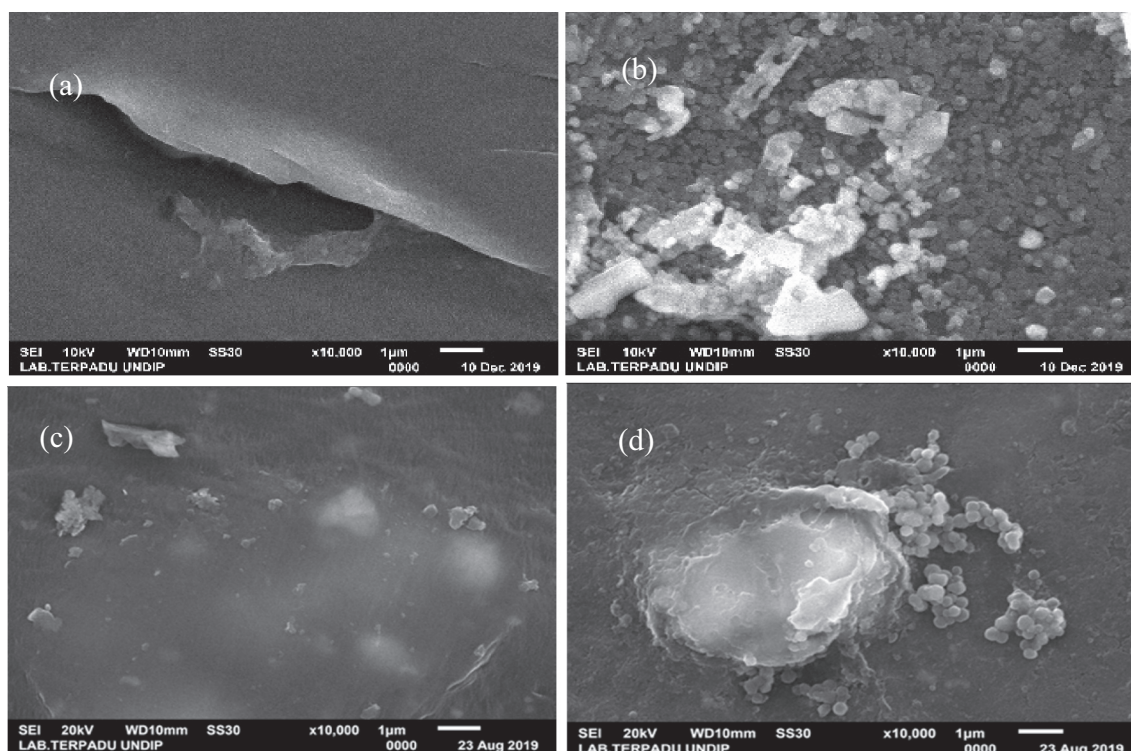

Figure 4. Microplastic microscopy with SEM (a) Oxidized HDPE (b) Oxidized HDPE with *D. salina* (c) Oxium control (d) Oxium with *D. salina*.

drives the idea of manufacturing oxo-degradable plastics so that microorganisms quickly degrade them. In the next stage, the plastic is ready to accept the attack of microorganisms (producers) that can produce oxygen as a product of photosynthesis and form a biofilm, which is a collection of microorganisms (reducing agents) surrounded by EPS on the plastic surface. The formation of biofilms is related to the secretion of EPS compounds, where EPS will form aggregates with plastics and enlarge the plastic pores so that the plastic becomes fragile. The microbial community has the potential to release various acidic compounds, including carboxylic acids and carboxylic acids. There is also a formation of alcohols other than assimilation products in $CO_2 + H_2O$ [32,55], and [56]. In this study, changes in the compounds in the plastic after degradation by *D. salina* are presented in the FTIR results.

The degradation process in HDPE is almost the same as that in other types of thermoplastics, where the initial changes that occur due to the oxidation process using peroxide are indicated by changes in the molecular structure due to chain cutting [56,57]. This is evidenced by the change in the intensity of the peaks at 2914 cm^{-1} and 2848 cm^{-1} for the alkyl group (CH) and 1468 cm^{-1} for CH$_2$ bend [58]. As can be seen in Figure 5, there was a decrease in peak intensity at wavelengths of 2914 cm^{-1}, 2848 cm^{-1}, and 2848 cm^{-1}, indicating that the alkyl groups in HDPE were oxidized after treatment with *D. salina*. The same effect was also observed in oxium plastic, where there was a significant decrease in the intensity of the alkyl groups (CH and CH$_2$) after *D. salina* treatment at 2914 cm^{-1} (CH), 2848 cm^{-1} (CH), 1468 cm^{-1} (CH$_2$ bend), and 717 cm^{-1} (CH$_2$ rock). The reduction in the intensity of the two organic functional groups plays a significant role in degradation of HDPE plastic, which is also the primary material for oxide plastic. This indicates that the degradation process was in its early stages. Hadiyanto et al [59]., in their research on changes in the intensity of alkyl groups in polyethylene, reported the presence of covalent bonds in alkyl groups, indicating that polyethylene has high stability and a decrease in alkyl groups suggests that the plastic is undergoing degradation. Another change that was a result of degradation of oxidized HDPE by *D. salina* was an increase in the concentration of carboxylic acid,
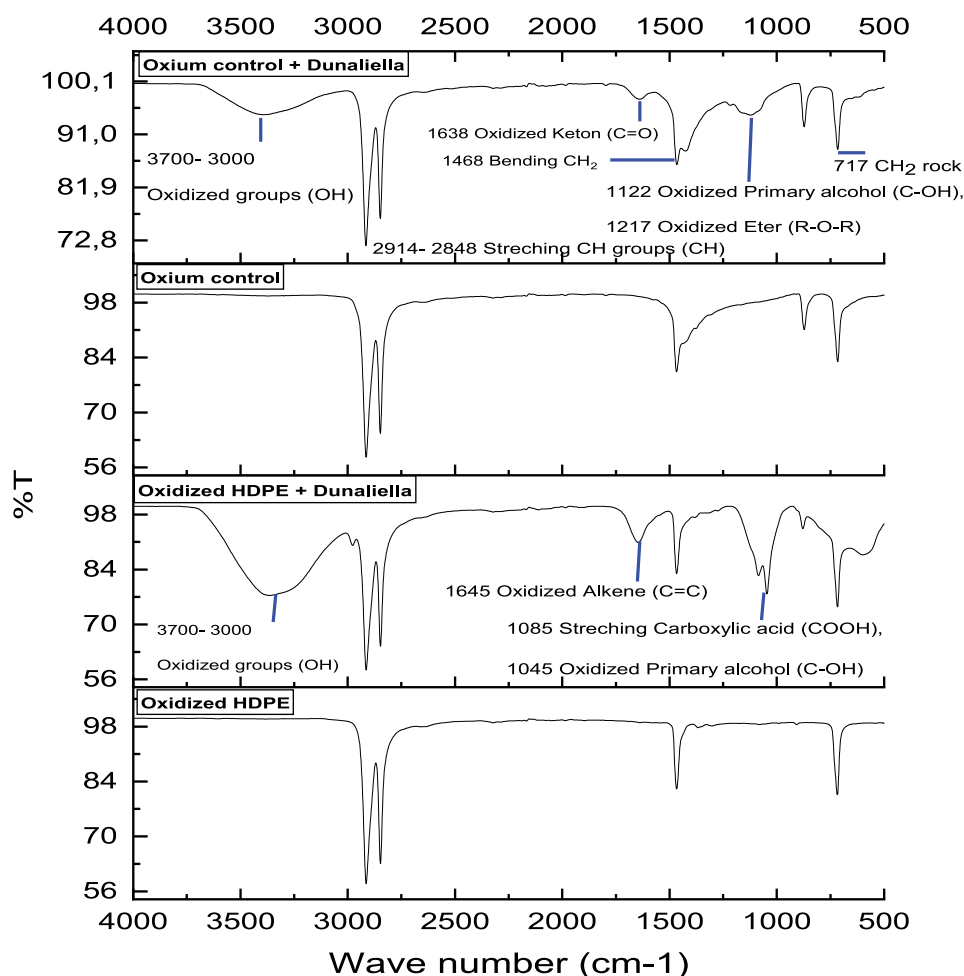
Figure 5. Comparative FTIR spectrum of oxidation of HDPE before being treated, HDPE being oxidized after being treated with *D. salina*, Oxium before being treated, and Oxium after being treated with *D. salina*.

as indicated by an increase in peak intensity at a wavelength of 1085 cm^{-1} (CO), followed by the emergence of a new group of alkenes (C = C) at 1645 cm^{-1} and a primary alcohol (C-OH) group at 1045 cm^{-1} [60]. The presence of new functional groups in HDPE oxidized by *D. salina* treatment is responsible for the formation of alcohol, which is one of the important groups resulting from the degradation process by microorganisms [61,62].

Kershaw et al [63] explained the role of bacteria and fungi that utilize EPS enzymes from microorganisms, especially microalgae, which allow an oxo-biodegradable process, and this process is proven to be able to destroy plastics and reduce the molecular weight of hydrocarbons in plastics that have undergone a process. This is indicated by the appearance of functional groups, such as carboxylic acids (COOH), alcohols (CO), and ketones. For example, the intensity of alcohol formation in oxium with *D. salina* treatment was characterized by very sharp peaks at wavelengths of 1122 cm^{-1} for primary alcohols, 1217 cm^{-1} for ethers, and 1638 cm^{-1} for ketones (C = O). A very significant increase in alcohol concentration and the formation of ketone groups with sharp intensity on oxium plastic after being treated with *D. salina* is caused by the ability of microorganisms to degrade oxidized HDPE or oxium over a very long period. The increase in the hydrophilicity of the plastic due to the cutting of the polyethylene polymer ring will facilitate the entry of bacteria and fungi through the pores of the plastic surface and allow dissolution of the plastic with the help of oxygen produced in the metabolic processes of these microorganisms [62]. Furthermore, the addition of additives (starch) to the oxium manufacturing process makes it easier for microorganisms to exert biodegradation functions by utilizing starch as a food source [13,64]

3.4. Investigation of changes in crystallinity characteristics

The crystallinity of the solid material was determined by XRD. The crystallinity of a material classified as good should be characterized by sharp peaks and high intensities, while the non-crystalline or amorphous materials do not exhibit sharp peaks. Crystals are solids that are composed of ordinary atoms or molecules. An amorphous solid has an irregular atomic or molecular arrangement [65]. Plastic materials are generally composed of crystalline atoms [65]. These properties play important roles in the natural degradation of plastics. The XRD results are shown in Figure 6, which indicate that HDPE and oxium plastics are crystals.

Figure 6 shows that HDPE and oxium plastics are in crystal form. The crystalline nature of the atoms that make up HDPE and oxium is evidenced by the appearance of peaks in the XRD spectra of the two plastic materials. The peaks in the HDPE spectrum have a greater intensity than the peaks in the oxium spectrum, indicating that HDPE has better crystalline properties than Oxium. Further, it indicates that HDPE is more difficult to degrade naturally than Oxium. It takes hundreds of years for HDPE to decompose naturally. Oxium naturally decomposes in nature in less than ten years.

Based on Figure 6, the HDPE oxidation spectrum had the best crystallinity properties compared to the other spectra before treatment. This is indicated by the appearance of a peak at an angle of 2θ 21.46180 °with an intensity (> 17,500). The crystalline properties of the oxidized HDPE spectrum also appeared at angles of 2θ 23,790 and 43,990 at intensities (≈5000) and (<5000), respectively. The crystallinity of HDPE was lower than that of the oxidized HDPE. The HDPE + *D. salina* oxidation spectra exhibited peaks at angles of 2θ 21.7180, 2θ 24.0460, and 44.00120 at intensities (≈5000), (<2500),
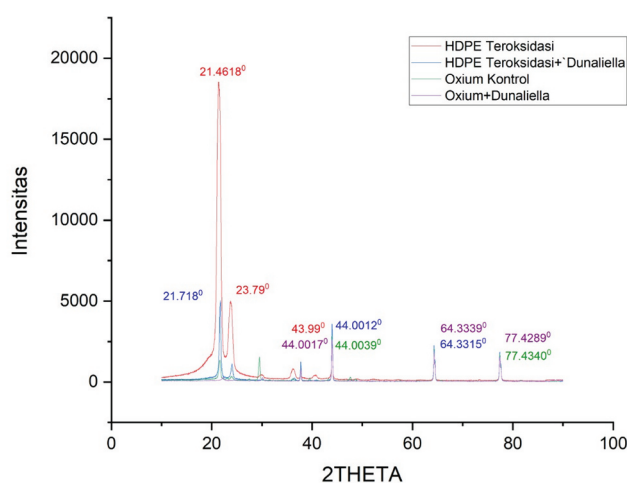
Figure 6. Comparative XRD spectrum of oxidized HDPE before treatment, oxidized HDPE after treatment *D. salina*, and Oxium before and after treatment with *D. salina*.

and (<5000), respectively. This decrease in crystallinity indicates significant degradation of oxidized HDPE by *D. salina*. Furthermore, the Oxium spectrum before treatment exhibited crystallinity properties that were not much different from those of Oxium after treatment with *D. salina*. The crystallinity of the oxide before treatment appeared at angles of 2θ 44,00390 and 77.43400 at intensities (approximately 2500) and (<2500), respectively. The crystallinity properties of Oxium + *D. salina* appeared at angles of 2θ 44.00170 and 77.42890 at intensities (~ 2500) and (<2500), respectively. This similarity in crystallinity indicates that the degradation of Oxium by *D. salina* was not significant. Therefore, it can be concluded that HDPE has better crystallinity than Oxium, but *D. salina* can significantly reduce HDPE crystallinity, indicating that *D. salina* can degrade HDPE significantly. In contrast, *D. salina* did not significantly reduce Oxium crystallinity, indicating that *D. salina* did not degrade Oxium substantially.

4. Conclusion

In this study, we investigated the degradation of oxium and oxidized HDPE microplastics. From the observation of the growth rate of *D. salina*, the most significant decrease in growth rate occurred in the treatment of *D. salina* with oxidized HDPE and the addition of microplastics. This confirmed the effect of the hydrophobicity of the oxidized HDPE microplastics on their ability to enter the cell membranes of *D. salina* was better than oxium microplastics. Pre-treatment of HDPE plastic, which is oxidized using peroxide, also decreases the physical and chemical properties of HDPE plastic. From the surface morphology analysis of microplastics using SEM, it was observed that more severe damage with erosion occurred on the surface of oxium microplastics, which had better hydrophilicity than oxidized HDPE. The increase in the hydrophilicity of the Oxium microplastics due to addition of the pro-oxidant increases the porosity of the Oxium microplastic surface, thus supporting the work of bacteria in damaging the Oxium microplastic surface.

Furthermore, important changes that indicate the occurrence of degradation were also observed in the FTIR analysis results. Several important functional groups, such as carboxylic acids and ketones, increased in intensity, accompanied by the appearance of alcohol functional groups on oxium microplastics after treatment with *D. salina*. In oxidized HDPE, the loss of the alkene functional group and appearance of the alcohol functional group are important indicators of the change in the organic molecular structure of the polymer without ignoring the increase in the intensity of the carboxylic acid functional group, confirming biodegradation. Finally, XRD investigation proved that degradation process of oxidized oxium and HDPE microplastics by treatment with *D. salina* decreased the crystallinity of both types of microplastics. However, the most significant reduction in crystallinity was observed in oxidized HDPE when comparing the crystallinity before and after treatment with *D. salina*. The results of all investigations indicate that microalgae can contribute to the bio-decomposition process of microplastics with the help of reducing microorganisms. However, microplastics themselves have a negative impact on the inhibition of microalgae growth owing to the high concentrations of carbon and pollutants adsorbed onto microalgae cells. We suggest that the pre-treatment of the HDPE oxidation process using hydrogen peroxide must be further studied, particularly its oxidizing ability and impact on microalgae, using the results of this study as a reference for comparison.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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