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Polyethylene Terephthalate Degradation by Microalga *Chlorella vulgaris* Along with Pretreatment

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Abstract: *The current research explored the potential of microalgal species Chlorella vulgaris and Pretreatment to remediate plastic waste. It was concluded from the results that Pretreatment had a marked effect on the cracking and alteration of plastic polymer, which helped to grow microbial species on the cracked surface as evident by Compound Microscopy (CM), Scanning Electron Microscopy (SEM), and Fourier Transformed Infrared Spectroscopy (FTIR) analysis. FTIR data also supported the notion that in the absence of any pretreatment, the microbial species were not able to mediate plastic biodegradation efficiently as the nature of functional groups was different in the presence and absence of Pretreatment. GCMS analysis revealed that the microbial specie could produce the biodegradation products which were likely to be found in the structure of PET, including alkanes ester, fatty acids, benzoic acid, and aromatics and the most toxic product of biodegradation is Bis (2-Ethyl hexyl phthalate), which is the biodegradation product of toxic ingredient of plastics that is phthalic acid.*

Keywords: *Polyethylene Terephthalate, degradation, Chlorella vulgaris, microbial remediation*

1. Introduction

Polyethylene terephthalate (PET) is semi-crystalline thermoplastic polyester. Several different companies produce it under separate trade names. It may also be referred to by the brand names Terylene in the UK, Lavsan in Russia and the former Soviet Union, and Dacron in the US PET is strong and durable, chemically and thermally stable, has low gas permeability, and is easy to handle and manage. This combined effect of properties makes PET a valuable product for various applications and a considerable global plastic usage [1-3]. More than 50% of the synthetic fabrics generated globally consist of PET, and global usage of PET has been reported to exceed $17 billion per year. PET is mainly used as sheets and film, fabrics and more precisely, is used in the food and beverage packaging (especially soft drinks and water bottles), appliances, auto parts, home appliances, lighting products, power tools, sports good, photography devices, X-ray sheets and textiles based on the actual use and the desired properties, PET can be manufactured to specifications by controlling the polymerization conditions [4-12].

Plastic consumption is increasing day by day, and plastic pollution has amplified manifold. About 100 million tons of plastics are manufactured around the world every year. It causes adverse impacts to the environment because of its recalcitrant nature. The accumulation of plastic in the water bodies is considered the central problem and may undergo mechanical and chemical breakdown, causing water pollution and a threat to aquatic life. Its direct contact with the ultraviolet rays from sunlight and disintegration by the ocean waves; breaks plastic into more toxic form (i.e., micro and nano form), which can quickly enter the food chain and cause environmental as well as health problems. The studies suggest that the plastic present in the oceans is challenging to degrade due to environmental conditions (i.e., exposure to UV radiations and its conversion into micro and nano form). The high-density polyethylene

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usually sinks into the water, undergoing torpid degradation and changing into microplastics, causing a massive threat to marine biota, while the low density like polyethylene and polypropylene floats on water surface [13-20].

Degradation of these polymers into monomers can be done as a result of any physical and chemical changes. In the degradation process, many procedures are involved. The plastic degradation includes all processes (either natural or synthetic) accounting for changing plastic properties. Depending upon composition and type, the degradation time of plastics varies like plastic bottles require 400 To 500 years, and grocery bags require 10 to 1000 years to degrade. Plastics can be degraded by several physical, chemical, and biological methods. Several procedures like chemical, photo, and thermal degradation can be used for enhancing biodegradation of polythene. Ecological factors such as humidity, pH, temperature, salinity, aerobic, anaerobic conditions, sunlight, water, pressure, and plant conditions not only affect the degradation of polymers but also have a critical impact on the microbial population and enzymatic activity. Carbon and energy sources for microorganisms like bacteria and fungi include polymeric substances [21-26].

Biodegradation can be influenced by the molecular weight of the polymer, type of microbe used, biofilm formation, polymer addition, solar radiation, geographic location, time of the day and year, ozone, the intensity of radiation, presence of nutrients and exoenzymes. Biodegradation involves the initial breakdown of polymers into simple biodegradation products, which degrade into biomass, carbon dioxide, and methane by anaerobic microbes. This is a comparatively more suitable method than physical and chemical degradation alone. It has the least hazardous impacts on the environment and has relative.ly efficient degradation potential and is an environmentally friendly technique but has not been practically applied at commercial scale. In our natural environment, the biodegradation process is performed by different microbes, but their polymer consumption rate is slow. Some microbial strains have the potential to degrade the plastic. Thus, such strains can be employed degradation of polymer by providing an appropriately controlled environment [27-29].

It is a common observation that plastic bags or other plastic materials are carried to the aquatic environment and remain as it is for a very long duration. Personal observations by the corresponding author revealed that many aquatic microalgal species could attack such plastic materials. Upon literature review, it was revealed that microalgal species, mainly *Chlorella vulgaris* has not been investigated to degrade the plastic materials so far. Being PET, a high-density polymer with inherent toxic properties takes a very long time to be degraded naturally. The PET was chosen to study biodegradation by integrated physicochemical and microalgal treatment to devise a low-cost biodegradation strategy.

Previous researches were focused on the physical, chemical or biological treatment of plastic waste alone. A combination of the physicochemical treatment for plastic degradation has not been applied along with biological treatment by *C. vulgaris*. The current research was designed to explore the potential of cohesive methods for remediation of plastic waste by *C. vulgaris*. The purpose of this study was to explore the role of *C. vulgaris* in plastic (Polyethylene Terephthalate, i.e., PET degradation along with physicochemical methods.

2. Materials and methods

2.1. Substrate preparation

Polyethylene Terephthalate (PET) is an ordinary high-density plastic - the most common thermo-plastic polymer resin of the polyester family. It is used in fibers for clothing, containers for liquids and foods, thermoforming for manufacturing, and in combination with glass fiber for engineering resins. PET bottles were cut in small strips (uniform length and width, i.e., 2 x 2 cm) using sterile scissors and were transferred into a sterile beaker with distilled water and stirred for 1 h. Further, they were aseptically placed to ethanol solution 70% v/v for 30 min. Then, the polyethylene films were transferred to a sterile Petri dish. Finally, the plastic strips were air-dried and were weighed in fixed mass [26, 29-34].

2.2. Pretreatment

Plastic films were pretreated by three processes: physical treatment (ultraviolet and temperature, i.e., 100ºC for 48 h) and chemical pretreatment (90% concentrated nitric acid, for five days at ambient temperature). No pretreatment was provided for control, in order to compare the degradation percentage.

2.3. Biological treatment

Microalgae were isolated and identified morphologically in the laboratory; the most dominant group of microalgae were selected for the biological treatment of Polythene terephthalate (PET). Algal samples were taken to the laboratory and were analyzed using the trinocular microscope Euromax (Euromex Microscope Spain), and the photomicrographs were also taken from the attached digital camera. The most dominant microalgae were Chlorella (single-celled green algae) belonging to the division Chlorophyta. They were further isolated and grown using BG11 (blue-green algae) media. BG11 medium (1500 mgL^{-1} NaNO3; 40 mgL^{-1} $K_2HPO_4.3H_2O$, 75 mgL^{-1} $MgSO_4.7H_2O$, 36 mgL^{-1} $CaCl_2.2H_2O$, 6 mgL^{-1} citric acid, 6mgL^{-1} Ferric ammonium citrate, 1 mgL^{-1} EDTA, 20 mg L^{-1} Na_2CO_3, 1mLL^{-1} A5 + Co solution. The A5 + Co solution (per 1000 mL) is composed of 286 mg H_3BO_3, 181 mg $MnCl_2.4H_2O$, 22 mg $ZnSO_4.7H_2O$, 39 mg $Na_2MoO_4.2H_2O$, 7.9 mg $CuSO_4.5H_2O$ and 4.9 mg $Co(NO_3)_2.6H_2O$, PH 7.0+0.5).

The *p*H was measured after treatment using a pH meter. PE films were measured for their initial weight and weight after 30 days of incubation. Weight loss in percentage was calculated and compared with control after an incubation period of 30 days as below:

$$\text{Weight loss (\%)} = ((\text{Weight loss}) / (\text{Initial weight})) \times 100 \quad (1)$$

2.4. Experimental treatment setup

Plastic films were treated with microalgae in two different setups. In one experiment, physical and chemical were given to plastic films and then treated with both the cultures while in another experiment, no physical or chemical treatment was given to plastic before biological treatment with microalgae. Three processes pretreated degradation with Pretreatment Plastic films: physical treatment (ultraviolet and temperature, i.e., 100 ºC for 48 h) and chemical pretreatment (60% concentrated nitric acid, for five days at ambient temperature). About 800 mL of culture media were prepared in 1000 mL flasks and transferred to 50 mL flask; each was autoclaved at 121°C, 15 psi for 15 min. The selected microalgal culture (Chlorella) was inoculated 2 mL) to their respective culture media and incubated under light illumination for 1 month at room temperature. The degradation percentage of polyethylene films after biological treatment was determined gravimetrically and recorded. The average weight of 2 x 2 cm PET films was measured before treatment. After the biological treatment, the algal mass was gently wiped off and dried and then weighed gravimetrically to analyze the percentage of degradation. Samples were analyzed through scanning electron micrographs (SEM) and Fourier-transform infrared spectroscopy (FTIR).

2.5. Degradation without pretreatment

In this experimental setup, no pretreatment was provided to compare the degradation percentage with and without Pretreatment.

2.6. Biological treatment of polyethylene Terephthalate films

Polyethylene Terephthalate bottles were chosen for the biological treatment and are then trimmed to (uniform length and width, i.e., 2 x 2 cm) and were transferred into a sterile beaker with distilled water and stirred for one h. Polyethylene films were used to treat biologically and separately with microalgae and macroalgal culture using Blue-green algae bg11 media and placed in a photobioreactor for one month.

3. Results and discussions

3.1. Algal identification

The cells of microalgae were identified as *Chlorella vulgaris* and were presented in Figures 1 and 2. Figure 2 also shows the *C. vulgaris* cells growing on plastic.

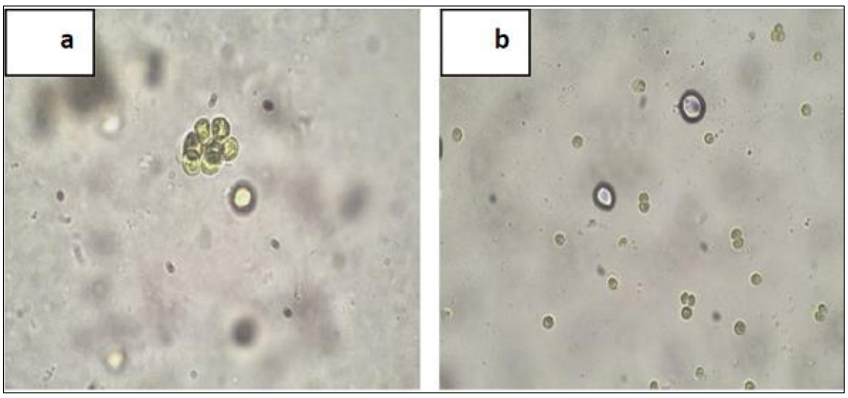

Figure 1. Identification of microalgae (Chlorella) by compound microscopy (a and b)

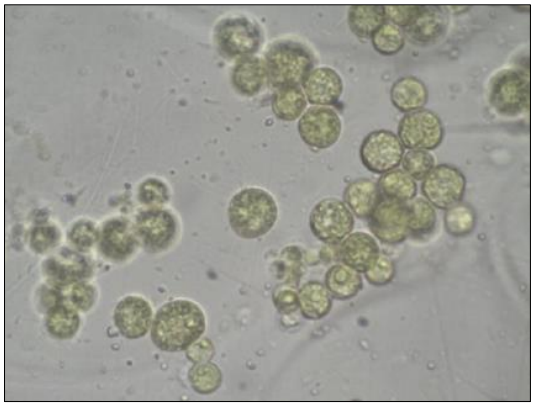

Figure 2. Visual observation of microalgal (*Chlorella vulgaris*) and physicochemical treatment of plastic film by compound microscopy

The effect of biodegradation using microalgae species *Chlorella vulgaris* accompanied by the Pretreatment of plastics with ultraviolet rays' temperature and nitric acid have been presented in Figure 3. It was evident that the overall percentage of biodegradation has not such a difference that is 5.57%, as compared to 5.45% without giving any pretreatment (Tables 1 and 2). However, providing Pretreatment caused some damage to the structure of plastics, which assisted algal specie to make biofilm and degrade plastic more efficiently. Microscopic image analysis supports this observation, and FTIR analysis has been presented (Figure 4).

Table 1. Microalgae with Pretreatment

Microalgae	Days of treatment	Initial wt	Final wt	% weight loss
Chlorella vulgaris	30	0.4984	0.4427	5.57
Control (no microbe)	30	0.4533	0.4533	0%

Table 2. Microalgae without Pretreatment

Microalgae	Days of treatment	Initial wt	Final wt	% weight loss
Chlorella vulgaris	30	0.4397	0.4157	5.45
Control (no microbe)	30	0.4533	0.4533	0%

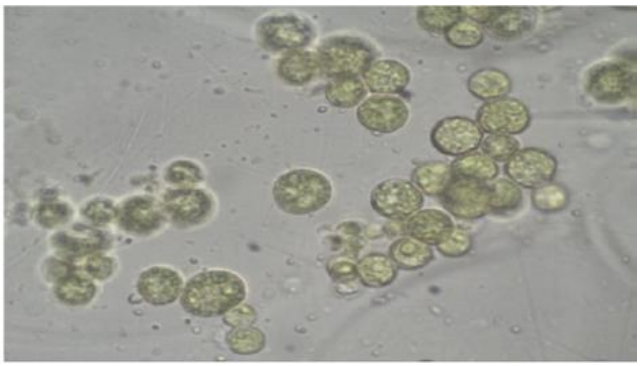

Figure 3. Visual observation of microalgal (*Chlorella vulgaris*) and physicochemical treatment of plastic film by compound microscopy at 100X magnification

3.2. FTIR analysis

In the Control sample, there is the presence of carboxylic acid, Sulfonyl chloride, aromatic amines, and aromatic esters. FTIR Analysis showed that the PET had various functional groups in 1340 to 1370 wavelength cm^{-1}. PET sample treated by Bacillus spp. R29 showed that the maximum functional group was lying between the wavelength range of 3750 to 4000 cm^{-1}, which were possibly primary amines, secondary amines, and carboxylic acids compared with control. Figure 4 describes the FTIR result of PET sample treated with *Chlorella vulgaris* after pretreatment. It was evident that the pretreatment had very prominent effect on biodegradation by *Chlorella vulgaris*. There was a big peak area between 2800 to 4000 cm^{-1} wavelength representing primary amines secondary amines and carboxylic acids. A number of peaks were observed at wavelength range of 1500 to 2000 cm^{-1} suggesting the presence of aromatic anhydrides, Conjugated acids halides

3.3. GCMS Analysis

The products of biodegradation by using *C. vulgaris* showed that the common compounds of biodegradation included Pentanoic acid, 4-methyl-, d-Galactono-1,4-lactone,5,6-O-(ethylboranediyl)-, Hexanoic acid, 1-cyclopentylethyl ester, 2-[Pentafluorophenyl]-4-[N-aziridyl]-2-butanol, 5,8,11-Heptadecatrien-1-ol, Benzenemethanol,3-hydroxy-5-methoxy-, 5,8,11-Heptadecatrien-1-ol, pentadecyl Ester, octadecyl ester, 1-Monolinoleoylglycerol trimethylsilylether.

The FTIR results of Chlorella treated PET sample without any pretreatment, also evident that the biodegradation rate was significantly less in the absence of physicochemical treatment (Figure 4) as only some peaks were evident at 3000 to 4000 cm^{-1} wavelengths.

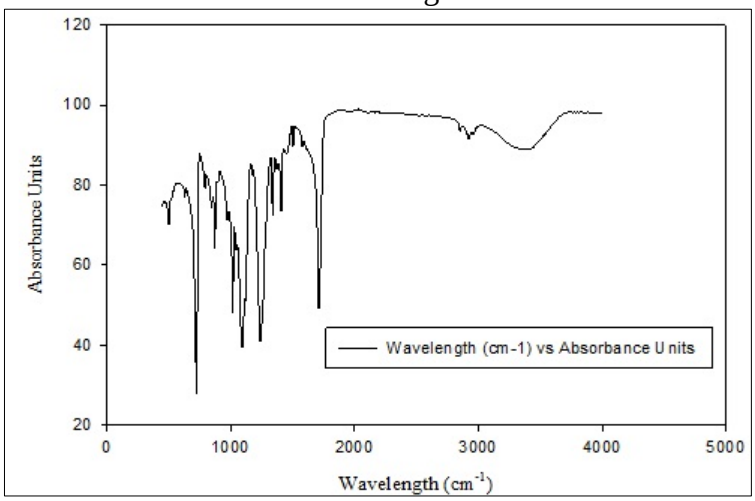

Figure 4. FTIR Analysis of Plastic Samples Treated with *Chlorella vulgaris* without Pretreatment

3.4. Scanning electron microscopy

Figure 5 (A-C) describes the scanning electron micrographs (SEM) of PET samples treated with *C. vulgaris* with or without pretreatment along control. It was evident that in control (Figure 5A) no visible cracks were evident. However, in Figure 5B, SEM showed growth of *C. vulgaris* without any visible signs of PET degradation. An interesting observation was evident in Figure 5 C in which SEM showed visible cracks and growth of *C. vulgaris*.

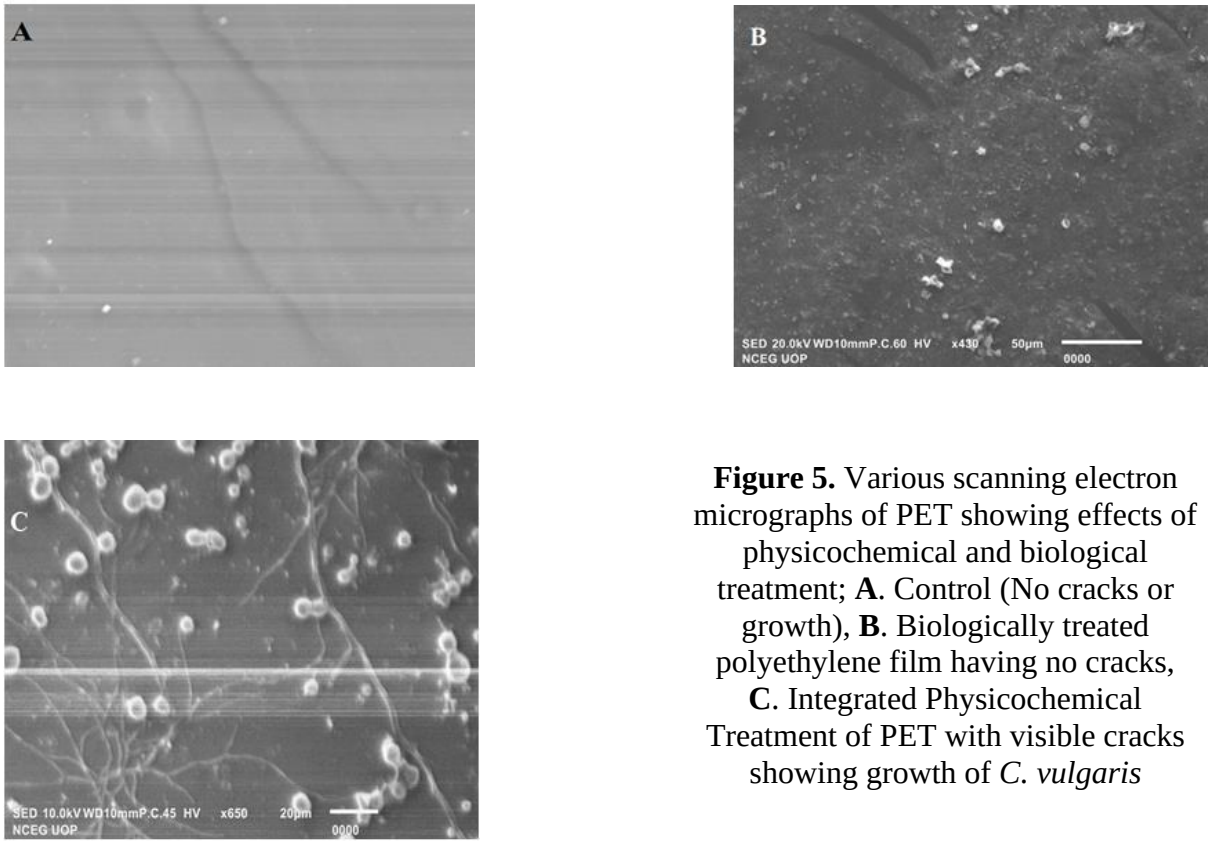

Figure 5. Various scanning electron micrographs of PET showing effects of physicochemical and biological treatment; **A**. Control (No cracks or growth), **B**. Biologically treated polyethylene film having no cracks, **C**. Integrated Physicochemical Treatment of PET with visible cracks showing growth of *C. vulgaris*

Plastic accumulation in our environment is a grave concern; its accumulation in the environment causes long-term problems to the living organism and their habitat. It destroys the natural habitat of flora and fauna, especially living in the aquatic environment [26]. Degradation of plastic mainly occurs by three processes: physical-chemical and biological degradation process [27]. The current research was aimed to compare the plastic biodegradation with or without Pretreatment. It was anticipated that physical treatment might enhance the biodegradation rate [28].

Many previous investigations also reported that providing Pretreatment enhanced the rate of biodegradation. UV, nitric acid, and temperature exposure are the best ways for physicochemical degradation. Different microorganisms like algae, fungi, and bacteria produce different chemicals like mucilaginous substances by algae, while bacteria and fungi produce laccases, hydrolases, PETases, peroxidases, lipases, which help in cleaving the polymer structure into more straightforward and available form for microbes. Laccases decompose the hydrocarbon group of polyethylene when plastics films are incubated with them; the average molecular weight of polymer declines up to 10 to 20 % as an outcome [28, 29, 35].

Microbes have a special plan for the utilization of plastic as it acts as a carbon and energy source for them. Degradation of plastic waste using a microbe is the most expedient method of degradation. It is based generally on two steps; in the first step adhering of enzyme-substrate (polyethylene) occurs, and the second involves the hydrolytic cleavage as fungus and bacteria produce intra and extracellular enzymes that further cause the degradation of polymer. Recently, it has been reported that the most

dominant microalgae were *Scenedesmus dimorphus* (Green microalga), *Anabaena spiroides* (blue-green alga), and *Navicula pupula* (Diatom). It was shown that polyethylene sheet showed the proliferation of microalgae in both outer and inner sides of the polyethylene sheet, and the erosion cum degradation was apparent [36].

The most prominent microbes that have the capability of polymer degradation are fungi and bacteria, but both have entirely different mechanisms of biodegradation, and both require different conditions for their growth. The effects of these microbes have been described on the physicochemical properties of these polymers, including changes in crystalline, molecular weight, sample topography, and functional groups found on the polythene layer. Several scientists have demonstrated the biodegradation of polyethylene, the enzymes involved, and the mechanisms associated with these phenomena remain unclear [37].

The effect of biodegradation using lichens accompanied by the Pretreatment of plastics with ultraviolet ray's temperature and nitric acid showed that the overall percentage of biodegradation was somehow more significant 6.78%, as compared to 0.31% without giving any pretreatment. It is suggested that providing Pretreatment cause some damage to the structure of plastics, which helped bacterial specie to make biofilm and degrade plastic more efficiently [34-37].

It was evident that the microbial species produced biofilms on the surface of PET plastic during the current study and the growth of microbes on plastic films was efficient when provided with Pretreatment. It was evident from compound microscopy that microbes could grow on cracks and fishers created during Pretreatment. That is why smooth surfaces of plastic without any pretreatment were not suitable for the growth of microbes [38-44].

There are very few reports on the biological degradation of PET or its utilization to support microbial growth. Regarding PET degradation by microalgae, a recent report has shown that diatoms (A group of microalgae) can degrade PET by producing the enzyme PETase under mesophilic marine conditions. Another aspect of plastic biodegradation is the development of efficient closed- or open-loop recycling strategies for TPA (and EG) to synthesize new PET from its degradation products up to the point of further metabolic engineering of the microalgal metabolism in order to generate cells capable of completely metabolizing PET and use it as a carbon source [45]. The same authors suggested that the enzymes like PETase produce can efficiently biodegrade physically treated PET by microalgae like diatoms. Once an enzyme-like PETase or laccase has initiated the PET degradation. It is speculated that its byproducts can be further acted upon by some other enzymes produced by other microbial consortia present in the same environment [45-54].

3.5. Products of microbial degraded/treated plastics

The literature demonstrates that CO_2 gas is a significant product released during the biodegradation of polythene. The generation of aldehydes, ketones, and carboxylic acids was reported in LDPE film extrusion smoke in the extrusion coating. *Rhodococcus ruber* (C208) generated polysaccharides and proteins using polythene as a carbon source in another study, *Rhodococcus ruber* ATCC29672 (Bacterium) and Clados produced polysaccharides and proteins. *Rhodococcus ruber* (C208) formed polysaccharides and proteins using polythene as a carbon source [55].

In another study, *Rhodococcus rhodochrous* ATCC29672 (Bacterium) and *Cladosporium cladosporoid* ATCC 20251 (Fungus) utilized polyethene'sq to generate polysaccharides and proteins, while *Nocardia asteroids* GK911 (Bacterium) generated proteins only [56, 57].

4. Conclusions

It was concluded from the result; Pretreatment had a marked effect on the cracking and alteration of plastic polymer, which helped to grow microbial species on cracked surfaces, as evident by Compound microscopy Scanning electron microscopy FTIR analysis. FTIR data also supported the notion that in the absence of any pretreatment, the algal species were not able to biodegrade plastic structure efficiently as the nature of the functional group was different in the presence and absence of Pretreatment. GCMS

analysis revealed that the microbial specie could produce biodegradation products that were likely to be found in the structure of PET. More prolonged biodegradation investigation on plastic using selected microbes should be carried out to understand the full mechanism of plastics degradation over a more extended period.

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