Identification, Quantification and Biodegradation of Microplastics in Aquatic Environment by Metabolism of Microorganisms

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Abstract

Microplastics pollution in aquatic environment has become a global environmental problem. However, technologies for identification, quantification and especially for efficient degradation of microplastics remain largely underdeveloped. This work presents a protocol which can identify and biodegrade microplastics based on the metabolizing of microorganism. Raman spectroscopy and FTIR spectrometry identify the main substance of the separated microplastics is polyolefin. CLSM images stained with Nile red reveal the shape and size of the microplastics. EDS elemental maps suggest the C/O ratio in the microplastics. The microorganism used without domestication by microplastics exhibit an efficient performance in degrading of microplastics by metabolizing. The microorganism grew and inhabited on or in the microplastics, and then metabolized them. 0.22 g of microplastics on the filter paper (d=50mm) can be degraded completely after 33 days.

1 Introduction

Microplastics are plastic debris with an effective diameter less than 5 mm[1], which have raised large attention due to their widespread existence in aquatic environment [2]especially in the ocean[3–5]. Microplastics are composed of primary microplastics and secondary microplastics. It has been estimated that most of primary microplastics are originating from rinse-off of household cosmetic products such as mildy wash, eye cream and hand cream. Secondary microplastics are generated through breakdown of macroplastic fragmentation[6–8].

In recent years, Microplastics can be detected frequently in aquatic organisms and the corresponding senior predators[9, 10] in aquatic and terrestrial systems which are feed of them leading to false satiety and pathological stress, reducing growth and reproductive rate[11]. Studies have shown that microplastics with smaller size are more easily ingested by organisms and will express more toxic effects on the organisms[12, 13]. Therefore, quantification and monitoring of microplastics contamination in the environment is currently required in the main economic entity.

However, technologies for quantification especially for efficient degradation of microplastics with reliable, reproducible, rapid, and inexpensive methods are still in the infancy[14–17]. Microplastics, as a new contaminant, are difficult to be quantified due to a lack of methods which are sensitive and allow high-throughput quantification[18] as well as be removed by traditional sewage treatment technology[19]. Accordingly, it is of vital significance to investigate methods on characterization, quantification and removal of microplastics in aquatic environment.

Under natural conditions, microplastics can persist in aquatic environment up to decades since they can resist degradation. However, it is interesting to find that microplastics can be decomposed by some kind of microorganisms[20–22]. Microorganisms own an inherent and excellent ability to adapt to varied environmental conditions[23, 24] and possess the potential to decompose various compounds to synthesize their necessary materials for survival and growth[25, 26]. More importantly, using indigenous

microorganisms to biodegrade microplastics will do no harm to the original environment[27–29]. Therefore, Microorganisms are promising candidates for degradation of microplastics. However, with our current state of knowledge, few microorganisms have been isolated for this application[30], and the interactions between microorganisms and MPs still not known.

In this work, we utilize Raman spectroscopy and FTIR spectrometry to identify the separated microplastics, CLSM images stained with Nile red to reveal their shape and size, EDS elemental maps to suggest the C/O ratio in them. Then, we separated the microorganism from soil and cultivated several generations. The species of microorganism in soil are similar to those in water due to the great exchange of substance between soil and water. We found that the microorganism separated showed a good ability of biological degradation on microplastics without domestication by the microplastics in advance.

2 Materials And Methods

2.1. Chemicals and reagents

Microorganism Medium: Sodium nitrate-N($NaNO_3$), citric acid($C_6H_5Na_3O_7$), sodium phosphate dibasic(Na_2HPO_4), potassium phosphate monbasic (KH_2PO_4), magnesium sulfate($MgSO_4$). Ethylenediaminetetraacetic acid(EDTA), zincsulfate($ZnSO_4$), calcium chloride($CaCl_2$), manganese chloride($MnCl_2$), iron(□) sulfate ($FeSO_4$), copper sulfate($CuSO_4$), cobalt chloride($CoCl_2$).

2.2 Separation of the microplastics

The microplastics studied in this work were acquired from mildy wash. 2 g of Mildy wash was dissolved into 200 ml purified water, stirred 24 h and then filtrated using 1μ filter membrane.

2.3. Characterizations of the microplastics

Scanning electron microscopy (SEM) and Energy dispersive spectrometer (EDS) images were obtained using Gemini 500. Fourier transform infrared spectrometry (FTIR) was characterized using a Nicolet 6700. Confocal laser scanning microscope (CLSM) was obtained using TCS SP8 Bright green fluorescence. Raman spectra were characterized using XploRA PLUS.

3 Results And Discussion

3.1 Identification of the separated microplastics

3.1.1 Raman spectra

The Raman spectra of the standard microplastics and the separated microplastic were shown in Fig. 1. It is found that the separated microplastic exhibits nearly the same peaks with the standard microplastics. The two peaks at about 2846 and 2881 cm^{-1} can be identified as stretching vibrations of CH_2 and peaks

at 1293 and 1438 cm^{-1} are the bending vibrations of CH_2. Furthermore, stretching vibrations of C-C at 1060 and 1127 cm^{-1}, and stretching vibrations of C=O at 1734 cm^{-1} are distinct. Therefore, based on the Raman spectra, the separated microplastic can be identified as polyolefin preliminarily.

3.1.2 FTIR spectra

The result was further verified by FTIR spectrum shown in Fig. 2A. The intense peaks at 2800 cm^{-1}-3000 cm^{-1} indicate stretching vibrations of CH_2, the peaks at 1680-1800 cm^{-1} are stretching vibrations of C=O and the broad peak at 1010 is stretching vibrations of C-O. The existence of C□C can be confirmed by the peaks at about 1640 cm^{-1} and wag vibrations at 874 and 911 cm^{-1}. Fig. 2B shows the FTIR spectra of separated microplastic with different filtration volume (10, 30 and 50 ml) of mildy wash, which indicate that intensity of the peaks at 2800 cm^{-1}-3000 cm^{-1} are enhanced with the increase of filtration volume confirming that quantity of microplastic increased with filtration volume. The result verified that the separated microplastic can be identified as polyolefin.

3.1.3 Confocal laser scanning microscope (CLSM)

Confocal laser fluorescence microscope has been proved as an effective tool for characterizing microplastics using Nile red as fluorescently label[31, 32]. Fig. 3(A, B) shows the confocal laser fluorescence microscope images of the microplastics' fragment stained with Nile red at green fluorescence (excitation/emission 460/525 nm). Fig. 3(C, D, E) give the three versions of the same microscope field of the microplastics'pile: C: bright field images, D: fluorescent image at green fluorescence and E: fluorescent image at red fluorescence (excitation/emission 565/630 nm). Based on those, it is found that the microplastics appear irregular shape and the fragments of the microplastics are in micron scale. Furthermore, we found that green fluorescence is the most effective one to label this kind of microplastics.

3.1.4 SEM images and EDS elemental maps

Fig. 4(A, B) shows the SEM images of the microplastics indicating the surface is relatively flat and smooth like the surface of standard plastics[33]. In addition, EDS was employed to give the elemental composition of the microplastics. Fig. 4 (C, D) are the EDS elemental maps of the microplastics; Fig. 4E is the C map and Fig. 4F is the O map. It is found that C and O element are in uniform distribution. Fig. 5 gives the signal intensity of C and O element relatively. The intensity of C element is 580 cps/ev and that of O element is 45 cps/ev. Therefore, the C/O ratios of the microplastics corresponds with that of the standard plastics[18, 33].

3.1.5 The density

The quality increment of the microplastics with the filtrating volume of mildy wash from 5 to 50 ml was shown in Fig. 6. We measured the thickness of the microplastics by filtrating each 10 ml on the filter membrane (d=50 mm) and calculated the volume of the microplastics. Then, we calculated the density of the microplastics being 0.95g/cm^3 which is nearly the same with that of standard PE.

3.2 Degradation of the separated microplastics by metabolism of microorganism

The microorganisms were separated from soil of Tianjin and cultivated several generations. Then, we put the filter membrane (d=50 mm) with 0.22 g microplastics into the microorganism medium to get rid of the microplastics. The whole reaction time is 33 days and it is worth mentioned that the microorganism was used without domestication by microplastics. If the microorganism were domesticated by microplastics for several generations, the whole reaction time is supposed to be shorter.

FTIR spectra of the microplastics before and after degradation were shown in Fig. 7, which indicate that the intense peaks resulting from the stretching vibrations of CH_2 at 2800 cm^{-1}-3000 cm^{-1} were weakened distinctly with the reaction time. Based on the FTIR spectra, it is found that the vibrations of CH_2 at 2800 cm^{-1}-3000 cm^{-1} are the most typical characteristic for the separated microplastics[18, 33]. It is well knowledged that the intensity of the peak in the FTIR spectra depends on the content of substance. Therefore, it is inferred that the separated microplastics was degraded greatly with time and almost completely after 33 days. From the group marked in Fig. 7, we found that the peaks of C=C, C-O and C=O arose or being enhanced with the degradation of microplastics, which means that with the vanishing of CH_2, the content of C□C, C-O and C=O were increased. It can be concluded that CH_2 has been converted to C□C, C-O and C=O by metabolizing of the microorganism (aerobic microorganism) to synthesize necessary materials for survival and growth. Furthermore, there was no CO-NH_2 in the original microplastics (0 day), while the peak of CO-NH_2 at 1750 cm^{-1} arose after degradation. The emergence of CO-NH_2 confirms that the microorganism has grown and inhabited on or in the microplastics. We tested the FTIR spectra of the microorganism medium before and after degradation shown in Fig. 8 to confirm that the vanishing of microplastics is degradation instead of dropping in the microorganism medium. The microorganism medium was filtrated using 1μ filter membrane (the same with that used in mildy wash filtration). As shown in Fig. 8, there is no typical characteristic peeks of CH_2 at 2800 cm^{-1}-3000 cm^{-1} which confirms that the vanish of microplastics is degradation instead of dropping in the microorganism medium.

Then, we tested the Raman spectra of the microplastics before and after degradation to confirm the above result as shown in Fig. 9, which present that the intense peaks resulting from the stretching vibrations of CH_2 about 2846 and 2881 cm^{-1} were weakened distinctly with the reaction time. Based on the Raman spectra, the vibrations of CH_2 at 2846 and 2881 cm^{-1} are also the most typical characteristic for the separated microplastics[18, 33]. It is well knowledged that the intensity of the peak in the Raman spectra depends on the content of substance. Accordingly, it is confirmed that the microplastics was degraded greatly along with time and almost completely after 33 days. Furthermore, from the group marked in Fig. 9, we found that the peak of C=O at 1734cm^{-1} arose with the beginning of degradation but the peak of C-C at 1060 cm^{-1} was weakened. The results confirm that the microorganism (aerobic microorganism) can metabolize CH_2 and C-C to synthesize living substance consist of C=O.

Moreover, we tested the electron microscopes of the microplastics before (Fig. 10A) and after (Fig. 10B) degradation. It is obvious that there were lots of microplastics on the filter membrane before degradation, while the microorganisms have become overgrown on the filter membrane after degradation. Fig. 10C and 10D are the partial enlargement of Fig. 10A and 10B, which indicate that the microplastics were red, yellow and blue granules before degradation but which can't be found after overgrown of microorganism. The results also confirm that the microorganism can degrade microplastics.

Finally, we tested the CLSM of the microplastics stained with Nile red before and after degradation. Fig. 10 (A, B) show 3D spatial structure map and cross section map before degradation and Fig. 10 (C, D) give the comparison after degradation. It is well known that with stained by Nile red, the intensity of signals are positive correlated with the content of the microplastics. It is worth mentioned that the lipids in the microorganism can also be stained by Nile red, and therefore there are still a certain intensity of signals after degradation. However, the intensity were weakened distinctly by comparison of Fig. 10A with Fig. 10C and Fig. 10B with Fig. 10D, which verify that the microplastics has been degraded greatly.

4 Conclusion

This work investigates the removal of microplastics in aquatic environment and provides important information on identifying by series of characterizations and biodegrading using microorganism. The emergence of CO-NH_2 confirms that the microorganism has grown and inhabited on or in the microplastics and then metabolize them. The results confirm that the microorganism (aerobic microorganism) can metabolize CH_2 and C-C to synthesize living matters consist of C=C, C-O and C=O. 0.22 g of microplastics on the filter paper (d=50mm) can be degraded completely after 33 days by metabolizing of the microorganism without domestication. If the microorganism were domesticated by microplastics for several generations, the whole reaction time is supposed to be shorter. The FTIR spectra of the microorganism medium before and after degradation verify that the vanishing of microplastics is degradation instead of dropping in the microorganism medium.

Declarations

Acknowledgments

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- Ethics approval and consent to participate

Not applicable

- Consent for publication

Not applicable

- Availability of data and materials

All data generated or analysed during this study are included in this published article [and its supplementary information files].

- Competing interests

The authors declare that they have no competing interests

- Funding

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- Authors' contributions

Hongyu Liu and Huan Zhang designed the experiment. Junfu Wei analyzed the practicability. Jun Zhang, Yuchen Sun and Liwei Liu performed the experiment, and was a major contributor in writing the manuscript. All authors read and approved the final manuscript.

- Acknowledgements

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Figures

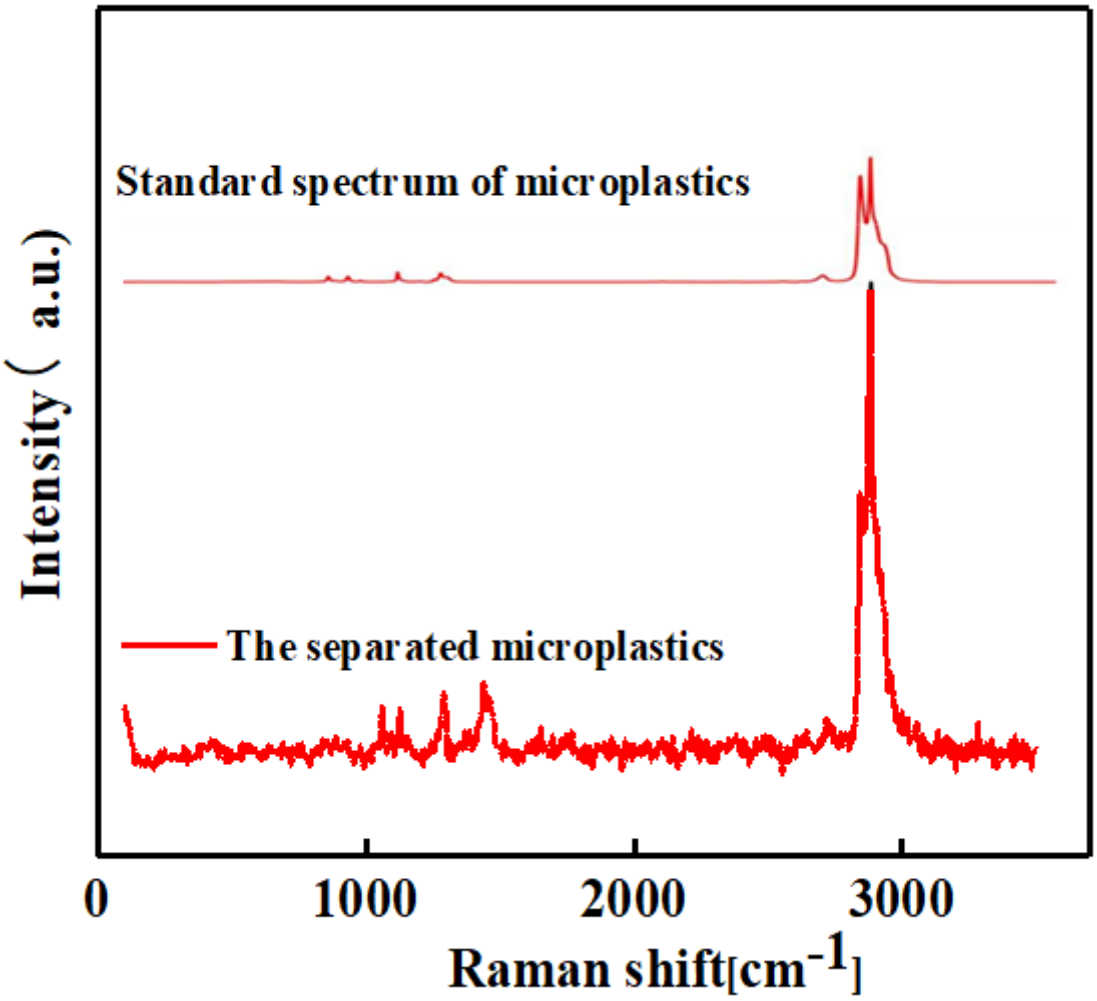

Figure 1

Raman spectra of the standard spectrum of microplastics[18] and the separated microplastics.

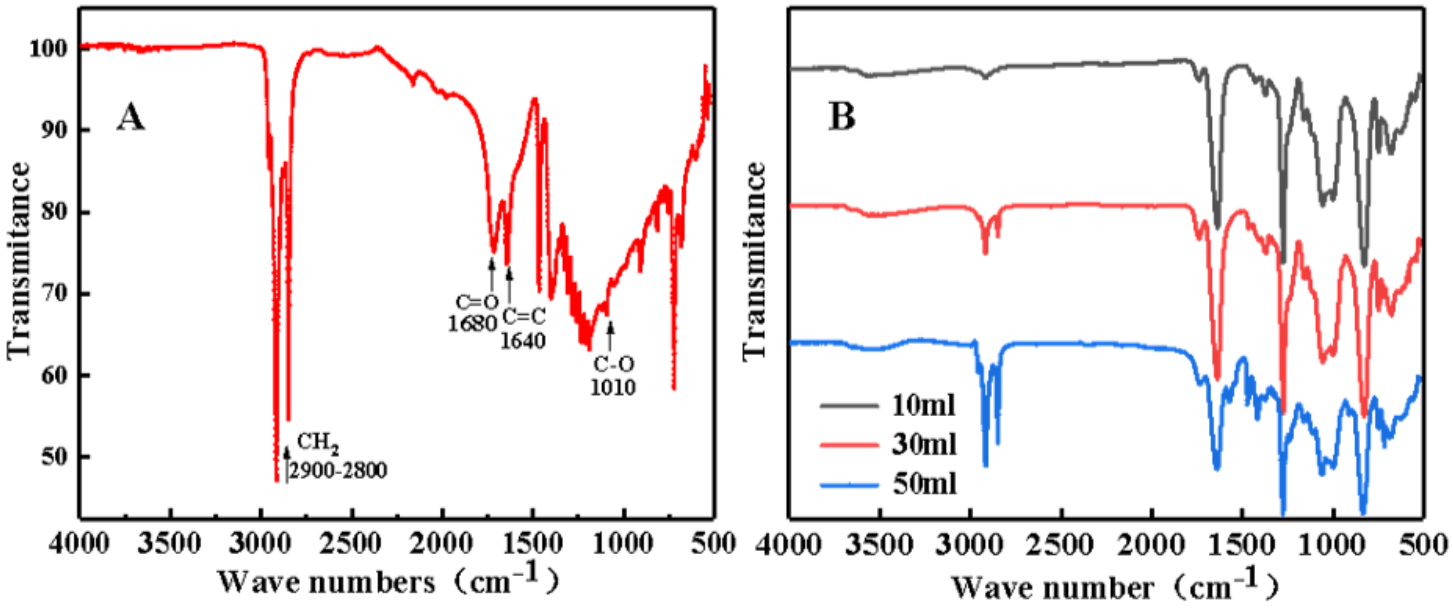

Figure 2

FTIR spectra of the separated microplastics

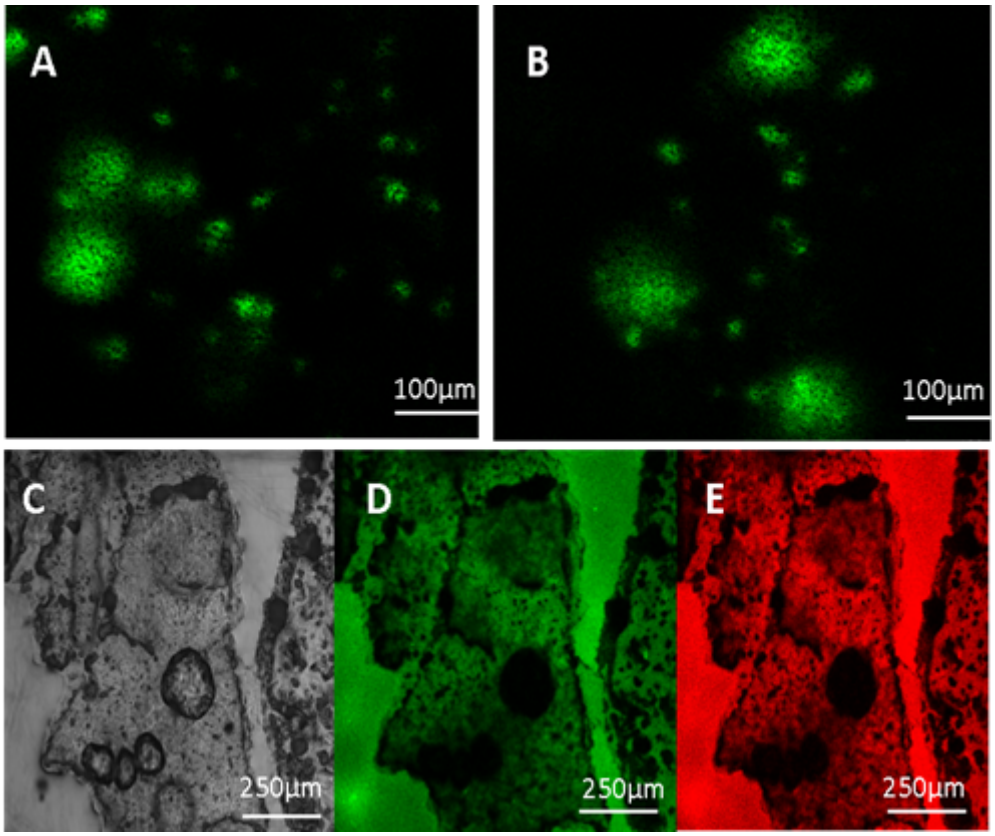

Figure 3

Confocal laser fluorescence microscope images of the separated microplastics stained with Nile red.

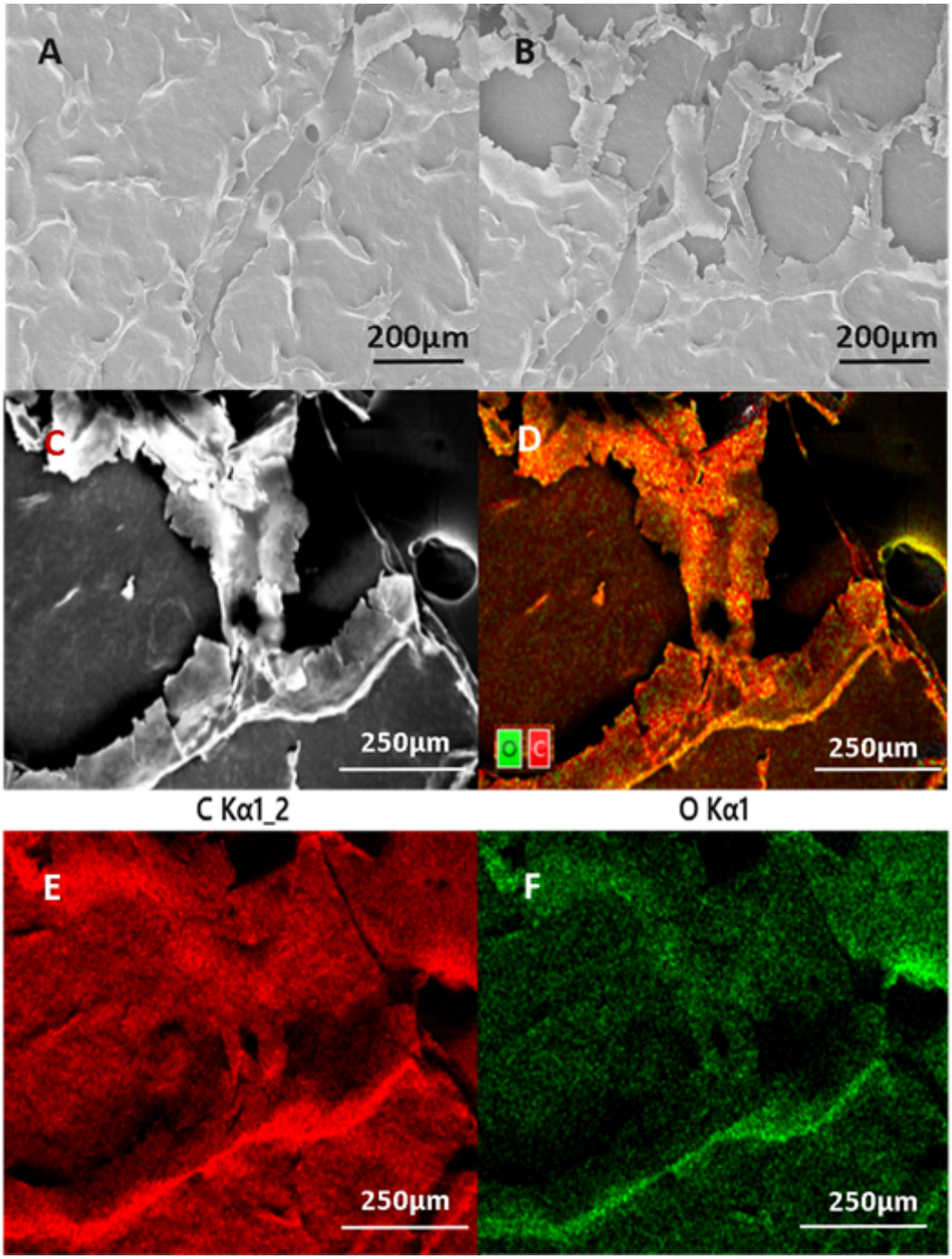

Figure 4

SEM images (A, B) and EDS elemental maps (C, D) of the microplastics, E: C map; F: O map.

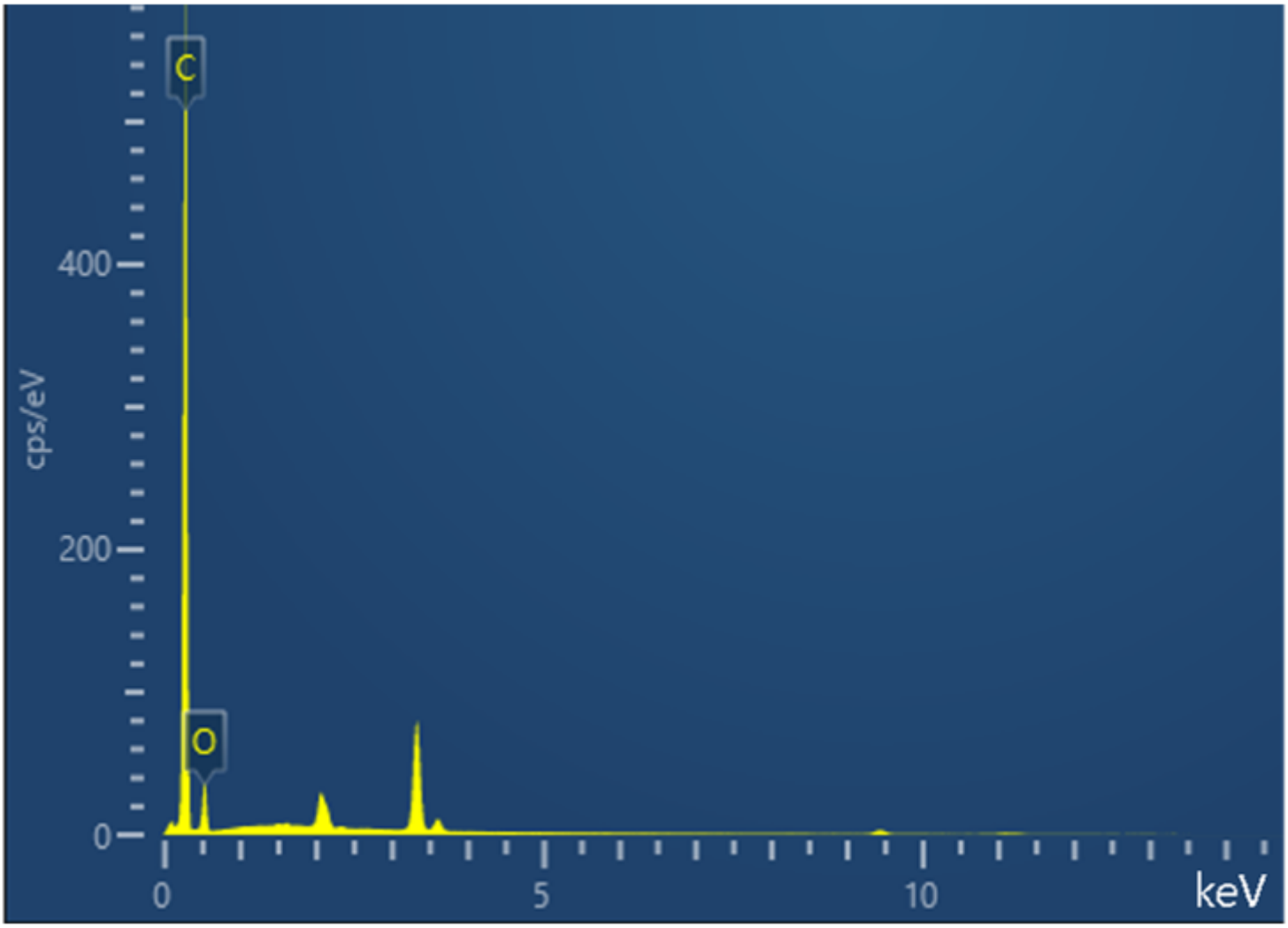

Figure 5

EDS image of C and O content of the microplastics.

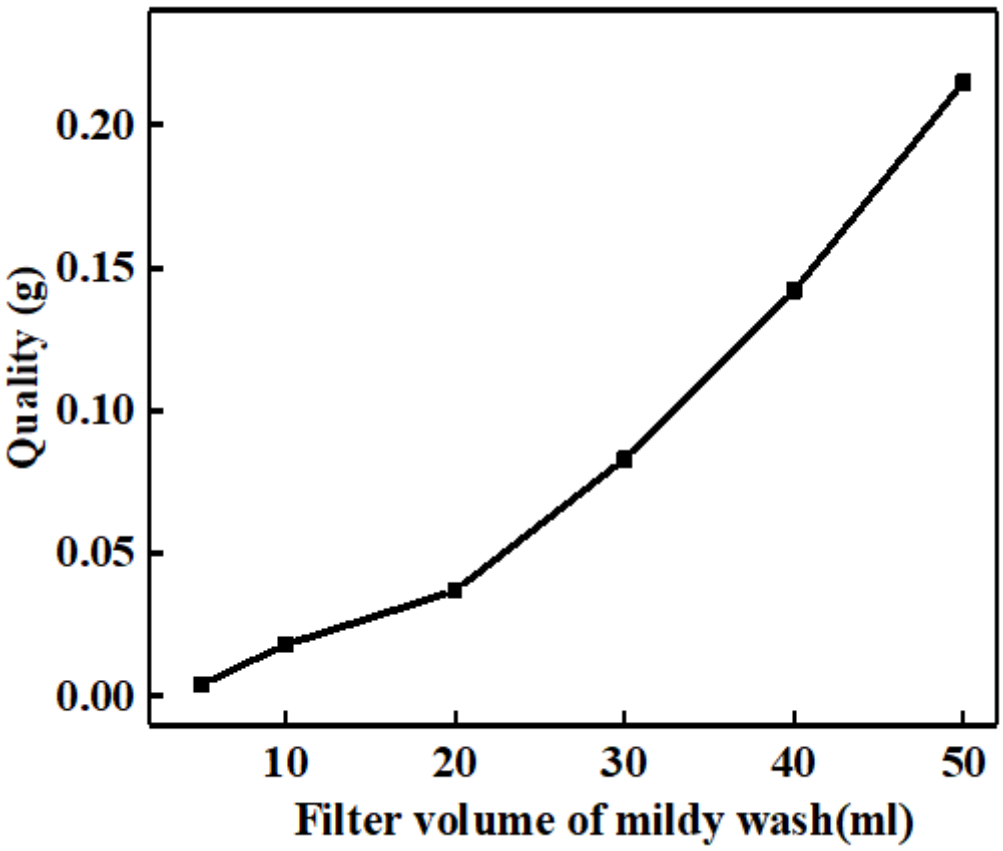

Figure 6

The quality increment of the microplastics with the filtrating volume of mildy wash.

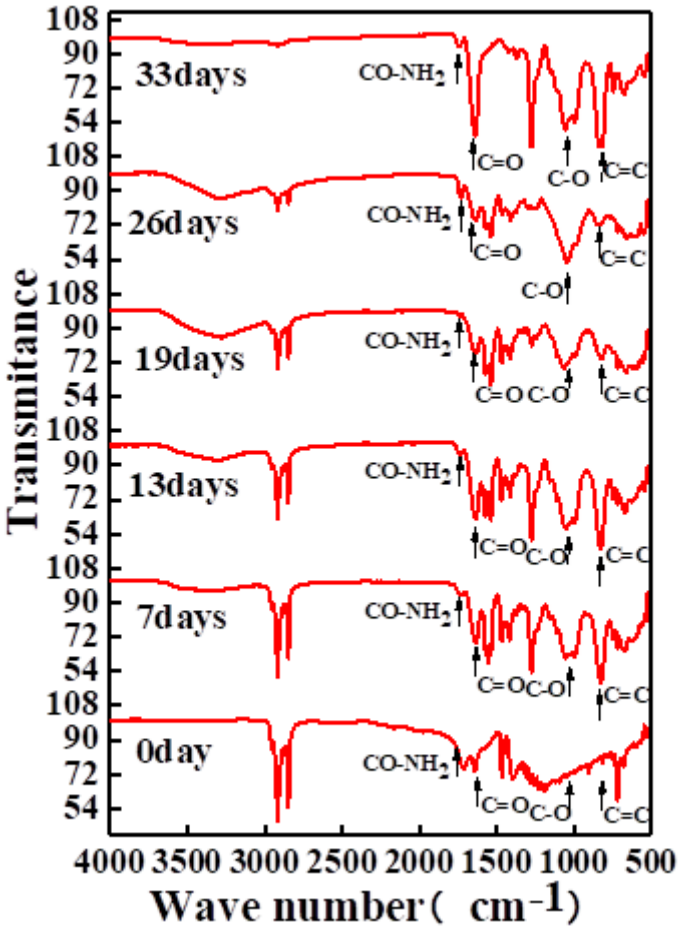

Figure 7

FTIR spectra of the microplastics before and after degradation.

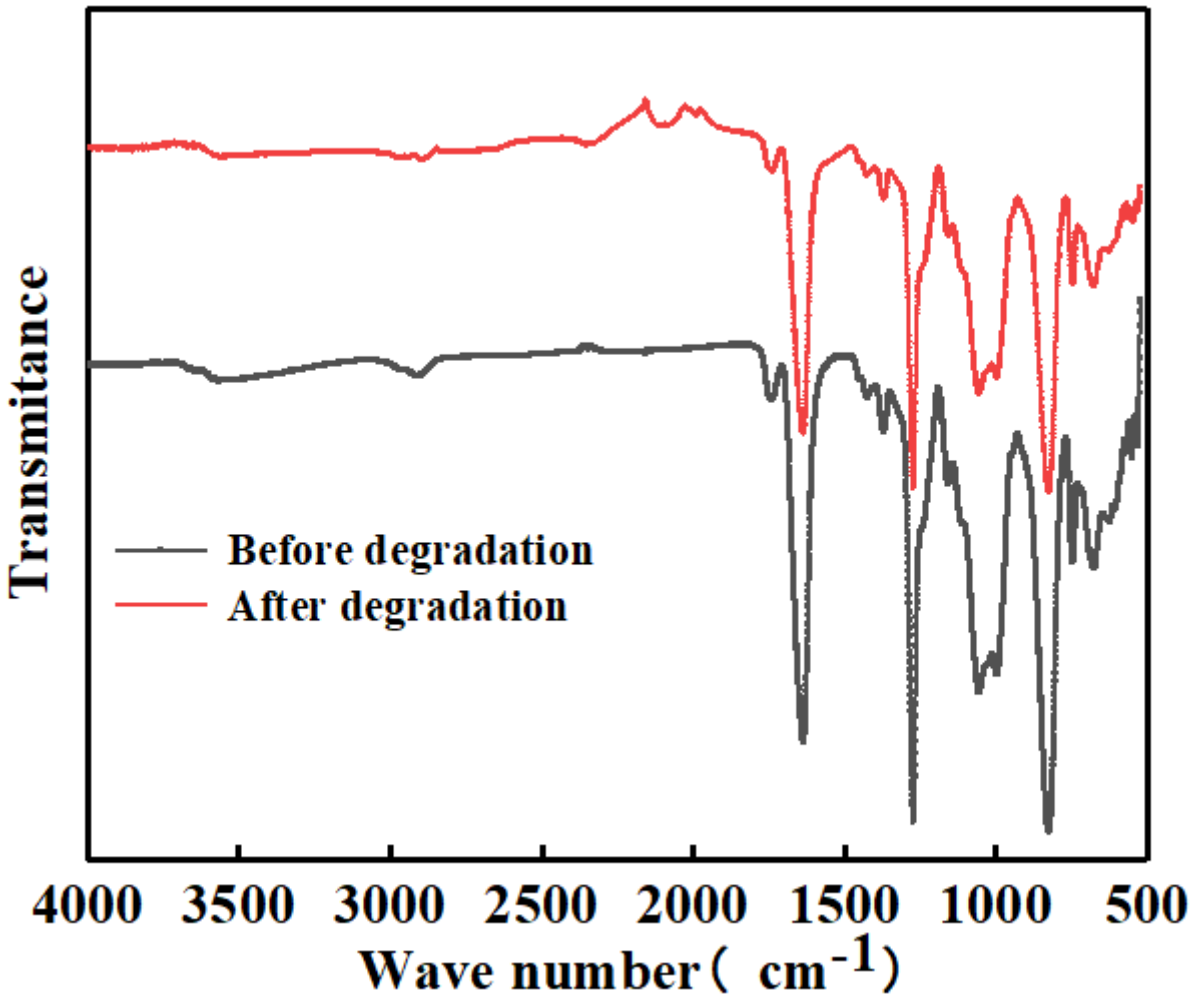

Figure 8

FTIR spectra of the microorganism medium before and after degradation.

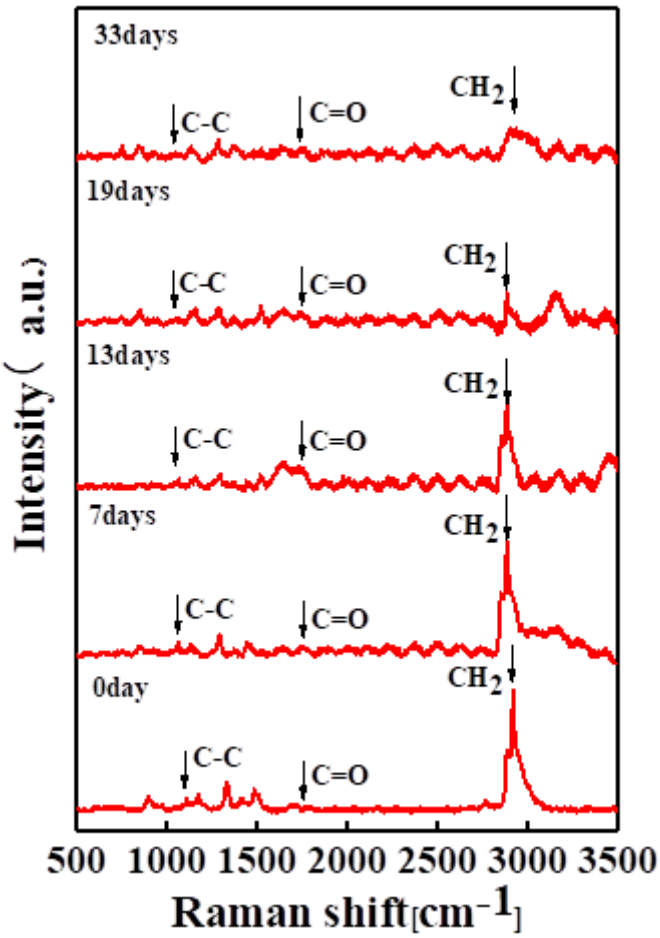

Figure 9

Raman spectra of the microplastics before and after degradation.

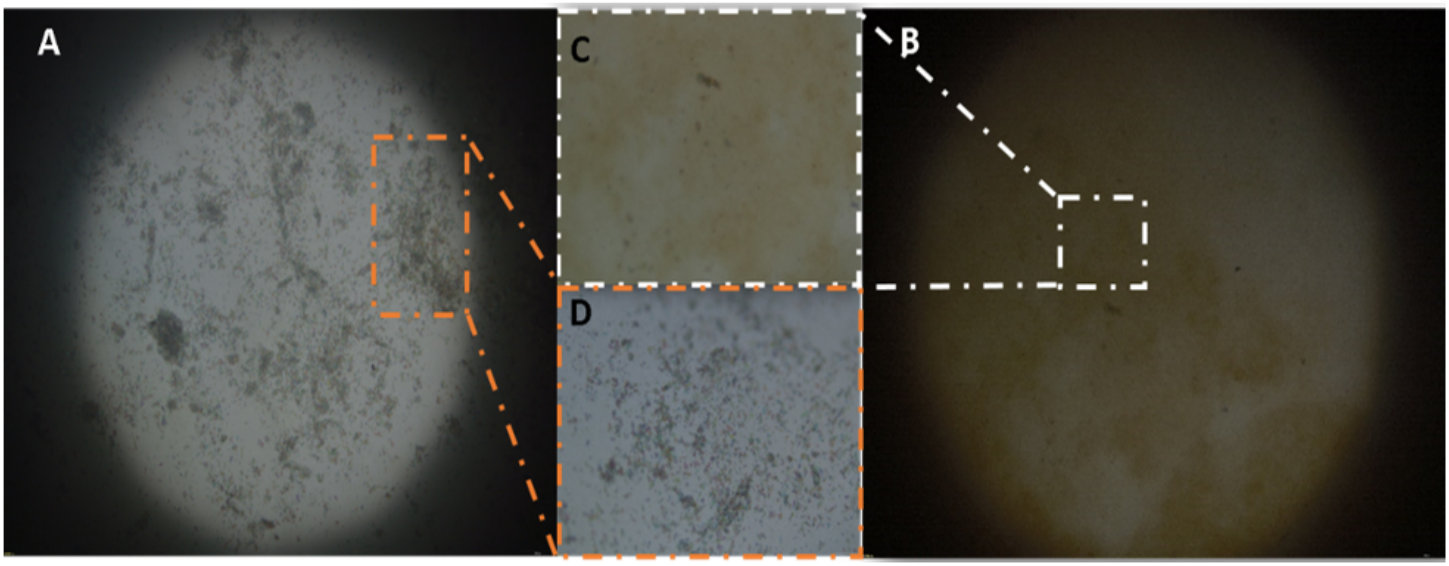

Figure 10

Electron microscopes of the microplastics before (A) and after (B) degradation.

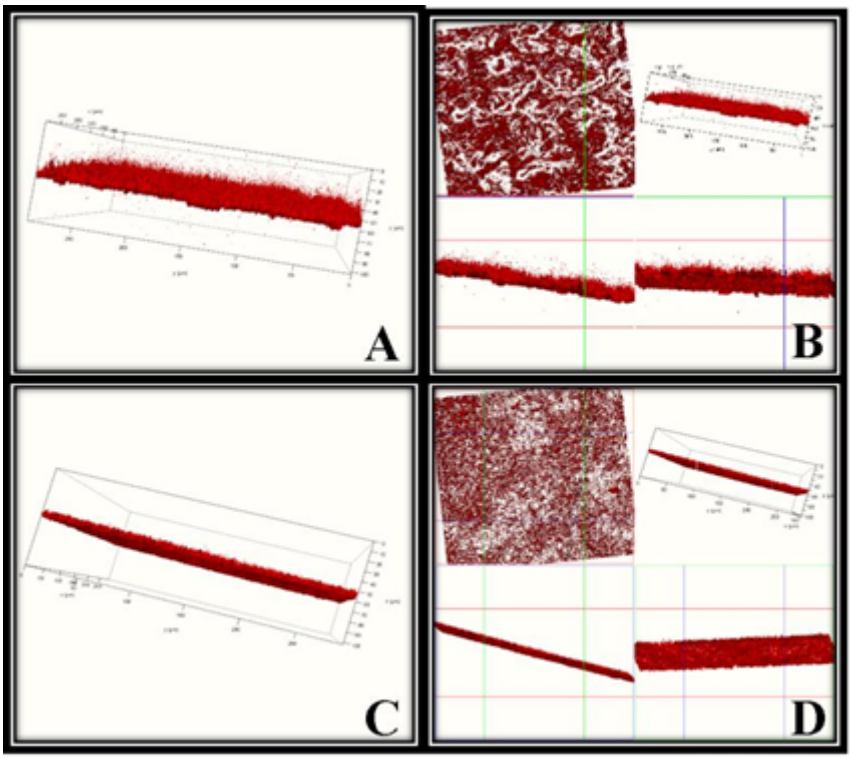

Figure 11

CLSM maps of the microplastics before (A, B) and after (C, D) degradation stained with Nile red.

Supplementary Files

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