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Reduction of Primary Microplastic in Nitrifying Medium Under Closed System

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ABSTRACT

Currently, microplastic is considered a major concern worldwide and noteworthy among the researcher and authorities. Microplastic has spread ubiquitously in the environment, particularly in the aquatic system, due to its tiny size. This microplastic is indispensable to treat since it poses hazards to marine life, human, and soil-plant. This research paper aims to investigate the performance of polyethylene (PE), polypropylene (PP), polyethylene terephthalate (PET), and polystyrene (PS) microplastic in a closed system. This microplastic has been biodegraded in the batch culture system using a colony of bacteria acquired from landfill leachate as a carbon source. The percentage of microplastic removal after the incubation period (7, 14, and 21 days) was determined. Moreover, the analysis of chemical properties, morphology surfaces of microplastic, and ammonia-nitrogen for each batch culture were evaluated. The findings revealed that all microplastic could be degraded after the incubation period. However, PE microplastic showed the highest percentage weight loss (8.8%) compared with other microplastic. Analysis by Fourier transform infrared spectroscopy demonstrates that the chemical structure of each polymer has changed, which involved the formation of C=O in PP and PE. The observation by scanning electron microscope indicated the alteration on the surface in each microplastic, such as fractures and rough surfaces. Besides that, PP microplastic indicated the maximum ammonia-nitrogen removal after 16 days incubation period (97.41%). This method can be applied in the leachate treatment system to achieve a higher quality of

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effluent. Furthermore, extending the incubation period for microplastic biodegradation can attain better optimal results in further research.

Keywords: Batch culture, biodegradation, environment, incubation, microplastic

INTRODUCTION

In 2019, the plastic generation worldwide nearly reached 370 million tonnes compared to 359 million tonnes in 2018. From the amount, the highest contributions of plastics demand are polyethylene—PE (29.8%), polypropylene—PP (9.4%), polyvinyl chloride—PVC (10%), followed by polyurethane—PUR, polyethylene terephthalate—PET, and polystyrene—PS (less than 10% for each material) (Plastics, 2020). The high requirement for plastic products in various applications has become a comprehensive environmental concern as these plastics are recalcitrant to dispose of and its non-biodegradable polymer materials. As mentioned in a previous review (Emadian et al., 2017), a large amount of plastic waste was dumped in landfills, ultimately leading to leachate and greenhouse gases production. Furthermore, the drawbacks of non-biodegradable plastic accumulation will cause carbon dioxide (CO_2) emissions over a long-time. In addition, the plastic waste that persists in the environment will result in the generation of microplastic. Recently, microplastic in the environment has become a global concern (Zhang et al., 2020).

Microplastic is a tiny plastic particle with a size of less than 5mm (Gras et al., 2021). Microplastic mainly comes from two sources which are primary and secondary. Primary microplastics are small-size particles manufactured for commercial use, such as plastic pellets used in industry, textiles, personal care products, and marine activities. Secondary microplastic emerged from fragmented large plastic items under the natural weathering process, such as plastic bags, water bottles, and fishing nets. The breakdown of larger plastic is generally due to exposure to ultraviolet radiation (UV) from sunlight and ocean waves (Emadian et al., 2017; Gras et al., 2021). This exposure can cause alterations of the physical and chemical properties of plastic materials. In addition, waste would be intricated by microplastic once this material is discharged into the environment due to its size, diversity, and wide range of properties (Belone et al., 2021). Microplastics have been discovered in freshwater systems (Li et al., 2018), coastal environments (Abayomi et al., 2017), sediment (Vaughan et al., 2017), and wastewater (Prata, 2018). In addition, this substance existed in toothpaste (Bråte et al., 2018), digestive tracts of fishes (Pegado et al., 2018), and the human placenta (Ragusa et al., 2021).

Microplastics in the ecosystem are abundant, and it takes a longer time (up to a thousand years) to degrade naturally. In addition, microplastics created various problems, particularly to the aquatic environment and humans. For example, marine organisms will easily ingest microplastics as these particles mimic the food they consume. Due to the smaller dimension

of this material, the microplastic has been absorbed in the aquatic organism, which reduces energy production, leading to premature death (Silva & Sousa, 2021). Reinold et al. (2021) found the existence of microplastics in the gastrointestinal tracts of cultivated European sea bass (*Dicentrarchus labrax*), located in the coastal water of Tenerife (Canary Island, Spain). The result had shown that 53 (65%) of 83 individuals sampled in fish have gradually ingested the microplastics. The higher presence of microplastics in the European sea bass was due to the massive plastic pollution discovered washed ashore on the coastlines and floating in the coastal waters of the Canary Islands.

Moreover, the toxicity of microplastic affects agriculture, particularly on plants and soil constituents. For example, previous authors have investigated the influence of macro and micro-size- polyethylene (PE) and biodegradable plastic mulch films on wheat (*Triticum aestivum*) growth (Qi et al., 2018). The result revealed that these plastic residues contributed an adverse effect on the top and ground parts of the wheat plants. As a result, both vegetative and reproductive growth of the wheat has been affected (Qi et al., 2018). In addition, the substance has clogged at the surface pores of the root due to the absorption process. Therefore, it affects the subsuming of nutrients (Zong et al., 2021).

Researchers have become concerned about the issues of microplastic degradation, and several microplastic treatments have been investigated to address the microplastic challenges. The effects of polyethylene and polypropylene microplastics on *Spirulina sp.* microalgae have been investigated (Bai et al., 2021; Hadiyanto et al., 2021). After a 30-day interaction, *Spirulina sp.* microalgae caused cracks on the surface of microplastics and the formation of new functional groups (hydroxyl, carbonyl, carboxylic acid). EDX analysis revealed that microplastic reduces carbon in polyethylene (1.62%) and polypropylene (1.08%). These results have proven that the polyethylene and polypropylene microplastics were degraded by *Spirulina sp.* microalgae (Hadiyanto et al., 2021). The biodegradation of polyethylene microplastics by marine fungus *Zalerion maritimum* recorded approximately more than 43% of microplastic removal after 14 days incubation periods (Paço et al., 2017). This finding shows that the *Zalerion maritimum* could consume polyethylene as a substrate in controlled conditions, reducing pellet mass and size.

Another study has shown the bacterial strains *Pseudomonas sp.* ADL15 and *Rhodococcus sp.* ADL36, isolated from Antarctic soil, has utilized polypropylene microplastics as their carbon source in 40 days, with the weight lost at 17.3% and 7.3 %, respectively (Habib et al., 2020). In addition, biodegradation of polymers using isolated bacteria from the soil grove (*Lysinibacillus sp.*) demonstrated the ability to degrade polyethylene and polypropylene by 4% and 9% over 26 days (Jeon et al., 2021). Besides that, *Bacillus cereus* and *Bacillus gottheilii* are the two *Bacillus* strains isolated from mangrove ecosystems were used to degrade several types of microplastics (Auta et al., 2017). Based on the observations of microplastic particles after 40 days incubation period,

B. gottheilii has recorded the percentage weight loss of polyethylene (6.2%), polystyrene (5.8%), polypropylene (3.6%), and polyethylene terephthalate (3.0%) after 40 days. On the other hand, *B.cereus* shows the percentage weight loss of polystyrene (7.4%), polyethylene terephthalate (6.6%), and polyethylene (1.6%). These findings validate the ability of bacterial strains isolated from mangrove ecosystems to degrade microplastics after 40 days of incubation. Recent research on microplastic biodegradation has mainly focussed on isolated bacteria from soil, marine, and mangrove systems. Here, it can be denoted that limited studies on the possible degradation rate of microplastics in leachate inoculum have been investigated so far (Silva et al., 2021).

This paper covers the analyses of the reduction for primary microplastic in the nitrifying medium with landfill leachate source of bacteria under a closed system. These bacteria assisted in removing the primary microplastics under optimum conditions. Therefore, this research study was conducted with four different types of primary microplastic (PP, PS, PET, and PS) in three different incubation periods (7, 14, and 21 days), respectively. The research focused on the changes in the properties of microplastics in terms of percentage weight loss of each type of microplastics, changes of the chemical structure, surface morphology, and ammonia-nitrogen removal in the batch culture systems.

MATERIALS AND METHODS

Materials

The materials for the experiment consist of four (4) types of polymers [Sigma Aldrich Chemical Co. (USA)]; polypropylene (PP), polystyrene (PS), polyethylene terephthalate (PET), and polyethylene (PE) **(**Table 1**)**. For the batch experiment, polypropylene (PP) and polystyrene (PS) were ground and sieved (600 µm) to acquire a size between 250 µm to 1000 µm for the range of microplastics (Paço et al., 2017). The primary sources of microplastics used in this batch experiment are shown in Figure 1.

Table 1

Raw polymer used for the batch experiment (Sigma Aldrich)

Polymer	Density (g/mL)	Descriptions
PP	0.9	Granules (white, spherical)
PS	1.59	Pellets (white/spherical)
PET	1.68	Granules (granular/milky white)
PE	0.94	Powder with 75 µm particle size

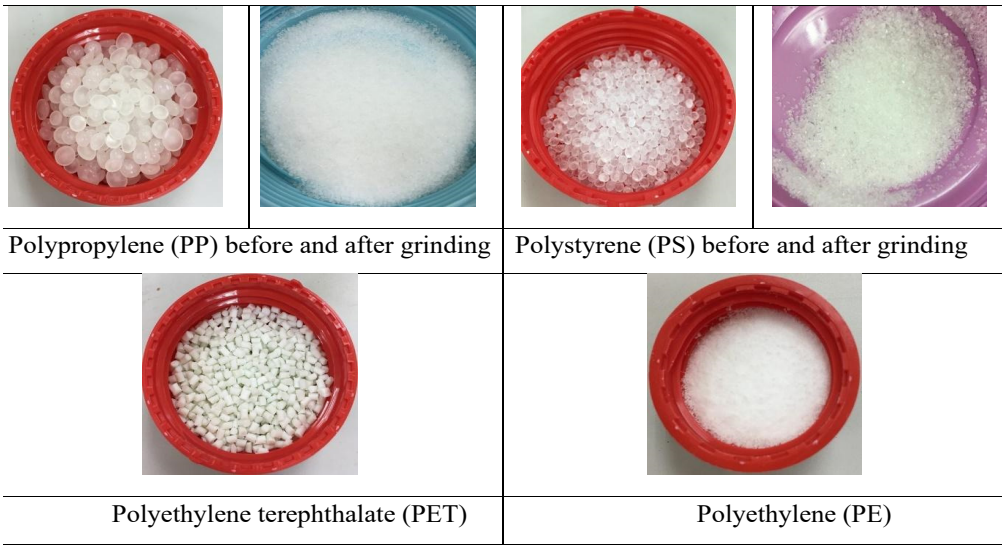


Figure 1. Microplastics polymer used in the batch experiment

Closed System (Batch Culture Experiment)

For this study, the primary source of microplastics was degraded under a closed system (batch culture experiment). This experiment employed five sets of flasks with a capacity of 250 ml each. First, synthetic wastewater was prepared in a Duran bottle for 1 L (deionized water) using the constituents displayed in Table 2 (Tarmizi et al., 2019). Then, the stock solution of $(NH_4)_2SO_4$ was prepared by adding 5g $(NH_4)_2SO_4$ into 1L (deionized water) to acquire the final concentration of 0.5g/L (Kasmuri & Lovitt, 2018). The flasks and solutions in the Duran bottles were then placed into an autoclave for 2 hours of sterilization.

Table 2

Chemicals were used for preparing synthetic wastewater (Kasmuri & Lovitt, 2018)

Chemicals	Weight (g)
Na_2HPO_4	13.5
KH_2PO_4	0.7
$NaHCO_3$	0.5
$MgSO_4.7H_2O$	0.1
$FeCl_3.6H_2O$	0.014
$CaCl_2.2H_2O$	0.18

As the enrichment culture for the microorganisms, inoculum leachate was taken from Air Hitam Landfill, Puchong, Selangor. Subsequently, 100 ml of synthetic wastewater, 100 ml of stock solution of $(NH_4)_2SO_4$, and 5.0 ml inoculum of leachate were poured into four (4) sets of flasks with a working volume of 205 ml each. The remaining flask was filled with 100 ml of synthetic wastewater, 100 ml of stock solution of $(NH_4)_2SO_4$, and no inoculum of leachate was added, as it was used as the control of the experiment and labelled as 'Blank.' Next, each set of four flasks was added with 5.0 g of polypropylene (PP), polystyrene (PS), polyethylene terephthalate (PET), and polyethylene (PE) microplastic, respectively. These primary microplastics were inserted as the sole carbon source or substrate to these microbes (Farzi et al., 2019).

These batch culture experiments were performed for an incubation time of 7, 14, and 21 days. The batch flasks were placed in the stackable incubator shaker at a constant temperature of 28°C and stirred at a uniform speed at 180 rpm (Tarmizi et al., 2019). Throughout the incubation periods, aeration was supplied in each flask. The analysis for ammonia-nitrogen uptake, nitrite-nitrogen, and nitrate-nitrogen detection was performed daily for the batch experiment. The procedure for determining NH_3-N, NO_2-N, and NO_3-N was done following the standard methods (APHA, 2005).

Determination of Microplastic's Dry Weight After Incubation in the Batch Culture Experiment

After the incubation, the micro-plastic polymers went through filtration and dried in an oven at 100°C overnight to determine the residual weight. The preincubated microplastics were used as the initial weight. Later, the reduction rate of each microplastic was calculated in terms of percentage weight loss using the following formula of Equation 1 (Taghavi et al., 2021);

$$Weight\ loss\ (\%) = \frac{W_0 - W}{W_0} \times 100 \quad [1]$$

Where;
W_0 is the initial weight of the microplastic (g)
W is the residual weight of the microplastic (g)

Microplastics Analysis Using Fourier Transform Infrared (FTIR)

The alterations in the structures of all types of microplastics were analyzed using Spectrum One Fourier Transform Infrared (FTIR) Spectroscopy (Perkin Elmer, TGA/SDTA 851, USA) in the range of 4000 cm^{-1} - 515 cm^{-1} (Paço et al., 2017). This analysis was carried out before and after the experiment of batch culture.

Microplastics Analysis Using Scanning Electron Microscopy (SEM)

The changes in surface morphology for each type of microplastic polymer were examined using Scanning Electron Microscopy (SEM) (Thermo Scientific, Phenom XL, Netherlands). All the microplastic samples were placed on double-sided tape and positioned on the SEM stubs. The PP, PS, PET, and PE microplastics were sputter-coated with a gold layer at 60 mA using a sputter coater instrument (Baltec, SCD 005 Sputter Coater, United States). The microplastics were visualized under the SEM at 10 kV resolution and magnification of 1500x (Thermoscientific, 2018). This analysis was carried out before and after the batch culture experiment.

RESULT AND DISCUSSION

Weight Loss of Microplastic Polymers in Batch Culture Experiment

The experimental observation on the reduction of primary microplastics in a batch culture system under aerobic conditions. It has been denoted that the reaction of reduction (biodegradation rate) in aerobic conditions was observed by Chinaglia et al., 2018 as follow:

$$C_{polymer} + O_2 \rightarrow CO_2 + H_2O + C_{biomass}$$

Microorganisms have assimilated the carbon of the polymer ($C_{polymer}$) (into $C_{biomass}$), which is then either rapidly mineralized into CO_2 and H_2O or consume for growth and reproduction (more $C_{biomass}$). $C_{biomass}$ is mineralized for the long-term due to the following turnover of the soil microbial population or storage polymers, which results in the generation of CO_2. More description of the biodegradation reaction is the $C_{polymer}$ converted into $C_{biomass}$ (Chinaglia et al., 2018). In this research study, PP, PS, PET, and PP microplastics are the $C_{polymer}$ that microorganisms have utilized as a carbon and energy source for their growth. Subsequently, the weight of microplastics has been reduced after the incubation period.

Figure 2 reveals the percentage weight loss of microplastics after 7, 14, and 21 days incubated in the batch culture experiment. The incubation period selected for this study is directly related to the initial surface properties of biofilms development by bacteria on microplastics (Ramsperger et al., 2020). After seven days of incubation, the highest percentage weight loss (3.03%) was obtained by PE, followed by PP, PS, and PET microplastics. Moreover, after 14 days of incubation, the weight loss of 3.10%, 2.72%, 0.38%, and 3.46% was recorded for PP, PS, PET, and PE, respectively. The sole carbon sources of PP, PS, PET, and PP microplastics have potentially degraded after 21 days incubated in the batch culture experiment. In supporting this statement, the microplastics showed an increase of percentage weight loss compared with 14 days incubation period. Again, PE microplastic (8.8%) showed greater weight loss, followed by PP, PS, and PET microplastics, which were 6.96%, 4.74%, and 0.92%, respectively.

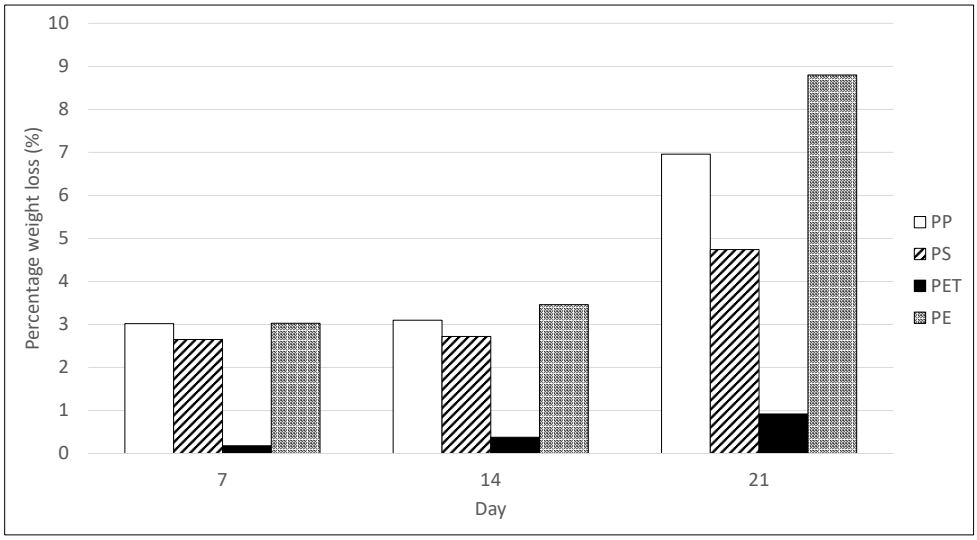


Figure 2. Percentage weight loss of PP, PS, PET, and PE microplastics after 7, 14, and 21 days incubated in the batch culture experiment.

Based on the results obtained, the size and shape of the microplastics have influenced the degradation rate. The physical properties of PE microplastic are powder with fine particle size compared to other pellets and granules sizes. The degradation of this polymer accelerated at the surface, where the mass loss rate is intimately correlated to (and often proportional to) the surface area of the plastic fragments (Chamas et al., 2020). It indicated that the shape of plastic specimens affects their fragment behavior in the ocean and that tiny pieces with low aspect ratios disintegrate rapidly as their isotropic motion hinders biofilm from forming on the surface of the materials (Chamas et al., 2020). Therefore, PET shows the lowest rate due to the physical size and the higher density than other polymers (Table 1). It can be denoted that PET is particularly resistant to biodegradation in the environment due to its dense structure and is slowly degraded by microbes (Gewert et al., 2015).

After being inoculated in the new medium, bacteria did not reproduce rapidly, and the population remained unchanged in the preliminary stages (Rogers, 2020). Bacteria require a certain time to grow and reproduce; initially, the first phase observed under batch culture is the lag phase, whereby the growth rate is zero (Maier, 2009). During this lag phase, bacteria are metabolically active but not yet dividing (Bailey, 2018), which explains the results obtained in the duration period of 7 and 14 days.

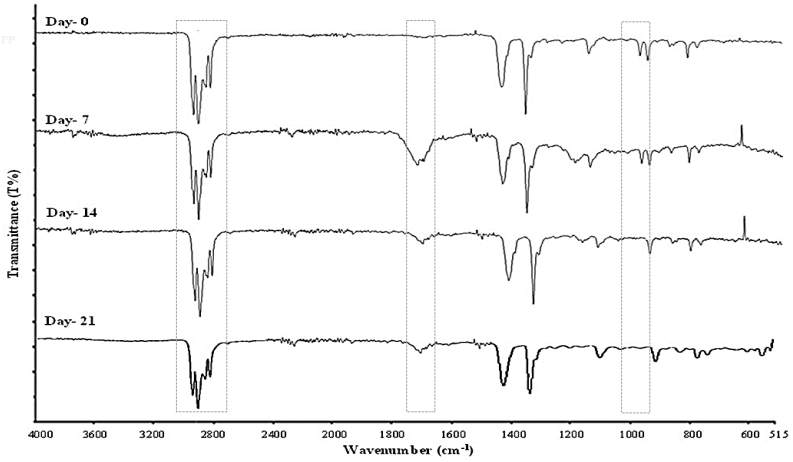
Moreover, the visual observations found that microplastic polymers turned yellow after incubation. In a previous study, Auta et al. (2017) stated that the yellowing color of the polymer indicated the initial phase of the degradation process and can be characterized as the colonization of bacteria on the polymer surface. Therefore, the weight loss of microplastics

and the changes in colors to yellow show that PP, PS, PET, and PE biodegraded in the batch culture systems. However, an extended incubation period is needed to achieve the optimal result of microplastics reduction and to evaluate the microbial activity during the degradation process.

Analysis from Fourier Transform Infrared (FTIR) of Microplastic Polymers

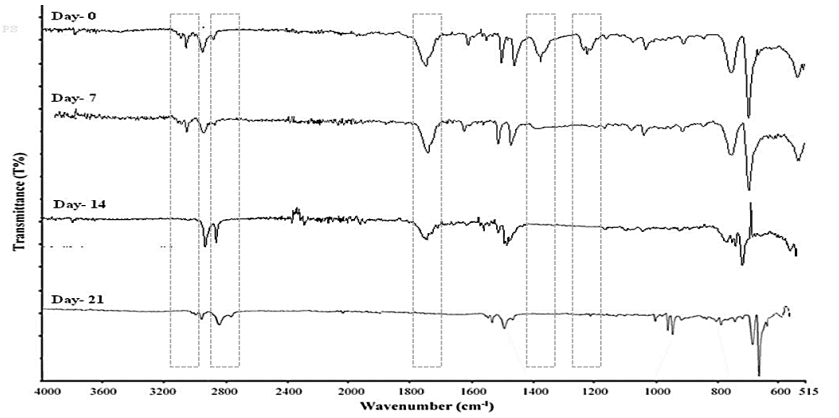
This experiment detected the absorbance peak of microplastics polymer using Fourier Transform Infrared (FTIR). This analysis intended to observe the alteration of chemical structures for verification of the biodegradation process. The absorption peak was referred to as the IR spectrum table (Merck, 2021).

Figure 3(a) shows the FTIR spectrum of PP microplastic at day-0 and after the reduction for 7, 14, and 21 days in the batch culture experiment. A new functional group of C=O at the peak of 1735 cm^{-1} was formed after seven days of incubation. C=O as a carbonyl group makes PP microplastics more susceptible to deterioration (Sudhakar et al., 2008). The previous report has mentioned that the appearance of carbonyl groups might cause the cutting of PP polymer rings (chain scission) and cross-linking while decreasing the hydrophobic properties of PP (Hadiyanto et al., 2021). In addition, the peak has been detected missing at 2867 cm^{-1} in the PP (day-21), attributed to the C-H. The absorption peak at 997 cm^{-1} (C=C) also was absent towards 14 and 21 days of the incubation time. This research study demonstrated the formation of a new carbonyl group as becoming a factor of degradation in PP microplastic.



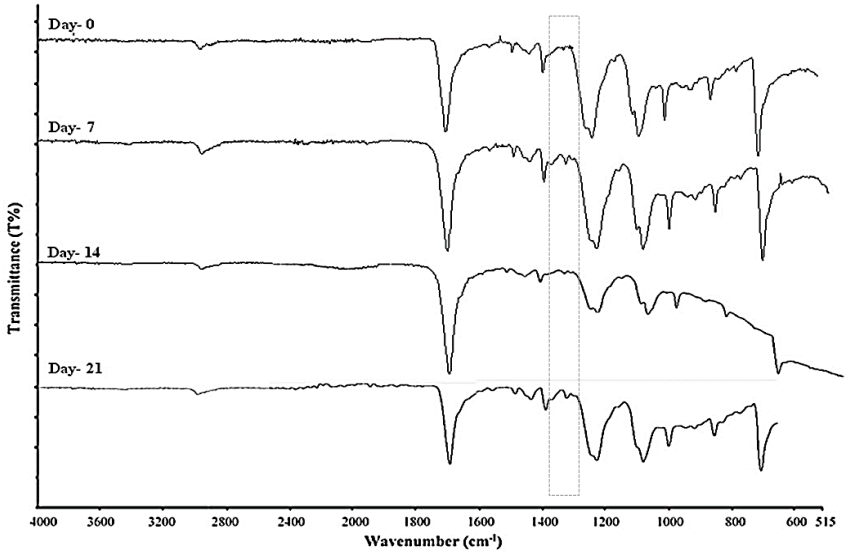
- Formation C=O, 1735 cm^{-1} at day-7
- Disappearance C-H, 2867 cm^{-1} at day-21
- Disappearance C=C, 997 cm^{-1} at day-14 and 21

(a) FTIR analysis of PP microplastic



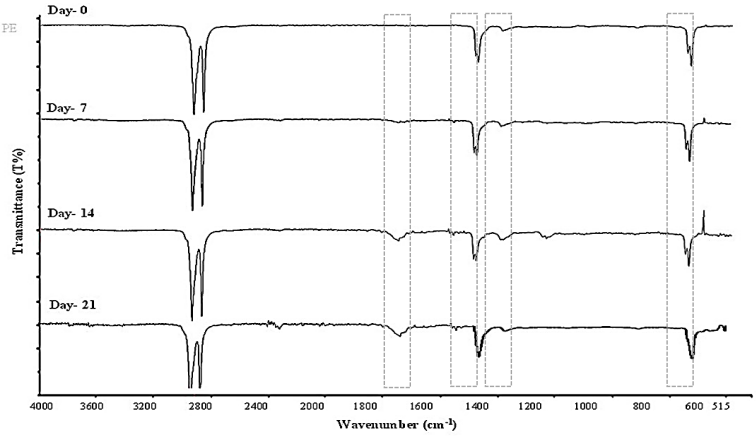
- Disappearance C-H, 3025 cm^{-1} at day-7,14 and 21
- Formation O-H, 2848 cm^{-1} at day-14 and 21
- Disappearance C=O, 1738 cm^{-1} at day-21
- Disappearance C-O, 1366 cm^{-1} and C-O, 1216 cm^{-1} at day-7, 14 and 21

(b) FTIR analysis of PS microplastic



- Formation O-H, 1339 cm^{-1} at day-7,14, and 21

(c) FTIR analysis of PET microplastic



- Formation C=O, 1735 cm^{-1} at day-7, 14 and 21
- Reduction O-H, from 1377 to 1367 cm^{-1} at day-14
- Disappearance C-H, 1471 cm^{-1} and 729 cm^{-1} at day-21

(d) FTIR analysis of PE microplastic

Figure 3. The FTIR analysis of microplastics before and after the incubation

Besides that, the chemical structure changes on PS microplastic in the biodegradation process, as shown in Figure 3(b). The absorption peaks at 3025 cm^{-1} assigned to C-H were absent in the batch culture experiment at 7, 14, and 21 days of the incubation period for PS. It has been noticed that the appearance of a new absorption peak at 2848 cm^{-1} represents the O-H of the carboxylic group on 14 days and 21 days of incubation for PS microplastic. The degradation by nitrifying bacteria leads to the production of the carboxylic group of the PS microplastic. The C=O (1738 cm^{-1}) disappeared after 21 days of incubation. Lastly, 1366 cm^{-1} assigned to C-H and 1216 cm^{-1} assigned to C-O were absent on 7, 14, and 21 days of the incubation period for PS in the batch culture experiment. These changes indicated that the chemical structure of PS microplastic was altered due to PS being degraded by microorganisms under aerobic conditions.

Figure 3(c) shows the trend of the FTIR spectrum of PET microplastic in chemical structure changes. The FTIR spectrum of PET did not show a significant change in chemical structures. PET plastics consist of heteroatoms in the main chain that is susceptible to deterioration by biodegradation. As PET degrades, O-H carboxylic group was developed (1339 cm^{-1}) on days 7-, 14- and 21-days degradation. This process accelerates the production of tiny fragments and the functional group of carboxylic (Gewert et al., 2015).

The changes in the chemical structure of PE microplastic reduction in batch culture experiments are depicted in Figure 3(d). The new absorption peak at 1735 cm^{-1} assigned to the C=O has formed after a 7-days,14-days, 21-days incubation period. In PE, the emergence of C=O is related to biological oxidation reactions that are frequently required to enhance the hydrophilicity of the polymer by introducing a functional group, such as alcohol or carbonyl groups, which can strengthen the bacterial attachment and degradation (Wilkes & Aristilde, 2017). Next, the absorption peak at 1377 and 1367 cm^{-1} attributed to the O-H experienced a reduction after 14 days incubation period. This degradation process could be due to the microbial activity and the formation of biofilms on the surface of microplastics. The two absorption peaks, 1471 and 729 cm^{-1}, assigned to the C-H, vanished after 21 days of the batch culture experiment. Hexadecane, which has the same basic chemical structure as PE, was used as a model compound for studying PE biodegradation (Montazer et al., 2020). The early step employed hydroxylate C-C bonds to produce primary or secondary alcohols, oxidized to aldehydes or ketones, and carboxylic acid. As a result of the development of carboxylic acids, microbial oxidation reduces the number of carbonyl groups (Montazer et al., 2020). Therefore, the change in the functional group of PE microplastics involves a new formation C=O, and even a decrease of the absorption peaks indicates the PE microplastic has been degraded.

Based on the findings, the modification of functional groups involved the formation, disappearance, and reduction of absorption peaks due to the exposure and interaction between microorganism colony and microplastic (Hadiyanto et al., 2021). When PP, PS, PET, and PE microplastics were added to the batch culture medium, the microorganisms have utilized this substance as their carbon source throughout the incubation period. It explained the alteration of the FTIR profiles for each microplastic.

The polymeric biodegradation mechanism indicated that during the initial stage of microorganism's consumption, the emission of extracellular enzymes for polymer oxidation or hydrolysis process has occurred. This scenario has developed functional groups which can improve the polymer hydrophilicity. Therefore, enhance the microbial adherence to the polymer matrix. The effects of enzymes on the sample surface released by the microorganisms produce estrangement of the polymer chain. Besides that, the reduction of the absorption peak after the inoculation with leachate microbes is related to the biofilm development on the microplastics' surface. The creation of biofilms is the growth and initial colonization phase of microorganisms (de Oliveira et al., 2020). During the incubation period, microbial activities inside or outside the polymer surface play vital roles in altering microplastic's chemical structures. Hence, these findings indicated the effect of the biodegradation process involving the emergence of a new functional group such as the formation of C=O, which have developed in PP and PE, O-H have formed in PS, and PET microplastics.

Analysis from Scanning Electron Microscopy (SEM) of Microplastic Polymers

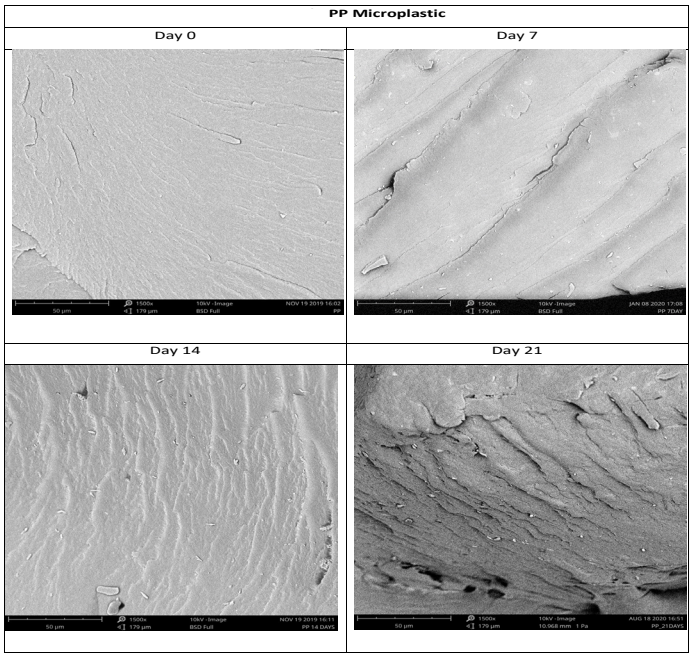
After the degradation in batch culture, the SEM analysis has displayed the images of morphology on the surface of PP, PS, PET, and PE (Figure 4). The character of PP microplastic [Figure 4(a)] shows a cracked and corrugated exterior after seven and 14 days of incubation. The surface of PP appeared to be rough lines with holes and pores after 21 days of incubation.

For the PS microplastics [Figure 4 (b)], after seven days of degradation, deep lines of grooves have formed. In addition, the narrow cut appeared on the surface, which started to break down after day 14. In the meantime, the PET [Figure 4 (c)] microplastics did not show any significant morphological changes after seven days of fragmentation. However, after 14 and 21 days, the surface of PET became rough, with grooves, cuts, and cracks emerging on the texture of the microplastic outer layer. Consequently, the deep gap and brittle exterior existed after 21 days of incubation.

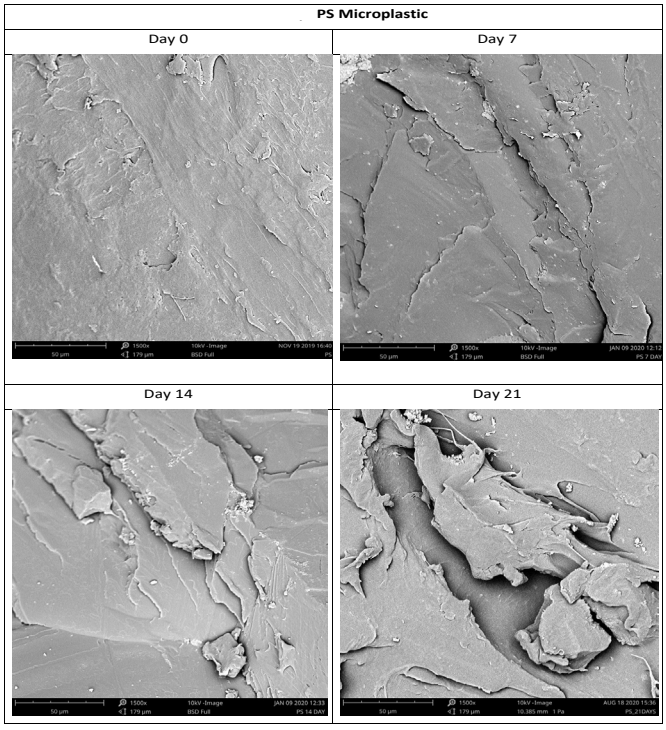
Figure 4(d) shows the SEM image of PE microplastic degradation in batch culture. The surface of PE microplastics was not altered after seven days, whereas only a small formation of holes became obvious. However, more crack has developed on the surface of the PE microplastic, with the unsmooth side following the next 14 days. Furthermore, after 21 days, the PE microplastic experiences greater extension of crack with holes, and the surface of the PE turns into more fractured with lines and a rough exterior.

The obvious morphological surface changes based on the observation of SEM analysis have confirmed that these microplastics had undergone the fragmentation process. After the incubation period in batch culture, the surface morphology of the microplastics was rougher than before, with visible lines, grooves, holes, cracks, and fractures. These changes of morphology surface of microplastic are due to PP, PS, PET, and PP being utilized by the microorganisms in the batch culture experiment.

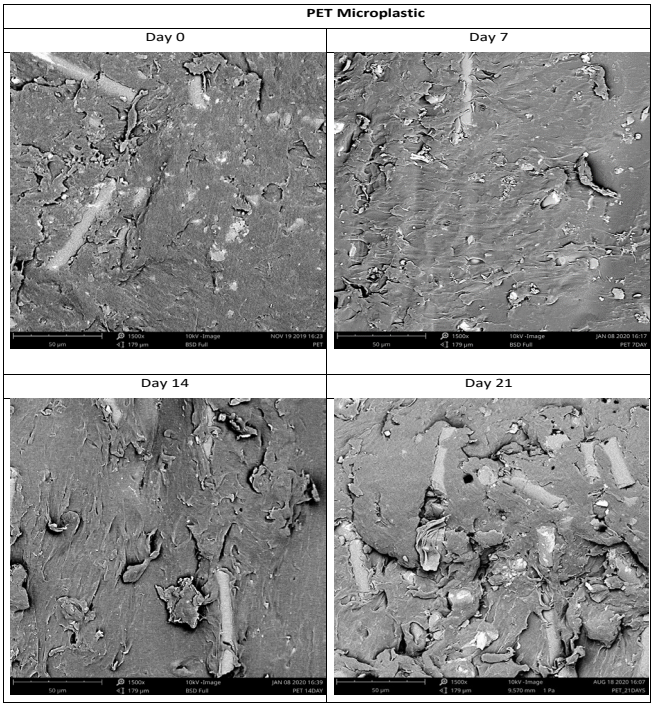
Moreover, the friction during the grinding and mixing process might also cause changes in the surface morphology of the microplastics. It is denoted that; microorganisms need substrate for their growth development. Carbon from polymer will provide energy for the metabolism of the bacteria (Chinaglia et al., 2018). Sun et al. (2021) stated that the rough surface of microplastic made it easier for the microbes to attach to the polymer surface. The degradation process has taken place (showed by the evidence of crack on the surface) by an extracellular polymeric substance (EPS) coming from the biofilm accumulation on the surface of microplastic (Hadiyanto et al., 2021).


PP Microplastic
Day 0
Day 7
Day 14
Day 21

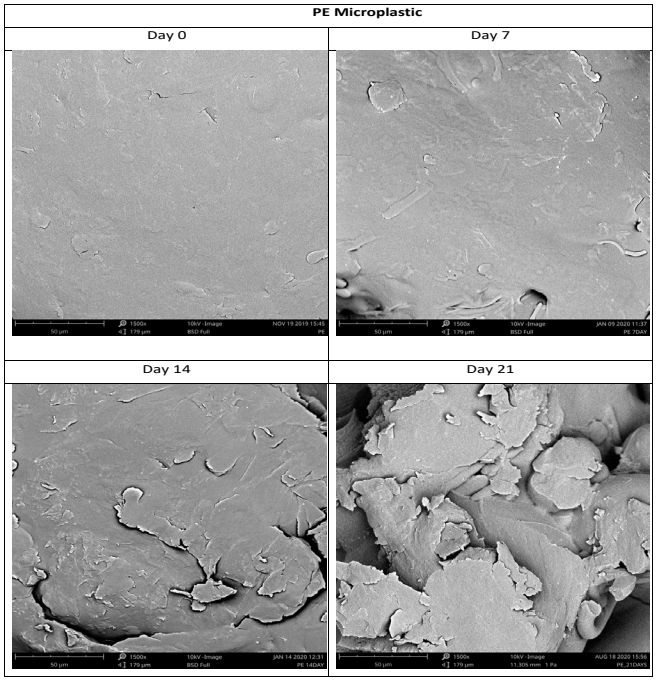
(a)


PS Microplastic
Day 0
Day 7
Day 14
Day 21

(b)



(c)



(d)

Figure 4. SEM images of four types of microplastics after biodegradation in batch culture; (a)PP; (b)PS; (c) PET; (d) PE

Ammonia-Nitrogen (NH_3-N), Nitrite-Nitrogen (NO_2-N), and Nitrate-Nitrogen (NO_3-N) Consumption in Batch Culture Experiment

The nitrification medium has been prepared for the batch culture experiment. It is to accumulate the growth of nitrifying bacteria from the inoculum of the landfill leachate. The reduction of ammonia-nitrogen (NH_3-N) will identify the existence of the nitrifying bacteria and production of the nitrite-nitrogen (NO_2-N) and the nitrate-nitrogen (NO_3-N) (Kasmuri & Lovitt, 2018). From the batch culture experiment, the concentration of NH_3-N, NO_2-N, and NO_3-N was analyzed using the standard method (APHA, 2005). The assays were done daily until 21 days of the microplastics incubation period.

It can denote that the initial ammonia–nitrogen concentration was approximately 25 mg/L in each set of flasks, respectively. Figure 5(a) shows the concentration of NH_3-N, NO_2-N, and NO_3-N for PP microplastics in the incubation period. The concentration of NH_3-N in the batch culture experiment was reduced from 24.38 mg/L (day 0) to 0.63 mg/L (day 16) with a percentage removal of 97.41%. Next, the concentration of NO_2-N was increased from 1.0 mg/L (day 0) to 14.88 mg/L (day 16). However, the concentration of NO_2-N was decreased to 12.71 mg/L on day 16. Lastly, the concentration of NO_3-N was slightly increased from 0 mg/L to 23.80 mg/L (day 16).

From Figure 5(b), the concentration of NH_3-N, NO_2-N, and NO_3-N of PS microplastics have been displayed until 21 days of incubation. The concentration of NH_3-N in batch culture was lowered from 24.38 mg/L (day 0) to 4.44 mg/L (day 21), indicating a removal rate of 81.79%. Next, the concentration of NO_2-N has risen from 1.0 mg/L (day 0) to 14.60 mg/L (day 17). However, after 21 days, the declination of NO_2-N to 11.77 mg/L was observed. The 13.88 mg/L for the concentration of NO_3-N was obtained (day 21).

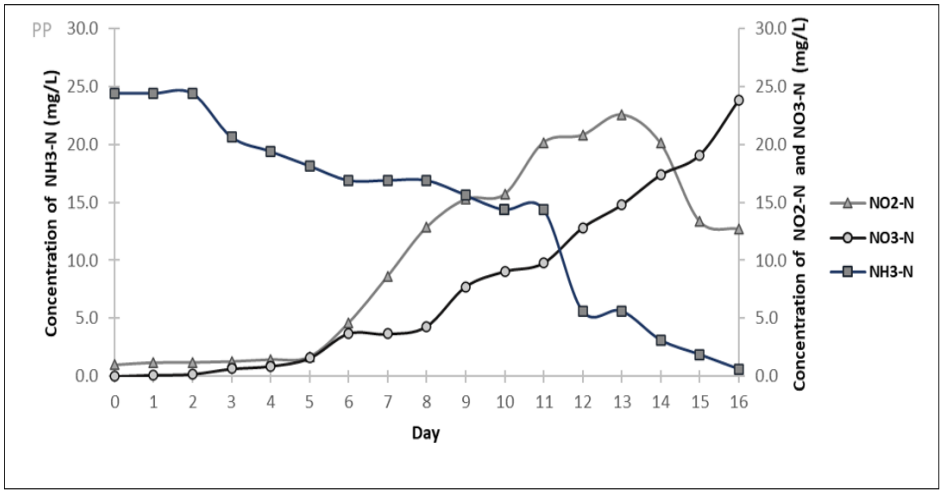
Figure 5(c) shows the concentration of NH_3-N, NO_2-N, and NO_3-N of PET microplastics in the incubation period. The concentration of NH_3-N in the batch culture was diminished from 24.38 mg/L (day 0) to 5.63 mg/L (day 21) with the removal of 76.91% of ammonia-nitrogen. Next, the concentration of NO_2-N was accelerated from 1.0 mg/L (day 0) to 13.06 mg/L (day 20). However, the concentration of NO_2-N had declined to 11.72 mg/L on day 21. Later, the concentration of NO_3-N was proportionally surged from 0 mg/L to 13.27 mg/L (day 21).

Figure 5(d) shows the concentration of NH_3-N, NO_2-N, and NO_3-N of PE microplastics in the incubation period. From the graph, 24.38 mg/L (day 0) to 0.63 mg/L (day 18) represent the concentration of NH_3-N in the batch culture experiment with a removal rate of 97.41%. The concentration of NO_2-N was raised from 1.0 mg/L (day 0) to 16.87 mg/L (day 13). However, the concentration of NO_2-N had declined to 7.39 mg/L on day 18. Later, marginally boosted the concentration of NO_3-N from 0 mg/L to 21.90 mg/L (day 18).

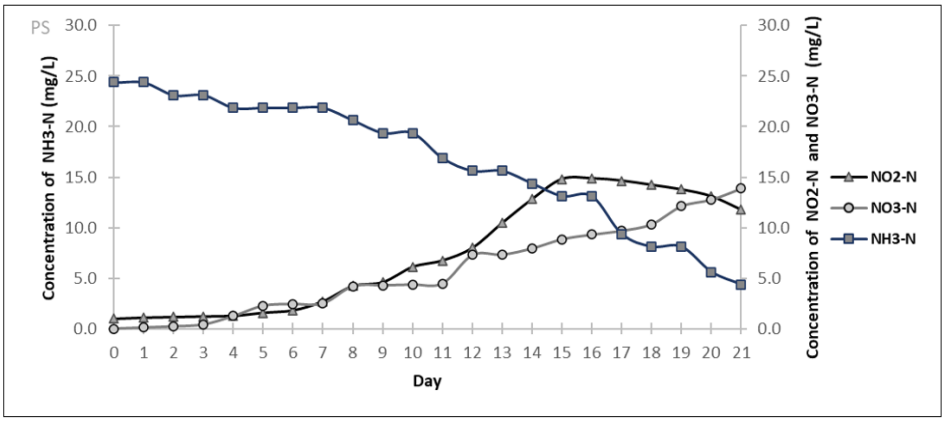
Biological nitrification involves converting ammonia-nitrogen to nitrite-nitrogen and nitrate-nitrogen in the presence of O_2 for the oxidation process (Park & Dho, 2018). The previous study explained that the nitrification process involves two steps: the ammonia-nitrogen reduction to nitrite-nitrogen and further oxidizing to nitrate-nitrogen. Two autotrophic microorganisms performed these actions: the first phase by ammonia-oxidizing bacteria (AOB), while the second stage by nitrite-oxidizing bacteria (NOB) (Kasmuri & Lovitt, 2018). Therefore, the ammonia-nitrogen in water bodies needs to be treated before discharge as the ammonia's toxicity will impair aquatic life, human health, and environmental surroundings. In addition, the high amount of ammonia-nitrogen indicates the toxic pollutant that would enter the blood and tissue by inhaling the ammonia, consequently leading to death.

According to Qin (2015), microorganisms produce and consume ammonia-nitrogen in a healthy ecosystem. In aerobic conditions, AOB, which is chemolithoautotrophic, utilizes and reduces nitrogen (primarily ammonia) as an energy source. Moreover, AOB can oxidize alternative substrates, in this case, microplastic—the process of cometabolism. Besides that, in aerobic conditions, microbes utilize oxygen to oxidize carbon and produce carbon dioxide as one of the main metabolic end products (Shah et al., 2008).

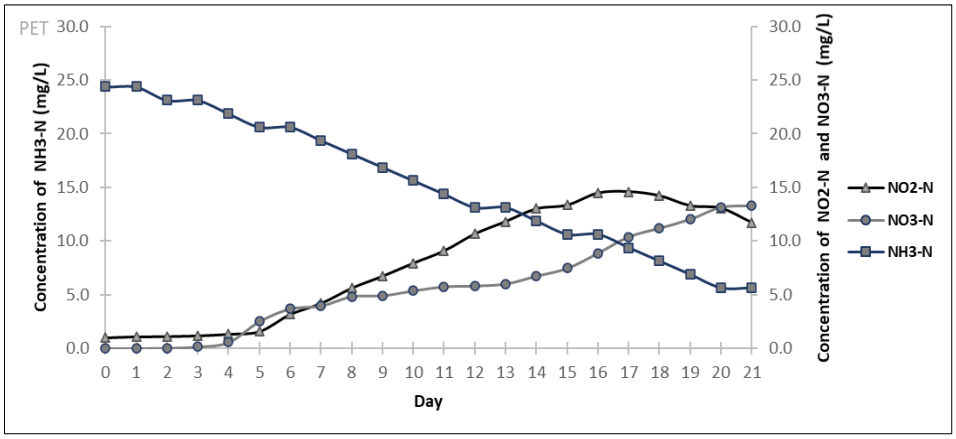
As the findings, the degradation process had greatly affected the weight loss, transformation in chemical structure, and alteration of surface morphology of microplastics related to the bacteria consumption and microorganism activity. In addition, the biodegradation of microplastic can also reduce the ammonia nitrogen through cometabolism.



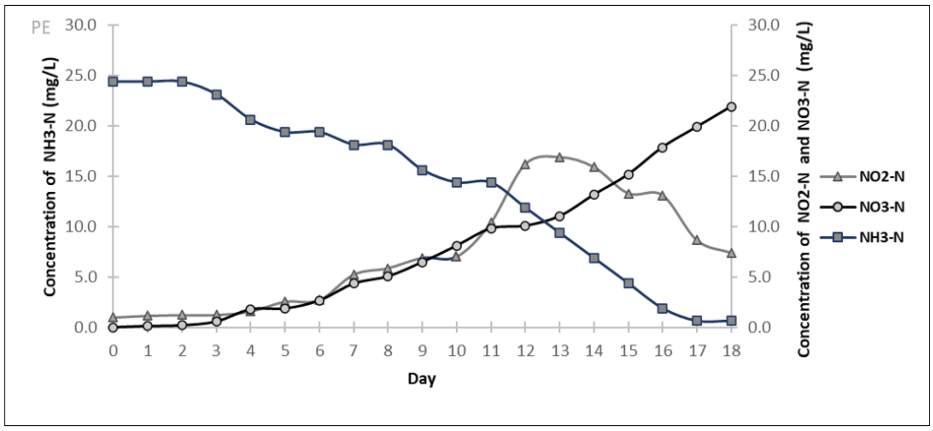
(a) PP microplastic



(b) PS microplastic



(c) PET microplastic



(d) PE microplastic

Figure 5. The concentration of NH_3-N, NO_2-N, and NO_3-N of four types of microplastics in batch culture experiment; (a) PP; (b) PS; (c) PET; (d) PE

CONCLUSION

It can deduce that the batch culture system can degrade PP, PS, PET, and PE with the reduction of PE microplastic, which revealed the highest percentage weight loss (8.8 %) in 21 days incubation period. In addition, the changes in the chemical structure of each polymer demonstrated the formation of a new peak after the incubation periods. The chemical C=O bond has formed in PP and PE, the O-H bond have been developed in PS, and PET. Furthermore, SEM detected obvious morphological changes due to microbial activities on the surface of microplastics. In contrast, the characteristic of these substances experienced lines with grooves, holes, rough surfaces, and cracked exterior texture. PP microplastic showed rapid ammonia-nitrogen (NH_3-N) removal of 97.41% after 16 days in the batch culture experiment. The results showed that leachate inoculum could reduce the primary microplastics in the batch culture. This study also found that the activity of the microorganism's population plays a significant role in the degradation of microplastic. Moreover, this biological treatment can be considered best management practice in reducing the impacts of microplastics on the ecosystem. Hence, further studies on the biological mechanisms in degrading this pollutant with a longer incubation period are required to achieve the best possible outcomes.

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