Micro-plastic Characteristics and Removal of Ammonia-Nitrogen in Batch Culture

Nur Aliah Ahmad Tarmizi, Norhafezah Kasmuri, Nor Hazelah Kasmuri

Abstract*: Plastic waste has become a sensitive issue in the world since this material needs a longer time to degrade. This material will take a month to a thousand years to decompose. Thus, would contribute to the environment pollution, which will affect human health and aquatic life. This research study focused on the biodegradation process of micro-plastic (PE, PP, PET and PS) in the batch culture system using a colony of bacteria obtained from leachate in Ayer Hitam Landfill, Puchong. After the batch experiment of micro-plastic degradation, percentage removal of ammonia-nitrogen, chemical structure and percentage weight loss were examined and evaluated. Succeeding through the incubation of micro-plastic in batch culture for fourteen (14) days period, biodegradation was verified by the estimation of the dry weight loss. From the result obtained, dry weight loss of polyethylene (PE) is the highest (3.46%) in 14 days and polyethylene (PE) shows the greater removal of ammonia nitrogen (NH_3-N) (44.17%). Besides that, polystyrene (PS) micro-plastic showed a significant change in chemical structural which was obtained by Fourier Transform Infrared (FTIR). Here, the new absorption peak C=O (aldehydes) was present in PS micro-plastic. Furthermore, PS micro-plastic has a high percentage mass loss in the second stage of thermal degradation by Thermogravimetric (TGA) analysis. It can be concluded that incubation time is needed to optimize the micro-plastic in the biodegradation process.

Keywords: Ammonia-nitrogen, batch culture, biodegradation, micro-plastic

I. INTRODUCTION

Recently, the usage of plastic is increasing from time to time due to the high demand for this material. However, a significant effect of plastic waste accumulation would generate higher waste in the landfill. Plastic waste commonly can be found in the surrounding ecosystem and environments. Besides that, the extensive use of plastic contributes to negative impacts on the environment as it could create plastic pollution, deterioration of human health, disrupting the aquatic system, flora and fauna. The impact of plastic waste will disturb the water environments such as lakes, rivers, and ponds if plastic waste is not well managed [1]. The micro-plastic has been considered as a crucial issue in the world that cause environmental contamination. This waste issue has gained greater attention among the public, researcher and the local authorities. Furthermore, the research study related to the micro-plastic sources, distribution, occurrence and adverse effect on the environment has increased tremendously [2]. For example, in a recent research study, an amount of nearly 300 micro-plastic particles were found from the digestive tract of 26 fish specimens representing 14 species. From the total amount of micro-plastic particles, 92.1 % were discovered in the stomach of 21 fish specimens that belong to 11 species and the remaining (7.9 %) were detected in the intestines of fish specimens [3].

The definition of micro-plastic is treated differently among researchers. Generally, micro-plastic is defined as a synthetic polymer which has a size less than 5 mm [4] or tiny particles in a range of 100 nm to 5 mm in size that can be abundantly found in the environment [5]. In a study of micro-plastic in the coastal environment of the Arabian Gulf [6], the result showed that micro-plastic is a piece of plastic which can be found in surface water. Due to the smaller size, this micro-plastic easily consumed by the organism.

Micro-plastic is originating from different sources known as primary micro-plastic and secondary micro-plastic. Primary micro-plastic is made purposely for manufactures specific in domestic and industrial application comprise with raw polystyrene (PS), polyethylene (PE), polypropylene (PP) and polyethylene terephthalate particle (PET) [7]. The primary micro-plastic commonly used for cosmetics, cleanser in personal care product, toothpaste, cleaning product, textiles, clothing, and plastic industries [1]. This primary micro-plastic can enter the aquatic system through the household sewage discharge, industrial effluent, human activities and treatment plant [4]. Consequently, this primary micro-plastic can contribute to environmental pollution because the micro-plastic is hard to degrade naturally due to its high molecular weight and does not dissolve or absorb in the water [2].

Secondary micro-plastic is one of the highest contributors to the environmental pollution that emerged from the process of degradation from bigger plastic into smaller plastic debris. This is due to the exposure under ultraviolet light at the ocean and terrestrial [7]. The high possibility of plastic turns to micro-plastic when the plastic exposed to the sunlight and bring a negative impact to the environment. Another study [8] stated secondary micro-plastic takes place in the environment because plastic has undergone breakdown process.

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This material has turn into a smaller fragment and changed in various physical, chemical and biological processes that downgrade the plastic debris performance. The examples of secondary micro-plastic in the ocean-based source are commercial fishing (fishing nets), vessel, plastic debris, and other activities in the marine environment. However, terrestrial sources are the major contributors to the secondary micro-plastic that comes from domestic items, air blasting process, inappropriate of dumping plastics and leachate at the disposal area [4]. Secondary microplastics are like primary micro-plastic that have difficulty to bio-degrade. Furthermore, this micro-plastic takes a long time from months to thousands of years to be degraded.

Currently, the existing degradation of micro-plastic treatment is mainly categories into three; which are physical, chemical, and biological treatment. The physical treatment involves the incineration process, UV treatment, dumping of plastic waste in sanitary landfills, and degradation of polymer waste in the presence of oxygen to reduce the molecular weight of the polymer [9]. The process of incineration includes the burning and combustion that release a dangerous gaseous combine with a substance such as dioxins and mercury into the environment [10]. These physical treatment leads to air pollution and brings severe impact on human health. Next, the chemical treatment majorly depends on the chemicals used or certain additives to break the plastic polymeric chain and causes non- hazardous forms. The use of this chemical in degradation process also has some difficulty in the disposal. Lastly, the third choice is biological treatment that involves microbes to degrade plastic waste [9]. Biodegradation or microbial degradation is the major process in the natural deterioration and biological conversion of organic compounds. This process is done by a living organism that comprises the attachment followed by the colonization of microorganisms on the surface. The plastic biodegradation is a natural process where this microorganism would consume the plastic as their food sources for the energy and development in the natural ecosystem [11]. The biological treatment is an eco-friendly and not dangerous as this treatment is a natural process without any significant effect on human, aquatic and environment. A recent study [12], has verified the microbes; *Bacillus sp.* strain 27 and *Rhodococcus sp.* strain 36 isolated from Matang mangrove were able to support the bio-degradation process for the polypropylene (PP) micro-plastic in 40 days. The results showed the percentage weight loss of PP micro-plastic in the bio-degradation process cause by *Bacillus sp.* strain 27 was 4.0 % and by *Rhodococcus sp.* strain 36 was 6.4 %.

To overcome the issues related to plastic waste, this study was conducted to determine the reduction of micro-plastic in synthetic wastewater in batch culture using inoculum from leachate obtained in Ayer Hitam Landfill. This experiment was incubated for 14-days to see the performance of the polymers in the degradation process done by microbes. Four (4) different micro-plastic polymers such as polypropylene (PP), polystyrene (PS), polyethylene terephthalate (PET) and polyethylene (PE) were added in the batch culture separately with the addition of ammonia-nitrogen as substrate. This paper analyzed the chemical bioprocess of percentage weight loss, chemical structures, thermal degradation of micro-plastic (PP, PS, PET and PE) and percentage removal of ammonia-nitrogen, accumulation in nitrite-nitrogen and nitrate- nitrogen by the microbes.

II. METHODS

A. Polymer Material

For this study, four (4) different micro-plastic raw polymer materials such as polypropylene (PP), polystyrene (PS), polyethylene terephthalate (PET) and polyethylene (PE) or known as primary micro-plastic are used for the biodegradation experiment (Table-I). These polymer materials were purchased from Sigma Aldrich Chemical Co. (USA). The raw polymer of polypropylene (PP), polystyrene (PS) and polyethylene terephthalate (PET) were crushed using a blender and pass through 600 µm sieve to obtain the micro-plastic range size between 250 µm to 1000 µm [13] for the degradation experiment.

Table- I: The polymer materials used in the bio-degradation process purchased from Sigma Aldrich Chemical Co. (USA).

Polymer material	Density (g/mL)	Temperature (°C)	Descriptions
PP	0.9	25	Granules (white, spherical)
PS	1.59	25	Pellets (white/spherical)
PET	1.68	25	Granules (granular/milky white)
PE	0.94	25	Powder with 75 µm particle size

B. Batch Culture Experiment

A batch culture comprises of five Erlenmeyer flasks (250 mL) are used for this biodegradation experiment. Synthetic wastewater was prepared contained; Na_2HPO_4, 13.5 g: KH_2PO_4, 0.7 g; $NaHCO_3$, 0.5 g; $MgSO_4.7H_2O$, 0.1g; $FeCl_3.6H_2O$, 0.014 g; $CaCl_2.2H_2O$, 0.18 g and dissolved with 1000 mL distilled water in Duran bottle. Afterward, all the batch culture flask and Duran bottle comprised of synthetic wastewater were sterilized using Hirayama autoclave for 2 hours.

The $(NH_4)_2SO_4$ stock solution was prepared by the mixed of 5g $(NH_4)_2SO_4$ with 1000 mL of distilled water to make the final concentration of 0.5g/L [14]. An inoculum of leachate obtained from Ayer Hitam Landfill, Puchong, Selangor was taken as the enrichment culture for the microorganisms. In this research study, the leachate has been initially characterized. However, these results were not shown here.

After the autoclave process was completed for the flasks and synthetic wastewater, these four conical flasks were filled with 100 mL of stock solution $(NH_4)_2SO_4$, 100 mL of synthetic wastewater and 5.0 mL inoculum of leachate. Meanwhile, one of the flasks contained 100 mL of stock solution $(NH_4)_2SO_4$ and 100 mL of synthetic wastewater without leachate was used as the control flask.

Lastly, each 5.0 g micro-plastics of polypropylene (PP), polystyrene (PS), polyethylene terephthalate (PET) and polyethylene (PE) were added separately in each flask. The flasks were then incubated at control temperature of 28°C and stirred with a constant speed at 180 rpm for 14 days [14] in the incubator shaker. Aeration was given in each flask during the incubation for 14 -days period to obtained aerobic condition. Further analysis of ammonia nitrogen (NH_3-N), nitrite-nitrogen (NO_2-N) and nitrate-nitrogen (NO_3-N) concentrations were taken daily within the 14 days to determine the removal rate of ammonia-nitrogen in the batch culture system. The method for calibration of NH_3-N, NO_2-N and NO_3-N followed standard method [15].

C. Determination of Dry Weight After Batch Culture Process

The dry weight of each micro-plastic polymers was obtained through a filtration and drying process from the synthetic medium in a hot air oven at 100°C overnight. The initial weight for each micro-plastic polymer at zero-day was measured and used as a reference. The percentage of weight loss of each polymer due to degradation was calculated using Equation (1):

$$(\%)\text{weight loss} = \left(\frac{W_o - W}{W_o} \times 100\right) \quad (1)$$

where;

W_0 is the initial weight of the micro-plastic (g)
W is the residual weight of the micro-plastic (g)

D. Fourier Transform Infrared (FTIR) Analysis of Micro-Plastic Polymers

The change in the structures of the micro-plastic polymers before and after the batch culture experiment was analyzed using FTIR Spectroscopy (Perkin Elmer, TGA/SDTA 851, USA) in the range of 4000 cm^{-1} - 515 cm^{-1}[13].

E. Thermogravimetric Analysis (TGA) of Micro-Plastics Polymers

Thermogravimetric analysis (TGA) of PE, PP, PET and PS polymer before and after biodegradation process was carried out using TGA Instruments (Mettler Toledo, TGA 851/LF/1600, Switzerland) in the temperature range of 25 to 900 °C [16]. A quantity of twenty (20) mg of each micro-plastic samples with a constant nitrogen gas flow at 50 mL/min and heating rate (10 °C min^{-1}) were set.

III. RESULT AND DISCUSSION

A. Weight Loss of Micro-Plastic Polymers

The potential of micro-plastic biodegradation process was determined by calculating the percentage of dry weight loss in 14-days incubation period. Based on the calculation obtained, PE micro-plastic show the highest percentage weight loss (3.46%) compared with other micro-plastic. Subsequently, the percentage weight loss of PP and PS was 3.10% and 2.72%, respectively. Lastly, PET micro-plastic showed the lowest percentage weight loss which is 0.38% after 14 days of the bio-degradation process.

B. Fourier Transform Infrared (FTIR) Analysis of Micro-Plastic Polymers

The analysis of FTIR in this study purposely to determine the changes in the chemical structures of the micro-plastic polymers due to the bio-degradation process in batch culture. Therefore, to confirm the bio-degradation process, the result of micro-plastic that was analysed using FTIR were compared between before (zero-day) and after 14 days of micro-plastic being treated in the batch culture experiment. Table-II shows a summary of the peaks wavenumber that was identified based on the FTIR analysis [17].

Referring to Fig. 1, it shows the FTIR spectrum of PE micro-plastic polymers before and after 14-days bio-degradation process. The absorption peaks of PE micro-plastic have taken place in chemical change after undergone deterioration process. The absorption peak 2915 $cm^{-1,}$ 1471 cm^{-1} and 729 cm^{-1} were attributed to C-H stretch (alkanes), C-H bend (alkanes) and C-H bend (aromatics), respectively were disappeared after the 14 days of the bio-degradation process. Moreover, new peak absorption 1735 cm^{-1} (C=O (aldehydes)) was formed at day 14 and absorption peak at 1377 cm^{-1} (day 0) was reduced to 1367 cm^{-1} (day-14) that assigned to C-H rock (alkanes) of the functional group.

The overall trends of PP micro-plastic were shown in Fig.2. The two absorption peaks were disappeared after the bio-degradation process in a batch culture which is 2917 cm^{-1} and 997 cm^{-1} that attributed to the C-H stretch (alkanes) and =C-H bend (alkenes), respectively.

Furthermore, the alteration of the chemical structure of PET micro-plastic was presented in Fig.3, where the peak absorption reduced from 2962 cm^{-1} to 2959 cm^{-1}, assigned to C-H stretch (alkanes). The peak absorption of 724 cm^{-1} also was reduced to 662 cm^{-1} attributed to C-H bend (aromatics). The two new functional groups of PET micro-plastic appeared after 14-days degradation of PET, that are 1339 cm^{-1} and 935 cm^{-1} assigned to N-O symmetric stretch (nitro compound) and O-H bend (carboxylic acids), respectively.

Lastly, the chemical structure of PS micro-plastic has a significant change in the bio-degradation process (Fig.4) The absorption peak at 2918 cm^{-1} (C-H stretch (alkanes)) has reduced to 2848 cm^{-1} (C-H (aldehydes)) after 14-days period in incubation. Besides that, the new absorption peak at 1734 cm^{-1} (C=O (aldehydes)) appeared and the peak absorption of 1492 cm^{-1} (C-C stretch (aromatics)) was absent in day 14. The peak absorption at 1451 cm^{-1} (day 0) to 1463 cm^{-1} (day 14), both assigned to C-H bend (alkanes) had experienced elongation. Moreover, the functional group of C-H bend (aromatics) and C-Br stretch (alkyl halides) were disappeared after 14-days of degradation, consequently verified the bio-degradation process.

C. Thermogravimetric Analysis (TGA) of Micro-Plastics Polymers

Fig.5 shows thermogravimetric analysis of biomass micro-plastic polymers which were obtained using TGA analyzer.

Three major stages of thermal degradation that comprises of moisture loss, high pyrolytic stage or devolatilization and low pyrolytic stage or char formation have been gained through this experiment. The first stage as a starting point of the thermal degradation of the micro-plastic polymers, moisture and evaporation has taken place up to the temperature of 150°C.

Table- II: Functional group of micro-plastic polymers

Polymer materials	Before (day 0)		After (day 14)	
	Wavenumber (cm^{-1})	*Functional groups*	*Wavenumber (cm^{-1})*	*Functional groups*
Polyethylene (PE)	2915	C-H stretch (alkanes)	2848	C-H (aldehydes)
	2848	C-H (aldehydes)	1735	C=O (aldehydes)
	1471	C-H bend (alkanes)	1463	C-H bend (alkanes)
	1463	C-H bend (alkanes)	1367	C-H rock (alkanes)
	1377	C-H rock (alkanes)	718	C-H bend (aromatics)
	729	C-H bend (aromatics)		
	718	C-H bend (aromatics)		
Polypropylene (PP)	2949	C-H stretch (alkanes)	2949	C-H stretch (alkanes)
	2917	C-H stretch (alkanes)	2838	C-H (aldehydes)
	2838	C-H (aldehydes)	1735	C=O (aldehydes)
	1735	C=O (aldehydes)	1455	C-H bend (alkanes)
	1455	C-H bend (alkanes)	1375	CH_3 bend
	1375	CH_3 bend	1166	C-O stretch (carboxylic acids)
	1166	C-O stretch (carboxylic acids)	972	=C-H bend (alkenes)
	997	=C-H bend (alkenes)	840	C-H bend (aromatics)
	972	=C-H bend (alkenes)		
	840	C-H bend (aromatics)		
Polyethylene terephthalate (PET)	2962	C-H stretch (alkanes)	2959	C-H stretch (alkanes)
	1712	C=O (carboxylic acid)	1712	C=O (carboxylic acid)
	1505	N-O stretch (nitro compound)	1505	N-O stretch (nitro compound)
	1452	C-C stretch (aromatics)	1452	C-C stretch (aromatics)
	1408	C-C stretch (aromatics)	1408	C-C stretch (aromatics)
	1242	C-O stretch (carboxylic acids)	1339	N-O symmetric stretch (nitro compound)
	1096	C-O stretch (carboxylic acids)	1242	C-O stretch (carboxylic acids)
	1016	C-O stretch (carboxylic acids)	1096	C-O stretch (carboxylic acids)

	872	C-H bend (aromatics)	1016	C-O stretch (carboxylic acids)
	724	C-H bend (aromatics)	935	O-H bend (carboxylic acids)

Table- III: Functional group of micro-plastic polymers (*continued*)

Polymer materials	Before (0-day)		After (14-days)	
	Wavenumber ***(cm^{-1})***	***Functional groups***	***Wavenumber*** ***(cm^{-1})***	***Functional groups***
Polyethylene terephthalate (PET)			872	C-H bend (aromatics)
			662	C-H bend (aromatics)
Polystyrene (PS)	2918	C-H stretch (alkanes)	2848	C-H (aldehydes)
	1492	C-C stretch (aromatics)	1734	C=O (aldehydes)
	1451	C-H bend (alkanes)	1463	C-H bend (alkanes)
	748	C-H bend (aromatics)	695	C-H bend (aromatics)
	695	C-H bend (aromatics)		
	539	C-Br stretch (alkyl halides)		

Next, the second stage that comprises the devolatilization which begins at temperature 150°C to 600°C. Basically, in this stage, decomposition of organic materials such as cellulose and hemicellulose, and maximum hot volatiles was discharge comprised of condensable and non-condensable gases (high pyrolysis stage). Besides that, the weight loss of micro-plastic polymers at the second stage due to the fast decomposition of these organic materials. Lastly, at the third stage, the temperature that reached more than 500°C has gained the biomass thermal stability due to the existing of the hydroxyl phenolic compounds and the temperature approached more than 600°C, lignin was decomposed at a slower rate [16]. The summary of the micro-plastics percentage weight loss in three-stage of thermal degradation is shown in Table III.

D. Ammonia - nitrogen (NH_3-N), Nitrite - Nitrogen (NO_2-N) and Nitrate - Nitrogen (NO_3-N) of Micro-Plastic Polymers

The result of the NH_3-N, NO_2-N and NO_3-N absorbance were acquired from the calibration test that has been done using standard method [15] during the biodegradation process of micro-plastic (PE, PP, PET, and PS) in batch culture system for 14 days.

Fig.6 shows the concentration of PE micro-plastic in 14 days of biodegradation in batch culture. The concentration of NH_3-N in bacth culture was decreased by 44.17% from 120.36 mg/L (day 0) to 67.20 mg/L (day 14). Next, the concentration of NO_2-N was rise 95.55% from day 0 (0.052 mg/L) to day 7 (1.16mg/L). But after day 7, the concentration NO_2-N was reduced to 68.85% (0.69mg/L) at day 14. Also, the concentration NO_3-N was slightly increased by 53.76% from 3.50 mg/L to 7.58 mg/L.

Besides that, Fig.7 shows the concentration of PP micro-plastic in 14 days of biodegradation in batch culture. The concentration of NH_3-N in batch culture was decreased by 39.18% from 120.36 mg/L (day 0) to 73.20 mg/L (day 14).

Next, the concentration of NO_2-N was rise 95.84% from day 0 (0.052 mg/L) to day 7 (1.24 mg/L). But after day 7, the concentration NO_2-N was reduced to 59.17% (0.78 mg/L) at day 14. The concentration NO_3-N was slightly increased by 55.63% from 3.36 mg/L to 7.57 mg/L.

Next, Fig.8 shows the concentration of PET micro-plastic in 14 days of biodegradation in batch culture. The concentration of NH_3-N in bath culture was decreased by 37.52% from 120.36 mg/L (day 0) to 75.20 mg/L (day 14). The concentration of NO_2-N was rise 95.02% from day 0 (0.052 mg/L) to day 7 (1.04 mg/L). But after day 7, the concentration NO_2-N was reduced to 26.40% (0.82 mg/L) at day 14. Further, the concentration NO_3-N was slightly increased by 54.65% from 3.36 mg/L to 7.64 mg/L.

Lastly, Fig.9 shows the concentration of PS micro-plastic in 14 days of biodegradation in batch culture. The concentration of NH_3-N in bath culture was decreased by 33.86% from 120.36 mg/L (day 0) to 77.20 mg/L (day 14). Next, the concentration of NO_2-N was rise 94.75% from day 0 (0.052 mg/L) to day 7 (0.98 mg/L). But after day 7, the concentration NO_2-N was reduced to 18.23% (0.83mg/L) at day 14. Hence, the concentration NO_3-N was slightly increased by 61.33% from 3.36 mg/L to 8.69 mg/L.

The changes of NH_3-N, NO_2-N, and NO_3-N concentration in 14-days period of bio-degradation due to the nitrification. Nitrification is a two-step process of converting ammonia-nitrogen to nitrite-nitrogen and then to nitrate-nitrogen. The biological degradation process of nitrification was carried out by two autotrophic microorganisms knows as ammonia-oxidizing bacteria (AOB) and nitrite-oxidizing bacteria (NOB) [18]. The reduction of ammonia-nitrogen in day 0 to day 14 due to ammonia-oxidizing bacteria (AOB) consumed the ammonia-nitrogen. At day 7, the concentration of nitrite-nitrogen in PE, PP, PET, and PS in batch culture was reduced until day 14.

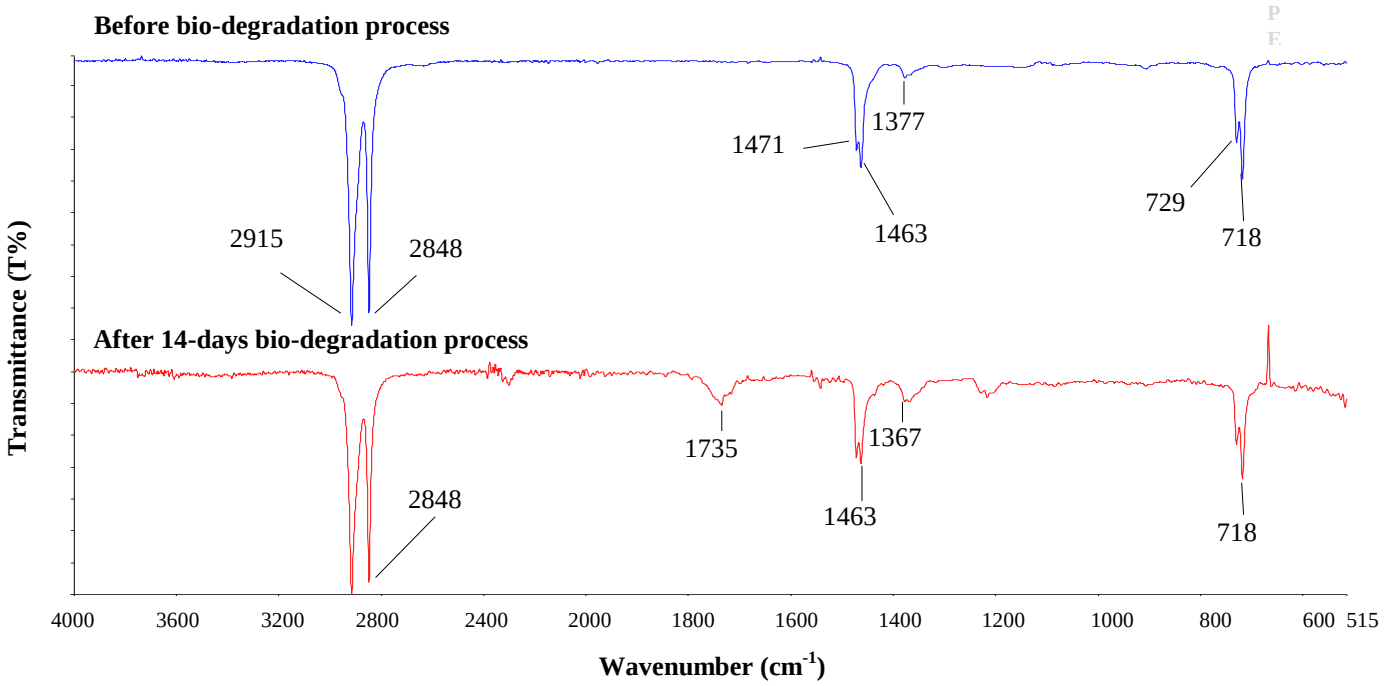

Fig. 1. FTIR spectrum of PE micro-plastic polymers

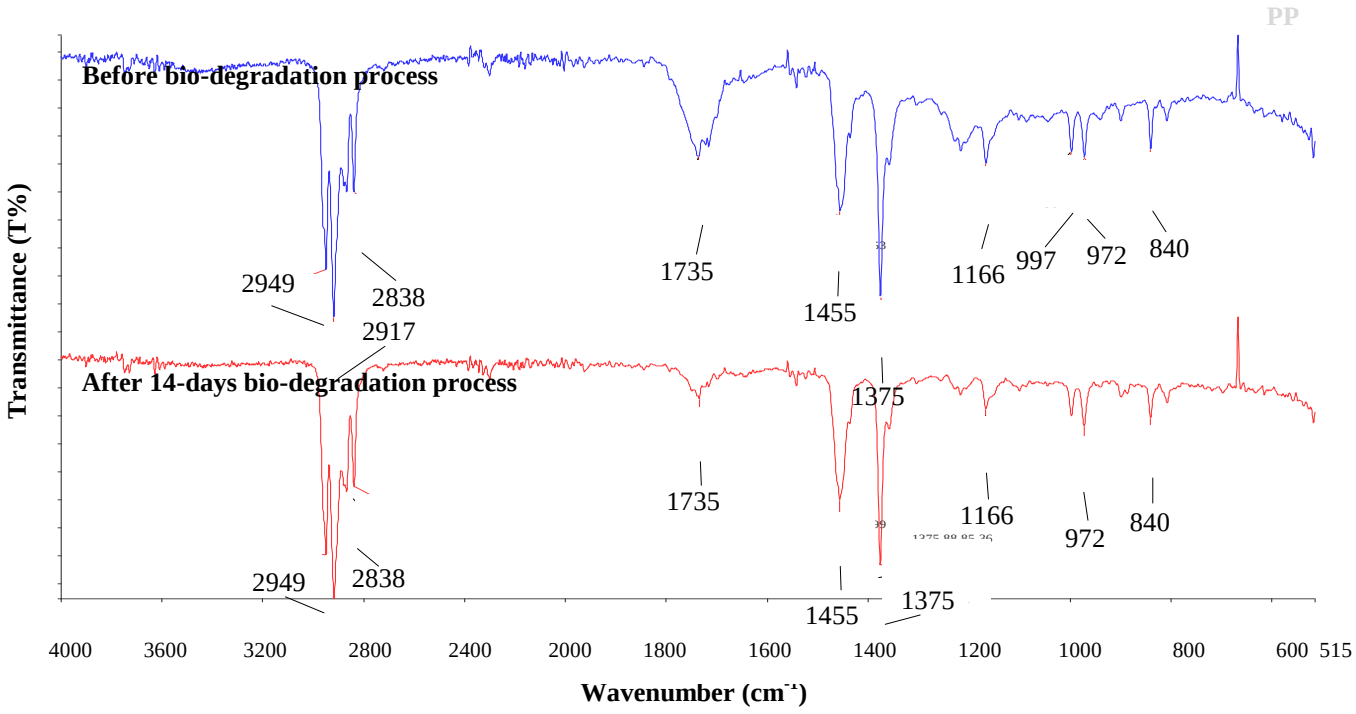

Fig. 2. FTIR spectrum of PP micro-plastic polymers

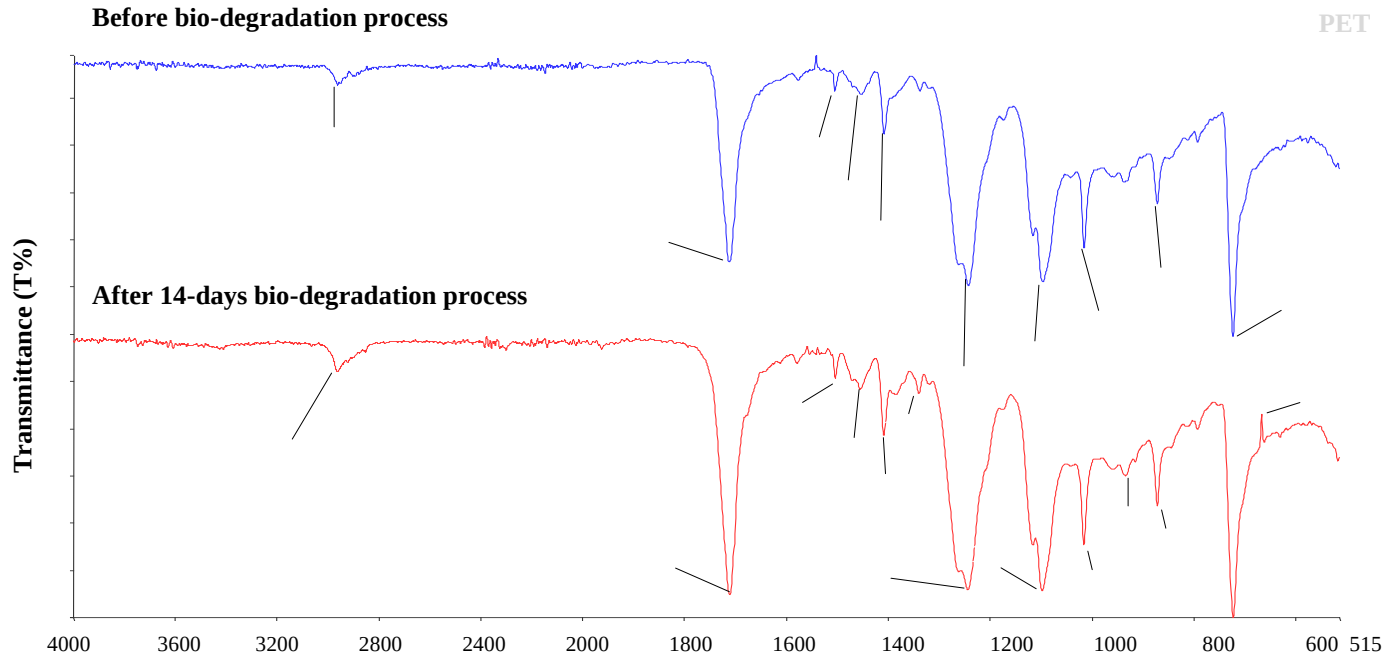

Fig. 3. FTIR spectrum of PET micro-plastic polymers

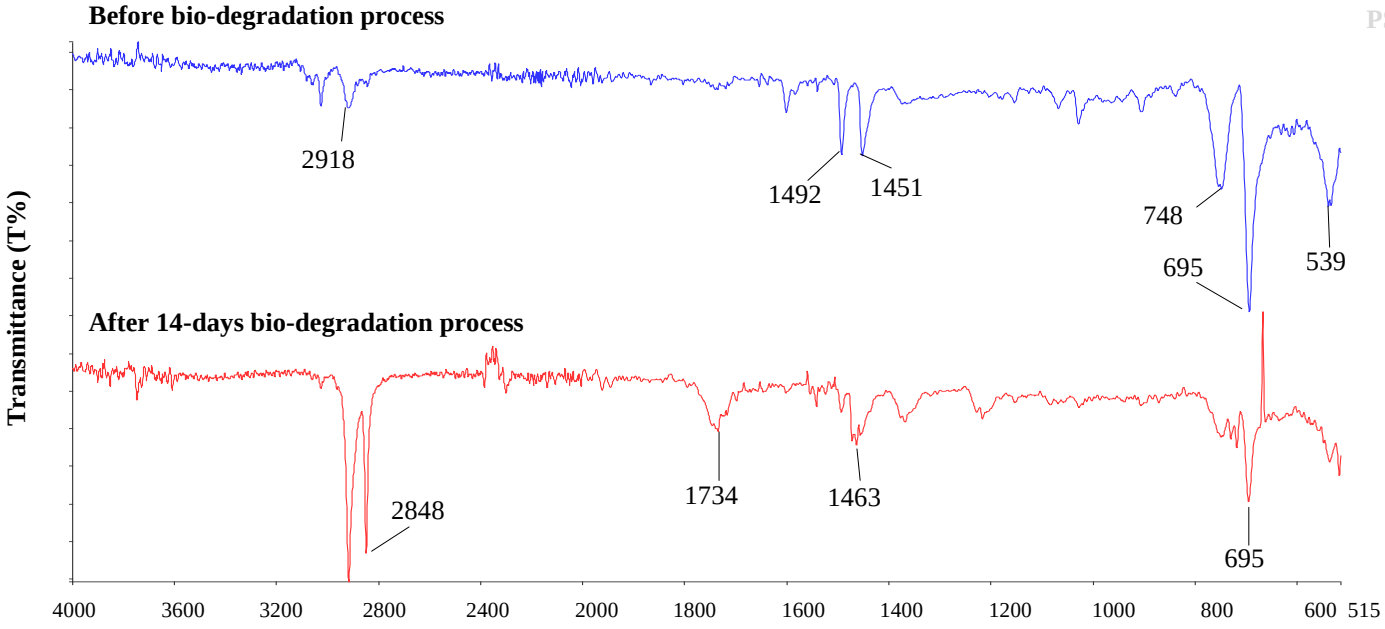

Fig. 4. FTIR spectrum of PS micro-plastic polymers

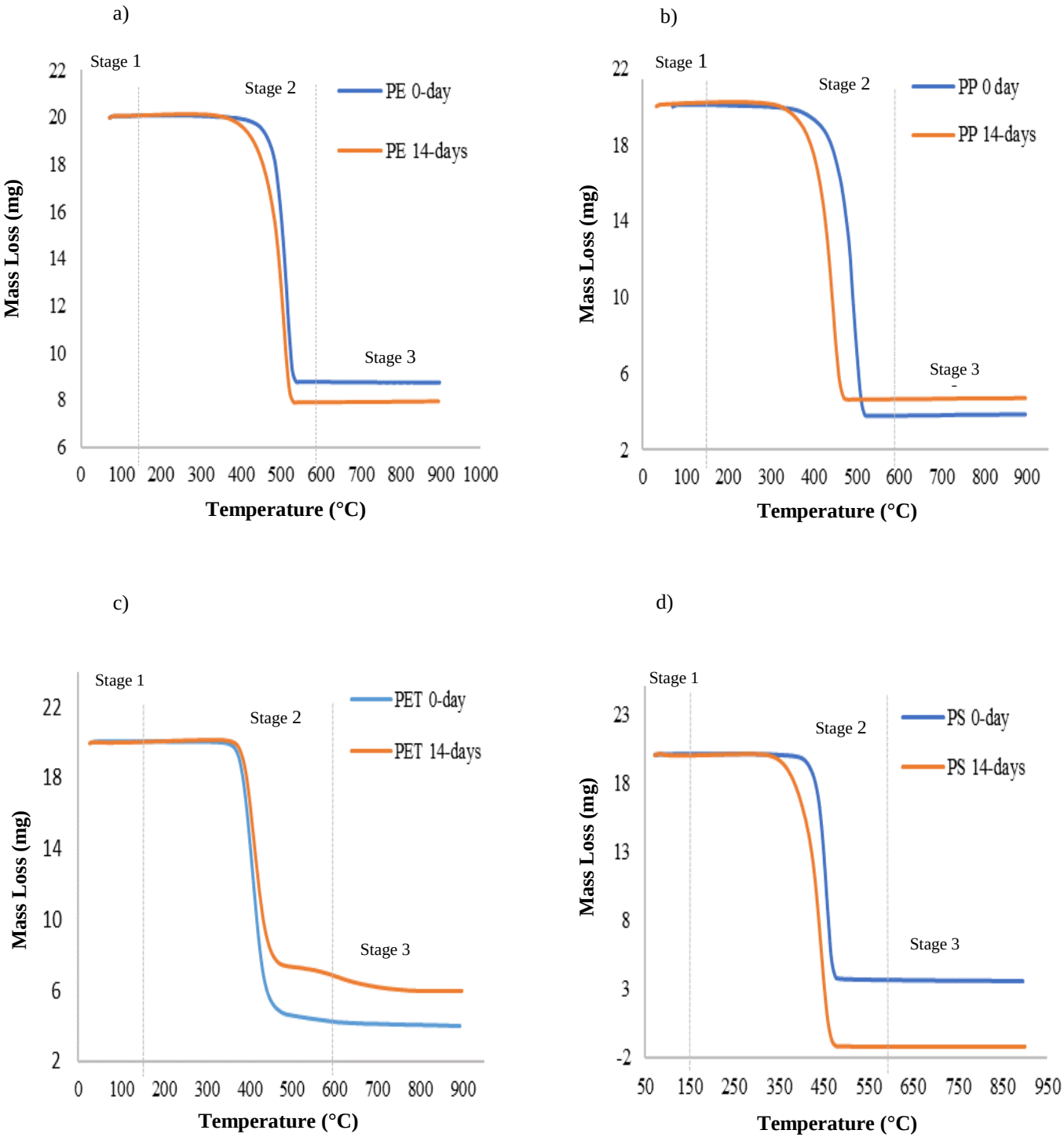

Fig. 5. Thermogravimetric Analysis (TGA) of micro-plastics polymers before and after degradation process, a) polyethylene (PE), b) polypropylene (PP), c) polyethylene terephthalate (PET), d) polystyrene (PS).

Table- III: The weight loss in three stages of micro-plastics thermal degradation

Polymers	Day 0			Day 14		
	Stage			*Stage*		
	1	*2*	*3*	*1*	*2*	*3*
	Percentage weight loss (%)			*Percentage weight loss (%)*		
PE	0.5	56.5	0.1	0.7	60.7	0.5
PP	0.3	81	1.5	1.0	76.9	1.1
PET	0.3	79	5.7	3.8	65.6	12.9
PS	0.4	81.9	2.8	0.1	94	2.5

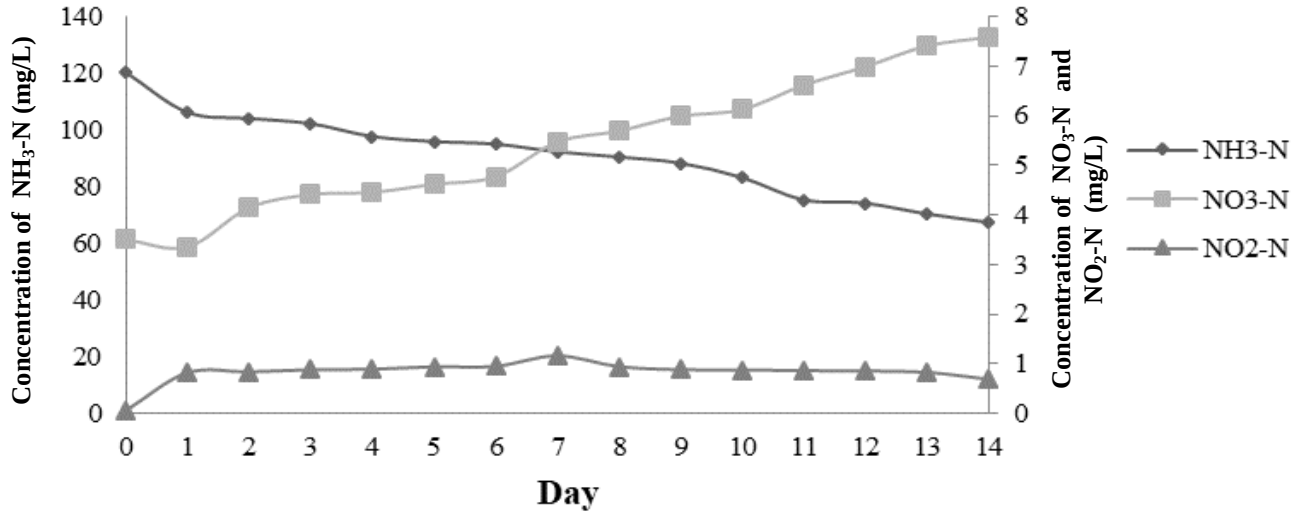

Fig. 6. The concentration of NH $_3$-N, NO_2-N, and NO_3-N in the batch culture containing PE micro-plastic

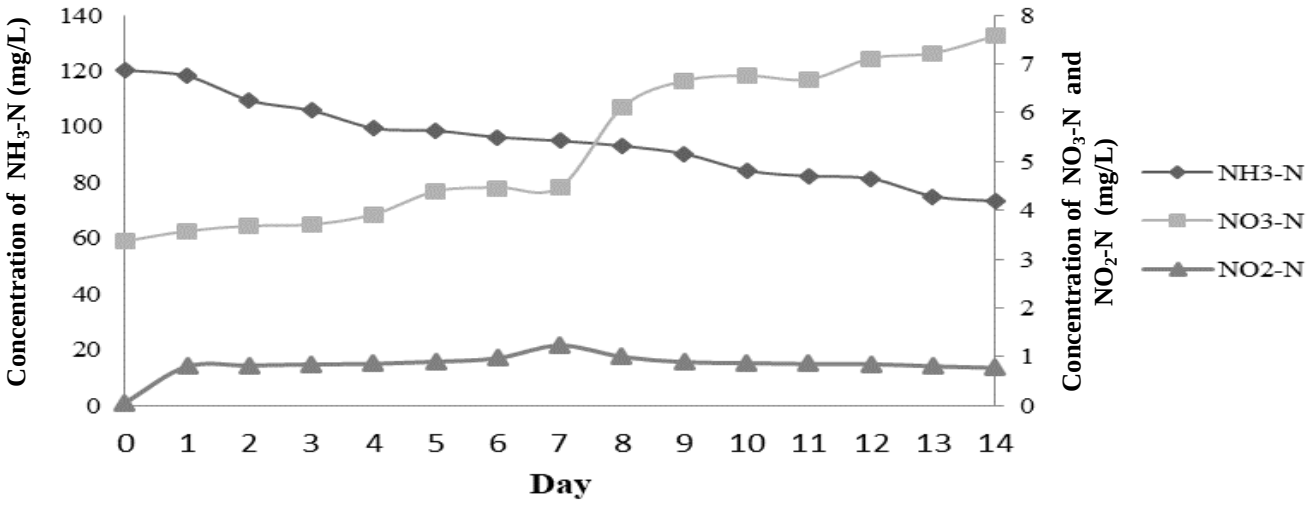

Fig. 7. The concentration of NH_3-N, NO_2-N, and NO_3-N in the batch culture containing PP micro-plastic

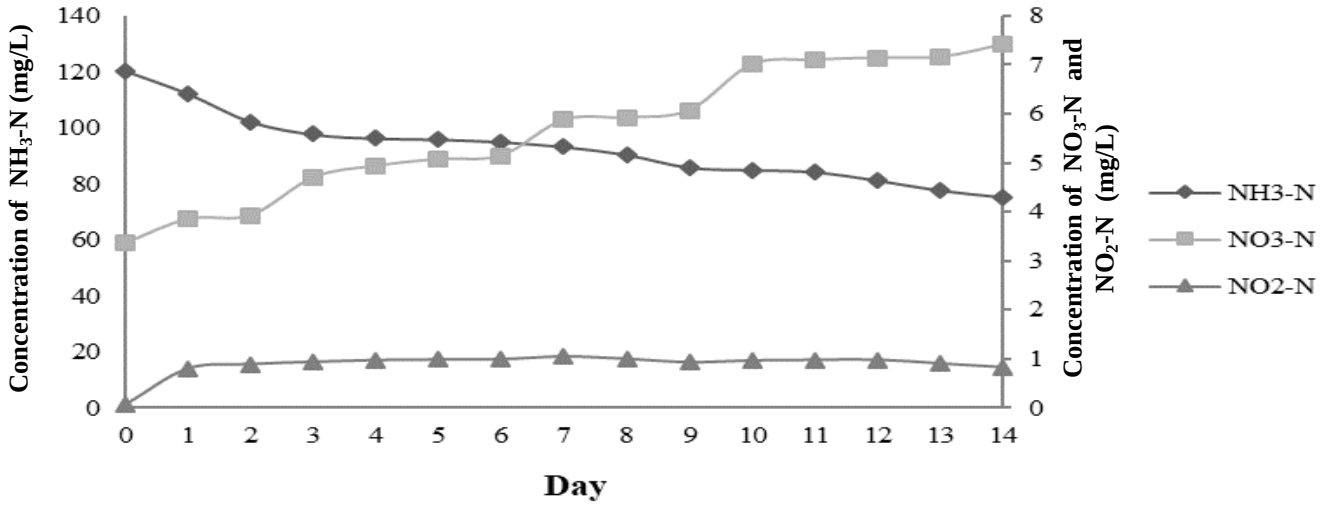

Fig. 8. The concentration of NH_3-N, NO_2-N, and NO_3-N in the batch culture containing PET micro-plastic

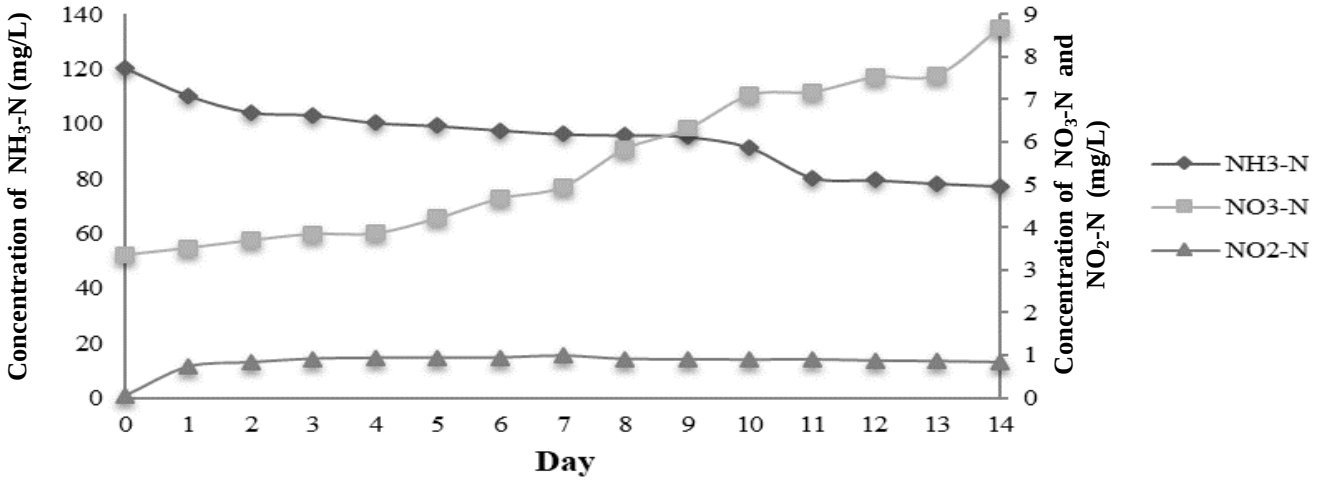

Fig. 9. The concentration of NH_3-N, NO_2-N, and NO_3-N in the batch culture containing PS micro-plastic

In the meantime, the concentration of nitrate-nitrogen slightly increased from day 0 to day 14 caused by the nitrite-oxidizing bacteria (NOB) utilized the nitrite-nitrogen to nitrate- nitrogen [18]. The high amount of ammonia-nitrogen in the water bodies will affect the water quality and resulting in an adverse impact on the aquatic organism. The exceed of ammonia-nitrogen also will disturb human health and contribute to the environment pollution. Thus, the ammonia-nitrogen need to be reduced before it enters the water body.

IV. CONCLUSION

Based on the result obtained, the micro-plastic (PP, PS, PET, and PE) in batch culture system can be degraded based on the percentage weight loss, removal of ammonia-nitrogen, changes of chemical structure and decomposition of micro-plastic polymers after 14-days incubation period. From the calculation of weight loss, PE micro-plastic showed the highest percentage of biodegradation in a batch culture which is 3.46 % compared with other micro-plastics. Besides that, PE micro-plastic also showed the highest removal of ammonia-nitrogen (NH_3-N) (44.17%) after 14 days in batch culture experiment. Besides, the micro-plastics effectively biodegraded after 14 days in batch culture, whereby all the chemical structure were changed involving the elongation, reduction, disappeared and the formation of a new peak has been detected. Furthermore, PS micro-plastics showed a greater thermal degradation by 81.9% (day 0) and 94 % (day 14) in the second stage of TGA analysis contrast with three other micro-plastic polymers. From this result, it can be observed that the micro-plastics have changed their chemical structure due to the biodegradation process. Longer incubation time is needed to evaluate more changes in the weight loss, removal of ammonia nitrogen (NH_3-N), chemical structure, thermal degradation and properties of the micro-plastic.

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