Marine Pollution Bulletin 127 (2018) 15–21

Contents lists available at ScienceDirect

Marine Pollution Bulletin

journal homepage: www.elsevier.com/locate/marpolbul

Growth kinetics and biodeterioration of polypropylene microplastics by *Bacillus* sp. and *Rhodococcus* sp. isolated from mangrove sediment

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ARTICLE INFO

Keywords:
Polypropylene
Rhodococcus sp. strain 36
Microplastics
Bacillus sp. strain 27
Biodegradation

ABSTRACT

Interest in the biodegradation of microplastics is due to their ubiquitous distribution, availability, high persistence in the environment and deleterious impact on marine biota. The present study evaluates the growth response and mechanism of polypropylene (PP) degradation by *Bacillus* sp. strain 27 and *Rhodococcus* sp. strain 36 isolated from mangrove sediments upon exposure to PP microplastics. Both bacteria strains were able to utilise PP microplastic for growth as confirmed by the reduction of the polymer mass. The weight loss was 6.4% by *Rhodococcus* sp. strain 36 and 4.0% by *Bacillus* sp. strain 27 after 40 days of incubation. PP biodegradation was further confirmed using Fourier-transform infrared spectroscopy and scanning electron microscopy analyses, which revealed structural and morphological changes in the PP microplastics with microbial treatment. These analyses showed that the isolates can colonise, modify and utilise PP microplastics as carbon source.

1. Introduction

Microplastics are tiny ubiquitous plastic particles that are < 5 mm in size and originate from two sources: primary microplastics (plastic manufactured intentionally for particular domestic and/or industrial application, such as exfoliating facial scrubs, resin pellets used in the plastic industry and toothpaste) and secondary microplastics (formed from the breakdown of larger plastic items under ultraviolet (UV) radiation or mechanical abrasion) (IMO, 2015). Microplastics enter the marine environment through several activities on land and sea. Microplastic beads in cosmetics, such as facial cleansers, synthetic clothing, toothpaste and scrubs, reach the marine ecosystem through domestic and industrial drainage systems and wastewater treatment plants (Auta et al., 2017; Murphy et al., 2016). Moreover, macroplastics that have been broken down into smaller fragments from waste dumps can be transported into seas and lead to microplastic pollution (Alomar et al., 2016). Polypropylene (PP) is one of such synthetic plastic materials. PP is a thermoplastic polymer made up of a carbon–carbon backbone built of carbon atoms (Gewert et al., 2015). It is a tough and flexible material used in both industrial and household applications, including textiles, labelling, stationery, packaging, automotive components and laboratory equipment. PP is the second most widely produced and used plastic polymer globally, after polyethylene. The global market for PP was reported to have hit approximately 55 million tonnes in 2013 (Market Study, 2014), which equated to a consumer market of $ 65 billion. In addition to being one of the most versatile polymers with applications of both as a fibre and plastic, PP adapts to various fabrications, and this ability enables PP to stand out as an invaluable material for a wide range of applications (Longo et al. 2011). In the five subtropical gyres (North Pacific, South Pacific, North Atlantic, South Atlantic and the Indian ocean), the Mediterranean Sea, Bay of Bengal and Coastal Australia, plastic particles were found at concentrations of 5.25 million particles weighing approximately 270,000 t (Eriksen et al., 2014). Plastic polymers are recalcitrant, are not easily susceptible to microbial degradation and, therefore, persist in the environment (Longo et al., 2011; Cacciari et al., 1993). Microplastics pose a danger to aquatic biota that ingests them and thereby add pressure to the already vulnerable ecosystem. PP is used in the production of cosmetics (Weinstein et al., 2016), and microplastics consisting of PP have been identified in submerged sediments (Ballent et al., 2016; Nor & Obbard, 2014). PP microplastics have been extracted from marine environments and organisms in the aquatic ecosystem. In some cases, PP microplastics have been extracted from the digestive tract of commercial fish (Neves et al., 2015), gooseneck barnacles (Goldstein & Goodwin, 2013), sea turtles (Caron et al., 2016) and whales (Fossi et al., 2016; Lusher et al., 2015).

In marine environments, microplastics also portray a novel ecological niche for microorganisms by offering support for microbial

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https://doi.org/10.1016/j.marpolbul.2017.11.036
Received 25 April 2017; Received in revised form 16 November 2017; Accepted 16 November 2017

colonisation and growth (Dussud & Ghiglione, 2014). Microorganisms that colonise plastic polymers instigate biodegradation by adhering to the surface of the polymer and forming biofilms. These microorganisms sometimes excrete extracellular enzymes that act on the polymer materials (Eich et al., 2015; Sekhar et al., 2016). Laboratory experiments have shown that plastic degradation by microorganisms begins within a few days to weeks. Biofilm is involved in surface biodegradation of plastics and possibly plays a significant role in microplastic biodegradation (Eich et al., 2015; Jeon and Kim, 2016; Sheik et al., 2015).

Considering the excessive use of plastics and the increasing pressure placed on capacities available for the disposal of plastic waste, the microbial degradation of synthetic plastic polymers, especially microplastics, is an important consideration to understand what is necessary for their biodegradation. The interaction that occurs between the microorganisms and the microplastic material and the biochemical changes involved needs to be elucidated. Enzymes produced by microbes are the most powerful tools for the biodegradation of plastic polymers. Microbes that can grow in different conditions and under specific stressful conditions and use plastic polymers as energy source need to be investigated.

Therefore, this study was aimed at demonstrating the growth and biodegradation ability of bacterial isolates from marine sediments in the degradation of synthetic PP microplastic. This study aims to identify a remedial option for the disturbing accumulation of microplastics in the marine environment.

2. Materials and methods

2.1. Microplastics

Isotactic PP microplastic granules (white, spherical) with a density of 0.9 g/ml at 25 °C, molecular weight of 250,000 Mw, average Mn of 67,000 and CAS number 9003-07-0 were obtained from Sigma Aldrich Chemical Co. (USA). For the degradation experiments, the PP microplastics were obtained by grating or cutting the PP granules and commercial PP plastic materials obtained from plastic-producing industries. These materials were treated using UV light (UV treated) and stored for further use. The sizes of the prepared plastic debris were measured using an optical microscope (IX71, Olympus, Japan) equipped with × 4 lens (Olympus).

2.2. Sample collection and bacterial isolation

Sediment samples were collected from the Matang mangrove area in Perak (4°50′25.8″N, 100°38′9.60″E) and Cherating mangrove area in Pahang (4°7′36.15″N, 103°23′29.46″E) in Peninsular Malaysia. The environmental temperature, pH and salinity of the mangrove sediments were 28.9 °C, 6.89 and 12.37 ppt for the Matang mangrove area and 28.4 °C, 6.20 and 21.50 ppt for the Cherating mangrove area, respectively. Samples were collected from different depths in the sediment at 1 cm to 5 cm. The bacterial cultures were isolated on nutrient agar (NA) plates by using serial dilution at 25 °C. All morphologically distinct colonies were purified through subculturing (thrice) and preserved on slants.

2.3. Identification of isolates

Extraction of the genomic DNA from the bacterial culture was performed using a standard phenol chloroform extraction procedure. The bacterial 16S rDNA was amplified using the primers 1492R and 27F. The following were the PCR conditions: 1 cycle (95 °C for 5 min) for initial denaturation, 30 cycles (95 °C for 30 s, 51 °C for 15 s and 72 °C for 2 min) for annealing and extension and 1 cycle (72 °C for 5 min) for the final extension of the amplified DNA. The PCR products were purified using standard methods and sequenced with primers 518F and 800R by using BigDye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems). The similarity search was conducted in silico using the BLAST database of NCBI. The phylogenetic and molecular evolutionary analyses were conducted using MEGA version 5. Bootstrap analysis with 100 replicates was performed to estimate the confidence of the tree topologies (Saitou and Nei, 1987; Tamura et al., 2004; Kumar et al., 2016).

2.4. Screening of bacterial isolates for PP degradation

The bacterial isolates were screened for their ability to make use of PP microplastics as sole carbon source. Each individual isolate was grown in Bushnell Haas (BH) medium (containing 0.20 g $MgSO_4$, 0.02 g $CaCl_2$, 1.0 g KH_2PO_4, 1.0 g K_2HPO_4, 1.0 g NH_4NO_3 and 0.05 g $FeCl_3$ per litre of distilled water) that was infused with 0.5 g PP microplastics and incubated for a period of 4 weeks at room temperature. Control set was maintained (inoculation on media without PP microplastics) simultaneously, and all media were observed for growth (Harshvardhan & Jha, 2013). All experiments were performed in triplicate.

2.5. Microbial inoculum preparation and assay for PP microplastic biodegradation

The bacteria isolated and identified as microplastic degraders were regrown as pure cultures on freshly prepared NA before inoculation into nutrient broth and were allowed to grow in a rotating shaker at 29 °C until cultures attained log phase (absorbance of 1.09 at 600 nm). The cell densities of the inoculum were adjusted to 3.8×10^8 CFU/ml (implying the concentration of bacterial cells at the beginning of the experimental set-up). Ten percent of the log phase cultures were inoculated into Erlenmeyer flasks containing 270 ml of BH medium and 0.5 g of sterilised PP microplastics (2.4 mm in diameter) for the biodegradation assay. For the control, un-inoculated BH broth supplemented with PP microplastics was maintained under similar conditions. The optical density (OD), pH and microbial counts were monitored every 10 days for a period of 40 days. All experiments were performed in triplicates.

2.6. Determination of weight loss of residual PP microplastics

The PP microplastics were recovered from the broth through filtration and sieving. The bacterial films colonising the plastic particles were removed by washing (four-step washing, with incubation time of 2 min for each step) the microplastics with 70% ethanol and then dried in a hot-air oven at 50 °C overnight. The residual polymer weight was determined to measure the extent of degradation by using the Sartorius ENTRIS 224-1S analytical balance with a readability of 0.0001 g (Mohan et al., 2016). The initial weights of the pre-incubated PP microplastic particles were also measured. The weight loss of the PP microplastics in percentage was determined using Eq. 1:

$$\%\text{weight loss} = \left(\frac{W_0 - W}{W_0}\right) \times 100 \quad (1)$$

where W_0 is the initial weight of the polymer (g) and W is the residual weight of the polymer (g).

2.6.1. Determination of polymer reduction rate

The data were further processed to determine the rate constant of PP microplastic reduction by using the first-order kinetic model based on the initial and final weights along specific intervals (10 days) (Alaribe & Agamuthu, 2015). Eq. (2) is as follows:

$$K = -\frac{1}{t}\left(\ln \frac{W}{W_O}\right) \quad (2)$$

where K is the first-order rate constant for PP microplastic uptake per day, t is time in days, W is the weight of residual PP microplastics (g)

and W_O is the initial weight of PP microplastics (g).

This model was adopted, because it gives a constant fraction per unit time present/removed within the microplastics.

2.7. Fourier transform infrared (FTIR) analysis of microplastic polymers

The changes in the structure of the microplastic polymers with subsequent bacterial incubation were analysed using FTIR spectroscopy (PerkinElmer 400 FTIR/FTFIR) in the frequency range of 4000–450 cm^{-1}. The analysis was conducted on PP microplastic samples incubated with bacterial strains and on the un-inoculated control PP microplastic samples.

2.8. Scanning electron microscopy (SEM) of PP microplastics

The morphology of the degraded PP microplastics was viewed after the experimental time (40 days) with the aid of SEM (Leica EM SCD005, Austria). The samples were sputter coated with a gold layer at 25 mA under an Argon (Ar) atmosphere at 0.3 MPa and visualised using SEM at magnification of 3,500 × (Sekhar et al., 2016).

2.9. Statistical analysis

The statistical analysis of data was conducted using analysis of variance (ANOVA) in the SPSS software 21.0 with the LSD post-hoc test at P-value = 0.05.

3. Results and discussion

3.1. Bacterial screening and identification

Mangrove environments are habitats characterised with microbial distribution and occurrence. They are highly productive ecosystems and home to unexplored microbial diversity, including bacilli and actinobacteria (Hong et al., 2009), which play vital roles in the degradation of organic and inorganic molecules (Chantarasiri, 2015). A global need exists to remedy plastic pollution, especially microplastics. One of the potential solutions is the use of microbes given that microorganisms are capable of utilising and metabolising a wide variety of organic and inorganic molecules. From the present study, six different bacteria were isolated from mangrove sediments. Out of the six isolates, two isolates (isolates 27 and 36) were capable of growing on PP microplastic-infused media and indicated significant clear zone diameter on the media after incubation. This finding implied that the two bacterial isolates possibly possessed the enzymatic components needed to degrade PP and were considered as possible PP microplastic degraders. Studies have demonstrated that bacteria from mangrove environments possess the ability to degrade plastics but at a slow rate (Kathiresan, 2003). Further identification of isolate 27 showed 99% homology with *B. cereus* and *B. thuringiensis*, and isolate 36 showed 99% homology with *Rhodococcus rube*r, as revealed by the 16S rRNA phylogenetic analysis in Fig. 1. In light of this, isolates 27 and 36 were referred to as *Bacillus* sp. strain 27 and *Rhodococcus* sp. strain 36, respectively.

3.2. Determination of weight loss and reduction rate of PP by bacterial isolates

The changes that occurred as a result of microbial action were assessed qualitatively by measuring the weight loss of the PP microplastics after inoculation with *Bacillus* sp. strain 27 and *Rhodococcus* sp. strain 36. The results are presented in Fig. 1. The weight loss of PP was 6.4% and 4.0% after 40 days of biodegradation assessment with *Bacillus* sp. strain 27 and *Rhodococcus* sp. strain 36, respectively. The highest weight loss was observed in the degradation of PP microplastics by *Rhodococcus* sp. strain 36 (6.4%), although *Bacillus* sp. strain 27 also depicted good degradation capacity (4.0%). This finding implied the ability of the bacterial isolates to excrete specific enzymes that can putatively attack UV-treated PP microplastics and consequently cause partial UV PP microplastic biodegradation after treatment with both isolates. The isolates possibly catalysed metabolic reactions that contributed to the adsorption, desorption and breakdown of the PP microplastics. In a different study, the degradation of polystyrene and polyethylene by *R. ruber* recorded a weight loss of 12.4% and 7.5% after 42 and 56 days, respectively (Sivan et al., 2006). The high weight loss exhibited by *Rhodococcus* sp. strain 36 could be due to the discrete potential of the microbe to partially biodegrade PP microplastics. Microorganisms possess different characteristics; hence, degradation varies from one microbe to another (Bhardwaj et al., 2012). No weight loss of PP microplastics was observed in the control (un-inoculated) flask. The weight loss of PP microplastics after incubation could be a result of microbial activity and indicated not only the percentage decrease in weight but also the loss of certain properties, hence hinting at the physical breakdown and degradation of PP microplastics by the microbes (Board, 2006).

This study further determined the removal rate constant (K) of PP microplastics per day by the two isolates by using the first-order kinetics and the half-life ($t_{1/2}$) (i.e. the time it will take for half of the PP microplastics to be reduced). Table 1 shows that between the two isolates, *Rhodococcus* sp. strain 36 recorded a higher PP microplastic uptake rate of 0.002 day^{-1} and a shorter half-life of approximately 346 days. The higher uptake rate by *Rhodococcus* sp. strain 36 was possibly be due to the genetic make-up of the microbe and its greater tolerance for the PP microplastics. *Bacillus* sp. strain 27 recorded a removal rate of 0.001 day^{-1} and a longer half-life of approximately 693 days. The calculated rate constants and corresponding half-lives further bolstered the degree of activities within the PP microplastic-infused BH medium.

3.3. Growth pattern of Bacillus sp. strain 27 and Rhodococcus sp. strain 36 on exposure to PP microplastics

The growth pattern exhibited by the microbes was evaluated, and the results are presented in Fig. 2. *Rhodococcus* sp. strain 36 showed significant growth upon exposure to UV-pretreated PP microplastics. An exponential growth response was observed from day 0 (0.251 OD600) to day 10 (0.903 OD600). However, increasing growth rate on the 10th day did not imply high response and performance of the isolate upon exposure to PP microplastics. The increase showed that the period was favourable for the interaction between the bacterial cell membrane and the PP microplastics and consequently allowed for rapid metabolism. The growth of the bacteria accelerated towards a positive growth pattern from 0.903 OD600 to 1.01 OD600 on the 20th day, which depicted the highest growth of the isolate. OD increase coincided with increasing bacterial counts. The highest bacterial cell counts (2.4×10^{11} CFU/ml) were also recorded on the 20th day of the experiment, followed by a reduction in the number of cells. *Bacillus* sp. strain 27 exhibited an exponential increase in growth from set-up time (0.25 OD600) up to the 10th day (1.38 OD600), which was the highest. A similar trend was observed in bacterial cell counts during the same period, with the highest counts (7.3×10^{11} CFU/ml) recorded on the same day. Afterward, the bacterial cell counts continued to decrease up to the last day of the experiment. Increasing microbial biomass was possibly be due to substrate utilization by both isolates, which could have resulted in the biodegradation of the PP microplastics (Okpokwasili & Nweke, 2005). A sharp decline in the growth of the isolate on the 20th day up until the 40th day on exposure to the PP microplastics was observed. The decline phases attained by the isolates was possibly be due to the lysis of cells, nutrient depletion or presence of inhibitory products in the culture media. Generally, *Rhodococcus* sp. strain 36 exhibited more tolerance between the 10th and 20th days of exposure to the PP microplastics than *Bacillus* sp. strain 27 even though the microbe recorded the highest growth on the 10th day (1.38 OD600). The growth pattern of the

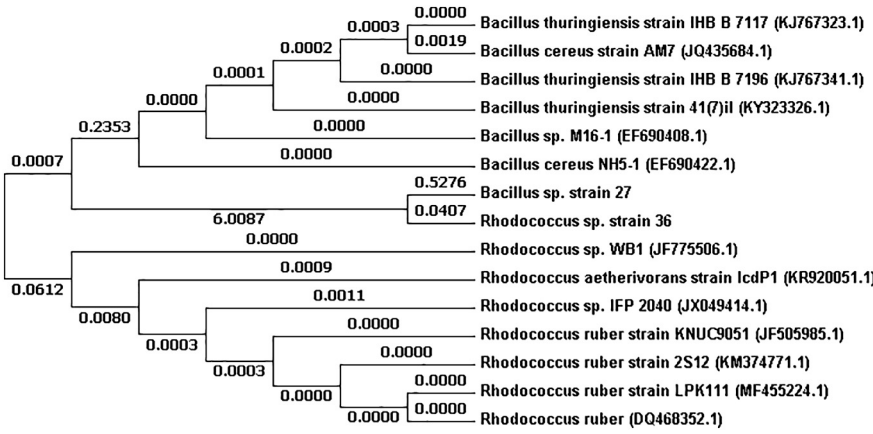

Fig. 1. Phylogenetic dendrogram of the relationship between the 16S rRNA gene sequences retrieved from GenBank and the 16S rRNA of the best degradative *Rhodococcus* sp. strain 36 and *Bacillus* sp. strain 27. The evolutionary relationship was inferred using the neighbour-joining method. The evolutionary distances were computed using the maximum composite likelihood method and presented in the units of the number of base substitutions per site. The analysis involved 15 nucleotide sequences, and ambiguous positions with gaps were removed. All evolutionary analyses were conducted in MEGA7.

isolates did not significantly differ ($P > 0.05$) during the different days of incubation. The differences in the metabolic rates and associated genetic alterations within bacterial species were possibly responsible for the varied responses exhibited by the bacterial isolates. The higher tolerance of *Rhodococcus* sp. strain 36 to PP exposure than *Bacillus* sp. strain 27 was possibly due to the potential of *Rhodococcus* sp. strain 36 to significantly influence the bonds of the PP microplastics. Hence, *Rhodococcus* sp. strain 36 was able to maintain its metabolic activities. Furthermore, the ability of *Rhodococcus* sp. strain 36 to tolerate the PP microplastics was evident along the lag phase because its reduction in OD was not as steep as that demonstrated by *Bacillus* sp. strain 27 on exposure to the same PP microplastics. Therefore, *Rhodococcus* sp. strain 36 exhibited a higher metabolic potential on the PP than *Bacillus* sp. strain 27 based on the observed tolerance that was higher than that of *Bacillus* sp. strain 27.

The population count of *Bacillus* sp. strain 27 and *Rhodococcus* sp. strain 36 during the biodegradation period is presented in Fig. 3. The counts of *Bacillus* sp. strain 27 increased from the initial count (3.8×10^8 CFU/ml) at the start of the experiment to 7.3×10^{11} CFU/ml on the 10th day. This finding depicted that the microbe adapted to the culture conditions of the PP microplastic culture media and were probably utilising the PP microplastics as a carbon source for growth. The counts of *Rhodococcus* sp. strain 36, however, decreased (1.1×10^8 CFU/ml) with counts that were lower than the initial count recorded at the start of the experiment. This outcome was possibly due to the inability of the microbe to completely adapt to the culture conditions or the presence of degradation metabolites that might have rendered the culture media unfavourable for growth and multiplication. Vásquez-Murrieta et al. (2016) reported that when a population is introduced into a foreign environment, the population count tends to decrease with time due to biotic or abiotic factors. On the 20th day of biomonitoring, the count of *Bacillus* sp. strain 27 decreased, whereas the count of *Rhodococcus* sp. strain 36 increased to 2.4×10^{11} CFU/ml. This finding indicated that some degradation metabolites were possibly produced that made the culture environment favourable for the growth and proliferation of the isolate. On the 30th and 40th day, the counts of both microbes further decreased. The *Rhodococcus* sp. strain 36 recorded the highest count of 4.2×10^{10} CFU/ml and 4.5×10^7 CFU/ml on the 30th and 40th day, respectively.

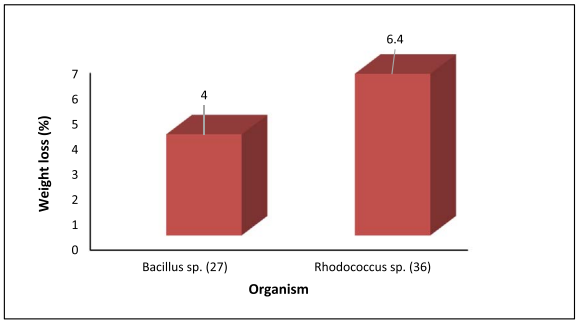

Fig. 2. Weight loss of microbially treated PP microplastics after incubation for 40 days. Maximum weight loss (6.4%) was obtained for PP microplastic inoculated with *Rhodococcus* sp. strain 36.

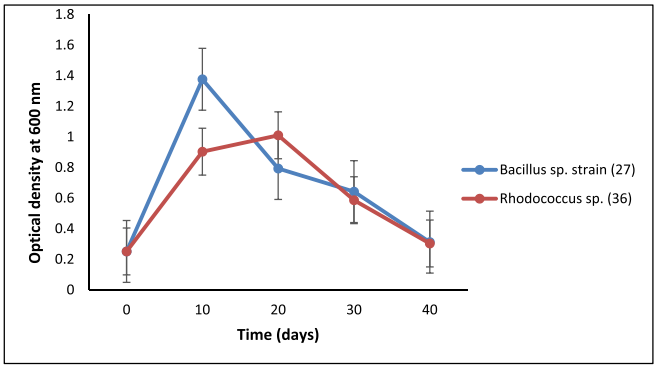

Fig. 3. Growth curve of *Bacillus* sp. strain 27 and *Rhodococcus* sp. strain 36 during biodegradation assay.

3.4. Changes in the pH of the aqueous media during biodegradation assay

pH is a key factor for the survival and activity of microorganisms as it has crucial influence on microbial population, enzyme activity and the rate of degradation (Xu et al., 2011; Gu, 2003). Fig. 4 illustrates the changes in the pH of PP microplastic-infused culture mineral media during the 40 days of biodegradation assay with *Bacillus* sp. strain 27

Table 1
Mass reduction efficiency of *Bacillus* sp. strain 27 and *Rhodococcus* sp. strain 36 in PP microplastic-infused BH media.

Organism	Initial weight (g)	Final weight (g)	Weight loss (%)	F-value	R^2	Sig.	Removal constant (K) day^{-1}	Half-life (days)
Control	0.500	0.500	0				0	∞
Rhodococcus sp. strain 36	0.500	0.468 ± 0.009	6.4	2.658	0.8866	0.149	0.002	346.5
Bacillus sp. strain 27	0.500	0.480 ± 0.013	4.0		0.582		0.001	693

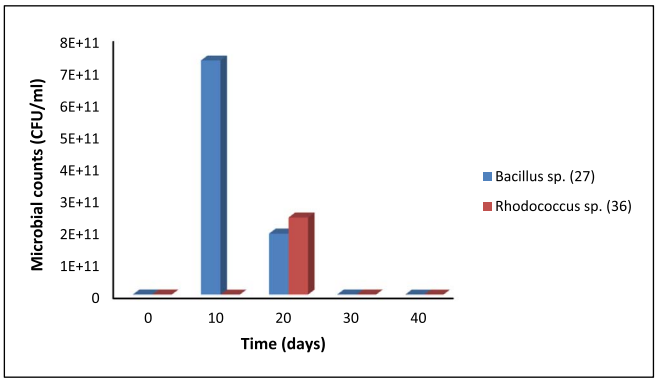

Fig. 4. Counts of *Bacillus* sp. strain 27 and *Rhodococcus* sp. strain 36 in PP microplastic-infused media during 40 days of biodegradation. Bars indicate standard error (n = 3).

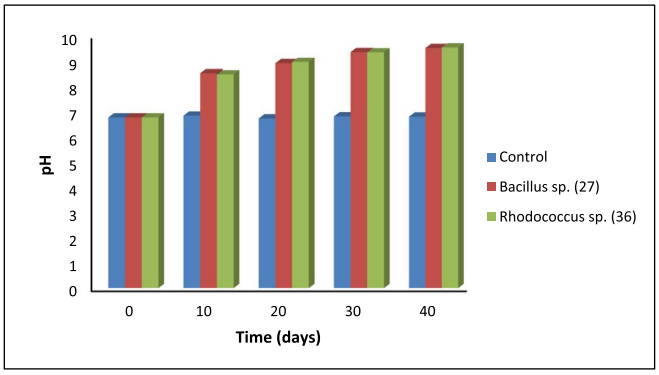

Fig. 5. pH changes of PP culture media inoculated with *Bacillus* sp. strain 27 and *Rhodococcus* sp. strain 36 during the 40 days of incubation.

and *Rhodococcus* sp. strain 36. The degradation of the PP polymers significantly increased the pH of the aqueous media towards alkalinity. Similar pH variations were observed for both microbes. The pH progressed from the initial neutral 6.76 to 8.51 and 8.46 for *Bacillus* sp. strain 27 and *Rhodococcus* sp. strain 36, respectively, on the 10th day. The pH increased continuously up to the 40th day, which recorded the pH values of 9.51 and 9.53 for *Bacillus* sp. strain 27 and *Rhodococcus* sp. strain 36, respectively. The optimum growth of *Bacillus* sp. strain 27 (1.376 OD600) was achieved on the 10th day when the pH reached 8.51, whereas *Rhodococcus* sp. strain 36 achieved optimum growth (1.01 OD600) on the 20th day at pH 8.96. This outcome implied that these pH values were the optimum pH for the growth of the isolates. Increasing in pH towards the alkaline range with time can be attributed to the production and accumulation of basic aromatic compounds and/or other metabolites in the PP microplastic-infused media and suggested that pH-modulating metabolites were produced by the isolates. Hence, progressive degradation occurred that changed the microstructure of the polymer matrix. Further increase beyond the stated pH values led to a decline in the growth of the microbes. Xu et al. (2011) observed rapid microbial degradation of polylactic acid at pH 8.0. (See Fig. 5.)

3.5. FTIR spectroscopy analysis of microplastic polymers

The changes in the microplastic structures with subsequent bacterial inoculation were determined using FTIR spectroscopy (PerkinElmer 400 FTIR/FTFIR) in the frequency range of 4000–450 cm^{-1}. The FTIR spectra of the pristine PP microplastic, UV-treated PP microplastic, PP microplastic incubated with *Bacillus* sp. strain 27 and PP microplastic inoculated with *Rhodococcus* sp. strain 36 for a period of 40 days in aqueous media are shown in Fig. 6 (a), (b), (c) and (d), respectively. In

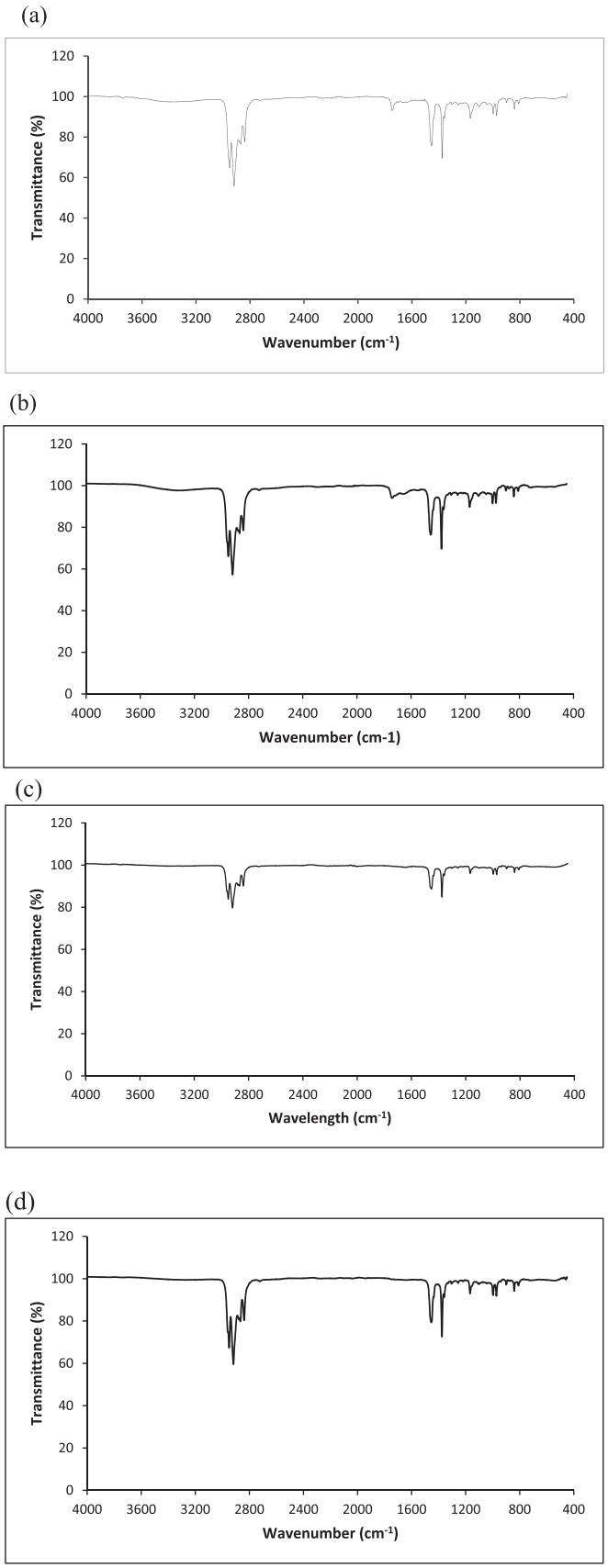

Fig. 6. (a) FTIR spectrum of pristine PP microplastic, (b) UV-treated PP microplastic (c) PP microplastic inoculated with *Rhodococcus* sp. strain 36 and (d) PP microplastic inoculated with *Bacillus* sp. strain 27.

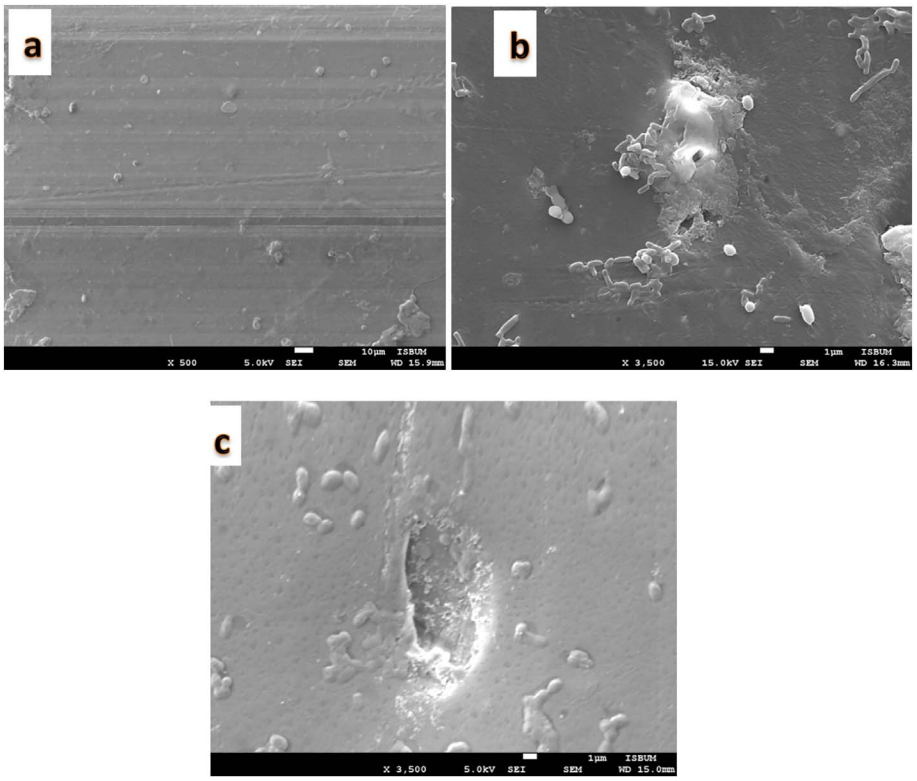

Fig. 7. SEM micrographs of PP microplastics: (a) un-inoculated (control) PP microplastic, (b) PP microplastic inoculated with *Bacillus* sp. strain 27 and (c) PP microplastic inoculated with *Rhodococcus* sp. strain 36.

pristine PP microplastics (not exposed to UV radiation), absorption peaks were present at 1745 cm^{-1} and 1167 cm^{-1}, which were attributed to the C = O carbonyl and C–O phenolic bands, respectively. The peaks at 2839–2951 cm^{-1} were attributed to the C—H alkyl stretch, whereas those at 1456 cm^{-1} and 1376 cm^{-1} to the C—H bend of the methylene (CH_2) group and C—H bend of the methyl group (CH_3), respectively. The peaks at 899 cm^{-1}, 841 cm^{-1} and 809 cm^{-1} were assigned to the C—H alkyl bend. When PP microplastics were treated under UV radiation, a new absorption peak appeared at 3310 cm^{-1} attributable to the O—H of the hydroxyl band. The peaks at 1739 and 1167 cm^{-1} were attributed to the C═O stretch of a carbonyl group and C—O phenolic stretch, respectively. The bands at 2839–2950 cm^{-1} were for the C—H alkyl stretch, whereas those at 1456 cm^{-1} and 1376 cm^{-1} were attributed to the C—H bend of the methylene (CH_2) group and C—H bend of the methyl group (CH_3), respectively. The peaks at 899 cm^{-1}, 841 cm^{-1} and 809 cm^{-1} were attributed to the C—H alkyl bend. A new peak was also formed at 876 cm^{-1}, which was assigned to the C—H alkyl bend. On treatment with *Bacillus* sp. strain 27, the C═O carbonyl bands at 1745 cm^{-1} and 1739 cm^{-1} in pristine PP microplastics and UV-treated PP microplastics, respectively, disappeared. In addition, the O—H hydroxyl peak at 3310 cm^{-1} and C—H alkyl bend at 876 cm^{-1} in PP(B) disappeared in PP microplastics treated with *Bacillus* sp. strain 27. However, other absorption peaks at 1456 cm^{-1} and 1376 cm^{-1}, which were attributed to the C—H bend of the methylene (CH_2) group and C—H bend of the methyl group (CH_3), respectively, were present in PP microplastics treated with *Bacillus* sp. strain 27. Furthermore, the peaks at 899, 841 and 809 cm^{-1} assigned to the C—H alkyl bends were also present in PP microplastics treated with *Bacillus* sp. strain 27. The O—H hydroxyl band at 3310 cm^{-1} and C − H alkyl bend at 876 cm^{-1} in the spectrum of UV-treated PP microplastics disappeared on treatment with *Rhodococcus* sp. strain 36. The C═O carbonyl bands at 1745 cm^{-1} in the spectrum of pristine PP microplastics and 1739 cm^{-1} in UV-treated PP microplastics also disappeared. Other absorption peaks at 1456 and 1376 cm^{-1} assigned to the C—H bend of the methylene (CH_2) group and C—H bend of the methyl group (CH_3), respectively, and peaks at 899, 841 and 809 cm^{-1} assigned to the C—H alkyl bend were present in spectrum of PP microplastics treated with *Rhodococcus* sp. strain 36. The pretreatment of PP microplastics by UV radiation possibly decreased the hydrophobicity of PP microplastics and consequently increased their compatibility with the microbes. In addition, the introduction of C═O and O—H groups after UV treatment possibly increased the susceptibility of the PP microplastics to degradation by the isolates (Arutchelvi et al., 2008).

Furthermore, the presence of Mn metal ions in the PP microplastics may have aided the degradation given that the presence of metal ions in plastic polymers generate free radicals on the surface of the plastic polymer. The free radicals then react with oxygen to produce carbonyl groups (Jeyakumar et al., 2013). Oxidation decreases the hydrophobicity of the plastic, and this behaviour may have aided the attachment of the isolates on the PP microplastic surface. Loss or disappearance of peaks corresponding to ester linkages was similarly reported by Russel et al. (2011). Formation of new groups, such as hydroxyl and carbonyl groups, were also observed by Iwamoto and Tokiwa (1994) and Alariqi et al. (2006) in their study of the biodegradation of PP.

3.6. SEM observation of degraded PP microplastics

The gravity of biodegradation was validated by the morphological changes observed using SEM. After 40 days of incubation, the untreated (control) PP microplastics exhibited a smooth surface (Fig. 7a), whereas the microbially treated PP microplastics exhibited attachment or colonisation of the bacterial isolates on the surface of the microplastics (Fig. 7b). Various pores/pits and irregularities formed as a result of microbial activity (Fig. 7 (a) and (b)). These surface changes observed in the SEM micrographs indicated surface damage to PP treated with *Bacillus* sp. strain 27 and *Rhodococcus* sp. strain 36. This outcome implied that the isolates were able to adhere and colonise the surface of the PP polymer and cause surface damage. The formation of biofilms on the PP polymers may have resulted in the secretion of extracellular enzymes by the isolates. The enzymes could have enabled the isolates to enter the pores and grow inside, thus increasing the pore size and

provoking cracks that might have weakened the properties of the PP polymer. Consequently, the polymer was reduced. As a reference to earlier studies on the degradation of plastics, considerable research have reported similar morphological changes on LDPE and PP biodegradation by microorganisms (Gajendran et al., 2016; Jeon and Kim, 2016; Sheik et al., 2015; Syranidou et al., 2017). In a study conducted by Bonhomme et al. (2003) on the degradation of polyethylene, SEM analysis confirmed that microorganisms built up on the surface of polymer and the surface also became physically pitted and eroded after removal of the microorganisms.

4. Conclusion

This study highlighted the potential of marine bacteria, namely, *Bacillus* sp. strain 27 and *Rhodococcus* sp. strain 36, isolated from mangrove environments in the degradation of PP microplastics. The study responded to the current need for tests to be conducted on bacteria (especially those of marine origin), which possess properties that could degrade microplastics accumulating in the aquatic environment. The isolates were able to grow in aqueous synthetic media containing PP microplastics. *Rhodococcus* sp. strain 36 recorded a weight loss of 6.4%, whereas *Bacillus* sp. strain 27 recorded a weight loss of 4.0%. Growth of the isolates in the media led to changes in pH, which possibly resulted from the microbial degradation activities that influenced biodegradation. The biodegradation of the PP microplastics by the organisms was confirmed by the structural, morphological and chemical changes observed on the PP microplastics using SEM and FTIR analyses. These changes point to the ability of the isolates to utilise PP microplastics as a carbon source. This study contributed towards the possible environmental application of the isolates in the degradation of PP microplastics.

Acknowledgements

The authors thank the Postgraduate Research Fund (PPP) of the University of Malaya, Kuala Lumpur, for funding this research via grants (PG 135-2015B).

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