

Microbial colonization of microplastics in the coastal water of Jakarta Bay

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Abstract. Jakarta Bay's water and sediment had more microplastics than other Indonesian water regions during the Covid-19 pandemic. Those microplastics act like an artificial microbial reef, attracting many different communities of bacteria. In this study, we incubated two of the most prevalent types of microplastic polymers, polyethylene terephthalate (PET) and polyethylene (PE), in the coastal water of Jakarta Bay for two weeks to investigate the microbial communities that live in these particles and their potential uses. The 16S rRNA gene's amplicon sequencing revealed that the orders Bacillales, Eubacteriales, Lactobacillales, Flavobacteriales, and Hyphomicrobiales made up the majority of the bacterial populations on two types of microplastic particles and coastal water. We discovered considerable changes in the makeup of biofilm communities on PE and PET compared to coastal water, indicating that particular microbial communities prefer to colonize specific polymer types. Surprisingly, the findings reveal a significant diversity of sulfur-oxidizing bacteria in two examined plastic types, with *Sulfurovum* sp. dominating the overall bacterial community. However, their participation in the plastic-associated biofilm and potential significance in plastic degradation require additional investigation.

Key Words: biofilm, microbes, microplastics, seawater.

Introduction. Microplastic pollution has become a major environmental concern because of the increase in plastic waste during the COVID-19 epidemic. Since the pandemic began, the daily production of plastic trash has increased to 1.6 million tons. The daily expected waste due to the COVID-19 pandemic is 3.4 billion single-use facemasks or face shields. It was estimated that by the end of 2020, Indonesia, which has the world's fourth-largest population, will have produced 123 million discarded facemasks daily and over 21 million tonnes of plastic garbage (Benson et al 2021). Because of a combination of physicochemical and mechanical factors, such as exposure to ultraviolet light, temperature, hydrophobicity, wind, and current, or possibly due to microbial degradation, the polymer may degrade into smaller particles (Khoironi et al 2020). Recent studies on the identification and quantification of microplastics in Jakarta Bay coastal water and sediment during the COVID-19 pandemic revealed that the abundance of microplastics in Jakarta Bay was significantly greater than in other water regions in Indonesia, with polyethylene terephthalate (PET), polyethylene (PE), polystyrene (PS), and polyamide (PA) being the most prevalent microplastic polymers (Azizi et al 2022).

The potential for microplastics to be ingested and bioaccumulated by aquatic organisms, as well as the release of plasticizers and other additives such as pesticides, polychlorinated biphenyls, polycyclic aromatic hydrocarbons (PAHs), through leaching and subsequent microplastic particle disintegration, raises concerns about their presence in the environment (Wagner 2014). Microplastics also behave as an artificial microbial reef, attracting a variety of bacterial microbiota communities known as plastisphere (Vidal-Verdú et al 2022). It is unclear, however, if different polymers host and support distinct microbial communities, whether microbial communities on microplastic differ from those on other inert surfaces, and whether bacteria inside these biofilms have the ability to degrade the polymers.

In this study, we carried out a 2-week long colonization experiment of two most common microplastic polymers (PE and PET), in the coastal water of Jakarta Bay, Indonesia. Our objectives were to examine the microbial communities on PE and PET

particles along with the seawater, and to identify taxa possibly significant for plastic breakdown with an emphasis on genera including hydrocarbon degraders.

Material and Method

Description of the study sites. This study was conducted in July, 2022 on the Muara Angke coast, which is part of Jakarta Bay and has shallow waters with an average depth of up to 15 meters, at coordinates 6°6'13.71"S and 106°46'35.56"E (Figure 1). There are industrial zones, markets, merchant stalls, and maritime transportation terminals in the research area. Furthermore, a large amount of inorganic trash was discovered on the coast of Muara Angke in the form of plastic bags, styrofoam, and plastic bottles.

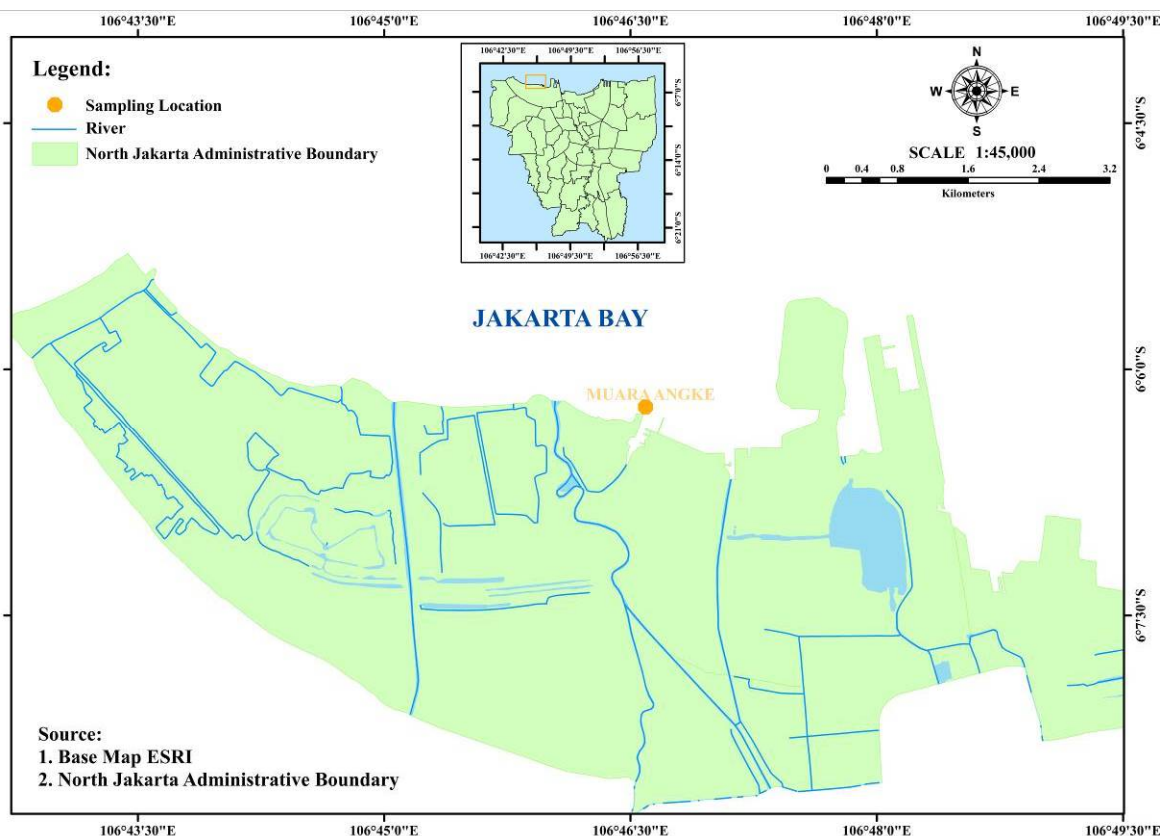


Figure 1. The microbial colonization site at Muara Angke, Jakarta Bay, Indonesia.

Microbial colonization. The colonization experiment has been conducted in the coastal waters of Muara Angke, Jakarta Bay. Approximately 1 g of each microplastic type (PE and PET) has been encased in nylon sachets (1 sachet per plastic) with a mesh pore size of 1 mm, anchored approximately 1 m below the surface at the base of the station's sensor platform approximately 3 m from shore, and sampled after 2 weeks for DNA extraction.

Sampling, DNA extraction, and pyrosequencing. PE and PET samples were taken from the seashore after 14 days incubation. Seawater as the original habitat was also subjected to DNA extraction and pyrosequencing. Prior to the DNA extraction, which was carried out using the PowerSoil DNA Isolation Kit, these samples were immediately placed in a freezer set to -20°C (MO BIO Laboratories, Carlsbad, CA, USA). Pyrosequencing was carried out with a few modifications to a methodology that was provided by Genetika Science Inc. (Jakarta, Indonesia). The DNA that was collected was put to use as a template in fusion PCRs that were performed on the hypervariable regions (V1–V3) of the genes that code for bacterial 16S rRNA. The primers for the bacterial sequences were V1-27F (5'-CCTATCCCCTGTGTGCCTTGGCAGTC-TCAG-AC-GAGTTTGATCMTGGCTCAG-3') (gene-specific sequences are underlined) and V3-518R (5'-

CCATCTCATCCCTGC GTGTCTCCGAC-TCAG-X-AC-WTTACCGCGGCTGCTGG-3'); the X barcode was uniquely designed for the sample, followed by the common linker AC.

PCRs were performed with the following settings: an initial denaturation at 94°C for 5 mins, followed by 30 cycles of denaturation at 94°C for 30 sec, annealing at 50°C, and elongation at 72°C for 90 sec, followed by a final elongation for 10 mins at 72°C. The amplicons were separated using a spectrophotometer after being purified with a QIAquick PCR Purification kit that was manufactured by Qiagen and located in Valencia, California, United States (NanoDrop Technologies, Wilmington, DE, USA). The purified PCR products (*1 µg of each sample) were utilized for the pyrosequencing process. All of the pyrosequencing procedures, such as the construction of a single-stranded DNA library, emulsion PCRs, and pyrosequencing reactions, were carried out by Genetika Science Inc. (Jakarta, Indonesia) using a Roche/454 GS Junior system in accordance with the instructions provided by the manufacturer.

Pyrosequencing data analyses. Data obtained through pyrosequencing were examined using methods that have been published earlier (Jeon et al 2013). In a summary, the demultiplexing process consisted of separating the raw data from each sample using their respective barcodes, and then excluding from further analysis any reads that were deemed to be of poor quality based on the average quality score. Pairwise sequence alignments and the hmm-search tool that is included in the HMMER 3.0 package were utilized in order to trim the primer sequences in accordance with the profile of the 16S rRNA V1–V3 regions (Eddy 2011). The sequencing mistakes were fixed by selecting the representative sequences from within each cluster of trimmed sequences and used those for taxonomy classification. Individual reads' taxonomic locations were calculated based on the reads with the highest pairwise similarity among the top five BLASTN hits made against the EzTaxon-e database (<http://eztaxon-e.ezbiocloud.net>). Chimeric sequences were eliminated using UCHIME (Edgar et al 2011). Alpha diversity indices were calculated by the MOTHUR Package (Schloss et al 2009).

Results

Microbial diversity and abundance. A total of 203,383 sequences were produced via DNA sequencing. There were 83,966 sequences in PE samples, 58,683 sequences in PET samples, and 60,734 sequences in seawater samples. Seawater and PET samples both contain a quite similar number of sequences, with the PE sample having the most sequences. According to Table 1, the Shannon Wiener index of PE has the highest index value of 4.107 ($H' > 3$) when compared to PET and seawater. This demonstrates a significant degree of diversity. This high diversity suggests that there are more species and that their relative abundance is greater. PE particles also had the greatest Simpson index 0.972 ($0.60 > D > 1.00$) when compared to PET and seawater. All three samples have Simpson index values close to one, indicating that bacterial species predominate.

Table 1
Alpha diversity indices of the bacterial 16S rRNA gene sequences from the pyrosequencing

Sample	Valid reads	Simpson (D)	Shannon Wiener (H')
PE	83966	0.972	4.107
PET	58683	0.970	4.089
Seawater	60734	0.968	3.929

There were 41 phyla identified at the phylum level for PE and PET samples, and 36 phyla identified for seawater samples. According to the table above, the input data has a relative abundance percentage value greater than 1%. Proteobacteria had the largest relative abundance in seawater samples, accounting for 54.55%, 47.83% in PE samples, and 45.27% in PET samples. The percentage of Actinobacteria in PE and PET samples is 8%, while ocean samples had a percentage of 4%. Other phyla with abundances more than 1% were discovered, including Actinobacteria, Bacteroidetes, Cyanobacteria,

Firmicutes, Planctomycetes, and Spirochaetes, with percentages ranging from 1.17 to 22.85%. The most prevalent phylum is the Proteobacteria. The findings of investigations on the microbial communities on the surface of marine microplastics (Amaral-Zettler et al 2015) also support this. Proteobacteria are typically Gram-negative bacteria, can have or not have flagella, have spherical, spiral, or rod-shaped cells, and can be facultatively or actively anaerobic or aerobic (Holt 2000).

The relative abundance at the order level revealed 164 orders in the PET sample, 168 orders in the PE sample, and 145 orders in the seawater sample. The information entered above is based on a percentage number greater than 1%. Bacillales had the largest relative abundance in seawater samples at 5.54 percent, in PE samples at 6.88 percent, and in PET samples at 7.86 percent, followed by Eubacteriales and Lactobacillales. In addition, several orders were discovered to have abundances greater than 1%, ranging from 1.15 to 6.91%. Changes of the bacterial communities at the ordo level are displayed in Figure 2 and Table 2.

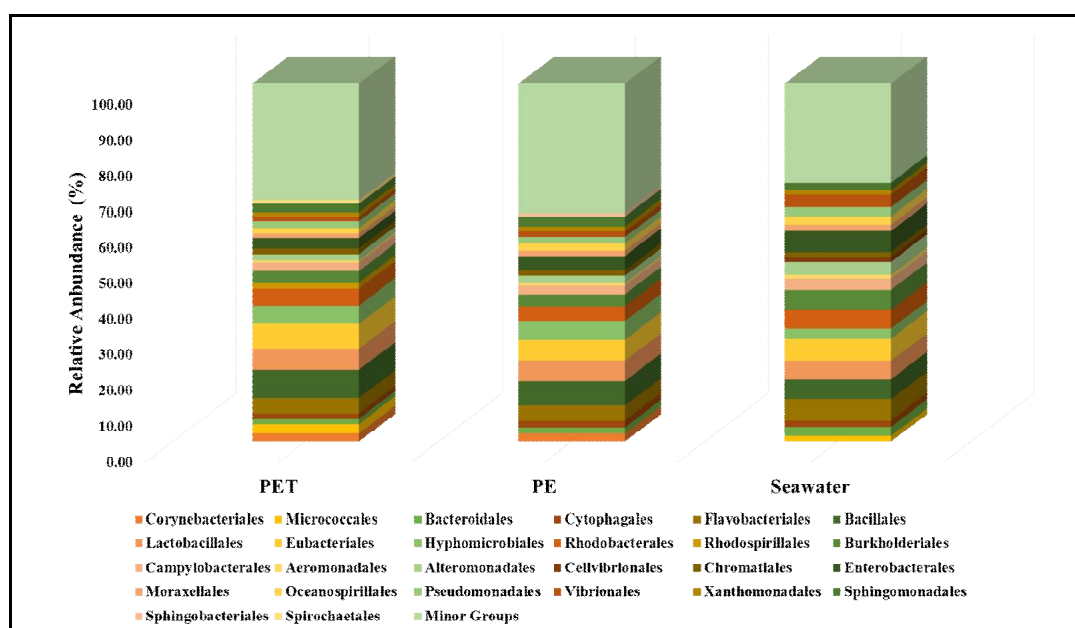


Figure 2. Changes of the bacterial communities at the ordo level.

Table 2

Changes of the bacterial communities at the ordo level

Phylum	Class	Ordo	%			
			PET	PE	Seawater	
Actinobacteria	Actinomycetia	Corynebacteriales	2.30	2.29	0.54	
		Micrococcales	2.60	2.46	1.59	
Bacteroidetes	Bacteroidia	Bacteroidales	1.45	1.66	2.52	
		Cytophagia	1.50	1.91	1.75	
Firmicutes	Bacilli	Flavobacteriales	4.30	4.16	5.98	
		Bacillales	7.86	6.88	5.54	
		Lactobacillales	5.91	5.69	5.15	
Proteobacteria	Alphaproteobacteria	Clostridia	6.91	5.82	6.20	
		Hyphomicrobiales	5.11	5.18	2.80	
		Rhodobacterales	4.75	4.21	5.26	
		Rhodospirillales	1.70	1.65	0.93	
		Sphingomonadales	2.30	2.76	2.08	
		Betaproteobacteria	Burkholderiales	3.25	3.31	5.54
		Epsilonproteobacteria	Campylobacteriales	2.40	2.38	3.18
Gammaproteobacteria	Aeromonadales	Aeromonadales	1.60	1.72	1.04	
		Alteromonadales	1.70	2.08	3.67	

		Cellvibrionales	0.95	0.97	1.21
		Chromatiales	1.60	1.53	1.54
		Enterobacterales	2.85	3.99	5.87
		Moraxellales	1.25	1.44	1.75
		Oceanospirillales	1.60	1.95	2.30
		Pseudomonadales	1.95	1.87	2.58
		Vibrionales	1.15	1.61	3.51
		Xanthomonadales	1.30	1.36	1.26
	Sphingobacteriia	Sphingobacteriales	0.85	1.02	0.87
Spirochaetes	Spirochaetia	Spirochaetales	1.05	0.89	0.82
		Minor group ($< 1\%$)	29.81	29.21	24.52
Total			100	100	100

The predominance of many *Sulfurovum* species was observed in this study including *Desulfobacula toluolica*, *Sulfurovum* sp. NBC37-1, and *Sulfurovum lithotrophicum* (Table 3). *Sulfurovum* was also the dominant microbe on PET microplastics in previous study (Vidal-Verdú et al 2022). Sulfur-oxidizing chemolithoautotrophic bacteria of the genus *Sulfurovum* are important producers in marine sediment communities (Mori et al 2018), and in some places they have even been identified as the dominant taxon in seafloor sediments (Sun et al 2014).

Table 3
Predominant bacterial communities at the species level

Species	%		
	PET	PE	Seawater
<i>Sulfurovum lithotrophicum</i>	30.36	25.27	1.39
<i>Sulfurovum</i> sp. NBC37-1	13.66	10.74	0.43
<i>Desulfosarcina widdellii</i>	1.72	2.01	0.01
<i>Trichodesmium erythraeum</i>	1.43	0.91	0.02
<i>Desulfatibacillum aliphaticivorans</i>	1.34	1.59	0.02
<i>Desulfobacula toluolica</i>	1.13	2.74	0.06

As may be seen in Table 4, the survey did not find any physiochemical properties typical of hydrothermal vents. However, hydrocarbon pollutants including gasoline, diesel, and mineral oil are present here in small quantities. Sediment buildup in the lee of the breakwater is thought to play a significant role in intercepting harbor runoff, including oil spills. *Sulfurovum* sp., as is commonly believed, is a natural bacteria found in volcanic regions and deep hydrothermal vents. Metabolic adaptability may play a significant role in managing hydrocarbon pollutants for survival, which may help explain why *Sulfurovum* sp. is so abundant in the studied location (Marziah et al 2016).

Table 4
Environmental parameters at colonization site

No	Parameter	Unit	Result
1.	pH	-	7.34-7.80
2.	Temperature	°C	26-27.2
3.	Salinity	ppt	21.5-22.5
4.	Dissolved oxygen (DO)	mg L ⁻¹	3.2-5.4
5.	Ammonia	mg L ⁻¹	0.8
6.	Nitrate	mg L ⁻¹	0.03
7.	Sulfate	mg L ⁻¹	2.220-2.224

According to Jacquin et al (2019), Gammaproteobacteria and Alphaproteobacteria are typically responsible for the colonization and biodegradation of plastics in the water, with Bacteroidota eventually becoming a significant group in their biofilms. Plastic materials are produced using a polymerization process that utilizes monomer basic ingredients, which are arranged into a link that fuses into a polymer. Plastics also contain a number of additives required to alter their chemical characteristics. Low-molecular-weight inorganic or organic substances are referred to as non-plastic components, which are introduced as additives. These additives can serve as colorants, antioxidants, UV absorbers, and anti-sticking agents (Hansen et al 2013).

Bacterial colonies on the plastic surface will form a biofilm (Das & Kumar 2014). These bacteria that degrade plastic will either convert the carbon in the polymer chain to carbon dioxide or incorporate it into proteins. This biodegradation process leads plastic to become brittle and split into smaller pieces, resulting in a low molecular weight plastic polymer chain that can be digested by microorganisms (Webb et al 2013).

Depolymerization, or the breakdown of complex chains, is a crucial phase in the polymer biodegradation process. Polymers are complex compounds that prohibit microorganisms from transporting polymers across cell membranes and thereby excreting extracellular enzymes to break down polymers outside the cell. Extracellular and intracellular depolymerase enzymes are involved in polymer biodegradation (Mohanani et al 2020).

Exoenzymes from microbes can degrade complicated polymers into short chains or simpler molecules like water-soluble oligomers, dimers, and monomers. These simple molecules will be used as a source of carbon and energy after passing through a semi-permeable membrane. Following that, it is introduced into the cell and formed assimilation to produce the end product of CO₂, CH₄, H₂O, and biomass. This entire process is called as mineralization (Arutchelvi et al 2008).

Potential of microbes in degrading polymers. According to earlier studies, a number of common bacteria detected in samples of PE and PET microplastics have the potential to break down polymers. Despite some bacterial domination, more studies are needed to demonstrate their capacity to break down polymers. Based on literature investigations, the following are some examples of microbial dominance in microplastics that can breakdown polymers (Table 5).

Table 5

A review on several examples of microbial dominance in microplastics that can degrade polymers

<i>This study</i>				<i>Previous study</i>
<i>Phylum</i>	<i>Ordo</i>	<i>Genus</i>	<i>Percentage (%)</i>	<i>Description</i>
Proteo bacteria	Campylo bacterales	<i>Sulfurovum</i>	PET 1.57 PE 1.68	<i>Sulfurovum</i> is a genus that also dominates PET samples in studies conducted in the Mediterranean Sea (Vidal-Verdú et al 2022).
Proteo bacteria	Pseudo monadales	<i>Pseudomonas</i>	PET 1.2 PE 1.21	<i>Pseudomonas</i> spp. can produce enzymes capable of degrading plastic polymers, such as serine hydrolase, esterase, and lipase. If there are no inhibitors in the environment that can interfere with enzyme function, the process of plastic degradation by this enzyme can take place optimally (Mohanani et al 2020).
Proteo bacteria	Vibrionales	<i>Vibrio</i>	PET 0.96 PE 1.46	<i>Vibrio</i> are Gram-negative bacteria with a short rod structure, a flagellum, and the ability to breathe aerobically. Plastics can be degraded by these bacteria (Foulon et al 2016).

Proteo bacteria	Moraxellales	<i>Acinetobacter</i>	PET 0.8 PE 1.03	The mass weight and molecular weight of Polystyrene (PS) particles were significantly reduced after 60 days of incubation with <i>Acinetobacter</i> sp AnTc-1. The results showed that the isolated strain of <i>Acinetobacter</i> sp. AnTc-1 has the ability to degrade PS (Wang et al 2020).
Firmicutes	Eubacteriales	<i>Clostridium</i>	PET 1.64 PE 2.03	<i>Clostridium</i> are spore-producing Gram-positive, rod-shaped, non-motile anaerobic bacteria. <i>Clostridium botulinum</i> , for example, can digest plastic trash under anaerobic conditions (Ghosh et al 2013).
Firmicutes	Bacillales	<i>Brevibacillus</i>	PET 1.94 PE 2.03	<i>Brevibacillus</i> spp. may break down polymers of the PE type to provide carbon sources. A biofilm is produced on the plastic surface by <i>Brevibacillus</i> species. The dry weight of the plastic will fall by 37.5% as a result of this plastic deterioration within 3 weeks (Nanda & Sahu 2010).
Firmicutes	Lactobacillales	<i>Streptococcus</i>	PET 1.33 PE 1.57	Filayani (2020) reports that utilizing the Winogradsky column method, <i>Streptococcus thermophilus</i> and <i>Lactobacillus bulgaricus</i> decompose polyethylene plastic. Within 20 days of incubation, this plastic degradation process results in the highest percentage of degradation, up to 19.47%.

Conclusions. Our 16S rRNA amplicon gene sequencing findings demonstrate that various microbial communities flourish on two types of types of microplastic polymers, polyethylene (PE) and polyethylene terephthalate (PET) and seawater. We found significant differences in the microbial community composition of biofilms on PE and PET from those in coastal water, suggesting that some microbial populations prefer to colonize particular polymer types. Sulfur-oxidizing bacteria were found to be abundant in both plastic types tested, with *Sulfurovum* sp. dominating the bacterial community as a whole. Future research should concentrate on isolating individual bacteria to assess their capacity to degrade plastics in order to better understand plastic degradation and the possible involvement of specific microbes in plastic degradation. This latter option would also facilitate additional research into their biochemical properties, genetic potential, and enzymatic utility.

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Conflict of interest. The authors declare that there is no conflict of interest.

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