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THE OCCURRENCE OF MICROPLASTICS ON THE START-UP PROCESS OF AN ANOXIC BIOFILM BATCH REACTOR

*Ansiha Nur[1], Mhd. Fauzi[1], Prayatni Soewondo[1], Ahmad Soleh Setiyawan[1], Katharina Oginawati[1]

[1]Faculty of Civil and Environmental Engineering, Institut Teknologi Bandung, Indonesia

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ABSTRACT: Seed sludge from several sources is often used to initiate bacterial growth at the start-up of the biological treatment system, and it contains microplastics contaminants. Currently, there is no information regarding the presence of microplastics in a biological reactor during the start-up process. This study aimed to investigate the occurrence of microplastics during the start-up process in an anoxic fixed-biofilm batch reactor. Three units of 42 Laboratory-scale reactor feed with synthetic biodegradable substrates were inoculated with fecal sludge residential areas were filled with PET media with three different specific surface areas (SSA). The results showed that the stable biofilm formation rates were associated with the removal of COD after 75 days of operation. The source of microplastics in the reactors during the start-up process comes from fecal sludge ($\bar{x}$ ± SD, n = 3) 7.666,67 ± 513.16 MP/kg (w/w). Approximately 7.96 – 9.24% of microplastics were adsorbed on the biofilm. No PET as secondary microplastics was found during the start-up process. Two types of microplastics found in the reactor were fibers and fragments with the amount of 80.87 ± 44.8% and 19.13 ± 10,1%, respectively. The types of polymers detected by the Attenuated Total Reflection-Fourier Transform Infrared (ATR-FTIR) spectroscopy were PES, PE, PET, and PS. SSA affects the adhesion of microplastics on PET media. In a larger SSA, more microplastics are adsorbed on the biofilm.

Keywords: Anoxic, Fecal sludge, Fixed-biofilm batch reactor, Microplastics, the Start-up process

1. INTRODUCTION

In recent years, the biological wastewater treatment process is still the most widely used method to remove organic pollutants and nutrients because of its high treatment efficiency and cost-effectiveness [1,2]. It has been proven by many types of research that fixed-bed biofilm reactor (FBR) is a promising technology over moving bed biofilm reactor (MBBR), such as greater diversity in the population of microorganisms [3], stability, and long retention time of microorganisms in removing pollutants, much less surplus biomass or sludge with good settling properties [4].

Inoculation refers to the initial biofilm adhesion to supporting media (bio-carrier) in attached growth systems during the start-up of the process. The start-up process is defined as the time required for the biofilm development rate to achieve a steady-state, which can range from 1 – 6 weeks [5,6]. Numerous laboratory-scale biological reactor experiments mostly employ residential fecal sludge or sewage-activated sludge as inoculum [7], due to its exceptional VSS value. Furthermore, some researchers have investigated MP in sewage sludge, which is a pathway for entering the environment. However, the existence of microplastics as a result of the use of residential fecal sludge as an inoculum during the anoxic reactor start-up procedure is still unknown. Currently, publications on microplastics raised concerns in the food chain as potential intake by humans and harm health [8,9]. Schwabl [10] have been investigated microplastics in human stool was 20 particles per microplastics in human feces found 1 particle/g to 36 particles/g (size 20 – 800 µm) in Beijing, China [11]. Estimation of microplastics consumption within the food and beverage range 39.000 to 52.000 particles annually by humans in America [8]. All of the metabolic products of the human body will end up in the form of feces as the final product.

Besides, numerous communal wastewater treatment plants in Indonesia use polyethylene terephthalate (PET) plastic bottles waste as a bio-carrier for microorganisms during the wastewater treatment process. Several investigations show that PET can be degraded through physical [12], chemical [13,14], and biological processes [15,16]. Microplastics have also been identified in PET mineral bottled water in other research [17-19]. As a result, it is critical to understand the potential for PET degradation as a secondary microplastic in the reactor. Microplastics are an emerging environmental pollutant less than 5 mm in size [20]. Based on the origin, the microplastics are classified as primary and secondary. Primary microplastics are originally manufactured in very small sizes, such as microbeads (facial scrub cleansers), industrial 'scrubbers' used to blast clean surfaces, and plastic powders used in molding [21]. Secondary microplastics are the result of decomposed, degraded, and fragmented larger

plastics into sizes smaller than 5 mm due to environmental mechanical, photo-oxidative, physicochemical, and biotic factors [22].

All this means that more data and mapping studies are needed worldwide to improve our knowledge of the occurrence of MP on the start-up process. To date, there have been limited studies on the occurrence of microplastics during the start-up of fixed attached growth systems. Most studies are still focused on bacteria accumulation, biomass adhesion, biofilm growth to support media to reduce the start-up duration within the reactor. Addressing this challenge, the present study has been carried out to identify the occurrence of microplastics in the fixed-bed reactor during the start-up process as a result of the use of fecal sludge as inoculum and PET degradation as secondary microplastic during the start-up process.

2. MATERIAL AND METHODS

2.1 Lab-scale Set-up

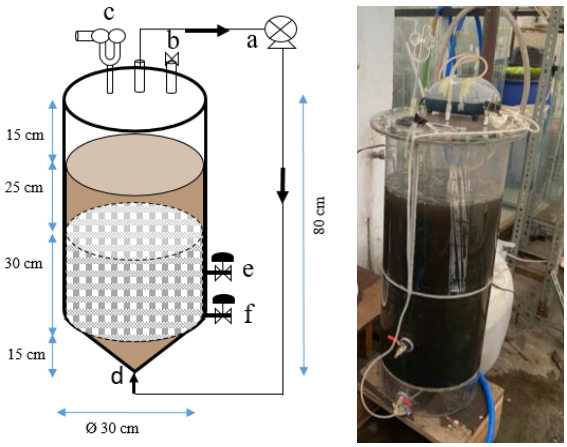
The lab-scale fixed-bed circulating batch reactor (CBR), was made from transparent acrylic material with a working volume of 42 L (Fig. 1). The CBR reactor was equipped with a recirculation pump to ensure that substrate and seed sludge were completely mixed well, allowing the biomass attached to the PET surface during the start-up process. Three units of CBR were employed under anoxic conditions. Each reactor was filled with PET modules with three different specific surface areas (SSA); Anx-1 (SSA-1; 555.78 m^2/m^3), Anx-2 (SSA-2; 444.53 m^2/m^3), and Anx-3 (SSA-3; 374.68 m^2/m^3) as shown in Fig. 2.

To obtain anoxic conditions in the CBR, the DO level in the wastewater was artificially removed by injecting N_2 gas into the bottom reactor for 15-20 minutes [23]. The N_2 gas injection pressure was set at ± 4.5 lbf/in^2. When the DO was less than 0.5 mg O_2/L, the air was pumped into the bottom of the reactor through a perforated plate using a recirculation pump. This design was set up to prevent sludge from settling at the reactor's bottom. During the experiment period, the operating temperature was kept in a constant temperature room. The microplastics mass balance was determined in the initial liquid (day 0) and at the end of the start-up process to identify the presence of microplastics in the reactor after 75 days of operation.

2.2 Synthetic Wastewater and Inoculum

Synthetic wastewater consisted of glucose as a source of carbon, NH_4Cl as a source of nitrogen, and KH_2PO_4 as a source of phosphate, and tap water of the Water Quality Research Laboratory, Environmental Engineering Institut Teknologi Bandung (ITB) as mixing water was used. Before use, the characteristics of tap water were checked for organic, nutrient, and microplastics content. The result showed that tap water was free from organic, nutrients, and microplastics. The C:N:P substrate ratio was used for optimum bacterial growth conditions, 100:5:1 for anoxic [25].

Inoculum or seed sludge was taken from a septic tank of one household, Bandung City. To monitor bacterial growth, VSS and COD were measured routinely according to SMEWW (Standard Methods for Examination of Water and Wastewater) 2540 E and 5220 I. During the start-up process, the optimum pH for bacterial growth was maintained at 6.5 – 7.5 [25,26] by checking the pH periodically and operating the reactor at room temperature, 25 – 27 ^{0}C. Anoxic DO levels were kept below 0.5 mg/L.



a = resirculation pump, b = feeding valve, c = siphon tube, d = perforated plate, e = sampling point, f = discharge valve

Fig. 1 Anoxic Circulating Batch Reactor

2.3 Identification and Quantification of Microplastics

There is no standard method for the quantification and identification of microplastics in a sample. However, various sample processing for identification and quantification methods for microplastics were employed. There were 3 types of samples processing for microplastics; synthetic wastewater, sludge, and biofilm. To remove natural organic from samples, 30% hydrogen peroxide (H_2O_2) solution was added to the samples, digesting using a magnetic stirrer under heat 60^{0}C until H_2O_2 fully evaporated [27].

Synthetic wastewater was filtered using a glass microfiber filter Whatman GF/C 1,2 µm pore size diameter 4,5 cm and dried in an oven heat 60 ^{0}C for approximately 30 minutes. Different from artificial

wastewater, sludge and biofilm samples were dried in an oven at 60°C for 24 hours before being digested with 30% H_2O_2 [27]. When it was dried, it mixed with distilled water. The next step was density separation for 24 hours with $ZnCl_2$ solution (density 1,5 g/cm^3) [28]. Both supernatants were filtered under a vacuum pump onto Whatman GF/C glass microfibre filter paper. Filter papers were observed by visual observation using an Olympus CX-21 light binocular microscope with 100x magnification coupled with Optilab Viewer 3.0 software. Observations on the surface of the filter paper in a zigzag manner [29]. The measurement of the particle by using Image Raster 3.0 software. Finally, all possible microplastics were collected with tweezers (BK-V9 SS-SA stainless steel), then the chemical structure was identified using ATR-FTIR (Bruker Alpha II Platinum ATR) at the Environmental Engineering Instrument Laboratory, ITB.

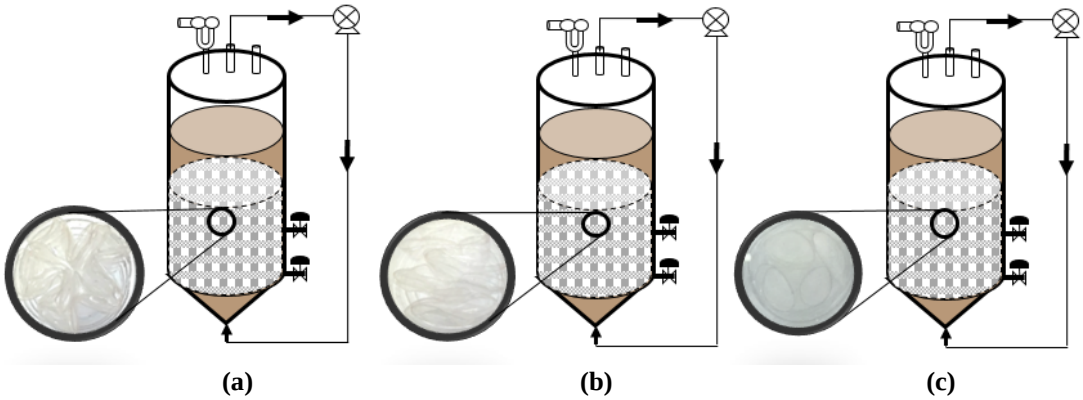


Fig. 2 Different SSA on CBR. a) Anx-1, b) Anx-2, c) Anx-3

3. RESULTS AND DISCUSSION

3.1 Seed Sludge as a Source MP in the Reactor

Although fecal sludge provided bacterial seeds for the start-up process, microplastics were widely detected in all of the sampled seed sludge during the start-up process in an anoxic batch reactor. The average of microplastics in the seed sludge ($\bar{x} \pm$ SD, n = 3) was 7,666.67 ± 513.16 MP/kg (w/w). It should be noted that if the residual sludge from seeding were released into laboratory drainage channels or plumbing systems, it contributes to the contribution of microplastic pollutants to the aquatic environment.

Figure 3 shows the distribution of size, shape, polymer, and color of microplastics in the seed sludge used. The dominant microplastics characteristics were less than 300 μm in size, in the form of fibers (80.87 ± 44.8 %), transparent (55%) in color, and polyester (PES) polymer (39%). PES types are mostly found in this activated sludge, then polyethylene terephthalate (PET) and polystyrene (PS) types. This was because its density was greater than water, so a lot of it settled in the sludge.

Density of PS 1.04 – 1.1 g/cm^3, Polyethylene (PE) 0.92 – 0.97 g/cm^3, PET 1.37 – 1.45 g/cm^3, PES 1.24 – 2.3 g/cm^3 and water 1.0 g/cm^3. Feces is a waste product of the human digestive. One of the potential routes of microplastics into the human body is through the ingestion process, consumption of food and beverages [30,31]. The food chain is strong evidence of the transfer of microplastics to humans [32]. Wright and Kelly [33] recently reviewed the effects of microplastic uptake on human health via gastrointestinal absorption. Microplastics cause health hazards if they enter the human body. Many factors influence microplastic absorption and translocation, and smaller particles transmit more. Larger plastics (>2 mm) easily enter the digestive system. The presence of microplastics found in human feces in Beijing 1 – 36 particles/gr (20 – 800 μm), PET (83.3%), polystyrene (PS) (50%), polyethylene (50%) [11], human feces in Europe and Asia, an average of 20 particles/10 g (50 – 500 μm) [8]. Schwabl [10] found sizes 50 – 500 μm and polypropylene (PP) (62.8%), PET (17%) polymers were the most commonly found. Its similar size with microplastics obtained in this study, less than < 500 μm. Also, a similar size of the human stool was found by Schwabl [10] and Zhang [11]. Compared with the previous study, the differences in polymers that were found in this study, as an effect of the difference in food and beverage consumption in a country.

Several studies on investigating the potential sources of microplastics from various types of food and beverage have been reported. Microplastics were found in foods and beverages commonly consumed by humans such as sea salt, lake salt [34],

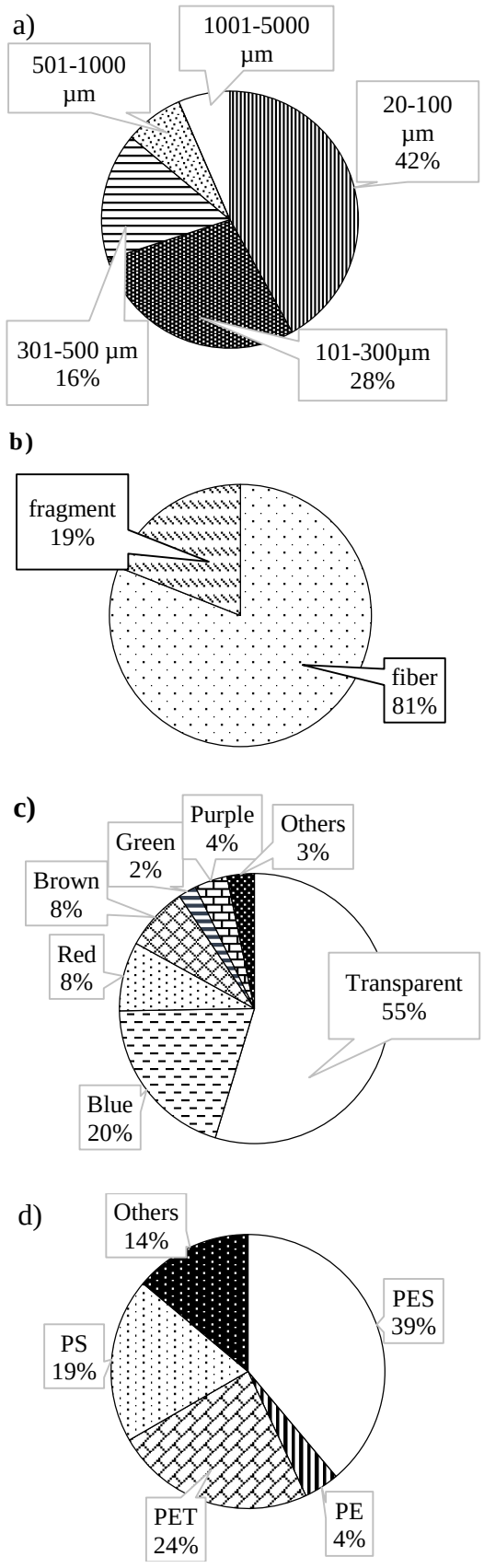


Fig. 3 Distribution of microplastics characteristics in activated sludge. a) size, b) shape, c) color, d) polymer

seafood [35,36], fish [37], vegetables and fruit [38-39], piped water [40,41], drinking water [42], mineral water from various countries, such as Indonesia, Thailand, China, India, Mexico, Brazil, USA, Thailand, Lebanon [43]. Microplastics found in PET bottled water were < 10 m [44,45]. Meanwhile, and more concerningly, we notice that sample collection procedures, pretreatment methods, and detection techniques utilized in the literature are not uniform, and that individual approaches may result in false-positive results.

3.2 Mass Balance Microplastics in the Reactor

The occurrence of PET as secondary microplastics as long as the start-up period was detected from the mass balance of the presence of microplastics in the reactor (Fig 4). As illustrated in Fig. 4, no microplastics were added at the end of the start-up phase. This suggests that no secondary microplastic from PET media was fragmented in a short period (75 days). SSA affects the adherence of microplastics to PET media, as illustrated in Figure 4 and Table 1. More microplastics were adsorbed on the biofilm in bigger SSA. At the beginning of the start-up process, all microplastics were suspended and completely mixed in the synthetic wastewater. At the end of start-up, 7.96 – 9.24% of microplastics were adsorbed on the biofilm attached to the PET surface. Despite the lack of comparative data are available in the bibliography for this kind of study, the results seem to be very logical, as PET, PE, PES, PS were the most widely found on a human stool. A recent study [46] has evidenced that sewage sludge application is a vehicle for microplastics. These findings must be evaluated in light of several considerations.

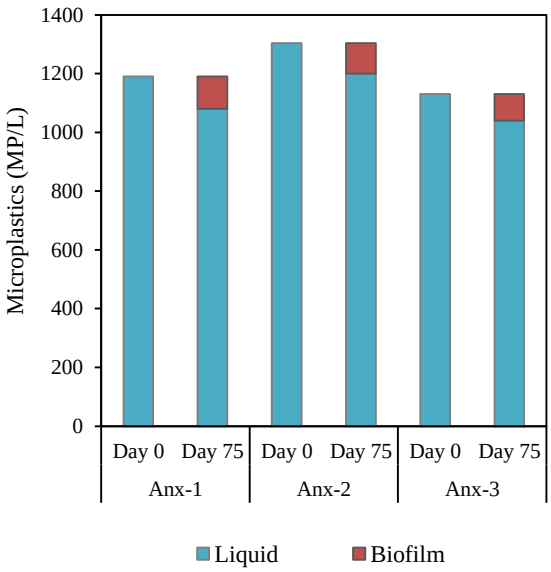


Fig.4 The occurrence of microplastics during the start-up process

3.3 Identification of PET Polymer

To our surprise, by far the mass balance is known that there was no fragmentation of PET as secondary microplastics during the start-up process, identification of PET from bottles using ATR-FTIR was still carried out by matching it with the standard spectrum of PET from plastic bottles. For this purpose, a standard spectrum of PET bottles was made, then stored in a spectrum library, making it easier to identify PET polymers from samples of microplastic particles that were read by ATR-FTIR. The sample spectra were matched with the spectrum library, as well as the information that appeared on the monitor screen showing the molecular formula and images of the PET functional groups. The PET functional groups that appear on the monitor screen are adapted to the PET functional groups described by Andardy [47]. For the first time, our study demonstrates the seed sludge as a potential source of microplastics in the environment.

Figure 5 shows the types of microplastic polymers found in ATR-FTIR. The types of microplastic polymers identified by ATR-FTIR in the seeding reactor samples were polyethylene (PE), polystyrene (PS), polyester (PES), and PET. In addition, other types of polymers such as polypropylene (PP), polyamide (PA) were also found in small amounts. The red spectrum graph shows the sample spectrum and the blue one shows the standard spectrum in the library. Figure 6 shows the polymer distribution in the anoxic reactor during the seeding process in the liquid phase and biofilm. Differences in density, shape, and size of each particle in the sludge affect their presence in the reactor during the start-up process. According to Li [48], 63 % of microplastics in sewage sludge were fibers. PET fibers were the most commonly detected microplastic in sludge and comprised between 60 and 100% of the total detected microplastics in each sample for all wastewater treatment plants [27,49].

PET polymer was found 17.38 ± 5.72% in the anoxic reactor, less abundant than PES and PE polymers. The difference in particle density affects its distribution in the liquid and biofilm. PE polymer found in biofilms is more dominant than others, because its density is smaller than water, so it's easier adsorbed on biofilms.

4. CONCLUSION

The source of microplastic in the anoxic reactor for 75 days of start-up process came from fecal sludge taken from housing with a concentration of 7,666.67 ± 513.16 MP/kg (w/w). At the end of seeding, around 7.96-9.24% of microplastics were adsorbed on the biofilm. The most identified types of microplastics by the ATR-FTR were PES and PET polymers. Based on the mass balance calculation, there was no PET fragmentation at the end of the start-up process, meaning that no PET was fragmented as secondary microplastics during the start-up process. From this analysis, it can be concluded that fecal sludge should be evaluated in future research. Future studies on the toxicity of microplastics and their impact on aquatic environments will be necessary to evaluate the possible harmful effects of microplastics.

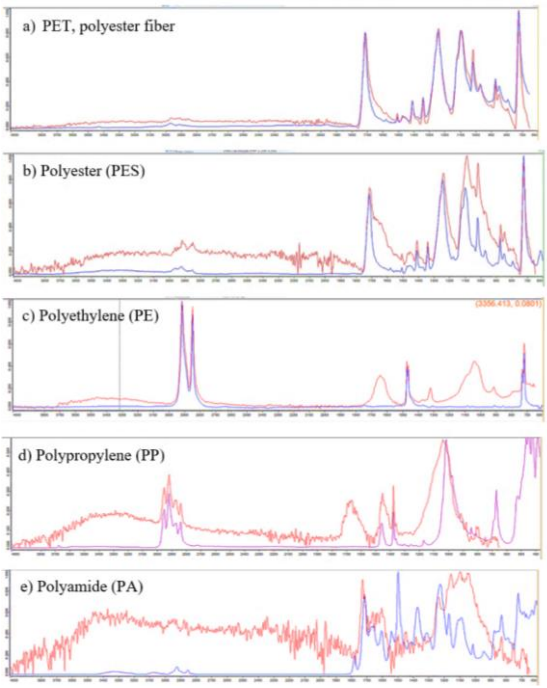


Fig. 5 Types of microplastic sample polymer found by the ATR-FTIR

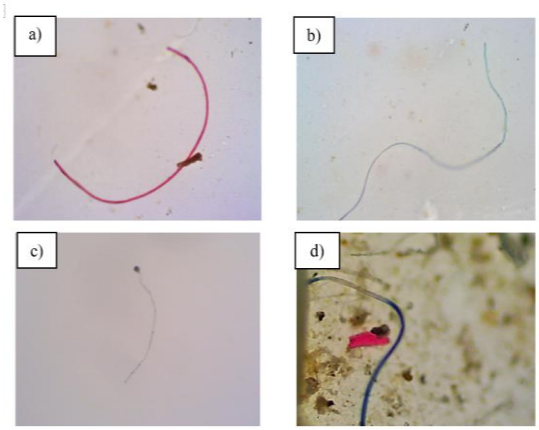


Fig. 6 Size, shape, and color of microplastics in the reactor; a) red fiber, b) blue fiber, c) transparent fiber, d) red fragment, and blue.

5. ACKNOWLEDGMENTS

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