



## Microplastics Removal Efficiencies by Non-Sewered and Sewered Wastewater Treatment Systems and Potentials of the Algal-Bacterial System

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### ABSTRACT

Wastewater treatment systems (WWTSSs) are a major source of microplastics (MPs) in the environment. In this study, the influent, effluent, and sludge samples from three non-sewered and two sewerred WWTSSs were analyzed. The samples were collected and filtered through two sized mesh sieves (5.6 and 0.3 mm). The retained residues were pretreated and observed under a digital microscope for their abundance and characteristics. Results showed that MPs concentrations in the influent of the non-sewered and sewerred WWTSSs were 3 – 6 and 1.6 – 2.0 particles/L, respectively, with the removal efficiencies ranging from 83 – 94 and 74 – 78 %. Due to their higher MPs loading rates and shorter HRT, the sewerred WWTSSs had less efficient in MPs removal than the non-sewered WWTSSs. Polyethylene terephthalate (PET) was the most polymer type found in all samples, while fiber accounted for about 70-95% of all samples. The results indicated that the MPs in wastewater could be partially removed and accumulated with the sludge in both non-sewered and sewerred WWTSSs. To minimize the impacts of MPs contained in the treated effluents, the results of the biosorption experiments showed the potential of the algal-bacterial system in which the operation time of 96 h had the highest biomass production of 220 mg/L and the MP removal efficiency and capacity of 88-90% and 587 mg/g, respectively. Because the algal-bacterial system operating at 96 h was effective in COD, TN and TP removal, it is strongly recommended for use in both MP removal and wastewater treatment.

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## INTRODUCTION

MPs are plastic particles with size smaller than 5 mm (Li et al., 2021; Piskula & Astel, 2022). MPs can be originated from plastic manufacturing for specific purposes, called “primary microplastics” (i.e., pellets and microbeads using in personal care products) and the fragmentating of larger plastics into smaller pieces which is called “secondary microplastics” (Sun et al., 2022). MPs enter to the aquatic environments mainly through the weathering processes of discarded macroplastics when they are exposed to solar radiation and microbial decomposition (aquatic-based source), as well as through the discharging of wastewater effluent (land-based source) (Li et al., 2021). Once the MPs reach the environments, the plastic particles trend to persist in all areas of the environments (i.e., air, water, and soil) (Azoulay et al., 2019; Li et al., 2021; Hu et al., 2022; Piskula & Astel 2022). In addition to their occurrence and distribution along the food chain, MPs can cause several adverse effects to aquatic organisms ranging from

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small planktons to big fishes (Mateos-Cárdenas et al., 2022). The aquatic organisms may have potential negative impacts from ingesting MPs from water sources, increasing MP accumulation and chemical adsorption on their bodies (Cverenkárová et al., 2021). Since humans are a top consumer in the food chain, MPs in human food may pose a potential threat to their health (Al Mamun et al., 2023).

With respect to this above situation, the objectives of this study were: 1) To evaluate efficiencies of MPs removal by non-sewered and sewerer WWTs including abundance, characteristics and plastic types in the influent, effluent, and sludge samples., 2) To investigate potential factors affecting MPs removal in the non-sewered and sewerer WWTs., 3) To recommend improvement on design and operation of the non-sewered and sewerer WWTs to minimize discharges of MPs and reducing potential impacts, and suggested potential of the algal-bacterial system for removing MPs.

## MATERIAL AND METHODS

### *Sample collection*

The influent, effluent and sludge samples were collected from three non-sewered WWTs, including Photobioreactor (PBR), Solar Septic Tank (SST) and Zyclonic™, treating domestic wastewater from a household, a dormitory, and public toilets, respectively. Two sewerer WWTs including Sequencing batch reactors (SBR) and Rotating Biological Contactor (RBC), treating wastewater from an academic institute and a hospital, respectively, were chosen for this study. All WWTs are located in Pathumthani province, central Thailand. These sampling locations were selected to investigate MP abundance and characteristics, as well as their removal efficiencies among different sources of wastewater in both the non-sewered and sewerer WWTs which may have different conditions and environments. Composite samplings of the influent, effluent and sludge samples were conducted three times during March 2021-January 2022. The samplings were done by using a 10-L stainless steel bucket and then filtered through two sized stainless steel mesh sieves (5,600 and 300 µm). The residues retaining on the mesh sieve sized 300 µm were collected in a cleaned wide-mouth glass bottle with aluminum cap prior to further analysis in laboratory. The collected samples were pre-treatment prior to digital microscopic analysis.

### *Pretreatment*

According to the modified methods of previous studies, the retained residues were pretreated by transferring the retained residues from the cleaned wide-mouth glass bottle to a cleaned and dried 250-mL beaker, then minimally rinsed with distilled water to ensure all residues were transferred (Michida et al., 2019; Wang et al., 2020; Vibhatabandhu & Srithongouthai, 2022). The beaker was dried in oven at 90°C for 24 hours. Organic matters in the retained residues were further degraded by adding 20 mL of 30% hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) and 20 mL of aqueous 0.05 M Fe<sup>2+</sup>solution into the beaker to generate hydroxyl radicals (HO·) from the Fenton oxidation process (FOP) to achieve this purpose. Subsequently, the mixed solution was boiled at 75°C for 30 minutes. Details of the pretreatment procedure and contamination prevention are described in Sections 2 and 3 of the Supplementary Material.

### *Analysis*

Each dried petri dish was visually observed under a digital microscope (Axiolab 5, Carl Zeiss Microscopy, USA) for determination of MPs abundance, shape, size, and color. The observed MPs were counted for their abundance in the unit of particles per L of sample (part/L) and photographed to determine characteristics: shape, size, and color. MPs shapes were categorized to fragments (similar width and length), fibers (length longer than width), and fiber bunches

(group of more than 5 fibers) (See Table S1 in the Supplementary Materials). The overall MPs dimension was measured based on length according to five categories:  $<100\ \mu\text{m}$ ,  $101\text{--}500\ \mu\text{m}$ ,  $501\text{--}1,000\ \mu\text{m}$ ,  $1,001\text{--}5,000\ \mu\text{m}$ ,  $>5,000\ \mu\text{m}$  (See Table S2 in the Supplementary Materials). Additionally, the color of MPs was visually classified as dark blue, light blue, red, green, white, black, and mixed colors (See Table S3 in the Supplementary Materials). About 6 MPs of each petri dish were picked up and transferred to a 1.5 mL glass vial and analyzed by fourier transform infrared (FT-IR) microscopes (Nicolet iN10, Thermo Scientific, USA). Spectra of MPs were analyzed and identified by Software Thermo Scientific OMNIC<sup>TM</sup> Picta<sup>TM</sup> in which the polymer types of MPs were confirmed with the matched polymer name (See Table S4 in the Supplementary Material).

#### *Evaluation of the MP removal by the algal-bacterial system*

Since MPs and nutrients (e.g. total nitrogen; TN and phosphorus; TP) mostly remained in effluent of the non-sewered WWTSSs, effluent of septic tank was used as a cultivation solution of the algal-bacterial system. The cultivating method of algal-bacterial biomass in this research (adapted from Chaiwong et al. (2021)) is described in Section 4 of Supplementary Material.

From the above cultivation phase, the optimum initial biomass concentration was applied in the biosorption experiment. A set of the biosorption experiment was conducted by using micro sized-polyester (PE) with a diameter less than  $200\ \mu\text{m}$ , which was added into three prepared cultivation solution flasks (each with a size of 200 mL) at concentrations of 40 (MP-40), 200 (MP-200), and 400 (MP-400) mgMPs/L, while another flask was setup as a control (no MP added). The HRTs of the four flasks, a main factor affecting the removal efficiencies of both wastewater contaminants (COD, TN and TP) and MPs of the WWTSSs, were varied at HRT of 24, 48, 72, and 96 h. All sets of the experiment were placed in a closed box to avoid environmental cross-contaminations and effects of ambient light.

After reaching each operational HRT, the average temperatures and light intensities were  $32.5\text{--}33.8^\circ\text{C}$  and  $142\text{--}154\ \mu\text{mol}/\text{m}^2/\text{s}$ , respectively. In addition, the soluble COD, TN and TP concentrations in the cultivation solution were 512, 59 and 19 mg/L, respectively. The set of experiment including four flasks was separately transferred to clean glass funnels for gravitational settling for 12 h. After the settling processes, the supernatant (upper layer) and sediment (lower layer) were separately collected to determine total biomass, chlorophyll *a* concentration, and MP removals. Details of analysis and determination methods, including the analysis of biomass growth, chlorophyll *a*, and wastewater samples, as well as the determination of MP removals and statistical analysis, are described in Section 5 of the Supplementary Material.

## RESULTS AND DISCUSSION

#### *MPs in influent, generation and loading rates*

Table 1 shows average MPs concentrations of the influent samples of both non-sewered and sewerer WWTSSs. For the non-sewered treatment systems, due to the different numbers of the users served among the three non-sewered WWTSSs, MPs generation rates from the three different sources which are a household, dormitory and public toilets were varied between 35–141 part/cap/d. Accordingly, the influent MPs concentrations were  $3 \pm 2$ ,  $3.8 \pm 0.3$ , and  $6 \pm 4$  part/L, respectively, resulting in the MPs loading rates to the PBR, SST, and Zyclonic<sup>TM</sup> systems of  $0.30 \pm 0.22$ ,  $0.14 \pm 0.01$  and  $4.15 \pm 2.90$  part/L/d, respectively. Based on wastewater flow rates and population served at the two sewerer WWTSSs, SBR and RBC, the ranges of MPs loading rates were found to be 2.1 - 27 part/L/d or 398 – 1,050 part/cap/d. The influent MPs concentrations of  $1.6 \pm 0.5$  and  $2.0 \pm 0.7$  part/L were observed in the SBR and RBC systems, similar to the previous studies of Maw et al. (2022), Tadsuwan & Babel (2021, 2022) which reported the influent MPs concentrations of conventional WWTSSs in Thailand to be 0.40 - 77 part/L.

**Table 1.** MPs concentrations in influent samples and generation rates and loading rates among different WWTSS

WWTSS	Source of WW	Flow rate, (L/d)	System Volume (L)	Pop. Served	Average MPs concentration (part/L)	MPs generation rate (part/cap/d)	MPs loading rate (part/L/d)
<b>Non-sewered WWTSS</b>							
PBR	Household	120	1,000	5	3 ±2	60 ±44	0.30 ±0.22
SST	Dormitory	110	1,000	1	3.8 ±0.3	141 ±10	0.14 ±0.01
Zyclonic™	Public toilets	300	400	48 <sup>a</sup>	6 ±4	35 ±24	4.15 ±2.90
<b>Sewered WWTSS</b>							
SBR	Academic institute	520,000	392,000	2,045	1.6 ±0.5	398 ±132	2.1 ±0.7
RBC	Hospital	2,062,500	165,000	4,288 <sup>b</sup>	2.0 ±0.7	1,050 ±329	27 ±8

**Note:** <sup>a</sup> Average number of users was counted during weekday and weekend.

<sup>b</sup> Average number of medical officers, in- and out- patients

Due to high wastewater flow rates from domestic activities (washing, laundry, toilets, etc.), MPs generation rates of the sewerer WWTSSs were found to be higher than the non-sewered WWTSSs which treated only toilet wastewater (Table 1). Wastewater from washing activities such as laundry machines were found to be the major MPs source of the sewerer WWTSSs, especially the hospital wastewater which has the MPs loading rate of 27 ±8 part/L/d (Table 1), most of MPs shapes were microfibers (Rathinamoorthy et al., 2022). Additionally, Periyasamy (2021) reported that PET could release up to 4,900,000 microfibers from the 1 kg wash load of PET jeans, and its number varied by washing conditions such as water temperature, washing time, number of rotations, and type of detergent. These microfibers were washed into water ways or wastewater treatment systems (Mishra et al., 2019; Galvão et al., 2020; Geyer et al., 2022).

#### *Characteristics of MPs in the influent samples and their sources*

The MPs characteristics were determined in terms of MPs size, shape, color, and polymer type. The MPs size distribution in the influent samples of both non-sewered and sewerer WWTSSs are showed in Fig. S5 of the Supplementary Material. The MPs sizes ranging from 500 to 5,000µm were accounted about 27-65 and 34-47% of the non-sewered and sewerer WWTSS, respectively, similar to some previous studies which reported the MPs over 500 µm to be the major size about 70% in the municipal influent wastewater of a conventional activated sludge system and a membrane bioreactor (Sun et al., 2019). The MPs sizes could be varied according to classification methods, the most sizes of MPs were ranged between two sized mesh sieves (300 and 5,600µm) used for sample collection (Simon et al., 2018; Sun et al., 2019). While smaller MPs (100-300µm) could sometimes reach up 40-70% of the inflow of a SBR and a media process (Lee & Kim 2018; Ding et al., 2020). The small microfibers could attach and remain after sieving through a 300 µm mesh sieve even if their size was smaller.

#### *MP shapes, colors, polymer types and their sources*

In comparison, there are no significant difference of portion of MPs shapes among wastewater sources ( $p = 0.01$ ), fiber was the most MPs shape which was accounted for about 70-91 and 77-95% of the non-sewered and sewerer WWTSS influents, while fragment was 9-30 and 22-30%, respectively. Additionally, dark-blue, light-blue and red fibers representing polymer types of PET, polyethylene (PE) and polycyclohexanediol terephthalate (PCDT), respectively, were the most common color found in influent samples. In contrast, there were various colors and polymer types of MP fragment found in influent samples of both WWTSSs. The MPs fragment

of the non-sewered WWTs influents were observed to be red, light-blue, and dark-blue colors representing polymer types of polypropylene (PP), PE, and PET, respectively; while some MPs fragments in white color were identified as poly (11-bromoundecyl acrylate) (PBA), polybutylene terephthalate (PBT), and polystyrene (PS) (see Fig. S6(a1-3) in the Supplementary Material). The fragments of PE (light-blue) and PE-PP co-polymer (green) were predominated in the influent samples of the sewer WWTs (see Fig. S6(b1-2) in the Supplementary Material).

Consequently, the MPs characteristics could be varied among different sources of influent wastewater. At household level of the non-sewered WWTs, the MPs could be originated from both personal care products (toothpastes, cleansers, etc.) and laundry, resulting both fragment and fiber shapes detected in the influent wastewater. However, the portion of fiber from laundry activity (70%) was much higher than that of fragment (30%) found in personal care products. Most of fibers were identified as polyester group (PET and PE) generated from synthetic textile products, while PE and PP fragments were found to be the main composition of microbead in facial cleanser (see Fig. S6(a-1) in the Supplementary Material). (Ziajahromi et al., 2017; Tang et al., 2020). Similar to a study in China which reported that the MPs fragment and fiber with an average particle size of  $612 \pm 110$  and  $850 \pm 114$   $\mu\text{m}$ , respectively, were mainly found in household wastewater, the MPs were detected mainly in blue, red, transparent, black, and green colors, representing the most polymer types of polyvinylchloride (PVC), PE, and PP (Tang et al., 2020). About 50% of the smaller sized fragment was identified as PBA which could be generated by daily products such as commercial shower gels, waterproof sunscreen, or as a gelling agent in lipsticks and paint particles (Bayo et al., 2020; Xu et al., 2020). More details of MP shapes, colors, polymer types and their sources, are described in Section 6 of the Supplementary Material.

#### *MP fates, removal efficiencies and mechanisms*

The MPs can enter and be present in all parts of both of the non-sewered and sewer WWTs. In the non-sewered WWTs, the MPs concentrations of the influent, sludge, and effluent samples were found to be 3 – 6, 12 – 246, 0.22 – 0.61 part/L, respectively, while the MPs concentrations of 1.6 – 2.0, 21 – 70, and 0.35 – 0.45 part/L were detected in the samples from the sewer WWTs, respectively (Table 1 and 2). The MPs characteristics of both non-sewered and sewer sludge and effluent samples were not significantly different, being mainly fiber (PET) in the sizes of 501–5,000  $\mu\text{m}$  (see Fig. S1 in Supplementary Material). The relatively high MPs concentrations in the sludge samples from both the non-sewered and sewer WWTs indicated that the MPs were mostly retained in sludge or sediment through adsorption and sedimentation. A recent study reported that the MPs abundance in sludge samples of  $2,302 \times 10^3$  part/kg dry sludge was found to be higher than other samples (e.g., scum) of a non-sewered WWT (septic tank) in Beijing, China (Liu et al., 2022). Additionally, Sun et al. (2019) confirmed that MPs concentrations in sludge samples of 1,500–170,000 part/kg dry sludge were significantly higher than that in the water samples from the sewer WWTs. Obviously, the highest MPs concentrations in the sludge samples from the non-sewered WWTs might be caused by long sludge ages or less frequency of sludge removal (Table 2), resulting in more solid and MPs accumulation (Mac Mahon et al., 2022).

Accordingly, the MPs removal efficiencies of 83 – 94% of the non-sewered WWTs were found to be higher than those of the sewer WWTs (74 – 78%) (see Fig. S7 in the Supplementary Material). The difference in the MPs removal efficiencies between the non-sewered and sewer WWTs were probably caused by the smaller unit size of the non-sewered WWTs and relatively less MPs generation rates and MPs loading rates (Table 1). Further details of MP fates, removal efficiencies and mechanisms, are described in Section 7 of the Supplementary Material.

**Table 2.** MPs concentrations in sludge and effluent samples, and removal efficiencies of both the non-sewered and sewer WWTSS

WWTS	Hydraulic retention time (HRT; d)	Frequency of sludge removal (year)	Average MPs concentration (part/L)		MPs removal efficiencies (%)
			Sludge	Effluent	
Non-sewered WWTS					
PBR	8.3	2	12 ±7	0.61 ±0.66	83 ±19
SST	9.1	3	246 ±166	0.22 ±0.25	94±6
Zyclonic™	1.3	0.5	15 ±7	0.30 ±0.03	93 ±6
Sewered WWTS					
SBR	0.1	0.2	21 ±1	0.35 ±0.18	74 ±20
RBC	0.08	1	70 ±23	0.45 ±0.04	78 ±9

### *Factors affecting MPs removal in the WWTSS*

Due to the relatively long HRT of the non-sewered WWTSS which resulted in more adsorption and sedimentation of the influent MPs, their MPs removal efficiencies were relatively higher than those of the sewer WWTSS (Table 2). The MPs found in wastewater can exist either as individual particles or as adsorption to larger particles such as paper, wood branches, or larger plastic fragments (see Fig.S2 in the Supplementary Material). During the sedimentation process, the majority of MPs adhered to these larger particles were effectively removed (Kwon et al., 2022). Because large quantities of MPs were found in the sludge samples (Table 2), if improperly managed, those MPs could be released from the sludge to the environment and possibly causing ecosystem impacts. Therefore, to avoid the negative impacts on the environment and human health, the removed sludge from the WWTSS should be managed in proper ways such as reuses for making concrete blocks or in energy recovery. In addition to the effect of HRT, other chemicals found in wastewater may also influence the efficiency of MP removal and should be further investigated.

### *Optimum operating conditions of the algal-bacterial system*

After 96 h of operation, the pH values were not significantly different among the control (pH = 8.2) and experimental groups ( $P>0.01$ ), which ranged from 7.9 to 8.2. At the initial stage of cultivation, the algal-bacterial biomass concentrations (without added MPs) at varied initial biomass were found to increase (exponential phase) and became stable (stationary phase) after 72 h of operation (see Fig. S8 in the Supplementary Material). Simultaneously, the chlorophyll *a* concentrations were also increased, becoming stable and slightly decreased at the last operating day when the system completed the growth cycle period (See Fig. S3a in the Supplementary Material). Due to the cell growth between the lag phase (initial) and stationary phases (steady), the specific growth rates of the varied initial biomasses were consistent with the determined biomass values, as shown in Fig. S3b in the Supplementary Material. The optimum initial concentration of 34 mgDCW/L resulting in the highest specific growth rate (average of 4.3 h<sup>-1</sup>), was applied in the biosorption experiment. To enhance mixing conditions, continuous air mixing was provided to the algal-bacterial systems, resulting in dissolved oxygen (DO) concentrations of about 0.4 ±0.2 mg/L.

### *Effects of MPs on growth of algal-bacterial biomass*

The growth of algal-bacterial biomass and chlorophyll *a* concentrations of the control and experimental flasks, exposed to variation of MP concentrations are illustrated in Fig. S8 of the Supplementary Material. The MP concentration of 40 mg/L showed the growth trend of the algal-bacterial biomass to be similar to the control. Simultaneously, the variation of

chlorophyll *a* concentrations represented the effects of MPs on photosynthetic activities of the algal biomass. Meanwhile, at the first 48 h of the operation, the algal-bacterial biomass concentrations were found to obviously decrease when exposed to the MP-200 and MP-400, indicating self-adjustment of the algal-bacterial cells to the added MPs (Song et al., 2020). Afterwards, the algal-bacterial biomass concentrations gradually increased to the highest values of 220 mgDCW/L at the 96 h of operation. During the self-adjustment period, the MP-200 and MP-400 showed the decreased of chlorophyll *a* concentrations at 48 h. Consequently, the increase of chlorophyll *a* concentrations was found at 72 h of the MP-200. A longer self-adjustment period was required for the higher MP concentration (MP-400), resulting in lower chlorophyll *a* concentrations than others (see Fig. S8 in the Supplementary Material). Similar to previous studies, exposing to more than 200 mgPE/L could enhance biomass growth and chlorophyll *a* concentration of both marine and freshwater microalgae (Chae et al., 2019; Song et al., 2020).

In addition, the MP-40, MP-200 and MP-400 showed significantly different specific growth rates from the control ( $P < 0.01$ ). The MP-200 and MP-400 presented the most significantly negative effects on specific growth rates of  $-0.023 \pm 0.012$  and  $-0.035 \pm 0.003 \text{ d}^{-1}$ , respectively, at 48 h of operation, resulting in inhibition ratios of  $47 \pm 4$  and  $55 \pm 3$  %, respectively (see Fig. S9(b) in the Supplementary Material). However, the inhibition ratios of the algal-bacterial biomass of the MP-200 and MP-400 were found to decrease after 72 and 96 h of the operation corresponding to the highest specific growth rates of  $0.128 \pm 0.012$  and  $0.247 \pm 0.008 \text{ d}^{-1}$ , respectively, at the 96 h of the operation (see Fig. S9(a) in the Supplementary Material). The maximum specific growth rate of the biomass of the MP-200 was found to be 2.5 times higher than those found in a previous study ( $< 0.05 \text{ d}^{-1}$ ) which were conducted at similar MP exposures; 74  $\mu\text{m}$ -PE and added 200 mgPE/L by using algal specie of *Chlorella* sp. L38 (Song et al., 2020). Furthermore, the increased MP exposure of MP-400 resulted in specific growth rate 2 times higher than the MP-200. Hence, the results of this study indicated that variation of MPs concentrations added to the algal-bacterial system had effects on the biomass growth and its self-adjustment period.

Consequently, the growth of algal-bacterial biomass affected the wastewater treatment performance in the algal-bacterial systems. At 48 h of operation, The TN and TP removal efficiencies of the MP-200 and MP-400 systems were observed to be lower than those of the MP-40 system (see Fig. S4(b,c) in the Supplementary Materials), corresponding to the specific growth rates and inhibition ratio of algal-bacterial biomass (see Fig. S9 in the Supplementary Material). Because of continuous air mixing in the algal-bacterial system suitable for bacterial degradation, the added MP concentrations did not affect the COD removal performance of the algal-bacterial systems at any operation time (see Fig. S4(a) in the Supplementary Material) (Chaiwong et al., 2021). Because the added MPs negatively affecting the prolonged self-adjustment period of the algal cells in photosynthetic reactions (Song et al., 2020), there was some decrease in chlorophyll *a* concentration (see Fig. S8 in the Supplementary Material) and specific growth rates (see Fig. S9(a) in the Supplementary Material) at 48 hours of operation time. However, the TN and TP treatment performances of MP-40, MP-200, and MP-400 showed an increasing trend at 72 hours, and were stable at 96 hours, resulting in average COD, TN, and TP removal efficiencies of  $67 \pm 11$ ,  $76 \pm 28$ , and  $60 \pm 20$  %, respectively (see Fig. S4 in Supplementary Material).

#### *MP removal efficiencies of the algal-bacterial system*

As shown in Fig. S10 of the Supplementary Material, the algal-bacterial system provided the potentials for MPs removals in which the maximum MP removal efficiencies of 75 - 90 % were achieved under the condition of all MP concentrations at 96 h of the operation time. Because longer operation times could provide higher growth of the algal-bacterial biomass and better aggregation between MPs and biomass including biosorption, the MP removal efficiencies

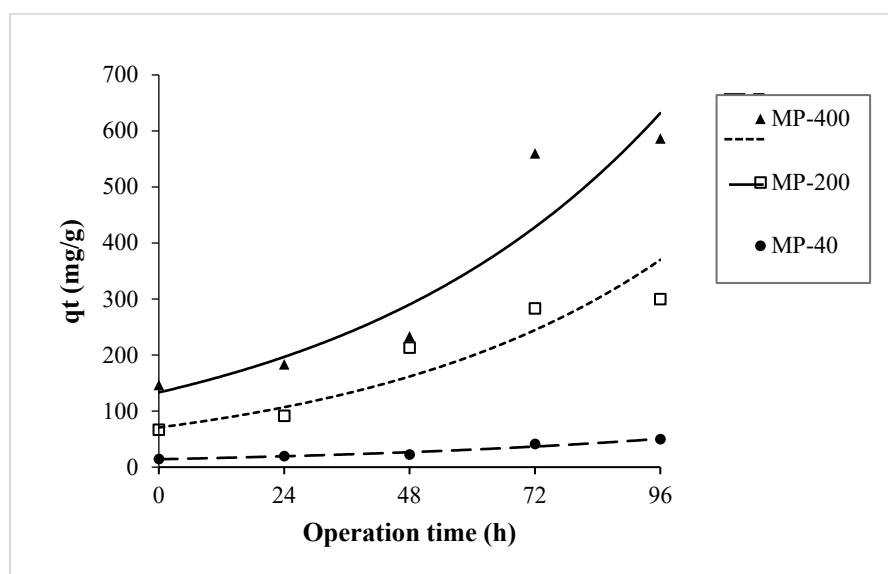


Fig. 1. The effects of operation time on MP removal capacity

were found to increase with increasing the operation times. When exposed to the MP-20 to MP-400, the highest MP removal efficiencies of 88 - 90 % were achieved by the maximum biomass concentration of 220 mgDCW/L, similar to Kim et al. (2023) reporting the biosorption efficiency of PS sized-430 nm of 92% by the bacterial biofilm (*Acinetobacter sp.*) (see more details in Section 8 of the Supplementary Material).

#### Effects of operation time on MP removal capacity

The results of biosorption experiments showed the MP removal capacities were found to increase with increasing operation times (Fig. 1). At final stage of operation (96 h), the maximum MP removal capacities of the MP-200 and MP-400 were 300, 587 mg/g, respectively. As a result of high biomass production and specific growth rates (see Fig. S8 and S9(a) in the Supplementary Material), the algal-bacterial biomass might have capacity in adsorbing and aggregating MPs. While the low biomass production of the MP-40 had the low MP removal capacity of 50 mg/g.

Hence, together with the data of Fig. 1, Fig. S4 and Fig. S10 in Supplementary Material, the operation time or HRT of 96 h is recommended for the algal-bacterial system to operate to achieve the high MP removal and wastewater treatment efficiencies. In addition, future challenges and perspectives of MP removals and management, are described in Section 9 of the Supplementary Material.

## CONCLUSIONS

Characteristics of the MPs in the influent wastewater of both the non-sewered and sewer WWTs were mainly polyester fibers (e.g., PE and PET). Due to their relatively longer HRT, the non-sewered WWTs were found to be more efficient in MP removal than the sewer WWTs. Because most of the removed MPs were found to accumulate in the sludge samples of both the non-sewered and sewer WWTs through adsorption and sedimentation, appropriate management of these MPs contaminated sludge is strongly recommended to avoid further environmental contamination. The algal-bacterial system had a potential for removing MPs in wastewater which was illustrated by the biosorption experiment. At the operation time of 96 h



the algal-bacterial system performed the highest biomass concentration of 220 mg/L, resulting in the MP removal efficiency and capacity of 90% and 587 mg/g, respectively. Accordingly, the algal-bacterial system is recommended an alternative technology for MP removal and wastewater treatment.

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## CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

## LIFE SCIENCE REPORTING

No life science threat was practiced in this research.

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