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Greywater treatment performance of a pilot-scale membrane bioreactor and characteristics of bacterial biofilm signatures in permeate effluent

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ABSTRACT

Treated greywater has a high potential for water reuse. However, the post-treatment processes of reclaimed greywater may face biofouling problems due to biofilm formation. In the present study, the greywater treatment performance of a pilot-scale membrane bioreactor (MBR) was evaluated, and the effect of a dominant bacterial biofilm growing on the Pall ring media placed in the permeate pipeline was investigated. The MBR was operated for 125 days with a constant hydraulic retention time of 2 days under complete sludge retention. The treatment performance in terms of the levels of organic compounds, ammonium nitrogen, suspended solids, total coliforms, and *Escherichia coli* was >90 %. During the operation, a high relative abundance (>70 %) of the following bacterial phyla was observed within the MBR and the bacterial biofilm: *Proteobacteria*, *Chloroflexi*, *Actinobacteria*, and *Planctomycetes*. Furthermore, the following bacterial orders were predominantly observed in the biofilm: *Sphingomonadales*, *Burkholderiales*, and *Planctomycetes*. *Acidovorax*, *Methylibium*, *Blastomonas*, and *Planctomyces* were the important genera identified in the biofilm. Interestingly, the present study revealed that these bacterial species were predominant and well adapted in treated greywater containing various surfactant and detergent components. Knowledge of the predominant bacterial species in the biofilm could enable to select the appropriate disinfection method and prevent the biofouling of the reverse osmosis (RO) membrane, which can facilitate further application of the MBR-RO process for water reuse.

1. Introduction

Greywater treatment and reuse for nonpotable requirements have received increasing attention in urban and peri-urban areas because of scarcity and deterioration of water resources [1]. Greywater is defined as any domestic wastewater other than toilet water [2]. Greywater usually constitutes 50 % to 80 % of the total domestic water use, and it has the most significant potential energy savings in household communities [3]. Treated greywater is perceived to be more suitable for water reuse than wastewater from toilets (blackwater) [4,5]. This is because the content of organic pollutants and pathogens in greywater is lower than that in blackwater. Therefore, greywater reuse is an alternative option for recycling wastewater. However, greywater includes a wide range of chemicals such as surfactants, detergents, cosmetics, pharmaceuticals, and numerous other compounds commonly present in household items [6–8]. Hence, the available treatment technologies have been modified to treat common organic compounds and remove specific pollutants to meet water reclamation guidelines [9]. Among the different treatment methods, such as coagulation, sequencing batch reactor, and membrane bioreactor (MBR), MBR has been proven and recommended to be the most efficient method for greywater treatment and reuse [9]. Wastewater treatment with MBR involves the combination of aerobic biological treatment of the dissolved organic matter and physical separation of suspended solids (SS) and pathogens, which can achieve excellent effluent quality and meet regulatory standards for reuse [10,11]. Although the MBR process can substantially remove organic pollutants and pathogens, some bacterial regrowth can occur in the reclaimed water, thereby impeding water reclamation and reuse [12,13]. Several studies have investigated the mechanisms of bacterial passage through microfiltration (MF) membranes, such as bacterial mobility [14] and size and shape-dependent filterability [15–17], which may result in bacterial growth in the storage and distribution systems. Some studies have also attempted to identify the formation of predominant biofilms in MBR effluent to determine the cause of biofouling in the reverse osmosis (RO) process [18,19].

In the present study, a pilot-scale MBR was developed for treating

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greywater; the MBR was continuously operated to generate greywater for nonpotable reuse and agricultural irrigation without any disinfection treatment. In addition to the evaluation of the treatment performance of the MBR, the present study also aimed to investigate the dominant microfilterable bacterial biofilm in the permeate effluent of the MBR. The characteristics of the bacterial biofilm and the suspended sludge in the aeration tank of the MBR were also evaluated. Understanding the dominant bacterial species in the biofilm and the related influential factors could give us more insights into the design of effective post-treatment methods and enable to develop approaches to improve water reuse quality for practical MBR operations.

2. Materials and methods

2.1. MBR set up and operation

The MBR pilot plant was installed and operated for treating greywater from a dormitory in Srinakharinwirot University, Nakhon Nayok, Thailand. Fig. 1 shows the schematic diagram of the MBR. The pilot plant comprised an equalization tank, a membrane compartment, an aerobic tank, a permeate tank, and several pumps. The membrane tank was equipped with submerged flat-sheet microfiltration membranes (nominal pore size: 0.4 μm; Kubota Corp, Japan) with a total membrane area of 8.0 m^2. Aeration was provided through air supply at the rate of 210 L/min. This value was selected based on several factors, including the oxygen demand of the biological processes, the requirement for efficient membrane scouring, and the maintenance of dissolved oxygen (DO) levels at >2 mg/L, all of which are crucial for effective wastewater treatment. A level sensor was connected with the feed pump by using an electrical controller to maintain a constant water level in the aerobic tank. The MBR was operated to treat up to 2.2 m^3 of wastewater per day for approximately 125 days with a constant hydraulic retention time (HRT) of 2 days without sludge withdrawal. Excess sludge from the conventional activated sludge process was added to the aeration tank as the seed sludge. The cyclic filtration time and relaxation time of the operation were 5 and 40 s, respectively. The average permeate flux was regulated at 11.25 litres per square meter per hour (LMH) during the operation.

2.2. Sampling and collection of water and activated sludge samples, and biofilm

The influent and effluent samples were collected on a biweekly basis and stored at 4 °C in the dark until analysis. Seed sludge sample was collected only at the start of the operation (day 0). Activated sludge samples were collected after the first, second, and fourth month of operation on days 26, 68, and 113. To investigate the potential biofilms inside the permeate greywater pipeline, the polyethylene Pall ring media (diameter: 25 mm; height: 12 mm; specific surface area: 500 m^2m^{-3}) were added in an expanded pipe located across the effluent flow (Fig. 1). The biofilm sample from the media was collected by a sterile technique on day 92 (the third month of the operation), nearly at the end of the experiment; the sample was collected from an approximate active surface area of 180–200 cm^2. All samples were transported on ice to the laboratory and stored at −20 °C until further analysis.

2.3. Water quality analysis

DO levels, pH, and temperature were measured on-site with an appropriate probe (HandyLab 680, Xylem Inc., Germany), while the other parameters were measured in the laboratory. Chemical oxygen demand (COD), ammonium nitrogen (NH_4-N), total nitrogen (TN), and total phosphorus (TP) levels were analyzed using Hach methods (Hach, USA). Biochemical oxygen demand (BOD), SS, total coliforms (TC), and *Escherichia coli* concentration were analyzed according to the standard methods [20]. The concentration of mixed liquor suspended solids (MLSS) was analyzed according to the standard methods [21].

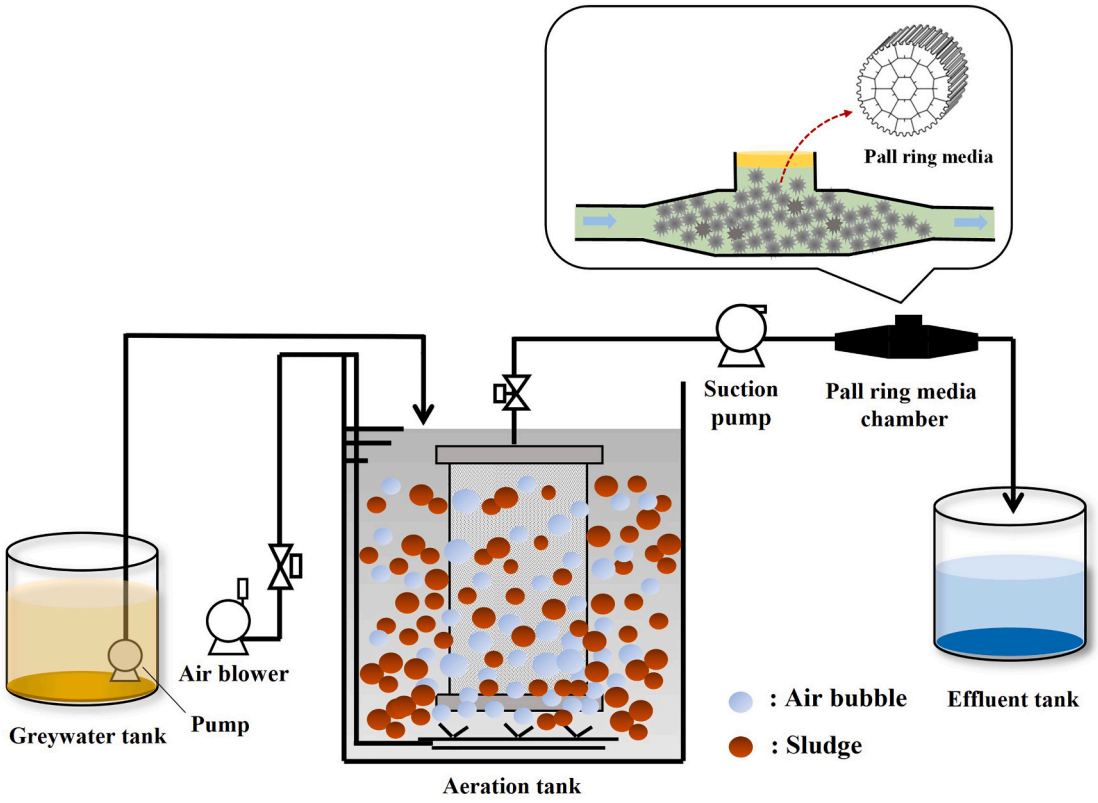

Fig. 1. Schematic diagram of the MBR pilot plant.

2.4. Micropollutant measurements

Four organic micropollutants were identified and quantified in the greywater. These micropollutants covered a broad range of chemicals from cosmetic ingredients to household detergents. These micropollutants included anionic surfactants (AS); linear alkylbenzene sulfonates (LAS) and sodium lauryl sulfate (SLS) and personal care products; triclosan (TCS) and triclocarban (TCC). LAS and SLS typically used as emulsifying cleaning agents in household products (laundry detergents, cleaners, and dishwasher detergents), while TCS and TCC are antibacterial compounds present in soaps, detergents, cosmetics, and many other personal care products [8,22]. To quantify the removal efficiencies of individual micropollutants during MBR treatment, we compared the concentrations of these pollutants in the influent and effluent greywater samples.

Micropollutant concentration in the water sample was determined using solid-phase extraction (SPE) cartridges (6 mL) containing 100 mg of nonpolar, octadecyl-bound, end-capped silica. The cartridges were preconditioned with 10 mL dichloromethane/methanol (1:9, v/v), followed by 10 mL methanol and 10 mL demineralized water. The samples were then passed through the SPE cartridges at the flow rate of 3 mL/min. The cartridges were then rinsed with 10 mL of demineralized water and dried by passing air under vacuum for 30 min. The eluents were evaporated to 2 mL under a gentle stream of nitrogen. The levels of the surfactants (LAS and SLS) were analyzed by reversed-phase high performance liquid chromatography (HPLC) using a C18 column and the SPD-20A UV-VIS detector (220 nm). Data acquisition and processing were performed using the LC solution system (Shimadzu, Japan). TCS and TCC levels were estimated using the GC–MS system (Shimadzu, Japan). The DB-5MS Ultra Inert column (30 m × 0.25 mm × 0.25 μm) was used. The GC column temperature was programmed from 60 °C (initial equilibrium time of 0.5 min) to 300 °C through a temperature ramp of 20 °C min^{-1} and maintained for 2 min. The injector port and the interface temperature were maintained at 290 °C. The samples were injected (2 mL) in the spitless mode [23–25].

2.5. DNA extraction, sequencing, and data processing analysis

DNA extraction and sequencing were performed as described by Ittisupornrat et al. [26]. Briefly, genomic DNA was extracted from each sample using the Taco™ Total DNA Extraction Kit (GeneReach Biotechnology Corp., Taiwan). The purified DNA concentration was quantified using a UV-VIS nano-spectrophotometer (NanoDrop 2000, Thermo Fisher, USA). A set of primers was used to amplify the hypervariable V3 and V4 regions of the bacterial 16S rRNA gene. The forward and reverse primers were 341F (5′-CCTACGGGNGGCWGCAG-3′) and 805R (5′-GACTACHVGGGTATCTAATCC-3′), respectively [27]. After amplification, the final PCR products were sequenced on an Illumina MiSeq at Omics Sciences and Bioinformatics Center (Chulalongkorn University, Bangkok, Thailand). The quality of sequencing reads was examined using FASTQC software. Chimeras were removed through the UCHIME method [28] with vsearch1.1.1 [29]. Taxonomic classification was conducted using the Greengenes database at 97 % identity (version 13). Relative bacterial abundance was characterized at the phylum

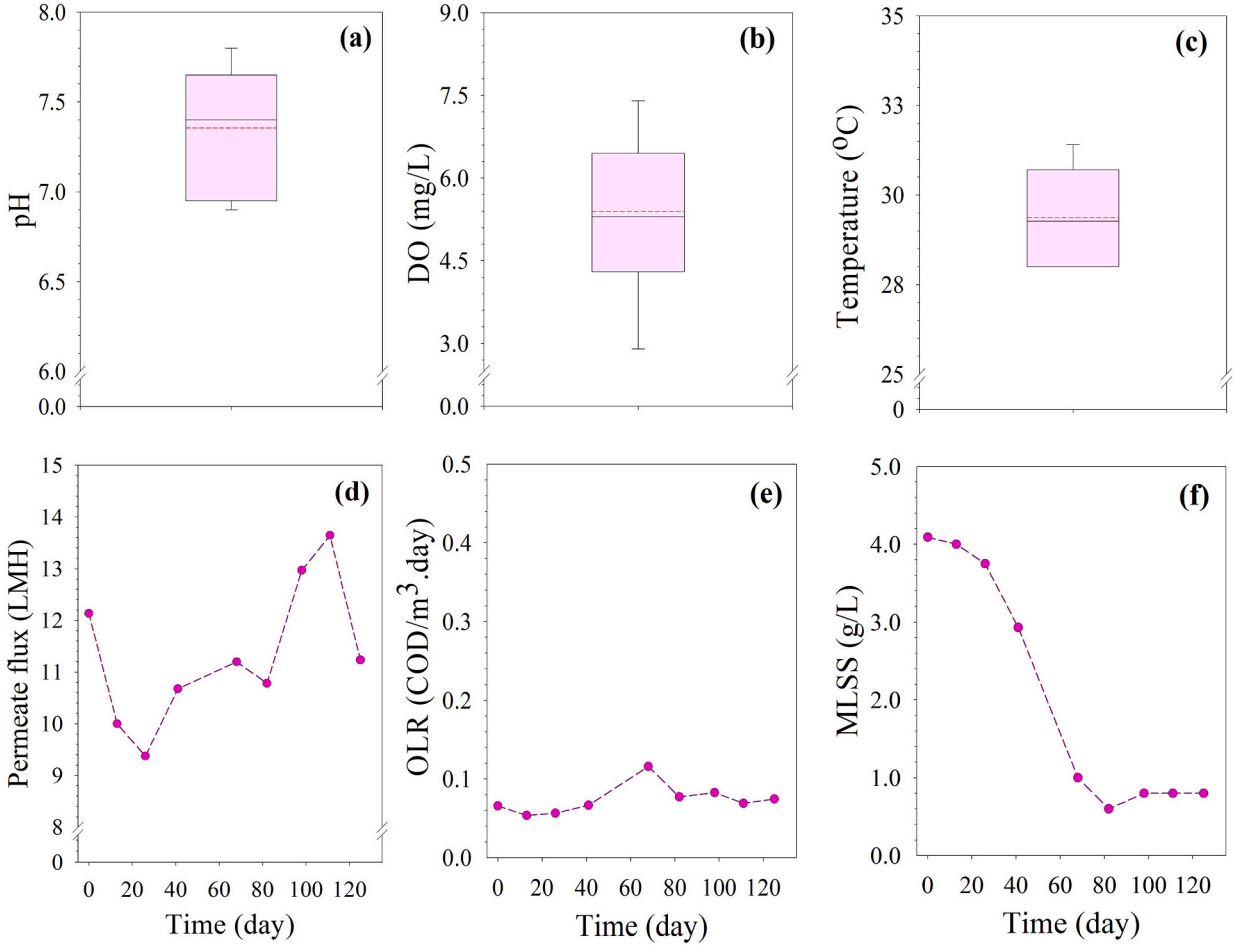

Fig. 2. Physical and chemical characteristics of the MBR operation (a) pH, (b) DO, (c) Temperature, (d) Permeate flux, (e) OLR, and (f) MLSS. The lower and upper whiskers are represented as the 5th and 95th percentiles, respectively. The lower and upper whiskers indicate the 10th and 90th percentiles, respectively, while the bottom and top of the box indicate the 25th and 75th percentiles. The horizontal line and red dashed line represent median and mean, respectively.

level, and a phylum with an abundance of <2 % was considered to be a minor phylum. Principal component analysis (PCA) was used for multivariate data analysis by using the FactoMineR software package [30], with the use of ggplot2 (RStudio Inc., San Francisco, CA, USA) for visualization.

3. Results

3.1. Operating conditions of the MBR

Fig. 2 shows the operating conditions of the MBR. The pH and DO levels were relatively constant with the average values of 7.3 ± 0.4 and 5.3 ± 1.6 mg/L (Fig. 2a and b), respectively, at the ambient temperature of 29.6 ± 1.4 °C (Fig. 2c). The permeate flux was monitored to examine the performance of membrane filtration. Fig. 2d shows alterations in the permeate flux during the MBR operation. The average permeate flux was stably maintained at 11.25 ± 1.67 LMH during the MBR operation. The organic loading rate ranged from 0.05 to 0.12 kg COD/m^3-d, with an average value of 0.07 ± 0.02 kg COD/m^3-d (Fig. 2e). A decrease in the MLSS concentration was observed throughout the monitoring periods. The MLSS concentration was below 1.0 g/L after 70 days of operation (Fig. 2f). This sharp decline, however, was not affected by the permeate flux.

3.2. Greywater treatment performances

Table 1 shows the greywater treatment performance of the MBR. The pH and temperature values of the influent and effluent were almost identical. The pH values were neutral during the operation at ambient temperature. The DO level in the influent was <0.6 mg/L, while the DO level in the effluent increased up to 5.0 mg/L because of the aeration in the MBR tank. The results showed that the average BOD and COD levels of the greywater were 62 and 149 mg/L, respectively. However, the BOD and COD levels in the reclaimed effluent were consistently lower at 0.4 and 13 mg/L, respectively, with an average treatment efficiency of 99.4 % and 91.2 %, respectively. The average SS level in the influent was 75 mg/L, and it was completely removed through the treatment.

The TN concentration was slightly reduced with a treatment efficiency of only 10.6 %. However, the average NH_4-N content in greywater was 4.5 mg/L; in contrast, NH_4-N was completely removed from the effluent, and its content was below the detection limit of 0.2 mg/L. TP concentration in the influent and effluent ranged from 1.4 to 7.5 mg/L and from 1.5 to 7.0 mg/L, respectively. A slight change in TP concentration was observed between the influent and effluent, with an average removal rate of 17.9 %. With regard to the pathogenic indicators of TC and *E. coli*, the removal efficiencies were > 99 % throughout the experimental period because the permeate effluent was filtered through the membrane with a nominal pore size of 0.4 μm. The concentration of micropollutants in the influent and effluent was measured to investigate their removal efficiency. LAS showed the highest anionic surfactant concentration in the influent (0.14 mg/L), followed by SLS (0.08 mg/L). The biocide concentrations of TCS and TCC in the influent were 1.24 and 0.07 μg/L, respectively. Although the concentrations of the micropollutants in the greywater was analyzed only one time, the MBR treatment could remove >85 % of LAS and TCS and 42–50 % of SLS and TCC.

Table 1
Water quality characteristics of greywater and MBR effluent.

Parameters	Greywater	MBR effluent	Removal efficiency (%)
pH	6.5	6.9	–
Temperature (°C)	29.2	30.0	–
DO (mg/L)	0.6	5.0	–
BOD (mg/L)	62	0.4	99.4
COD (mg/L)	149	13	91.3
SS (mg/L)	75	<0.2	100.0
TN (mg/L)	12.3	11.0	10.6
NH_4-N (mg/L)	4.5	<0.2	100.0
TP (mg/L)	3.9	3.2	17.9
Total coliform (CFU/100 mL)	4.7×10^4	4.0	99.9
E. coli (CFU/100 mL)	351	0.1	99.9
LAS (mg/L)	0.14	0.02	85.7
SLS (mg/L)	0.08	0.04	50.0
TCS (μg/L)	1.24	0.20	98.4
TCC (μg/L)	0.07	0.04	42.9

Note: the results reported here are given as mean ($n = 7$) except for surfactants ($n = 1$).

3.3. Bacterial community structure within the MBR

Bacterial community population at specific time periods was evaluated at the dominant phylum level, as shown in Fig. 3. The composition of bacterial community members was similar between seed sludge and biomass, but with a relative difference in abundance. In both seed sludge and biomass, the bacterial population was dominated by the phyla *Chloroflexi*, *Proteobacteria*, *Actinobacteria*, *Planctomycetes*, *SBR1093*, *Acidobacteria*, and *Bacteroidetes*.

During the operation period, the relative abundance of *Proteobacteria* in seed sludge increased from 16.4 % to 17.9 %, 19.2 %, and 35.1 % on days 26, 68, and 113, respectively. In contrast, the abundance of *Chloroflexi* decreased to 29.1 %, 22.8 %, and 14.3 % during the MBR operation period as compared to that in seed sludge (34.5 %). The abundance of *Planctomycetes* and *SBR1093* generally fluctuated in the range of 8.5–20.3 % and 2.9–6.0 %, respectively, throughout the operation. The abundance of *Bacteroidetes* (2.9–6.3 %) and *Acidobacteria* (3.0–4.4 %) seemed to be relatively constant from the start to the end of the operation. The abundance of *Actinobacteria* was slightly elevated during the MBR operation. Interestingly, the predominance of some phyla affiliated with *Verrucomicrobia* (4.5 %) and *TM6* (2.2 %) was partly detected in the MBR operation period, whereas the abundance of *OD1* (3.8 %) was observed only in seed sludge; this finding highlighted the sequential shift and adaptation of the bacterial community.

Fig. 4 (left panel) shows the distribution of the bacterial population at the class and order levels in each phylum. Within *Proteobacteria*, *Betaproteobacteria* was the most abundant in all samples, followed by *Alphaproteobacteria*, *Gammaproteobacteria*, and *Deltaproteobacteria*. The abundance of these classes was remarkably increased during the MBR operation when compared with that in seed sludge. *Planctomycetes* was also predominantly observed, and its abundance increased up to 19.4 % on day 68 of operation. On the other hand, *Anaerolineae* was the most dominant class in *Chloroflexi*, and its abundance seemed to gradually decrease during the MBR operation. As shown in the right panel of Fig. 4, *Calidinea* sp. was a dominant genus in seed sludge. Although the abundance of other classes showed slight fluctuations, these classes showed almost identical composition in seed sludge and biomass during the operation. Regarding the composition at the order level, the members of *Rhizobiales*, *Rhodobacterales*, *Rhodospirillales*, and *Sphingomonadales* in *Alphaproteobacteria* showed an identical composition in biomass. This result was similar to the composition of the members of *Myxococcales* and *Xanthomonadales* in *Deltaproteobacteria* and *Gammaproteobacteria*, respectively. The members of *Rhodocyclales* appeared to be higher than those of *Burkholderiales* in *Betaproteobacteria*. The members of *Gemmatales* were also higher than those of *Planctomycetales*. However, the dominant genus in these orders could not be identified.

3.4. Dominant bacterial members of the biofilm in the permeate of treated greywater

The bacterial community in the biofilm attached to the Pall ring media was investigated to determine the potential prominent bacterial population in the reclaimed effluent. As shown in Fig. 3, the biofilm formed on Pall ring media submerged in the pipeline of the reclaimed

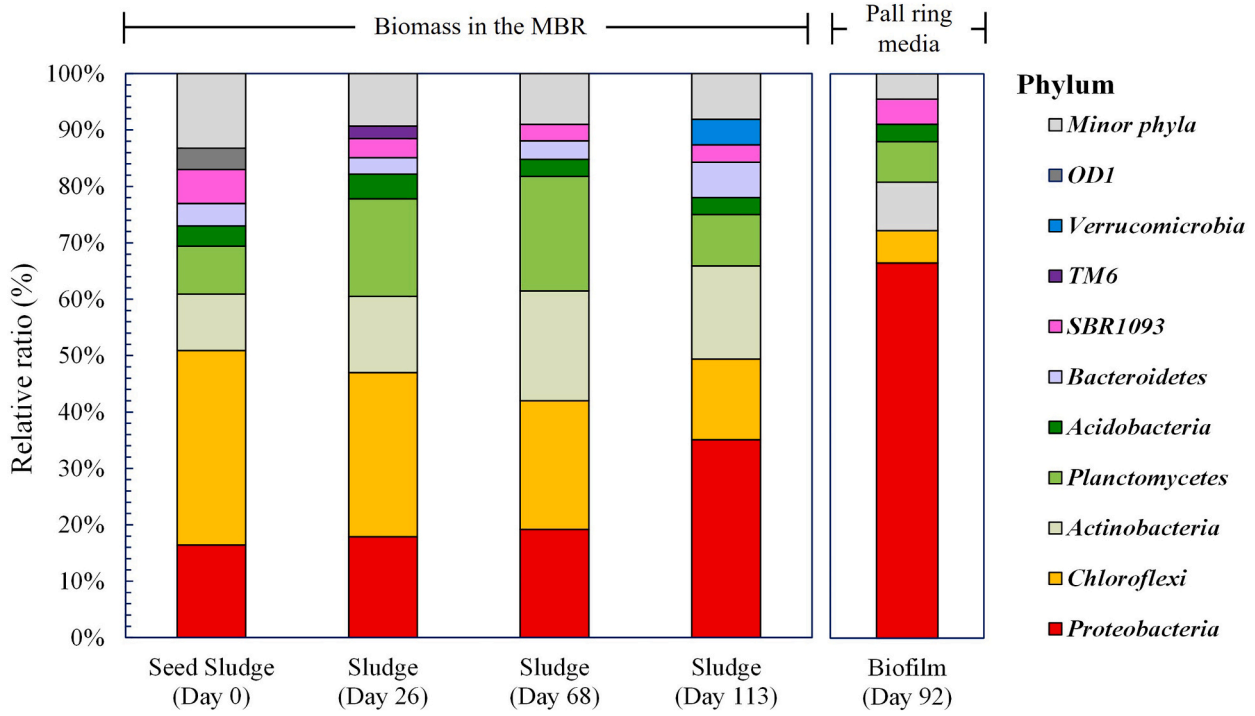

Fig. 3. Spatial times of the microbial communities composition at the phylum level of suspended sludge within the MBR and biofilm on the Pall ring media. Note: Abundance of phyla <2 % was termed as Minor phyla.

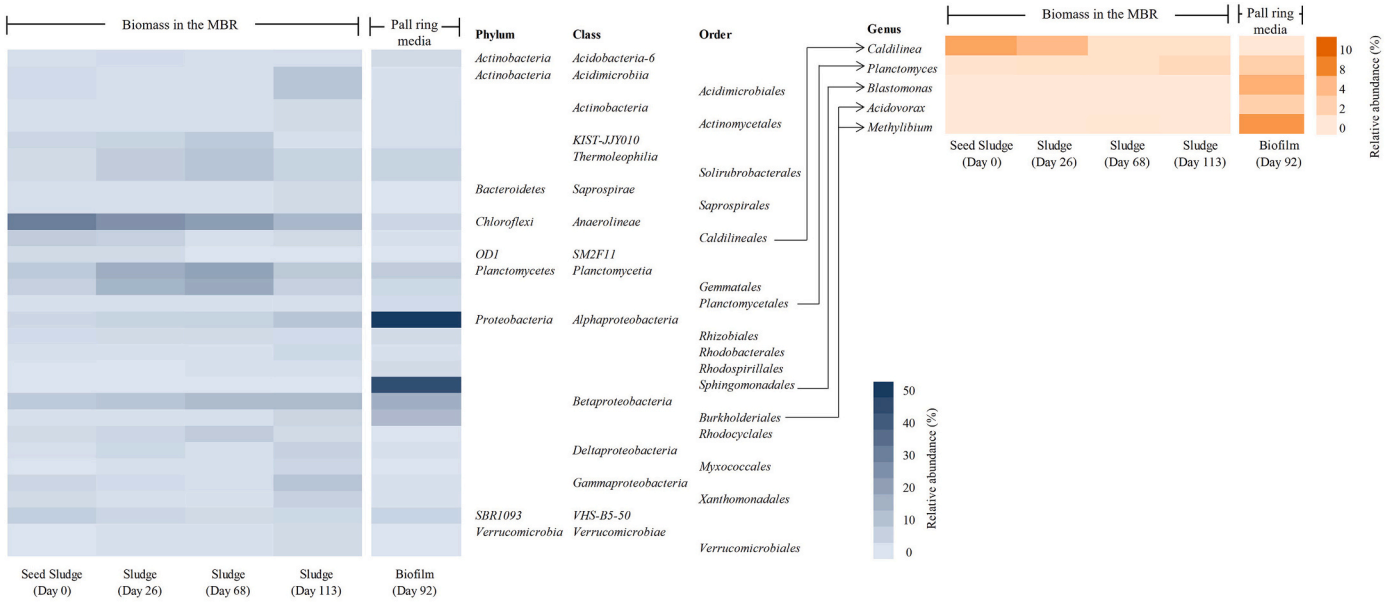

Fig. 4. Heatmap illustrating relative abundance of the dominant bacteria within the MBR and biofilm in the phylum, class, and order levels (blue heatmap on the left panel) and in the genus level which is considered of phyla with abundance higher than 10 % (orange heatmap on the right panel).

greywater contained six bacterial phyla (relative abundance >2 %). Although these phyla were also found in suspended sludge in the aeration tank, their relative abundances were remarkably different. *Proteobacteria* was the most abundant phylum, followed by the phyla *Actinobacteria*, *Planctomycetes*, *Chloroflexi*, *SBR1093*, and *Acidobacteria*. Surprisingly, *Proteobacteria* exhibited the highest relative abundance of 66.5 % in the biofilm, with two classes of *Alphaproteobacteria* (49.5 %) and *Betaproteobacteria* (15.4 %), as shown in Fig. 4 (left panel). Notably, *Sphingomonadales* belonging to *Alphaproteobacteria* was also a predominant order (43.8 %). However, the significant dominant genus in this order could not be identified. Only the abundance of *Blastomonas* sp. (4.9 %) could be identified, as shown in the right panel of Fig. 4. The order *Burkhoderiales* (12.4 %) was also a significant dominant order in *Betaproteobacteria*. Two genera, *Acidovorax* (2.1 %) and *Methylibium* (6.8 %), in this order were dominantly observed in the biofilm.

Fig. 5 shows the PCA ordination diagram for the suspended sludge and biofilm. A clear difference in bacterial community assemblages was observed at the genus level. *Blastomonas*, *Acidovorax*, *Methylibium*, and *Planctomyces* were the significant dominant genera in the biofilm.

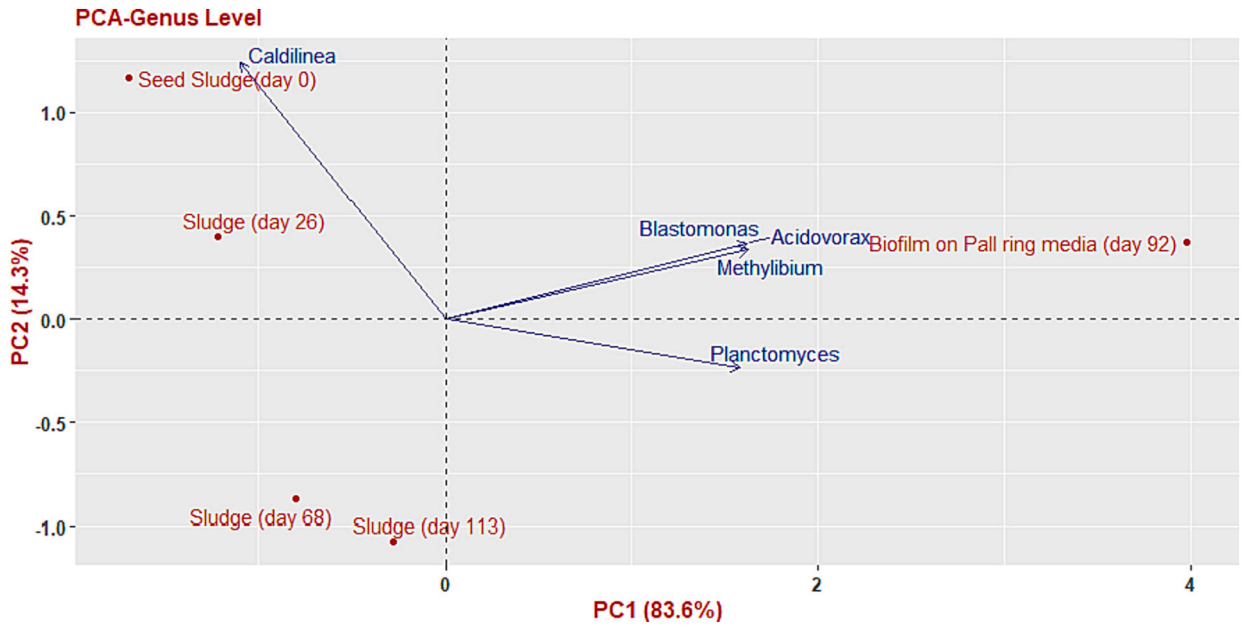

Fig. 5. Principal component analysis (PCA) biplot based on the relative abundance of all detected bacterial genera of sludge in aeration tank of MBR and biofilm on the Pall ring media.

4. Discussion

4.1. Greywater treatment performance of the MBR

The average BOD/COD ratio of the MBR-treated greywater was 0.42 (range: 0.31–0.71), thus indicating the good potential of the MBR for biological treatment [5]. The greywater used in the present study was acquired from university dormitory, and it mainly contained wastewater from bath, shower, and laundry sources; thus, this greywater had a low COD concentration (116–237 mg/L). This finding agreed with the result of Atanasova et al. who reported COD values in the range of 41–535 mg/L for greywater originating from a hotel facility during low and high occupancy seasons [31]. The MLSS concentration in the bioreactor reduced from 4.1 g/L and was stabilized at approximately 0.8–1.0 g/L. The low values of MLSS observed in the present study could be attributed to the long HRT (2 days) of the operation and a low-level concentration of organic components in the influent composition of the greywater affected food scarcity [32]. Various bactericidal substances from shampoos, body soaps, other cleaning agents, and some surfactants can inhibit bacterial growth [33,34].

Although the operation was conducted under low MLSS concentration, the treatment performance was higher than 90 %; this finding agreed with the results of Huelgas et al., except for TN and TP removal [35]. Because the MBR system was operated only under the aerobic condition, the nitrification process alone was promoted, and the nitrogen content was transformed from NH_4-N to nitrate nitrogen (NO_3-N); consequently, the amount of TN was exclusively based on the amount of NO_3-N present in the system [36,37]. In the same way, the process of enhanced biological phosphorus removal could not be supported [38,39]. Moreover, the low MLSS concentration might have led to the slight removal of TP through uptake in bacterial cells [26,40].

In terms of AS; LAS and SLS, high removal rate was attributed to the retention in MBR and the biodegradation capability [34]. Liu et al. who reported that the high AS removal was observed in the influent concentration up to 30 mg/L, indicating that there was no any inhibition of AS biodegradation in previous study [33]. In the same way, TCS and TCC can also remove well through biodegradation in the activated sludge process [41].

Based on these results, we think that the MBR effluent could be used for irrigation purposes in terms of TN and TP concentrations, as US EPA does not provide any specific guidelines regarding the nitrogen and phosphorus concentrations of the water reused for crop irrigation [42]. Some studies have reported that the membrane effluent has potential for nutrient recovery from wastewater that can be reused for agriculture purposes [43,44]. From the economic point of view, the application of the MBR can provide optimal water reuse and nutrient recovery. The investment and operational cost of MBR and other treatments were compatible in terms of the practical benefits gained through water footprint and water recycling [43].

4.2. Differences of bacterial community character between suspended sludge in MBR and biofilm in permeate of treated greywater

Bacterial communities in the MBR and biofilm were similar in terms of members; however, their abundance showed a gradual shift. The abundance of *Chloroflexi* gradually decreased because of the low concentration of organic compounds associated with a long HRT (2 days), which might enhance food scarcity [45]. This phylum also could not proliferate when the MBR was operated at low MLSS concentration [46]. *Proteobacteria* was the predominant phylum during the MBR operation [26,47,48]. This phylum was primarily responsible for the biodegradation of various organic compounds in the MBR [49,50]. Rehman et al. reported that *Proteobacteria* is the most dominant phylum in both sludge and biofilms in the MBR, and this phylum, particularly the *Alphaproteobacteria* class, plays an important role in biofilm formation on surfaces [51]. Biofilms were formed not only on the membrane surfaces in the MBR but also on the Pall ring media in treated greywater, in which *Sphingomonadales* belonging to *Alphaproteobacteria* and *Burkholderiales* belonging to *Betaproteobacteria* were the dominant orders. The members in these classes were more enriched in the biofilm than in suspended sludge [19,52]. This finding was consistent with the result of a previous study [53], which showed that the bacterial communities of *Comamonadaceae* and *Sphingomonadaceae* families belonging to *Burkhoderiales* and *Sphingomonadales* orders, respectively, could pass through the MF step and form a biofilm community on RO membranes.

Although the MF membrane acts as a physical barrier to prevent the leakage of bacteria into the permeate, the presence of bacterial biofilms on the surface of the Pall ring media was detected in the permeate

pipeline. According to Friedler et al., this phenomenon implies that bacteria can migrate from one place to another through aerosols and contaminated pipelines [54]. This type of contamination may occasionally occur in locations where the physical distance between the reactor tank and the permeate tank is minimal [55]. Another hypothesis is that bacterial leakage occurs by their passage through the membranes. Tsutsui & Urase reported the presence of *Acinetobacter junii* and *Microbacterium fluvii* in the permeate; these species could pass through the small pore size of the MF membrane [56]. Bacterial leakage might also occur through the deformation mechanism [57] and variations in the peptidoglycan layer of the cell wall [58]. The conditions of food scarcity in the treated greywater could reduce the size of bacterial cells, thereby allowing them to penetrate through the MF membranes [17,59]. These assumptions indicate that the bacteria that passed through the MF membranes showed regrowth and eventually formed a biofilm on the Pall ring media placed in the permeate pipeline in this study. Moreover, the MBR effluent possibly contained a high amount of assimilable organic carbon [60] that enhanced bacterial regrowth [61] to subsequently generate a biofilm community. In particular, *Sphingomonadales*, which were observed as a dominant community in this study, have previously been identified as significant members of biofilm communities on a surface membrane for water purification; the members of this order possess swarm motility and produce viscous exopolysaccharides, which collectively facilitate their adhesion and colonization of surfaces [14].

4.3. Dominant biofilm formation in the treated greywater permeate at the genus level

The predominant bacterial genera in biofilm formed on the Pall ring media in the permeate pipeline were *Acidovorax*, *Methylibium*, *Blastomonas*, and *Planctomyces*. These genera can adapt to the changes in the micro-ecological environment despite their predominance under famine conditions [62–64]. Some studies have reported that these bacteria can degrade various organic pollutants in the environment; for example, *Methylibium*, a methanotroph, can potentially degrade toxic compounds of methyl tertiary butyl ether [65–68]. This finding agrees with other reports, which state that *Sphingomonas* sp., *Hydrogenophaga pseudoflava*, and *Blastochloris viridis* were the key species in biofilms formed on RO membranes [18,19]. Although the bacterial genera that formed biofilm in the present study were different from those reported earlier, these genera were in the same orders of *Sphingomonadales* and *Burkholderiales* belonging to *Alphaproteobacteria* and *Betaproteobacteria*, respectively. The bacterial genera responsible for biofilm formation may vary depending on feed water characteristics and the survival behaviors of certain bacterial strains. Based on our results, the dominant bacterial members could resist toxic compounds (detergents and biocides) in the treated greywater and could form biofilm under food scarcity conditions. This study indicated an interesting aspect that the richness of the bacterial genera on biofilm is related to the potential bacterial regrowth ability and nutrient conditions in feed water. To the best of our knowledge, the present study is the first to report the different dominant bacterial communities at the genus level in the biofilm formed in the permeate pipeline of a pilot-scale MBR for treating greywater. The reason for the formation of this bacterial biofilm remains unclear and should be investigated in further studies. Moreover, because bacterial communities can regrow on the biofilm formed in the permeate pipeline, an effective post-treatment disinfection process should be developed for treated greywater reclamation. The prevention of biofouling on the RO membrane should also be considered for the MBR-RO process. Furthermore, the effluent quality before and after the Pall ring media set up should be evaluated for subsequent work to gain more understanding of its impact on bacterial regrowth.

5. Conclusions

The MBR was found to be efficient for greywater treatment. *Proteobacteria* was the most dominant bacterial phylum in the MBR. *Proteobacteria* and *Planctomycetes* were the dominant phyla in the biofilm formed on the Pall ring media placed in the permeate pipeline. The dominant bacterial members of *Acidovorax*, *Methylibium*, *Blastomonas*, and *Planctomyces* played a key role in biofilm community. In addition to confirming the excellent greywater treatment performance of the MBR, this study provides more insightful information on bacterial members and their abundance in the biofilm, which is fundamental to optimize reactor performance and prevent adverse biofouling during post-treatment processes.

Declaration of competing interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

Data availability

The sequence datasets generated during the present study are available at NCBI Sequence Read Archive (SRA) under BioProject PRJNA 530355: https://www.ncbi.nlm.nih.gov/bioproject/PRJNA530355. The BioSample accession numbers are SAMN11311902- SAMN11311906.

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