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Characteristics of sewer biofilms in aerobic rural small diameter gravity sewers

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ABSTRACT

Small diameter gravity sewers (SDGS) are extensively used to collect rural sewage as they are low in cost and quick to construct. However, the characteristics of biofilms in rural SDGS are still not clear. In this study, biofilms characteristics of aerobic rural SDGS were investigated using simulations in a lab under different flow conditions and slopes. Results indicated that the average thickness of aerobic rural SDGS biofilms was in the range of 350–650 μm, decreasing at locations with variable flow and high slopes. Protein was the most abundant substance in extracellular polymeric substance of SDGS biofilms. The most abundant bacteria, *Proteobacteria*, *Actinobacteria*, and *Bacteroidetes*, and functional bacteria showed different distributions when analyzed through Illumina HiSeq sequencing of 16S rRNA. The relative abundances of denitrifying bacteria, nitrite-oxidizing bacteria, and sulfate-reducing bacteria (SRB) were lower during variable flow than during stable flow. High slopes (15‰) decreased SRB presence, which could be used to mitigate H_2S accumulation in aerobic SDGS. Overall, this study describes the characteristics of aerobic rural SDGS biofilms and provides valuable suggestions for the optimal design of SDGS based on these characteristics.

Introduction

Sewer systems have been designed for the collection and transportation of sewage in cities, which prevents the rapid spread of diseases caused by randomly discharged sewage. The collection and treatment of sewage in rural areas have drawn extensive attention with the development of rural economies and increased environmental awareness. This is to reduce pollution and health risks caused by rural sewage. However, the construction of sewer systems in rural areas lags behind cities because they lack adequate financial support. Small diameter gravity sewers (SDGS), using smaller pipes (generally less than 200 mm in diameter) to transfer sewage by gravity, suitable for remote villages and communities have been constructed and applied successfully (Otis and Mara, 1985; Simmons and Newman, 1985; Hass, 2007a; b). Compared to conventional gravity sewers, SDGS are less expensive (save more than 20%) and can be constructed rapidly (Little, 2007; Gikas et al., 2017). The flow velocity is usually higher, i.e. lower hydraulic retention time (HRT), in SDGS than conventional sewers since the smaller diameter of

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pipes and higher slopes in SDGS. However, small diameter pipes of SDGS could lead to poor sewer ventilation because the space above sewage is limited compared with conventional sewers and odors caused by the accumulation of H_2S are produced (Dias and Matos, 2001).

Sewer biofilms, attached to the inner wall of sewage pipes, were broadly researched, especially about functional bacteria distributions, on account of their pretreatment functions and safety risks. In sewer biofilms, N-cycle and S-cycle functional bacteria were widely distributed, such as *Pseudomonas*, *Comamonas*, *Desulfomicrobium*, *Desulfovibrio*, and *Desulfonema* (Jin et al., 2018; Li et al., 2019). Further, influential factors of sewer biofilms were extensively researched such as sewage characteristics and shear stress (Marjaka et al., 2003; Liu et al., 2015; Ai et al., 2016; Xu et al., 2016; Jin et al., 2018; Li et al., 2019) while the influence of flow conditions is still not clear. Most research indicated variations of biofilm properties, microbial communities and their effects on sulfide formation in conventional municipal sewers (Sun et al., 2018; Liang et al., 2019b). Rural sewers have different characteristics compared to municipal sewers (e.g. sewage characteristics and flow conditions). Rural sewage usually only contains rural domestic sewage but no industrial wastewater which is usually contained in municipal sewage. Furthermore, the quantity of rural sewage flow is time-dependent; the peak flow is usually at mealtime and is nearly stagnant at night. Thus, rural SDGS may be filled with sewage during peak flow and be empty at midnight which is different with conventional municipal sewer and may cause influences on biofilm properties and microbial communities. Additionally, aerobic conditions, dissolved oxygen (DO) concentration more than 4 mg/L, are widespread within the first hundred meters of the sewer systems (Chen and Leung, 2000; Dias and Matos, 2001).

The facility consists of six groups of lab-scale aerobic SDGS used to explore the characteristics of aerobic rural SDGS biofilms. The morphology and composition of SDGS biofilms were analyzed thoroughly. The bacterial communities of SDGS were studied using Illumina Hiseq sequencing. Particularly, influences of flow conditions and sewer slopes are discussed in this paper. Therefore, this research provides a deeper understanding of aerobic rural SDGS biofilms and recommendations for SDGS applications.

1. Materials and methods

1.1. Experimental facilities and operational conditions

The experimental facility mainly included six groups of 50 mm-diameter transparent unplasticized polyvinyl chloride (UPVC) sewers (Flowcolour, China), UPVC tanks, submerged pumps (HQB-5000, SUNSUN, China), and temperature controllers (300W, YEE, China) (Fig. 1a), which were designed to explore the influence of flow and sewer slopes on SDGS biofilms. The total length of each sewer was 5 m (2 m of straight sewer) and the effective volume of the storage tank was 200 L (L × W × H = 0.8 m × 0.45 m × 0.6 m). The temperature of the facility was maintained at 20 °C and the average DO

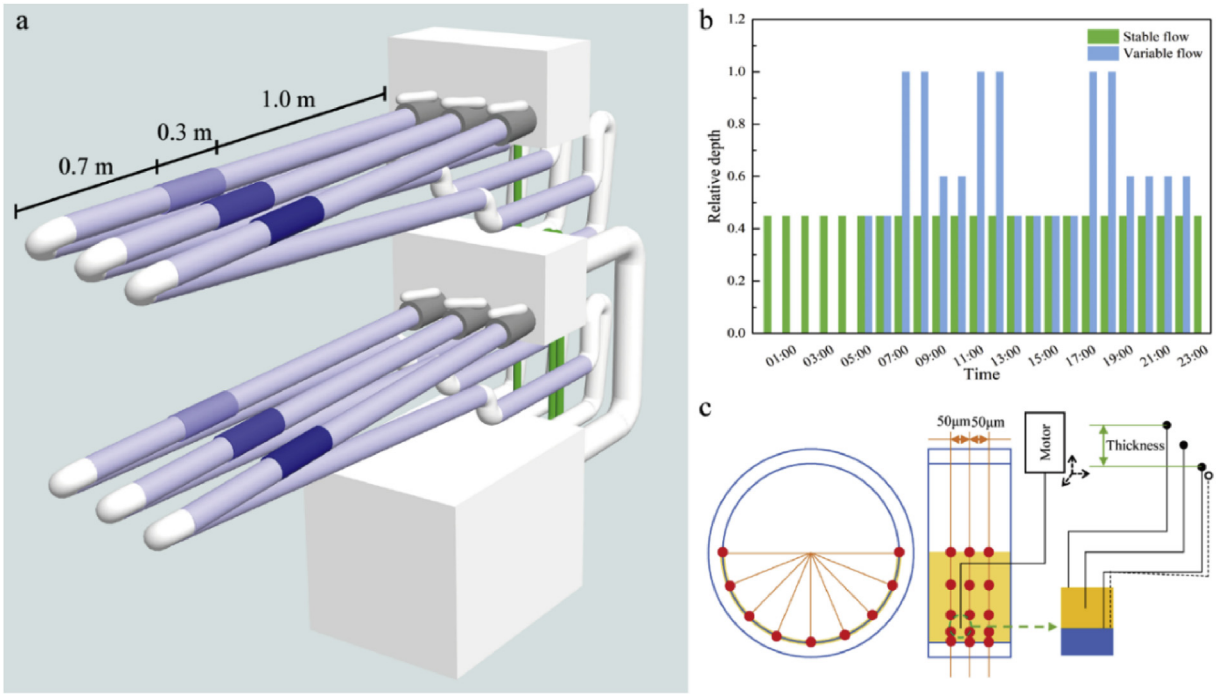

Fig. 1 – Schematic diagram of the experimental facility (a), flow conditions (b) and sampling positions (c). Note: six short blue pipes in (a) depict detachable sewers to biofilm sampling. The framework, submerged pumps (in bottom tanks) and temperature controllers (in bottom tanks) are not shown. Blue bars and green bars in (b) show variable flow conditions and stable flow conditions, respectively. In (c), the blue profiles are the detachable sewers, the brown areas are the position of biofilm sampling, and the red dots are the positions where the thickness was measured.

Table 1 – Operating parameters of the experimental facility.

Group of sewers	Flow condition	Slope (‰)
1	Stable	5
2	Stable	10
3	Stable	15
4	Variable	5
5	Variable	10
6	Variable	15

concentration was 4.09 ± 0.47 mg/L (Oxi 3310, WTW, Germany). The sewage used in this study was made of septic tank blackwater and synthetic greywater (Appendix A Table S1) with a volume ratio of 1:3 (Smolders et al., 1995; Panikkar et al., 2010; Zaharia, 2017) which was refreshed every two days to simulate the real inflow characteristics. The characteristics of sewage are shown in Appendix A Table S2, other operating conditions are shown in Table 1; stable flow implied that the depth ratio was fixed (green bars in Fig. 1b) and variable flow implied that the depth ratio was time-dependent (blue bars in Fig. 1b) which were controlled by pumps and valves. The mean flow velocity were 0.25, 0.28, and 0.38 m/s in the sewer of 1, 2, and 3, respectively. Moreover, the mean flow velocity were 0.00, 0.25, 0.27, and 0.32 m/s in the relative depth of 0.00, 0.45, 0.60, and 1.00 in sewer 4, respectively; the mean flow velocity were 0.00, 0.28, 0.32, and 0.35 m/s in the relative depth of 0.00, 0.45, 0.60, and 1.00 in sewer 5, respectively; the mean flow velocity were 0.00, 0.38, 0.39, and 0.40 m/s in the relative depth of 0.00, 0.45, 0.60, and 1.00 in sewer 6, respectively.

1.2. *Sampling methods*

Detachable sewers in the sewer experimental facility were utilized for SDGS biofilms sampling. Sewer biofilms matured after two months of operation (Marjaka et al., 2003; Ai et al., 2016), and were sampled thrice (named X-1, X-2, and X-3, shown below). Adhered biofilms were scraped off with sterile medicine spoon and cotton swabs, shown in Fig. 1c, in detachable sewers and carefully placed on sterile centrifuge tubes. The samples were transferred to the laboratory immediately and stored at 4 °C for morphology and substance analysis and −80 °C for bacterial community analysis.

1.3. *Morphology analysis*

The thickness of SDGS biofilms was characterized by microelectrodes systems (MM33 and LS18, Unisense, Denmark) and bent wire at a resolution of 50 μm (Fig. 1c), where sewer biofilms were submerged in sewage to simulate real conditions. Due to the heterogeneity of SDGS biofilms, each sewer was measured at 27 points, from 3 measurement layers and 9 measurements from each layer, shown in Fig. 1c. The arithmetic mean value from the measurements was considered the biofilm thickness. Scanning electron microscopy (SEM) was performed with JSM-5610LV (JEOL, Japan). Samples were fixed with 2.5% glutaraldehyde and stored at 4°C in the dark for 24 hr. Then, samples were gradually dehydrated by 25%, 50%, 75%, 95%, and 100% ethyl alcohol and freeze dried at −50 °C.

1.4. *Extracellular polymeric substance analysis*

The total solids (TS) and volatile solids (VS) of biofilms and sewage characteristics were tested using standard methods (APHA, 1998). Extracellular polymeric substance (EPS) was extracted with ultrasonic and centrifugal methods (Yu et al., 2009) and was quantified based on the amount of protein, humic substances, and polysaccharides. Protein and humic substances in EPS were measured using the modified Lowry method (Frølund et al., 1995) using bovine serum albumin and humic acid as the respective standards. Polysaccharides in EPS was measured using the anthrone method (Dubois et al., 1956) using glucose as the standard.

1.5. *DNA extraction and Illumina sequencing*

The DNA from the biofilm samples was extracted with the PowerSoil® DNA Isolation Kit (MoBio, USA). Universal PCR bacterial primer sets 338F (5′-ACTCCTACGGGAGGCAGCA-3′) and 806R (5′-GGACTACHVGGGTWTCTAAT-3′) were used to amplify bacterial 16S rRNA gene sequences. The PCR was performed in a total reaction volume of 20 μL: H_2O 13.25 μL, 10 × PCR ExTaq Buffer 2.0 μL, DNA template (100 ng/mL) 0.5 μL, prime 1 (10 mmol/L) 1.0 μL, prime 2 (10 mmol/L) 1.0 μL, dNTP 2.0 μL, ExTaq (5U/mL) 0.25 μL. After an initial denaturation at 95 °C for 5 min, it was amplified by 30 cycles of incubations for 30 sec at 95°C, 20 sec at 58°C, and 6 sec at 72°C, followed by a final extension at 72°C for 7 min. Then the amplified products were purified and recovered using 1.0% agarose gel electrophoresis. Finally, the Illumina HiSeq sequencing (HiSeq 2500, Illumina, USA) was conducted by Beijing Biomarker Technologies Co. Ltd., Beijing, China. The bioinformatic analysis in this study was partly performed using BMKCloud (www.biocloud.net). Raw tags obtained by merging the paired-end reads by FLASH (1.2.7), were filtered and clustered by Trimmomatic (0.33) and QIIME (1.8.0), respectively, and tags were regarded as operational taxonomic units (OTUs) with a similarity threshold of 97%. Taxonomy was assigned to all OTUs by comparing it with Silva databases using the RDP classifier within QIIME.

2. Results and discussion

2.1. *Morphologies and EPS analysis*

Macroscopic morphology was explored with photographs of the inner surface of sewers shown in Fig. 2, which illustrates the relatively uniform yellow-brown slime-like biofilms formed and attached to the inner surface of the sewers. SDGS biofilms were composed of a large amount of microorganism and EPS (Fig. 2). Bacteria were the most predominant microorganisms in sewer biofilms followed by fungi. Moreover, protozoa (e.g. *Gromia*) were observed in the surface of biofilms, indicating that complex micro-ecosystems existed in sewer biofilms and that SDGS biofilms matured in two months of operation.

The SDGS biofilms thickness data are shown in Fig. 3. The average thickness was in the range of 350 ± 100 μm to 650 ± 250 μm, similar to conventional sewers (Aesoy et al.,

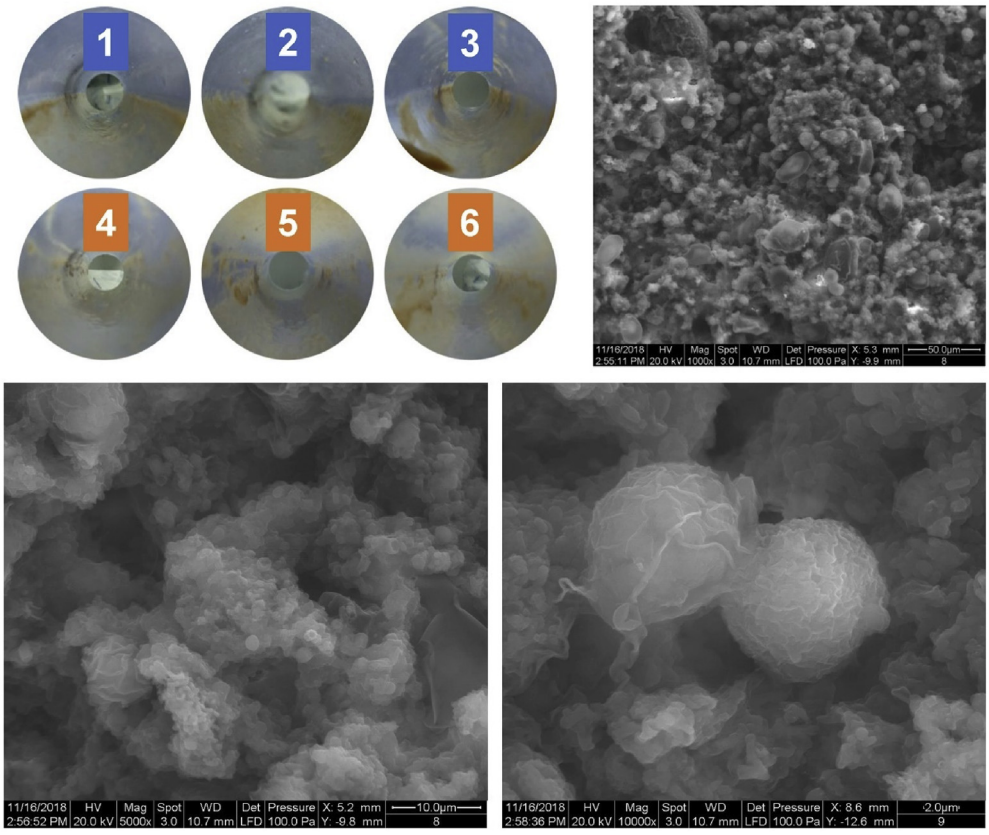

Fig. 2 – Photos and scanning electron microscopy images of small diameter gravity sewer biofilms. Note: Spherical and rod-shaped particles in images were identified as microbe (e.g. bacteria (about 1 μm in diameter) and fungi (more than 2 μm in diameter)), and irregular-shaped substances were extracellular polymeric substance in biofilms.

1997; Sun et al., 2014)._ENREF_11 Two kinds of flow conditions cause significant differences in the thickness of SDGS biofilms formed. The thickness of biofilms in stable flow (600 ± 400 μm to 650 ± 250 μm) was much larger than that in variable

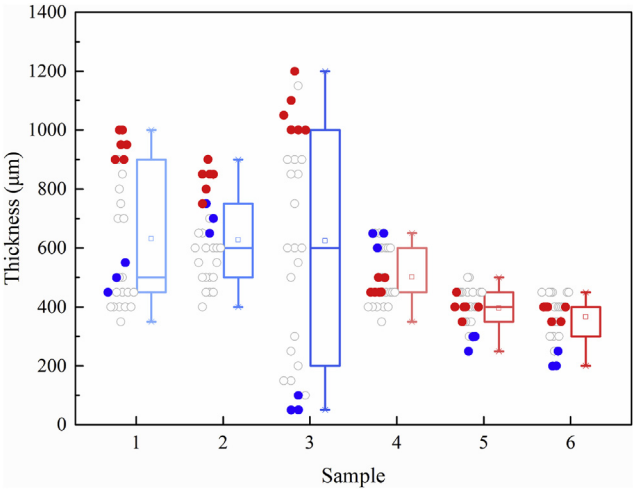

Fig. 3 – The thickness of small diameter gravity sewer biofilms. Note: Red and blue dots on the left of box charts were the thickness of biofilms in the water-air interface and bottom of sewers, respectively. White dots were the thickness of biofilms in other sampling positions.

(400 ± 100 μm to 500 ± 100 μm); variable flow leads to a time-dependent high shear stress in sewage flow which could scour the biofilms powerfully and decrease the thickness of SDGS biofilms. Additionally, cutoff conditions at night, one of situation of variable flow conditions, impeded the development of biofilms. With the increment of slope, the thickness of SDGS biofilms decreased, comparable to the research of Ai et al. (2016) to an extent. The slope of sewers impacted the thickness of SDGS biofilms in variable flow sewage sewers due to its much higher shear stress in full relative depth. Further, the thickness of biofilms had obvious spatial differences on the cross sections of sewers; the thickest biofilms were usually located on the water-air interface, which is due to the relatively lower shear stress and higher local DO concentration at the water-air interface (Wijman et al., 2007; Alihosseini and Thamsen, 2019).

Average TS and VS in this experiment (Appendix A Fig. S1a) were in the range of 0.022–0.033 g/cm^3 and 0.019–0.028 g/cm^3, respectively, similar to conventional sewers (Lemmer et al., 1994; Raunkjaer et al., 1997). The average ratio of VS/TS, representing the ratio of organic matter, reached the range of 0.872–0.907. The high ratio of VS/TS (>0.8) indicates that organics dominate the composition of biofilms, which justifies the high bioactivity of SDGS biofilms. Appendix A Fig. S1b shows protein to be the most abundant substance in EPS of SDGS biofilms, similar to Raunkjaer et al. (1997). Meanwhile, the humic compounds in EPS during stable flow were higher

than during variable flow, contrarily to polysaccharides. The amount of EPS was highest in the slope of 10‰ followed by that at 15‰. The relatively higher shear stress caused by the slope of 10‰ promoted the secretion of EPS but excessive shear stress caused by the slope of 15‰ restrained it.

2.2. Bacterial community characteristics

In total, 711,578 sequences were obtained from 18 samples collected from six sewers, assigned to 265 OTUs, shown in Appendix A Fig. S2. The rarefaction curves (Appendix A Fig. S2a) and Shannon diversity curves (Appendix A Fig. S2b) indicate that the sequencing depth was sufficient to reflect the bacterial community of samples. Additionally, Appendix A Fig. S2b shows that stable flow biofilms have more diversity in bacterial communities than variable flow biofilms. Almost all OTUs (242 OTUs) were shared between the treatments. The specific OTUs in stable flow biofilms were identified to *Bryobacter* sp., *Bdellovibrio* spp., and *Erythrobacter* sp., etc. while *Singulisphaera* sp., *Sphingopyxis* sp., and *Zymomonas* sp., etc. in variable flow biofilms. Different slopes in each flow condition caused slight differences in OTUs distribution shown in Appendix A Fig. S2c. The principal component analysis (PCA) in Appendix A Fig. S2d divided all samples into three clusters according to OTUs: sample 1 and 2, sample 3, and sample 4 to 6; nine samples with three slopes in variable flow conditions were similar and the bacteria communities with high slope (15‰) in stable flow were different from others.

The most abundant bacteria phylum was *Proteobacteria* (59.29% ± 5.57%), coinciding with previous research (Domingo et al., 2011; Jin et al., 2018), followed by *Actinobacteria* (23.50% ± 4.52%), *Bacteroidetes* (13.57% ± 4.13%), *Planctomycetes* (1.02% ± 0.46%), and *Firmicutes* (0.75% ± 0.28%) in SDGS biofilms (Fig. 4a). However, Jin et al. (2018) found the presence of *Firmicutes* to be higher than *Actinobacteria* in anaerobic sewer biofilms. The significantly different distributions in *Actinobacteria* and *Firmicutes* were due to DO concentrations. Note that *Actinobacteria* (mostly environmental-associated bacteria) thrives in aerobic environment and *Firmicutes* (mostly human-fecal-associated bacteria) in anaerobic. Fig. 4b indicates that *Arenimonas* (16.58% ± 4.49%), *Paenarthrobacter* (14.03% ± 4.18%), *Flavobacterium* (9.96% ± 4.69%), *Glutamicibacter* (8.87% ± 5.97%), and *Acidovorax* (8.04% ± 0.92%) were predominant in SDGS biofilms. *Arenimonas*, one genus of aerobic rod bacteria belonging to *Gammaproteobacteria*, is positive for the hydrolysis of casein and tyrosine, but negative for the hydrolysis of starch, urea, and cellulose (Soon-Wo et al., 2007). *Paenarthrobacter*, one genus of aerobiotic coccus form *Actinobacteria*, can hydrolyze starch and consume various organics for carbon (Busse, 2016). The wide distribution of heterotrophic bacteria (e.g. *Arenimonas* and *Paenarthrobacter*) in all samples indicates that aerobic SDGS have the ability to decrease COD in sewage.

Fig. 5a indicates that stable flow sewer biofilms had bacteria more evenly distributed than variable flow sewer biofilms. The abundance of *Arenimonas*, *Paenarthrobacter* and *Flavobacterium* was significantly higher in variable flow sewers, contrarily to *Glutamicibacter*. Redundancy analysis (RDA), based on Appendix A Table S3, shows the standard deviation of relative depth and thickness of sewer biofilms significantly influenced bacterial communities, while the influence of slopes was relatively weak (Fig. 5b). The high shear stress caused by variable flow during heavy flow could eliminate fragile bacteria and rebuild new micro-ecosystems which could resist the scouring of sewage. The ternary diagrams of two conditions shown in Appendix A Figs. S3a–b illustrate the primary parts of bacterial communities located in the center of ternary diagrams, which reveal that slopes of sewers could not cause obvious impacts on primary parts of bacterial communities compared to flow conditions. EPS, secreted by biofilms, may prevent the filtering process of mild shear stress caused by slopes, which could result in the minor differences between samples. Additionally, variable flow could make the distribution of primary genera more centralized in contrast with stable flow.

2.3. Functional bacteria

Functional bacteria in SDGS biofilms were classified into five, ammonia-oxidizing bacteria (AOB), nitrite-oxidizing bacteria (NOB), denitrifying bacteria (DNB), sulfur-oxidizing bacteria (SOB), and sulfate-reducing bacteria (SRB) (Matějů et al., 1992; Kowalchuk and Stephen, 2001; Schmidt et al., 2002; Abeliovich, 2006; Muyzer and Stams, 2008; Ge et al., 2015; Dong et al., 2017). Fig. 6 and Appendix A Fig. S4 indicate that *Nitrosomonas* (0.15% ± 0.05%) and *Nitrosospira* (0.10% ± 0.03%) were the detectable AOB, *Nitrospira* (0.21% ± 0.09%) was the only genus of detectable NOB, *Sphingomonas* (0.07% ± 0.02%) and *Acidiphilium* (0.01% ± 0.01%) were the detectable SOB, and, *Desulfobulbus* (0.13% ± 0.07%) and *Desulfomicrobium*

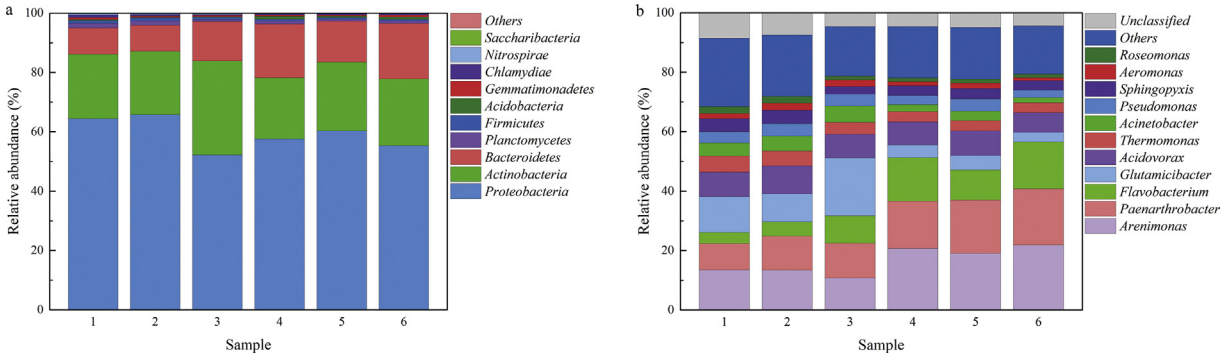

Fig. 4 – Relative abundance of bacteria at the phylum (a) and genus (b) level of small diameter gravity sewer biofilms.

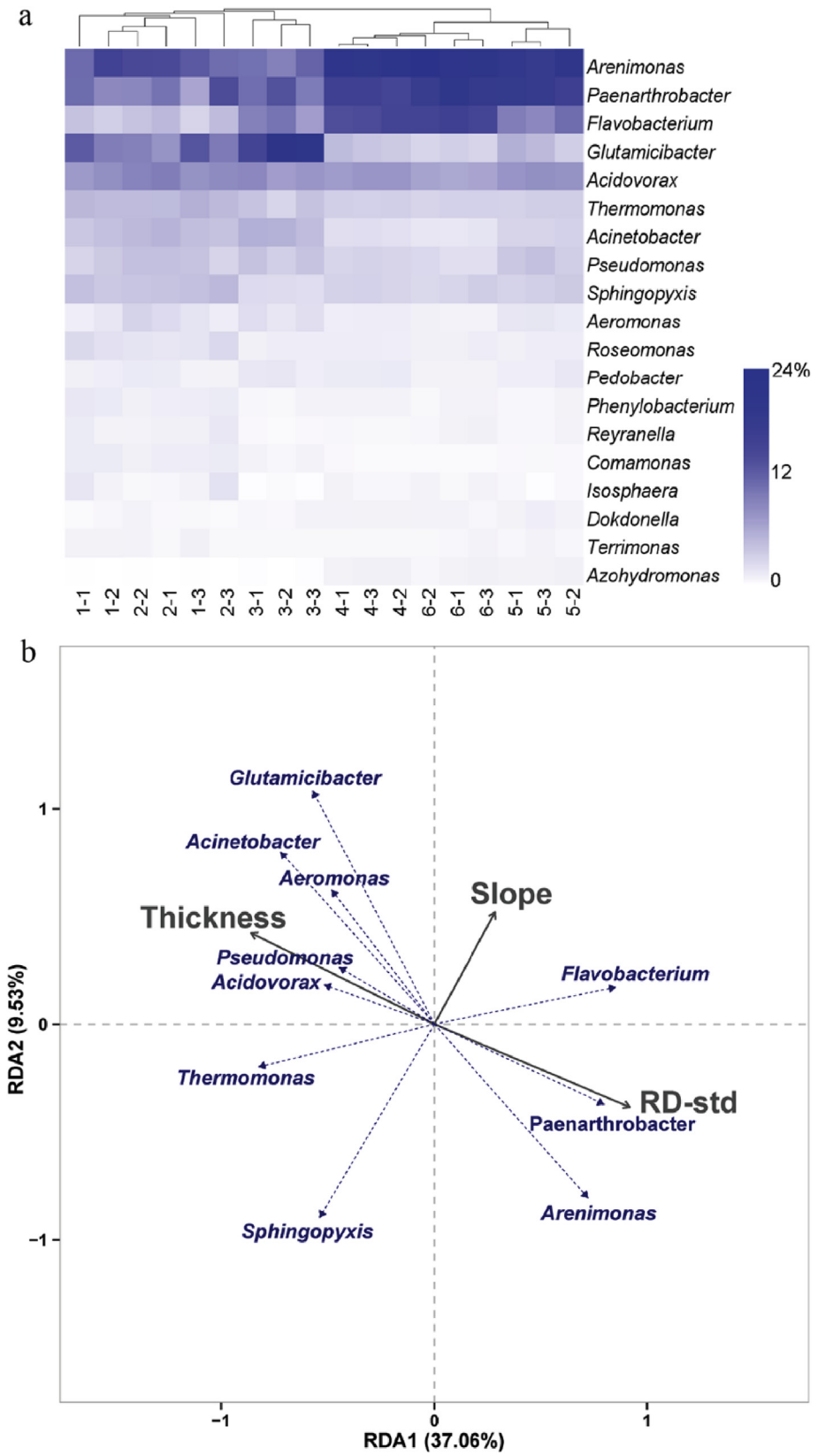

Fig. 5 – The heat map of the most abundant genera in samples (a), the redundancy analysis of bacteria at genus levels (b). Note: RD-std in (b) was the abbreviation of the standard deviation of relative depth in sewers.

(0.07% ± 0.03%) were the detectable SRB in SDGS biofilms. Further, 17 genera of DNB were detected in SDGS biofilms in which *Acinetobacter* (3.72% ± 1.43%) and *Pseudomonas* (3.63% ± 0.78%) were most abundant. The abundance of AOB and NOB in conventional municipal sewers are usually low since anoxic conditions and abundant organics in sewage can promote the growth of heterotrophic bacteria but restrain aerobic autotrophic bacteria (Li et al., 2019). However, the aerobic sewer condition in this study can promote the development of AOB and NOB to some extent. Additionally, the total abundance of AOB and NOB was similar in aerobic SDGS biofilms (Fig. 6b) which suggested full nitrification process could be achieved. *Desulfobulbus* exists widely in pipes and manholes of sewer systems and have high toleration for oxygen concentration which might cause the higher abundance in this study (Warthmann and Cypionka, 1990; Ito et al., 2002; Dong et al., 2017). The abundance of SRB in this experiment (about 0.2%) was lower than many previous researches (more than 2%) (Jiang et al., 2009; Sun et al., 2018; Liang et al., 2019b) which were focused on municipal sewer systems. The difference in SRB abundance is mainly due to sewage characteristics and operation conditions. Specifically, the sulfate concentration of sewage in Jiang et al. (2009) and Liang et al. (2019b) was obviously higher than this study and the results of Sun et al. (2018) were analyzed from a rising main sewer which was under anaerobic conditions. The aerobic sewer

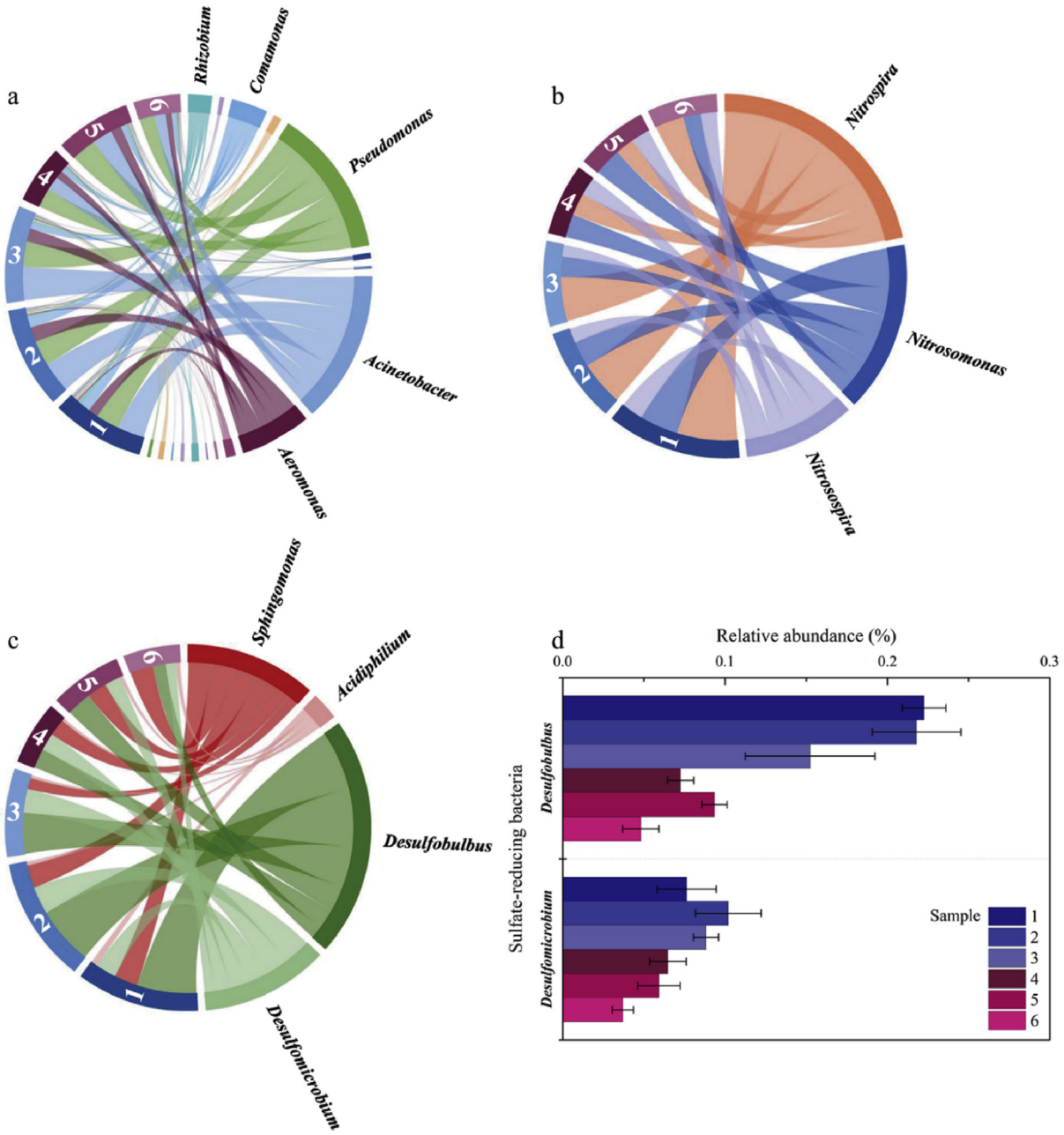

Fig. 6 – The distribution of functional bacteria in small diameter gravity sewer biofilms. (a) denitrifying bacteria; (b) ammonia-oxidizing bacteria and nitrite-oxidizing bacteria; (c) sulfur-oxidizing bacteria and sulfate-reducing bacteria; (d) relative abundance of sulfate-reducing bacteria.

conditions caused lower SRB abundance while the total abundance of SRB is still higher than SOB in this study (Fig. 6c).

Fig. 6 and Appendix A Fig. S5 indicate the distribution of AOB, NOB, DNB, SOB, and SRB were different in the different flow conditions. The abundance of NOB, DNB, and SRB were higher during stable flow than that in variable flow. However, the differences of AOB and SOB caused by flow conditions were relatively little. Organic loading in variable flow fluctuated and might cause adverse effects on heterotrophic functional bacteria (e.g. SRB and DNB), especially at stagnant period of SDGS. Biofilms exposed to air in variable flow at night could dramatically raise DO concentration and therefore inhibit the activity of SRB and some DNB. Moreover, variable flow conditions in daytime, especially at mealtime, which usually caused relatively lower HRT (similar to hydraulic flushing period in conventional sewers) in comparison to stable flow conditions could decrease the SRB abundance. Research focused on real municipal sewers found that low HRT, especially during hydraulic flushing, can effectively decrease the sulfide production rate and SRB abundance in sewer biofilms (Liang et al., 2019a, 2019b). Fig. 6d indicates that the decrease of *Desulfomicrobium* was less than *Desulfobulbus* in variable flow conditions. *Desulfomicrobium* existed extensively in many sewers (Sun et al., 2014; Dong et al., 2017; Li et al., 2019) that have widely adaptability in various conditions and might be tolerant to the change of flow conditions shown. Previous studies on biofilms during stable flow may overestimate the abundance of functional bacteria (e.g. NOB, DNB, and SRB) as most gravity sewers have variable flow. As a result of time-dependent sewage flow in rural areas, the flow condition is a factor to be considered in studies on sewer biofilms and the H_2S accumulation in rural sewers might less than municipal sewers to a certain extent.

Fig. 6 indicates that slopes could cause different degrees of influence on functional bacteria; the relative abundance of SRB in the 15‰ slope (Sample 6) was lower than the other two slopes while the relative abundance of SOB in it was higher during variable flow. Low abundance of SRB suggested H_2S generated by sewer biofilms was limited and could be further oxidized by high abundance of SOB. The different distributions of SRB and SOB implied that large slopes might reduce the risk of H_2S accumulation and odor generation in aerobic rural SDGS. However, it should be pointed out that using high slope to mitigate H_2S accumulation in aerobic SDGS might have practical limitation by topography and construction cost. Moreover, the pre-settled devices, septic tanks, are common facilities in SDGS systems globally but they are hotspots for H_2S accumulation (Zuo et al., 2019) and they decrease the DO concentration of SDGS. Low DO concentration promotes the growth and metabolism of SRB, causing more H_2S generation in SDGS. Therefore, if sewage was filtered effectively before emitted into SDGS, septic tanks should also be avoided in SDGS systems to reduce the accumulation of H_2S.

3. Conclusions

The thickness of aerobic rural SDGS biofilms was in the range of 350–650 μm and EPS was mainly consisted of protein. *Proteobacteria*, *Actinobacteria*, and *Bacteroidetes* were the most three abundant phylum and *Arenimonas*, *Paenarthrobacter*, and *Flavobacterium* were the predominant genus of aerobic rural SDGS biofilms. DNB, AOB, NOB, SRB, and SOB were detected and the abundance of DNB was apparently higher than other functional bacteria. Flow conditions and slopes caused obvious influence on morphology and bacteria community characteristic of aerobic rural SDGS biofilms and the influence of flow conditions was more obvious. Variable flow could decrease the thickness, bacteria diversity, and relative abundance of some functional bacteria of SDGS biofilms. Previous studies on biofilms during stable flow may overestimate the abundance of functional bacteria during true flow conditions. High slope (15‰) decreased the abundance of SRB and increased the abundance of SOB in variable flow SDGS biofilms and could help to lower the risk of H_2S accumulation and sewer odor generation.

Conflicts of interest

All the authors declare no competing financial interests and are aware of and accept responsibility for this manuscript.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jes.2019.10.019.

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