Mechanism and kinetics of biofilm growth process influenced by shear stress in sewers
Hainan Ai, Jingwei Xu, Wei Huang, Qiang He, Bingjie Ni and Yinliang Wang

ABSTRACT

Sewer biofilms play an important role in the biotransformation of substances for methane and sulfide emission in sewer networks. The dynamic flows and the particular shear stress in sewers are the key factors determining the growth of the sewer biofilm. In this work, the development of sewer biofilm with varying shear stress is specifically investigated to gain a comprehensive understanding of the sewer biofilm dynamics. Sewer biofilms were cultivated in laboratory-scale gravity sewers under different hydraulic conditions with the corresponding shell stresses are 1.12 Pa, 1.29 Pa and 1.45 Pa, respectively. The evolution of the biofilm thickness were monitored using microelectrodes, and the variation in total solids (TS) and extracellular polymer substance (EPS) levels in the biofilm were also measured. The results showed that the steady-state biofilm thickness were highly related to the corresponding shear stresses with the biofilm thickness of 2.4 ± 0.1 mm, 2.7 ± 0.1 mm and 2.2 ± 0.1 mm at shear stresses of 1.12 Pa, 1.29 Pa and 1.45 Pa, respectively, which the chemical oxygen demand concentration is 400 mg/L approximately. Based on these observations, a kinetic model for describing the development of sewer biofilms was developed and demonstrated to be capable of reproducing all the experimental data.

Key words | biofilm thickness, EPS, kinetic model, sewer, shear stress

INTRODUCTION

As an important part of urban sewerage system, sewers are responsible for collecting and transporting wastewater. In recent years, from the idea of recognizing the sewers as the reactions vessels to the observations on production of methane and H2S in the sewer (Guisasola et al. 2008; Foley et al. 2009; Gutierrez et al. 2009; Jiang et al. 2009; Jiang et al. 2010; Auguet et al. 2015; Liu et al. 2015), as well as corrosion of sewers (Nielsen & Hvitved-Jacobsen 1988; Vollertsen et al. 2008), increasing evidences demonstrated vast biochemical reactions occur in the sewers. These biochemical reactions are essentially driven by sewer biofilms. Therefore, understanding the growth process of sewer biofilm is critical for the potential mitigation of sewer emissions and corrosions. However, there is still not a study to fully describe the growth process of sewer biofilm in literature, particularly its relationship with the shear stress in sewer systems.

The shear stress is one of the major factors in biofilm growth, and it directly influences the growth and shedding of biofilm. The ratio of the surface loading rate to the shear stress of the biofilm might determine the structure of the biofilm, with a smooth and compact biofilm being obtained under suitable conditions (Gjaltema et al. 1997). Rittmann (1982) found that the shedding rate of the biofilm on a biological rotating disc is in proportion to 0.58 power of the shear stress as long as other parameters are constant, and the hydraulic shear force is the most important mechanism in the shedding of biofilm. Horn et al. (2003) found that the shedding of the biofilm resulted from a common function of the liquid shear stress and the strength of biofilm. Rochex et al. (2008) not only demonstrated a link between shear stress and composition of the microbial communities and high shear stresses decreased biofilm diversity but also shear stress would slow down biofilm maturation and tend to maintain a young biofilm. Etienne (Paul et al. 2012) found that for the same substrate, shear stress largely determined biofilm average thickness.

These various controversial findings suggested that a comprehensive study is desired to systematically investigate the impacts of shear stress on the growth of sewer...
biofilm and the related biofilm characteristics, e.g. microbial activity and extracellular polymer substance (EPS) production.

The aim of this study was to explore the mechanisms of the growth process of sewer biofilm through varying shear stress and substrate concentration in a sewer system. The evolution of the biofilm thickness under different conditions were monitored using microelectrodes, and the variation in total solids (TS) and EPS levels in the biofilm were assessed. A kinetic model for describing the development of sewer biofilms was also developed to interpret the experimental observations.

METHODS

Reactor setup and operation

As shown in Figure 1, the reactors mainly include tanks, sewers, magnetic pumps and a temperature controller. The laboratory setup used was designed to mimic the main features of sewer systems including (i) hydraulic features: shear stress and (ii) wastewater characteristics: chemical oxygen demand (COD) concentration, pH and temperature. The magnetic pumps were used to ensure continuous 24-hour operation. The temperature controller can maintain the temperature in the range of 20–25°C, consistent with that of an actual sewer.

The system consists of a three laboratory-scale gravity sewers operated in parallel (Figure 1), the slopes are 0.005, 0.008 and 0.010, respectively. Sewer reactor under each slope has a diameter of 57 mm and a length of 8 m. Sewer biofilms were developed on specially designed PVC chips (3 x 3 cm) that were installed in the sewer wall. In our previous study (Maolin 2017), the relationship between flow conditions and wall-shear stress in sewers was investigated by utilizing the particle image velocimetry technique combined with fluent software and the results showed that as the slopes are 0.005, 0.008 and 0.010 in this experiment, the corresponding shear stresses are 1.12 Pa, 1.29 Pa and 1.45 Pa, respectively.

In this study, a synthetic wastewater mimicking domestic wastewater was used. The composition of this synthetic wastewater is given in Table 1.

Experimental design

The experiment was divided into two parts. Part 1 used the COD concentration of 400 mg/L, approximately, and Part 2 used 200 mg/L, approximately, and both the shear stresses were 1.12 Pa, 1.29 Pa and 1.45 Pa, respectively, corresponding to system A, system B and system C.

The testing period for the system lasted approximately 45 days. The biofilm carriers were removed from the reactor rods regularly to determine the biofilm characteristics within the reactors.

Biofilm characteristics analytical methods

The biofilm thickness was measured with microelectrodes. Due to the heterogeneity of the local biofilms, nine measurements were made at each biofilm sample and calculated the average of the nine measurements. The average biofilm
thickness (δ, mm) at the periods was calculated from the values. The biomass on the tested sample was scraped off completely and its dry mass was weighed in terms of TS (g) and volatile solids (VS, g), according to the standard APHA methods (APHA 1998). The biofilm density, \( X' (g \cdot l^{-1}) \), of individual carrier, was calculated using the following equations:

\[
X' = \frac{TS}{\delta A_1} (gTSI^{-1}) \quad \text{or} \quad X' = \frac{VS}{\delta A_1} (gTSI^{-1}) \tag{1}
\]

where \( A_1 \) is the surface area of the tested biofilm sample.

The quantity of EPS in the biofilms was indicated by measuring the amount of protein, polysaccharides and humus in the EPS. EPS extraction was carried out by cation exchange resin (Dowex Marathon C, 20–50 mm mesh, sodium form, Fluka 91973) proposed by Frolund et al. (1996), and the analysis method was according to the description proposed by Gaudy (1962).

**Microbial diversity analysis**

**454 high-throughput pyrosequencing**

For a full understanding of the microbial community structure evolution resulting from shear stress variation, mature biofilm samples were analyzed by 454 high-throughput pyrosequencing when the biofilms reached their steady state. The biofilm samples taken from the chip were then transferred to 2.0 mL plastic centrifuging vials and transported to Sangon Biotech (Shanghai) Co., Ltd for contract analysis. The composition of the PCR products of 16S rRNA gene was determined by pyrosequencing using the Roche 454 GS-FLX Titanium sequencer (Roche 454 Life Sciences, Branford, CT, USA). Samples in this study were individually barcoded to enable multiplex sequencing. The results are deposited into the National Center for Biotechnology Information (NCBI) short reads archive database.

**Biodiversity analysis**

The operational taxonomic unit (OTUs) clustering was performed by setting a 0.03 or 0.05 distance limit (equivalent to 97 or 95% similarity) using the MOTHUR program (http://www.mothur.org/wiki/Main_Page). From the cluster file, rarefaction curves at \( \alpha \) of 0.03, chao1 richness estimator (http://www.mothur.org/wiki/Chao), Shannon diversity index (http://www.mothur.org/wiki/Shannon, Shannon diversity index is commonly used to characterize species diversity in a microbial community and it accounts for both abundance and evenness of the species present) and the Good’s coverage (http://www.mothur.org/wiki/Coverage) were generated in MOTHUR for each sample.

**Kinetic modeled**

Firstly, the univariate non-linear fitting of origin was used to identify the relationship between the biofilm thickness and time under different shear stress conditions. Then the corresponding single regression models were established according to the measured data, and the models went through statistical tests.

The relationship between the biofilm thickness and time obtained through the first step was utilized, combined with the measured data of EPS and TS, and then a multivariate regression model was established to describe the relationship of biofilm thickness with shear stress, TS, EPS and COD concentration. Then the confidence interval and credibility of the model was analyzed.

**RESULTS AND DISCUSSION**

**Variations of the biofilm thickness at varying shear stress**

The biofilm thickness data obtained from this experiment are shown in Figure 2. The biofilm thickness changes according to a similar pattern under different shear stresses. Firstly, biofilm thickness reaches a maximum value within 0–25 days. This means that the growth rate of the biofilm is greater than the shedding rate within this period. Within

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**Table 1** Composition of synthetic wastewater/ mg·L\(^{-1}\)

<table>
<thead>
<tr>
<th>Substance</th>
<th>Concentration/ mg·L(^{-1})</th>
<th>Substance</th>
<th>Concentration/ mg·L(^{-1})</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaAc·3H(_2)O</td>
<td>850 (COD ≈ 400)</td>
<td>NH(_4)Cl</td>
<td>114.6 (NH(_4^+) N ≈ 30)</td>
</tr>
<tr>
<td>NaH(_2)PO(_4)2H(_2)O</td>
<td>50.3</td>
<td>MgSO(_4)·7H(_2)O</td>
<td>180</td>
</tr>
<tr>
<td>KCl</td>
<td>72</td>
<td>CaCl(_2)</td>
<td>10.6</td>
</tr>
<tr>
<td>Peptone</td>
<td>5</td>
<td>NaHCO(_3)</td>
<td>225</td>
</tr>
<tr>
<td>FeCl(_3)-6H(_2)O</td>
<td>375</td>
<td>MnCl(_2)·4H(_2)O</td>
<td>30</td>
</tr>
<tr>
<td>H(_3)BO(_3)</td>
<td>37.5</td>
<td>ZnSO(_4)·7H(_2)O</td>
<td>30</td>
</tr>
<tr>
<td>CuSO(_4)·5H(_2)O</td>
<td>7.5</td>
<td>EDTA</td>
<td>30</td>
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<tr>
<td>KI</td>
<td>45</td>
<td>T((^\circ)C)</td>
<td>20–25</td>
</tr>
<tr>
<td>pH</td>
<td>7–8</td>
<td></td>
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</tr>
</tbody>
</table>

GS-FLX Titanium sequencer (Roche 454 Life Sciences, Branford, CT, USA). Samples in this study were individually barcoded to enable multiplex sequencing. The results are deposited into the National Center for Biotechnology Information (NCBI) short reads archive database.
5–10 days after the thickness reaches its maximum value, as the shedding rate continuously increases with an increase in thickness of the biofilm, it becomes higher than the growth rate. For this reason, the thickness of the biofilm decreases to a certain extent. Finally, as the growth rate and the shedding rate gradually reach equilibrium, biofilm thickness tends towards stability. Under the conditions of this experiment, the thicknesses of the biofilms were 2.4 ± 0.1 mm, 2.7 ± 0.1 mm and 2.2 ± 0.1 mm at shear stresses of 1.12 Pa, 1.29 Pa and 1.45 Pa, respectively. When the biofilm thickness tends towards stability, the removal of COD were 40%, 35% and 32%, respectively.

Guzman et al. (2010) indicated that shear stress within the range of 1.1–1.4 Pa is suitable for biofilm growth in a sewer, in accordance with established sewer wall shear stress mathematical models. It can be seen form Figure 2 that the maximum thickness of the biofilms was largest in the sewer with shear stress of 1.12 Pa, while the smallest maximum thickness was with shear stress of 1.49 Pa. Finally, the stable state thickness was the largest with the shear stress of 1.29 Pa.

In the later stage of biofilm growth, a steady state is gradually reached. Biofilm thickness in the steady state was \( T(F = 1.29\, \text{Pa}) > T(F = 1.12\, \text{Pa}) > T(F = 1.45\, \text{Pa}) \), which confirms the results of Guzman’s research.

**Variations of TS in biofilms at varying shear stress**

In this study, due to the use of artificial water, the inorganic materials in the biofilm can be ignored and the average mass density of the biofilm (TS) represents the microorganism quantity. TS increases constantly in the early period and reach to a maximum value of 92–126 kg/m² as Figure 3 shows because the biofilm grows rapidly and its structure is compact. Although the thickness of the biofilm increased constantly, TS began to decrease in the middle stage and was similar to that of the research carried out by Chen (Chen & Chen 2000). When the biofilm reached the stable period, its thickness reduces through shedding action, and corresponding TS increases slightly. In this period, the rate of shedding increases constantly because of the increasing thickness of the biofilm. When the rate of shedding exceeds the rate of growth, the loose part of the upper layer of the biofilm begins to shed and thickness reduces, so TS in the biofilm increases. Finally, when the growth of the biofilm reaches a steady state, TS in the biofilm also reaches a steady state.

In days 0–10, corresponding TS in the biofilm was largest for the shear stress of 1.45 Pa. Since shear stress of 1.45 Pa was greatest, the structure of the biofilm was the most compact, and the density of biofilm was largest. When the biofilm was in a steady state, the average mass density of biofilm was largest when the shear stress was 1.45 Pa, and the average mass density of the biofilm was smallest when shear stress was 1.12 Pa.

**Variations of EPS in biofilm at varying shear stress**

EPS is an important component in the biofilm. As Figure 4 shows, under different shear stress conditions, the amount of EPS in the biofilm demonstrates similar patterns of variation during the growth of biofilm. The effect of the shear stress on the EPS has two aspects. On the one hand, the transformation of oxygen and other substrates in the biofilm would be impacted by the shear stress and then the activity...
of the microorganism would be effected. The stronger the shear stress, the more difficult the transformation and the lower the activity of the microorganism, and the amount of the EPS would be less. On the other hand, the shear stress influence the surface scour of biofilm, and the stronger the shear stress, the stronger the erosion of the biofilm and the attachment of the EPS is more difficult. As the thickness of biofilm continuously increases, the amount of EPS in the biofilm gradually increases. After the amount of EPS reaches its peak, it reduces to a certain extent before reaching a steady state. Maximum values of EPS content varied with shear stress, with the highest maximum recorded at the shear stress of 1.12 Pa, and the lowest at the shear stress of 1.45 Pa. This indicates that, in the later period of growth of the biofilm, the erosive effect of shear stress is mostly directed at surface EPS.

**Relationship between biofilm growth, TS content and EPS characteristics**

*Figure 5* is a change tendency chart for thickness of the biofilm and content of TS and EPS in the biofilm at shear stress of 1.12 Pa. In days 0–10, as biofilm thickness and TS continuously increased, EPS content also became greater, but the rate of increase of EPS was clearly slower than that of TS and thickness. This shows that the growth of the biofilm consists mainly in the increase of cells. In days 10–23, biofilm thickness increased continuously to a maximum value and EPS content also increased continuously to a maximum value. This shows that while biofilm thickness increases, the biofilm as a whole is relatively loose. The cause of the sustained increase in biofilm thickness in this period is the large amount of EPS secreted during its growth. *Kreft & Wimpenny* (2001) indicated that EPS increases the porosity of the biofilm. The density of the biofilm is thereby reduced and its structure becomes loose, so that TS is also reduced. In the process of growth of the biofilm, as it increases in thickness it forms unevenly in the space. That is, it develops anisotropically. As the biofilm continuously thickens, the upper layer of the biofilm becomes loose under the influence of hydraulic shear stress and the requirements of microbial metabolism. For this
reason, although the thickness of the biofilm increases constantly, it reduces in density. In this period, the increase in biofilm thickness is caused by the large amount of EPS secreted during the growth of biofilm, so that the structure of the biofilm is loose and the density is reduced.

As part of the process of growth of the biofilm, the upper layer of the loose biofilm begins to fall off, and biofilm thickness is reduced to a certain extent. As thickness reduces, EPS in the biofilm decreases and TS increases to a certain extent. Change trends of EPS and biofilm thickness are identical. This may result from the large amount of EPS in the upper layer of biofilm. When the biofilm is shed because of loose structure caused by shear stress, EPS in the upper layer of biofilm also drops off, so the amount of EPS in the biofilm is reduced.

The amount of EPS in mature biofilms was measured at three different shear stresses, and the results are shown in Figure 6. The EPS of mature biofilms at different shear stresses is mainly composed of protein, humus and polysaccharides, with the main ingredients being protein and humus. In mature biofilms at shear stress of 1.12 Pa, 1.29 Pa, 1.45 Pa, EPS was, respectively, 132 mg/gVSS, 146 mg/gVSS and 115 mg/gVSS, including protein content of 42%, 45% and 39%, respectively (VSS: volatile suspended solids). The polysaccharides content was 11%, 11% and 15%, respectively, and the humus content was 47%, 44% and 46%, respectively.

**Kinetic modeling of growth of sewer biofilm**

**Univariate regression model**

The measured data of biofilm thickness, under different shear stress when the COD concentration is 400 mg/L, were analyzed by origin, and the regression models were established. The measured data and the corresponding simulation results are shown in Figure 7(a)–7(c). Furthermore, it can be found that the three models had the same pattern, and it could be described using Equation (2).

\[
T = 10,000A \exp \left( \frac{B}{C_1 t} \right) + 10,000C \exp \left( \frac{D}{C_1 t} \right) + 10,000E \exp \left( \frac{F}{C_1 t} \right)
\]

where
- \(T\) = biofilm thickness (um);
- \(t\) = time (day); and
- A, B, C, D, E, F = the constant values corresponding to different model.

**Multivariate regression models**

The biofilm thickness and biofilm TS changes over time in the growth process of biofilm from the previous analysis. As a consequence, the biofilm thickness can be correlated with biofilm TS and EPS. Gjaltema et al. (1997) found the effect of COD concentration and shear stress on biofilm growth. An assumption was made that the biofilm thickness can be correlated with the biofilm TS, EPS, COD concentration and shear stress.

The measured data were used to establish a regression Equation (3) to describe the biofilm thickness with the relationship of biofilm TS, EPS, COD concentration and shear stress, with shear stresses being 1.12 Pa and 1.29 Pa under COD concentration of 400 mg/L. In the same way, the Equation (4) was established with shear stresses being 1.29 Pa and 1.45 Pa under COD concentration of 400 mg/L. The simulation results are shown in Figure 8(a) and 8(b) and Figure 8(c) and 8(d), respectively.

\[
T = 2.6278C_{COD} \times F - 22.8914\alpha + 19.2461\beta
\]

\[
T = 2.1683C_{COD} \times F - 16.8541\alpha + 19.7765\beta
\]

where
- \(T\) = biofilm thickness (um);
- \(C_{COD}\) = chemical oxygen demand concentration (mg/L);
- \(F\) = shear stress (Pa);
- \(\alpha\) = biofilm TS (kg/m3); and
- \(\beta\) = biofilm EPS (mg/g VSS).
It can be seen from Equations (3) and (4) that the COD and shear stress are multiplied together. The influence of shear stress on biofilm thickness is not only reflected in the erosion effect on biofilm, but also reflected in the influence of the mass transfer process inside the biofilm. Therefore, this arrangement is reasonable.

In Equation (3), the confidence interval of each coefficient is \([0.9506, 4.3049] \), \([-32.5481, 13.2347] \) and \([16.2411, 22.2511] \) respectively. In Equation (4), the confidence interval of each coefficient is \([0.5406, 3.7960] \), \([-26.2450, 7.4632] \) and \([16.3958, 23.1571] \), respectively.

In order to validate Equations (3) and (4), the measured data with COD concentration is 200 mg/L was used. For instance, the fitting results are shown in Figure 9(a) and 9(b) with shear stresses of 1.29 Pa and 1.45 Pa under COD. The model’s predictions well agreed with the measurements. The relative errors of the model fall in 10–20%.

**Microbial community structure and diversity**

**Richness and diversity of bacteria phylotypes**

Three 16S rRNA gene libraries were constructed for the pyrosequencing of A, B and C communities with 11.689, 12.972 and 13.700 high-quality sequence tags, respectively. Table 2 shows the richness and diversity estimators of the bacteria phylotypes in the biofilms. By performing the alignment at a uniform length of 440 bp, 281, 300 and 303, OTUs were clustered at a 3% distance. The total number of OTUs estimated by chao1 estimator was 289, 318 and 314 with infinite sampling at a 3% distance, indicating that the biofilms under higher shear stress exhibited greater microbial richness than that under low shear stress. Considering the fact that the Shannon index of A (3.78) and B (3.85) were larger than that of C (3.60), it could be inferred that the biofilms in lower shear stresses were distributed with enriched microbial diversity and more evenly than those under...
Figure 8 | Comparison of simulated and measured development of the thickness with different shear stresses and COD concentration of 400 mg/L.

Figure 9 | Comparison of simulated and measured development of the thickness with shear stresses of 1.29 Pa and 1.45 Pa and COD concentration of 200 mg/L.
high shear stress. The coverages are all 0.998 representing that the amount of sample sequencing can be a true reflection of biofilm microbial community structure.

**Microbial community structure from the variation in shear stress**

Figure 10 shows the relative abundance ratio of predominant bacterial phylum in mature biofilms under different shear stresses. *Proteobacteria, Firmicutes, Bacteroidetes* and *Candidate_division_TM7* were four phyla that were abundant in all samples. The relative abundances of the predominant phylum *Proteobacteria, Bacteroidetes, Firmicutes* and *Candidate_division_TM7* in the shared OTUs were 49.47%, 20.04%, 1.80% and 20.16% in A, 34.36%, 19.12%, 9.12% and 23.64% in B, and 32.90%, 15.16%, 9.45% and 28.15% in C. The relative abundances of phylum *Proteobacteria* in B and C were decreased by 15.11% and 16.57% compared to that in A. It could be inferred that the phylum *Proteobacteria* decreases with increased shear stress. The relative abundances of phylum *Bacteroidetes* in C has a significant reduction compared to that in A and B. It could be considered that the phylum *Bacteroidetes* decreases with increased shear stress.

**Diversity and abundances of denitrifying bacteria**

The three reactors were detected in three phylums and eight genus denitrifying bacteria, and most belong to *Proteobacteria* (Table 3). The denitrifying bacteria populations are 7.90%, 9.81% and 6.89% with shear stresses of 1.12 Pa, 1.29 Pa and 1.45 Pa, respectively. As the shear stress increased from 1.12 Pa to 1.29 Pa, the denitrifying bacteria population increased by 1.91%, and then decreased to 6.89% compared to shear stress of 1.45 Pa. On the genus level, the most predominant bacteria was *Flavobacterium*, and the ratio has great difference according to various shear stress.

**Sulfate-reducing bacteria**

Table 4 shows the sulfate-reducing bacteria (SRB) distribution in mature biofilms under different shear stresses. The three reactors were detected in 12 genus *SRB*, suggesting that the *SRB* in sewer systems had a rich diversity. The *SRB* populations were 0.056%, 0.041% and 0.027% in mature biofilms with shear stresses of 1.12 Pa, 1.29 Pa and 1.45 Pa, respectively. The *SRB* significantly decreased in C compared to that in A and B, indicating that higher stress could decrease the *SRB* population in sewers. In order to release the yield of hydrogen sulfide gas, increasing the shear stress might be a feasible method.

**Methanogenic Archaea**

The *Archaea* in the biofilm was also analyzed. Table 5 shows the microbial community of *Archaea* in mature biofilms. Six genera were detected in A and five genera were detected
in both B and C. The DHVEG-6 (Deep-Sea-Hydrothermal-Vent-Gp-6-norank) and Methanospirillum were the predominant bacteria in the different biofilms. As the amount of Methanosphaerula, Methanoregula and Methanobacterium was much smaller than methanospirillum, the amount of methanogenic Archaea (MA) was represented approximately by methanospirillum. The MA population was 22.87%, 57.07% and 69.75% in mature biofilms with shear stresses of 1.12 Pa, 1.29 Pa and 1.45 Pa, respectively. The MA significantly increased with the increasing shear stress, indicating that higher stress could increase the MA population in sewers. In order to release the yield of methane gas, decreasing the shear stress might be an advisable way.

**CONCLUSIONS**

In this study, the microelectrode testing technology and 454 high-throughput pyrosequencing was applied to investigate the effect of shear stress and substrate concentration on the biofilm growth process and microbial community structure. The results showed that shear stress and substrate concentration were two important factors for biofilm growth. And the microbial community structure was strongly influenced by shear stress. The corresponding regression model indicated that the biofilm thickness could be correlated with the biofilm TS, EPS, COD concentration and shear stress. Roughness coefficient, a parameter of the fluent, needs to be changed if applied to real-scale sewers and the calculation results of shear stress will be corrected. In addition, the characteristics of the biofilm structure and biological function should be known well before controlling the hydrogen sulfide and methane in sewers, so the results can provide important information for future investigations.

**ACKNOWLEDGEMENTS**

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**REFERENCES**


| Table 5 | The microbial community of Archaea in mature biofilms |
|---------|-----------------|-----------------|-----------------|
| Genus    | A-ratio (%)      | B-ratio (%)     | C-ratio (%)     |
| Others   | 3.24             | 0.34            | 0.07            |
| DHVEG-6  | 73.15            | 42.53           | 29.86           |
| Methanosphaerula | 0.04         | 0.00            | 0.01            |
| Methanoregula  | 0.49          | 0.04            | 0.32            |
| Methanobacterium | 0.22          | 0.02            | 0.00            |
| Methanospirillum  | 22.87         | 57.07           | 69.75           |

<table>
<thead>
<tr>
<th>Table 4</th>
<th>Sulfate-reducing bacteria distribution in mature biofilms under different shear stresses</th>
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<tbody>
<tr>
<td>Genus</td>
<td>Sequence tags</td>
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<tr>
<td>SRB</td>
<td>1.12 Pa</td>
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<tr>
<td>Desulfarculus</td>
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<td>Desulfatirhabdium</td>
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<td>Total number of SRB</td>
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<td>11,689</td>
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<tr>
<td>SRB ratio</td>
<td>0.036</td>
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</table>


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