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Co-Variation between Distribution of Microbial Communities and Biological Metabolization of Organics in Urban Sewer Systems

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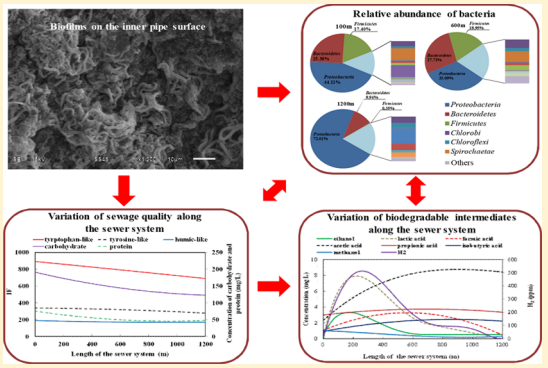
Supporting Information

ABSTRACT: Distribution characteristics and biodiversity of microbial communities were studied in a 1200 m pilot sewer system. Results showed that the dominant microorganisms, fermentation bacteria (FB), hydrogen-producing acetogen (HPA), sulfate-reducing bacteria (SRB) and methanogenic archaea (MA) changed significantly along the sewer systems, from start to the end. The distribution of the functional microorganisms could induce substrate transformation and lead to the accumulation of micromolecular organics (i.e., acetic acid, propionic acid and amino acid). However, substrate transformation induced by these microbes was affected by environmental factors such as oxidation−reduction potential, pH and dissolved oxygen. Changes in environmental conditions along the sewer resulted in the variation of dominant bioreactions. FB were enriched at the beginning of the sewer, while SRB and MA were found toward the end. Furthermore, based on Spearman rank correlation analysis of microbial communities, environmental factors and substrates, covariation between microbial community distribution and organics metabolization along the sewer was identified. This study could provide a theoretical foundation for understanding wastewater quality variation during transportation from sewers to treatment plants, therefore, promoting optimization of design and operation of wastewater treatment.



INTRODUCTION

The urban sewer system is an important component of urban water infrastructure for sewage collection and transportation.[1] As the starting point part of the entire urban sewage disposal system, urban sewer systems have been studied for decades.[2] In 1994, the IAWQ thematic sessions of sewage quality variation were convened in Denmark, opening a new chapter in the research on bioreactions in sewer system,[3−5] For example, in order to understand the different oxygen environment effects on microbial communities, Chen et al.[6] studied biofilms from sediments and the inner surface of sewers by cross-cutting biofilm samples and analyzing the bioactivity of microbial communities. In addition, Tanji et al.[7] studied the self-biopurification capacity of sewage by installing concrete blocks as carriers in sewer systems, and after 79 days, biofilms were found on the surface of the carriers, indicating that the inner environment of the sewer was suitable for biofilm growth.

In general, pollutants in urban sewage are easily degraded by the microbial community in the sewers, and with abundant carbon, nitrogen and other nutrients, urban sewage could provide substantial substrates for the growth and reproduction of microbes. Relevant study has indicated that hydrolysis and acidification were the dominant bioreactions for the decomposition of substrates in sewers.[8] Our previous study[9] demonstrated that larger organic molecules were transformed into products with smaller molecular weights and that unsaturated organics were converted into saturated organics, leading to the improvement in biodegradability of the sewage. Furthermore, the transformation of VFA into acetic acid with the production of CH_4 and CO_2 contributed to an obvious reduction in chemical oxygen demand (COD) along the sewer. However, due to a lack of understanding of the environmental factors in sewer systems, the correlation between substrate transformation and microbial community distribution is unknown.

The urban sewer is generally an anaerobic environment. Low dissolved oxygen (DO) could affect the distribution of microbial communities and then induce homologous substrate transformation. Liu et al.[10] investigated differences in microbial community distribution under anaerobic conditions, between manholes and sewage pipes. Results showed that the dominant microbial communities were significantly correlated to certain crucial anaerobic environmental factors (i.e., CH_4, sulfide). In

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addition, it is likely that changes in the content and composition of organics along the sewer would lead to changes in microbial communities, especially for fermentation bacteria (FB), hydrogen-producing acetogen (HPA) and methanogenic Archaea (MA). Therefore, in addition to the environmental effect on sewage quality, it is of particular significance to investigate the mechanism of how a variable environment influences microbial communities and what the correlation is between organics transformation and microbial community distribution. However, due to lack of relevant study, understanding remains poor regarding the distribution characteristics and diversity of microbial communities along the sewer system.

To investigate these mechanisms, a pilot experimental system consisting of a 1200-m-long sewer was built. The variation of substrates and environment in the sewer was monitored and the variation of microbial communities along the sewer was analyzed by high-throughput sequencing and quantitative polymerase chain reaction (qPCR) technologies. Furthermore, based on the Spearman analysis, the covariation between microbial community distribution and organic metabolization along the sewer was determined. In general, the sewage quality variation in sewer system resulted in the obvious differences between the design values of water quality and the actual influent quality in wastewater treatment plants. Therefore, this study provides a theoretical foundation for the understanding of wastewater quality variation during the transportation from sewer to treatment plant, and promotes information to optimize the operation of urban wastewater treatment plants.

MATERIALS AND METHODS

Experimental Setup and Raw Water Quality. The pilot urban sewer system was constructed from 1200 m of 40 mm-diameter PVC pipe and consisted of a total of 35 layers from the bottom to the top. Each layer was approximately 35 m in length. The sewage in the pilot sewer system was gravity fed. The experiment was conducted at room temperature (25 ± 2 °C) in a controlled environment where dissolved oxygen (DO) was maintained at 0.3 ± 0.05 mg/L (under anaerobic conditions), which was similar to that of actual sewer networks in China. During system operation, the flow rate was controlled at 0.6 m/s. To avoid sedimentation in the pipe, the pipe slope and depth ratio of sewage was adjusted to 5‰ and 0.6, respectively. Detailed information was provided by Jin et al.[9] Every 2−3 days during the pilot test, raw water was taken from a real sewer system in Xi'an, and the raw water quality (COD: 358 ± 17 mg/L; BOD_5: 260 ± 10 mg/L; TN: 45 ± 3 mg/L; NH_3−N: 38 ± 7 mg/L; TP: 7.9 ± 0.6 mg/L; pH: 7.3 ± 0.2) was analyzed. The data obtained from approximate 120 samples were averaged.

Sampling Method. For wastewater quality sampling and analysis, nine selected sampling points were located at 0, 30, 100, 200, 400, 600, 800, 1000, and 1200 m from the inlet in the sewer system. Five replicates at each location were taken. For each sampling point, water quality analysis included COD (chemical oxygen demand), BOD_5 (biochemical oxygen demand), NH_3−N (ammonia nitrogen), TN (total nitrogen), and TP (total phosphorus) among others.

For biofilm sampling, two slipknots, one at each end of the organic glass pipe in the sewer system were untied. Then, adhered biofilm was peeled off with sterile cotton swabs and carefully placed on disposable culture dishes. The samples were fully covered by dry ice during rapid transport to laboratory, and then stored at −40 °C. Due to the destructive nature of the sampling, the biofilm was only sampled for once.

Analysis of Methanol and Ethanol. The concentration of methanol and ethanol were measured by a gas chromatograph (GC-2014 Shimadzu, Japan) equipped with a flame ionization detector (FID), CB-5 capillary column and AOC-5000 automatic headspace sampler. The oven was maintained at 50 °C for 5 min; heated to 100 °C at a rate of 5 °C/min and held for 2 min; and then heated to 200 °C at a rate of 5 °C/min. Sample injection occurred at 200 °C, and the detector was operated at 280 °C. Nitrogen was used as the carrier gas at a flow rate of 5.0 mL/min. The split ratio was 2:1. The headspace operating conditions were: 30 min with strong shaking for sample equilibration at 80 °C with sampling probe held at 90 °C. The injection volume was 1.0 mL.

Fluorescence Excitation−Emission Matrix Analysis. A spectrofluorometer (FP-6500, Jasco, Japan) was used to analyze protein concentration in the treated samples. During measurements, the slit widths for excitation and emission were 5 nm with a scanning speed of 2000 nm/min. The excitation wavelength ranged from 220 to 480 nm with 2 nm intervals, and the emission wavelength ranged from 280 to 570 nm with 5 nm intervals. The scanning of distilled water was used to eliminate water Raman scattering.

Molecular Weight Distribution Analysis of Organic Matter. LC-2010AHF high-performance liquid chromatography (Shimadzu, Japan) with a Zenix SEC-100 7.8 × 300 mm gel column was used to determine the molecular weight. The wavelength of the ultraviolet detector was 254 nm. The mobile phase was a phosphate buffer solution with a pH of 7.0. The injection volume was 20 μL. The rate was set at 0.8 mL/min. Polystyrene sulfonic acid sodium salt was used as a standard.

Analysis of pH, DO, and ORP. The pH, DO and ORP of the sewage were measured by a HQ30d meter (HACH).

The pH, DO and ORP of the biofilm were analyzed by microelectrodes (Unisense Denmark). The tip diameters of the DO, pH and ORP microelectrodes were 10 μm. During measurement, the ultrapure water, bubbled by N_2, was used as the base fluid. Nylon nets immobilized the biofilm. The microelectrodes were controlled to penetrate the biofilm to analyze the microenvironment inside.

Analysis of VFAs, CH_4, Formic Acid and Other Wastewater Parameters. VFAs and CH_4 were measured by gas chromatography and formic acid was measured by high-performance liquid chromatography.[9] The measurements of COD, NH_3−N, NO_3^-TN and TP were processed according to the standard methods (State Environmental Protection Administration of China, 2002). Carbohydrates were measured by the anthrone method.[12] Protein was measured by Lowry method.[11] All measurements were performed for 3 times for each sample.

High-Throughput Sequencing. Illumina Miseq sequencing and 454 pyrosequencing were performed to analyze the distribution of bacteria and Archaea in this study.[13−15] Those sequencing were conducted by Shanghai Majorbio Biopharm Biotechnology Co. Ltd., Shanghai, China. The PCR reactions were performed three times for each sample and three parallel samples were sequenced in this study. In addition, the Spearman analysis was used in this study and the correlation of microbes and environment (p-value) are represented by gradient colors from dark blue to dark red, and the significant positive or negative correlation in this analysis are represented

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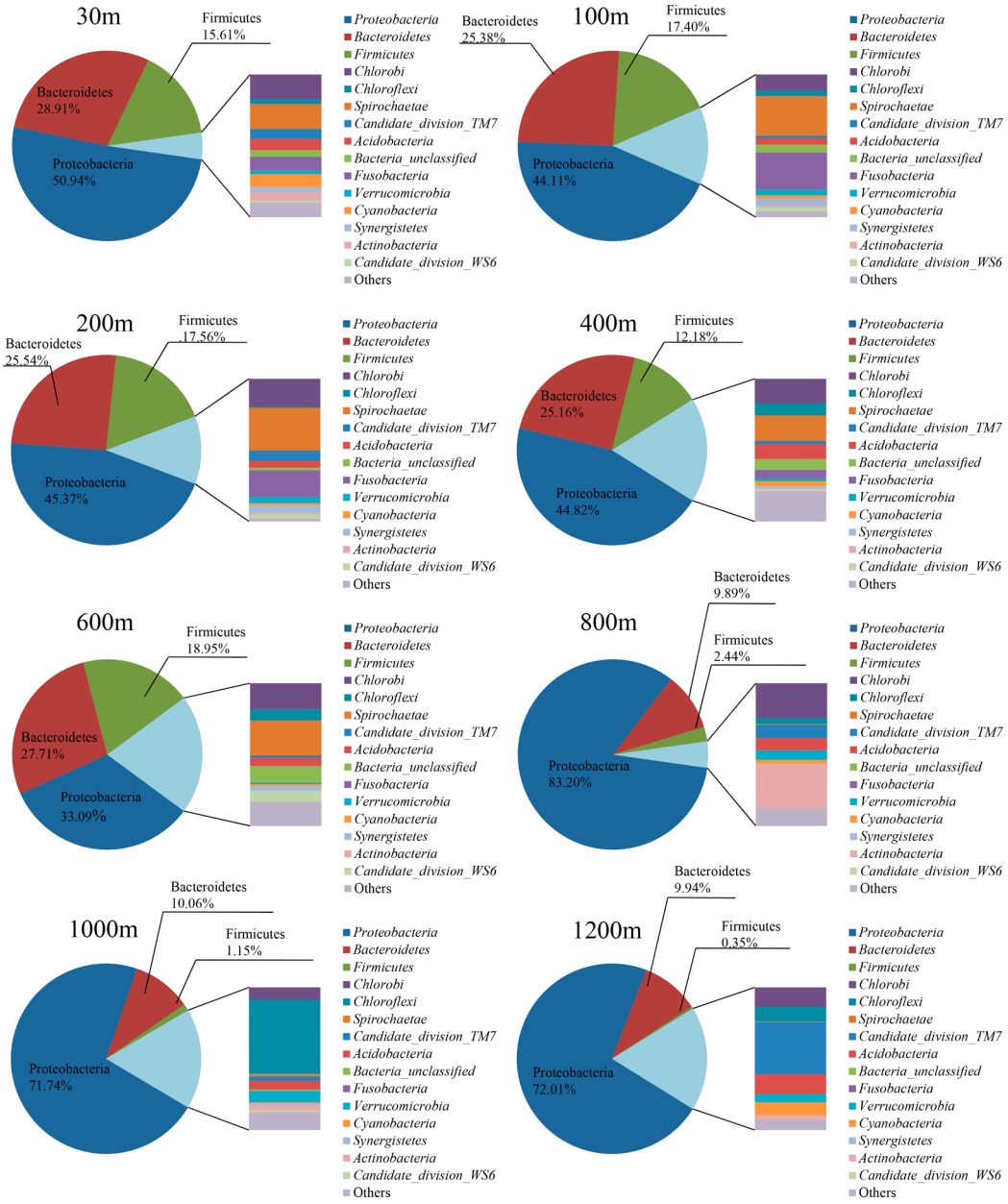

Figure 1. Relative abundance of microbial communities at the phylum level along the sewer (Others in each plot represented the unidentified bacteria).

by stars (inside the gridding). The NCBI accession number is SRP118971

Quantitative Polymerase Chain Reaction. Quantitative polymerase chain reaction (qPCR) was performed using an Applied Biosystems 7500 qPCR system (Applied Biosystems, Forster City, CA). To target the fermentation bacteria (FB), hydA-1290F (GGTGGAGTTATGGAAGCWGCHHT) and hydA-1538R (CATCCACCWGGRCAHGCCAT) were used as the forward and reverse primer, respectively.[16] To target hydrogen-producing acetogen (HPA), probes of S−S−S.wol-0223-a-R-19 (ACGCAGACTCATCCCCGTG), S−F−Synm-0700-a-R-23 (ACTGGTNTTCCTCCTGATTTGTA), S−S-MPOB-0222-a-R-19 (ACGCAGGCCCATCCCCGAA) were used.[17] To target the sulfate reducing bacteria (SRB), dsrA-290F (CGGCGTTGCGCATTTYCAYACVVT) and RH3-dsr-R′ (GTGGMGCCGTGCATGTT) were used as the forward and reverse primer, respectively.[16] To target the denitrifying bacteria (DNB), nirK 876 (ATYGGCGGVCAYGGCGA) and nirK 1040 (GCCTCGATCAGRTTRTGGTT) were used as the forward and reverse primer, respectively.[18] To target the methanogenic archaea (MA), mcrA-1430F (TTCTATGGT-TACGACTTVCAGGACCARTGYGG) and mcrA-1530R (TTCATTGCRTAGTTWGGRTAGTT) were used as the forward and reverse primer, respectively.[16] The analysis of blind samples was repeated 3 times. The 20 μL qPCR reaction mixture was prepared in triplicate using 6.8 μL of PCR-grade water, 10 μL TaKaRa SYBR Premix Ex Taq (TaKaRa BIO INC. Japan), 0.4 μL of each primer, 0.4 μL of 50 × ROX reference dye, 20 ng of template DNA. The thermal cycler protocol was as follows: initial step for 10 s at 95 °C followed by 40 cycles of 95 °C for 5 s, then maintained 56 °C for 10 s, finally maintained 72 °C for 27 s. The qPCR was tested for three times. The qPCR was conducted by the fluorochrome detection in PCR amplification production which differed

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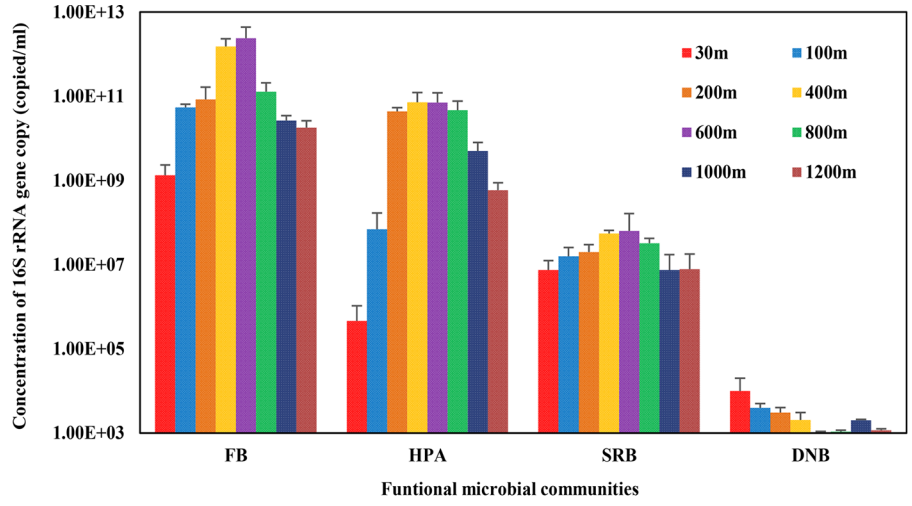
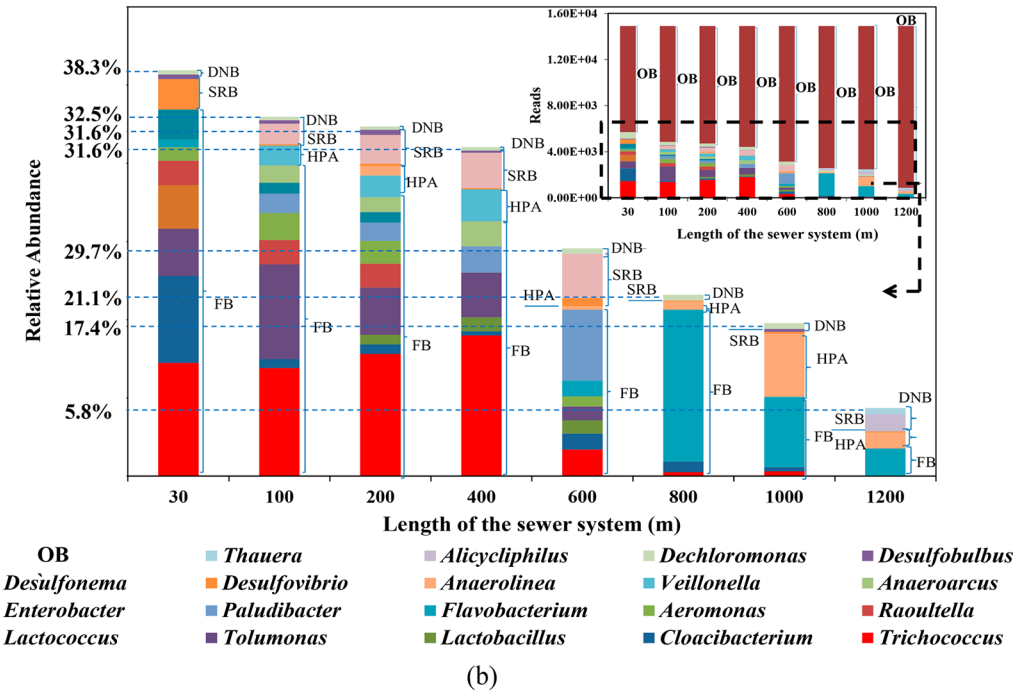


(a)



(b)

Figure 2. Variation of fermentation bacteria (FB), hydrogen-producing acetogen (HPA), sulfate reducing bacteria (SRB), denitrifying bacteria (DNB), and other bacteria (OB) along the sewer (a) 16S rRNA gene copy concentration of functional microbial communities (detected by qPCR); (b) relative abundance of functional microbial communities (detected by sequencing). (The data and detailed classification of each functional microbial community was shown in SI Tables 1 and 2).

with different sequencing methods (high throughput and high sequence depth), therefore, some results might not be identical.

Functional Microbial Community Classification Standard. To investigate bioreactions occurring in sewer system, the microbial communities (determined by sequencing results) were classified as fermentation bacteria (FB), hydrogen-producing acetogen (HPA), sulfate reducing bacteria (SRB), denitrifying bacteria (DNB), and other bacteria (OB). According to the three steps theory of anaerobic fermentation,[19] the microbial communities participating in the first step were represented by hydrolytic and fermentative communities. In this step, the microbial communities decomposed the macromolecular organic matters into micromolecular compounds (such as volatile fatty acids, alcohol among others), therefore these kinds of microbial communities were classified as fermentation bacteria (FB).[20,21] In the second step, hydrogen-producing and acetate-producing bacteria would consume the substrates produced in the first step and produce hydrogen and acetate, which could be available to methanogens for methane formation. Therefore, this type of bacteria was classified as hydrogen-producing acetogen (HPA).[22,23] Sulfate-reducing bacteria (SRB) obtain energy by oxidizing macromolecular organic compounds or molecular hydrogen (H_2) while reducing sulfate (SO_4^{2-}) to hydrogen sulfide (H_2S).[24] Denitrifying bacteria (DNB) are capable of performing denitrification as part of the nitrogen cycle. This kind of bacteria metabolizes nitrogenous compounds using the enzyme nitrate reductase, turning nitrogen oxides back to nitrogen gas

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or nitrous oxide.[25,26] Except for FB, HPA, DNB, and SRB, other bacteria were classified as OB.

RESULTS AND DISCUSSION

Analysis of the Biodiversity and Wastewater Quality in the Sewer System. The Shannon index decreased from the start to the rear along the sewer system. The Shannon index was 4.53−4.79 from 30 to 600 m along the sewer system; however, it decreased to 4 toward the end of the sewer from 800 to 1200 m. The reduction of the Shannon index showed that the biodiversity changed along the Sewer.

As shown in Figure 1, the relative abundance of *Proteobacteria* was 30.08−50.94% within the beginning 30−600 m of the sewer, however, from 600 to 800 m, *Proteobacteria* became dominant with a relative abundance of 71.7−83.2%. In contrast, in the first 600 m of the sewer, the relative abundance of *Bacteroidetes* was 25.2−28.9%, and, rapidly decreased to approximately 10% at 800−1200 m. Moreover, within 30−600 m, the relative abundance of *Fimicutes* was 12.2−18.9%, however, it dramatically decreased to less than 2.5% at 800−1200 m.

In fact, *Firmicutes*, *Bacteroidetes*, and *Proteobacteria* contain the fermentative genera which contribute to the fermentation processes in the anaerobic digestion system.[27] Moreover, the environmental conditions of the sewer system are dark and anaerobic, similar to anaerobic digestion systems, indicating that fermentation might occur in a sewer system. In general, hydrolysis and acidification are components of fermentation process. During hydrolysis, refractory organic matter is decomposed into degradable compounds, and macromolecular organic matter is decomposed into small molecules. On the other hand, acidification produces acid and gases. Kang et al.[28] found that *Proteobacteria* was the major phylum in hydrolysis and acidification during sludge fermentation, suggesting that the competitive capacity of *Proteobacteria* could be stronger at the end of the sewer, where acidification might be the primary process (accumulation of small-molecule organic matters as shown in Supporting Information (SI) Figure 3). It has been reported that *Bacteroidetes* and *Firmicutes* could hydrolyze protein and carbohydrates.[29,30] As the content of macromolecular organic compounds (molecular weight >10 000 Da and within 650−10 000 Da) decreased along the sewer (SI Figure 3), and the relative abundance of *Bacteroidetes* and *Firmicutes* similarly decreased, it can be inferred that the local environment became disadvantageous for *Bacteroidetes* and *Firmicutes* due to lack of metabolizable substrates.

To study the effect of sewer length on the microbial community (bacteria and Archaea) distribution, the beta-diversity as principal component analysis (PCA) plots were produced (SI Figure 1), and the microbial communities in each biofilm sample were represented as colored points. The results showed that significant separation of bacteria occurred in the samples from 0 to 200 m, 400−600 m and 800−1200 m (SI Figure 1(a)). It indicated that due to the variation of environmental factors along the sewer, the diversity (Beta) of bacteria was affected along the range of 400−600 m (diversity changed significantly in the beginning of the sewer). The separation groups of Archaea were found in the samples from 0 to 400 m, 600−800 m, and 1000−1200 m (SI Figure 1(b)). The results indicated that the diversity (Beta) of Archaea could be affected in the range of 600−800 m of sewer. The PCA analysis was in accordance with the results shown in Figure 1. The analysis of microbial community diversity and structures at the phylum level along the length of sewer could provide a profound understanding on microenvironments and the investigation of the diverse metabolism occurring in sewer system.

Distribution Characteristics of Functional Microbial Communities in the Sewer System. Because pyrosequencing only provided information on the relative abundance of microbial communities rather than the absolute quantity variation, quantitative polymerase chain reaction (qPCR) and pyrosequencing were used together to investigate the distribution characteristics of functional microbial communities along the sewer. The variation of the 16S rRNA gene copy concentration and the relative abundance at the genus level are shown in Figure 2. It was observed that the functional microbes in the sewer were mainly comprised of fermentation bacteria (FB), hydrogen-producing acetogen (HPA), sulfate reducing bacteria (SRB) and denitrifying bacteria (DNB).

As shown in Figure 2 (a), the 16S rRNA gene copy concentration of FB continuously increased along the 0−600 m range of the sewer and reached the maximum at 600 m (2.39×10^{12} copied/ml). It then began to decrease significantly from 600 to 1200 m. These results indicated that within 600−1200 m of the sewer, some communities of FB declined in population, and the microbial community structures and biometabolism processes might be altered in this region. To understand this phenomenon, pyrosequencing was utilized to reveal the variation of microbial community structures and then infer the bioreactions that occurred in the sewer system. In the first 30 m of the sewer system, *Trichococcus* and *Cloacibacterium* were the dominant genera. As the distance increased, *Cloacibacterium* was gradually eliminated. Previous studies indicated that *Trichococcus* could adapt to the environment where hydrolysis substrates, (especially macromolecular organic compounds) were rich.[31] Therefore, along the sewer from 0 to 400 m, due to the high content and complicated composition of macromolecular organic compounds (SI Figure 3), the environment might be more suitable for the growth of *Trichococcus* (which displayed high relative abundance in this region). From 600 to 800 m, the relative abundance of *Trichococcus* decreased dramatically, indicating that *Trichococcus* might no longer adapt to the changing environment. This result was highly consistent with the qPCR data which indicated that the 16S rRNA gene copy concentration of FB decreased significantly after 600 m. Moreover, it also indicated that the change of FB concentration was mainly due to the elimination of *Trichococcus*. However, the relative abundance of *Flavobacterium* significantly increased, making it the dominant genus in this region. This might be resulted from the accumulation of micromolecule compounds as substrates for its reproduction (SI Figure 3). After 800 m, as the substrates were gradually consumed, the relative abundance of *Flavobacterium* and the 16S rRNA gene copy concentration of FB continuously decreased along the sewer, indicating that the substrates were more readily utilized by other genera at the end of the sewer. Hence, these results indicated that the distribution characteristics of FB featured the dominant genus changing from *Trichococcus* to *Flavobacterium* within 600−800 m of the sewer system.

HPA mainly consumed hydrolysate for final acidification. The 16S rRNA gene copy concentration of HPA remained low in the beginning of sewer, with no accumulation of (hydrolytic products), and then it increased dramatically after 100 m as hydrolysis improved along the sewer (Figure 2 (a)). Moreover,

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the distribution characteristics of HPA changed in the sewer, i.e., the dominant HPA genus changed from *Veillonella* to *Anaerolinea* within the 600−800 m region. Therefore, the variation of the HPA structure also caused the decline of HPA concentration after 600 m (Figure 2 (a)). Previous studies have showed that *Veillonella* and *Anaerolinea* prefer to utilize lactic acid and small-molecule fatty acids, respectively.[32,33] Therefore, the accumulation of lactic acid at the beginning of the sewer (SI Figure 5) might be the reason for the high relative abundance of *Veillonella*. When the content of lactic acid decreased near the end of the sewer, the reproduction of *Veillonella* was limited, whereas *Anaerolinea* became dominant perhaps due to the stable concentration of small-molecule fatty acids (VFA).

As shown in Figure 2(b), the relative abundance of *Desulfovibrio* was higher than that of other SRB genera at 30 m. Previous reports indicated that *Desulfovibrio* preferred to utilize methanol and lactic acid as carbon source.[34] From 100 to 400 m, the relative abundance of *Desulfovibrio* decreased gradually, whereas *Desulfonema* became the dominant genus, which might be due to the accumulation of isobutyric acid, the preferable carbon source for *Desulfonema*, in this region (SI Figure 5).[35] However, due to the low content of SO_4^{2-}, the relative abundance of all SRB genera decreased beyond 600 m in the sewer, suggesting that the sulfate reduction process had gradually declined. This was also verified by the qPCR data that showed that the 16S rRNA gene copy concentration of SRB decreased after 600 m. These results suggest that the distribution characteristics of SRB changed along the sewer with the dominant genus changing from *Desulfovibrio* to *Desulfonema*.

Due to the anaerobic environment in sewer systems, nitrification was inhibited. Therefore, the content of NO_3^- was extremely low (SI Figure 4). Previous study has shown that *Dechloromonas* and *Alicycliphilus* can reduce NO_3^-,[36] however, due to the low content of NO_3^- in the sewer, the metabolism of these two dominant DNB genera might be weakened. Moreover, the total concentration of DNB was also low in the sewer.

Except for microbial genera discussed above, all other genera could be regarded as a whole microbial community, namely, other bacteria (OB). The relative abundance of OB varied from 60% to 95% along the sewer system, suggesting that the competition capacity of functional bacteria mentioned above were gradually weaker than OB, and the OB were the most dominant microbial communities at the end of sewer.

The production of methane in the sewer system indicated the existence of methanogenic Archaea (MA). Most likely due to lack of metabolizable substrates available to MA at the beginning of the sewer, MA was not found at the 30 m location (verified by both qPCR and pyrosequencing detection as shown in Figure 3). The 16S rRNA gene copy concentration of MA increased from 100 to 1000 m of the sewer, which indicated that methanogenesis could be gradually enhanced along the sewer. To acquire the specific information on methanogenesis, the MA taxa were identified. The dominant MA genera were *Methanosarcina*, *Euryarchaeota*, and *Methanobacteriaceae*, among which a transition from *Methanosarcina* to *Euryarchaeota* was clearly observed. It was reported that *Methanosarcina* was able to use aceticlastic and hydrogenotrophic pathways, as they are more adaptable to changes in nutritional conditions.[37] The relative abundance of *Methanosarcina* continuously increased from 100 to 800 m, and exceeded that of *Euryarchaeota* at 200 m while the relative

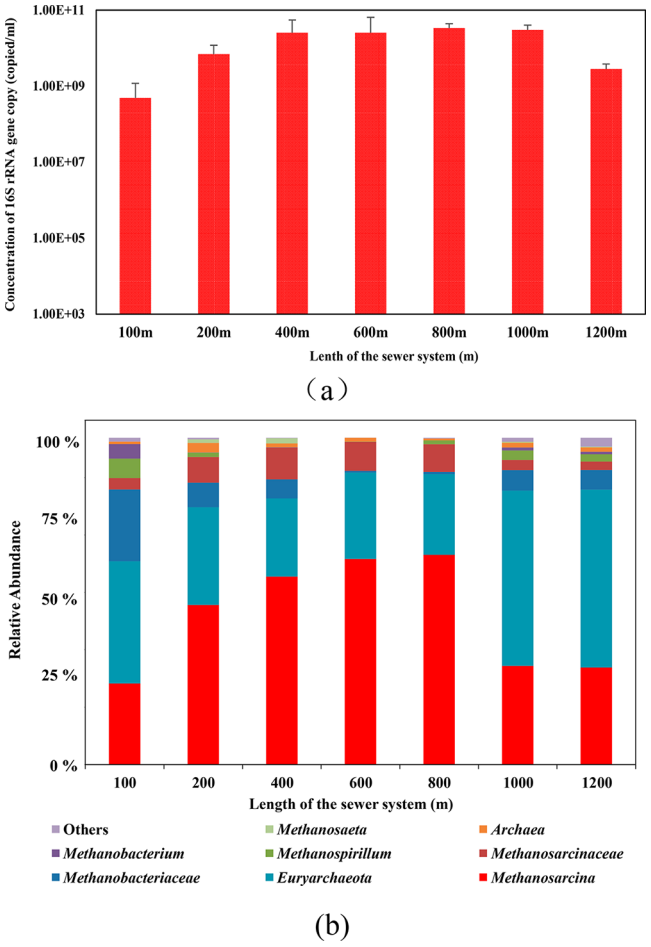

Figure 3. Variation of methanogenic Archaea (MA) along the sewer (a) 16S rRNA gene copy concentration of MA (detected by qPCR); (b) relative abundance of MA ((detected by sequencing).

abundance of *Euryarchaeota* decreased continuously along the sewer. This indicates that *Methanosarcina* adapted to grow in the environment where the substrates were rich in complex substrate conditions from 100 to 600 m. However, after 800 m, the relative abundance of *Methanosarcina* decreased, whereas that of *Euryarchaeota* increased along 800−1200 m. This suggested that the substrate conditions from 800 to 1200 m were more suitable for the growth of *Euryarchaeota*. Based on the analysis of the functional microbial communities distribution along the sewer discussed above, the metabolism can be inferred. The metabolism occurring in sewer would be affected by environmental factors, and therefore cause the substrate transformation. Therefore, the variation of nutrient substrate and environmental conditions along the sewer were further studied

The Variation of Nutrient Substrate and Environmental Conditions along the Sewer. To investigate the distribution characteristics of microbial communities, the wastewater quality along the sewer was analyzed. The molecular weight, fluorescence intensity (FI) and content of organic matters are shown in Figure 4. The content of carbohydrate and protein was high within 0−600 m. The macromolecular organics ranged from 198.56 mg/L to 148.46 mg/L, and from 81.74 mg/L to 49.29 mg/L, respectively, in the first 600 m of sewer. Moreover, the FI of amino acids, such as tryptophan-like substrate, showed the same declining trend

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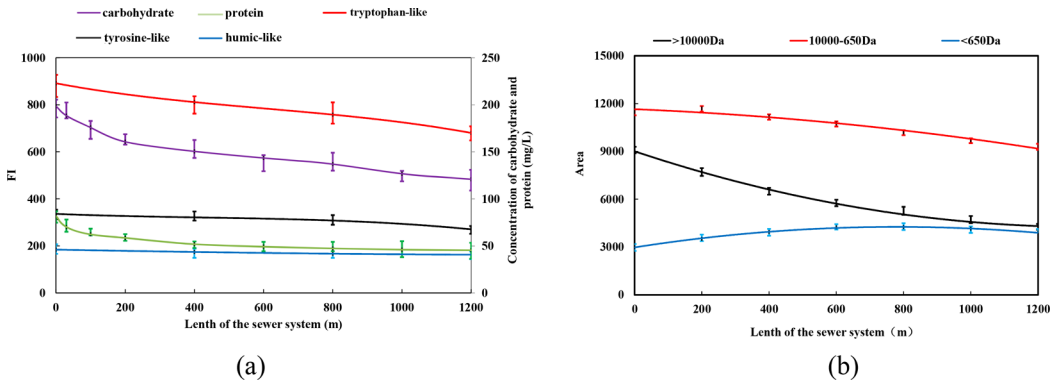

Figure 4. Variation of sewage quality along the sewer system (a) the substrate variation of the sewage along the sewer; (b) the molecular weight variation of the sewage along the sewer (concentration of substance component, excitation−emission matrix spectra, characteristics of DOM and molecular weight are shown in SI Table 3, Table 4, and Figures 2−4).

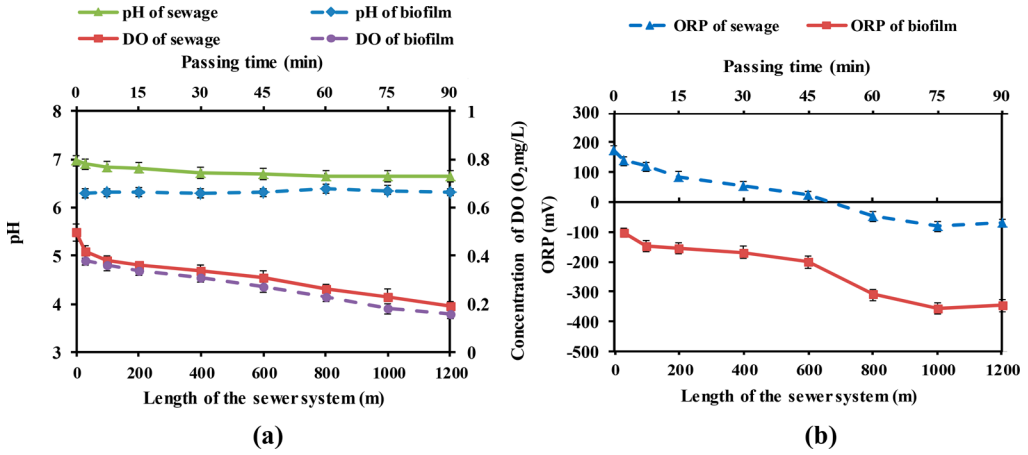

Figure 5. Variation of pH, DO and ORP along the sewer system (measured five times).

along the sewer, decaying from 891.5 to 811.5 in 0−600 m and from 758.2 to 680.4 in 800−1200 m. This result is in accordance with the distribution characteristics of *Trichococcus* and *Cloacibacterium* (FB), *Veillonella* (HPA), and *Desulfovibrio* (SRB) which mainly utilized macromolecular organics at the beginning of the sewer (Figure 2). From 800 to 1200 m, the content of organic compounds (>650 Da) continuously declined while the small-molecule organics (<650 Da) rose constantly. This indicated that the macromolecular organic compounds were continuously decomposed to small-molecule organics in the 800−1200 m region, which might retard hydrolysis and facilitate acidification. Thus, the environment might be more suitable for the growth of *Flavobacterium* (FB), *Anaerolinea* (HPA), *Desulfonema* (SRB), and *Euryarchaeota* (MA) near the end of sewer due to the enhancement of acidification.

To further study the influence of environmental condition on microbial communities in the sewer, pH, DO, and ORP of the sewage and biofilm were measured. As shown in Figure 5 (a), the concentration of DO in the sewage and biofilm decreased gradually along the sewer from 0.5 mgO_2/L to 0.19 mgO_2/L, and from 0.38 mgO_2/L to 0.16 mgO_2/L, respectively, whereas the pH values of the sewage and biofilm both remained stable (6.0−7.0). Therefore, the sewer was anaerobic. Moreover, the anaerobic conditions in both sewage and biofilm became more pronounced along the sewer. Figure 5 (b) presents the change of ORP in sewage and biofilm. The ORP of sewage decreased from 175 mV to −70 mV along the sewer. In the first 600 m, the ORP of the biofilm decreased steadily along the sewer, however, it decreased rapidly from −200 to −306 mV between 600 and 800 m. The rate of decrease slowed at the end of the sewer. The ORP variation in the biofilm affected biochemical reactions and therefore could influence the change of substrates in the sewer. As shown in Figure 5 (b), the ORP of biofilm ranged from −100 to −200 mV between 30 and 600 m in the sewer. As reported previously, the optimal ORP ranges for acidogenic fermentation and sulfate reduction are from −100 to −225 mV and from −50 to −250 mV, respectively.[38] Therefore, it can be inferred that acidogenic fermentation and sulfate reduction mainly occurred along the 30−600 m segment the sewer system. This result was verified by the distribution of acidogenic fermentation microflora (i.e., high relative abundance of FB and HPA) in the beginning of sewer (Figure 2). In contrast, despite of the favorable ORP condition for SRB reproduction, the relative abundance of SRB remained low probably due to the low content of SO_4^{2-} (less than 50 mg/L along the sewer, SI Figure 4). From 800 to 1000 m, the ORP of biofilm ranged from −300 to −350 mV, therefore, acidogenic fermentation and sulfate reduction was reduced and the relative abundance of the corresponding functional microbes was lower than that in the 0−600 m segment. Moreover, the suitable ORP for denitrification ranged from 50 to −50 mV,[38] therefore, along the entire sewer the relative abundance of DNB genera was lower than that of other functional microbes. In addition, the optimal range of ORP for the methane production is between −175 and −400 mV,[38] thus the ORP condition along

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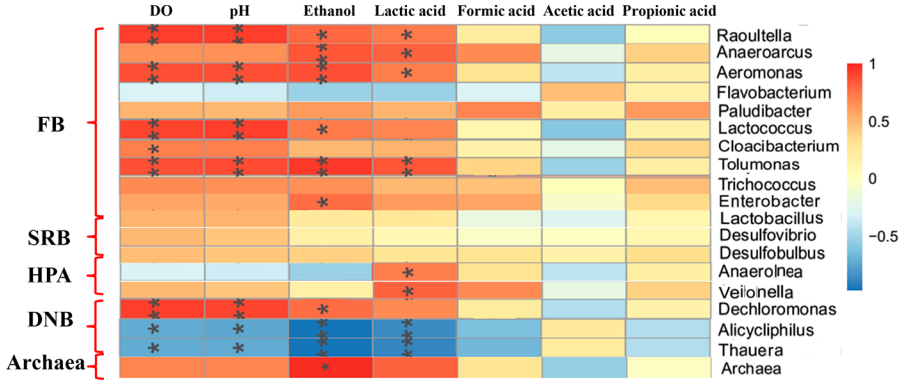

Figure 6. Correlations between microbiota abundances and environment conditions (based on Spearman' s correlation between the relative abundance of significantly functional microbes and the environment condition in the sewer. The color and the stars of each stripe indicate magnitude of correlation, with p-values superimposed on stripe (One star represents $p < 0.05$, and two stars represent $p < 0.01$); Subject groups were divided in FB, SRB, HPA, DNB, and Archaea; the alpha values and water quality along the sewer are shown in SI Table 5 and Table 6).

the majority part of the sewer was beneficial to methane production. As shown in Figure 4, from 100 to 800 m, the relative abundance of *Methanosarcina* was the highest among MA with ORP between −147 and −306 mV, suggesting that the ORP condition was highly suitable for *Methanosarcina* growth. When ORP decreased to −356 mV, the relative abundance of *Euryarchaeota* was the highest among the MA, indicating that the ORP condition was more beneficial for the growth of *Euryarchaeota*.

Within 0−200 m of the sewer, fermentation products continuously increased, including lactic acid, acetic acid, propionic acid and isobutyric acid, of which lactic acid and acetic acid are the dominant products and contribute to the highest ratio (SI Figure 5). The content of lactic acid reached its maximum value of 7.89 mg/L at 200 m. In the 200−600 m segment of the sewer, lactic acid and ethanol concentration was reduced while the content of propionic acid and isobutyric acid increased continuously. At the 600 m location, the content of acetic acid, propionic acid and isobutyric acid increased to 8.67 mg/L, 3.5 mg/L, and 2.5 mg/L, respectively, and it was corresponded with the analysis of molecular weight that demonstrated that the macromolecular organic compounds were gradually decomposed to small-molecule organic compounds along the sewer systems.

The Covariation between Environmental Condition, Substrate, and Microbial Communities. To assess the correlation between sewage quality variables and microbial communities on bioflims in the sewer, the Spearman analysis was conducted to visualize the correlation between functional microorganism and environmental factors. As shown in Figure 6, FB exhibited a significantly positive correlation (red cell with stars) with macromolecular organics, especially ethanol and lactic acid, therefore the levels of DO and pH in sewer could remarkably affect the metabolism of FB. These results were highly consistent with the previous discussion that FB became the dominant microflora as a result of the abundant macromolecular organics available as substrate in the beginning of sewer system while the relative abundance of FB gradually decreased along the sewer. Nevertheless, it should be noted that *Flavobacterium* (FB) showed positive correlation with small-molecule organics (acetic acid and propionic acid), and the bacteria could survived in the sewer despite variations in DO and pH. Therefore, FB could still be detected at the end of sewer even though their relative abundance was quite low. HPA and SRB exhibited a positive correlation with lactic acid (red cell), therefore, abundant lactic acid at the beginning of the sewer enabled HPA and SRB to be more competitive and display greater relative abundance. The utilization of lactic acid as a carbon source by *Veillonella* (the dominant genus of HPA) and *Desulfovibrio* (the dominant genus of SRB) led to the continuous decrease of lactic acid. Thus, beyond 600 m, due to the lower content of lactic acid, the distribution characteristics of HPA changed. Meanwhile, due to lack of SO_4^{2-}, the relative abundance of SRB decreased within 800−1200 m. DNB (*Alicycliphilus* and *Thauera*) exhibited significantly negative correlation with carbon availability (blue cell with stars) although *Dechloromonas* could use carbon for denitrification. This is due to the extremely low content of NO_3^- was in sewer. A complex substrate composition for MA was observed, which included methanol, lactic acid, formic acid and propionic acid. The relative abundance of *Methanosarcina* was higher before 800 m. However, beyond 800 m, acetic acid contributed nearly all of the substrate for MA and the relative abundance of *Euryarchaeota* became the highest among the MA. Thus, it can be inferred that *Methanosarcina* could adapt to the complex substrate conditions while *Euryarchaeota* tended to prefer acetic acid as a substrate.

In conclusion, this study revealed the distribution characteristics of different functional microbial communities (FB, HPA, SRB, DNB, MA). Due to the variation of ORP, DO, and pH, the dominant bioreaction changed from fermentation to methane production along the sewer. These results indicated that different environmental factors could affect the distribution of functional microbial community, and the change of dominant FB, HPA, SRB, DNB, and MA genera along the sewer could induce homologous transformation of organics. The distribution of microbial communities and organic metabolism are inseparably connected along sewer systems. This study could provide a theoretical foundation for an understanding of wastewater quality variation along sewer transport systems. Thus, these results could help to predict the actual influent quality of wastewater treatment plants, and promote optimization of wastewater treatment.

■ ASSOCIATED CONTENT

Supporting Information

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Relative abundance of functional microbial community; the variation of substrate concentrations; the variation of environmental factors along the sewer (PDF)

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Notes

The authors declare no competing financial interest.

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