



# Characterization of microflora and transformation of organic matters in urban sewer system



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## ABSTRACT

A study was conducted using a pilot sewer system consisting of 35 sequential sections, totalling 1200 m of gravity pipe. Urban sewage flowed into the sewer system at a constant flow rate until it reached physical and microbiological steady states. Microflora in the biofilm that attached to the inner surface along the pipe length were analysed. The organic compositions in both the liquid and gaseous phases of the sewer system were monitored. The results showed that typical fermentation bacteria, such as *bacteroidetes* and *bacillus*, were abundant in the system, indicating that the anoxic environment (DO = 0.3 mg/L) was suitable for fermentative bacterial growth. This resulted in a substantial reduction of the chemical oxygen demand (COD) along the pipe length and an increase of the biodegradable oxygen demand/chemical oxygen demand (BOD/COD) ratio from 0.68 at the beginning of the sewer system to 0.84 at the end of the sewer system; this was an indication of a transformation of organic matters from less-biodegradable to more-biodegradable products. Via molecular weight (MW) analysis, it was further identified that the larger organic molecules (MW > 10,000 Da) were transformed into products with smaller molecular weights. Regarding the fermentation products, the concentrations of the volatile fatty acids (VFAs) increased dramatically in the initial 600-m sections and then remained constant for the later sections except for the end section of the sewer; acetic acid was found to be the primary product of the VFAs. Gaseous carbon dioxide (CO<sub>2</sub>) and methane (CH<sub>4</sub>) were found to increase along the length of the sewer system, whereas the concentrations of ethanol, lactic acid, and hydrogen (H<sub>2</sub>) were high at the beginning of the sewer and then decreased in the rear sections of the sewer system. It could thus be concluded that in an urban wastewater sewer system, fermentative microflora could perform important roles in contributing to organic matter removal and/or improving the biodegradability of organic matter.

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## 1. Introduction

Traditionally, as an important part of urban infrastructure, an urban sewer network is primarily used for the effective collection and transportation of residential and industrial wastewater and rainwater (Liu et al., 2015). However, as people gain more knowledge about urban sewer networks, the function of urban sewer networks as “biochemical reactors” and their role as an integral part of wastewater treatment facilities has drawn increasing amounts of attention (Hvitved-Jacobsen et al., 1995; Warith et al., 1998). Studies have shown that the composition and contents of organics and nutritive salts can be changed or reduced in urban

sewer systems (Leu et al., 1996; Raunkjaer et al., 1997; Tanaka and Hvitved-Jacobsen, 1998; Tanaka and Takenaka, 1995). For example, Almeida et al. (2000) studied a sewer with a length of 7.2 km and found that after a hydraulic retention time (HRT) of 1.5 h, the soluble chemical oxygen demand (SCOD) removal in the wastewater due to fermentation of degradable organics by microbes was 19%, and approximately 6% of the ammonium nitrogen could be hydrolysed from nitrogen-containing compounds (e.g., urea, organic nitrogen) and removed by microbe assimilation. Additionally, sedimentation has been considered to be another important factor in sewer systems (Heaney et al., 1999). Studies have indicated that both sedimentation and biochemical actions of the biofilm that attaches to the inner pipe surface play important roles in the change of pollutant concentrations in sewer networks (Chen et al., 2003); however, researchers have typically paid more attention to the biochemical action in the sewer and its effect on wastewater quality (Warith et al., 1998).

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It is undeniable that biofilms with highly active biocenoses are often attached to the inner sewer surface (Lemmer et al., 1994). Studies have shown that the microflora in the biofilm contain large amounts of amphi-microbes with some obligate anaerobes and even methanogens at the end of the sewer (Jin et al., 2014). Those microflora produce poisonous and harmful gases such as hydrogen sulphide ( $\text{H}_2\text{S}$ ) and methane ( $\text{CH}_4$ ), which could cause pipe accidents. However, previous studies have primarily focused on describing the distribution characteristics of microbes in the biofilm along the length of the sewer. Luo et al. (2013) studied a 17.6-km long gravity sewer system and found that microbial morphologies changed along the length of the sewer; *Proteus hauseri* was found to form the majority of the microbes present, which was primarily *nitrospirae* at the starting section of the sewer and then evolved into *firmicutes* through the remaining 15.3 km of the sewer. Subsequently, the microbial flora tended to be stable, and *actinomyces* were found to be the majority of the microbes. Consequently, many studies investigated the behaviours of various pollutant changes in sewer systems. Raunkjaer et al. (1995) studied the changes in the biodegradable oxygen demand (BOD) in wastewater transportation in a 5-km-long gravity drainage pipe. The results showed that BOD removal in the flowing wastewater was approximately 30–40% at 25 °C, which followed the zero order reaction. Chen and Leung (2000) monitored the wastewater quality in a 1.5-km-long concrete drainage pipe and reported that 14% of the dissolved organic carbon (DOC) had been removed. Compared to sedimentation, these results showed the role of the biofilm that attached to the sewer was more significant (Chen et al., 2003; Chen and Leung, 2000). Additionally, based on the detection of adenosine triphosphate (ATP) in the wastewater, the microbes can also decompose sediments and thus enhance the pollutant transformation in the sewer system (Chen et al., 2001).

Previous studies have shown that pollutant concentrations can be decreased, and, ignoring sedimentation, it is often thought that sewer networks had the capability of removing organic matters and other pollutants by microbes (Vollertsen et al., 1999; Rudelle et al., 2009). However, the decrease in the concentration of organic matter, which is evaluated by the chemical oxygen demand (COD), does not mean that the organic matter decomposed due to inorganic carbonization; this reduction in the concentration of the COD may be a result of a transformation of the organic matters by microbial hydrolyzation and fermentation. Consequently, the macromolecular organic matter in a sewer can be degraded into organic matter with small molecular weight and methane ( $\text{CH}_4$ ), and unsaturated organics can be converted into saturated organics, resulting in improving the biodegradability of the sewage. To date, there has been no detailed information on the transformation of organic matter and how microflora act on the organic matter in urban sewer systems. To investigate the characteristics of the microflora in urban sewer systems, a pilot experimental system with a 1200-m-long simulated sewer was created, and the effects of microbes on the organic matter in the sewer were studied. Therefore, the mechanisms of the changes in the organic matter and the improvement in biodegradability of the sewage can be explored theoretically to describe the degradation pathways of the pollutants.

## 2. Materials and methods

### 2.1. Experimental setup

The simulated sewer system constructed for this study is shown in Fig. 1. The primary part consisted of a 40-mm-diameter PVC pipe, and the sewer's total effective length was 1200 m. The simulated sewer system consisted of a total of 35 layers from the bottom to

the top, and each adjacent layer was approximately 35 m and connected with a cylindrical inspection well that was 100 mm in diameter and 50 mm high and was made of organic glass, as shown in Fig. 1(b). As shown in Fig. 1(a), the sewage was raised 8 m by a submersible pump from water tank 0 to water tank 1, and the sewage flowed to the top layer via gravity. From the top layer, the sewage dropped into the cylindrical inspection well and into the right angle joint towards the adjacent layer. This design allowed the sewage to flow from the bottom layer and be discharged downstream through the sewer system.

To fully simulate the gravity flow state of an urban sewer, and ignoring any sedimentation that may occur in the system, the simulated sewer was designed with a slope of 5‰. The inner surface of the pipe was polished to a roughness of approximately 1.2 mm, which is similar to that of real reinforced concrete pipes, to ensure a proper resistance coefficient and Reynolds number, thus mimicking the flow characteristics of reinforced concrete pipes.

A sampling point was located in each layer of the sewer system. An organic glass pipe section 500 mm long was installed in each layer to observe the flow status in the pipe and the biofilm attached to the pipe's inner surface; this glass section was connected to the sewer system by two slipknots at each of its ends. These glass pipes were also polished to the same roughness as the sewer. The pipes were covered by a thickness of 2-cm thermal insulation material to ensure that they were in a dark environment and maintained a constant temperature.

### 2.2. Experimental conditions and raw water quality

The experiment was conducted at room temperature ( $25 \pm 2$  °C) in a controlled environment that kept the dissolved oxygen (DO) equal to  $0.3 \pm 0.05$  mg/L, which was similar to that of real sewer networks in China. During system operation, the flow rate was controlled to be equal to 0.6 m/s to avoid sedimentation in the pipe by adjusting the pipe slope and wastewater fullness degree to 5‰ and 0.6, respectively. Raw water was pumped from the real sewer of Xi'an, China to water tank 0, as shown in Fig. 1(a). Every 2–3 days, the water quality was evaluated; the characteristics of the raw water are listed in Table 1.

### 2.3. Sampling and analytical methods

#### 2.3.1. Sampling methods

For wastewater quality analysis, seven sampling points were selected in the sewer system and were located at the same interval of 200 m from the water inlet to the outlet of the sewer system. During more than 200 days of operation, the wastewater in the sewer was sampled from each sampling point for water quality analysis, which included DO, COD,  $\text{BOD}_5$ ,  $\text{NH}_3\text{-N}$  (ammonia nitrogen), TN (total nitrogen), TP (total phosphorous), and other indices.

For biofilm analysis and sampling, two slipknots at each end of the glass pipe in the sewer system were first untied; then, the biofilm that adhered to the glass pipe was sliced with sterile blades and carefully placed into a disposable culture dish. When the biofilms were sampled, the samples in the dish were covered by dry ice, transported to the laboratory immediately, and stored at  $-20$  °C.

#### 2.3.2. Fermentation products analysis

##### (1) Volatile fatty acids (VFAs) analysis

The wastewater samples were filtered through a 0.45- $\mu\text{m}$  filter before analysis. The concentration of the VFAs was analysed by gas chromatography (GC-2014 Shimadzu, Japan), which was equipped

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