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

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A B S T R A C T

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 steady physical and microbiological states. Microflora in the biofilm that attached to the inner surface along the pipe length were analysed. 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 (CO2) and methane (CH4) were found to increase along the length of the sewer system, whereas the concentrations of ethanol, lactic acid, and hydrogen (H2) 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.

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; Raunjaer 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 (H₂S) and methane (CH₄), 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 actinomycetes 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 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 ± 2 °C) in a controlled environment that kept the dissolved oxygen (DO) equal to 0.3 ± 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, BOD₅, NH₃-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-μm filter before analysis. The concentration of the VFAs was analysed by gas chromatography (GC-2014 Shimadzu, Japan), which was equipped.
with a flame ionization detector (FID) and a PE WAX ETR capillary column. The oven was kept at 100 °C for 2 min and then heated to 160 °C at a rate of 3 °C/min and held constant for 2 min. Nitrogen was used as a carrier gas at a flow rate of 20 mL/min. The liquid injection volume of the samples was 1 μL. The H2 flow rate was kept at 35 mL/min; the airflow rate was maintained at 350 mL/min; and the make-up gas (N2) flow rate was kept at 20 mL/min.

(2) Ethanol analysis

The concentration of ethanol was measured by a gas chromatograph (GC-2014 Shimadzu, Japan) equipped with a FID and a DB-FFAP capillary column. The oven was held at 40 °C for 5 min; heated to 90 °C at a rate of 10 °C/min and held for 5 min; and then heated to 150 °C at a rate of 10 °C/min and held for 2 min. The sample was then injected with 1 μL of liquid injection. Nitrogen was used as a carrier gas at a flow rate of 35.3 mL/min. The H2 flow rate was kept at 35 mL/min; the airflow rate was maintained at 350 mL/min; and the make-up gas (N2) flow rate was kept at 35.3 mL/min.

(3) Formic acid analysis

The concentration of formic acid was measured using a LC-2010AHT liquid chromatographic analyser (Shimadzu, Japan) with a Hypersil BDS C18 (250 mm × 4.6 mm × 5 μm) chromatographic column and an ultraviolet (UV) detector set to 210 nm. The flow phase was a 0.02 mol/L KH2PO4-methanol liquid with a volume ratio of 95:5. The injection liquid volume of the samples was 1 μL. The pH was adjusted to 2 using phosphoric acid, and the velocity was set to 1.0 mL/min. The column temperature was set to 30 °C.

(4) Lactic acid analysis

The concentration of lactic acid was measured using a LC-2010AHT liquid chromatographic analyser (Shimadzu, Japan) with a Hypersil BDS C18 (250 mm × 4.6 mm × 5 μm) chromatographic column and a UV detector set at 205 nm. The flow phase was a methanol-H2O liquid with a volume ratio of 10:90. The velocity was 0.7 mL/min; the column temperature was 35 °C; and the injection liquid volume of the samples was 20 μL.

2.3.3. Gaseous products analysis

(1) Carbon dioxide (CO2) analysis

CO2 was detected and analysed by a gas analyser with an infrared sensor (JSAS-CO2-IR, Shenzhen Jishun Technology Co., Ltd. China). The measurement range was 0–2000 ppm with a 1 ppm resolution; the device could only detect the gas in the sewer. In the pilot scale experimental system, seven testing ports were set at the same distance along the sewer system; they were located at a distance interval of 200 m from the water inlet port to the outlet of the sewer network with one testing port. A detection probe was then placed into each testing port. To ensure satisfactory detection accuracy, the device was sealed and calibrated with standard CO2 of different concentration monthly. When CO2 was detected, the device produced an electronic signal that was transmitted to the connected computer for data analysis.

(2) Methane (CH4) and hydrogen (H2) analysis

CH4 and H2 were measured with a gas chromatograph (GC-2014 Shimadzu, Japan) that was equipped with a thermal conductivity detector (TCD) and a TDX-01 packed column. The column temperature was 100 °C, which was retained for 10.0 min. The tail gas used was N2 at a flow rate of 10.0 mL/min. Argon was used as the carrier gas at a flow rate of 48 mL/min. The chromatograph was calibrated with a mixture of standard gases, which was composed of 37% CO2, 4% N2, 0.802% H2 and CH4.

2.3.4. Molecular weight distribution analysis of organic matter

High-performance liquid chromatography (HPLC) was conducted using a LC-2010AHT liquid chromatographic analyser (Shimadzu, Japan) with a Zenix SEC-100 7.8 × 300 mm gel chromatographic column and a UV detector set at 254 nm. The injection volume was 20 μL. The mobile phase was phosphate buffered solution (PBS) that had a pH of 7.0, and the velocity was set at 0.8 mL/min. The correlation between the MW and the retention
time was calibrated using polystyrene sulfonic acid sodium standards. To quantify the changes in the MW distribution, the chromatograms were deconvoluted into a number of Gaussian peaks using PeakFit v4.12 software with similar settings presented by Bazri et al. (2012).

2.3.5. Analysis of microflora in biofilm

The biofilms were sampled for microflora analysis at seven sampling points except at the water inlet of the sewer system. According to the methods of Auguet et al. (2015) and Satoh et al. (2009), 18 DNA samples were extracted from the biofilms using OMEGA E.Z.N.A. TM Soil DNA Kits (D5625-01), among which groups of three samples originated from the same piece of biofilm. DNA amplification was performed by a TP600 gradient PCR (polymerase chain reaction) amplification instrument. The universal primers GC-338F and 518R for prokaryotes were used. A 25-mer primer was used, consisting of a 2.5 μL of buffer, 2 μL of dNTP, 1 μL of 338F, 1 μL of 518R, 0.15 μL of exata enzyme, 17.35 μL of sterile ultrapure water, and 1 μL of a DNA template, was used for PCR amplification. The amplifying conditions were as follows: 35 cycles of a 94 °C preheating for 9 min, then a 94 °C denaturing for 30 s, then a 55 °C annealing for 30 s, and finally a 72 °C extension for 30 s. Each amplification was finished with a 72 °C extension for 7 min. The specificities of the PCR reaction products were examined by 1.5% agarose gel electrophoresis.

The denaturing gradient gel electrophoresis (DGGE) apparatus used was a DCode TM Universal Mutation Detection System (Biorad Co. U.S.A). Equal amounts of PCR products were loaded onto 8% (w/v) polyacrylamide gel with a urea denaturing gradient ranging from 40% to 70%. Electrophoresis was performed in a 1 x TAE buffer at 60 °C for 12 h at 80 V. After electrophoresis, the gel was stained with Gel Red for 30 min. Then, the electrophoresis results were observed under UV irradiation (Wu et al., 2013). After the dominant band was cut, PCR amplification was repeated for the DNA solution after gel extraction based on the above amplifying conditions. The amplified PCR products were cloned, and then ten DGGE fragments were excised from the gel, connected, transformed, and screened for blue white spots. At last, 3 white spots were randomly selected from each plate and sent to Shenggong Biological Engineering Co. Ltd, Shanghai, China for sequencing.

2.3.6. Analysis of the rate of organics degradation

The rate of organics degradation was obtained by measuring the oxygen utilization rate (OUR). Firstly, sludge collected from a biological tank was adequately aerated to achieve an endogenous respiration phase, washing sludge for 3 times and maintaining about 2500 mg/L concentration of the sludge VSS (volatile suspended solids). Then a mixed liquid of 1000 mL with water sample and sludge (v/v = 3:1) was put into a reaction vessel, and allylthiourea (ATU) solution was added to the mixed liquid, by which biological nitrification was inhibited; 2 mL of ATU reagent at a concentration of 500 mg/L was also added to the water samples per litre of water. During measurement, a thermostatic water bath was used to ensure that the temperature of the circulating water around the reaction vessel remained at 20 °C. Phosphate buffer was used to adjust the system pH to neutral. During the process of oxygenation monitoring on the airtight reaction vessel, the sludge was stirred with a magnetic stirrer to prevent sedimentation. The change in DO was monitored online by a dissolved oxygen meter. The aeration device started when the concentration of the dissolved oxygen dropped from 6 to 2 mg/L. When the concentrations of DO reached 6 mg/L, the aeration device was shut down and monitoring of the changes in DO continued. The contents of the rapidly biodegradable COD (RB COD) and the slowly biodegradable COD (SB COD) were calculated using MATLAB software and a correlation formula (Fall et al., 2014).

2.3.7. Analysis of other wastewater qualities

The COD, NH3–N, TN and TP in this experiment were processed according to the standard methods (State Environmental Protection Administration of China, 2002). The pH and dissolved oxygen (DO) were measured by a pH5-3C precision acidity meter (Shanghai Dapu Instrument Co., Ltd., China), and a portable magnetic-type dissolved oxygen meter (Hq30d, HACH, USA), respectively. BOD5 was measured by a BOD5 analyser (WTW, Germany) using the dilution inoculation method. All measurements were made in triplicate in all cases.

3. Results and discussion

3.1. Characteristics of the microflora in the sewer system

As shown in Fig. 2, the microflora grown in the biofilms were analysed with PCR-DGGE technology, which showed 10 dominant bands in the DGGE profile, indicating there were 10 dominant bacteria in the biofilm along the length of the sewer. There was a reduction in the intensity in bands 1, 3, and 5 as the length of the sewer increased; this suggested that these bacteria could not adapt to the changing environment and thus were gradually reduced. Conversely, bands 7 and 8 gradually increased in intensity as the sewer length increased, suggesting that the end of the sewer system was more suitable for the growth of these bacteria compared to
the beginning of the system and that these species eventually became the dominant bacterium. There was little variability in the intensities in bands 2, 4, 6, 9, and 10, which maintained a favourable expression along the length of the sewer system; these results indicated that these bacteria had a high adaptability to the environmental changes in the sewer network.

Gel extraction was performed in the dominant bands of the DGGE profile, and cloning and sequencing were conducted for the amplified PCR products. The ten sequence fragments obtained from sequencing were compared with those in an online database by applying the GenBank’s BLAST program to acquire the homology information of each sequence; also, using the DNAStar5.0 program, a phylogenetic tree of bacteria was constructed, as shown in Fig. 3. The results showed that the microbes corresponding to these bands of microflora primarily belonged to bacteroidetes, mesota, bacillus, thiomonas, trichococcus, and enterococcus. All ten bands showed high homologies (>90%) with the bacterial genes of known sequences; specifically, bands 5, 6, and 7 were 100% homologous to the bacterial genera of known sequences. A close relationship was shown within bands 3, 6, 7, and 10 and between bands 8 and 9. Bacteria that showed similar genetic relationships to those of bands 1 and 4 were difficult to locate on the phylogenetic tree; those bands may correspond to bacteria with unknown functions. However, bands 1, 3, 8, 9, and 10 belonged to bacteroidetes or bacillus, which are fermentation microflora. Usually, fermentative microflora play important roles in the transformation of organic matter.

To investigate the effects of microflora on the transformation of organic matter, the primers used were related to the fermentation bacteria specifically identified in this study, which are believed to be helpful in the decomposition of organic matter. Therefore, other previously reported microflora in sewers, such as sulphate-reducing bacteria, denitrifying bacteria and methanogens (Dizer and Hagendorf, 1991; Satoh et al., 2009; Sun et al., 2015), were not involved.

3.2. Fermentation products formation in the sewer system

The formation of fermentation products along the length of the sewer system was detected, and the results are shown in Fig. 4(a) and (b). As one of the principal products of hydrolyzation and fermentation of organic matter, the content of VFAs reflected the degree of fermentation of organics in wastewater (Wang et al., 2014). Fig. 4(a) shows that the VFAs in the sewer system primarily consist of acetic acid, propionic acid and isobutyric acid, of which the acetic acid content was the highest. Conversely, the contents of other VFAs (OVFA) were low in the sewer. In the first 600 m of the sewer, the content of all VFAs increased along the length of the sewer. Between 600 and 1000 m in the sewer, the content of several types of VFAs remained stable, and the content of TVFA reached its maximum value of 19.34 mg/L at 800 m. However, at the end of the sewer, the VFA content decreased significantly; this could be as a result of the rear end of the sewer being exposed to the air, and changes in the environmental conditions that were unsuitable for the fermentation of microflora growth. The significant variations in the content of different VFAs were related to the distribution of microflora in the sewer system.

Additionally, other fermentation products (e.g., ethanol, lactic acid, CH₄, CO₂, and H₂) were also detected in the study, as shown in Fig. 4(b). The results showed that the content of ethanol, lactic acid, and H₂ within 0–400 m at the beginning of the sewer increased quickly along the length of the sewer and then dramatically decreased to low concentrations between 600 and 1200 m along the sewer. However, variability was found in the content of CH₄ and CO₂; although these two gaseous products steadily increased along the length of the sewer, the difference found may be related to the utilization of the carbon source by the methanogens in the sewer. Previous reports indicated that methanogens favour acetic acids as their carbon source compared to other fermentation products (Martens and Klump, 1980). Therefore, combined with the variations in the acetic acid content and CH₄ along the length of the sewer, as shown in Fig. 4, it could be inferred that ethanol, lactic acid and H₂ may be converted into acetic acids by acetogenic and homoacetogenic bacteria at lengths greater than 400 m in the sewer. However, future studies are required to further explore this hypothesis. Regardless, the increasing content of CO₂ with the increasing length of the sewer system indicated that inorganic carbonization of some organic matter had occurred in the system.

Based on the fermentation processes involved in the generation of acetic acid, propionic acid, isobutyric acid, ethanol, lactic acid, H₂ and CO₂ in the sewer, and combined with the analysis results...
discussed above, the hydrolysis and fermentation of organic matter in the sewer by microbes were a "mixed acid fermentation" (Ren et al., 2008; Zhang et al., 2013), indicating several fermentation processes that produced ethanol, acetic acid, and butyric acid existed within the sewage transportation system of the sewer.

To understand the effects of the fermentation process on organic matter transformation, the TVFA and CH₄ concentration can be evaluated in COD base. Based on the calculation methods shown in previous studies (Wu et al., 2014; Hvitved-Jacobsen et al., 2013), the fractional changes of TVFA of the total COD (TCOD) and CH₄ in the reduction of the TCOD are shown in Table 2. When the biofilm was in a steady state between 200 and 1000 m in the sewer, approximately 8% TCOD was converted into VFAs. However, the fraction of CH₄ in the reduction of TCOD increased from 12% at the beginning of the sewer to 50% at the rear end of the sewer, which was the primary reason of the decrease of the concentration in COD along the length of the sewer system. From this calculation, it could be concluded that fermentation reactions played an important role in the total conversion process.

3.3. Variation in the molecular weight of organic matter in the sewer system

Generally, the assimilation by microbes can convert organic matter into macromolecular cellular substances. Exoenzymes that are constantly secreted from microbes can hydrolyse and ferment dissolved macromolecular organic matter into smaller molecular weight products, which are then utilized and consumed by microbes, reducing the amount of macromolecular organics.

Therefore, the variation in organic molecular weight in sewage due to biological treatment has been reported to result from the collective action of these processes. In this study, variations in molecular weight, as monitored by HPLC, in each interval along the length of the sewer system suggested the effect of microbes on the degradation of organic matter and the improvement of organic matter. As shown in Fig. 5, the content of organic matter with molecular weights greater than 10,000 Da showed a decreasing trend as the length of the sewer system increased. In addition, the

![Fig. 4. Variation of fermentation products along the length of the sewer system. (a) VFAs (b) other fermentation products and CH₄. (Note: Error bars represent standard error of 5 replicates).](image)

![Fig. 5. Variation of organic matter in MW along the length of the sewer system.](image)

### Table 2

<table>
<thead>
<tr>
<th>Length of the sewer (m)</th>
<th>TCOD (mg/L)</th>
<th>ΔTCOD (mg/L)</th>
<th>TVFA (mg COD/L)</th>
<th>ΔCH₄(g) (mg COD/L)</th>
<th>ΔCH₄(w) (mg COD/L)</th>
<th>ΔCH₄/ΔTCOD</th>
<th>TVFA/TCOD</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>370 ± 10.00</td>
<td></td>
<td>7.59 ± 1.1</td>
<td>2.83</td>
<td>0.58</td>
<td>0.12</td>
<td>0.021</td>
</tr>
<tr>
<td>200</td>
<td>342.42 ± 5.12</td>
<td>27.58 ± 2.30</td>
<td>20.59 ± 0.9</td>
<td>4.08</td>
<td>0.84</td>
<td>0.20</td>
<td>0.077</td>
</tr>
<tr>
<td>400</td>
<td>318.16 ± 9.20</td>
<td>24.26 ± 2.18</td>
<td>24.39 ± 0.25</td>
<td>4.28</td>
<td>0.88</td>
<td>0.23</td>
<td>0.082</td>
</tr>
<tr>
<td>600</td>
<td>296.12 ± 11.25</td>
<td>22.04 ± 1.83</td>
<td>24.06 ± 0.32</td>
<td>4.64</td>
<td>0.95</td>
<td>0.31</td>
<td>0.091</td>
</tr>
<tr>
<td>800</td>
<td>278.05 ± 4.53</td>
<td>18.07 ± 2.24</td>
<td>25.27 ± 0.7</td>
<td>4.97</td>
<td>1.02</td>
<td>0.40</td>
<td>0.076</td>
</tr>
<tr>
<td>1000</td>
<td>262.98 ± 7.20</td>
<td>15.07 ± 1.44</td>
<td>20.10 ± 1.1</td>
<td>5.40</td>
<td>1.11</td>
<td>0.50</td>
<td>0.054</td>
</tr>
<tr>
<td>1200</td>
<td>248.89 ± 5.12</td>
<td>13.09 ± 1.13</td>
<td>13.61 ± 0.8</td>
<td>5.80</td>
<td>1.15</td>
<td>0.65</td>
<td>0.054</td>
</tr>
</tbody>
</table>

Note: ΔTCOD represents the amount of reduction in COD, while ΔCH₄(g) and ΔCH₄(w) represent the amount of CH₄ production in the gas and water phase every 200 m length of the sewer respectively. 

ΔCH₄(g), ΔCH₄(w), ΔCH₄/ΔTCOD and TVFA/TCOD all represent the mean value of data of samples measured.
content of organic matter with molecular weights ranging from 650 to 10,000 Da increased between 0 and 200 m along the sewer system and decreased between 200 and 1200 m; conversely, that with molecular weights less than 650 Da increased between 0 and 800 m and then decreased between 800 and 1200 m. The results showed that during sewage transportation, the content of organic matter with higher molecular weights decreased, which indicated that the biodegradability of the sewage improved along the length of the sewer.

3.4. Effects of the fermentation process on wastewater quality

To assess the effects of the fermentation process on the organic matter, the specific ratio of BOD<sub>5</sub>/COD was monitored along the sewer system. As shown in Fig. 6, as the length of the sewer increased, the specific ratio of BOD<sub>5</sub>/COD continuously increased from 0.68 at the beginning of the sewer to 0.84 at 1200 m (i.e., the end of the sewer network). The variation of the specific ratio of BOD<sub>5</sub>/COD indicated that the effect of the fermentation of microbes on organic matter was beneficial in improving the biodegradability of the sewage.

Using activated sludge models (ASMs) as a reference, organic matter in urban sewage can be categorized as biodegradable COD (BCOD) or non-biodegradable COD (NBCOD). The BCOD can be further classified into rapidly biodegradable COD (RBCOD) and slowly biodegradable COD (SBCOD) based on the degradation rate (Vollertsen and Hvitved-Jacobsen, 1998). Using the oxygen utilization rate (OUR) and the calculation of the COD, the variations in the component and content of the TCOD, RBCOD, SBCOD, and NBCOD along the length of the sewer system were obtained and are shown in Fig. 6. The results showed that the content of the TCOD in the sewer system decreased by approximately 32% with increasing sewer length, which indicated a significant change in the content of organic matter after the microbes acted on the wastewater within the 1200-m length of the sewer system. The content of RBCOD gradually reduced with increasing sewer length by approximately 41%, whereas the content of SBCOD decreased with increasing sewer length by 26%. Generally, the primary components of RBCOD consisted of VFAs and other readily biodegradable matter, which likely had lower molecular weights (Henze et al., 1987). These substances may be utilized directly by the microbes either as a source of energy required for inorganic carbonization, which is vital for microbial activity, or for living organism synthesis (Aarrestad, 2011; Karahan et al., 2008). The decrease in RBCOD can directly result in the removal of COD in wastewater. However, the primary components of SBCOD are typically particle substances in sewage (Hu et al., 2002). As the sewage flowed through the sewer network, the fermentative microbes constantly hydrolysed and fermented these substances into soluble organic matter, and then converted them into RBCOD (Gujer et al., 1999; Aarrestad, 2011). Thus, the variation in BOD<sub>5</sub>/COD of these substances was actually caused by changes in their chemical structure and molecular weight distribution by hydrolysis and fermentation in the sewer system. Additionally, as shown in Fig. 6, the content of NBCOD continuously decreased by 9% as the length of the sewer increased. Due to hydrolysis and fermentation in the sewer system, some NBCOD can be slowly converted into SBCOD or RBCOD.

Based on the discussion above, a schematic graph describing the conversion of organic matter along the length of the sewer system was constructed, as shown in Fig. 7. The structure and composition of organic matters in the sewage were transformed by microflora in the biofilm attached to the inner surface of the sewer. At the initial section of the sewer system, organic matters, especially for the

![Fig. 6. Variation of TCOD, RBCOD, SBCOD, NBCOD and the ratio of BOD<sub>5</sub>/COD along the length of the sewer system. (Note: Error bars represent standard error of 5 replicates).](image1)

![Fig. 7. Schematic graph of the conversion mechanisms of wastewater quality in the sewer.](image2)
SBCOD fraction, were converted into soluble matters with small molecular weight by the microbes. The primary products were VFAs, lactic acid, ethanol, hydrogen (H₂), and methane (CH₄). Some RBCOD were decomposed to CO₂, causing in the decrease of COD. As the length of the sewer increased, the fermentation process was strengthened, and therefore lactic acid, ethanol, and hydrogen (H₂) were converted into acetic acid by microbes. Consequently, acetic acid became the primary fermentative product and was utilized by methanogens to further produce large amounts of CH₄ in the rear section of the sewer system. Therefore, due to microbial hydrolyzation and fermentation, the macromolecular organic matter in the sewer was decomposed into organic matter with small molecular, and the unsaturated organic matter was decomposed into saturated organic matter. Consequently, the biodegradability of the organic matter in the sewer was improved dramatically.

4. Conclusions

The characteristics of microflora and the transformation of organics in urban sewer networks were studied in a pilot sewer system with a total length of 1200 m. The key findings of this study include the following:

(1) Biofilms were found to be attached to the inner surfaces of sewer after the sewage flowed through it for a long period of time. Additionally, typical fermentation bacteria, such as bacteroidetes and bacillus, were found to be abundant in the system.

(2) In the sewer system, the chemical structure of organic matter could be changed via microbial hydrolyzation and fermentation: the macromolecular organic matter in the sewer decomposed into organic matter with small molecular, and the unsaturated organic matter decomposed into saturated organic matter.

(3) In the sewer system studied, the primary fermentation products were VFAs, gaseous CO₂, CH₄, and H₂, and ethanol, lactic acid. Most of these products tended to be converted into acetic acids.

(4) Fermentative microflora could be beneficial in organic matter removal and in improving the biodegradability of organics in urban sewer systems.

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