Original Article

Effects of Attached and Suspended Biomass on the Dynamics of the Microbial Community and Wastewater Characteristics in Sewers

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ABSTRACT

Various transformations in wastewater quality along sewers, such as that due to self-purification, have been reported. However, little is known about the contributions of the attached (sewer-wall) and suspended biomass originally existing in wastewater due to a lack of experimental fields. In this study, we examined the effects of attached and suspended biomass on the dynamics of the microbial communities in sewers by conducting recirculating batch tests in a pilot-scale sewer system equipped with sponge media and a lab-scale aerating batch test, respectively. The changes in the quantity and quality of organic matter indicated that the contribution of the attached biomass to self-purification was much larger than that of the suspended biomass, because the former was sufficiently acclimated to the wastewater. Moreover, the microbial community analysis by pyrosequencing suggested that there were two candidates responsible for self-purification: 1) Comamonadaceae and Rhodocyclaceae, which could immediately proliferate under attached conditions and become dominant (15% each) in the attached biomass, and 2) Pseudomonadaceae, which could proliferate under suspended conditions after a lag period of several hours and remain a small component (4%) of the attached biomass.

Keywords: attached biomass, organic matter, self-purification, sewer, suspended biomass

INTRODUCTION

Domestic wastewater is collected and transported by a sewer network and treated at a wastewater treatment plant (WWTP). Although end-of-pipe WWTPs are responsible for the final treatment, various changes in wastewater quality along sewers have been reported since the pioneer works in the 1970s [1,2]. In gravity sewers, the aerobic biodegradation of organic matter, or self-purification, is the dominant transformation process (as reviewed by [3]) because a considerable amount of oxygen can be supplied through the water's surface by reaeration [4]. So far, several investigations have demonstrated that organic matter degrades in full-scale gravity sewers by comparing wastewater samples collected

at points several kilometers apart [5–9]. In addition to degradation, a few studies have found that the characteristics of organic matter, such as the soluble/particulate fractions and specific components (volatile fatty acids, carbohydrates, lipids, and proteins), are affected by self-purification through modeling and/or experimental approaches [5,10,11]. The transformation of organic matter in terms of both quantity and quality should be carefully estimated, because it can have both positive and negative impacts on WWTPs: the degradation of organic matter can reduce the space, energy, and costs for COD removal [7,12–14], whereas the decrease in specific components, such as acetate, can deteriorate biological nutrient removal [3].

Sewers contain the suspended biomass originally existing

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in wastewater and attached (sewer-wall) biomass, which can contribute to self-purification depending on various factors [7]. However, it is difficult to characterize the effect of each type of biomass due to the lack of experimental fields or facilities. In full-scale, operational sewers, advective diffusion is a major barrier to evaluating changes in wastewater quality between distant sampling points [15], even if there is no input or bifurcation. Moreover, it is almost impossible to stably retain attached biomass on sewer walls. However, in laboratories, the long-term acclimation of attached biomass fed with actual wastewater is laborious enough that only one lab-scale study has been conducted [16] to the best of our knowledge. Although aerating batch tests (i.e., the aeration of collected wastewater) is an alternative approach [17,18], the target of these tests is limited to the suspended biomass originally existing in the wastewater. Presumably for the same reasons, several studies have assessed the microbial communities related to either suspended [19–21] or attached [22–24] biomass collected at specific points; however, little is known about the dynamics and interactions between the suspended and attached biomass in sewers.

One of the possible solutions to this is to develop recirculating facilities at an appropriate scale, as demonstrated by two groups [25,26] who operated pilot-scale channel reactors using actual wastewater. We also developed a pilot-scale recirculation system consisting of a modified sewer pipe (**Fig. 1a**) and facilities for either continuous or recirculating operation (**Fig. 1b**), which was originally intended to enhance self-purification by sponge media to stably retain attached biomass [27]. This experimental system was large enough to simulate self-purification in sewers (250 mm in diameter) and small enough to ignore advective diffusion under recirculating conditions (32 m in total length). Moreover, it was located in a WWTP serving a small residential area and had acclimated to fresh wastewater for over six months. Using these features, we simultaneously monitored the changes in wastewater characteristics and the dynamics of the microbial community in this study. For the wastewater characteristics, we fractionated organic matter (COD) in terms of its settleability [28] and biodegradability [29] to understand both the changes in quantity and quality. For the microbial community, the bacterial 16S ribosomal RNA genes in suspended and attached biomass were pyrosequenced. Overall, the aim of this study is to provide further insights into the mechanisms of self-purification in sewers based on interactions between wastewater characteristics, suspended biomass originally existing in the wastewater, and attached biomass.

MATERIALS AND METHODS

Setup and Operation of the Experimental Sewer System

We developed a modified sewer pipe with a double-deck structure, as reported in our previous study [27]. **Figure 1a** presents the cross-sectional view with upper and lower decks divided by a separator; the upper deck was mainly designed for smooth wastewater transportation and the lower deck was designed for self-purification by the attached biomass on the two rows of polyethylene sponge media. The separator also supplies more oxygen to the attached biomass by two processes: 1) reaeration by falling the wastewater from the upper deck to the lower deck when the wastewater is supplied to or recirculated in the pipe, mimicking high-flow conditions in full-scale sewers; and 2) direct exposure of the attached biomass to air when wastewater is not supplied to or recirculated in the pipe, mimicking low-flow conditions in full-scale sewers. As illustrated in **Fig. 1b**, the experimental system included the modified pipe (32 m in total length), pumps for influent supply and recirculation, and a tank for recirculation. The system was located in a WWTP that receives domestic wastewater from a small residential area (ca. 1,000 population equivalent) in Nagareyama, Chiba, Japan. The composition of the influent wastewater is summarized in **Table 1**. As the collection area was within ca. 500 m from the WWTP, the influent wastewater was very fresh, i.e., very little self-purification should have occurred before the wastewater reached the WWTP. The influent underwent no pre-treatment excluding coarse screenings at 50 and 20 mm immediately before the receiving well. The experimental system was usually operated in the normal mode (i.e., without recirculation) for acclimation, during which the wastewater was supplied intermittently (30 min on/off) to mimic flow changes in full-scale sewers. The flow rate was controlled to approximately 9 m³/h, corresponding to several minutes of hydraulic retention time (HRT) in the system.

Recirculating Batch Tests

The changes in wastewater characteristics and microbial community were examined through two types of 12-h recirculating batch tests. After the recirculation tank (ca. 1.2 m³) was filled with 1 m³ of influent I or II (**Table 1**), the recirculation pump was operated continuously or intermittently (30 min on/off) at 9 m³/h, and the pH (7–8), DO (6–9 mg/L during recirculation), and temperature (12–16°C) of the recirculation tank were monitored. The wastewater samples were collected from the recirculation tank every 2

(a) Cross-sectional view of the pipe

(b) Schematic of the experimental system

Fig. 1 Schematic diagrams of the experimental system reported in the previous study [27].

(a) Cross-sectional view of the pipe, which shows that the attached biomass can be intermittently exposed to air and wastewater corresponding to flow changes.

(b) Schematic diagram of the experimental sewer system with 32 m (in total length) of pipe. The system could be operated in either the normal mode for acclimation or the recirculation mode for batch tests.

h, screened through a 0.85-mm mesh to remove gross solids, and transferred to our laboratory for analysis as soon as possible (< 30 min). These tests were conducted at least six months after acclimation began, thus, a stably attached biomass for enhancing self-purification had already been retained [27].

Aerating Batch Test

In addition to the recirculating batch tests, a lab-scale aerating batch test was conducted to examine the effect of the suspended biomass originally existing in the influent wastewater. Influent wastewater III (Table 1) was collected

from the receiving well, and then screened and immediately conveyed to our laboratory in the manner mentioned above. The wastewater (10 L) was transferred to a glass bottle with a mixing apparatus and a diffuser to supply air at 0.3 L/min, which maintained the DO above 6 mg/L throughout the test. The batch test was conducted for 12 h at approximately 15°C. Samples were collected every 2 h and analyzed as soon as possible.

COD Fractionation

Settleability-based fractionation [28] was conducted with minor modification. In summary, a wastewater sample was

Table 1 Characteristics of the influent wastewater for acclimation and recirculating/batch tests.

(mg/L)	For acclimation ^a		For the batch tests ^b		
	average	SD	I	II	III
total COD	318	80	262	228	242
SB fraction ^c			40	42	48
uSB fraction ^d			125	137	141
B fraction ^e			165	179	189
BOD ₅	131	32	123	105	117
SS	116	41	100	73	73
NH ₄ ⁺ -N	13	3	13	9	12
NO ₃ ⁻ -N	0.7	0.3	0.9	1.1	1.0
PO ₄ ³⁻ -P	1.4	0.4	1.7	1.1	1.6
DNA (pg/μl)	—	—	263	244	302

^a The average was calculated from 27 wastewater samples obtained in this study (the influents I–III) and our previous study [27]. Most samples were collected at 7–8 a.m.

^b Influent I, II, and III were used for the continuously recirculating, intermittently recirculating, and aerating batch tests, respectively.

^c "SB fraction" represents the settleable (particulate) and biodegradable COD.

^d "uSB fraction" represents the unsetttable (soluble and colloidal) and biodegradable COD.

^e "B fraction" represents the biodegradable COD, i.e., the sum of the SB and uSB fractions.

settled in a 0.5- or 1-L measuring cylinder at 4°C for 2 h, which is a common HRT range for primary settlers. Half of the supernatant volume was collected as the unsetttable fraction (referred to as uS-COD), which mostly contained soluble and colloidal organic matter, after the top quarter that potentially contained scum was discarded. The settleable fraction (S-COD), which mostly contained particulate organic matter, was determined by subtracting the uS-COD from the total COD.

Biodegradability-based fractionation was subsequently conducted following the ultimate BOD-based method [29], which was also validated for different wastewaters in Japan [30]. In this method, the ultimate BOD (BOD_u) followed by biodegradable COD (B-COD) were calculated as follows:

$$\text{BOD}_u = \frac{1}{1 - \exp(-k_{\text{BOD}} \cdot t)} \cdot \text{BOD}_t \quad (1)$$

$$\text{B-COD} = \frac{1}{1 - f_{\text{BOD}}} \cdot \text{BOD}_u, \quad (2)$$

where t (d) is the incubation time, BOD_t is the BOD at day t , k_{BOD} (1/d) is the first order rate constant, and f_{BOD} (assumed to be 0.15, dimensionless) is a correction factor to compensate for the interaction of biomass growth and decay. The BOD_t was measured in triplicate or duplicate at $t = 2$ or 3, 5, 7, and 9 or 10. The BOD_u and k_{BOD} for a set of BOD_t

were simultaneously estimated by the AQUASIM simulation software [31]. The same methods were also applied to uS-COD to classify B-COD into the settleable (SB-COD) and unsetttable fractions (uSB-COD).

Chemical Analysis

The COD was measured using a commercially available kit (Hach, Loveland, USA). The BOD and suspended solids (SS) were analyzed according to Japanese Industrial Standard JIS K 0102 [32]. For the BOD assay, nitrification was inhibited by adding 10 mg/L of allylthiourea. Several ions were analyzed using an ion chromatograph Dionex ISC-3000 (Thermo Fisher Scientific, Waltham, USA) equipped with an AS-12A column (Thermo Fisher Scientific) for anions, or a TSKgel SuperIC-CR column (Tosoh, Tokyo, Japan) for ammonium after filtration through a 0.45-μm membrane.

Microbial Community Analysis

The first step of microbial community analysis involved mixing a wastewater sample (0.75 mL) with ethanol (0.75 mL) in a 2-mL plastic tube and storing it at −80°C until analysis. The frozen wastewater samples were thawed and the DNA was extracted using a sonicator Branson AD 250 (Emerson, St. Louis, USA) at 20% amplitude for 15 s, following our previous study [33]. For the attached biomass in the pipe (collected 2, 18, and 32 m from the inlet after all the batch tests), a small amount of the sponge media was collected in a 50-mL plastic tube, mixed with a comparable

volume of ethanol, and stored at -80°C . The thawed samples were adjusted to 30 mL by adding 50% ethanol, and the mixture was sonicated at 90% amplitude for 30 s to remove the biomass from the sponge. Then, 1.5 mL of the sonicated suspension was collected in a 2-mL plastic tube and further sonicated to extract the DNA under the same conditions as the wastewater samples. The extracted DNA concentrations were determined using a PicoGreen dsDNA Quantification Kit (Thermo Fisher Scientific). The extracts were diluted by 10 pg-DNA/ μL (around 20-fold) for subsequent PCR.

The PCR and pyrosequencing were conducted following our previous studies [33,34] with a minor modification. The DNA extracts were used as the template and amplified using a 27fmod primer [35], prepended with a 454 adaptor A sequence and a unique eight-base barcode sequence (described as NNN NNN NN) for each sample (3'-CCA TCT CAT CCC TGC GTG TCT CCG ACT CAG NNN NNN NNA GRG TTT GAT CMT GGC TCA G-5'), and a 519r primer prepended with a 454 adaptor B sequence (3'-CCT ATC CCC TGT GTG CCT TGG CAG TCT CAG GWA TTA CCG CGG CKG CTG-5'). The PCR product was purified with a QIAQuick PCR Purification Kit (Qiagen, Hilden, Germany), and its concentration was checked using a PicoGreen dsDNA Quantification Kit. The quality of the purified PCR products was checked using a 2100 Bioanalyzer (Agilent Technologies, Santa Clara, USA). The purified PCR products were pooled and subjected to standard pyrosequencing analysis using a 454 FLX pyrosequencer GS Junior (Roche, Basel, Switzerland). The obtained data were processed using the pipeline of QIIME Version 1.9.1 [36]. The sequences were de-novo clustered at a similarity of 97% using uclust, and taxonomy was assigned using uclust [37] and GreenGenes 13_8 [38]. The representative sequences have been deposited in the DNA Data Bank of Japan with the accession numbers SAMD00051527–00051547 (wastewater samples) and SAMD00051842–00051844 (attached biomass samples). From the pyrosequencing, we obtained several hundred reads for each wastewater or attached biomass sample after removing short or low-quality sequences. Considering the read numbers were relatively small, we confirmed the reliability of the dynamics of the major taxonomic groups based on the 95% confidence intervals calculated by the statistical package R (R Core Team).

RESULTS AND DISCUSSION

Changes in Organic Matter Characteristics

We will begin our discussion by comparing the continu-

ously recirculating and aerating tests to determine the effects of attached and suspended biomass on wastewater characteristics. As illustrated in **Figs. 2a–f**, all changes in organic matter indicated that self-purification was much more active in the continuously recirculating test than that in the aerating test. That is, the contribution of the attached biomass with the aid of the sponge media was much larger than that of the suspended biomass originally existing in the influent wastewater. For example, the biodegradable organic matter (B-COD, **Fig. 2d**) immediately declined to approximately half of its initial level within 6 – 8 h and then plateaued in the continuously recirculating test, while it decreased very little within the initial 6 – 8 h before exhibiting a slight decrease in the aerating test. Filtration by the attached biomass and sponge media themselves may have contributed to the immediate removal of the particulate or settleable fractions (SB-COD), even though they should have been almost saturated as they acclimatized for over six months. Considering that the concentrations of the soluble/colloidal or unsettlable fractions (uSB-COD) were approximately three times higher than those in particulate SB-COD (**Table 1**), a better explanation for these contrastive performances is that the suspended biomass was not acclimated to the organic matter contained in the influent wastewater and required a lag period of several hours to utilize it. The attached biomass, however, could immediately utilize the organic matter in the influent wastewater owing to the long acclimation. The decreasing patterns of the organic matter corresponded to the increasing patterns of the DNA concentration (**Fig. 2g**), which should represent the quantity of suspended biomass, i.e., increasing/decreasing for the initial 6 – 8 h then plateauing during the continuously recirculating test, while lagging for 6 – 8 h followed by a gradual increase/decrease during the aerating test. The increase in DNA (i.e., suspended biomass) in the wastewater samples collected from the recirculating test could be due to two different reasons: 1) the detachment of the attached biomass due to its (over) growth on the sponge media, or 2) the growth of the detached biomass under suspended conditions. These possibilities will be validated later when the results of the microbial community analysis are considered.

The recirculating batch tests under continuous and intermittent conditions will now be compared to elucidate the effects of flow changes, which are inevitable in full-scale sewers. In these recirculating tests, clear differences were observed in the changes in SS (**Fig. 2a**) and DNA (**Fig. 2g**). It is reasonable to assume that the pump restart in the intermittently recirculating test is comparable to a so-called

Fig. 2 Organic matter and DNA concentrations represented as the relative abundance to the initial concentrations in the continuously recirculating (●), intermittently recirculating (■), and lab-scale batch tests (▲).

“first flush” in combined sewers, resulting in the detachment of the captured SS and attached biomass from the sponge media. However, the effect of flow changes on the biodegradable fractions shown in **Figs. 2d–f**, especially on soluble/colloidal uSB-COD (**Fig. 2f**), was much smaller than those on SS and DNA. Although uSB-COD, by definition, does

not contain inert particulates that are strongly governed by the flow conditions, it should be noted that the wastewater was only in contact with the attached biomass for 50% of the test period during the intermittently recirculating test as most of the wastewater was located in the recirculation tank rather than the pipe when the recirculation was interrupted

Fig. 3 Phylum-level classification of the obtained DNA sequences. “Influent” indicates the average of influents I, II, and III, while “Attached biomass” indicates the average of biomass samples collected at 2, 18, and 32 m from the beginning of the experimental system. The wastewater samples were collected at the end of each batch test (12 h).

(Fig. 1). Therefore, it can be concluded that direct exposure of the attached biomass to air (gaseous oxygen) during the interruption contributed to self-purification at the same level as the contact with wastewater (containing enough DO) during recirculation. We observed a significant decrease in the concentration of gas-phase oxygen inside the pipe during the interruption in our previous study [27]. Overall, the results of the batch tests indicate that organic matter removal on/around the attached biomass was the most important factor for self-purification, regardless of the recirculating conditions.

Dynamics of Microbial Community

As the first step of characterizing the microbial community, the obtained sequences were roughly classified at the phylum/class levels, as illustrated in Fig. 3. In the influent wastewater (average of influents I, II, and III), three dominant phyla, Bacteroidetes (37%), Proteobacteria (34%), and Firmicutes (22%), accounted for more than 90% of the community. After the recirculating and aerating batch tests, although the increase in the proportion of Proteobacteria in the wastewater samples was a common feature, there were notable differences at the class level. After the continuously and intermittently recirculating batch tests, the wastewater samples were characterized by the proliferation of Betaproteobacteria (from 10% to 23 or 31%), and the microbial communities closely resembled those of the attached biomass,

however, after the aerating batch test, the wastewater sample was characterized by the proliferation of Gammaproteobacteria (from 12% to 22%). However, in contrast to some previous studies analyzing wastewater or sewer-wall biofilm communities [20,22–24], the proportion of Deltaproteobacteria, which mainly contains sulfate-reducing bacteria in sewers, was very low at 1 – 2% in the wastewater samples and 5% in the attached biomass sample. Thus, the consumption of organic matter coupled with sulfate reduction had little impact on the characteristics of wastewater in the experimental system.

The next point we should examine is the dynamics of the major bacterial groups during the batch tests. From the observed changes in the organic matter characteristics, we will discuss the dynamics from the following perspectives: 1) the identification of the attached biomass responsible for self-purification during the recirculating batch tests, 2) the identification of the suspended biomass responsible for self-purification after a lag period during the aerating batch test, and 3) the inability of the biomass originally existing in the influent wastewater to contribute to self-purification.

In the recirculating batch tests, two proliferated bacterial families, Comamonadaceae and Rhodocyclaceae of Betaproteobacteria, were observed (Figs. 4a and 4b). As the proportions increased during the initial 6 – 8 h, and then plateaued, corresponding to the changes in organic matter and DNA (Fig. 2), it can be concluded that these families

Fig. 4 Fate of the proliferated bacterial groups (at family level) in the continuously recirculating (●), intermittently recirculating (■), and aerating batch tests (▲). The bold line indicates the average of the attached biomass samples for comparison.

were responsible for self-purification as part of the attached biomass. Although these groups each constituted 5% of the community in all the influents, the proportions increased very little during the aerating batch test. These contrasting fates suggest that Comamonadaceae and Rhodocyclaceae proliferated as they could utilize the organic matter in the influent wastewater without a lag period, however, their attached conditions also aided their proliferation. Moreover, their inability to contribute to self-purification under suspended conditions support one the hypothesis that the biomass or DNA detected in the wastewater samples after the recirculating tests were mainly derived from the detachment of the attached biomass as a result of their (over) growth on the sponge media, rather than the growth of the detached biomass under suspended conditions.

However, during the aerating batch tests, only the Pseudomonadaceae family of the Gammaproteobacteria proliferated (**Fig. 4c**). As their proportion significantly increased after a lag period within the initial 6 – 8 h, corresponding to the changes in organic matter and DNA (**Fig. 2**), it can be concluded that this family was responsible for self-purification as part of the suspended biomass originally existing in the influent wastewater. Although they constituted ap-

proximately 4% of the community in all the influents and the attached biomass, their proportion did not increase during the recirculating batch tests. Thus, Pseudomonadaceae could utilize organic matter in the influent wastewater, however, their requirement for a lag period of several hours and/or their possible preference towards suspended conditions were major barriers to their contribution to self-purification as attached biomass. Nevertheless, the existence of these bacterial groups also suggests that the contribution of suspended biomass to self-purification is more significant if the retention time of the tested wastewater in the sewers is long enough to compensate for the lag period. Thus, the reports that self-purification can immediately occur (i.e., without a lag period) from aerating batch tests [18] and that the contributions of suspended and attached biomass can be similar [7] do not necessarily contradict the observation of our aerating test using fresh wastewater.

In the influent wastewater, the predominant bacterial orders were Bacteroidales (32%) and Clostridiales (18%), which are obligatory anaerobic bacteria. Several researchers have also reported that these anaerobic groups were abundant in influent wastewaters collected in large-scale WWTPs, and most of them were probably derived from the human gut flora via

Fig. 5 Fate of the deteriorated bacterial groups in the continuously recirculating (●), intermittently recirculating (■), and aerating batch tests (▲). The bold line indicates the average of the attached biomass samples for comparison.

feces [19,21]. As illustrated in **Figs. 5a** and **5b**, the proportions of Bacteroidales and Clostridiales slightly decreased during all the batch tests, presumably due to the strict aerobic conditions ($\text{DO} > 6 \text{ mg/L}$). However, this does not necessarily represent their rapid death or decay considering the increase in total DNA concentrations (**Fig. 2g**), especially during the recirculating batch tests. Thus, we also calculated the relative abundance of their DNA concentrations (**Figs. 5c** and **5d**) assuming that the DNA concentrations of each bacterial group correspond to the products of the total DNA concentration and the proportions of the detected sequences. The trends shown in **Figs. 5c** and **5d** suggest that 1) these groups did not grow during all batch tests, 2) the strict aerobic conditions were not necessarily toxic enough to cause their rapid death or decay, and 3) Clostridiales were partially removed during the recirculating batch tests. From a self-purification viewpoint, it can be concluded that anaerobic groups, such as Bacteroidales and Clostridiales, were not contributors, but rather a part of the targeted organic matter by self-purification in the majority of the suspended biomass originally existing in the influent wastewater.

Mechanism of Self-purification by Attached Biomass

From the above discussion, we can propose mechanisms for the degradation of organic matter in the experimental sewer system. The biodegradable and particulate fraction represented as SB-COD is captured on/around the attached biomass with the aid of the sponge media during recirculation (*i.e.*, when wastewater comes into contact with the attached biomass), and then degraded by the biomass while either DO or gaseous oxygen is consumed depending on the recirculation conditions. Degradation was mainly attributed to the attached biomass, especially to Comamonadaceae and Rhodocyclaceae, rather than the suspended biomass originally existing in the influent wastewater, because the former was sufficiently acclimated to the organic matter. The attached biomass and media can also retain organic matter and biomass even when recirculation is interrupted. These advantages of attachment may correspond to previous reports that biofilms could be more suitable for degrading macromolecules by microbial communities in wastewater [39,40].

Very little of the biodegradable and soluble/colloidal frac-

tion represented as uSB-COD should be captured on/around the attached biomass as a result of physical interaction. However, many researchers have reported that several bacterial groups can store organic matter as intracellular substrates, such as polyhydroxyalkanoate (PHA), and subsequently oxidize them, especially when they are cultivated under so-called “feast and famine” conditions (as reviewed by [41]). As acclimation with an intermittent wastewater supply and the structure of the modified pipe (sponge media and separator) should have created feast and famine conditions for the attached biomass, a significant proportion of the bacterial community was quite likely to utilize organic matter via storage. Although we could not analyze the storage of PHA or other substrates in the attached biomass during the testing of the experimental sewer system, Comamonadaceae and Rhodocyclaceae were two of the three dominant PHA-accumulating families in activated sludge taken from municipal WWTPs that used the conventional (fully aerated) process [42]. Thus, it is reasonable to assume that uSB-COD was also degraded by subsequent reactions, immediately taken up by the attached biomass, such as Comamonadaceae and Rhodocyclaceae, during recirculation, and then degraded by consuming either DO or gaseous oxygen, depending on the recirculation conditions.

The proposed mechanisms and dominant bacterial groups should have been affected by the features of the experimental sewer system, including the sponge media, separator, and intermittent wastewater supply. However, these features also apply to specific, but not rare conditions or environments, in conventional sewers considering that sediments and the sewer wall, can serve as attached media [43] and that fluctuations in flow levels and organic loads are inevitable, even in separated sewers. Although it has been recognized that the sequential removal and oxidation of organic matter, especially nonoxidative removal, can serve as aerobic wastewater treatment [44], very few studies have focused on this mechanism in sewers. However, it was assumed that a very high yield value (0.79 g-biomass/g-acetate, corresponding to over ca. 0.9 g-COD/g-COD) observed during aerobic acetate consumption by a sewer biofilm indicated luxury uptake as storage products [16]. The contribution of such nonoxidative organic matter removal should be evaluated to better understand the transformation of wastewater characteristics, including self-purification, along sewers.

CONCLUSIONS

The contributions of attached and suspended biomass to self-purification in a sewer were investigated by recirculating and aerating batch tests. From the changes in the wastewater characteristics and microbial community during the tests, the following conclusions were obtained.

1) The contribution of the attached biomass was much larger than that of the suspended biomass, as only the attached biomass was sufficiently acclimated to organic matter.

2) The attached biomass enabled self-purification, even when they were temporarily exposed to air rather than wastewater, suggesting that, once organic matter was captured or stored by the attached biomass, it could be oxidized regardless of flow conditions.

3) Comamonadaceae and Rhodocyclaceae, which are reportedly capable of storing organic matter, were responsible for self-purification as they constituted a significant proportion of the attached biomass. However, these families could not proliferate under suspended conditions.

4) A small part of the suspended biomass, containing the Pseudomonadaceae family of the Gammaproteobacteria, could contribute to self-purification after a lag period of several hours.

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