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Thiothrix eikelboomii interferes oxygen transfer in activated sludge

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ABSTRACT

This study revealed that, *Thiothrix eikelboomii*, a well-known filamentous bacterium that causes sludge bulking, could also interfere oxygen transfer during wastewater treatment. The volumetric oxygen transfer coefficient (K_La) in filamentous-bulking sludge (FBS) was found to be 43% lower than that in floc-forming sludge (FFS) at similar biomass concentrations, partially because the filamentous bacteria had increased the sludge apparent viscosity. The K_La value for FBS, however, was still significantly lower than that for FFS even if both sludges had similar apparent viscosity. Numerous tiny and free-swimming filaments were observed to attach on the air bubble surface, presumably reducing the liquid film renewal and increasing the liquid film thickness. Moreover, the filaments were co-coated with extracellular polymeric substances of protein and polysaccharide, which could make them performing like "amphiphilic molecules" of surfactants to hinder oxygen transfer. Therefore, the particular surface property of filaments and their interaction with air bubbles could also impact oxygen transfer. *Thiothrix eikelboomii* was identified to be the responsible filamentous bacterium that lowered the K_La value, while other filamentous bacteria with short filaments did not interfere oxygen transfer. This study implies that controlling sludge bulking benefits not only sludge settling but also oxygen transfer.

1. Introduction

Aeration is needed to provide sufficient oxygen for microbes during aerobic wastewater treatment. The low solubility of oxygen in water makes aeration an energy intensive process. Currently, more than 50% of the energy consumption in wastewater treatment plants is used for aeration (Henriques and Catarino, 2017; Li et al., 2017). Therefore, improving oxygen transfer efficiency (OTE) is critically important for improving wastewater treatment sustainability (Daigger and Boltz, 2018). The effects of mixed liquor suspended solid (MLSS) concentration, surfactants, mixing intensity, salts, temperature, and viscosity on the oxygen transfer from air to water have been intensively studied (Baquero-Rodríguez et al., 2018; Germain et al., 2007; Nittami et al., 2013; Painmanakul et al., 2005; Vogelaar et al., 2000). However, very little attention has been paid to the impact of microbial community in activated sludge on the oxygen transfer.

During our previous study on nitrification under low dissolved oxygen (DO) conditions, we observed that the OTE in the activated sludge reactor decreased by approximately 50% when filamentous bulking occurred (Liu et al., 2018). Filamentous microorganisms were hypothesized to be the troublemakers. Unfortunately, the mechanisms behind this phenomenon were not clear. Filamentous microorganisms occur in the activated sludge process commonly, which are notorious for decreasing sludge settling ability. However, their effect on the oxygen transfer has been rarely reported. The significant decrease in the OTE along with the occurrence of filamentous bulking could result from the dissolved contents and/or the sludge solids. The shift in the dominant microbes in activated sludge could change the type and concentration of soluble microbial products (SMPs). The SMPs, e.g., biosurfactants, could impact the oxygen transfer significantly (Capodici et al., 2015). Filamentous microorganisms have long filaments, which may physically change the activated sludge rheological properties, e.g., increasing the apparent viscosity. Moreover, filamentous microorganisms could have different cell surface properties due to higher production of extracellular polymeric substances (EPS), which may directly or

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indirectly impact the oxygen transfer (Germain and Stephenson, 2005; Meng et al., 2006; Wagner et al., 2015).

Previous studies noticed a new issue associated with filamentous bulking in term of oxygen transfer. Unfortunately, this issue has not been well studied. In this study, the oxygen transfer characteristics, SMP production, EPS content and distribution, apparent viscosity, and microbial communities in the filamentous bulking sludge (FBS) and floc-forming sludge (FFS) were comparatively investigated. Our goals were to (1) further characterize the interference of filamentous microorganism on oxygen transfer; (2) explore the major mechanisms; and (3) identify the type of filamentous microorganism that will interfere the oxygen transfer.

2. Materials and methods

2.1. Activated sludge samples

Sludge samples for FBS and FFS were respectively collected from two pilot-scale dynamic membrane bioreactors, which were fed with simulated domestic wastewater. The effluent COD, turbidity, and ammonia for both reactors were below 20 mg/L, 1 Nephelometric Turbidity Unit (NTU), and 1 mg/L, respectively. The activated sludge in one reactor had very good settling ability with sludge volume index (SVI) of less than 80 mL/g-MLSS, while the sludge in the other reactor had poor settling ability with a SVI value of greater than 200 mL/g-MLSS. The MLSS concentrations for FBS and FFS in the corresponding bioreactors were approximately 1800 mg/L and 3200 mg/L, respectively. More operation information for both reactors are given in Table S1. Filamentous microorganisms were present in the FBS sludge (Fig. 1), which was observed using an inverted fluorescence microscope (Olympus-IX53, Tokyo, Japan).

2.2. Oxygen transfer tests

The oxygen transfer characteristics (i.e., the overall volumetric oxygen transfer coefficient, K_La, and α-factor) for both sludge samples were determined using a dynamic testing method (Metcalf and Eddy, 2003). To evaluate the effect of the soluble substrates, e.g., SMPs, the oxygen transfer tests were also conducted for the effluent from both reactors and the tap water.

All of the oxygen transfer tests were conducted in measuring cylinders with an effective volume of 2.5 L (87 mm in diameter and 475 mm in height). A fine bubble diffuser was placed at the bottom of the measuring cylinder for aeration. When testing the oxygen transfer in activated sludge, the samples were aerated sufficiently in advance to remove the residual soluble COD and ammonium. After that, the decrease in the DO concentration along with time were recorded using a YSI 550A DO meter. Since the activated sludge was in endogenous respiration phase, the oxygen uptake rate was a constant value, which could be determined based on linear regression. When the DO concentration became below 1.0 mg/L, aeration at a fixed airflow rate started, and the change in the DO concentration as a function of time was recorded. During the oxygen transfer tests for the effluent and tap water, moderated amount of sodium sulfite and cobalt chloride were initially added to deplete the DO in the water before aeration. The solution temperature was also recorded for each test, which was used to correct the effect of temperature on the K_La values.

The oxygen transfer characteristics for both types of sludge were firstly tested at a similar MLSS of approximately 1800 mg/L under different volumetric airflow rates of 4.8, 9.6, and 19.2 L-air/L-water·h. In addition, the oxygen transfer characteristics in both sludge samples were tested under different MLSS concentrations between 900 and 8000 mg/L for the same aeration intensity of 9.6 L-air/L-water·h. In these tests, the sludge samples were diluted with bioreactor effluent or concentrated by gravity settling or membrane filtration. The K_La values for tape water under different airflow rates were also tested to estimate the α-factors in the activated sludge and reactor effluents. The equations used to determine the $K_La_{(20)}$ values and α-factors were described in the Supporting Information. The K_La values determined in the measuring cylinders were not referred to the ordinary hydraulic depth in the real wastewater treatment processes. However, they were effective to compare the oxygen transfer property in FBS and FFS.

2.3. Apparent viscosity measurement

The apparent viscosity of both sludge samples with various MLSS concentrations from 900 to 8000 mg/L and the effluents from both reactors were measured by a rotational viscometer (NDJ-5S, Bonsai Instrument Technology Co., Ltd, Shanghai, China) with spindle #0. The measurement was conducted under a room temperature of approximately 22 °C. During the test, the spindle was inserted into a breaker (114 mm in diameter and 155 mm in height) that contained one litter sample. Before measurement, the level of the viscometer was adjusted to ensure that the instrument was in horizontal condition and the spindle mark was parallel with the liquid level of the test sample. According to the operation manual (Table S2), a rotating speed of 60 rmp was used when the measured viscosity was less than 10 mPa s. When it was between 20 and 50 mPa s, a rotating speed of 12 rmp was used. The shear rate was proportional to rotating speed. According to the correlation between the shear rate and the rotating speed provided by the manufacture and reference (Nittami et al., 2013), the shear rates were 61.8 s^{-1} and 12.4 s^{-1} at the rotating speeds of 60 and 12 rpm, respectively. Each measurement was conducted in triplicates.

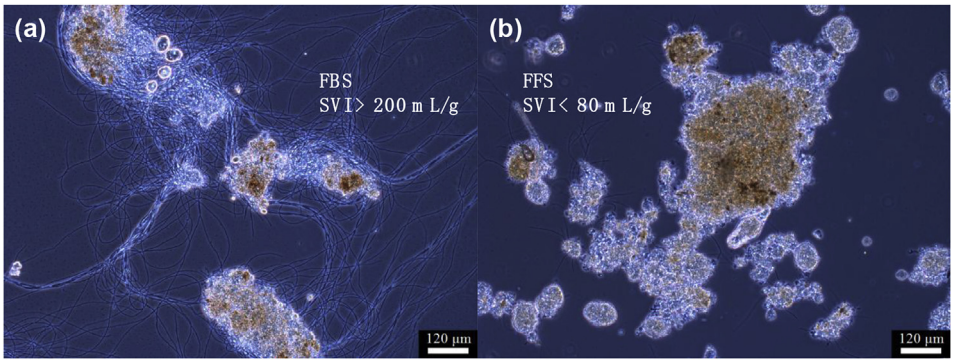

Fig. 1. Representative microscope images for (a) filamentous-bulking sludge (FBS) and (b) floc-forming sludge (FFS).

Because the activated sludge with a solids concentration above approximately 2% exhibits non-Newtonian behavior (Eftekharzadeh et al., 2007), the measured viscosity of sludge samples represented the apparent viscosity (μ_{ap}).

2.4. SMP and TB-EPS analysis

The contents of SMP and tight-bound extracellular polymer substances (TB-EPS) in both FBS and FFS were measured. Heat treatment method was used to extract EPS, which was recognized as one of the most effective methods with less disruption to cells (Chang and Lee, 1998). The detailed extraction protocols was described previously (Ding et al., 2015). In brief, the activated sludge samples were firstly centrifuged at 4000 rpm for 15 min. The supernatant was then taken out for SMP analysis. The remaining biomass was re-suspended with saline water (0.9% NaCl solution) and then centrifuged at 4000 rpm for another 15 min. After discharging the supernatant, the left biomass was re-suspended again with 0.9% NaCl solution and heated in a water bath at 80 °C for 30 min. Finally, the mixed liquor was centrifuged again at 4000 rpm for 15 min and the supernatant was used for TB-EPS analysis after filtration by a 0.45 μm membrane.

In this study, the contents of SMP and EPS were normalized as the sum of carbohydrate and protein since they are the main components in both SMP and EPS (Liu and Fang, 2002). The carbohydrate in SMP and EPS was measured using the H_2SO_4/Anthranone oxidation method with glucose as standard (Dubois et al., 1956). The protein was determined by using a modified Lowry method with BSA (bovine serum albumin, Sigma fraction V, 96%) as the standard (Frolund et al., 1996).

2.5. Confocal laser scanning microscopy (CLSM) analysis

Fluorescein isothiocyanate (FITC), Concanavalin A (ConA), and Calcofluor white (CW) were used to stain and probe the proteins, α-D-glucopyranose polysaccharides, and β-D-glucopyranose polysaccharides, respectively (Yu et al., 2011; Yuan et al., 2015). Both types of activated sludge were washed with phosphate-buffered saline (PBS) solution to remove SMP. Then 50 μL of mixed liquor was placed in a 200 μL centrifuge tube. The sludge sample was first stained with 50 μL of FITC solution (10 g/L) for 30 min, before which a bit of sodium bicarbonate (1 M) buffer was added to maintain pH of 9, and to keep the amine group in a non-protonated form. Next, 50 μL of Con A solution (0.25 g/L) was added into the centrifuge tube and incubate for 30 min again. Finally, 50 μL of Calcofluor white (0.3 g/L) was added and incubate for another 30 min. After each staining step, PBS solution was utilized to wash the labeled sample several times to remove the extra probes.

A scanning microscopy (CLSM, Zeiss LSM880, Germany) was applied to capture the structure of sludge samples and the distribution of EPS components. The specific operating conditions and parameters are shown as Table S1. The fluorescence intensity of different contents was analyzed and calculated using Image Pro-Plus 6.0 and ZEISS confocal software (ZEN 2.0).

2.6. Observing the contact of sludge floc with air bubble

It will be ideal to directly observe the interaction between the floc and the rising air bubbles. Unfortunately, a suitable approach was not found. In this study, the contact of sludge floc with air bubble was observed under a motionless condition. A NEST glass bottom dish was used to hold the mixed liquor. The external diameter was 35 mm and the bottom dish had a diameter of 15 mm. When the bottom dish was filled with the mixed liquor, an air bubble (3–5 mm in diameter) was developed using a pipette. Then a coverslip was used to cover the bottom dish and trap the air bubble inside the mixed liquor. Then the contact of sludge floc with the air bubble in FBS or FFS was observed by an inverted fluorescence microscope (Olympus-IX53, Tokyo, Japan).

2.7. Microbial community analysis

The bacterial community for each sludge was analyzed in duplicate. Considering some filamentous microorganisms may be fungi, the fungal community was also characterized based on the ITS1 region. Power soil DNA Isolation kit (Anbiosic, Shenzhen, China) was used to extract the DNA. The quality and quantity of the DNA were checked using gel electrophoresis and Nanodrop-2000 micro-spectrophotometry (Thermo Scientific, USA), respectively. Subsequently, the extracted DNA was used as a template for polymerase chain reaction (PCR) amplification. The hypervariable V3–V4 region of the bacterial 16S rRNA genes were amplified using the universal primers of 338F and 806R. The ITS1 region of 18S rRNA genes were amplified with primers of ITS1F and ITS2 (Lindahl et al., 2013; Nilsson et al., 2009; Xu et al., 2017; Zhou et al., 2017). PCR amplification for both 16S and 18S were conducted in a Veriti FAST (ABPCR, USA) under the following condition: initial denaturation at 95 °C for 5 min; 25 cycles at 95 °C for 30 s, 50 °C for 30 s and 72 °C for 3 replicates 40 s; and final extension at 72 °C for 7 min. After purification using AMPure XP beads (Vazyme, Nanjing, China), the PCR amplicons were amplified for another round. Finally, equal concentrations of purified DNA fragment for each sample were sequenced by the Illumina Hiseq 2500 platform, which was conducted by Biomarker Technologies (Beijing, China).

After Hiseq sequencing, the reads for each sample were merged using FLASH software, version 1.2.7 (Magoc and Salzberg, 2011) to obtain raw tags. Then the raw tags were purified by filtering out the low-quality or ambiguous sequences using Trimmomatic software, version 0.33 (Bolger et al., 2014) and obtain the clean tags. Finally, UCHIME software, version 4.2 (Edgar et al., 2011) was used to authenticate and exclude chimeras sequences to get effective tags for further analyses. The effective tags were clustered into operational taxonomic units (OTUs) at a similarity threshold of 97% using UCLUST methods (Edgar, 2010) embraced in QIIME (Caporaso et al., 2010). The taxonomic classification of OTUs was assigned based on the Ribosomal Database Project (RDP) classifier with SILVA and UNITE databases for bacterial and eukaryotic communities, respectively. The data for the abundance of bacteria and fungi at different levels were processed on the BMK cloud online platform (Biomarker Technologies, Beijing, China).

3. Results and discussion

3.1. Oxygen transfer characteristics in FBS and FFS

Two membrane bioreactors were operated in our laboratory. In one reactor, the OTE decreased abruptly without any changes in the aeration conditions. Meanwhile, the sludge settling ability became poor with SVI values of greater than 200 mL/g-MLSS. Excessive filamentous microorganisms were found in this activated sludge (Fig. 1(a)). The OTE in the other reactor kept normal and the sludge settled well with SVI value of less than 80 mL/g-MLSS. Therefore, we inferred that the decreasing OTE was associated with the filamentous bulking.

As expected (Fig. 2(a)), both activated sludges had poorer oxygen transfer characteristics than the tape water and effluent did, suggesting that the activated sludge would hinder oxygen transfer during aeration. At a similar MLSS concentration of approximately 1800 mg/L, however, the DO concentration increased significantly faster in the FFS than that in the FBS. The $K_La_{(20)}$ values for FBS and

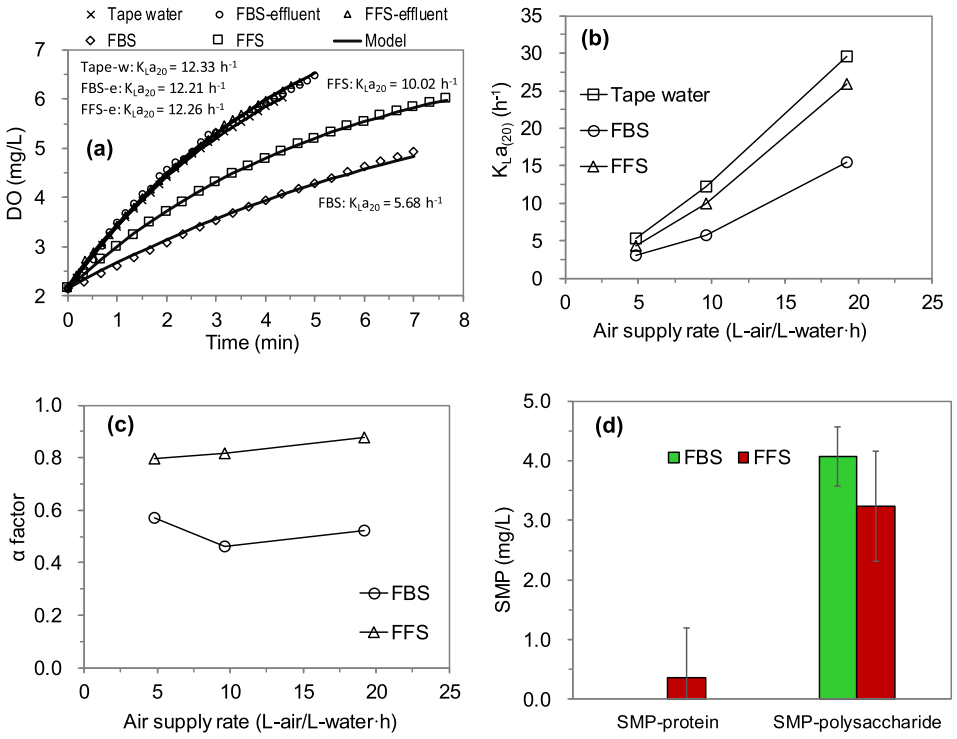

Fig. 2. (a) The DO concentration changes during the oxygen transfer batch tests for the filamentous bulking sludge (FBS), floc-forming sludge (FFS), FBS effluent, FFS effluent, and tape water under the same air supply rate of 9.6 L-air/L-water·h; (b) The estimated K_La values and (c) α factors at 20 °C for FBS and FFS under different aeration intensity (The MLSS concentrations for FBS and FFS were approximately 1800 mg/L). (d) Soluble microbial products (SMPs) in FBS and FFS (The SMP-protein for FBS was under detection limit).

FFS were determined to be 5.68 and 10.02 h^{-1}, respectively. This indicates that the overall oxygen mass transfer rate in the FBS was 43.3% lower than that in the FFS under the same aeration condition. Fig. 2(b) shows that increasing the volumetric airflow rate had significantly increased the $K_La_{(20)}$ values for both sludges. However, the $K_La_{(20)}$ values and α-factors in FBS were always lower than those in FFS under the same aeration intensity (Fig. 2(a) and (c)). This confirmed that the FBS with excessive filamentous microorganisms had much worse oxygen transfer property than FFS did.

3.2. Effect of dissolved contents on oxygen transfer

The differences in the oxygen transfer characteristics in FBS and FFS could result from the dissolved contents produced by the microbes and/or the biomass solids. As shown in Fig. 2(a), both reactor effluents had very similar $K_La_{(20)}$ values with tap water, suggesting that the dissolved contents in both sludge samples had insignificant impact on the oxygen transfer. SMPs, mainly including protein and polysaccharide, are the major soluble constituents for well-treated effluent (Barker and Stuckey, 1999; Wang and Zhang, 2010; Xie et al., 2012). Their combined concentrations in both reactor effluents were similar and less than 5 mg/L (Fig. 2(d)), which were too low to pose negative impact on oxygen transfer (Wei et al., 2015). Though the biosurfactants were not measured, the similar $K_La_{(20)}$ values in both effluents and the tape water suggested that the microbes in both sludge samples did not synthesize sufficient biosurfactants that could negatively impact the oxygen transfer. These results suggested that the differences in the oxygen transfer characteristics for FFS and FBS mainly resulted from the biomass solids.

3.3. Effect of biomass solids on oxygen transfer

The FBS and FFS sludge with MLSS concentrations ranging from 900 to 7000 mg/L (possible concentrations in municipal sewage plants) were examined to explore the correlation between MLSS concentration and oxygen transfer characteristic ($K_La_{(20)}$ and α-factor). For both sludges, the values of $K_La_{(20)}$ (and so as the α-factor) at MLSS greater than 3000 mg/L were significantly lower than those at MLSS less than 2000 mg/L. It was reported that an exponential correlation could be used to describe the $K_La_{(20)}$ value or α-factor as a function of MLSS concentration for the same type of activated sludge with an extended MLSS range from 5000 to 30,000 mg/L (Germain et al., 2007). For the data presented in Fig. 3, obviously, one exponential-like equation could not fit the $K_La_{(20)}$ data for both FBS and FFS. Under a similar MLSS concentration, the FBS always had significantly lower $K_La_{(20)}$ values than FFS did. This suggested that, in addition to biomass concentration, other properties of biomass, e.g., microbial types, floc morphology, apparent viscosity, and biomass surface properties, might have impacted the oxygen transfer.

A few exponential correlations between α-factor and MLSS concentration had been reported (Germain et al., 2007; Günder, 2001; Krampe and Krauth, 2003; Muller et al., 1995; Rosenberger, 2003). Their correlations, however, varied significantly from case to case (Fig. S1). These variations could partially result from the differences in testing conditions. The aeration intensity, diffuser types, reactor dimension, and mixing intensity could impact the measured K_La values. Even with the same testing conditions, inconsistent relationships between α-factor and MLSS concentration were observed for the sludge from different sources (Nittami et al., 2013). Henkel (2010) proposed that the difference in α-factor at a given MLSS concentration could be due to the difference of sludge characteristics and origins. In conclusion, both literature reviews and this study suggested that, in addition to MLSS concentration, other properties of the activated sludge, e.g., the presence of filamentous microorganisms, also had a great impact on the

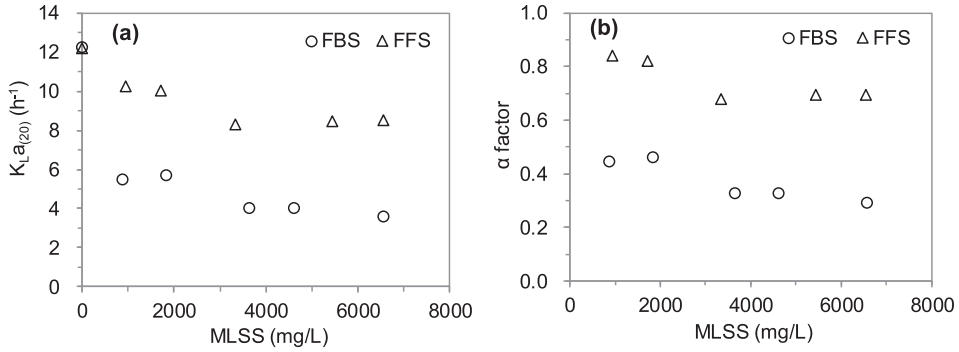

Fig. 3. Correlations of (a) $K_La_{(20)}$ and (b) α factor with the MLSS concentrations for filamentous bulking sludge (FBS) or floc-forming sludge (FFS), air supply rate = 9.6 L-air/L-water·h.

oxygen transfer.

3.4. Correlations of apparent viscosity with MLSS and oxygen transfer

The results shown in Figs. 2 and 3 evidenced that the FBS with excessive filamentous microorganisms had significantly poorer oxygen transfer characteristics than FFS did. Our previous study additionally observed that the OTE in the reactor decreased with increasing density of filamentous microorganisms (Liu et al., 2018). Therefore, the occurrence of filamentous microorganisms in FBS had interfered the oxygen transfer.

The filamentous microorganisms in FBS had long filaments (Fig. 1), which could impact the oxygen transfer by changing the apparent viscosity of the activated sludge. Therefore, the apparent viscosity of FBS and FFS under various MLSS concentrations were measured, with results shown in Fig. 4(a). Under similar MLSS concentrations, the apparent viscosity of FBS was significantly greater than that of FFS. Moreover, when the MLSS concentration was greater than 3000 mg/L, the apparent viscosity of FBS increased exponentially. However, the effluents from both reactors had very similar apparent viscosity with tap water, indicating that the dissolved contents in the mixed liquor did not impact the apparent viscosity. Therefore, the filamentous microorganisms in FBS had enhanced the apparent viscosity of the mixed liquor.

The K_La value decreased with increasing viscosity, which has been well documented (Badino et al., 2001; García-Ochoa et al., 2000; Özbek and Gayik, 2001). A greater apparent viscosity will lead to greater air bubbles, which significantly reduce the specific interfacial area for oxygen transfer (Amaral et al., 2017; Badino et al., 2001; García-Ochoa et al., 2000; Martín et al., 2010; Özbek and Gayik, 2001). The increase in the apparent viscosity would

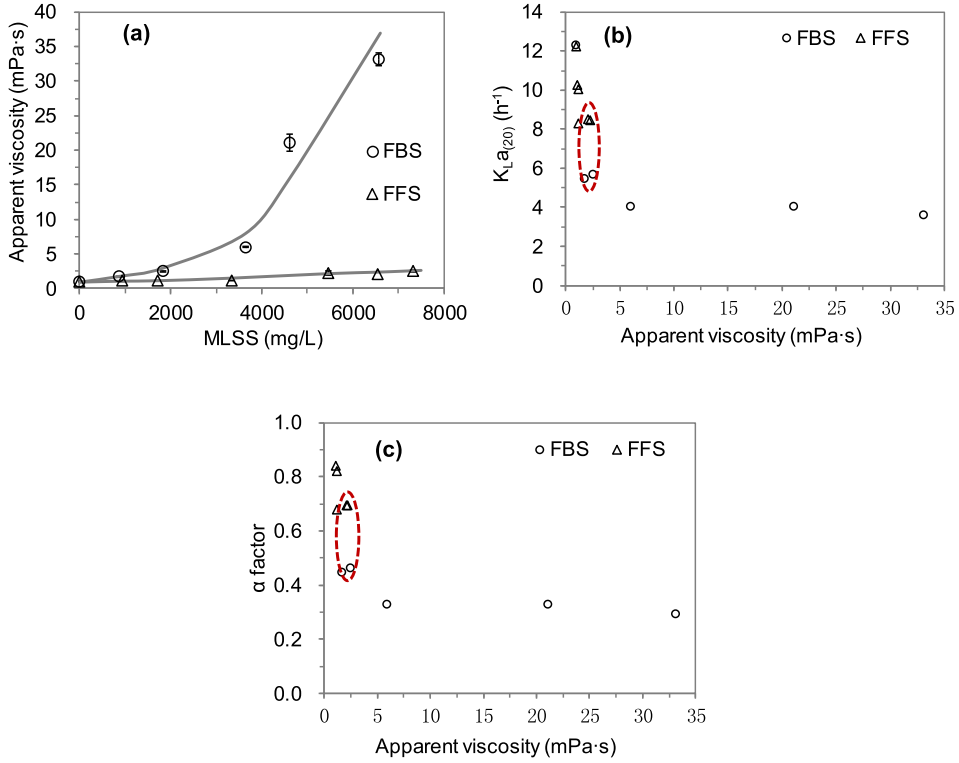

Fig. 4. (a) Correlation of apparent viscosity with the MLSS concentration for filamentous bulking sludge (FBS) or floc-forming sludge (FFS) (when the apparent viscosity was < 10 mPa s, a shear rate of 61.8 s^{-1} was used; when it was between 20 and 50 mPa s, a shear rate of 12.4 s^{-1} was used); Correlations of (b) $K_La_{(20)}$ and (c) α factor tested under the air supply rate of 9.6 L-air/L-water·h with the apparent viscosity of FBS or FFS.

reduce the liquid film renewal by reducing the turbulence induced by rising air bubbles or stirrers, which negatively impact the oxygen diffusion at the air-liquid interface. On the other hand, the increasing apparent viscosity could promote oxygen transfer by reducing the bubble rising velocity due to a higher bubbles drag coefficient (Durán et al., 2016; Mena et al., 2005). However, this positive contribution could not offset the negative effects of increasing apparent viscosity on oxygen transfer and, as result, the K_La value decreased with increasing apparent viscosity. Therefore, filamentous microorganisms could hinder oxygen transfer by enhancing the apparent viscosity of activated sludge.

Unlike the relationship of $K_La_{(20)}$ value (or α-factor) with MLSS presented in Fig. 3, a consistency existed between apparent viscosity and $K_La_{(20)}$ value for both sludge samples, as shown by Fig. 4(b). When the apparent viscosity was less than 5 mPa s, the $K_La_{(20)}$ values (and so as α-factors) decreased rapidly with increasing apparent viscosity. Further increasing the apparent viscosity of activated sludge had less impact on the $K_La_{(20)}$ values or α factors. This exponential-like correlation was in agreement with some other studies (Günder, 2001; Krampe and Krauth, 2003; Nittami et al., 2013). Fig. 4(b) also indicated that the apparent viscosity, instead of MLSS concentration, was the dominant factor that impacts the oxygen transfer in activated sludge. Undeniably, a greater MLSS concentration could enhance the apparent viscosity of the mixed liquor. The presence of excessive filamentous microorganism, however, would additionally enhance the apparent viscosity.

3.5. EPS distribution and contact of floc with air bubble

The FFS with MLSS concentrations of 5500–6500 mg/L (apparent viscosity = 2.09–2.20 mPa s) had a similar apparent viscosity with FBS at MLSS of 800–1800 mg/L (apparent viscosity = 1.7–2.53 mPa s). Under a similar apparent viscosity, the $K_La_{(20)}$ value for FBS was still significantly lower than that in FFS, which was marked by a red oval in Fig. 4(b). This indicated that, in addition

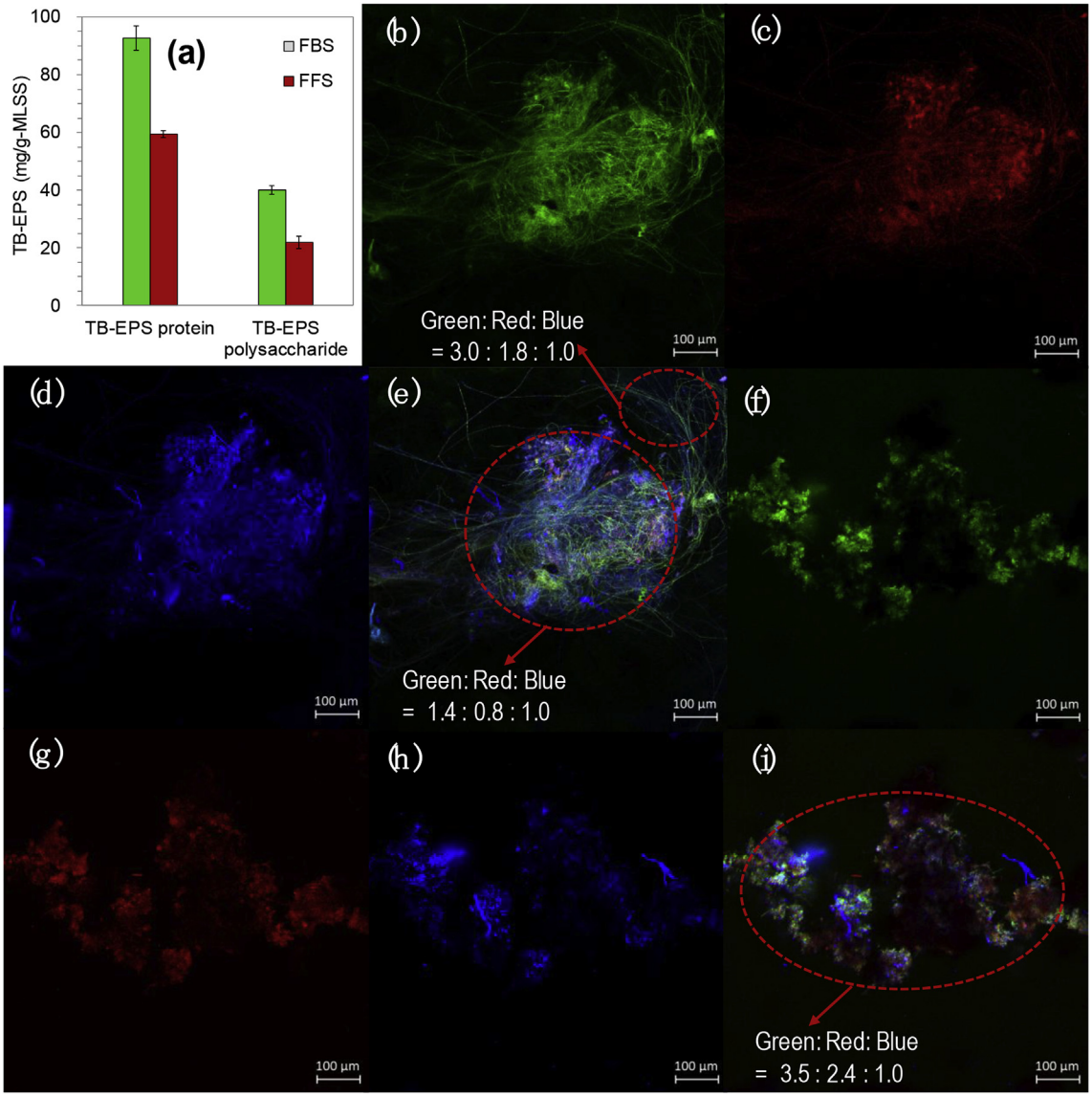

Fig. 5. (a) Contents of tightly bound extracellular polymeric substances (TB-EPS) in filamentous bulking sludge (FBS) and floc-forming sludge (FFS); confocal laser scanning microscopy (CLSM) images of FBS for (b) proteins (FITC), (c) α-polysaccharides (calcofluor white), (d)β-polysaccharides (Con A), and (e) integrated picture; and CLSM images of FBS for (f) proteins (FITC), (g) α-polysaccharides (calcofluor white), (h)β-polysaccharides (Con A), and (i) integrated picture. The ratio of Green: Red: Blue represents the averaged fluorescence intensity for different EPS components. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

to increasing the apparent viscosity, the filamentous microorganism also interfered the oxygen transfer with other pathways.

Proteins and carbohydrates are the main components of EPS, which make up the largest fraction of sludge floc. They locate at or outside the cell surface (Wang et al., 2014; Xu et al., 2013; Zhu et al., 2015). Their presence could significantly impact biomass surface properties and flocculability (Liu et al., 2010; Yu et al., 2009). As shown in Fig. 5(a), in the FBS with poor oxygen transfer, both the contents of EPS-protein and EPS-polysaccharide were almost doubled compared to those in FFS. Moreover, the contents of protein in both sludge samples were significantly greater than polysaccharide. This indicated that the filamentous bulking sludge could produce more EPS than FFS did, which was in agreement with other studies (Germain et al., 2007; Meng et al., 2006; Wagner et al., 2015). A negative correlation between α-factor and the content of EPS in sludge was also proposed by some studies (Fan et al., 2017; Ferreira et al., 2010).

Fig. 5(b–i) present the distribution of EPS components in the sludge floc for both type of sludge. In the core areas of FBS flocs where both filaments and regular bacteria were co-existed, the fluorescence intensity ratio for protein/α-polysaccharides/β-polysaccharides was equal to 1.4/0.8/1.0. Fig. 5(b–d) and the enlarged CLSM images (Fig. S2) clearly shows that the filaments of filamentous microorganisms were also coated by EPS. On the swimming-free filaments (extending from the floc), that ratio was 3.0/1.8/1.0, suggesting that they were mainly coated by protein and α-polysaccharides. For the FFS, the three major EPS components are also co-existed (Fig. 5(i)) and the fluorescence intensity of protein and α-polysaccharide were significantly greater than that of β-polysaccharide. Some studies suggested that EPS-polysaccharide are solely hydrophilic substances, while the EPS-protein was believed to be more hydrophobic (Liu and Fang, 2003; Xie et al., 2010). The co-coated protein and polysaccharides might have made the massive free-swinging filaments acting like “amphiphilic molecules”, e.g., surfactants.

Fig. 6(a) shows that numerous filaments directly attached on the air-water interface. The filaments were too tiny and soft to impale the air bubble. The wide distribution and great hydrodynamic volume of filaments in FBS made the attachment easier. In addition, the coated proteins could facilitate the attachment of filaments on

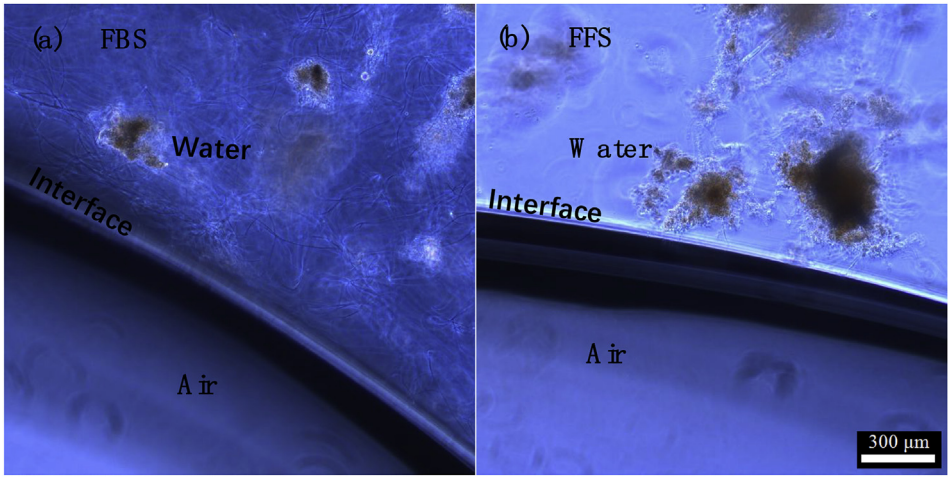

Fig. 6. Contact of sludge floc and air bubble in (a) filamentous bulking sludge (FBS) and (b) floc-forming sludge (FFS) under static conditions.

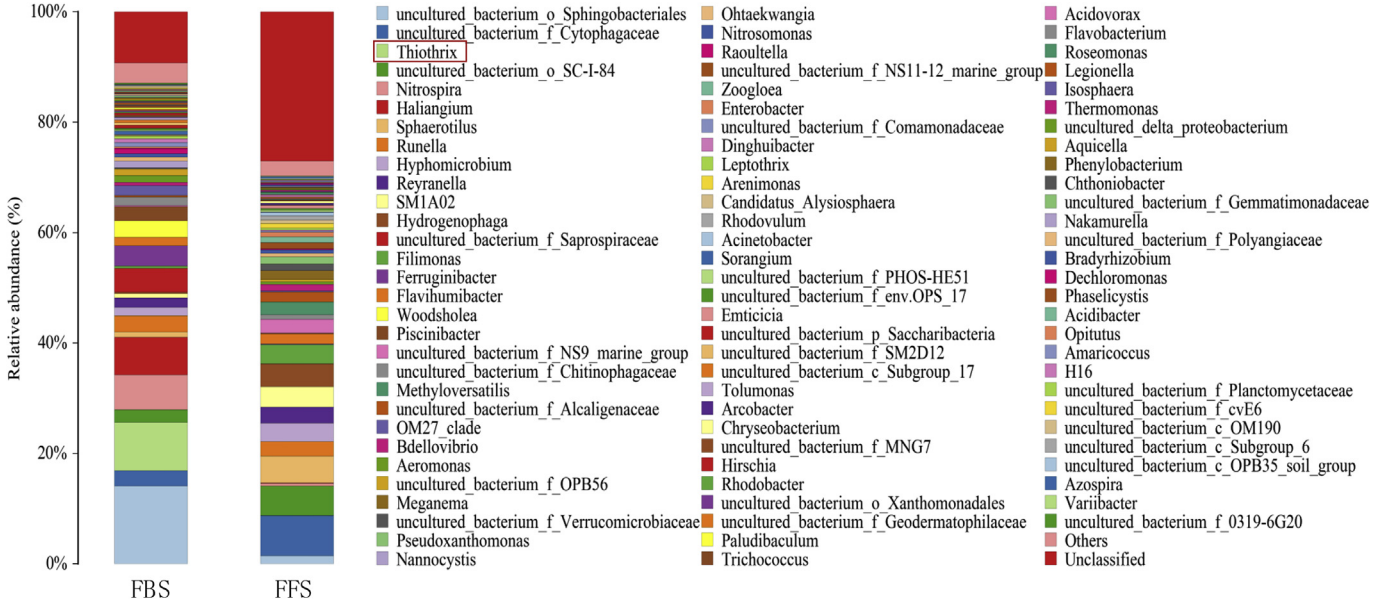

Fig. 7. Relative abundance for the top 90 bacterial genera in the filamentous bulking sludge (FBS) and floc-forming sludge (FFS).

the interface as well, because hydrophobic substances are more likely to attach to the air bubble surface compared to hydrophilic substances. This suggests that, during aeration, the very long but tiny filaments can attach on the gas-liquid interface, which will reduce the liquid film renewal, increase the liquid film thickness, and directly block the oxygen mass transfer. Then the liquid film mass transfer coefficient (K_L) will be reduced. In FFS, the floc had a certain shape and only one side or angle could touch the interface of air bubble, as shown Fig. 6(b). As a result, the specific contact area between floc and air bubble in FFS is much smaller than that in FBS.

The motionless observation only represented a specific moment for the contact of sludge flocs with air bubble. The changes in the bubble size, bubble shape, and the dynamic interaction between air bubbles and flocs during aeration, however, were not observed. It will be ideal to directly observe the interaction between the swimming-free filaments and the rising air bubbles in the bulk solution. Unfortunately, a suitable approach was not found. Moreover, no references were available on the effect of interfacial properties of sludge floc, e.g., hydrophilicity and hydrophobicity, on oxygen transfer and the interaction between sludge floc and air bubble. This study suggested that, in addition to interfering oxygen transfer by enhancing the apparent viscosity, the direct interaction between the filaments and air bubbles could also play a significant role in oxygen transfer. However, more studies are needed to further explore the effect of biomass surface properties on the oxygen transfer.

3.6. Microbial communities

As shown in Fig. 7, though both type of sludges shared many bacterial genera, their dominant genera were different. The uncultured bacteria, *Cytophagaceae* and *Sphingobacteriales,* were the most dominant genera in FFS and FBS, respectively. Both genera are commonly found in wastewater treatment systems. With a relative abundance of greater than 3%, these two sludges did not share any bacterial genus. According to the database for bulking bacteria summarized by Nielsen et al. (2009) and Guo and Zhang (2012), ten related filamentous genera with relative abundance of greater than 0.1% were identified in both samples, which included *Thiothrix, Sphaerotilus*, *Runella*, *Meganema*, *Leptothrix*, *Chryseobacterium*, *Trichococcus*, *Flavobacterium*, *Isosphaera*, and *Planctomycetaceae*. As shown in Fig. 8, FBS was dominated by *Thiothrix* with a high relative abundance greater than 8.5%, while *Thiothrix* was nearly not detected in FFS. Both *Sphaerotilus* and *Runella* had a relatively high abundance in FBS, while they both also existed in FFS with a relatively high abundance of 4.9% and 2.6%, respectively. Fortunately, their presence did not significantly impact the sludge settling, apparent viscosity, and oxygen transfer efficiency in FFS. This might because both *Sphaerotilus* and *Runella* had short filaments that would not pose negative impact on the sludge settling ability and apparent viscosity. Alternatively, their short filaments could work as bones to form large floc (Fig. 1). *Thiothrix* in FBS, however, had very long filaments (0.5–1.5 μm in diameter and a few millimeters in length), which could bridge the flocs. As a result, the sludge settling ability decreased and the apparent viscosity increased. Therefore, not all of filamentous bacteria had negative effects on the sludge settling ability and apparent viscosity. The relative abundances for other filamentous bacteria were less than 0.6% and might not have a significant impact on sludge properties. The high-throughput sequencing analysis for eukaryotic community indicated that there were only a few non-filamentous fungi existed in both FBS and FFS (data not shown). This indicated that the filamentous microorganisms in the experimental sludge were bacteria. Consequently, we can infer that *Thiothrix* was the major filamentous genus in FBS that had caused significantly negative impact on the oxygen transfer.

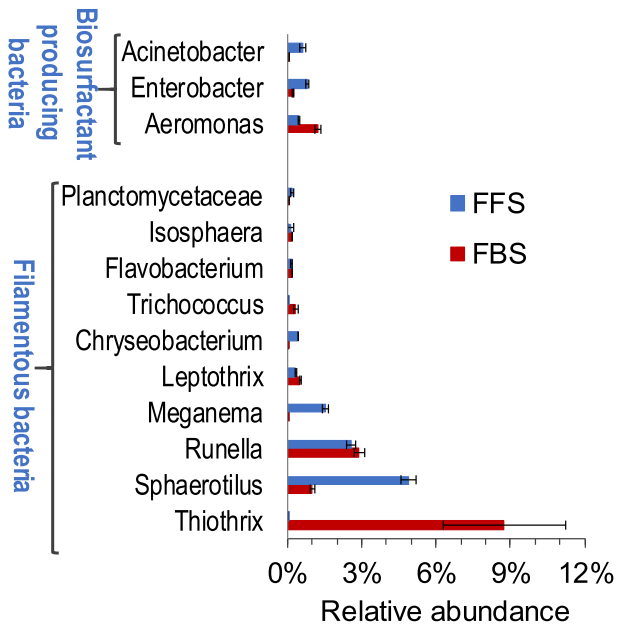

Fig. 8. Relative abundance for filamentous bacteria and biosurfactant producing bacteria in the filamentous bulking sludge (FBS) and floc-forming sludge (FFS).

Based on diversity analysis at specie level (Fig. S3), *Thiothrix eikelboomii* was identified as the predominant species. *Thiothrix eikelboomii* is a member of Eikelboom Type 021N bacteria (Aruga et al., 2002; Nielsen et al., 2000). Unlike other *Thiothrix* ssp., e.g., *T. nivea* JP2^T and *T. unzii* A1^T, they can grow heterotrophically without requiring reduced sulfur (Aruga et al., 2002). Therefore, *Thiothrix eikelboomii* was frequently found in the bulking sludge of municipal wastewater treatment process and some other *Thiothrix* ssp. were more frequently found in the industrial wastewater treatment systems (Aruga et al., 2002; Guo and Zhang, 2012; Howarth et al., 1999). Previously, they are well-known for decreasing sludge settling property. However, no reports are found about their impact on the apparent viscosity and oxygen transfer in activated sludge. Other *Thiothrix* ssp. may also have negative impact on the apparent viscosity and oxygen transfer since they have similar morphology with *Thiothrix eikelboomii* (Aruga et al., 2002). In addition to *Thiothrix* species, another popular filamentous bacterium in activated sludge that has long filaments, i.e., *Microthrix parvicella*, may also impact the oxygen transfer negatively (Wagner et al., 2015).

More than 30 typical biosurfactant producing microbes were summarized by Shekhar et al. (2015) and Ndlovu et al. (2016). Only three types of biosurfactant producing microbes, i.e., *Aeromonas*, *Enterobacter*, and *Acinetobacter*, were detected in our experimental sludge (Fig. 8). However, their relative abundances in FBS were low, 1.23%, 0.24%, and 0.08%, respectively. In addition, the relative abundances of *Enterobacter* and *Acinetobacter* in FBS were even significantly lower than those in FFS (0.81% and 0.63%, respectively). The low abundances of biosurfactant producing bacteria might not produce sufficient biosurfactants to impact the oxygen transfer. Therefore, community analysis also supported that biosurfactants were not the troublemakers for the reduced oxygen transfer performance in FBS.

4. Conclusions

FBS had significantly poorer oxygen transfer characteristics than

FFS did even at similar biomass concentrations. Filamentous bacteria of *Thiothrix eikelboomii* were determined to be the troublemakers. They had long filaments and could significantly enhance the apparent viscosity of activated sludge. However, the K_La value for FBS was still lower than that for FFS even if the apparent viscosity of FFS was increased to a similar level by concentrating the sludge. The additional impact of *Thiothrix eikelboomii* on oxygen transfer could be caused by their particular cell surface property and interaction with air bubbles. Sludge bulking especially the growth of filamentous bacteria with long filaments have to be controlled even in the membrane bioreactors not only for improving sludge settling but also for energy conservation.

Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.watres.2018.12.019.

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