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# Formation of filamentous microorganisms impedes oxygen transfer and decreases aeration efficiency for wastewater treatment

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#### A R T I C L E I N F O

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### ABSTRACT

A low operational dissolved oxygen (DO) concentration in activated sludge improves oxygen transfer efficiency. However, it also can promote the growth of filamentous microorganisms that adversely affect sludge settling. In this study, filamentous microorganisms were found to additionally impede oxygen transfer; a previously unrecognized problem. We found that, when the operational DO of the complete-mix activated sludge reactor was reduced from 2.0 to 0.5 mg/L, the improvement in the oxygen transfer efficiency (OTE) was less than expected. Further investigation revealed that the change in OTE was highly correlated to the abundance of filamentous microorganisms and the excessive growth of filamentous microorganisms could reduce the OTE by 50%, even under the same operational DO condition. It was hypothesized that filamentous microorganisms impeded oxygen transfer mainly by increasing the mixed liquor viscosity due to their long filamentos microorganisms in activated sludge process must be controlled for improving aeration efficiency and more studies are needed to better understand the effect of microbe types in activated sludge on the oxygen transfer.

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# 1. Introduction

Energy conservation and resource recovery are essential considerations of the sustainable wastewater treatment (McCarty et al., 2011; Henriques and Catarino, 2017; Torregrossa et al., 2017). In the United States, the municipal wastewater treatment industry accounted for 0.8% of the country's electricity demand in 2011, approximately 30.2 billion kWh (WRF and EPRI, 2013). For existing wastewater treatment facilities, aeration contributes to more than 50% of the total plant energy use (Li et al., 2017; Henriques and Catarino, 2017). Reducing the operational dissolved oxygen (DO) during aeration is a logical consideration to reduce the aeration energy use because it can increase the oxygen deficit which consequently enhances the oxygen transfer from air to water. However, nitrifiers are very sensitive to low DO. Therefore, the operational DO in the aeration tank is usually controlled at approximately 2 mg/L to achieve complete nitrification (Metcalf

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and Eddy, 2003). This DO level allows oxygen to penetrate deep into the microbial aggregate, allowing aerobic conditions to predominate, which is critical for chemolithoautotrophic respiration.

Interestingly, recent studies indicate that complete nitrification is achievable under long-term low DO conditions (<0.5 mg/L) (Park and Noguera 2004, 2007; Bellucci et al., 2011; Liu and Wang, 2013, 2015a; Arnaldos et al., 2013; Fitzgerald et al., 2015; Keene et al., 2017). The low DO could inhibit the endogenous decay of nitrifiers, significantly increasing nitrifier population size and the nitrification capacity (Liu and Wang, 2013, 2015a). The increase in nitrifier population size compensates for the adverse effect of the low DO on individual nitrifiers. As a result, long-term low DO does not significantly impact the nitrification performance at the community level. In addition, the long-term low DO improves the oxygen affinity of nitrifiers by a natural selection process or enhancing heme protein expression, which could improve oxygen delivery to cells (Liu and Wang, 2013; Arnaldos et al., 2013; Fitzgerald et al., 2015). The change in oxygen affinity could also be a result of impacts to oxygen mass transfer in the system (Manser et al., 2005; Sarioglu et al., 2009) or a conformational change in the cellular enzymatic equipment utilized to transport electrons to the terminal







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electron acceptors (Stein et al., 2007). Our studies showed that the half-velocity constant of oxygen ( $K_{DO}$ ) for the growth of nitriteoxidizing bacteria (NOB) under steady conditions decreased from 0.39 to 0.08 mg/L as the operational DO decreased from greater than 2 mg/L to less than 0.4 mg/L (Liu and Wang, 2015a). This low  $K_{DO}$  indicates that the nitrite oxidation rate is not inhibited by a DO as low as 0.2 mg/L. These recent findings strongly suggest that it is feasible to operate an activated sludge process with a low DO to achieve complete nitrification.

In the activated sludge process, the aeration need is mainly dependent on the total oxygen consumption and oxygen transfer efficiency (OTE). With an operational DO of less than 0.5 mg/L (instead of 2.0 mg/L), the theoretical overall OTE will increase by approximately 18% at a water temperature of 20 °C primarily due to the greater DO deficit that drives oxygen transport (Metcalf and Eddy, 2003). However, the actual aeration energy savings and oxygen transfer performance under long-term low DO have not been well studied. The total oxygen consumption is determined by the substrate oxidation and biomass production. Under long-term low DO conditions, the biomass production could increase mainly due to the inhibitions of low DO on the hydrolysis of cell debris and on the endogenous decay of active biomass (Liu and Wang, 2015b). Assuming the total substrate oxidation is the same under long-term low DO, the total oxygen consumption will decrease with the increase in biomass production. Therefore, the anticipated aeration saving under the long-term low DO conditions should be greater than 18% that saved by a greater DO deficit. However, long-term low DO may cause variations in microbial community structure and floc morphology and their effects on the oxygen transfer have rarely been studied. Notably, the low DO operation of the activated sludge system could cause excessive growth of filamentous microorganisms which have greater specific surface area, faster growth coefficient, and better oxygen affinity (Rossetti et al., 2005). The effect of excessive growth of filamentous microorganisms on oxygen transfer is not clear.

During our study of long-term low-DO activated sludge processes, we observed that the actual savings in aeration need was less than theoretical estimates based on the increased DO deficit. More interestingly, we observed that the fluctuation in OTE was highly correlated to the abundance of filamentous microorganisms, which was an incidental finding not reorganized previously. The possible mechanisms for the inhibition of filamentous microorganisms to oxygen transfer are also discussed.

## 2. Materials and methods

#### 2.1. Reactor setup and operation

Three bench scale complete-mix flow reactors with the same dimension  $(L \times W \times H = 50.8 \text{ cm} \times 20.3 \text{ cm} \times 30.0 \text{ cm})$  were used for this research (Fig. S1). They were fed continuously with the same synthetic wastewater containing chemical oxygen demand (COD) and ammonia nitrogen (NH<sub>3</sub>–N) concentrations of 180 mg/L and 48 mg-N/L, respectively. The COD and ammonia in the influent were provided with glucose and ammonium bicarbonate, respectively. In addition, trace elements were provided in the influent, which was described in a previous paper (Liu and Wang, 2013). The three reactors were operated with solids retention times (SRT) of 10, 20, to 40 days, respectively. The hydraulic retention time was 12 h, the pH was controlled in the range of 7.0–7.5, and the water temperature was approximately 20.5  $\pm$  0.6 °C, for all reactors.

The treatment performance and aeration needs were tested under different DO levels. The fine bubble diffusers utilized were 2ft long Bio-Weave diffuser hose (Pentair Aquatic Eco-systems, Cary, NC) capable of providing 0.2 to 0.6 SCFM air per foot at 20 inches of head loss. The diffuser hoses were fashioned in a semi-circular shape and installed in the middle of each reactor. Mechanical mixing was provided to promote particle suspension and prevent denitrification under all tested DO levels. The radial flow impeller mixer was operated at a constant speed of 20 rpm.

During the long-term test, the operational DO, feed rate, and air flow rate for each reactor were carefully checked and recorded multiple times daily. The feed rate and airflow rate were manually adjusted within preselected limits as needed to maintain the preselected operational duty point. Because the inflow loading and the experimental temperature were very consistent, a relatively constant DO in the reactors was easily maintained without using an automatic DO control device. A YSI 58 DO meter with a YSI 5239 probe was used to measure DO. Regulation and adjustment of airflow was performed utilizing variable area rotometer (Key Instruments, Hatfield, PA), which had been calibrated at standard conditions for use with air. To build the accurate correlation of oxygen consumption rate, operational DO, and air supply rate, the concentrations of effluent COD, ammonia, nitrite, and nitrate, and MLSS and mixed liquor volatile suspended solid (MLVSS) were measured only when both the inflow rate and DO concentration in each reactor was sequentially detected in the control ranges. Analytical methods for MLSS, MLVSS, COD, ammonia, nitrite, and nitrate were described previously (Liu and Wang, 2012, 2017). A microscope (Olympus-CKX41, Center Valley, PA) fitted with  $10 \times$ ocular lens and  $10\times$ ,  $20\times$  and  $40\times$  phase contrast objective lenses was used to observe the sludge floc morphology regularly. Images were captured utilizing a Lumenera (Ottawa, ON) Infinitity 2-1C, 1.4 megapixel CCD array and stored for subsequent evaluation. Under each tested DO, the reactor was operated for at least 2 to 3 SRTs. The effluent quality, biomass nitrification capacity, and MLSS concentration were used to assess steady operations within the system (Liu and Wang, 2013).

#### 2.2. Oxygen consumption calculation

In activated sludge, the oxygen consumption for organic biodegradation and nitrification, and the oxygen-use reduction due to biomass synthesis determines the total oxygen consumption (Metcalf and Eddy, 2003). Since no non-biodegradable COD was included in the influent, the influent and effluent COD concentrations were used to estimate the oxygen consumption for organic biodegradation. The oxygen consumption for COD biodegradation was calculated using Eq. (1):

$$R_{\rm COD} = Q(S_{0,\rm COD} - S_{\rm COD}) \tag{1}$$

where  $R_{COD}$  = the oxygen consumed for COD biodegradation, g-O<sub>2</sub>/ d; Q = the inflow rate, m<sup>3</sup>/d; S<sub>0, COD</sub> = the influent COD concentration, mg/L; S<sub>COD</sub> = the effluent COD concentration, mg/L. Because the experimental SRTs and DO levels did not impact COD removal significantly, the effluent COD was not monitored as frequently as effluent nitrogen species. The average effluent COD under different DO levels was used to estimate the oxygen consumption for organic biodegradation.

The oxygen consumption for nitrification was estimated using Eq. (2):

$$R_{\rm N} = 4.57 Q S_{\rm NO_3^-} + 3.43 Q S_{\rm NO_2^-} \tag{2}$$

where  $R_{\rm N}$  = the oxygen consumed for nitrification, g-O<sub>2</sub>/d;  $S_{\rm NO_3^-}$  = the effluent nitrate concentration, mg-N/L;  $S_{\rm NO_2^-}$  = the effluent nitrite concentration, mg-N/L; 4.57 = oxygen demand for complete oxidation of ammonia into nitrate, g-O<sub>2</sub>/g-N; 3.43 = oxygen

demand for complete oxidation of ammonia into nitrite, g-O<sub>2</sub>/g-N. The reduction in oxygen consumption due to biomass synthesis

$$R_{\rm S} = -1.42 P_{\rm Bio} \tag{3}$$

where  $R_S$  = the oxygen-use reduction due to biomass production, g-O<sub>2</sub>/d;  $P_{\text{bio}}$  = the biomass production, g-VSS/d; 1.42 = averaged oxygen demand for complete oxidation of biomass, g-O<sub>2</sub>/g-VSS.

Under low DO conditions, simultaneous nitrification and denitrification may occur. However, mass balance calculations revealed no significant nitrogen loss indicating that denitrification was nominal under the low DO conditions (Liu and Wang, 2013). Therefore, the reduction in oxygen consumption by denitrification was not considered. The total oxygen consumption was calculated using Eq. (4):

$$R_T = R_{COD} + R_N + R_S \tag{4}$$

where  $R_{\rm T}$  = the total oxygen consumption, g-O<sub>2</sub>/d.

#### 2.3. Oxygen transfer efficiency estimation

In the activated sludge system, the OTE describes the percentage of oxygen used by microorganisms with respect to the total oxygen supplied and can be calculated using Eq. (5).

$$OTE = \frac{Q(C - C_0) + R_T}{O_{\text{sup}}}$$
(5)

where  $O_{sup} =$  rate of total oxygen supply,  $g-O_2/d$ ; C = DO in the mixed liquor and the effluent stream, mg/L;  $C_0 = DO$  in the influent, mg/L.

The OTE indicates the overall oxygen transfer performance and is proportional to (a) the oxygen mass transfer coefficient of the mixed liquor for a given aeration tank, aeration devices, mixing intensity, and operation temperature, and (b) the actual DO deficit. Under the same operational DO, the OTE is mainly impacted the oxygen mass transfer property of the mixed liquor.

# 3. Results

# 3.1. Oxygen consumption under different SRTs and DO

The detailed treatment performance under various SRTs and DO levels was presented and discussed previously (Liu and Wang, 2013, 2015a). For convenience, the nitrification performance and the MLSS data are also presented in Figs. S2 and S3, and Table 1. Under all tested conditions, both COD degradation and nitrification were completed (Table S1). With a similar operational DO, a longer SRT would lead to a greater total oxygen consumption since it had a

lower sludge production. When the DO was reduced from 4 to 2, and then 1 mg/L in the 10- and 20-day SRT reactors, the total oxygen consumption did not change significantly. Compared to the data under a high DO of greater than 2 mg/L, the total oxygen consumption under a low DO of less than 0.5 mg/L for all reactors were reduced by roughly 10%. For the 10-, 20-, and 40-day SRT reactors, the biomass production increased by 22.5%, 15.7%, and 39.0% under the low DO, respectively (Table 1). This suggested that the reduction in the total oxygen consumption under low DO was mainly caused by the increased biomass production.

# 3.2. Aeration needs under different SRTs and DO

Fig. 2 shows the air supply rate to different reactors, with average values under steady conditions exhibited in Table 1. When the DO was reduced from approximately 4 to 2 mg/L in the 10- and 20-day SRT reactors, there was no change in the oxygen consumption; however, the air supply rate was reduced by approximately 45%, which confirmed that the aeration efficiency was improved significantly by a greater DO deficit. Reduction in the DO from 2 to 1 mg/L in the 10-day SRT reactor reduced air supply rate by 12.7%. When the DO was further reduced from 1 to 0.37 mg/L, the air supply rate was reduced by approximately 7%. For the 20day SRT reactor, the air supply rate varied marginally when the DO was reduced from 2 to 1 mg/L, indicating that other factors had offset the benefit of an increased DO deficit for the oxygen transfer. Compared to the air supply rate at a DO of 2 mg/L in the 10-day SRT reactor, average reductions of 19.3%, 12.1%, and 17.1% aeration were achieved when the operational DO decreased to less than 0.5 mg/L, for 10-, 20-, and 40-day SRT reactors, respectively (Table 1).

The long-term low DO could additionally reduce the oxygen consumption by increasing the biomass production (Liu and Wang, 2015b), further reducing the aeration need. As previously discussed, the total oxygen consumption was reduced by roughly 10% under low DO conditions compared to that at a high DO of 2–4 mg/ L. Therefore, under long-term low DO conditions, both increased DO deficit and decreased oxygen consumption contributed to the reduction in the aeration needs. Based on the theoretical calculation, the aeration need at 20 °C could be reduced by approximately 18% with a low DO of 0.5 mg/L compared to that at a regular DO of 2 mg/L if the total oxygen consumption was the same. Considering the realistic reduction in total oxygen consumption due to the increased biomass production under low DO (<0.5 mg/L), the aeration need could be reduced by approximately 28%. However, the observed reduction in air supply rate was much less than what had expected, indicating that other factors had impacted the oxygen transfer under low DO conditions.

## 3.3. OTE under different SRTs and DO

The OTE estimated based on Eq. (5) could indicate the effect of

Table 1

Summary of biomass production, total oxygen consumption, aeration needs, and OTE under steady-state condition in the reactors with different DO and SRTs.

SRT(day)	DO(mg/L)	Biomass production(g/d)	Total oxygen consumption(g-O <sub>2</sub> /d)	Aeration needs(m <sup>3</sup> -air/d)	OTE
10	$4.0 \pm 0.4$	$3.05 \pm 0.18$	$18.7 \pm 0.2$	5.76	$1.17 \pm 0.01\%$
	$2.1 \pm 0.3$	$3.12 \pm 0.23$	$18.6 \pm 0.3$	$3.22 \pm 0.17$	$2.11 \pm 0.15\%$
	$1.0 \pm 0.3$	$3.07 \pm 0.20$	$18.2 \pm 0.8$	$2.81 \pm 0.09$	$2.25 \pm 0.12\%$
	$0.37 \pm 0.09$	$3.79 \pm 0.14$	$17.1 \pm 0.9$	$2.60 \pm 0.11$	$2.37 \pm 0.11\%$
20	$3.9 \pm 0.4$	$2.58 \pm 0.13$	$19.8 \pm 0.3$	5.76	$1.19 \pm 0.02\%$
	$2.0 \pm 0.4$	$2.81 \pm 0.22$	$19.6 \pm 0.4$	$3.24 \pm 0.17$	$2.10 \pm 0.11\%$
	$0.98 \pm 0.30$	$3.17 \pm 0.12$	$18.4 \pm 0.6$	$3.22 \pm 0.87$	$1.98 \pm 0.09\%$
	$0.28 \pm 0.16$	$2.97 \pm 0.36$	$17.9 \pm 0.7$	$2.83 \pm 0.26$	$2.28 \pm 0.48\%$
40	$4.2 \pm 0.5$	$1.86 \pm 0.06$	$21.7 \pm 0.4$	$6.06 \pm 0.99$	$1.18\pm0.10\%$
	$0.43 \pm 0.27$	$2.59 \pm 0.11$	$19.0\pm0.5$	$2.67 \pm 0.44$	$2.31\pm0.13\%$

was calculated using Eq. (3):

DO and the mixed liquor property on oxygen transfer performance in the reactors. Because the difference between the influent DO and effluent DO had negligible impact on the calculation, it was discarded during the OTE calculation. As shown in Table 1, when the DO was at the similar level, the OTEs under different SRTs were similar. These results suggest that the geometry, accuracy of air flow meters, performance of diffusers and mixing devices, etc. for all reactors were similar, and when looking at the overall trends. the SRT did not play a significant role in OTE. For the 10-day SRT reactor, the OTE at a DO of 2, 1, and 0.4 mg/L were 2.1%, 2.3%, and 2.4%, respectively, indicating that the oxygen transfer under lower DO conditions was improved only slightly. For the 20-day SRT reactor, the OTE decreased by 5.7% at a DO of 1 mg/L and increased by 9.0% at a DO of less than 0.5 mg/L, compared to the OTE at 2 mg/ L. This indicates that lower DO may not always improve the overall OTE. Other factors have impacted the oxygen transfer.

More interestingly, between 300-day and 440-day for the 20day SRT reactor operated at a low DO of 0.28 mg/L, the air supply rate fluctuated dramatically between 1.87 and 3.67 m<sup>3</sup>/d (Fig. 2(b)). This is also reflected in the OTE value shown in Fig. 3(b). On 301day, the OTE was 1.8% and it increased to 3.4% on 375-day. However, the OTE decreased gradually to approximately 1.7% between days of 398–414. Surprisingly, the OTE increased to 3.1% on 423day, while it decreased again to 1.9% on 440-day. Notwithstanding, the total oxygen consumption at 20-day SRT, as shown in Fig. 1(b), changed marginally during this period, indicating that the aeration needs and OTE were significantly affected by the oxygen transfer process.

Also, as shown in Fig. 2(c), when the DO was reduced from 4 to less than 0.5 mg/L for the 40-day SRT reactor, the aeration need decreased from  $5.2 \text{ m}^3/\text{d}$  to approximately  $1.3 \text{ m}^3/\text{d}$ . The reduced total oxygen consumption (Fig. 1(c)) due to incomplete nitrification



Fig. 1. Total oxygen consumption and oxygen consumption for COD degradation, nitrification, and biomass yield under different DO concentrations in the (a)10-, (b)20-, and (d) 40- day SRT reactors.

and increased DO deficit resulted in the decrease in aeration need. Interestingly, the aeration need increased gradually from 116-day to 200-day and finally stabilized at about  $3.9 \text{ m}^3/\text{d}$  (Fig. 2(c)) for the low DO operation period. The increase in the aeration need was partially due to recovery of complete nitrification. The oxygen consumption decreased from average  $21.7 \text{ g-O}_2/\text{d}$  to  $16.7-21.2 \text{ g-O}_2/\text{d}$  (from day 116-140) and subsequently recovered to a regular level at 400-day. After reducing the DO to 0.43 mg/L at 40-day SRT, the OTE decreased gradually from initially 5.4% to finally 2.4% after 215-day, indicating that the increase in the aeration need was mainly caused by the decrease in OTE. Because the DO was not changed, the decreases in OTE were a result of changes in oxygen transfer properties of the mixed liquor.

#### 3.4. Correlation between OTE and MLSS

As previously discussed, the OTE in the 20-day SRT reactor fluctuated significantly from 300-day to 440-day with an operational DO of 0.28 mg/L (Fig. 3(b)) and the OTE in the 40-day SRT reactor decreased by approximately 50% from 115-day to 215-day. During both periods, no changes in the tank dimensions, aeration devices, influent wastewater characteristics and loading, the operational DO, and temperature were made for each reactor. Therefore, the significant changes in the OTEs must be caused by alternations in the mixed liquor characteristics.

The biomass concentration could impact the OTE by changing the viscosity of mixed liquor (Krampe and Krauth, 2003; Germain et al., 2007; Henkel et al., 2009). Fig. 3(S)(b) presents that the MLSS concentration also fluctuated significantly from 300-day to



Fig. 2. A eration needs under different DO concentrations in the reactors with (a) 10-, (b) 20-, and (c) 40-day SRT.

440-day in the 20-day SRT reactor. In the 40-day SRT reactor from 115-day to 255-day, the MLSS concentration increased from 2.4 g/L on the 116-day, to 3.6 g/L on the 248-day, as shown in Fig. S3(c). Therefore, the change in OTE during both periods could be caused by the change in MLSS concentrations. The correlations between OTE and MLSS during both periods are made and given in Fig. 4(a). which likely supports this hypothesis. However, at a similar operational DO, the OTE values under different MLSS concentrations associated with different SRTs were very similar (Fig. 4(b)), suggesting that the experimental MLSS concentration was not the major cause for OTE change. Moreover, previously developed correlations between  $\alpha$  factor and MLSS indicated that the MLSS would impact the oxygen transfer when it was greater than 5 g/L (Muller et al., 1995; Germain et al., 2007; Henkel et al., 2009). Our experimental systems had a much lower MLSS concentration and, therefore, the increase in the MLSS concentration could not be the primary cause for the significant decrease in OTE during the low DO aeration.

#### 3.5. Filamentous microorganisms reduce oxygen transfer

The changes in biomass morphology in the 20-day SRT reactor from 300-day to 440-day, and those in the 40-day SRT reactor from 115-day to 255-day are presented in Figs. 5 and 6, respectively. For the 20-day SRT reactor, the high OTE is clearly correlated to the low abundance of filamentous microorganisms. For the 40-day SRT reactor, the OTE gradually decreased while the density of filamentous microorganisms gradually increased after reducing the DO to 0.43 mg/L. Both Figs. 5 and 6 strongly suggest that the bloom



Fig. 3. Oxygen transfer efficiency (OTE) under different DO concentrations in the (a) 10-, (b) 20-, and (c) 40-day SRT reactors.



**Fig. 4.** (a) Correlation between oxygen transfer efficiency and MLSS concentration in the 20-day SRT reactor (from 300-day to 440-day) and 40-day SRT reactor (from 115-day to 255-day); (b) Correlation between oxygen transfer efficiency and MLSS concentration from different SRTs under the stabilized condition.

of filamentous microorganisms significantly reduced the oxygen transfer efficiency, which is a contradiction to the intuitive understanding that the filamentous microorganisms might enhance oxygen transfer due to their much greater specific surface area and greater exposure to the bulk solution than floc forming microorganisms.

Interestingly, by comparing Fig. 5 with Fig. 3(S)(b) and Fig. 6 with Fig. 3(S)(c), it was found that MLSS concentration was

positively correlated with the density of filamentous microorganisms. In activated sludge, the filamentous microorganisms and flocforming bacteria have similar theoretical biomass yield coefficient if using the same substrate (Lau et al., 1984). In our systems, the COD was nearly completely oxidized under both high and low DO conditions and so that the amount of biomass yield due to cell synthesis did not change with the operational DO. However, filamentous microorganisms generally have lower endogenous decay coefficients than the floc-forming bacteria do; they range between 0.03 and 0.05 d<sup>-1</sup> and 0.08–0.15 d<sup>-1</sup>, respectively (Lau et al., 1984; Lou and leong, 2015). This suggests that, in addition to the inhibition of low DO to active biomass decay and cell debris hydrolysis, the observed increases in the MLSS (or sludge production) were probably also caused by the lower endogenous decay coefficients of the filamentous microorganisms.

# 4. Discussion

More than 50% of energy is used for aeration in the wastewater treatment plant (Li et al., 2017; Henriques and Catarino, 2017). Low DO aeration strategy could save aeration energy and also achieve simultaneous nitrification and denitrification, which have attracted the attention of many researchers (Daigger and Littleton, 2014; Liu and Wang, 2013; Fitzgerald et al., 2015; Keene et al., 2017). Both greater DO deficit and higher biomass production under low DO could contribute to the saving in aeration. Unfortunately, the formation of filamentous microorganisms under low DO could inhibit the oxygen transfer, which partially offset the benefit of low DO operation in energy saving. Therefore, the filamentous sludge bulking must be controlled through better integrating the selectors process and other means, to realize the anticipated energy saving.

The mechanisms of filamentous microorganisms resisting oxygen transfer are unclear. As shown in Figs. 5 and 6, the filamentous microorganisms in our reactors had long filament length, like Type 021N filamentous bacteria. In addition, the filamentous microorganisms could produce excessive extracellular polymeric substances (EPS) (Meng et al., 2006; Germain et al., 2007; Wágner et al., 2015). Both the long filaments and excessive EPS might had increased the viscosity of the mixed liquor. The increased viscosity not only directly reduces the oxygen diffusion at the air-mixed liquor interface (diffusion coefficient is inversely proportional to the mixed liquor viscosity), but also enhances air bubble coalescence, resulting in increased bubble size. Greater air bubble size reduces



Fig. 5. (a) OTE and MLSS concentration, and (b to f) typical microscope images for the sludge floc in the 20-day SRT reactor from 300-day to 440-day with an operation DO of 0.28 mg/L (the number in above images are the day when it they were taken).



**Fig. 6.** (a) OTE and MLSS concentration, and (b to j) typical microscope images for the sludge floc in the 40-day SRT reactor from day 115–250 after reducing the DO concentration to 0.43 mg/L (the number in above images are the day when it they were taken).

the air-water interface area and air-water contact time (through increasing the rising velocity), negatively impacting the oxygen transfer (Garcia-Ochoa et al., 2000; Badino et al., 2001; Ozbek and Gayik, 2001). The increase in the viscosity also decreases the turbulence induced by rising air bubbles or stirrers, negatively impacting oxygen transfer.

Unfortunately, the viscosity of the mixed liquor was not measured because this was an incidental finding during our study of long-term low-DO activated sludge system. However, in a separate experiment under a constant DO of 2 mg/L, we have also found that the OTE is negatively related to the sludge settling ratio (SV%), or the abundance of filamentous organisms (data not shown). We also find that the viscosity is positively related to the SV%. Therefore, we speculate that, the presence of filamentous organisms will increase the viscosity that reduces the OTE.

In addition, filamentous microorganisms in activated sludge have higher cell surface hydrophobicity than floc-forming bacteria do (Nielsen et al., 2002; Henze et al., 2008). A positive correlation was observed between the total filament density and the relative hydrophobicity of the sludge (Meng et al., 2006). The hydrophobic cell surface enables filamentous microorganisms better attract hydrophobic substrates like lipids, long-chain fatty acid, and other nonpolar substrates (Rossetti et al., 2005). Hydrophobic particles will tend to accumulate at the gas-liquid interface, blocking the oxygen mass transfer (Ferreira et al., 2010; Mena et al., 2011). Moreover, it is also possible that the filamentous microorganisms in the systems had synthesized unknown biosurfactants, which could significantly reduce the practical solubility of oxygen at the airwater interface (Capodici et al., 2015; Crittenden et al., 2012).

The adverse effect of the filamentous microorganisms on oxygen transfer was an incidental finding during our study of long-term low-DO activated sludge system. More research is necessary to validate the hypotheses. The growth of filamentous microorganism is a common problem in the activated sludge process. They were previously notorious for decreasing the sludge settling ability. However, their effect on the rheological properties of activated sludge and then the oxygen transfer process have rarely been noticed. This study presents a new issue associated with the filamentous microorganism, in addition to decreasing with the sludge settling ability. Generally, optimizing aeration efficiency in the activated sludge processes has been focused on the aeration devices, tank dimension, operational DO and surfactants (Metcalf and Eddy, 2003; Germain and Stephenson, 2005). Moreover, MLSS concentration was also found to impact the oxygen transfer and a high MLSS could decrease the OTE in membrane bioreactors (Germain et al., 2007; Henkel et al., 2009; Krampe and Krauth, 2003). However, little attention has been paid to the impacts of the biomass morphology and microbial types on aeration efficiency (Sarioglu et al., 2009). This study demonstrated that the excessive growth of filamentous microorganisms could reduce the OTE by 50% and then doubled the aeration need. This suggests, under the same MLSS concentration and aeration conditions, the activated sludge contained excessive filamentous microorganism will have poorer oxygen transfer performance, i.e., lower  $\alpha$  factor.

# 5. Conclusions

Previous studies usually focus on the optimization of aeration devices and operation strategy for improving aeration efficiency and reducing energy use for wastewater treatment. However, the effect of microbe type in activated sludge on oxygen transfer has rarely been investigated. In this study, we accidentally discovered that the excessive growth of filamentous microorganisms under low DO could resist the oxygen transfer and increased the aeration need. As a result, the low DO operation saved less aeration need than anticipated. Filamentous microorganisms were hypothesized to resist oxygen transfer mainly by increasing the mixed liquor viscosity due to their long filaments and excessive production of extracellular polymeric substances. The results from this study imply that, to optimize the aeration efficiency in the activated sludge process, the growth of filamentous microorganisms must be controlled. In addition, more studies are suggested to better understand the correlations between the microbe types in activated sludge and the oxygen transfer performance.

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#### Appendix A. Supplementary data

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