Effects of hydraulic retention time on process performance of anaerobic side-stream reactor coupled membrane bioreactors: Kinetic model, sludge reduction mechanism and microbial community structures

Lu-Man Jiang, Zhen Zhou, Tianhao Niu, Lingyan Jiang, Guang Chen, Hongjian Pang, Xiaodan Zhao, Zhan Qiu

Abstract

An anoxic/oxic membrane bioreactor (AO-MBR) and three anaerobic side-stream reactor (ASSR) coupled MBRs (ASSR-MBR) were operated to investigate the effects of hydraulic retention time of ASSR (HRTA) and to elucidate sludge reduction mechanisms in ASSR-MBRs. Increasing HRTA from 3.3 to 6.6 h improved nitrogen removal, and enhanced sludge reduction from 8.0% to 40.9% in ASSR-MBR. The sludge decay coefficient was 0.0221 d$^{-1}$ in MBRs, and 0.0231–0.0345 d$^{-1}$ in ASSRs. The measured lysis rate coefficient of heterotrophic biomass was 0.083–0.112 d$^{-1}$ in MBRs and 0.079–0.111 d$^{-1}$ in ASSRs. The hydrolysis rate coefficient of inactive particulate organic matters (POMs) in ASSRs significantly exceeded that in the MBR. At HRTA of 6.6 h, POMs hydrolysis in ASSR (38.6%) is the dominant route of sludge reduction, and cell lysis occurred principally in aerobic tanks. Illumina-MiSeq sequencing showed ASSR-MBRs enriched hydrolytic and fermentative bacteria, and confirmed that anaerobic hydrolysis contributed most to sludge reduction.

1. Introduction

The treatment and disposal of waste activated sludge (WAS) generated during biological process has become a major challenge for wastewater treatment plants (WWTPs) (Wang et al., 2013). Handling the large amount of WAS accounts for 25–65% of the total plant operating costs (Niu et al., 2016). In situ sludge reduction technology showed advantages over conventional sludge treatment and disposal by

Keywords:
- Sludge reduction
- Anaerobic side-stream reactor
- Decay
- Hydrolysis
- Microbial community
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-consuming sludge within the process and producing much less amount of WAS. One of the most promising in situ sludge reduction choices is to install an anaerobic side-stream reactor (ASSR) in the sludge return line to construct anoxic-settling-anaerobic (OSA) like system, so sludge enters the aerobic and anaerobic environments alternately. Therefore, effective sludge reduction is achieved by microbial anabolism and catabolism uncoupling without affecting effluent quality and microbial activity (Saby et al., 2003; Ye et al., 2008). Despite the increasing interest in the application of ASSR in full-scale WWTPs (Wang et al., 2013), the hydraulic retention time of ASSR (HRTA) required for effective sludge reduction is still comparable to the main stream treatment system (Semblante et al., 2016; Ye et al., 2008). Therefore, the key for practical application of ASSR is to understand its sludge reduction mechanism, and to increase sludge reduction rate and shorten HRT through physiological and ecological control of microorganisms.

Various theoretical hypotheses have been proposed to explain the possible mechanisms underlying sludge reduction, including energy uncoupling (Troiani et al., 2011), cell lysis-cryptic growth (Foldatori et al., 2015; Li et al., 2017), and sludge decay (Chen et al., 2003). Systematical evaluation by Chen et al. (2003) identified that anaerobic sludge decay under a low oxidation-reduction potential (ORP) condition was the main mechanism for sludge reduction. Sludge decay occurs in both aerobic and anaerobic conditions and is composed of two pathways, hydrolysis of particulate organic matter (POM) and lysis of bacterial biomass (Niu et al., 2016). Foldatori et al. (2015) evaluated the contribution of the two pathways to sludge reduction by determining viable and dead bacteria, and found that anaerobic hydrolysis of in-active POM and anaerobic bacteria lysis contributed equally to sludge reduction. Martínez-García et al. (2016) compared the kinetics of endogenous residues biodegradation and heterotrophic biomass decay under different conditions by batch digestion tests, and found that operating intermittent aeration cycles achieved the fastest biodegradation and decay rate. The intensive efforts mentioned above are helpful to explore kinetics and effective pathways of sludge reduction, but most studies were independent batch test with synthetic wastewater. Recently, researchers have used the conventional activated sludge model (ASM) (Henze et al., 2000) and its modification to simulate sludge production in sludge reduction process for wastewater treatment (Amanatidou et al., 2015). More detailed research is needed using real wastewater during long-term operation to better understand effects of HRTA on sludge reduction, and to correlate ASM and sludge decay kinetics to microbial community structure.

In this study, an anoxic/oxic membrane bioreactor (AO-MBR) and three ASSR-MBRs were operated in parallel to investigate the effects of HRT, on sludge reduction and pollutants removal of ASSR-MBR. The kinetic parameters related to sludge decay, active biomass lysis and inactive POM hydrolysis were estimated to evaluate contribution of different routines to sludge reduction. High-throughput sequencing analyses were applied to correlate microbial community to sludge reduction kinetics in ASSR-MBRs under different HRTs. The results of this study are expected to elucidate sludge reduction mechanisms in ASSR-MBRs for real wastewater treatment.

2. Materials and methods

2.1. System setup and operation conditions

Three pilot-scale ASSR-MBRs, as shown in Fig. 1, and an AO-MBR (for control) were operated for 167 days at the Donggu municipal WWTP in Shanghai, China, and fed with wastewater from the grit chamber at flow rate of 7.5 L/h. The effective volumes were 16.7 and 50 L for anoxic and aerobic zone in all the four MBRs, and 25, 37.5 and 50 L for the three ASSRs. Four flat-sheet polyvinylidene fluoride membranes with an average pore size of 0.2 μm (300 × 280 mm) and a total effective filtration area of 0.25 m² were mounted vertically in the aerobic zone with air diffuser below the membrane module. Agitators were installed in the anoxic zone and ASSR. The effluent was obtained by a peristaltic pump connected to membranes with constant permeate flux of 24 L/(m²-h) and intermittent filtration mode (Cheng et al., 2017). The mixed liquor recirculation (MLR) was controlled at 250% for AO-MBR and 150% for ASSR-MBR. Activated sludge was delivered into ASSR with a side-stream ratio (f) of 100%, and thus HRTA was controlled at 3.3 (A L-MBR), 5.0 (A M-MBR) and 6.6 h (AH-MBR). Therefore, the four MBRs achieved an identical recirculation ratio of 250%. Mechanical cleaning and in-place chemical cleaning with 0.5% NaClO solution were conducted for membrane module once the trans-membrane pressure exceeded 30 kPa. Dissolved oxygen (DO) in the four MBRs was 4.0–5.0 mg/L, while temperature was 21.4–30.2 °C during operation. The concentration of mixed liquor suspended solids (MLSS) in MBR was maintained at about 6000 mg/L by discharging WAS from the MBR.

2.2. Theoretical model for sludge reduction

2.2.1. Mass balance model for sludge reduction

A steady-state sludge mass balance in an ASSR-MBR is established as follows:

\[ \Delta X_{d,a} + \Delta X_{d,m} + \Delta X_w = \Delta X_{g,m} + \Delta X_{g,a} \]  

(1)

where \( \Delta X_{d,a} \) and \( \Delta X_{d,m} \) are sludge decay in the main stream (i.e. anoxic and aerobic tank) and ASSR, g/d; \( \Delta X_w \) is sludge variation item of the system, including WAS discharge, effluent loss and sludge accumulation in reactors, g/d; \( \Delta X_{g,m} \) and \( \Delta X_{g,a} \) are sludge generated in the main stream and ASSR, g/d.

Sludge decay happens in both ASSR and main stream reactor. Therefore,

\[ \Delta X_{d,a} + \Delta X_{d,m} = K_{d,a} V_a X_{s,a} + K_{d,m} V_m X_{s,m} \]  

(2)

where \( K_{d,a} \) and \( K_{d,m} \) are decay coefficients of ASSR (using subscript ‘a’) and the main stream reactor (using subscript ‘m’), d⁻¹; \( V_a \) and \( V_m \) are volumes of ASSR and the main stream reactor, L; \( X_{s,a} \) and \( X_{s,m} \) are MLSS in ASSR and the main stream reactor, g/L. Combining Eqs. (2) and (1), sludge decay can be expressed as

\[ K_{d,a} V_a X_{s,a} + K_{d,m} V_m X_{s,m} = \Delta X_{g,m} + \Delta X_{g,a} - \Delta X_w \]  

(3)

In the main stream reactor, biomass growth occurs concurrently with the oxidation of organic matters from influent wastewater and effluent of ASSR, and thus

\[ \Delta X_{g,m} = Y_{g,m} [Q_{0} + Q_{a} - (Q_{e} + Q_{s})] \]  

(4)

where \( Y_{g,m} \) is net biomass yield in the main stream, g SS/g COD, a value of 0.55 was recommended (Chen et al., 2003); \( Q_{0}, Q_{a}, \) and \( Q_{e} \) are flow rate of influent, ASSR effluent and effluent, L/d; \( S_0, S_a \) and \( S_e \) are
substrate concentration in influent, ASSR effluent and effluent, mg COD/L.

Sludge generated in ASSR is related to anoxic growth of biomass (Eq. (5)).

$$\Delta X_{\text{fa}} = X_{\text{fa}}(2.86-\Delta \text{NO}_3-N + 1.71-\Delta \text{NO}_2-N)$$  \hspace{1cm} (5)

where $X_{\text{fa}}$ is net biomass yield in ASSR, g SS/g COD, a recommended value of was 0.55 (Chen et al., 2003); $\Delta \text{NO}_3-N$ and $\Delta \text{NO}_2-N$ are denitrified nitrate nitrogen (NO$_3$-N) and nitrite nitrogen (NO$_2$-N) in ASSR, mg N/L. If two or more sludge reduction systems are operated in parallel and assuming that $K_{d,m}$ values are equal, $K_{d,m}$ and $K_{d,a}$ can be estimated by substituting Eq. (4) and (5) in Eq. (3).

2.2.2. Estimation of sludge reduction

The observed sludge yield ($Y_{\text{obs}}$, g VSS/g COD) is estimated by the ratio of cumulative generated sludge to cumulative consumed substrate, according to Eq. (6):

$$Y_{\text{obs}} = \frac{\Delta X_{\text{fa}}}{Q_{\text{in}} \cdot (X_{\text{fa}} - X_{\text{fo}})} = \sum \frac{V_{i} (X_{\text{fa}} - X_{\text{fo}})}{Q_{\text{in}}} + \sum \frac{(Q_{\text{in}} X_{\text{fa}} + Q_{e} X_{e}) \Delta t}{(Q_{\text{in}} X_{\text{fa}} + Q_{e} X_{e}) \Delta t}$$  \hspace{1cm} (6)

where $X_{\text{fa}}$ and $X_{\text{fo}}$ are initial and terminal sludge concentrations in reactor, respectively, mg/L; $V_{i}$ is the volume of reactor $i$, L; $Q_{\text{in}}$ is flow rates of WAS, L/d; $X_{\text{fa}}$ and $X_{\text{fo}}$ are sludge concentrations in WAS and effluent, mg/L; $\Delta t$ is duration of the operation period, d. The sludge reduction efficiency (SRE) is calculated by Eq. (7).

$$SRE = 1 - Y_{\text{obs}} \text{ASSR}/Y_{\text{obs, control}}$$  \hspace{1cm} (7)

2.2.3. Mechanism model of sludge reduction

In an ASSR-MBR without considering biological phosphorus removal, mixed liquor volatile suspended solids (MLVSS) consist of three components: (1) active biomass (heterotrophic and autotrophic, $X_{\text{H/AUT}}$), (2) endogenous residue and (3) POMs from influent. Based on ASM (Henze et al., 2000), the endogenous residue was incorporated into POMs, and $X_{\text{AUT}}$ was considerably lower than $X_{\text{H/AUT}}$. Therefore, $X_{\text{v}} = f_{\text{CV}} \text{MLVSS} = X_{\text{H/AUT}} + X_{\text{IV}}$

where $X_{\text{v}}$, $X_{\text{H/AUT}}$ and $X_{\text{IV}}$ are MLVSS, heterotrophic biomass and inert POM concentrations in terms of COD, g COD/L; $f_{\text{CV}}$ is conversion factor for MLVSS to COD, g COD/g VSS, a value of 1.48 was recommended (Water Research Commission, 1984). Based on the recommended $f_{\text{CV}}$ value, the active fraction $f_{\text{v}}(X_{\text{H/AUT}})$ and inert fraction $f_{\text{v}}(X_{\text{IV}}/X_{\text{v}})$ in the sludge can be calculated.

MLVSS decreased through $X_{\text{H/AUT}}$ lysis and $X_{\text{IV}}$ hydrolysis, namely

$$\frac{dX_{\text{H/AUT}}}{dt} = -K_{a} X_{\text{H/AUT}} = -(1-f_{\text{v}}) f_{\text{v}} X_{\text{H/AUT}} - K_{a} X_{\text{IV}}$$  \hspace{1cm} (9)

where $b_{\text{v}}$ and $K_{a}$ are rate coefficients of $X_{\text{H/AUT}}$ lysis and $X_{\text{IV}}$ hydrolysis, d$^{-1}$; $f_{\text{v}}$ is inert residue fraction, with default value of 0.20 (Henze et al., 2000). With $K_{a}$ and $b_{\text{v}}$ estimated by mass balance model, contributions of biomass lysis and POM hydrolysis to sludge decay and the kinetic coefficients can be calculated if $X_{\text{H/AUT}}$ and $b_{\text{v}}$ can be determined by batch tests.

2.3. Determination of concentration and decay coefficient of $X_{\text{H/AUT}}$

2.3.1. Basic theory

According to ASM (Henze et al., 2000), $X_{\text{H/AUT}}$ lysis can be expressed as

$$\frac{dX_{\text{H/AUT}}}{dt} = -b_{\text{H/AUT}} X_{\text{H/AUT}}$$  \hspace{1cm} (10)

If the metabolism of $X_{\text{AUT}}$ is inhibited by allylthiourea (ATU), oxygen uptake rate (OUR) under the famine state depends on $X_{\text{H/AUT}}$ lysis, as shown in Eq. (11).

$$\text{OUR} = (1-f_{\text{v}}) b_{\text{H/AUT}} X_{\text{H/AUT}}$$  \hspace{1cm} (11)

Combining integral solution of Eq. (10) and Eq. (11), under a given boundary condition of $t = 0$, $X_{\text{H/AUT}} = X_{\text{H/AUT}}$, then taking logarithmic transformation gives

$$\ln(\text{OUR}) = \ln[(1-f_{\text{v}}) b_{\text{H/AUT}} X_{\text{H/AUT}}] - b_{\text{H/AUT}} t$$  \hspace{1cm} (12)

2.3.2. Aerobic and anaerobic digestion batch tests

Mixed liquor samples were collected from each MBR and ASSR on Day 155 for digestion batch tests to determine $X_{\text{H/AUT}}$ and $b_{\text{v}}$. The samples were placed in a 1.21 batch reactor at temperature of 20 ± 1 °C and pH of 7.0–8.0. The reactor was operated for 4–6 d with OUR determined at an interval of 0.5–1.0 d. For MBR samples, the reactor was aerated at DO of 4–5 mg/L with 20 mg/L ATU dosed. For SSR samples, the reactor was maintained anaerobic conditions. At an interval of 0.5–1.0 d, 200 mL of activated sludge was withdrawn from the reactor, rinsed by deionized water thrice, then transferred to an aerobic batch reactor and aerated for 30 min for OUR determination.

2.4. Microbial community analysis

Seven samples were collected from three ASSRs (ASSRs$_{1}$, ASSRs$_{2}$ and ASSRs$_{3}$) and four MBRs (AO-MBR, MBR$_{1}$, MBR$_{2}$ and MBR$_{3}$) on Day 155 for MiSeq pyrosequencing to investigate the effects of HRT$_{\alpha}$ on microbial structures and community evolution. DNA extraction, PCR amplifications and amplicons purification were conducted according to the reported methods (Cheng et al., 2017). After purification, amplicons from the samples were sequenced using the Illumina-MiSeq platform (Majorbio Bio-Pharm Technology, China). The 16S rRNA sequences were clustered into operational taxonomic units (OTUs) with an average length of 396 bp by setting a 3% distance limit ($\alpha$). Based on cluster information, parameters referring to species richness (Chao1 and Ace), community diversity (Shannon and Simpson) and sequencing depth (Good’s Coverage) were calculated by Mothur software.

2.5. Analytical methods

Concentrations of COD, ammonium nitrogen (NH$_4$-N), NO$_3$-N, NO$_2$-N, total nitrogen (TN) and total phosphorus (TP) in influent and effluent were analyzed every two days. MLSS and MLVSS in MBRs and ASSRs were determined twice a week. Samples from the influent, anaerobic, ASSR influent (ASI), ASSR effluent and effluent were collected for dissolved organic matters (DOM) and nitrogen species analysis. DO, pH and ORP were monitored using an HQ30d meter (Hach, USA). The inert particulate COD in the influent was determined according to Henze et al. (2000) in thrice.

Differences of pollutants in the effluent were compared by one-way factor analysis of variance (ANOVA). The correlations between released substrate and HRT$_{\alpha}$, between relative abundance of hydrolytic bacteria and reaction rate were determined by Pearson’s rank correlation.

3. Results and discussion

3.1. Process performance

As shown in Table 1, the four MBRs were equally efficient in COD removal (91.6–92.0%) and NH$_4$-N removal (97.8–98.1%). The one-way ANOVA results showed that COD removal and nitrification capacity of AO-MBR were not impaired after inserting ASSRs. Compared to AO-MBR with TN removal of 49.0%, ASSR-MBR achieved higher nitrogen removal efficiency, which increased from 56.0% to 69.5% with HRT$_{\alpha}$ rising from 3.3 to 6.6 h. ANOVA ($\alpha = 0.05$) showed that TN concentrations in effluents of the four MBRs were significantly different, and decreased with the rising HRT$_{\alpha}$. Nitrogen removal in ASSR-MBR was enhanced by utilizing DOM release from sludge decay in ASSR for denitrification (Cheng et al., 2017). The decrease of NO$_3$-N in Table 1 also confirmed denitrification was improved with increasing HRT$_{\alpha}$. 
Phosphorus removal efficiency was slightly improved after inserting ASSR, which was attributed to anaerobic phosphorus release in ASSRs (Chen et al., 2003).

According to Eq. (6), the $Y_{\text{obs}}$ was 0.088 g SS/g COD for AO-MBR, which was close to the reported values of 0.10 g SS/g COD at SRT of 80 d (Wang et al., 2013). It was attributed to the lower volumetric load and longer solid retention time (SRT) (111 d). After inserting ASSR with HRT of 3.3, 5.0 and 6.6 h, ASSR-MBR obtained $Y_{\text{obs}}$ of 0.081, 0.062 and 0.052 g SS/g COD, and reduced sludge production by 8.0%, 29.5% and 40.9% in comparison to AO-MBR, respectively. The SRE was in the commonly claimed range for OSA and ASSR process (0.2–66%) (Cheng et al., 2017; Semblante et al., 2016). The SRE increased with the rising HRTA, and achieved the maxima at HRTA of 6.6 h, which is close to the optimal value obtained by Ye et al. (2008) (6–7 h) and Coma et al. (2013) (5.9 h).

### 3.2. Pollutants transformation and estimation of $K_d$:

Fig. 2a and 2b show variations of soluble COD (SCOD) and NH$_4$-N among different modules of the four MBRs. Compared to that in ASI, SCOD in ASE rose by 15.2, 26.7 and 39.5 mg/L, while NH$_4$-N increased by 0.13, 0.31 and 0.34 mg/L with HRTA of 3.3, 5.0 and 6.6 h, respectively. The net release of SCOD and NH$_4$-N was linearly correlated with HRTA with correlation coefficient ($r$) of 0.937 and 0.929, respectively, indicating that longer HRTA contributed to more complete destruction of sludge flocs. The released SCOD was used to calculate $\Delta X_{g,m}$ according to Eq. (4), with results shown in Table 2.

Denitrification in ASSRs resulted in the enhancement of both nitrogen removal (Saby et al., 2003) and anoxic growth of $X_{H.\text{N O}_2-N}$ concentrations in all the samples were below 0.1 mg/L, thus the data were not illustrated here but considered in the calculation. As shown in Fig. 2c and 2d, in the three ASSRs, TN decreased by 3.99, 5.25 and 3.88 mg/L, while the denitrified NO$_3$-N were 2.52, 4.54 and 4.83 mg/L with HRTA of 3.3, 5.0 and 6.6 h, respectively. The release of SCOD and denitrification of oxidized nitrogen species increased with the rising HRT. This confirmed our results in Table 1 that TN concentration in AO-MBR effluent was the highest, and nitrogen removal increased with rising HRTA. With $\Delta$NO$_2$-N and $\Delta$NO$_3$-N, $\Delta X_{g,a}$ were calculated according to Eq. (5) and are shown in Table 2.

Based on data in Table 2, a matrix equation was constructed according to Eq. (3) for the calculation of $K_d$ values.

\[
\begin{bmatrix}
139.4 & 0 & 0 & 0 \\
374.0 & 255.9 & 0 & 0 \\
377.5 & 0 & 216.8 & 0 \\
403.9 & 0 & 0 & 176.3 \\
\end{bmatrix}
\begin{bmatrix}
K_{d,m} \\
K_{d,a} \\
K_{d,AM} \\
K_{d,HH} \\
\end{bmatrix}
\begin{bmatrix}
8.61 \\
12.36 \\
12.63 \\
15.13 \\
\end{bmatrix}
\]

(13)

By solving Eq. (13), the obtained $K_{d,m}$ of the four MBRs was 0.0211 d$^{-1}$, and $K_{d,a}$ values were 0.0231, 0.0306 and 0.0345 d$^{-1}$ for ASSRL, ASSRM and ASSRH, respectively. The results indicated that prolonging HRTA increased anaerobic $K_d$, and ASSR with HRTA of 6.6 h achieved the highest $K_{d,a}$. Chen et al. (2003) estimated $K_d$ at 0.11 and 0.05 d$^{-1}$ in

<table>
<thead>
<tr>
<th>Items</th>
<th>Influent</th>
<th>Effluent</th>
<th>AO-MBR</th>
<th>ASR-MBR</th>
<th>ASR-MBR</th>
</tr>
</thead>
<tbody>
<tr>
<td>COD</td>
<td>197.3 ± 165.4</td>
<td>16.1 ± 7.2</td>
<td>15.8 ± 7.6</td>
<td>15.8 ± 7.3</td>
<td>16.6 ± 6.8</td>
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<tr>
<td>NH$_4$-N</td>
<td>19.78 ± 7.22</td>
<td>0.42 ± 0.19</td>
<td>0.41 ± 0.17</td>
<td>0.37 ± 0.14</td>
<td>0.43 ± 0.27</td>
</tr>
<tr>
<td>NO$_2$-N</td>
<td>1.08 ± 1.48</td>
<td>12.3 ± 4.39</td>
<td>10.8 ± 3.00</td>
<td>8.56 ± 3.34</td>
<td>7.90 ± 2.96</td>
</tr>
<tr>
<td>NO$_3$-N</td>
<td>0.047 ± 0.031</td>
<td>0.016 ± 0.003</td>
<td>0.006 ± 0.003</td>
<td>0.007 ± 0.006</td>
<td>0.014 ± 0.013</td>
</tr>
<tr>
<td>TN</td>
<td>25.70 ± 6.15</td>
<td>13.1 ± 5.53</td>
<td>11.3 ± 4.28</td>
<td>9.42 ± 4.51</td>
<td>7.85 ± 4.87</td>
</tr>
<tr>
<td>TP</td>
<td>2.86 ± 1.22</td>
<td>2.11 ± 1.04</td>
<td>2.09 ± 0.68</td>
<td>1.97 ± 0.87</td>
<td>1.84 ± 0.87</td>
</tr>
</tbody>
</table>

Table 1

Influent and effluent characteristics of the four MBRs (Unit: mg/L).

Influent Anoxic MBR/ASI ASE Effluent

<table>
<thead>
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Fig. 2. Variation of organic and nitrogenous matters of AO-MBR and ASSR-MBRs.
an anaerobic tank (ORP of -250 mV) and aeration tank. The obtained $K_{da}$ in this study was lower than 0.05 d$^{-1}$ and that of conventional aeration tank (Tchobanoglous et al., 2003), but close to An and Chen (2008) with $K_a$ of 0.021 d$^{-1}$. The obtained $K_{da}$ was comparable to that in activated sludge processes (0.02–0.04 d$^{-1}$) (Tchobanoglous et al., 2003), but lower than that in OSAs (An & Chen, 2008; Chen et al., 2003). It could be attributable to different sludge fractions and microbial population between ASSR-MBR and OSA.

### 3.3. Parameter estimation for sludge reduction in ASSR-MBR

#### 3.3.1. Estimation of kinetic parameters for biomass lysis and hydrolysis

Variations of OUR with time in batch tests are shown in Fig. 3. As shown in Table 3, $b_1$ values obtained were 0.095, 0.112, 0.098 and 0.083 d$^{-1}$ in the four MBRs. The results were close to the aerobic decay rate of total bacteria (0.07–0.11 d$^{-1}$) measured in anaerobic–aerobic sequence at single-cell level by flow cytometry (Foladori et al., 2015). It is noteworthy that the obtained $b_1$ is generally lower than the widely recognized value of 0.20 d$^{-1}$ in ASMs (Henze et al., 2000) and reported values in MBRs (Ramdani et al., 2012). Nevertheless, Martínez-García et al. (2016) obtained a much smaller $b_1$ (0.02–0.05 d$^{-1}$) by sludge from an ASSR coupled sequencing batch reactor (SBR). The $b_1$ values were 0.0785, 0.0953 and 0.111 d$^{-1}$ in ASSR$_{A}$, ASSR$_{M}$ and ASSR$_{H}$, respectively. Results showed that cell lysis rate was not particularly improved in ASSRs, which was also confirmed by Troiani et al. (2011) and Habermacher et al. (2015).

Although slowly biodegradable POM only accounts for a small proportion in $X_{IV}$ (Henze et al., 2000), the inactive organic fraction was also biodegradable with decay rates of 0.005–0.012 d$^{-1}$ under anaerobic and alternating aerated/non-aerated conditions, respectively (Ramdani et al., 2010). For aerobic hydrolysis of $X_{IV}$, $k_{h}$ values were 0.0160, 0.0190, 0.0182 and 0.0219 d$^{-1}$ in the four MBRs. The $k_{h}$ value in AO-MBR was the same order of magnitude as Ramdani et al. (2010), but was 8 times of aerobic $k_{h}$ obtained by Martínez-García et al. (2016). This gap was because completely biodegradable synthetic influent was used in literatures and $X_{IV}$ only contained endogenous residue, while $X_{IV}$ in this study was composed of POM from real wastewater and endogenous residues. The anaerobic $k_{h}$ in ASSR was linearly correlated with $HRT_A$ ($r = 0.996$), indicating that higher hydrolysis rate was obtained under its lower ORP at longer $HRT_A$ (Saby et al., 2003). It was also shown that alternating anaerobic and aerobic conditions (ASSR-MBR) resulted in a higher hydrolysis rate than aerobic conditions (AO-MBR).

#### 3.3.2. Estimation of $X_{III}$ and $X_{IV}$

As shown in Table 3, the fraction of $X_{III}$ ($f_{III}$) in AO-MBR was greatly lower than the fraction (0.595–0.684 g/g VSS) in an MBR fed with synthetic wastewater at a volumetric load ($N_v$) of 0.74 g COD/(L·d) (Ramdani et al., 2012). In this study, AO-MBR was fed with wastewater containing a proportion of inert particulate COD (61.3 ± 4.2 mg/L) at a lower $N_v$ of 0.53 g COD/(L·d), which caused accumulation of inactive fraction in the sludge. Lower $f_{III}$ values were also observed in full-scale WWTP in Shanghai (0.268 g/g VSS) with $N_v$ of 0.53 g COD/(L·d) (Wu et al., 2008) and the simulated AO process in ASM1 (0.454 g/g VSS) with $N_v$ of 0.89 g COD/(L·d) (Henze et al., 2000). In ASSR-MBR, $f_{IV}$ decreased in MBRs and further declined in ASSRs. Higher SRT of ASSR-MBRs is a major reason for the lower $f_{IV}$ which decreased with the rising SRT (Ramdani et al., 2012).

#### 3.3.3. Contribution of lysis and hydrolysis to sludge reduction

Estimating reaction rate and the contribution of $X_{III}$ lysis and $X_{IV}$ hydrolysis under different conditions are very important for understanding sludge reduction mechanism. In the aerobic condition of AO-MBR, the reaction rate of cell lysis was 1.87 times of $X_{IV}$ hydrolysis (Fig. 4a). In the three A-MBRs, the difference between reaction rates of cell lysis and POM hydrolysis was not as large as in AO-MBR, which should be attributed to the decreasing $X_{II}$ fraction in sludge and the increasing $k_h$ after inserting ASSR (Table 3). In ASSRs, cell lysis rate was 46.4%-61.7% of that in MBRs. The fact that active biomass decayed faster under aerobic than anaerobic conditions was also observed in a side-stream reactor coupled SBR (2015). It should be noted that hydrolysis rates in the three ASSRs were enhanced by 1.12–1.85 times to those in MBRs, and were 2.2–3.6 times of cell lysis rate in the corresponding ASSR.

The contribution of cell lysis and POM hydrolysis to sludge decay can be estimated by reduction mass shown in Fig. 4b. Cell lysis and POM hydrolysis accounted for 65.2% and 34.8% of sludge decay in AO-MBR. The total reduction masses of cell lysis and POM hydrolysis in the
three A-MBRs were almost the same, thus changing HRT only affected sludge decay in ASSR. With HRT increased, the contribution of sludge decay in MBR decreased, whilst anaerobic hydrolysis in ASSR increased to 38.6%. The contributions of anaerobic cell lysis to sludge reduction were below 11%. The results indicated that at HRT of 6.6 h, anaerobic hydrolysis of X_SS in ASSR dominated in sludge decay, and cell lysis occurred principally in aerobic tanks. Similar results that anaerobic hydrolysis contributed majorly to sludge reduction were recently observed in a full-scale ASSR (Foladori et al., 2015). Therefore, improving X_V hydrolysis in ASSR is critical to enhance sludge decay.

### 3.4. Microbial evolution

#### 3.4.1. Richness and diversity of bacteria phylotypes

After reads normalization, samples from ASSR-MBRs obtained significantly higher OTUs (1312–1395) than that from AO-MBR (Table 4), indicating that ASSR-MBR had richer microbial community. Chao and Shannon estimators also showed that inserting ASSR greatly enhanced microbial richness and diversity, while prolonging HRT only had a slight effect on microbial community structure. Further enhancing HRT and decreasing r caused microbial community structure evolution. Cheng et al. (2017) reported that ASSR-MBR with r of 20% and HRT of 25.0 h obtained more abundant and diverse microbial community than that with r of 100% and HRT of 6.0 h. In this study, high r (100%) promoted sludge interchange and resulted in microbial community distribution more evenly between ASSR and MBR, and thus decreased the difference among the three ASSR-MBRs.

#### 3.4.2. Taxonomic complexity of the bacterial community

The top 20 abundant genera in a total of 472 genera for the 7 samples were selected and compared with their abundances in Fig. 5. *Dechloromonas* and *Nitrospira* were the most dominant genera in MBRs and ASSRs. The relative abundance of *Dechloromonas*, a slow grower correlated with ammonia oxidation and enriched in sludge reduction module (Li et al., 2017; Niu et al., 2016), was higher in ASSR-MBR than that in AO-MBR, and increased with the rising HRT, *Nitrospira*, conventionally belonging to γ-Proteobacteria nitrite oxidizing bacteria, contributed to the unexpected higher abundances compared with ammonia oxidizing bacteria in ASSR-MBR. Koch et al. (2015) found that besides aerobic nitrate oxidation, *Nitrospira* had the ecophysiological flexibility to survive during nitrite or oxygen deprivation by reducing nitrate using organic products of fermenting organisms, and thus enriched in ASSR. The relative abundance increased from 3.8% to 6.2% in ASSR with HRT rising from 3.3 to 6.6 h, indicating that higher anaerobic time generated more fermentation products for the growth of *Nitrospira*. The total relative abundance of another five predominant genera of denitrifying bacteria, *Azospira*, *Halomonas*, *Sulfuritalea*, *Thauera* and *Bacillus*, which were also classified as slow growers (Guo et al., 2013; Wu et al., 2016), were 3.7%, 7.7% and 5.8% in ASSR at HRT of 3.3, 5.0 and 6.6 h, respectively. This suggests that at longer HRT, ASSR-MBR had higher denitrification potential and lower sludge yield from the microbiological perspective.

POM hydrolysis and cell lysis are achieved by enriching hydrolytic

### Table 3

**Estimation of sludge fractions and sludge decay parameters for the four MBRs.**

<table>
<thead>
<tr>
<th>Reactor</th>
<th>k_0 (g COD/L·d)</th>
<th>X_ss (g COD/L)</th>
<th>SS (g/L)</th>
<th>X_V (g COD/L)</th>
<th>k_v (g COD/L·d)</th>
<th>h_v (g/L)</th>
<th>f_s (g/g VSS)</th>
<th>f_v (g/g VSS)</th>
<th>Coverage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AO-MBR</td>
<td>0.0948</td>
<td>1.221</td>
<td>2.9</td>
<td>3.098</td>
<td>0.0221</td>
<td>0.0160</td>
<td>0.283</td>
<td>0.717</td>
<td></td>
</tr>
<tr>
<td>A1-MBR</td>
<td>0.112</td>
<td>0.785</td>
<td>3.33</td>
<td>4.145</td>
<td>0.0221</td>
<td>0.0190</td>
<td>0.159</td>
<td>0.841</td>
<td></td>
</tr>
<tr>
<td>A2-MBR</td>
<td>0.0978</td>
<td>1.109</td>
<td>3.74</td>
<td>4.148</td>
<td>0.0221</td>
<td>0.0182</td>
<td>0.201</td>
<td>0.799</td>
<td></td>
</tr>
<tr>
<td>A3-MBR</td>
<td>0.0834</td>
<td>1.061</td>
<td>3.36</td>
<td>3.905</td>
<td>0.0221</td>
<td>0.0219</td>
<td>0.214</td>
<td>0.786</td>
<td></td>
</tr>
<tr>
<td>ASSR</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ASSR_k</td>
<td>0.0785</td>
<td>0.696</td>
<td>2.84</td>
<td>3.443</td>
<td>0.0268</td>
<td>0.0452</td>
<td>0.166</td>
<td>0.834</td>
<td></td>
</tr>
<tr>
<td>ASSR_m</td>
<td>0.111</td>
<td>0.45</td>
<td>2.63</td>
<td>3.443</td>
<td>0.0194</td>
<td>0.0256</td>
<td>0.115</td>
<td>0.885</td>
<td></td>
</tr>
<tr>
<td>ASSR_s</td>
<td>0.0953</td>
<td>0.528</td>
<td>2.78</td>
<td>3.583</td>
<td>0.0198</td>
<td>0.0373</td>
<td>0.128</td>
<td>0.872</td>
<td></td>
</tr>
<tr>
<td>ASSR_h</td>
<td>0.0785</td>
<td>0.696</td>
<td>2.84</td>
<td>3.443</td>
<td>0.0268</td>
<td>0.0452</td>
<td>0.166</td>
<td>0.834</td>
<td></td>
</tr>
</tbody>
</table>

### Table 4

**Richness and diversity indices of microbial communities in the four MBRs (α = 0.03).**

<table>
<thead>
<tr>
<th>Samples</th>
<th>Reads</th>
<th>OTU</th>
<th>Chao</th>
<th>Shannon</th>
<th>Coverage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Raw Normalization</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AO-MBR</td>
<td>26160</td>
<td>19171</td>
<td>1268</td>
<td>1574</td>
<td>5.48</td>
</tr>
<tr>
<td>A1-MBR</td>
<td>33880</td>
<td>19171</td>
<td>1362</td>
<td>1644</td>
<td>5.73</td>
</tr>
<tr>
<td>A2-MBR</td>
<td>28647</td>
<td>19171</td>
<td>1395</td>
<td>1676</td>
<td>5.64</td>
</tr>
<tr>
<td>A3-MBR</td>
<td>24010</td>
<td>19171</td>
<td>1334</td>
<td>1695</td>
<td>5.70</td>
</tr>
<tr>
<td>ASSR_k</td>
<td>24171</td>
<td>19171</td>
<td>1370</td>
<td>1741</td>
<td>5.72</td>
</tr>
<tr>
<td>ASSR_m</td>
<td>27173</td>
<td>19171</td>
<td>1350</td>
<td>1604</td>
<td>5.65</td>
</tr>
<tr>
<td>ASSR_s</td>
<td>26363</td>
<td>19171</td>
<td>1312</td>
<td>1644</td>
<td>5.69</td>
</tr>
<tr>
<td>ASSR_h</td>
<td>22763</td>
<td>19171</td>
<td>1268</td>
<td>1574</td>
<td>5.48</td>
</tr>
</tbody>
</table>

![Fig. 4. Sludge reduction rate (a) and sludge reduction mass (b) in the four MBRs.](image-url)
bacteria and endogenous metabolism of biomass (Cheng et al., 2017). The enrichment of predatory bacteria in sludge reduction processes is considered as an enhancement of cell lysis. Furthermore, hydrolysis converts POM to soluble compounds and simple monomers that are used by bacteria performing fermentation (Tchobanoglous et al., 2003). Thus, total relative abundance of fermentative bacteria can be adopted to reflect hydrolysis efficiency of sludge reduction processes. Therefore, functional bacteria responsible for hydrolysis, fermentation and predation were chosen to generate a heat map (Fig. 6).

The slow grower *Dechloromonas*, a solid-phase denitrifier degrading lignocellulosic carriers as carbon sources (Feng et al., 2017), was the most dominant hydrolytic bacteria enriched in ASSR-MBRs (3.4–5.0%) (Fig. 6). In AO-MBR, *Cytophagaceae_uncultured*, responsible for both aerobic and anaerobic hydrolysis of proteins and polysaccharides (Weissbrodt et al., 2014), displayed significantly higher relative abundance (3.9%) than in ASSR-MBR (0.5%-1.7%). *Haliangium*, responsible for hydrolysis of large molecular organic substances (Crossman et al., 2012), was the third abundant genus enriched in MBRs (1.9%) and AO-MBR (1.2%). In ASSRL, ASSRM and ASSRH, the total relative abundance of hydrolytic bacteria was 8.8%, 10.4% and 12.0%, respectively, which was positively proportional to hydrolysis reaction rate in Fig. 4a ($r = 0.993$). The total relative abundance of hydrolytic bacteria in MBR also increased with the rising HRTA and reached the maxima of 11.7% at HRTA of 6.6 h. In AO-MBR, a comparable relative abundance of hydrolytic bacteria (11.4%) was obtained owing to the enrichment of *Cytophagaceae_uncultured, Haliangium* and *Rhizobacter*, indicating that the evolution of microbial community structures occurred in MBRs after inserting ASSR.

The dominant fermentative bacteria enriched in MBRs included *Comamonadaceae_unclassified*, *Anaerolineaceae_uncultured*, and *Chitinophagaceae_uncultured*. The total relative abundance of fermentative bacteria was 5.0%, 5.2% and 5.5% in ASSRL, ASSRM and ASSRH, respectively. In MBRs, their relative abundance in A-MBR (4.7%-5.7%) was greatly higher than that in AO-MBR (3.2%). The results indicated that ASSR-MBR had more efficient hydrolysis and generated more organic substrate for fermentative bacteria growth. Furthermore, the increased relative abundance of fermentative bacteria with rising HRTA provided more carbon source for denitrification, and thus induced higher TN removal (Table 1).

The enrichment of predatory bacteria in ASSR-MBR facilitated lysicryptic growth and resulted in lower $Y_{obs}$ (Niu et al., 2016). In the four MBRs, the predatory bacteria mainly included *Bdellovibrio, Polyangiaceae_unclassified* and *Neochlamydia*, but showed insignificant variation trend. It is difficult to draw firm conclusion on the role of
predatory bacteria owing to their small relative abundance (0.8–1.2%). The results indicated that cell lysis of \( X_h \) themselves rather than the predation was the major cause for sludge reduction of the active fraction.

4. Conclusions

Three pilot-scale ASSR-MBRs and an AO-MBR were operated to investigate sludge reduction mechanisms. HRT\(_A\) rising from 3.3 to 6.6 h improved TN removal and enhanced SRE from 8.0% to 40.9%. \( k_d \) was 0.0221 d\(^{-1}\) in MBR and increased with HRT\(_A\) in ASSRs. The measured \( b_H \) was 0.083–0.112 d\(^{-1}\) in MBRs and 0.079–0.111 d\(^{-1}\) in ASSRs, while \( k_h \) in ASSRs was greatly higher than MBRs. \( X_H \) hydrolysis in ASSR was the dominant route of sludge reduction (38.6% at HRT\(_A\) of 6.6 h), and cell lysis occurred principally in aerobic tanks. Illumina-MiSeq sequencing confirmed that ASSR-MBRs enriched hydrolytic and fermentative bacteria.

Acknowledgments

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References


