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**Characterization of the refractory dissolved organic matters (rDOM) in
sludge alkaline fermentation liquid driven denitrification: Effect of HRT on
their fate and transformation**

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Abstract: Enhanced biological denitrification for nitrogen removal using sludge alkaline fermentation liquid (SAFL) as an alternative carbon source has been widely reported in previous studies, while limited studies focused on the degradation of the organics presented in SAFL. In this study, an SAFL driven anoxic denitrification sequencing batch reactor (SBR) was established, the mechanism of organics utilization was characterized and the refractory dissolved organic matters (rDOM) was identified. Denitrification could rapidly proceed with the presence of volatile fatty acids (VFAs) initially, while the denitrification rate largely decreased after the VFAs depleted. A great deal of rDOM, which was hard to be utilized by denitrifying microorganism, was found in the effluent. A prolonged hydraulic retention time (HRT) led to the further transformation of particles and colloids to smaller colloids and soluble organics. Extended HRT promoted the degradation of soluble microbial by-product (SMP), but had minor effect on the removal of humic-like, and fulvic acid-like substances. The characterization of the effluent demonstrated the building blocks, were dominated in the rDOM (43.79%-48.78%), followed by high molecular weight protein (HMW-PN) (13.37%-17.39%), HMW polysaccharide (HMW-PS) (12.84%-15.9%), low molecular weight (LMW) neutrals (11.28%-13.65%), and hydrophobic dissolved organic carbon (HO-DOC) (8.0%-12.62%). Moreover, it was found that the building blocks were relatively easy to be degraded with the extension of HRTs, followed by LMW-PS, LMW-PN, LMW neutrals, HMW-PN, and HMW-PS. However, further extended HRT > 24 h could not improve the removal of building blocks, LMW-PS and LMW neutrals. This study, for the first time, provided insights into the transformation of organic matters produced by SAFL in a denitrification system and acted as a guide for the subsequent advanced treatment.

Key words: Denitrification, Sludge alkaline fermentation liquid (SAFL), Size-fractionated dissolved

46 organic matters (DOM), Liquid Chromatography coupled to Organic Carbon and Organic Nitrogen
 47 Detection (LC-OCD-OND), Hydraulic retention time (HRT)

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Abbreviations				MLVSS/VS	Mixed liquor volatile suspended solids
				S	
A/O	Anoxic/oxic			NRE	Nitrogen removal efficiency
BNR	Biological nutrient removal			P-COD	Particulate chemical oxygen demand
BSA	Bovine serum albumin			PN	Protein
COD	Chemical oxygen demand			PS	Polysaccharide
DOC	Dissolved organic matters			rDOM	Refractory dissolved organic matters
Em	Emission			SAFL	Sludge alkaline fermentation liquid
Ex	Excitation			SBR	Sequencing batch reactor
FID	Flame ionization detector			SEC	Size exclusion chromatography
FS	Fulvic acid-like substances			SC-COD	Small colloidal chemical oxygen demand
HMW	High molecular weight			S-COD	Soluble chemical oxygen demand
HI-DOC	Hydrophilic	dissolved	organic	SMP	Soluble microbial by-product
	carbon				
HO-DOC	Hydrophobic	dissolved	organic	3D-EEM	Three-dimensional fluorescence
	carbon				excitation-emission matrix
HRT	Hydraulic Retention Time			T-COD	Total chemical oxygen demand
HS	Humic-like substances			TOC	Total organic carbon
LC-COD	Large	colloidal	chemical oxygen	tVFAs	Total volatile Fatty Acids
	demand				
LC-OCD-ON	Liquid Chromatography coupled to			VFAs	Volatile Fatty Acids
D	Organic	Carbon	and Organic	WAS	Waste activated sludge
	Nitrogen Detection				
LMW	Low molecular weight			WRP	Water reclamation plant
MLSS/SS	Mixed liquor suspended solids			WWTPs	Wastewater treatment plants

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1. Introduction

Biological denitrification, which involves in a series of reduction steps from nitrate to nitrogen gas, has been widely used to remove nitrogen from wastewater (Lu et al., 2014). In order to achieve a high-efficiency nitrogen removal, external carbon source is generally required due to its shortage in wastewater (Li et al., 2011). The commercial carbon sources, such as methanol, ethanol, acetic acid, and sodium acetate, are usually supplemented (Modin et al., 2007; Fernández-Nava et al., 2010). The addition of these commercial carbon sources will increase the operational cost and has a potential risk in transportation (i.e. ethanol) and human health (i.e. methanol). It is thus necessary to find an alternative carbon source for more sustainable application.

Waste activated sludge (WAS), which is largely produced in wastewater treatment plants (WWTPs) contains massive organic compounds (i.e. proteins and carbohydrates). The particulate organic matters in WAS can be effectively released and transformed into volatile fatty acids (VFAs) and other soluble form of carbon source via anaerobic fermentation process (Wu et al., 2010; Li et al., 2011; Liu et al., 2016; Zhang et al., 2016; Guo et al., 2017). The usage of carbon source from sludge fermentation for enhancing the biological denitrification is a sustainable approach, as it can simultaneously reduce the sludge amount.

To enhance the fermentation process, alkaline fermentation has been developed and demonstrated to be an effective method (Chen et al., 2007; Zhao et al., 2015; Chen et al., 2017; Liu et al., 2018). The mechanism of high VFAs accumulation during sludge alkaline fermentation is mainly caused by the highly efficient solids solubilization, which provides sufficient substrate (i.e. protein - PN, polysaccharide - PS) for acidification (Wu et al., 2009; Chen et al., 2007). On the other hand, it was reported that a lot of refractory compounds would also be generated simultaneously, which may be non-biodegradable or even inhibitory (Li et al., 2016; Lu et al., 2018). It is known that organic matters discharged from biological nutrient removal (BNR) system are of significant concern, as they not only affect the effluent quality but also negatively impact on the subsequent advanced

treatment (i.e. membrane and advanced oxidation) (Shon et al., 2006; Meng et al., 2011; Chen et al., 2017; Wang and Chen, 2018). Up to now, most studies mainly focused on the performance of denitrification enhanced by sludge alkaline fermentation liquid (SAFL) (Liu et al., 2016; Li et al., 2011; Sun et al., 2016), Sun et al. (2016) reported that the biological nitrate removal efficiency with SAFL was higher than that with sludge thermal hydrolysis liquid. Few studies have been carried out on the transformation and consumption of organic matter, especially the fate of the refractory dissolved organic matters (rDOM). Hence, it is crucial to obtain a detailed characterization of the organic compounds in the denitrification effluent driven by SAFL. It is also desired to investigate the effects of operating conditions on the rDOM transformation.

In this study, size exclusion chromatography (SEC) coupled to organic carbon and organic nitrogen detection (SEC/LC-OCD-OND) were employed to characterize the dissolved organic matters (DOM) in the denitrification process, which was assisted with SAFL as carbon source. The main objectives of this study were to (1) track the transformation behavior of organic matters in SAFL driven denitrification, (2) examine the composition and distribution of size-fraction organic matter in effluent, (3) identify the rDOM in denitrification effluent, and (4) explore their fate under different operating conditions, namely, varied hydraulic retention time (HRT). The results would offer an insight into the residual DOM from the SAFL driven denitrification process and thus provide essential information for the design of subsequent advanced treatment.

2. Materials and methods

2.1 Set-up and operation of a lab-scale denitrification reactor

In order to provide an alternative carbon source for nitrogen removal from nitrate contained wastewater, a lab-scale sequencing batch reactor (SBR) (dimension: 7 cm (internal diameter) × 28 cm (height)) with a working volume of 0.8 L was used to perform the denitrification (Fig.S1). The SBR was equipped with an influent pump, effluent pump and a magnetic stirring apparatus (300 rpm)

for the complete mixing during the reaction. The operation of the SBR was controlled by the on/off timer switches. Each cycle included 10 min feeding, 240-1200 min stirring, 60 min setting, 10 min discharging and 50-170 min idling. During the whole operation period, four HRTs (12 h, 16 h, 24 h, and 48 h) were designed to evaluate the degradation of organic matter from SAFL. The actual reaction phase was 4 h, 6 h, 10 h and 20 h in each HRT, and the operation period of each HRT was 7 d, 8 d, 10 d, and 15 d, respectively. The SBR was operated at room temperature (26-28°C). In each cycle, after 60 min setting, 0.4 L of the supernatant was discharged and replaced with the fresh nitrate wastewater, resulting in the volume exchange ratio of 50%. The SBR effluent was sampled daily and filtered through 0.45 µm nylon membrane filters for further analysis.

2.2 Sludge and wastewater source

The seeding sludge of SBR was collected from a local water reclamation plant (WRP) operated with anoxic/oxic (A/O) process. The initial mixed liquor suspended solids (MLSS) and mixed liquor volatile suspended solids (MLVSS) in SBR were 1.6 g/L and 1.3 g/L, respectively. In order to reduce the potential refractory organics accumulated in the SBR due to the adsorption by the sludge, the MLVSS was maintained at around 1.2 g/L during the whole operation, with the sludge retention time (SRT) about of 16 d. Synthetic wastewater was prepared with NaNO₃ 0.73 g (120 mg N/L), KH₂PO₃ 0.05 g, CaCl₂ 0.40 g, MgSO₄·7H₂O 0.10 g and trace elements solution 1 mL in every one-liter water (Du et al., 2015). The composition of trace elements were: 1.5 g/L FeCl₃ 6H₂O, 0.12 g/L ZnSO₄ ·7H₂O, 0.18 g/L KI, 0.15 g/L H₃BO₃, 0.15 g/L CoCl₂ 6H₂O, 0.03 g/L CuSO₄ 5H₂O, 0.12 g/L MnCl₂ 4H₂O, 0.06 g/L Na₂MoO₄ 2H₂O and 10 g/L ethylene diamine tetraacetic acid (EDTA). SAFL was used to provide the electron donor for denitrification, and added to the synthetic wastewater with chemical oxygen demand (COD) of 600 mg/L, resulting in a COD to NO₃⁻-N (COD/NO₃⁻-N) ratio of 5.0 (Li et al., 2013). The feed for the denitrification SBR was prepared daily.

The SAFL used in this study was produced from WAS that was collected from the same WRP. A commercial fermenter with a working volume of 10 L was operated at room temperature (26-28°C)

for fermentation process. Mixed liquor suspended solids (MLSS) of the WAS was 11.8 g/L and MLVSS was 9.5 g/L. The pH for the alkaline anaerobic fermentation was controlled at 10.0 in initial 4 days with 2 mol/L NaOH or 2 mol/L HCl online, and no NaOH was added in following days as the pH was self-regulated in the reactor. The alkaline fermentation was carried out for 7 days according to our pre-test, which produced the maximum level of VFAs. After 7-days operation, the mixture was discharged from the fermenter and centrifuged immediately at 7000 rpm for 10 min. The supernatant was collected and stored in 4°C refrigerator for experiment use. The major characteristics are presented in Table S1.

2.3 COD fractionation

COD can be divided into particulate, colloidal and soluble fractions. A long HRT may induce the transformation of particulate and/or colloidal fractions to soluble fraction. In order to reveal HRT effects on the refractory compounds properties, COD in SBR effluent was fractionated according to [Zhang et al., \(2018\)](#). Four fractions were considered within tCOD: particulate COD (P-COD), large colloidal COD (LC-COD), small colloidal COD (SC-COD) and soluble COD (S-COD) ([Fig.S2](#)). The effluent firstly passed through a 12-25 µm paper filter (Whatman Black Ribbon 589/1) to remove the suspended solids and measured as the T-COD. Then it was subsequently passed through a 1.5 µm glass-fiber filter and 0.45 µm nylon membrane filters. P-COD was defined as the difference between T-COD and filtered COD that passed through a 1.5-micron filter. LC-COD was defined as the difference between filtered COD that passed through a 1.5-micron filter and filtered COD that passed through a 0.45-micron filter. SC-COD was defined as the difference between the filtered COD that passed through a 0.45-micron filter and S-COD. The S-COD fraction was measured directly using the flocculation-filtration (ZnSO₄ as flocculant, 0.45 mm filtration) method ([Hu et al., 2002](#)), which is also called ffCOD. The concentration of PN and PS in each COD fraction was also determined to quantify their abundance and distribution in particles, colloids, and soluble fractions.

2.4 Batch tests in determining the transformation of DOM during denitrification

In order to further explore the degradation mechanism of organic matter from sludge alkaline fermentation liquid, batch tests were conducted to determine the evolution of DOM. The denitrification sludge was withdrawn from the SBR and washed three times with the Milli-Q water to remove the residual substrates. Washed sludge was resuspended in four 0.5-L conical flasks placed at room temperature, with the initial MLVSS around 650 mg/L. The nitrate concentration was 60 mg N/L and the dosages of SAFL were COD/NO₃⁻-N of 5.0, 7.5, 10.0 and 15.0, respectively. The tests were carried out for 720 min, samples were taken at 30 min intervals in the initial 120 min, and a relatively longer interval of 60 min, 120 min, and 240 min were adopted in the following 600 min due to the low degradability of the rDOMs. Samples were immediately centrifuged for 30 s at 4000 rpm to remove the solids, and the supernatant was passed through 0.45 µm filter and stored in 4°C refrigerator for analysis.

2.5 Three-dimensional excitation-emission matrix (3D-EEM) analyses

3D-EEM measurement was carried out by a Cary Eclipse Fluorescence Spectrophotometer (Agilent, USA). Excitation (Ex) scans were progressively increased from 200 nm to 400 nm at 10 nm intervals. The emission (Em) spectra were scanned from 280 to 550 nm at 0.5 nm steps for each excitation wavelength (Chen et al., 2003). The spectra were recorded at a scanning speed of 1200 nm/min with a 10 nm band-pass of excitation and emission slit. The threshold of fluorescence emission intensity was 1000 a.u. The exported EEM data were plotted with Origin 8.5 (OriginLab Inc., USA).

2.6 LC-OCD-OND

The size-exclusion chromatography-organic carbon detect inorganic nitrogen detection (LC-OCD-OND) (DOC-LABOR, Karlsruhe, Germany) analysis was performed to characterize the DOM in effluent according to the method in Huber et al., (2011). The water sample was passed through a size exclusion chromatographic column with a separation range of 20 kDa-100Da. A phosphate-buffer (12.5 g KH₂PO₄ and 7.5 g Na₂HPO₄ · 2H₂O to 5 L of Milli-Q water) was used as

mobile phase with a constant flow rate of 1 mL/min, and acidification solution was prepared with a flow rate of 23 mL/h (2.5 g potassium peroxodisulfate to 5 L, then added 20 mL phosphoric acid). Samples were diluted to have TOC less than 5 mg C/L.

Five peaks with different molecular weights (MW) could be detected, namely biopolymers, humic substances, building blocks, low molecular acids, and low molecular neutrals. Biopolymers consist of HMW-PN and HMW-PS. The concentration of HMW-PN in biopolymers could be calculated according to OND result (Jacquin et al., 2017). And the concentrations of LMW-PN and LMW-PS were calculated by subtracting HMW-PN and HMW-PS from total PN and PS respectively. Hydrophobic dissolved organic carbon (HO-DOC), was defined as the difference between total DOC concentration (the value measured in the column bypass) and hydrophilic dissolved organic carbon (HI-DOC) concentration (the value obtained for the hull curve of the chromatogram) (He et al., 2016; Huber et al., 2011).

2.7 Analytical methods

NO_3^- -N, NO_2^- -N, COD, NH_4^+ -N, PO_4^{3-} -P, MLSS, and MLVSS, were measured according to the standard method (APHA, 1998). PS was determined using the phenol-sulfuric acid method with glucose as the standard, and PN was measured using the Lowry-Folin method with bovine serum albumin (BSA) as standard (Raunkjaer et al., 1994). VFAs were analyzed by a gas chromatograph (GC7890A, Agilent, USA) equipped with a flame ionization detector (FID) and DB-FFAP fused-silica capillary column. Dissolved organic carbon (DOC) was measured by a TOC analyzer (Shimadzu Co., Kyoto, Japan). 1 g of PN and PS was equal to 0.497 and 0.444 g of equivalent carbon based on their proposed chemical formulae.

3. Results and discussion

3.1 Reactor performance at different HRT

3.1.1 Nitrogen removal

Fig.1 displays the effluent concentration of nitrate and nitrite, and total nitrogen removal efficiency at different HRT. Incomplete denitrification was observed in all HRT applied. With the increase of HRT from 12 to 48 hours, the effluent nitrate concentration decreased from 28.4 to 20.9 mg N/L. Besides nitrate, nitrite was also accumulated in the effluent (Fig.1b). Similar results were reported in the previous study that nitrite can be easily accumulated in the denitrification with sludge fermentation liquid as carbon source (Liu et al., 2016; Cao et al., 2013). The concentration of nitrite in effluent reduced with the increased HRT, which varied from 33.2 mg N/L (HRT of 12 h) to 15.2 mg N/L (HRT 48 h). The nitrogen removal efficiency (NRE) was thus increased with the HRT, which varied from 48.7% at HRT of 12 h to 69.9% at 48 h. The sludge fermentation liquid generally contains a certain amount of ammonia that is released during the hydrolytic acidification process (Zhang and Chen, 2009). The accumulated nitrite in the SAFL driven denitrification provided a new pathway for simultaneous removal of the ammonia and nitrite via combining with the anammox process (Du et al., 2019). However, it was calculated that nitrite reduction rate was higher than that of nitrate (0.4388 mg N/L/h vs. 0.2056 mg N/L/h), indicating nitrite was a more competitive electron acceptor with refractory organics for denitrification (Zhang et al., 2016). In other words, long HRT was not beneficial to the nitrite accumulation. Hence, a relatively low HRT is suggested if considering the combination of anammox process for total nitrogen removal.

The organic matter demand for nitrate reduction to nitrogen gas is 2.86 g COD/g N theoretically. A COD/NO₃⁻-N ratio of 5.0 is generally enough for complete denitrification (Li et al., 2013), the nitrate removal efficiency was as high as 99% in a denitrifying automatic circulate reactor with methanol as carbon source. Nevertheless, in this study, excess nitrate and nitrite remained. Besides, a relatively long reaction time was required. These were likely due to complex organic matter composition in SAFL, which cannot be directly used by denitrifiers. In fact, complete denitrification can be obtained if COD/N ratio is further increased. But the trade-off and consequences should be noted that DOM in the effluent would be even higher. In order to explore the degradability of these

complex organics, detailed organic matter transformation was discussed in the following sections.

Fig.1

3.1.2 Organic matters degradation

The SAFL mainly contained VFAs, PN, and PS (Table S1). The results show VFA was hardly detected in the effluent at all HRT condition, indicating that VFA could be easily utilized by denitrifiers. The concentration of COD, PN, and PS in the effluent at different HRT is shown in Fig.2a. At HRT of 12 h, COD concentration in the effluent was as high as 244.9 mg/L, with the removal efficiency of 49.4% only, which demonstrates large amount of slow degradable and/or refractory organic matter existed in SAFL and effluent. These compounds can be further degraded with the increased HRT. The COD removal efficiency improved to 73.1% at HRT of 48 hours.

Soluble protein and polysaccharides were also found in the effluent. With the increase in HRT, both compounds were gradually reduced, with the removal efficiency being around 70% for both. The results indicate both PN and PS can be further transformed and utilized in the system given longer reaction time. Furthermore, longer HRT was more beneficial to the degradation of PS, as its removal efficiency was largely improved from 24.2% at HRT=12 h to 69.9% at 48 h. In comparison, the removal efficiency of PN increased from 37.0% to 70.3% correspondingly.

Fig.2

3.2 Size-fractionated effluent organics

The organic substance in SAFL was mainly derived from the destruction and solubilization of sludge (Chen et al., 2017; Li et al., 2016), the subvisible particles and colloidal organics were expected to be presented in the SAFL. In order to have a better understanding of the effluent DOM, the size-fractionated organics removal at different HRT was evaluated.

The concentration of organics in terms of COD, PN and PS in the four fractions is displayed in Fig.3. As expected, the denitrification effluent contained relatively high particles and colloidal organics, despite the soluble fraction was dominated. Generally, the SC-COD fraction was higher

than that of LC-COD and P-COD. For example, at the HRT of 12 h, S-COD was 167.5 mg/L, and SC-COD, LC-COD, and P-COD were 112.5, 77.0 and 33.5 mg/L, respectively. In addition, PN and PS were the main organic compounds in the COD fraction of colloids (Fig.3). This likely resulted from the degradation of high molecular weight organics. It was observed, with the increase of HRT, COD, PN, and PS in the four fractions all decreased (Fig.3), suggesting the degradation of organics in particles and colloids could be also enhanced, thus providing the electron donor for denitrification.

Fig.3

The relative proportions of the four fractions were further calculated. It was found the proportions varied with the HRTs (Fig.S3), indicating a cross-transformation of organics among these four fractions may take place. Specially, an obvious reduction of P-COD and LC-COD was observed when the HRT extended from 24 h to 48 h. On the other hand, a corresponding increase of SC-COD and S-COD occurred. Similar results were obtained for the variation of PN and PS. These results clearly demonstrate the occurrence of the transformation of particles and large colloids into smaller colloids and soluble matters.

3.3 Transformation of DOM during batch denitrification experiment

The results show that the variation of nitrogen and organics followed very similar trends regardless of COD/N ratios. Thus, COD/NO₃⁻-N ratio of 5.0 was taken as an example, and the results are showed in Fig.4. Two reaction periods were clearly observed in the 720 min reaction.

VFA, which is a preferred carbon source for denitrification, was reduced rapidly and depleted in 240 mins (period I). The amount of VFA reduced corresponded to 41.5 mg/L COD consumption. While 122.5 mg/L COD was consumed in the 240 mins, this suggests other readily used carbon source was also removed. The concentration of PN was relatively stable when readily COD was present while that of PS was slightly increased (initial 60 min). Following which, PN decreased at consistent rate in the following 480 min (period II), providing the electron donor for denitrification, while there was minor change of PS concentration, indicating PN was easily utilized by denitrifiers

in comparison to PS.

The kinetics of nitrate reduction and organics degradation at different COD/NO₃⁻-N ratios are presented in Table 1. At the COD/NO₃⁻-N of 5.0, a relatively high nitrate reduction rate (0.0545 mg N/L/min) was accompanied with a high organics degradation rate (0.5019 mg COD/L/min) in the initial 240 min (Period I), and a relatively low nitrate reduction (0.0094 mg N/L/min) was obtained with a low organics degradation rate (0.0135 mg COD/L/min) in the following 480 min (Period II). Similar results were also obtained with other COD/NO₃⁻-N ratios (Table 1). These clearly demonstrate that part of organics in SAFL was difficult to degrade under anoxic condition.

Fig. 4

Table 1

3.3.1 Fluorescent spectra of the DOM

Once SAFL was added into the batch reactors, samples were taken at pre-designed time intervals for 3D-EEM analysis. The EEM fluorescence spectra are displayed in Fig. S4, and the peak intensity of compounds are compared in Table 2.

Four peaks were easily identified in the EEM fluorescence spectra (Fig.S2). The first peak was located at excitation/emission wavelengths (Ex/Em) of 280/336–366 nm (Peak A), and the second, third and fourth peaks were detected at Ex/Em of 310-330/402-420 nm (Peak B), 250/444-450 nm (Peak C) and 220/368 nm (Peak D), respectively. According to Chen et al. (2003), Peak A was described as SMP-like substance (tryptophan & protein-like group), Peak B belonged to humic-like substances (HS), Peak C pertained to fulvic acid-like substances (FS) and Peak D generally referred as tyrosine protein-like substances. At initial stage, SMP-like substance (Peak A) and tyrosine protein-like substances (Peak D) were the two abundant components, which was primarily resulted from the SAFL, and produced from cellular lysis and transformed from EPS. The intensity of Peak D was as high as 649.38, while it was hardly detected after 30 mins. This explains the tyrosine protein-like substances were easily transformed. The intensity of Peak A was gradually reduced with

the operation, suggesting the SMP-like substance can be also transformed, but the rate was relatively slow. Peak B and peak C were not detected in initial stage but appeared in the later operation stage. These suggest HS and FS were produced during the reaction. HS and FS are generally correlated to non-biodegradable compounds (Chai et al., 2012; Rodríguez et al., 2014). The generation of these two substances was likely caused by the stabilization of the refractory organic substances through mineralization. It was reported that the fluorescence zone of Peak D from DOM was accessible and easily biodegradable organics; fluorescence zones of peak A was biodegradable compounds in slowly accessible fraction, and fluorescence zones of peak B and peak C were correlated to the least biodegradable and bio-accessible compounds (Jimenez et al., 2014).

In addition, the location of Peak A was gradually blue shifted with the reaction in terms of the emission wavelength. Such shift is normally associated with the structural change of organic substances, such as fragmentation of large molecules into small molecules or decrease of conjugated bonds in a chain structure or aromatic rings (Świetlik and Sikorska, 2004; Cai et al., 2016). A red-shift (longer excitation and emission wavelengths) was observed in Peak B and Peak C. This red-shift can be attributed to the greater apparent molecular weight or to the higher degree of aromatic condensation of humic acids in comparison with fulvic acids (Rodríguez et al., 2014).

Table 2

3.3.2 LC-OCD-OND fractions of the DOM

EEM technique can only detect fluorescence active compounds, whereas other components such as polysaccharides cannot be detected. Therefore, a characterization by LC-OCD-OND was performed to obtain more fractions of DOMs. The evolution of the sub-fractions of the organic substances during the denitrification is displayed in Fig.5.

Most of the sub-fractions of the DOMs decreased during the denitrification process. Remarkable reduction can be found in HO-DOC, LMW neutral, LMW acids, and LMW PS. This is reasonable that LMW compounds are generally easier to be removed compared to HMW compounds.

It should be noted that the concentration of HO-DOC and LMW-PS was found to increase at the beginning. This may result from the degradation of the HMW substances, such as the building blocks, biopolymers. It was found the degradation of the building blocks, HMW-PN, HMW-PS and LMW-PN showed a linear regression during the whole reaction period. It seemed the degradation rates of above compounds were limited by certain process, likely hydrolysis. After 240 mins reaction, the degradation rate of HO-DOC slowed down and the concentration of LMW-PS and LMW neutrals even increased. This is in corresponding to the turning point of denitrification (Fig. 4), which indicates some portion of newly produced LMW-PS and LMW neutrals may not be biodegradable. Thus, LMW-PS and LMW neutrals were the major compounds remained at the end of the denitrification. These compounds were further evaluated in the long-term experiment to track their transformation and trends.

Fig. 5

3.4 Effect of HRT on the rDOM in the parent reactor effluent

The short-term batch experiment results demonstrate a large amount of DOM stayed in denitrification effluent. A long HRT generally could enhance the denitrification, and thus the degradation of organic matter. In this study, the composition of the DOM in denitrification effluent was identified, and the fate of these organic matter in various HRT was also investigated.

3.4.1 Fluorescent spectra of the rDOM

The three-dimensional EEM spectra of the rDOM in the SBR effluent at different HRTs are displayed in Fig.S5. The fluorescence parameters including specific peak location and fluorescence intensity of the three peaks identified at different HRTs are summarized in Table 3. The intensity of Peak A was found to gradually decrease with the increase of HRTs, suggesting a longer reaction time can enhance the degradation of SMP-like substance. The intensity of Peak B and Peak C, which represent HS and FS compounds, increased with the HRTs, which was opposite to other organics. This was likely attributed to the transformation of other refractory organics over longer reaction time.

350 And the peak D of tyrosine protein-like substances, which was rich in the SAFL was not detected,
351 confirming its easy biodegradability. These results are in line with the batch tests.

352 **Table 3**

353 **3.4.2 LC-OCD-OND fractions of rDOM at different HRTs**

354 The rDOM was further investigated with more specific fractions revealed by LC-OCD-OND.
355 The results present that building blocks, which are the by-product of HS degradation, were dominated
356 in the rDOM (43.79%-48.78%), followed by the HMW-PN (13.37%-17.39%), HMW-PS
357 (12.84%-15.9%), LMW neutrals (11.28%-13.65%), HO-DOC (8.0%-12.62%), and LMW acids
358 (0.57%-1.11%) (Fig.6). They were maintained at a relatively stable proportions under different HRTs.
359 LMW acids were found to very low across the operation period. It should be noted that the result is
360 different from previously reported in anaerobic sludge digestion system, which indicates the
361 components of residual DOMs were mainly HS, HO-DOC and LMW neutrals (Lu et al., 2018; Li et
362 al., 2016). This suggests the hydrolysis step on HMW compounds under anoxic condition was not as
363 effective as anaerobic condition.

364 Fig.7 displays the variation of the sub-fractions of rDOM at different HRTs. The concentration
365 of HO-DOC in the effluent maintained stable regardless of HRTs, which suggests the prolonged
366 reaction time would not enhance the degradation of HO-DOC. This result is in line with Fig.5, which
367 also shows HO-DOC was not further utilized by denitrifier over a certain time. Building blocks can
368 be reduced with a relatively long HRT of 12-24 h. However, the further increased HRT to 48 h did
369 not show positive effect on the reduction. Similar phenomenon was also observed with the LMW
370 neutrals and LMW-PS, suggesting further extended reaction time was not beneficial for their removal.
371 With respect to the biopolymers, HMW-PN, HMW-PS, and LMW-PN gradually decreased with the
372 increase of HRTs.

373 **Fig.6**

374 **Fig.7**

Besides, it was found that the building blocks was relatively easy to be degraded under the anoxic condition with longer HRTs. Its degradation rate was -0.58 mg C/L/h, which was higher than that of LMW-PS (-0.43 mg C/L/h), LMW-PN (-0.29 mg C/L/h), LMW neutrals (-0.22 mg C/L/h), HMW-PN (-0.12 mg C/L/h), and HMW-PS (-0.1 mg C/L/h). It is widely believed that the organic matter with high molecular weight generally contributes a low metabolic activity. Building blocks were mainly in the molecular weight range of 300-500 Da, which is likely the main reason for a relatively high removal rate. In comparison, HMW-PN and HMW-PS had a molecular weight far more than 20 kDa, thus a relatively low removal rates were realized for those compounds. The degradation of HMW-PS was slower than that of HMW-PN, and similar results were also found in previous reported anaerobic digestion systems (Lu et al., 2018). This is likely due to the more complex structure of HMW-PS than that of HMW-PN.

This study clearly demonstrated a great deal of refractory dissolved organic matters could not be reduced by anoxic denitrification. Non-/low- degradation of HS and FS was discovered and they were accumulated during the anoxic denitrification. Building blocks was the major component in rDOM, followed by the HMW-PN, HMW-PS, LMW neutrals, HO-DOC, and LMW neutrals. Prolonged HRT could further reduce certain effluent organics, such as the biopolymers, HMW-PN, HMW-PS and LMW-PN (Fig.7), and induce the transformation of particulate and colloidal organics to the soluble form (Fig.S3). However, the total organics removal efficiency was still low. Furthermore, the longer HRT led to the accumulation of HS and FS (Table 3).

The SAFL can act as an alternative carbon source for municipal or industrial nitrate contained wastewater treatment via biological denitrification. However, in order to have complete denitrification, higher COD/N ratio should be considered. The concentration of COD and refractory organics in effluent is thus high. Based on the above results, a subsequent advanced treatment may be adopted to further decrease the effluent organics and meet the strict discharge standard. A series of advanced wastewater processes, such as coagulation, membrane process, ozonation, and advanced

oxidation processes (AOPs), have been used to reduce the effluent organics (Shon et al., 2006; Chen et al., 2017; Ly et al., 2018; Meng et al., 2011). Among these approaches, membrane process has currently received an increasing interest due to its obvious benefits (i.e. the higher effluent quality) (Guo et al., 2012; Shon et al., 2006). This study revealed the denitrification effluent contained a large amount of particulate and colloidal organics, as well as biomacromolecules (BMM) (i.e. HMW-PN, HMW-PS), which could be effectively rejected by the membrane. Soluble organic matters can also be reduced by MBR under aerobic conditions. In addition, if partial denitrification is allowed, the accumulated nitrite in the SAFL driven denitrification may provide a new pathway for simultaneous removal of the ammonia and nitrite via combining with the anammox process. In this case, remaining organic matters can be converted. Further studies are required to develop new sustainable approach to integrate all the factors.

4. Conclusions

The effluent from SAFL driven denitrification process contained a great deal of refractory organics, with building blocks most dominated (43.79% - 48.78% of DOM). Prolonged HRT could enhance the anoxic degradation of refractory organics, improve total nitrogen removal and solubilization of particulate organics. It was revealed that building blocks showed a relatively higher degradation rate than that of LMW-PS, followed by LMW-PN, LMW neutrals, HMW-PN, and HMW-PS during denitrification. However, the accumulation of humic-like and fulvic acid-like substances was discovered with increased HRT. Moreover, increasing the HRT may not be a cost-effective approach to further degrade the refractory organics. A subsequent advanced treatment should be considered.

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455 **References**

- 456 American Public Health Association, 1998. Standard Methods for the Examination of Water and Wastewater, twentieth
457 ed. Washington, DC, USA.
- 458 Cai, W., Liu, J., Zhang, X., Ng, W.J., Liu, Y., 2016. Generation of dissolved organic matter and byproducts from
459 activated sludge during contact with sodium hypochlorite and its implications to on-line chemical cleaning in
460 MBR. *Water Res.* 104, 44-52.
- 461 Cao, S., Wang, S., Peng, Y., Wu, C., Du, R., Gong, L., Ma, B., 2013. Achieving partial denitrification with sludge
462 fermentation liquid as carbon source: The effect of seeding sludge. *Bioresour. Technol.* 149, 570-574.
- 463 Chai, X., Liu, G., Zhao, X., Hao, Y., Zhao, Y., 2012. Fluorescence excitation-emission matrix combined with regional
464 integration analysis to characterize the composition and transformation of humic and fulvic acids from landfill at
465 different stabilization stages. *Waste Manag.* 32, 438-47.
- 466 Chen, W., Westerhoff, P., Leenheer, J.A., Booksh, K., 2003. Fluorescence Excitation–Emission Matrix Regional
467 Integration to Quantify Spectra for Dissolved Organic Matter. *Environ. Sci. Technol.* 37, 5701-5710.
- 468 Chen, Y., Jiang, S., Yuan, H., Zhou, Q., Gu, G., 2007. Hydrolysis and acidification of waste activated sludge at
469 different pHs. *Water Res.* 41, 683-689.
- 470 Chen, Y., Jiang, X., Xiao, K., Shen, N., Zeng, R.J., Zhou, Y., 2017. Enhanced volatile fatty acids (VFAs)
471 production in a thermophilic fermenter with stepwise pH increase – Investigation on dissolved organic matter
472 transformation and microbial community shift. *Water Res.* 112, 261-268.
- 473 Chen, Z., Li, M., Wen, Q., Ren, N., 2017. Evolution of molecular weight and fluorescence of effluent organic
474 matter (EfOM) during oxidation processes revealed by advanced spectrographic and chromatographic tools.
475 *Water Res.* 124, 566-575.
- 476 Du, R., Cao, S., Li, B., Zhang, H., Wang, S., Peng, Y., 2019. Synergy of partial-denitrification and anammox in
477 continuously fed upflow sludge blanket reactor for simultaneous nitrate and ammonia removal at room
478 temperature. *Bioresour. Technol.* 274, 386-394
- 479 Du, R., Peng, Y., Cao, S., Li, B., Wang, S., Niu, M., 2015. Mechanisms and microbial structure of partial denitrification
480 with high nitrite accumulation. *Appl. Microbiol. Biot.* 100(4) 2011-2021.
- 481 Fernández-Nava, Y., Marañón, E., Soons, J., Castrillón, L., 2010. Denitrification of high nitrate concentration
482 wastewater using alternative carbon sources. *J. Hazard. Mater.* 173, 682-688.

483 Guo, W., Ngo, H.H., Li, J., 2012. A mini-review on membrane fouling. *Bioresour. Technol.* 122, 27-34.

484 Guo, Y., Guo, L., Sun, M., Zhao, Y., Gao, M., She, Z., 2017. Effects of hydraulic retention time (HRT) on
485 denitrification using waste activated sludge thermal hydrolysis liquid and acidogenic liquid as carbon sources.
486 *Bioresour. Technol.* 224, 147-156.

487 He, W., Chen, M., Park, J., Hur, J., 2016. Molecular diversity of riverine alkaline-extractable sediment organic
488 matter and its linkages with spectral indicators and molecular size distributions. *Water Res.* 100, 222-231.

489 Hu, Z., Chandran, K., Smets, B.F., Grasso, D., 2002. Evaluation of a rapid physical-chemical method for the
490 determination of extant soluble COD. *Water Res.* 36, 617-24.

491 Huber, S.A., Balz, A., Abert, M., Pronk, W., 2011. Characterisation of aquatic humic and non-humic matter with
492 size-exclusion chromatography--organic carbon detection--organic nitrogen detection (LC-OCD-OND). *Water*
493 *Res.* 45, 879-85.

494 Jacquin, C., Lesage, G., Traber, J., Pronk, W., Heran, M., 2017. Three-dimensional excitation and emission matrix
495 fluorescence (3DEEM) for quick and pseudo-quantitative determination of protein- and humic-like substances
496 in full-scale membrane bioreactor (MBR). *Water Res.* 118, 82-92.

497 Jimenez, J., Gonidec, E., Cacho Rivero, J.A., Latrille, E., Vedrenne, F., Steyer, J., 2014. Prediction of anaerobic
498 biodegradability and bioaccessibility of municipal sludge by coupling sequential extractions with fluorescence
499 spectroscopy: Towards ADM1 variables characterization. *Water Res.* 50, 359-372.

500 Li, D., Zhou, Y., Tan, Y., Pathak, S., Majid, M.B.A., Ng, W.J., 2016. Alkali-solubilized organic matter from sludge and
501 its degradability in the anaerobic process. *Bioresour. Technol.* 200, 579-586.

502 Li, W., Zheng, P., Wang, L., Zhang, M., Lu, H., Xing, Y., Zhang, J., Wang, R., Song, J., Ghulam, A., 2013.
503 Physical characteristics and formation mechanism of denitrifying granular sludge in high-load reactor.
504 *Bioresour. Technol.* 142, 683-687.

505 Li, X., Chen, H., Hu, L., Yu, L., Chen, Y., Gu, G., 2011. Pilot-Scale Waste Activated Sludge Alkaline
506 Fermentation, Fermentation Liquid Separation, and Application of Fermentation Liquid To Improve
507 Biological Nutrient Removal. *Environ. Sci. Technol.* 45, 1834-1839.

508 Liu, F., Tian, Y., Ding, Y., Li, Z., 2016. The use of fermentation liquid of wastewater primary sedimentation sludge
509 as supplemental carbon source for denitrification based on enhanced anaerobic fermentation. *Bioresour.*
510 *Technol.* 219, 6-13.

511 Liu, H., Han, P., Liu, H., Zhou, G., Fu, B., Zheng, Z., 2018. Full-scale production of VFAs from sewage sludge by

512 anaerobic alkaline fermentation to improve biological nutrients removal in domestic wastewater.
513 *Bioresour.Technol.* 260, 105-114.

514 Lu, D., Xiao, K., Chen, Y., Soh, Y.N.A., Zhou, Y., 2018. Transformation of dissolved organic matters produced
515 from alkaline-ultrasonic sludge pretreatment in anaerobic digestion: From macro to micro. *Water Res.* 142,
516 138-146.

517 Lu, H., Chandran, K., Stensel, D., 2014. Microbial ecology of denitrification in biological wastewater treatment.
518 *Water Res.* 64, 237-254.

519 Ly, Q.V., Nghiem, L.D., Cho, J., Hur, J., 2018. Insights into the roles of recently developed coagulants as
520 pretreatment to remove effluent organic matter for membrane fouling mitigation. *J. Membr. Sci.* 564, 643-652.

521 Meng, F., Zhou, Z., Ni, B., Zheng, X., Huang, G., Jia, X., Li, S., Xiong, Y., Kraume, M., 2011. Characterization of
522 the size-fractionated biomacromolecules: Tracking their role and fate in a membrane bioreactor. *Water Res.* 45,
523 4661-4671.

524 Modin, O., Fukushi, K., Yamamoto, K., 2007. Denitrification with methane as external carbon source. *Water Res.*
525 41, 2726-38.

526 Raunkjaer, K., Hvitved-Jacobsen, T., Nielsen, P.H., 1994. Measurement of pools of protein, carbohydrate and lipid
527 in domestic waste-water. *Water Res.* 28(2), 251-262.

528 Rodríguez, F.J., Schlenger, P., García-Valverde, M., 2014. A comprehensive structural evaluation of humic
529 substances using several fluorescence techniques before and after ozonation. Part I: Structural characterization
530 of humic substances. *Sci. Total. Environ.* 476-477, 718-730.

531 Shon, H.K., Vigneswaran, S., Snyder, S.A., 2006. Effluent organic matter (EfOM) in wastewater: Constituents,
532 effects, and treatment. *Crit. Rev. Env. Sci. Tec.* 36, 327-374.

533 Sun, J., Sun, M., Guo, L., Zhao, Y., Gao, M., She, Z., 2016. The effects of denitrification with sludge alkaline
534 fermentation liquid and thermal hydrolysis liquid as carbon sources. *Rsc. Adv.* 6, 72333 - 72341.

535 Świetlik, J., Sikorska, E., 2004. Application of fluorescence spectroscopy in the studies of natural organic matter
536 fractions reactivity with chlorine dioxide and ozone. *Water Res.* 38, 3791-3799.

537 Wang, M., Chen, Y., 2018. Generation and characterization of DOM in wastewater treatment processes.
538 *Chemosphere* 201, 96-109.

539 Wu, H., Gao, J., Yang, D., Zhou, Q., Liu, W., 2010. Alkaline fermentation of primary sludge for short-chain fatty
540 acids accumulation and mechanism. *Chem. Eng. J.* 160, 1-7.

541 Wu, H., Yang, D., Zhou, Q., Song, Z., 2009. The effect of pH on anaerobic fermentation of primary sludge at room
542 temperature. *J. Hazard. Mater.* 172, 196-201.

543 Zhang, C., Chen, Y., 2009. Simultaneous Nitrogen and Phosphorus Recovery from Sludge-Fermentation Liquid
544 Mixture and Application of the Fermentation Liquid To Enhance Municipal Wastewater Biological Nutrient
545 Removal. *Environ. Sci. Technol.* 43, 6164-6170.

546 Zhang, H., Jiang, J., Li, M., Yan, F., Gong, C., Wang, Q., 2016. Biological nitrate removal using a food
547 waste-derived carbon source in synthetic wastewater and real sewage. *J. Environ. Manage.* 166, 407-13.

548 Zhang, Q., Vlaeminck, S.E., DeBarbadillo, C., Su, C., Al-Omari, A., Wett, B., Pümpel, T., Shaw, A., Chandran, K.,
549 Murthy, S., De Clippeleir, H., 2018. Supernatant organics from anaerobic digestion after thermal hydrolysis
550 cause direct and/or diffusional activity loss for nitrification and anammox. *Water Res.* 143, 270-281.

551 Zhang, Y., Wang, X.C., Cheng, Z., Li, Y., Tang, J., 2016. Effect of fermentation liquid from food waste as a carbon
552 source for enhancing denitrification in wastewater treatment. *Chemosphere* 144, 689-696.

553 Zhao, J., Wang, D., Li, X., Yang, Q., Chen, H., Zhong, Y., Zeng, G., 2015. Free nitrous acid serving as a
554 pretreatment method for alkaline fermentation to enhance short-chain fatty acid production from waste
555 activated sludge. *Water Res.* 78, 111-20.

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Tables and Figures captions.

Table 1 Nitrate reduction and organics degradation kinetics during the anoxic denitrification using the first-order kinetic equation.

Table 2 Fluorescence spectral parameters of the samples taken in the denitrification process at a different time point.

Table 3 Fluorescence spectral parameters of the effluent samples at different HRTs.

Fig.1 Performance of denitrification with SAFL as carbon source at different HRT. (a) mean effluent nitrate concentration, (b) mean effluent nitrite concentration and (c) total N removal efficiency.

Fig.2 Variation of (a) effluent COD, PN, and PS concentration and (b) removal efficiency of COD, PN, and PS at different HRT.

Fig.3 Concentration of COD, PN and PS in the size-fractionated effluent at different HRTs. (a) HRT=12 h, (b) HRT=16 h, (c) HRT=24 h and (d) HRT=48 h.

Fig. 4 Evolution of (a) nitrate, nitrite, COD, and (b) tVFAs, PN and PS in denitrification at COD/NO₃⁻-N=5.0.

Fig. 5 Evolution of the sub-fractions of the organic substances during the denitrification. (a) HO-DOC, (b) building blocks, (c) LMW neutrals, (d) LMW acids, (e) Biopolymers, (f) HMW-PN, (g) HMW-PS, (h) LMW-PN and (i) LMW-PS.

Fig.6 Relative proportions of the sub-fractions of effluent rDOM. (a) HRT=12 h, (b) HRT=16 h, (c) HRT=24 h, (d) HRT=48 h.

Fig.7 Variation of the sub-fractions of effluent rDOM at different HRTs.

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Table 1 Nitrate reduction and organics degradation kinetics during the anoxic denitrification using the first-order kinetic equation.

COD/NO ₃ ⁻ -N	Period I			Nitrite concentration (mg N/L) ^c	Period II		
	Time (min)	r _{NO3} ^a	r _{COD} ^b		Time (min)	r _{NO3} ^a	r _{COD} ^b
5.0	0-240	0.0545	0.5019	12.94	240-720	0.0094	0.0135
7.5	0-240	0.1129	0.549	14.98	240-720	0.0101	0.0381
10.0	0-360	0.1321	0.5788	20.26	360-720	0.0107	0.0487
15.0	0-360	0.1608	0.8538	21.02	-	-	-

^a r_{NO3} represented the nitrate reduction rate (mg N/L/min);

^b r_{COD} represented the COD degradation rate (mg COD/L/min);

^c Denoted the maximum accumulation amount during the whole denitrification.

Table 2 Fluorescence spectral parameters of the samples taken in the denitrification process at a different time point.

Time (min)	Peak A		Peak B		Peak C		Peak D	
	Ex/Em	Intensity	Ex/Em	Intensity	Ex/Em	Intensity	Ex/Em	Intensity
0	280/362	417.85	-	-	-	-	220/368	649.38
30	280/362	370.86	-	-	-	-	-	-
60	280/366	355.30	-	-	-	-	-	-
120	280/366	333.72	-	-	-	-	-	-
240	280/362	287.19	310/402	71.43	-	-	-	-
360	280/360	178.10	320/412	44.57	-	-	-	-
480	280/336	77.66	330/418	45.72	250/444	51.21	-	-
720	280/336	59.00	330/420	43.52	250/450	49.49	-	-

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Table 3 Fluorescence spectral parameters of the effluent samples at different HRTs.

HRT	Peak A		Peak B		Peak C	
	Ex/Em	Intensity	Ex/Em	Intensity	Ex/Em	Intensity
12h	280/340	309.03	340/434	246.56	250/458	255.45
16h	280/342	225.25	340/424	274.18	250/460	273.70
24h	280/340	205.12	340/426	264.70	250/462	274.36
48h	280/342	145.81	340/426	268.43	250/456	281.93

Fig.1 Performance of denitrification with SAFL as carbon source at different HRT. (a) mean effluent nitrate concentration, (b) mean effluent nitrite concentration and (c) total N removal efficiency.

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Fig. 5 Evolution of the sub-fractions of the organic substances revealed by LC-OCD-OND during the denitrification.

Fig.6 Relative proportions of the sub-fractions of effluent rDOM revealed by LC-OCD-OND. (a) HRT=12 h, (b) HRT=16 h, (c) HRT=24 h, (d) HRT=48 h.

Fig.7 Variation of the sub-fractions of effluent rDOM revealed by LC-OCD-OND at different HRTs.