Endogenous respiration process analysis between aMBR and UV/O_3-aMBR for polluted surface water treatment

Lu Li, Kang Song and Chettiyappan Visvanathan

ABSTRACT

The microbial endogenous respiration process is very important in biological water treatment processes. This study analyzed and compared the endogenous respiration process in an attached growth membrane bioreactor (aMBR) system and a UV/O_3 integrated aMBR system (UV/O_3-aMBR) in treating polluted surface water with COD_{Mn} around 10 mg/L. The endogenous respiration activity of heterotrophic microbes and autotrophic nitrifiers activity in both systems was analyzed and compared. Results show that heterotrophic bacteria and autotrophic nitrifiers enter endogenous respiration at 6 h aeration in an aMBR and 0 h in a UV/O_3-aMBR system. Biomass amount on PVA-gel in aMBR was higher than in UV/O_3-aMBR in terms of specific respiration rates $SOUR_t$, $SOUR_H$, and $SOUR_A$. Substrate remained on PVA-gel in the aMBR system, but no substrate remained on PVA-gel in the UV/O_3-aMBR system. Higher species of microbes, including recoverable and irrecoverable components, existed in the aMBR system as compared to the UV/O_3-aMBR system. The UV/O_3-aMBR system could make full use of the advanced oxidation process (AOP) and biological process, leading to a higher treatment performance, and has the potential to mitigate total energy demand. Thus, the UV/O_3-aMBR system can be used as a new technology for treating polluted surface water with the co-contribution of biological process and AOP treatment.

Key words | attached growth membrane bioreactor (aMBR), endogenous respiration, polluted surface water, respiration map, UV/O_3-aMBR

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INTRODUCTION

Surface water quality is facing increasing organic contamination due to human activities worldwide (Ma *et al.* 2018; Utaaker *et al.* 2019). Overload of organic matter pollution in surface water leads to challenges during treatment using conventional drinking water treatment plants. These challenges include higher consumption of coagulants and higher disinfection by-product formation potential. The water treatment technology should be able to treat the polluted surface water and reach the drinking water quality standard, which is a big challenge for the world's drinking water supply. Table 1 demonstrates the drinking water national standards of some selected countries and institutions. Improving current wastewater treatment technology or developing high efficiency water treatment technology can be the solution to the polluted surface water issue. A previous study has reported that in an attached growth membrane bioreactor (aMBR) for treating polluted surface water with COD_{Mn} around 10 mg/L, over 50% COD_{Mn} removal was achieved (Li *et al.* 2017). The aMBR system works as an enhanced natural purification process, and polyvinyl alcohol gel (PVA-gel) was used as a bio-carrier to immobilize bacteria for the degradation of organic pollutants from polluted surface water. The aMBR system was operated without adding activated sludge and,

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Table 1 | Drinking water quality index of different countries

	Water Quality Convention Index and Limiting Value							
Country/Organization	**NO_3-N (mg/L)**	**NO_2-N (mg/L)**	**TOC (mg/L)**	**Turbidity (NTU)**	**TDS (mg/L)**	**COD_{Mn} (mg/L)**	**NH_3-N (mg/L)**	**pH**
China	10	1	5	1	1,000	5	0.5	6.5–8.5
US-EPA	10	1	–	0.5	500	–	–	6.5–8.5
Japan	10		3	2	500	3	–	5.8–8.6
EC	10		–	1	–	5	0.5	–
WHO	–	–		10	–	–	1.5	6.5–8.5
Australia	3		–	5	600	–	0.5	6.5–8.5

EC, European Commission.

as a result, membrane fouling was largely mitigated, and PVA-gel was used as the main contributor to organic removal in the system. In order to improve the recalcitrant organic compounds' removal, advanced oxidation processes (AOPs) were integrated into the aMBR system as a polishing step (UV/O_3-aMBR), to partially degrade recalcitrant pollutant and improve its biodegradability.

The organic loading rate in the aMBR and UV/O_3-aMBR system was much lower than in conventional wastewater treatment plants, because the COD_{Mn} concentration was only around 10 mg/L. The endogenous respiration of bioprocess with higher organic loading rate, such as wastewater, was reported by earlier studies (Fall *et al.* 2014; Li *et al.* 2014, 2018a; Ordaz *et al.* 2017; Park *et al.* 2017). The bioprocess in the aMBR system is expected to be quite different from conventional activated sludge processes or other biological treatment processes. The AOPs combined with an aMBR system could also lead to changes in substrate utilization and microbe activities. In addition, the bioprocess between aMBR and UV/O_3-aMBR is expected to be quite different and thus needs to be compared and analyzed.

Bioprocess aerobic respiration commonly includes exogenous respiration and endogenous respiration (Hao *et al.* 2010). Endogenous respiration always occurs once no substrate remains for the microbes to use, and the active cell starts utilizing intracellular substances (Gujer *et al.* 1999). Endogenous respiration has been commonly regarded as an evaluation method for active microorganisms, and can be tested quickly and simply with oxygen uptake rate methods (Spanjers & Vanrolleghem 1995). The widely used oxygen uptake rate analysis method is one type of respirometry method, which detects the dissolved oxygen (DO) concentration with a DO meter providing real-time data, to intuitively reflect the substrate utilization by microbes (Blok 1976; Chandran & Smets 2005; Capodici *et al.* 2014; Li *et al.* 2018b).

There has been limited research on the membrane bioreactor (MBR) in treating polluted surface water, let alone the biological process in the MBR in this sector. This study analyzed and compared the endogenous respiration process in aMBR and UV/O_3-aMBR systems in treating polluted surface water by the respirometry method. The microbe activities, including heterotrophic and autotrophic entry time of endogenous respiration, and ability in anti-decay, were also compared and discussed. This study could provide useful information for the understanding and practical application of aMBR and UV/O_3-aMBR processes in treating polluted surface water for drinking purposes in a more environmentally friendly way.

MATERIALS AND METHODS

System setup

A schematic and flowchart of aMBR and UV/O_3-aMBR parallel operation system setup are shown in Figure 1(a) and 1(b), respectively. Polyvinyl alcohol gel (PVA-gel) was used as a bio-carrier with filling ratio of 5% (volume to volume, V/V) in the carrier side of both systems. Hollow fiber PTFE UF membrane with pore size 0.1 μm and surface area 0.1 m^2 was submerged in the membrane side. Hydraulic retention time (HRT) and flow rates controlled in both systems were 2.5 h and 2 L/h, respectively. The aMBR and

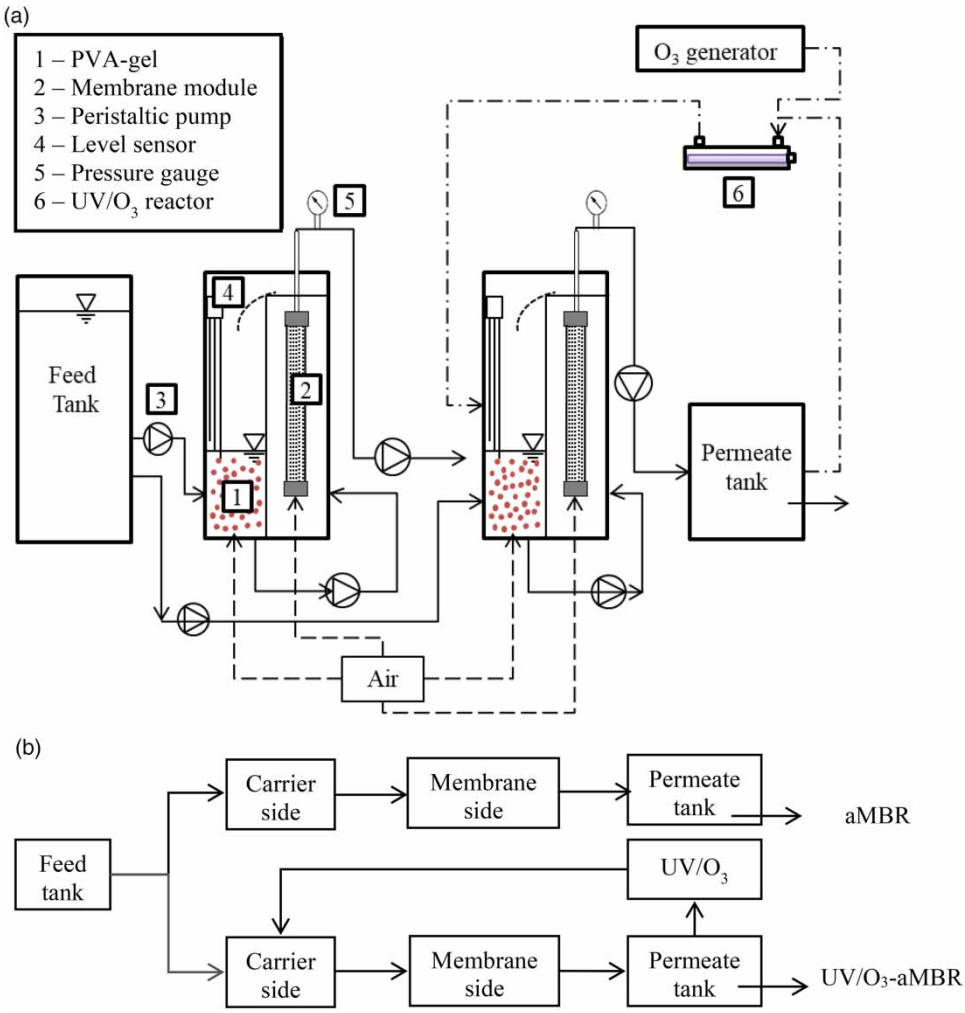

Figure 1 | Schematic (a) and flowchart (b) of aMBR and UV/O_3-aMBR parallel systems.

UV/O_3-aMBR parallel system inlet and permeate pump were maintained by a timer (8 min on and 2 min off). The transmembrane pressure (TMP) of both systems was recorded by a digital pressure gauge. The UV/O_3-aMBR system consisted of an aMBR system with UV/O_3 set in a recirculation stream as a polishing step, as shown in Figure 1(a). aMBR permeate with a recirculation ratio of 60% was pumped into the UV/O_3 reactor to be partially treated and then sent back to the aMBR carrier side. The contact time in the UV/O_3 reactor was based on optimal biodegradable organic carbon (BDOC) results as 7 min. UV light 8 W with a wavelength of 254 nm was used and an O_3 generator (Nippon Ozone Co., Ltd, Japan) with concentration of 1.5 mg/L was used.

Experimental materials

PVA-gel used as a bio-carrier in both systems was taken out for endogenous respiration analysis from the stable operated aMBR and UV/O_3-aMBR system, respectively. The removal performance of basic parameters including COD_{Mn}, DOC, UV_{254}, specific UV absorbance (SUVA), and total nitrogen (TN) was tested before taking out the PVA-gel from both systems. The suspended solids concentration in the carrier side of both systems was less than 100 mg/L and was not taken into consideration for endogenous analysis.

A respirometer was set up with an automatically recording DO meter (HQ40d, HACH, USA) and in a room at constant temperature; the schematic of the system is as

shown in Figure 2. During the oxygen uptake rate test, 50 mg/L NH_4Cl was used as the nitrogen source and 100 mg/L CH_3COONa was used as the carbon source. DO values were automatically recorded every 30 seconds for 15 min in all conditions tested. The temperature in all tests was maintained at 25.7 ± 0.2 °C. The pH was 7.79 ± 0.04 in the aMBR system and 7.90 ± 0.03 in the UV/O_3-aMBR system.

Analytical methods

Respirometric analysis calculation

A respirometric method with a DO meter recording the DO concentration changes in the aqueous system was used. Oxygen uptake rate was calculated from the DO values. The PVA-gel bio-carrier amount in each condition was counted after the test, and the specific oxygen uptake rate was calculated accordingly. The respiration rate was composed of exogenous respiration and endogenous respiration, among which, exogenous respiration included autotrophic respiration and heterotrophic respiration as is shown in Equation (1):

$$OUR_T = OUR_{en} + OUR_A + OUR_H \quad (1)$$

Equation (1) divided with OUR_T can produce Equation (2):

$$1 = \frac{OUR_{en}}{OUR_T} + \frac{OUR_A}{OUR_T} + \frac{OUR_H}{OUR_T} \quad (2)$$

where the OUR_T is theoretic total respiration rate, OUR_{en} is real tested endogenous respiration rate, OUR_A is real tested autotrophic respiration rate, OUR_H is real tested heterotrophic respiration rate, and OUR_t is real tested total respiration rate. OUR_{en}/OUR_T represents the ratio of endogenous respiration to the theoretical total respiration rate, while OUR_A/OUR_T represents the ratio of autotrophic respiration to the theoretical total respiration rate, and OUR_H/OUR_T represents the ratio of heterotrophic respiration to the theoretical total respiration rate. $SOUR_T$ is specific total respiration rate, $SOUR_{en}$ is specific endogenous respiration rate, $SOUR_A$ is specific autotrophic respiration rate, and $SOUR_H$ is specific heterotrophic respiration rate.

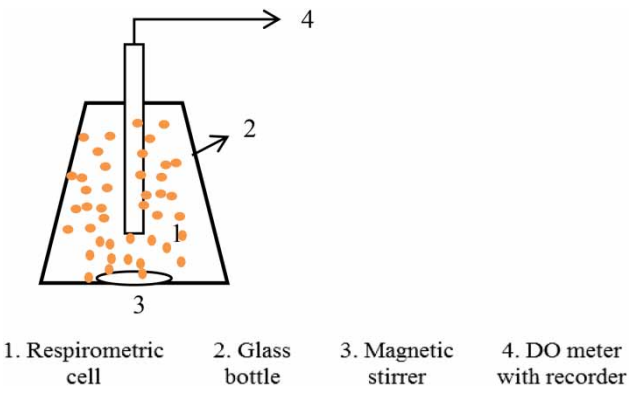

Figure 2 | Schematic of respirometer used in this study.

$$SOUR = OUR/amount\ of\ PVA - gel\ tested\ (pcs) \quad (3)$$

Respirometric test setup

One hundred and forty milliliters of PVA-gel were sampled from the aMBR and UV/O_3-aMBR systems, of which 20 mL from each system was taken out for testing at an aeration time of 0 h, and the remaining 120 mL PVA-gel was placed in 1.5 L of reverse osmosis (RO) water and aerated in an aeration vessel separately. After that, 20 mL PVA-gel was sampled from the aeration vessel for testing after 6, 12, 24, 48, 96, and 120 h of aeration. The aeration time of 6 to 120 h was selected based on earlier research indicating that activated sludge enters endogenous respiration in 1 h to 5 days (Henze *et al.* 2000; Friedrich & Takács 2013).

The respiration test for one set of aeration time was carried out as follows. (1) 20 mL carrier was put into 400 mL RO water in a respirometer, and OUR_{en} was tested in this situation. (2) The solution in the respirometer was then discharged and the respirometer filled with 400 mL RO water. Ten mL of 2,000 mg/L NH_4Cl was then added to obtain a concentration of 50 mg/L, and OUR_N was then tested. (3) The solution in the respirometer was then discharged and the respirometer filled with 400 mL RO water. Ten mL of 4,000 mg/L CH_3COONa was added to obtain 100 mg/L of a CH_3COONa concentration, after which OUR_c was then tested. (4) The solution in the respirometer was discharged and it was filled with 400 mL RO water, 10 mL of 4,000 mg/L CH_3COONa and 10 mL of 2,000 mg/L NH_4Cl was added to obtain 100 mg/L CH_3COONa concentration and a NH_4Cl concentration of 50 mg/L, then

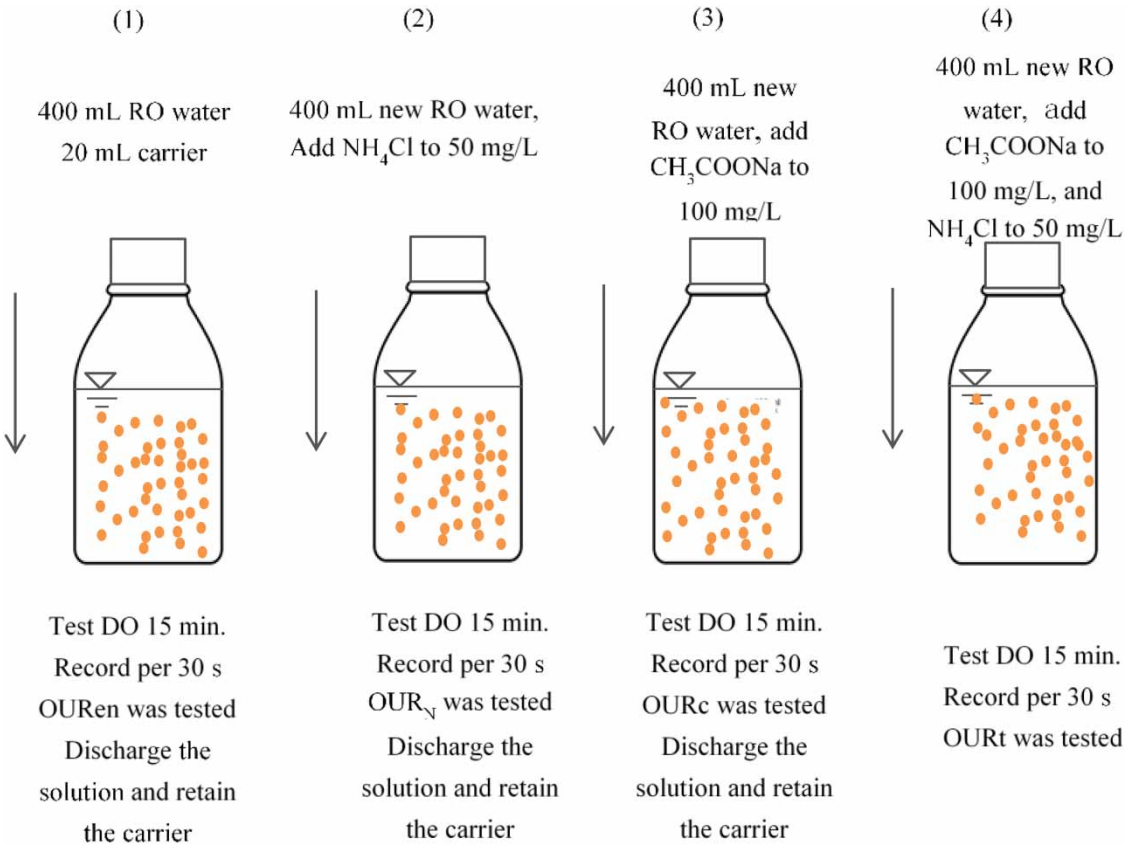

Figure 3 | Respiration map testing procedure for one cycle.

OUR_t was tested. The flowchart of this process is shown in Figure 3.

OUR_{en} was obtained when no substrates were added, OUR_N was obtained when only nitrogen source was added, OUR_c was obtained when only carbon source was added, and OUR_t was obtained when both nitrogen source and carbon source were added:

$$OUR_N = OUR_{en} + OUR_A \quad (4)$$

$$OUR_c = OUR_{en} + OUR_H \quad (5)$$

Due to the low organic loading rate at around 0.096 kg·COD_{Mn}/(m^3 d) in feed, with corresponding low biomass in both systems, the unit of *SOUR* value used in this study was mg/L d pcs, as shown in Figures 4 and 5. To maintain the accuracy of the analysis, *SOUR* was used instead of *OUR*. Even though 20 mL of PVA-gel as carrier was used for testing in all conditions, the accurate amount was counted (as pcs) after each test and used as specific volume parameter to minimize the error.

RESULTS AND DISCUSSION

The removal performance of aMBR and UV/O_3-aMBR systems

The removal performance of the stable operated aMBR and UV/O_3-aMBR system is shown in Table 2. The UV/O_3-aMBR system has higher removal performance, including both COD_{Mn}, DOC, UV_{254}, SUVA, and TN as compared with the aMBR system. With the adding of UV/O_3 as a polish step in the recirculation stream of the aMBR system, the removal performance of recalcitrant organic matters was largely increased, as shown by the UV_{254} results. The SUVA index shows that UV/O_3-aMBR system-treated water has higher hydrophilicity compared with the aMBR system,

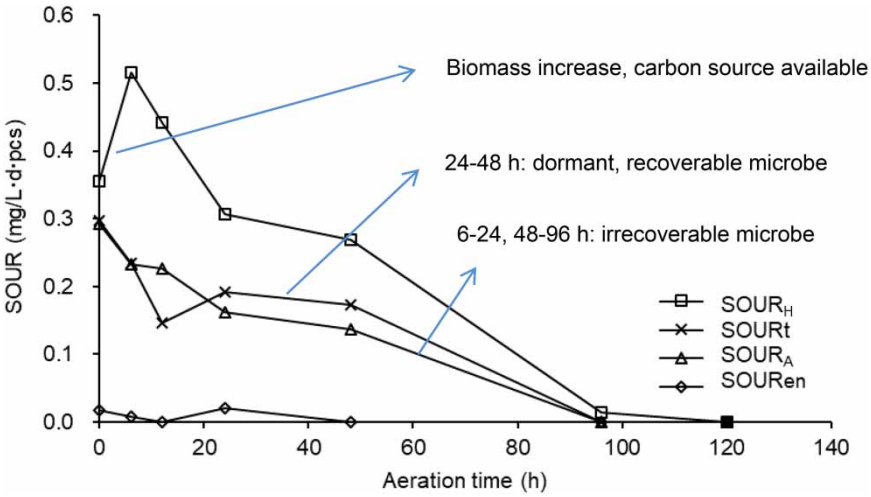

Figure 4 | Specific respiration rate in endogenous respiration processes of carriers from the aMBR system.

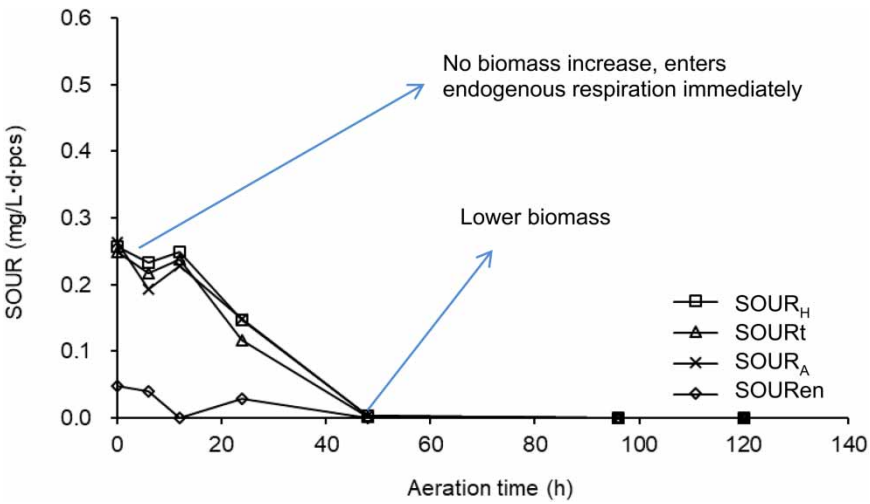

Figure 5 | Specific respiration rate in endogenous respiration processes of carriers from the UV/O_3-aMBR system.

Table 2 | Removal rate of basic parameters in aMBR and UV/O_3-aMBR system

Parameters	Feed	aMBR permeate	UV/O_3-aMBR permeate
COD_{Mn} (mg/L)	10.44 ± 1.88	4.66 ± 0.75	2.91 ± 0.34
DOC (mg/L)	10.33 ± 2.05	6.78 ± 0.87	3.73 ± 0.55
UV_{254} (cm^{-1})	0.20 ± 0.02	0.15 ± 0.02	0.05 ± 0.01
SUVA (L/mg · m)	1.97 ± 0.45	1.93 ± 0.51	1.10 ± 0.21
TN (mg/L)	14.45 ± 3.65	13.26 ± 3.75	8.54 ± 1.21

and this could be the reason why the UV/O_3-aMBR system shows higher organic removal. The contribution of the microbes in both systems was worth analyzing.

The *SOUR* changes in endogenous respiration process

$SOUR_{en}$ value was similar and low in both aMBR and UV/O_3-aMBR systems. Within 0–6 h, the $SOUR_H$ of aMBR was increased sharply, which indicated that the biomass was increasing, and shows the carbon source was available on the PVA-gel. The PVA-gel sampled from the aMBR system had substrate on it, which could be extracellular polymer substrates, organic matter from polluted surface water adsorbed on it, or intracellular storage (Li & Yang 2007). The decrease of $SOUR_N$ ($SOUR_{en} + SOUR_A$) indicated that no nitrogen source remained on the PVA-gel.

This also could suggest that sufficient ammonia oxidation bacteria existed on the PVA-gel in the aMBR system. According to Figure 4, 6 h is a turning point, where $SOUR_H$ starts to decrease after increasing, thus, 6 h can be regarded as the point where heterotrophic bacteria enters endogenous respiration in the aMBR system. As shown in Figure 5, the *SOURt*, $SOUR_H$, and $SOUR_A$ were continuously decreasing, with low increasing turning point observed at 12 h. This shows that, in the UV/O_3-aMBR system, no substrates remained on the PVA-gel surface and the increase observed during 6 h to 12 h may be contributed to by the intracellular storages of the microbes (Ramdani *et al.* 2010). This phenomenon further indicates that the microbes on PVA-gel enter endogenous respiration immediately when taken out from the UV/O_3-aMBR system. The results also suggest that in the UV/O_3-aMBR system, substrate biodegradability was higher than in the aMBR system.

As shown in Figure 4, the decrease of $SOUR_H$ curve showed a rapid decreasing period, 6–24 h and 48–96 h, and a slow decreasing period 24–48 h. This indicated that heterotrophic bacteria can be divided into two components, recoverable and irrecoverable. When there was enough substrate, the respiration rate did not recover at all during 6–24 h and 48–96 h. This indicated that the irrecoverable heterotrophic microbes had decayed. During 24–48 h, the slow decreasing approach to a stable situation indicated that recoverable bacteria component regained activities when enough substrate was added. The $SOUR_A$ curve was decreased with three fast decreasing and two slow decreasing periods and no increasing period was observed. This indicates autotrophic bacteria also included recoverable and irrecoverable components. When substrate is not enough, the recoverable component will start to become dormant, and regain activities once sufficient substrate is provided (Roszak & Colwell 1987). The stable or slow decreasing parts are defined as the dormant period, as shown in Figure 4.

For the aMBR system, $SOUR_t$ shows lower value than $SOUR_H$ and $SOUR_A$ in 0–12 h, and during 0–6 h, it even shows a similar value to $SOUR_A$. This indicated that when there was sufficient carbon source and nitrogen source provided at the same time, autotrophic bacteria respiration dominated and heterotrophic bacteria were probably inhibited in the aMBR system. During 6–12 h, $SOUR_t$ decreased with a similar trend as $SOUR_H$, and $SOUR_A$ decreased quite slowly due to recoverable autotrophic bacteria respiration. $SOUR_t$ was slightly higher than $SOUR_A$ in the 24–96 h period and this might be due to the decrease of effects from autotrophic bacteria inhibition.

The microbe respiration includes endogenous and exogenous respiration, with endogenous respiration showing more stable characteristics as compared with exogenous respiration. Thus, the endogenous respiration to total respiration rate could be used as the microbe activity index (Park *et al.* 2017; Li *et al.* 2019). The $SOUR_{en}/SOUR_t$ in aMBR and UV/O_3-aMBR was increased with increasing aeration time, which indicated that the endogenous respiration will increase when facing adverse environmental conditions such as lacking substrate. The higher endogenous respiration rate implied a higher oxygen consumption required for microbe fundamental metabolism, which also presents a higher operation cost demand of the system. The $SOUR_{en}/SOUR_t$ value in aMBR was lower than in UV/O_3-aMBR, which indicated that the microbe activity was higher in the aMBR system as compared with the advanced oxidation process (AOP) integrated system.

The changes of respiration ratio in endogenous respiration process

$SOUR_H$ quickly decreased by 14.15% from 6 to 12 h, 16.21% from 12 to 24 h, 7.48% from 24 to 48 h, 49.60% from 48 to 96 h, and 2.56% from 96 to 120 h. This indicated that over 90% of the heterotrophic bacteria were irrecoverable in the aMBR system. $SOUR_A$ continuously decreased by 20.52% from 0 to 6 h, 1.71% from 6 to 12 h, 22.09% from 12 to 24 h, 8.68% from 24 to 48 h, and 47% from 48 to 96 h, until it stopped decreasing at 96–120 h. This indicated that some recoverable autotrophic bacteria regained activity from 6 to 12 h and 24 to 48 h, until after 96 h of aeration, all the autotrophic bacteria decayed.

Calculated from the theoretical $SOUR_T$, heterotrophic bacteria occupied around 60% and autotrophic bacteria 40% in the aMBR system. As shown in Figure 4, it can also be seen from the system that the $SOUR_H$ was always higher than $SOUR_A$ throughout the aeration time tested. Also, as shown in Figure 4, there is no absolute stability of $SOUR_H$, $SOUR_A$, and $SOUR_t$ during 120 h aeration and this indicated that the microbes' activity in the aMBR system is tough and

active enough. Thus, the addition of any substrates can motivate their respiration processes within 120 h aeration.

The respiration map of bacteria growth on PVA-gel in the UV/O_3-aMBR system is shown in Figure 5. Compared with Figure 4, the values obtained for $SOUR_t$, $SOUR_H$, and $SOUR_A$ were lower than the values obtained from the aMBR system in all samples tested during 120 h aeration. This shows that the biomass amount on PVA-gel in the UV/O_3-aMBR system was much lower than in the aMBR system. During 0–6 h, the $SOUR_t$, $SOUR_H$, and $SOUR_A$ were decreased, which indicated that no extra substrate remained on the PVA-gel, regardless of the presence of carbon source and nitrogen source in any form. Thus, there was no increase in biomass in any form. During 6–12 h, it was observed that there was a slight increase in $SOUR_t$, $SOUR_H$, and $SOUR_A$, which might be due to a part of the microorganisms using the decayed part as substrates. It can also be contributed to by the variation of carriers used, since the difference is quite small, based on the $SOUR$ unit. After 12 h, the $SOUR_t$, $SOUR_H$, and $SOUR_A$ values continued to decrease until 0 at 48 h of aeration and remained at 0 until 120 h. This indicated that both heterotrophic and autotrophic bacteria in the UV/O_3-aMBR system were quite low or there were no recoverable components. Thus, even if sufficient substrates were provided, all $SOUR_t$, $SOUR_H$, and $SOUR_A$ values were decreasing continuously, and there was no steady or dormant period found in its respiration map.

As shown in Figure 5, the $SOUR_t$, $SOUR_H$, and $SOUR_A$ values were quite similar, which indicated that the contribution of the heterotrophic and autotrophic bacteria were nearly equal in the system. However, when the presence of carbon source or nitrogen source is too high, autotrophic bacteria would be the dominant microbes. This is also due to the feed water quality from the parallel aMBR and UV/O_3-aMBR systems with both COD_{Mn} value and NH_4^+-N value of around 10 mg/L. Nitrogen source was sufficient as compared to carbon source in the system, thus the amount of autotrophic bacteria dominated the system compared to the heterotrophic bacteria.

Bacteria were all decayed after 48 h aeration in the UV/O_3-aMBR system and 96 h aeration in the aMBR system as shown in Figures 4 and 5. This also indicated that less biomass and substrate were attached on carriers in UV/O_3-aMBR than aMBR. It is also probable that UV/O_3-aMBR has less species of microbes compared to the aMBR system; attached specific heterotrophic bacteria and autotrophic bacteria provide a high performance in consuming all available substrates, or the integration of the UV/O_3 process had accelerated the biodegradability of organic substrates in the UV/O_3-aMBR system. It is also shown in Figures 4 and 5 that the $SOUR_t$ in the two reactors was nearly the same, and this indicated that, even with higher biomass in the aMBR system, it provides a similar degree of bioactivity as the UV/O_3-aMBR system when sufficient nitrogen source and carbon source were provided.

Practical applications and future perspectives

The membrane-based technology could guarantee the permeate water quality in drinking water treatment. The biological process is an environmentally friendly treatment technology for dealing with organic matter in polluted surface water. With the deterioration of surface water quality, the combination of membrane technology and biological technology-membrane bioreactor system has the potential to be developed as an effective alternative technology in drinking water supply. However, very limited work has been done in illustrating the MBR related system in the treatment of polluted surface water. Polluted surface water was defined as water quality with COD_{Mn} around 10 mg/L and NH_3-N around 3 mg/L. This is much lower as compared with the municipal wastewater, while being higher than the common source water that is used for drinking water supply. The increase of organic matter in surface water is an inevitable trend, which has gradually brought challenges to the conventional coagulation and flocculation drinking water treatment process. The integration of an AOP into the aMBR system as a polishing step to improve the permeate water biodegradability and then to be treated by biological processes could largely improve the recalcitrant organic matter removal. More work has to be focused on the investigation of the MBR technology application in the treatment of polluted surface water.

The biological process has played an important role in the MBR system for treating polluted surface water. The illustration and comparison of the biological process in the aMBR and UV/O_3-aMBR systems could provide detailed

information for a future pilot-scale system design. The endogenous respiration map method used in this study is a simple and accurate one. The use of an endogenous respiration map could provide information about microbes in the system, and provide guidance in practical application. It is also recommend to build up a small program to generate a corresponding respiration map simply with the input from the necessary parameters.

CONCLUSIONS

This study investigated the endogenous respiration process in aMBR and UV/O_3-aMBR in treating polluted surface water. Results indicated that microbes enter endogenous respiration at different times in the two systems. With the integration of the UV/O_3 process into the aMBR system, the biomass on the bio-carrier was changed. Higher abundance of microbes including recoverable and irrecoverable components existed in the aMBR system compared to the UV/O_3-aMBR system. The use of AOPs in aMBR for treating polluted surface water could improve recalcitrant removal, while the design of AOP conditions also has to be carefully involved to guarantee the contribution of the biological process.

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