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Influence of carbon sources on nutrient removal in A²/O-MBRs: Availability assessment of internal carbon source

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ARTICLE INFO

Article history:

Received 31 July 2015

Revised 12 November 2015

Accepted 18 December 2015

Available online 19 March 2016

Keywords:

Carbon source

Nutrient removal

Mass balance

Cost analysis

ABSTRACT

Both internal carbon source and some external carbon sources were used to improve the nutrient removal in Anaerobic-Anoxic-Oxic-Membrane Bioreactor (A²/O-MBRs), and their technical and cost analysis was investigated. The experimental results showed that the nutrient removals were improved by all the carbon source additions. The total nitrogen and phosphorus removal efficiency were higher in the experiments with external carbon source additions than that with internal carbon source addition. It was found that pathways of nitrogen and phosphorus transform were different dependent on different carbon source additions by the mass balance analysis. With external carbon source addition, the simultaneous nitrification and denitrification occurred in aerobic zone, and the P-uptake in aerobic phase was evident. Therefore, with addition of C-MHP (internal carbon source produced from sludge pretreatment by microwave-H₂O₂ process), the denitrification and phosphorus-uptake in anoxic zone was notable. Cost analysis showed that the unit nitrogen removal costs were 57.13 CNY/kg N of C-acetate addition and 54.48 CNY/kgN of C-MHP addition, respectively. The results indicated that the C-MHP has a good technical and economic feasibility to substitute external carbon sources partially for nutrient removal.

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Introduction

Nowadays, the effluent standards are required to meet Standard A of “Discharge Standard of Pollutants for Municipal Wastewater Treatment Plant (GB 18918–2002)” in China. As the effluent standards are getting more and more stringent, improvement of the nutrient removal efficiency in wastewater treatment plants (WWTPs) becomes an urgent issue. A significant factor in the success of the biological nutrient removal process is the availability of suitable carbon source, and some literatures suggested that the C/N ratio should be

above 6 to achieve satisfactory effluent quality in biological wastewater treatment (Sun et al., 2010; Fu et al., 2009a). However, the domestic wastewater is always low in C/N ratio, sometimes lower than 3 in southern China (Wu, 2010). A most common method for increasing the C/N ratio is adding extra carbon. External carbon sources such as methanol, acetate, glucose and ethanol are used commercially as extra carbon to improve nutrient removal (Wang et al., 2012, 2013a; Kargi and Uygun, 2003; Bracklow et al., 2010). However, the external carbon source addition not only raise the operational cost but also increase the excess sludge production significantly

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(Mokhayeri et al., 2009), and the produced sludge increased further treatment cost (Metcalf and Eddy, 2004).

The readily biodegradable organic and volatile fatty acids (VFAs) are the preferred carbon source for nutrient removal microbes. There is an amount of organics in sludge, and VFA generation from sludge pretreatment has been suggested as alternative carbon source for nutrient removal. Various chemical, physical and biological methods are developed for sludge pretreatment, such as alkaline fermentation (Tong and Chen, 2009; Gao et al., 2011; Zhao et al., 2015; Wang et al., 2013b), thermochemical pretreatment (Rajesh Banu et al., 2009; Galí et al., 2006; Do et al., 2009), ozonation pretreatment (Cui and Jahng, 2004), mechanical disintegration (Kampas et al., 2007) and microwave pretreatment (Wang et al., 2009; Wang, 2009). Among these technologies, microwave irradiation has gained widespread popularity as an effective thermal method due to its rapid and selective heating and energy efficiency (Tyagi and Lo, 2013; Remya and Lin, 2011).

In previous study, microwave- H_2O_2 process (MHP) was proved to be an efficient way for sludge breakage, by which some of biodegradable organics could be released from solid content to the bulk liquid phase of sludge (Wang et al., 2009; Xiao et al., 2012), and sludge reduction was significant in a pilot study based on microwave (Xiao, 2012). A subsequent research found that the organics released from sludge pretreated by MHP can be used as the internal carbon source to enhance the nutrient removal in an Anaerobic-Anoxic-Oxic-Membrane Bioreactor (A^2/O -MBR) (Xu et al., 2014).

However, in the application of solubilized sludge as internal carbon source to improve the biological nutrient removal, it is important to note that the addition of internal carbon source includes not only VFAs, but also amount of nitrogenous and phosphorous compounds. Thus, it is necessary to analyze effect of carbon source on nitrogen and phosphorus transformation, and to evaluate the technical and economical availability of the internal carbon source for nutrient removal in A^2/O -MBR.

In the present paper, the effects of the internal carbon source produced by MHP (C-MHP) and some common external carbon sources, as methanol and acetate, on the nutrient removal were studied in A^2/O -MBRs. The influence of nitrogenous and phosphorous compounds in the internal carbon

source on nutrient removal was analyzed and evaluated, and then the pathways of nitrogen and phosphorus removal were investigated by mass balance calculation. Finally, the cost assessment of internal and external carbon source was studied.

1. Materials and methods

1.1. Experimental set-up

The A^2/O -MBR experimental setup was made up of an anaerobic tank, an anoxic tank and an oxic tank with submerged membrane modules in (Fig. 1). The working volume was 58.3 L, including anaerobic tank 8.3 L, anoxic tank 16.7 L and aerobic tank 33.3 L, respectively. The Hydraulic Retention Time (HRT) of anaerobic, anoxic and aerobic basins were 2, 4 and 8 hr, respectively. There are two internal recycle (R). R1 ($Q_1 = Q$) connected anoxic and anaerobic and R2 ($Q_2 = 2Q$) was between aerobic and anoxic. Anaerobic and anoxic tanks were gently mixed with low speed mixer. Air was introduced via perforated pipe placed at the bottom of membrane module. The Sludge Retention Time (SRT) of A^2/O -MBR was set at 22 days.

Five pieces of flat-sheet Polyvinylidene Fluoride (PVDF) membranes (SINAP membrane S&T Co., Shanghai, China) with 0.2 μm pore size and 0.1 m^2 filtration area of each element were used in each oxic tank of A^2/O -MBR. The operation mode was adopted at a constant permeate flux maintained by peristaltic pump in two A^2/O -MBRs. The membranes were operated in a cycle mode of 6 min-filtration at 10 $L/(m^2 \cdot hr)$ (LMH) and 1 min-off, no backwashing of the membrane was required.

For examining the influence of different carbon sources on nutrient removal systematically and for the contrast test, two same experimental setups as presented above were set, and the experiment was divided into two stages. At stage 1, C-MHP was added in one A^2/O -MBR, and another contrast setup as a control system was without any carbon source addition. At stage 2, the two setups were added methanol and acetate as extra carbon sources, respectively. In the two stages, the carbon sources were added in anoxic tank, and the C/N ratio of influent was improved to 6.

Fig. 1 – Schematic of lab-scale Anaerobic-Anoxic-Oxic-Membrane Bioreactor (A^2/O -MBR) reactors.

1.2. Internal C-MHP process

Sludge was obtained from Xiaohongmen Wastewater Treatment Plant with A²/O process in Beijing, and thickened to 10–12 g/L for MHP pretreatment. A microwave reactor (Julong Co. Ltd. Baoding, China) used to treat the sludge was a device with 2450 MHz of microwave, 25 L of cavity volume, a lift mixer and a thermocouple temperature sensor. The microwave reactor was operated at 600 W and 50 r/min of the agitation rate, respectively. Before pre-treating the sludge, the pH value of the sludge was adjusted to 10.0 ± 0.5 , then it was heated to 80°C with microwave irradiation, adding H₂O₂ (30%, W/W) with a ratio of H₂O₂:MLSS (mixed liquor suspended solid) at 0.6, finally, the sludge was heated to 100°C continuously with microwave irradiation (Xu et al., 2014). FeSO₄·7H₂O was used as precipitant to remove phosphorus from the supernatant of sludge pretreated by MHP. After phosphorous removal, the content was added to the anoxic tank as the internal C-MHP for denitrification. The characteristics of C-MHP were listed in Table 1.

1.3. Wastewater characteristics and operation conditions

The feed water was domestic wastewater taken from the sewer at residential district of Research Center for Eco-Environmental Sciences, Chinese Academy of Science. Wastewater characteristics and operation conditions were shown in Table 2. The influent was fed into A²/O-MBRs by the pumps controlled by the level controllers and the carbon sources to be tested were added into anoxic tank to maintain C/N at 6.

1.4. Analytical procedures

The operation parameters of the A²/O-MBR system were constantly monitored including DO, temperature, pH, Oxidation Reduction Potential (ORP) and transmembrane pressure (TMP), in which DO, pH and ORP were measured using Mult 3410 (WTW). The sCOD (soluble COD) was filtered (0.45 µm, cellulose acetate membrane), sCOD and COD were measured using HACH protocols. The concentrations of TN (total nitrogen), TP (total phosphorus), NH₄⁺-N, PO₄³⁻-P, MLSS, MLVSS (mixed liquor volatile suspended solid) were measured according to APHA methods (APHA, 2005). The concentrations of protein and polysaccharide were measured by Lowry method (Lowry et al., 1951) and the phenol-sulphuric method (Dubois et al., 1956), respectively. Humic acid was measured as previous studies (Wang, 2009).

Table 1 – Characteristics of internal carbon source extracted from MHP.

Characteristics	Value
COD (mg/L)	5956.8 ± 585.7
TN (mg/L)	360.10 ± 94.56
TP (mg/L)	200.51 ± 51.81, 44.65 ± 6.79 ^a
pH	7.49 ± 0.27
Protein (mg/L)	827.13 ± 218.55
Carbohydrate (mg/L)	357.38 ± 81.75
Humic acid (mg/L)	1151.57 ± 396.61

MHP: microwave-H₂O₂ process; TN: total nitrogen; TP: total phosphorus.

^a TP concentration after chemical precipitation.

2. Results and discussion

2.1. Nutrient removal under different carbon sources

2.1.1. COD removal

Fig. 2 shows the COD removal during the operation period with different carbon source additions. The fluctuation of COD in influent was evident and sometimes very low COD concentration was detected. The low concentration of influent COD may drop the MLSS concentration and weaken nutrient removal. It was observed that the effluent COD was lower than 50 mg/L, fluctuations of the influent COD did not alter the effective removal of organics in the system. In the initial phase, the COD effluent of C-methanol addition was higher than 200 mg/L due to the toxicity of high concentration methanol. However, the COD removal gradually stabilized later, which showed that, through the early acclimation, the addition of methanol did not cause long-term ham on the performance of the A²/O-MBR system.

Compared with external carbon source addition, the concentrations of COD in aeration basin and effluent were significantly influenced by C-MHP ($p < 0.05$). The sCOD in the aerobic basin was in the range of 18–45 mg/L and 42–82 mg/L with the addition of the other carbon sources and C-MHP, respectively. The carbon source addition was along with influent COD concentration, and the lower concentration of influent COD could cause more internal carbon source to be added in the A²/O-MBR. But unfortunately, due to some refractory organics contained in internal carbon source, the addition of more internal carbon source will lead to effluent quality deteriorate. Such results agreed with that presented in some reports for study on other sludge pretreatment (Yoon et al., 2004; Sakai et al., 1997).

2.1.2. Nitrogen removal

Fig. 3 explains TN removal of the A²/O-MBR system under different carbon source additions. It is evident that the nitrogen removal efficiency was significantly improved by adding carbon sources. The average nitrogen removal efficiency in each run was 47.62% for control, 57.08% for C-MHP addition, 68.17% for C-methanol addition and 69.66% for C-acetate addition, respectively. The t-test shows that there are significant differences among different carbon source additions. Neglecting the influent TN variations in each run, it can be summarized that TN removal efficiency could be improved significantly by carbon source additions, and compared to exogenous carbon sources (C-methanol and C-acetate), the C-MHP had a limit effect on nitrogen removal.

In the case of the nitrification process, it was observed that a complete nitrification was initiated in each run as seen from the low ammonia nitrogen concentration measured in oxic reactor. Contrast to C-methanol and C-acetate addition, C-MHP addition limited the nitride removal. Two reasons could account for this result. First, in previous study, we found that the biodegradable substance in C-MHP was lower than 50%, and there was not enough carbon source for denitrification. Second, there was an amount of organic nitrogen such as

Table 2 – Wastewater characteristics and operation conditions.

Carbon source	Stage 1		Stage 2	
	Control	C-MHP	C-methanol	C-acetate
Fed domestic wastewater characteristics				
COD (mg/L)			262.90 ± 108.78	
NH ₄ ⁺ -N (mg/L)	84.59 ± 13.71		71.14 ± 13.39	
TN (mg/L)	96.49 ± 13.71		74.25 ± 13.31	
TP (mg/L)	8.22 ± 2.51		10.21 ± 4.10	
C/N ratio	3.89 ± 1.77		3.48 ± 1.19	
Sludge characteristics				
MLSS (mg/L)	7635.67 ± 1071.14	8022.00 ± 609.55	7047.43 ± 1737.30	8397.25 ± 3444.02
MLVSS/MLSS	0.79 ± 0.01	0.77 ± 0.01	0.86 ± 0.06	0.87 ± 0.06
Viscosity (mPa·sec)	3.1 ± 1.5	7.79 ± 0.65	4.94 ± 1.42	6.53 ± 1.76
C-MHP: internal carbon source produced from sludge pretreatment by Microwave-H ₂ O ₂ process; TN: total nitrogen; TP: total phosphorus; MLSS: mixed liquor suspended solid; MLVSS: mixed liquor volatile suspended solid.				

protein in C-MHP, which increased the N loading, and the organic nitrogen was difficult to be removed from the system.

2.1.3. Phosphorus removal

In the process of MHP, the bound phosphorus in the biosolids was solubilized and released into the solution, which could have a negative influence on A^2/O -MBR system. Thus, the phosphorus removal in the solution was carried out by adding a precipitant ($\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$). After precipitation, TP in the

supernatant was decreased from (200.51 ± 51.81) to (44.65 ± 6.79) mg/L, and the supernatant was used as C-MHP.

Fig. 4 shows the phosphorus removal under different carbon source additions. It is clear that the phosphorus removal was enhanced by both the exogenous carbon source and internal carbon source additions. The average TP removal efficiency in each run was about 31% for control, 59% for C-MHP addition, 82% for C-methanol addition and 86% for C-acetate addition, respectively. Statistical analysis suggested

Fig. 2 – Chemical Oxygen Demand (COD) removal with different carbon source additions.

Fig. 3 – Nitrogen removal with different carbon source additions.

that there were significant differences between the additions of exogenous carbon source and internal carbon source. In Enhanced Biological Phosphorus Removal (EBPR) processes, phosphorus removal is based on the enrichment of activated sludge with polyphosphate-accumulating organisms (PAOs) by introducing alternating anaerobic-aerobic conditions.

During anaerobic periods the substrate is taken up by PAOs and stored as polyhydroxyalkanoates (PHAs) within the cells, which then release phosphate into the liquid phase. In the anoxic and aerobic phases, PHA is used as carbon and energy sources for growth and replenishment of the internal glycogen and poly-P pools. An amount of phosphate higher than

Fig. 4 – Phosphorus removal with different carbon source additions.

that released is taken up by PAOs and the poly-P pools are restored. Phosphorus is removed from the system through the wastage of excess sludge with high poly-P content (Puig et al., 2008). In present study, difference carbon sources added in the A²/O-MBR system affected the transformations of PHAs and glycogen, and finally influenced phosphorus uptake by microorganisms and phosphorus removal efficiency (Bracklow et al., 2010; Ahmed et al., 2008; Chen et al., 2015).

Compared with the control, by adding C-MHP, the TP removal was improved although a part of residual phosphorus was recycled back to the A²/O-MBR system. However, the TP removal efficiency was lower than C-methanol and C-acetate addition. Residual phosphorus in C-MHP may be a reason for that.

2.2. Nitrogen and phosphorus mass balance in A²/O-MBR

As shown in Table 1, the C-MHP contained a relatively high COD/N ratio (16.54 g COD/g N) and COD/P ratio (133.4 g COD/g P). However, compared with exogenous carbon sources, C-MHP has limitation to enhance nutrient removal. Because only part of its COD could be used for nutrient removal, and residual nitrogen and phosphorus returned into A²/O-MBR system, thus it is necessary to employ mass balance analysis to explore the transformation of nitrogen and phosphorus. Schematic diagram of nitrogen and phosphorus mass balance in A²/O-MBRs was shown in Fig. 5. The detail of nitrogen and phosphorus mass balance calculation was expressed in Appendix A. Supplementary data.

2.2.1. Nitrogen mass balance

The nitrogen in the whole system was considered as two components: NH₄⁺-N and NO₃⁻-N, and the variation of nitrogen include the transformation among NH₄⁺-N, NO₃⁻-N and N₂. Under aerobic conditions, nitrification process occurred, NH₄⁺-N can be transformed into NO₃⁻-N or NO₂⁻-N by nitrifiers. While under anoxic conditions, the denitrification process occurred, denitrifiers utilized biological organic substrate as

electron donor, and NO₃⁻-N or NO₂⁻-N as electron acceptor was transformed into N₂ (Wiesmann, 1994). Besides, simultaneous nitrification and denitrification process (SND) can be occurred under certain conditions, which relied on concurrent aerobic NH₄⁺-N oxidation and anaerobic denitrification under identical operating conditions (Fu et al., 2009b). According to Fig. 5, average concentration of NH₄⁺-N and NO₃⁻-N were used for mass balance calculation. The nitrogen balance analysis under different carbon source additions was shown in Table 3.

From Table 3, all the NH₄⁺-N in final effluents of the A²O-MBRs are quite low under different carbon source additions, which indicated that the nitrification processes were all in excellent conditions, and almost all the influent NH₄⁺-N into aerobic tank was completely oxidized into nitrate. The denitrification rates in anoxic basins were improved by carbon source additions. It is noteworthy that the denitrification rate was above 70% with C-MHP addition, even higher than exogenous carbon source additions. This phenomenon shows that C-MHP could enhance the denitrification significantly. By adding C-methanol and C-acetate, parts of nitrogen were removed in oxic zone, which means the SND occurred (Helmer and Kunst, 1998). In oxic zone, NH₄⁺-N could be transformed into NO₃⁻-N. If the completely nitrification process occurred, the variation of NH₄⁺-N and production of NO₃⁻-N will be almost equal. Compared the NO₃⁻-N production and NH₄⁺-N variation in oxic zone, it can be found that the NO₃⁻-N production was higher than NH₄⁺-N variation especially under C-MHP addition, and this nitrogen shortfall was reached to 1 g/day. The transformation of organic nitrogen to nitrate under aerobic condition may be the main reason for that. What is more, the transformation of organic nitrogen increased the nitrogen loading and led to the final low nitrogen removal efficiency.

Nitrogen could be removed by both assimilation into biomass and biological nitrification–denitrification process. For the influent nitrogen in A²/O-MBR system, there are five pathways for nitrogen discharge, they are effluent, excess

Fig. 5 – Schematic diagram of nitrogen and phosphorus mass balance in Anaerobic-Anoxic-Oxic-Membrane Bioreactor (A²/O-MBR) (S: concentration of different components in different zones; Q: flow; Q₁, Q₂: internal recycle; Q_c: flow of carbon source addition; Q_{sludge, D}: the flow of sludge discharge).

Table 3 – Nitrogen balance analysis of Anaerobic-Anoxic-Oxic-Membrane Bioreactor (A²/O-MBR).

Carbon source	Reaction zone	NH ₄ ⁺ -N				NO ₃ ⁻ -N				Nitrogen removal in oxic zone (g/day)
		INF (g/day)	EFF (g/day)	ΔN (g/day)	N rate	INF (g/day)	EFF (g/day)	ΔDN (g/day)	DN rate	
C-control	AN	8.45	8.52	−0.07	−0.80%	1.27	0.56	0.71	55.82%	–
	DN	8.67	7.70	0.97	11.23%	8.24	6.01	2.22	27.00%	–
	N	5.77	0.22	5.55	96.19%	5.33	11.39	−6.06	–	–
C-MHP	AN	8.28	7.86	0.42	5.08%	0.66	0.46	0.20	30.29%	–
	DN	8.63	8.33	0.30	3.01%	6.67	1.95	4.72	70.75%	–
	N	6.25	0.46	5.79	92.57%	1.46	8.25	−6.79	–	–
C-methanol	AN	10.90	9.99	0.90	8.30%	0.77	0.26	0.51	66.02%	–
	DN	10.26	9.76	0.50	4.88%	5.47	1.88	3.59	65.64%	–
	N	7.32	0.34	6.98	95.34%	1.41	7.88	−6.47	–	0.511
C-acetate	AN	10.87	10.65	0.22	2.00%	0.73	0.46	0.27	36.35%	–
	DN	11.06	9.65	1.42	12.80%	5.32	1.72	3.60	67.67%	–
	N	7.24	0.50	6.74	93.15%	1.29	7.35	−6.06	–	0.683

AN: anaerobic basins; DN: anoxic basins; N: oxic basins; INF: influent; EFF: effluent; ΔN: the variation of nitrification; ΔDN: the variation of denitrification; N rate: the variation rate of nitrification; DN rate: the variation rate of denitrification; –: no reaction occurred; C-MHP: internal carbon source produced from sludge pretreatment by microwave-H₂O₂ process.

sludge, nitrogen gas converted by the denitrification in anaerobic and anoxic basins, SND in oxic zones and consumption by microbe assimilation, respectively. To clarify the influence of different carbon source additions further, the mass balance for nitrogen (Table 4) was analyzed based on Table 3.

As shown in Table 4, the nitrogen in sludge discharge was considered with a nitrogen concentration coefficient 0.1 g N/g VSS (volatile suspended solid) (Wentzel et al., 1998). Considering nitrogen transformation in all phases of A²/O-MBR, it was observed that carbon source addition improved denitrification significantly especially the C-MHP addition, while the process of simultaneous nitrification and denitrification happened under C-methanol and C-acetate addition. Another phenomenon worthy of note is that the nitrogen balances were both higher than 94% in the period of C-control and C-MHP, while in the period of C-methanol and C-acetate, they were only 89.29% and 90.3%, respectively. That means the former had a higher nitrogen balance. The influence of organic nitrogen may be one of the reasons for these discrepancies. The substrates are selective used by microbes, and the readily biodegradable organics will be used in preference of endogenous substrate (Kargi and Uygur, 2003; Puig et al., 2008). As the C-methanol and C-acetate addition, the microorganisms chose to firstly utilize external carbon sources rather than endogenous substrate. On the contrary, under the condition of C-control and C-MHP addition, there was not enough readily biodegradable organics. Microbes had to use endogenous organics such as some protein or other nitrogen-containing organics, and these nitrogen-containing groups were released into the system in the utilization process, thus the nitrogen balance was improved. For the sludge discharge, there are more nitrogen removal with external carbon source additions, which was in accordance with the sludge yield, amount of nitrogen was consumed by microbe assimilation. While the nitrogen concentration coefficient may be low for the sludge with external carbon source additions, thus nitrogen balances were low for external carbon source additions. The last reason for the nitrogen balance discrepancy may be the influence of computing data. We chose

the average value of different nitrogen compounds, and did not consider the nitrite, which may cause some deviation for the calculation of nitrogen balance.

The above analysis shows that there were differences on the pathways of nitrogen transformation among different carbon source additions, and these discrepancies may be resulted from the difference of microbial community (Ahmed et al., 2008). It was no doubt that C-MHP could improve the nitrogen removal. Nevertheless, the organic nitrogen will increase the nitrogen loading, and that will lead to a negative effect on nitrogen removal.

2.2.2. Phosphorus mass balance

Phosphorus removal is by means of release of P in anaerobic basin and luxury uptake of P in aerobic basin. For a successful biological phosphorus process, the P-release in anaerobic phase and luxury P-uptake in anoxic or aerobic phase are crucial, and carbon source influences the biological phosphorus removing process performance (Ahmed et al., 2008; Puig et al., 2008). Thus it is necessary to expound the P-release and P-uptake under different carbon source additions. The phosphorus variations were shown in Table 5.

It was observed that the P-release in anaerobic phase were significantly enhanced by both C-MHP and external carbon source addition, the P-release efficiencies were 106.7% of C-MHP addition, 109.14% of C-methanol addition and 93.8% of C-acetate addition, respectively. In previous study, we found that the readily biodegradation substrate was low in C-MHP (Xu et al., 2014). Therefore, compared with external carbon source, the organic substrate in C-MHP had a similar effect on improving the PHA store and P-release by PAOs in this study. It is speculated that as C-MHP recycling from anoxic zone to anaerobic zone, some complex substrate was hydrolyzed into readily biodegradable substrate as C-MHP recycling from anoxic zone to anaerobic zone, which can be used to promote the P-release.

However, there are distinct differences on P-uptake. The P-uptake in aerobic phase was significantly improved under

Table 4 – Material balance for nitrogen.

Period	Influent		Denitrification		SND		Effluent		Sludge discharge		Nitrogen balance
	INF (g/day)	E	R (g/day)	E	R (g/day)	E	EFF (g/day)	E	D (g/day)	E	
C-control	7.12	100.0%	2.93	41.21%	–	–	3.51	49.30%	0.51	7.19%	97.70%
C-MHP	8.57	100.0%	4.92	57.43%	–	–	2.75	32.08%	0.77	8.96%	98.48%
C-methanol	9.25	100.0%	4.10	44.34%	0.51	5.52%	2.67	29.68%	0.90	9.76%	89.29%
C-acetate	9.25	100.0%	3.87	41.81%	0.68	7.38%	2.49	29.62%	1.06	11.49%	90.30%

SND: simultaneous nitrification and denitrification; E: efficiency; R: removal; D: discharge; –: no reaction occurred; INF: influent; EFF: effluent; C-MHP: internal carbon source produced from sludge pretreatment by microwave-H₂O₂ process.

C-methanol and C-acetate addition, the P-uptake efficiencies were 60% and 77.05%, respectively. While with C-MHP addition, the P-uptake was really quite low, it was only 17.51%. Nevertheless, it was obviously seen that the P-uptake was occurred in anoxic phase. The denitrifying phosphorus removing bacteria (DPB) can use nitrate as an electron acceptor, therefore, allowing simultaneous denitrification and phosphorus uptake (Sorm et al., 1996). Thus this phenomenon was improved by C-MHP addition in this study.

There are two pathways for influent phosphorus discharge, effluent and sludge discharge. As shown in Table 6, material balance for phosphorus was calculated. The phosphorus in sludge discharge was considered with a concentration coefficient 0.04 g P/g VSS (Barlindhaug and Odegaard, 1996). From the phosphorus content in effluent, it was observed that carbon source addition improved phosphorus removal significantly especially the external carbon source addition. However, the phosphorus balance in the period of carbon source addition was lower than control, and the phosphorus balance was about 80% for both C-MHP addition and external carbon source addition. It was indicated that the value of phosphorus concentration coefficient was too low for this study.

2.3. Economic assessment of internal carbon source

Based on the nutrient removal and mass balance analysis, the addition of external carbon sources, both C-methanol and

C-acetate, could improve nutrient removal significantly, and the C-MHP was available to improve nutrient removal. What is more, the sludge was consumed to produce C-MHP, and the cost for further sludge treatment was reduced. Thus, it is necessary to consider the whole cost from the whole sewage treatment process to assess the availability of C-MHP, and we chose C-acetate to be the contrast for assessment.

For C-acetate, unit nitrogen removal equivalent was 4.6 g COD/g N, and the cost of unit nitrogen converting into nitrogen gas was about 50 CNY (Sun et al., 2010). In present study, firstly, the unit nitrogen removal equivalent of internal carbon source was measured by the average value determined in the experiment, and it was 11.6 kg COD/kg N (Eq. (S16)). In previous study, when sludge MLSS is 15 g/L, the sludge treatment cost for extracting C-MHP was 0.06 CNY/L (Xiao, 2012) and the extracted COD concentration in internal carbon source was about 6000 mg/L. Thus, the cost of unit nitrogen removal can be determined, and according to calculation in Eq. (S17), the cost of unit nitrogen converting into nitrogen gas was about 116 CNY, meanwhile 1.94 m³ of sludge with MLSS at 15 g/L was consumed.

For sludge production, the sludge yield was 0.65 g VSS/g COD for C-acetate addition, and in this study, the sludge yield was 0.28 g VSS/g COD for C-MHP addition. In previous pilot study, the sludge was transported out and then further treated with landfill method (Xiao, 2012), and the cost of sludge disposal was 2.38 CNY/kg SS. With reference to this

Table 5 – Phosphorus balance analysis of A²/O-MBR.

Carbon source	Reaction zone	INF (mg/day)	EFF (mg/day)	Variation (mg/day)	Variation efficiency
C-control	AN	1535.3	2436	–900.7	–58.67%
	DN	3584	2980	604	16.85%
	N	2235	1891	344	15.395%
C-MHP	AN	1298.5	2684	–1385.5	–106.7%
	DN	3268	2022	1246	38.13%
	N	1516.5	1251	265.5	17.51%
C-methanol	AN	1466	3066	–1600	–109.14%
	DN	3464	3200	264	7.62%
	N	2400	960	1440	60%
C-acetate	AN	2033	3940	–1907	–93.80%
	DN	4364	5468	–1104	–25.30%
	N	4101	941	3160	77.05%

INF: influent; EFF: effluent; AN: anaerobic basins; DN: anoxic basins; N: oxic basins; C-MHP: internal carbon source produced from sludge pretreatment by microwave-H₂O₂ process.

Table 6 – Material balance for phosphorus.

Period	Inflow		Outflow		Phosphorus balance	
	INF (g/day)	E	EFF (g/day)	E	Sludge discharge (g/day)	E
C-control	0.86	100.00%	0.57	65.47%	0.26	29.66%
C-MHP	1.04	100.00%	0.32	30.58%	0.51	48.77%
C-methanol	0.82	100.00%	0.15	18.25%	0.51	61.70%
C-acetate	0.82	100.00%	0.13	16.22%	0.53	65.07%

INF: influent; E: efficiency; EFF: effluent; C-MHP: internal carbon source produced from sludge pretreatment by microwave-H₂O₂ process.

value, the sludge disposal cost was 7.13 CNY/kg N for C-acetate addition and 7.73 CNY/kg N for C-MHP addition, respectively (Eq. (S18)). With regard to the sludge consumption of C-MHP, the sludge disposal cost was revised to 6.93 CNY/kg N. Combine the expenses of nitrogen removal and sludge disposal, the unit nitrogen removal cost was 57.13 CNY of C-acetate addition and 54.48 CNY of C-MHP addition, respectively (Eq. (S19)). As discussed above all, with focus on sludge reduction and utilization, the C-MHP has a good technical and economic feasibility to be used to partial substitute external carbon source for nutrient removal.

3. Conclusions

Compared with nitrogen removal efficiency 47% and phosphorus removal efficiency 31% for the control, the nutrient removals were improved by both external and internal carbon source additions. The nitrogen removal efficiency was 57.08% for C-MHP addition, 68% for C-methanol addition and 70% for C-acetate addition, respectively. The phosphorus removal efficiency was 59% for C-MHP addition, 82% for C-methanol addition and 86% for C-acetate addition, respectively. By mass balance analysis, it was found that the nitrogen and phosphorus transform and removal pathways were different under different carbon source additions. With external carbon source additions, the simultaneous nitrification and denitrification occurred in aerobic zone, and the P-uptake in aerobic phase was evident. Under C-MHP addition, the denitrification and phosphorus uptake in anoxic zone was notably, and the denitrification rate was above 70%, even higher than external carbon source additions. Some organic nitrogen in C-MHP could be utilized and transformed, which will increase the nitrogen loading and lead to a negative effect on nitrogen removal. Economic assessment was conducted with regard to sludge reduction and utilization, and the results showed that the unit nitrogen removal cost was 57.13 CNY for C-acetate addition and 54.48 CNY for C-MHP addition, respectively. The C-MHP has a good technical and economic feasibility to be used to partial substitute external carbon source for nutrient removal.

Acknowledgments

This work was supported by the National Natural Science Foundation of China (No. 51278483), the National Major Science & Technology Projects for Water Pollution Control and Management (No. 2012ZX07202-005) and the project of Watershed Ecosystem Health Assessment of Guangxi.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <http://dx.doi.org/10.1016/j.jes.2015.12.031>.

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