Bioresource Technology 113 (2012) 148–153

Contents lists available at SciVerse ScienceDirect

Bioresource Technology

journal homepage: www.elsevier.com/locate/biortech

Effect of supplementary carbon addition in the treatment of low C/N high-technology industrial wastewater by MBR

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ARTICLE INFO

Article history:
Received 14 October 2011
Received in revised form 21 December 2011
Accepted 21 December 2011
Available online 27 December 2011

Keywords:
Membrane bioreactor
Supplementary carbon
Wastewater treatment
Denitrification
C/N ratio

ABSTRACT

The effect of supplementary carbon addition for the treatment of high-technology industrial wastewater in a membrane bioreactor (MBR) was investigated. The MBR was operated for 302 days under different C/N (BOD_L/NH_4^+-N) ratios, i.e. 0.9–1 to 20 days, 1.6–21 to 42 days, 2.9–43 to 82 days, 3.6–83 to 141 days, 4.8–165 to 233 days and 9.3–240 to 302 days. Irrespective of the C/N ratios investigated, SS and BOD_5 removal efficiencies were above 95% and above 80% COD removal efficiency was observed. In addition, complete nitrification was observed throughout the investigation. However, denitrification and total nitrogen removal efficiencies reached their maximum values at the highest C/N ratio (9.3) investigated. Real-time PCR analysis revealed 10 times higher ammonia oxidizing bacteria to total bacteria ratio under the highest C/N ratio condition (9.3) compared to the low C/N ratio condition (1.6).

1. Introduction

High-technology industries play a very important role in the economic system of Taiwan. In Hsinchu Science Park, semiconductor and optoelectronics are the main industries. These industries play greater role in Taiwanese economic development; nevertheless, wastewater produced in these industries is complex and hazardous. In order to meet the effluent disposal standards in the science park, the wastewater is pretreated in the respective industries before its discharge. Subsequently, the pretreated wastewater is collected into wastewater treatment plant. The SS, COD, BOD_5 and NH_4^+-N of wastewater were around 100, 100, 50 and 60 mg/L, respectively. Carbon to nitrogen ratio of the wastewater is very low, which has an impact on total nitrogen removal in the biological nutrient removal process (BNR).

Membrane bioreactor (MBR) technology is the combination of membrane filtration and activated sludge process. The secondary clarifier of conventional activated sludge (CAS) process is replaced by membrane module. The advantages of MBR include operation at higher mixed liquor suspended solids (MLSS) concentrations, reduced sludge production, independent control of solids and hydraulic retention time (SRT and HRT, respectively), compact and modular system requirement (i.e. small plant foot print), excellent performance and effluent quality (Judd, 2008). Since the MBR can be operated under longer SRT's, nitrification could be more effective and complete due to the space for the growth of nitrifiers. Moreover, carbonaceous organic matter removal efficiency is also excellent in MBR. Besides, the MBR can be modified with anaerobic, anoxic and oxic operations. Recent years, MBR is widely used in many countries owing to its elastic manipulations and excellent effluent quality. The MBR market value doubled in the 5 years between 2000 and 2005 to reach $217 and is expected to increase its market value from $296 million in 2008 to $488 million by 2013. Besides, the MBR technology is also efficient in treating numerous small domestic wastewaters apart from industrial and municipal wastewaters (Kraume and Drews, 2010; Santos and Judd, 2010).

Conventional biological nitrogen removal process utilizes the activity of nitrifiers and denitrifiers for removing nitrogen from wastewater. As a first step in nitrogen removal, NH_4^+-N is transferred to NO_3^--N by nitrifiers under aerobic condition and subsequently, NO_3^--N is transferred to N_2 by denitrifiers under anoxic condition. Several factors can affect directly the biological nitrogen removal efficiency, one of the most critical parameters is C/N ratio. Theoretically, the stoichiometric C/N ratio required for the denitrification process is 2.86 (COD/NO_3^--N). However, some authors reported that the practical C/N ratio required is greater than 2.86 in a combined nitrification/denitrification system. In anoxic/oxic

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doi:10.1016/j.biortech.2011.12.102

MBR (A/O MBR), The TN removal efficiency reached around 80%, when the C/N ratio of the system was maintained greater than 8 (Fu et al., 2009a,b; Tan and Ng, 2008). Carrera et al. (2004) have reported that complete denitrification was achieved when the C/N ratio was greater than 7.1 in pre-denitrification process. Choi et al. (2008) treated wastewater by intermittently aerated MBR (IAMBR) and observed that the TN removal efficiency reached a maximum of 89.1% when the C/N ratio was controlled at 7. The IAMBR experiment was also carried out by Yeom et al. (1999), where they obtained 90.7% TN removal efficiency at a C/N ratio of 11.6. Although the nitrifiers use inorganic carbon as electron donor, the C/N ratio still has influence on nitrification because of the growth competition between nitrifiers and heterotrophic microorganism. Komorowska-Kaufman et al. (2006) reported that nitrification is more stable when the C/N ratio less than 4 and reported a maximum TN removal efficiency of 95% at the stable condition.

In our previous study, we identified complete nitrification of the high-tech industrial wastewater in MBR without extra carbon source addition. However, the NO_3^--N concentration in the effluent was around 60 mg/L, which reveals that denitrification has not taken place. This is likely due to the characteristic of low C/N high-tech industrial wastewater. Therefore, the main aim of this investigation is to study the influence of C/N ratio in the treatment of low C/N high-technology industrial wastewater in a MBR system. The experiments were carried out under different C/N ratios of the wastewater in a pilot of anoxic/oxic (A/O) MBR.

2. Methods

2.1. Membrane bioreactor

A pilot scale A/O MBR was constructed in the premises of the wastewater treatment plant (WWTP) of Hsinchu Science Park in Hsinchu, Taiwan. The MBR system consists of anoxic and membrane tanks of 576 L and 1344 L capacity, respectively. The schematic diagram of the MBR is shown in Fig. 1. A submerged MBR module made up of PVDF (polyvinylidene difluoride) hollow fiber with a pore size of 0.05 μm and a membrane area of 3 m^2 was installed in the membrane tank. The feed water was pumped into the anoxic tank for denitrification and subsequently routed into the membrane tank for nitrification. The mix liquid suspended solids were recycled between aerobic and anoxic tanks with a recirculation ratio of 2.9 *Q*. Permeate from the MBR was withdrawn for 15 min, followed by settling and back wash times of 1 min each. During settling of solids no permeate outflow and back washing were done, which is useful for the physical relaxation of the membrane and also for relax cleaning of the solids by the membrane itself on its surface pores to certain extent. The operating cycle was automated by a programmable logic controller.

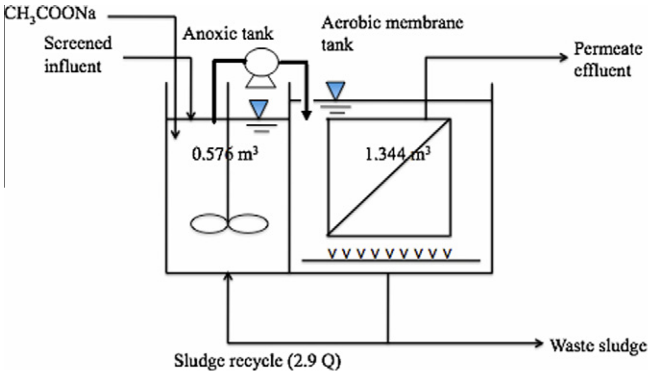

Fig. 1. Schematic diagram of the MBR system.

2.2. Operating conditions of the MBR

The pretreated high-technology industrial wastewater using the coarse screen was fed into the anoxic tank first. The characteristics of the high-technology industrial wastewater used in the present study are shown in Table 1. The flow rate were 14.85, 13.97, 13.47, 15.24, 13.83 and 15.06 m^3/day for days 1–20, days 21–42, days 43–82, days 83–141, days 165–233 and days 240–302, respectively. Mix liquid suspended solid from the anoxic tank was pumped into the aerobic MBR tank. The total HRT of the wastewater in the MBR system (including anoxic and aerobic tank) was controlled at 3.2 h. The effluent from the MBR system was taken out using a suction pump at 27 LHM and this constant flux was maintained throughout this investigation. However, the flux was increased to 29 LHM during days 297–303 owing to the breakage of membrane module during handling. The membrane module breakage has resulted from loosening of membrane module and it damaged the surface of membrane. This evidently increased the SS concentration in the effluent. The membrane adopted for the experiment is an assembly of multiple pieces of membranes, i.e. module, and every single piece has an individual valve. In this experiment, the pilot operated with a constant flow rate (*Q*). However, during the process of finding the membrane breakage, individual modules were tested one by one by closing the valves. While closing the valves, the permeate flux has increased to a certain extent. The air compressor was installed under the membrane module to scour the membrane fibers, and keep the DO above 2 mg/L in aerobic tank. Whereas, the DO in the anoxic tank was maintained below 0.3 mg/L. Sodium acetate was used as the source for supplementary carbon and the C/N ratio of the MBR system was varied from 1 to 9.3 by supplying sodium acetate under various flow rates. During days 1–19, MBR system was operated without supplementary carbon source. The various C/N ratios investigated in the present study, their time and the corresponding influent wastewater characteristics are shown in Table 2. The pH of the system was maintained between 7 and 7.5 by supplying sodium bicarbonate. During days 1–102, the MLSS and MLVSS concentrations were around 6600 and 2600 mg/L, and increased to 8500 and 4200 mg/L, respectively, between days 127 and 232. Subsequently, the MLSS and MLVSS concentrations were increased rapidly to maximum of 16,000 and 9000 mg/L, respectively, when the C/N was increased to 9.3 during days 240–269. In the final stage (after day 276), the MLSS and MLVSS concentrations maintained at a stable level, i.e. 11,000 and 6000 mg/L, respectively.

2.3. Quantification of bacteria by real-time PCR

As a first step, DNA was extracted using the FastDNA SPIN Kit for Soil (MP Biomedicals, Solon, OH, USA), in accordance with the manufacturer's instructions. The concentrations of DNA were

Table 1
Characteristics of high-technology industrial wastewater.

Parameters	Range	Mean
	mg/L	
SS	18–196	84
VSS	2–66	33
COD	36–108	64
BOD_5	27–73	36
NH_4^+-N	45–85	63
NO_2^--N	0–4	0.5
NO_3^--N	0.5–18	7
TKN	48–124	68
TN[a]	47–94	71

[a] The sum of NH_4^+-N, NO_3^--N and NO_2^--N.

Table 2
Characteristics of influent, effluent and removal efficiencies at different C/N ratio.

C/N[a]	Period (d)	Influent characteristics, mg/L								Effluent characteristics, mg/L (maximum removal efficiency)						
		SS	BOD_5	COD	NH_4^+-N	NO_2^--N	NO_3^--N	TN	Supplementary BOD_L, kg/day	SS	BOD_5	COD	NH_4^+-N	NO_2^--N	NO_3^--N	TN
1	1–19	99	42	68	62	1	7	69	–	2 (100)	<1 (100)	8 (91)	2 (100)	N.D.	74	76 (11)
1.6	20–42	76	43	63	66	<1	7	74	0.55	<1 (100)	<1 (100)	7 (93)	2 (100)	N.D.	66	69 (26)
2.8	43–82	89	43	66	56	<1	9	65	1.20	<1 (100)	<1 (100)	6 (97)	2 (100)	N.D.	61	63 (24)
3.6	83–164	74	43	64	67	<1	7	74	2.13	<1 (100)	<1 (100)	7 (96)	6 (100)	<1	53	60 (44)
4.8	165–233	98	47	64	71	<1	6	77	4.30	<1 (100)	<1 (100)	6 (98)	2 (100)	N.D.	45	48 (67)
9.3	240–302	74	42	57	57	<1	8	68	6.52	1 (100)	1 (100)	7 (91)	3 (100)	N.D.	10	13 (98)

[a] C/N ratio is based on the average value.

Table 3
Primers used in this study.

Target gene	Primer	Nucleotide sequence (5′–3′)	References
Bacterial *amoA* gene	*amoA*-1F *amoA*-2R	GGGGTTTCTACTGGTGGT CCCCTCKGSAAAGCCTTCTTC	Rotthauwe et al. (1997)
Archaeal *amoA* gene	Arch-*amoAF* Arch-*amoAR*	STAATGGTCTGGCTTAGACG GCGGCCATCCATCTGTATGT	Francis et al. (2005)
Nitrospira 16S rRNA gene	EUB338f Ntspa0685 M	ACTCCTACGGGAGGCAGC CGGGAATTCCGCGCTC	Regan et al. (2002)
Nitrobacter 16S rRNA gene	EUB338f NIT3	ACTCCTACGGGAGGCAGC CCTGTGCTCCATGCTCCG	Regan et al. (2002)
nirS gene	cd3aF R3 cd	GTSAACGTSAAGGARACSGG GASTTCGGRTGSGTCTTGA	Throback et al. (2004)
Bacterial 16S rRNA gene	Eub338 Eub518	ACTCCTACGGGAGGCAGCAG ATTACCGCGGCTGCTGG	Fierer and Jackson (2005)

determined with a NanoDrop ND-2000 spectrophotometer (Nanodrop Technologies, Wilmington, DE, USA). Subsequently, a LightCycler 2.0 system (Roche Diagnostics, Mannheim, Germany) was employed for the real-time PCR to quantify bacterial and archeal *amoA*, total *Nitrospira* and *Nitrobacter* and total bacterial 16S rRNA, and *nirS* genes. Table 3 and Table 4 show the primer information and real-time PCR conditions, respectively. The PCR reaction mixture (20 μL) contained 10 μL of 2 × SYBR® Premix Ex Taq™ (Perfect Real Time; TaKaRa Bio, Otsu, Japan), each primer and 20 ng of DNA extract. Melting curve analysis of the PCR products was conducted following each real-time PCR to confirm that the fluorescence signal originated from specific PCR products and not from primer to dimers or other artifacts.

Quantitative PCR efficiency for all standard curves of the six genes was more than 1.89 and the R^2 value for all six standard curves was more than 0.99. When converting copy number to cell number, assumed gene copy number/cell is 2 for bacterial *amoA* gene, 1 for archaeal *amoA* gene, 1 for *Nitrospira* 16S rDNA, 1 for *Nitrobacter* 16S rDNA, 1 for *nirS* gene, and 3.6 for bacterial 16S rDNA (Degrange and Bardin, 1995; Philippot, 2002).

2.4. Analytical methods

The concentrations of SS, VSS, COD, BOD, NH_4^+-N, NO_2^--N and NO_3^--N in the wastewater were analyzed using the Standard Methods (APHA, 1999). The SS concentration was measured by drying the sample at 103–105 °C; and subsequently, the residue was ignited at 550 °C to calculate the VSS concentration. The COD was measured by open reflux method. The BOD of the system was measured by the 5-day BOD test and the membrane electrode method was used to determine DO content in the samples. The NH_4^+-N, NO_2^--N and NO_3^--N concentrations were measured spectrophotometrically using a UV spectrophotometer (Shimadzu, Japan). From the analytical data, nitrification rate was calculated from the ammonia removal rate. Similarly, denitrification percentage was workout from the amount of total nitrate removed in the system (i.e. including the removal of nitrogen which produced from the oxidation of ammonium nitrogen).

Nitrification rate and denitrification percentage were calculated using the Eqs. (1) and (2), respectively. Denitrification percentage was defined as the nitrogen removed from oxidized ammonium nitrogen:

$$R_{\mathrm{Nitrification}} = Q_{\mathrm{in}}([NH_4^+\text{-N}]_{\mathrm{in}} - [NH_4^+\text{-N}]_{\mathrm{out}})/[VSS]_{\mathrm{reactor}}V_{\mathrm{reactor}} \quad (1)$$

$$\begin{aligned}\text{Denitrification }(\%) = \{&([NH_4^+\text{-N}]_{\mathrm{in}} - [NH_4^+\text{-N}]_{\mathrm{out}}) - [NO_3^-\text{-N}]_{\mathrm{out}} \\ &- [NO_3^-\text{-N}]_{\mathrm{in}})/([NH_4^+\text{-N}]_{\mathrm{in}} + [NO_3^-\text{-N}]_{\mathrm{in}} \\ &- [NH_4^+\text{-N}]_{\mathrm{out}})\} \times 100\% \end{aligned} \quad (2)$$

3. Results and discussion

3.1. MBR pilot performance

3.1.1. TMP

The variation of TMP in the pilot scale MBR is shown in Fig. 2. In the whole period, TMP was increased gradually with a constant flux. During days 1–120, TMP increased from 5.04 to 10.95 cm-Hg. Subsequently, the TMP shoot-up to a level a 50 cm-Hg on day 121 owing to some operational problems and the first chemical cleaning was carried out and it proceeded for 3 days. After chemical cleaning, the TMP returned to the initial value, i.e. around 5 cm-Hg. In the following period, the TMP situation cycled as above.

3.1.2. Influent and effluent characteristics

The characteristics of the high-technology industrial wastewater used in the present study are shown in Table 1. The concentrations of SS, VSS, COD, BOD_5, NH_4^+-N, NO_2^--N and NO_3^--N were 84, 33, 64, 36, 63, 0.5 and 7 mg/L, respectively. The BOD_5/NH_4^+-N ratio is around 0.6. The concentrations of NH_4^+-N and TKN were almost similar, which reveal that organic protein content in the wastewater is petite.

Table 4
Real-time PCR conditions used in this study.

Target gene	Primer conc. (μM)	Hot start		Cycle No.	Denature		Annealing		Extension	
		Temp.(°C)	Time (s)		Temp. (°C)	Time (s)	Temp. (°C)	Time (s)	Temp. (°C)	Time (s)
Bacterial *amoA* gene	0.2	95	20	50	95	7	55	20	72	20
Archaeal *amoA* gene	0.2	95	20	50	95	10	53	20	72	30
Nitrospira 16S rRNA gene	0.2	95	20	40	95	10	58	20	72	20
Nitrobacter 16S rRNA gene	0.1	95	20	40	95	7	60	20	72	40
nirS gene	1.0	95	10	30	95	5	57	20	72	15
Bacterial 16S rRNA gene	0.2	95	10	30	95	5	53	20	72	15

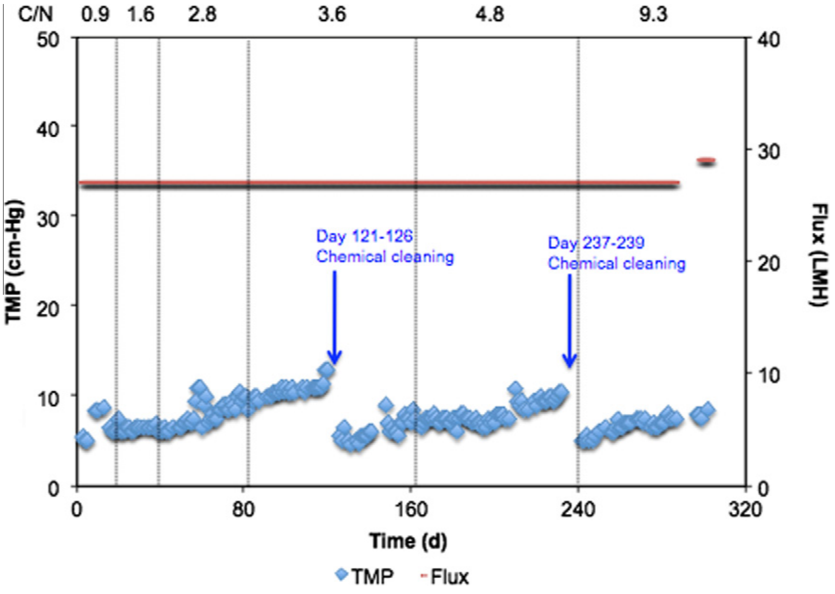

Fig. 2. TMP profile with time.

Table 2 shows the characteristics of the influent and effluent, and the removal efficiencies of various parameters in each period. The SS concentration in the influent was above 70 mg/L under all periods and less than 1 mg/L was observed in the effluent and the corresponding removal efficiencies were around 100%. This is attributed to the removal of solids by the membrane pores (Lerner et al., 2007). The BOD_5 in the influent was around 44 mg/L. After the treatment, the effluent concentration and removal efficiency were less than 1 mg/L and 100%, respectively, at C/N ratios below 4.8. The COD concentration of influent was around 65 mg/L and less than 8 mg/L in effluent under all the C/N ratios investigated, which shows the COD removal efficiency above 91%. The NH_4^+-N concentration in the influent and effluent were around 63 and 3 mg/L, respectively, indicating 100% removal efficiency. The NO_2^--N concentration in the influent was less than 1 mg/L and most of the time the NO_2^--N concentration in the effluent was 0 mg/L, indicating that all the NH_4^+-N in the influent was converted to NO_2^--N and subsequently to NO_3^--N. The NO_3^--N concentration in the influent was less than 9 mg/L, and its effluent concentration was above 60 mg/L when the C/N ratio was below 3.6. However, the NO_3^--N concentration decreased gradually while at higher C/N ratios (>3.6). These results demonstrate that the variation of C/N ratio has no significant effect on the removal efficiencies of SS, BOD_5, COD and NH_4^+-N. Moreover, these observations reflect that organic carbon and nitrification were highly successful irrespective of the various C/N ratios adopted. However, the removal of NO_3^--N in the MBR system, i.e. denitrification, was highly dependent on the C/N ratio and it was successful only when the ratio is maintained above 3.6. The lowest NO_3^--N concentration observed in the effluent was 10 mg/L at a C/N ratio 9.3.

3.2. Effect of C/N ratio on pollutant removal

3.2.1. Effect of C/N ratio on removal efficiencies

The sodium acetate as extra organic carbon source was used to vary the C/N ratio, and converted to BOD_L to determine the dosage. The relationship between C/N ratio and the removal efficiencies of COD, BOD_5, NH_4^+-N and TN were illustrated in Fig. 3. The COD, BOD_5, NH_4^+-N removal efficiencies were stable and good, even the C/N ratio was varied from 0.9 to 9.3. The experimental outcomes indicate that above 90% BOD_5 removal and above 80% for COD removal could be achieved in the MBR system. Moreover, the NH_4^+-N removal efficiency was higher than 80% throughout the experiment irrespective of the various C/N ratios adopted for the investigation. On contrary, a significant relationship between TN removal and C/N ratio was observed. The TN removal efficiency increased with the increase in C/N ratio, i.e. more than 60% TN removal efficiency was observed when the C/N ratio higher than 8. However, a maximum TN removal efficiency of 98% was achieved at a C/N ratio of 9.3. This result is in good agreement with the previous investigations (Tan and Ng, 2008).

3.2.2. Effect of C/N ratio on nitrification and denitrification

Fig. 4 shows the variation of nitrification rate and denitrification percentage with different C/N ratios. The nitrification rate was relative high when the C/N ratio was less than 4. The highest denitrification rate of 0.59 g of N/g of VSS-d was observed at a C/N ratio of 2.5. The nitrification rate was always below 0.2 g N/g VSS d when the C/N ratio was greater than 5 (Komorowska-Kaufman et al., 2006). In general, the nitrification rate is expected to

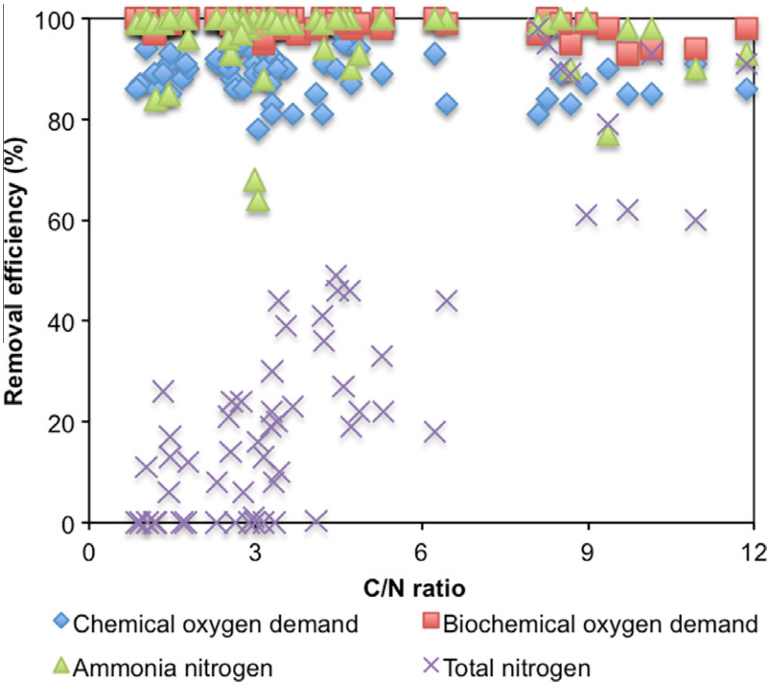

Fig. 3. COD, BOD_5, NH_4^+-N and TN removal efficiencies with different C/N ratio in the MBR.

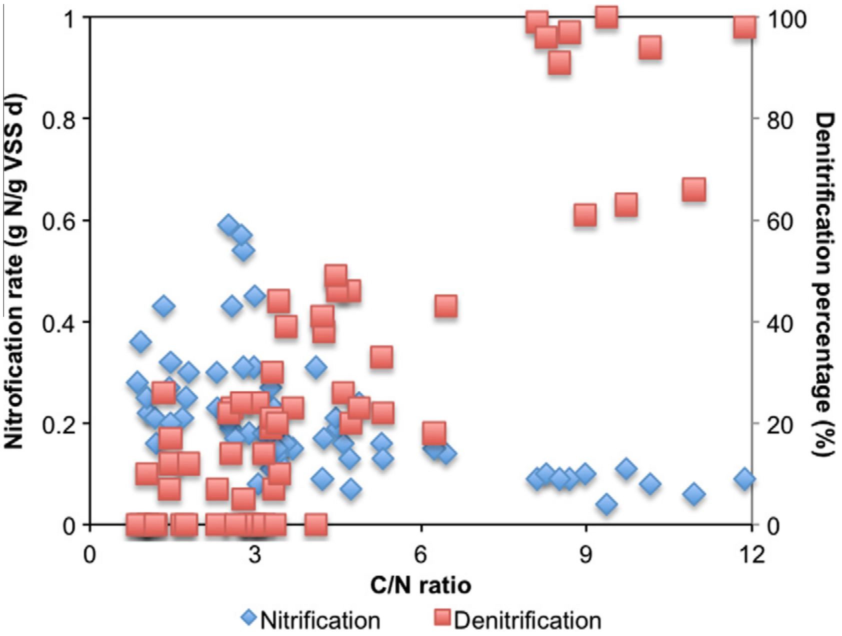

Fig. 4. Nitrification rate and denitrification percentage with different C/N ratio in the MBR.

decrease with the increase in the C/N ratio owing to the competitive growth of heterotrophic organisms in the system.

The experimental result also pointed out that denitrification was not significant when the C/N ratio was less than 3. However, it increased considerably when the C/N ratio was higher than 3. This could be due to the insufficiency of the organic source for the growth of heterotrophic denitrification. An average value of 83% denitrification was reached with C/N ratio above 8, and achieved completely at a C/N of 9.3. Carrera et al. (2004) reported complete denitrification at a C/N ratio of 7.1 in a pre-denitrification process. Fu et al. (2009a,b) obtained a complete denitrification with C/N ratio of 9.3 in an A/O MBR. These results also support the present investigation.

3.3. Quantification of bacteria by real-time PCR

The quantification results show that the proportion of ammonia oxidizing bacteria to total bacteria in the high C/N ratio (C/N = 9.3) sludge is around 0.05%, which is 10 times lower than that in the low C/N ratio sludge (C/N = 0.9). It is suggested that the bountiful carbon source in the high C/N ratio sludge enriched the growth of heterotrophic microorganisms and then slightly inhibited the growth of ammonia-oxidizing bacteria. *Nitrospira* is the major nitrite-oxidizing bacteria observed in both high and low C/N ratio sludge, and its magnitude was two orders higher than *Nitrobacter*. The nitrite concentration in the influent supplied to MBR was <1 mg/L throughout the experimental period which maintained a low nitrite condition. It is anticipated that under such a low nitrite condition, *Nitrospira* will be the predominant organisms in the MBR system. Since *Nitrospira* has high substrate affinity, which is useful for its predominance in the low nitrite environments (Schramm et al., 1999). However, no significant difference in denitrifier population was observed between high and low C/N ratio sludge samples.

4. Conclusions

The effect of supplementary carbon source in treating high-technology industrial WW by using MBR was investigated under various C/N ratios. The removal efficiencies of SS, COD and BOD_5 were not affected by the change in C/N ratio. Almost complete SS and BOD_5 removal efficiencies were observed throughout the experimental period, and above 80% COD removal efficiency was observed under all the C/N ratios investigated. However, the TN removal efficiency increased with the increase in C/N ratio and a maximum of 98% TN removal was obtained at a C/N of 8.1. Nitrification rate decreased with the increase in C/N ratio and a highest rate of 0.59 g of N/g of VSS d was obtained at a C/N of 2.5, and decreased quickly while C/N ratio greater than 5. On the other hand, the denitrification percentage increased with the increase in C/N ratio and a complete denitrification (no nitrate remained in effluent) was observed when the system C/N ratio was maintained at 9.4. In addition, ammonia oxidizing bacteria to total bacteria under low C/N ratio condition is 10 times higher than that observed in the high C/N ratio condition. The whole experimental results reveal that a C/N ratio of 8 and above is most appropriate for the occurrence of effective simultaneous nitrification and denitrification.

Acknowledgements

This research was funded by AXLON International Engineering Corporation. We would like to express our gratitude for staves of AXLON for assisting in the pilot scale MBR work and also for solving the technical issues in MBR plant operation during the course of this work. We also thank Century Environtech Co. Ltd. and AquaLab Co. Ltd. for providing the membrane modules.

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