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EFFECT OF COD/N RATIO ON PERFORMANCE OF A SEQUENCING BATCH REACTOR TREATING SALINE WASTEWATER

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Abstract

The performance and microbial community of an anoxic-aerobic sequencing batch reactor (SBR) treating saline wastewater were investigated at different influent COD/N ratios. The average COD removal efficiencies at steady states were 87.46%, 73.75%, 64.87% and 88.34% at the COD/N ratios of 20, 10, 6 and 30, respectively. The average NH_4^+-N removal efficiencies were 79.74%, 74.34%, 64.55% and 89.29% at the COD/N ratios of 20, 10, 6 and 30, respectively. No obvious accumulation of NO_2^--N and NO_3^--N in the effluent was found during the whole operational period. The specific ammonium oxidation rate (SAOR), specific nitrite oxidation rate (SNOR) and specific oxygen uptake rate (SOUR) increased with the decrease of COD/N ratio from 20 to 6, whereas the specific nitrate reduction rate (SNRR) decreased. The diversity indices of microbial community in the SBR were 2.19, 2.10, 2.17 and 2.07 at the COD/N ratio of 20, 10, 6 and 30, respectively. Some bacteria were present at all the COD/N ratios, such as *Nitrosomonas* sp., *Ohtaekwangia kribbensis* and *Propionicimonas paludicola*, suggesting these bacteria could adapt to the shock of influent COD/N ratio.

Key words: COD/N ratio, microbial community, saline wastewater, sequencing batch reactor, specific ammonium oxidation rate

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1. Introduction

The saline wastewater mainly derives from seafood processing, mariculture, brewing, tanning, petroleum, paper making, pharmaceutical process and chemical production (Lefebvre and Moletta, 2006; Tawfik et al., 2017). The saline wastewater is often rich in carbonaceous and nitrogenous contaminants as well as high amount of inorganic salt. When the saline wastewater is discharged into the environment without proper treatment, it can result in severe environmental pollution in surface water and groundwater. The physical-chemical treatment processes, including thermal techniques, coagulation-flocculation, ion exchange and membrane technology, are usually used to treat saline wastewater (Lefebvre et al., 2006; Pelaez et al., 2016). However, their startup and running cost are relatively high, and they consume large amounts of energy. Biological treatment processes have many advantages over physical-chemical treatment processes due to their low operational cost and high efficiency, and many biological treatment technologies have been successfully used to treat saline wastewater (Boardman et al., 1995; Gregory et al., 2012; Jang et al., 2013). SBR is known to be particularly robust and to withstand extreme conditions, which has often been used to treat saline

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wastewater (Lefebvre et al., 2006). The performance of SBR can be affected by many influencing factors, such as COD/N ratio, anoxic/aerobic phase fraction, salinity, solid retention time, and hydraulic retention time. Among the above-mentioned influencing factors, COD/N ratio is regarded as one of the key factors affecting functional microbial population and bioreactor performance (Fu et al., 2009; Kong et al., 2016; Li et al., 2016; Saba et al., 2017). Carrera et al. (2004) found the increase of influent COD/N ratio led to the decrease of nitrification rate. Li et al. (2017) indicated that low COD/N ratio could impact the stability of partial nitritation/anammox process. Wu et al. (2012) demonstrated that low COD/N ratio promoted the nitrifying ability and nitrifying bactiera population. Hao et al. (2016) reported that the variation of COD/N ratio could obviously affect the sludge property in a membrane bioreactor. However, little information is found in evaluating the effect of COD/N ratio on microbial activity and microbial community of an anoxic-aerobic SBR treating saline wastewater.

The main aims of the present research were (a) to evaluate the effect of COD/N ratio on COD and nitrogen removal in an anoxic-aerobic SBR treating saline wastewater, (b) to analyze the variation of SOUR, SAOR, SNOR and SNRR at different COD/N ratios, (c) to investigate the change of microbial community at different COD/N ratios by polymerase chain reaction-denaturing gradient gel electrophoresis (PCR-DGGE).

2. Material and methods

2.1. Reactor set-up and operation

A lab-scale plexiglass SBR with 7.7 L effective volume was applied in the present research. The SBR had 14 cm internal diameter of and 55 cm total height. A peristaltic pump was used to feed the influent into the reactor. The effluent was drawn at a height of 15 cm from the bottom by a solenoid valve, and the volume exchange rate very cycle was 70 %. The mixed liquor in the SBR at the anoxic stage was mixed by a magnetic stirrer (RH basic 1, IKA, Germany), and air was introduced at the aerobic stage by two air diffusers at the bottom of the reactor.

The anoxic-aerobic SBR was operated in a cycle of 12 h. One cycle consisted of 0.25 h of influent addition, 7 h of aerobic stage, 3 h of anoxic stage, 1.5 h of settling and 0.25 h of effluent withdrawal. The system was operated at room temperature (20-30 ºC). The dissolved oxygen (DO) concentration at the aerobic stage was over 2.0 mg/L, and that at the anoxic stage was below 0.5 mg/L.

2.2. Seed sludge and wastewater composition

The seed sludge was obtained from the recycled sludge of the secondary clarifier in Licunhe municipal wastewater treatment plant in Qingdao City, China. The initial mixed liquid suspended sludge (MLSS) in the SBR was 3050 mg/L. The composition of synthetic saline wastewater was as follows (mg/L): glucose, 384; NH_4Cl, 60-200; Na_2HPO_4, 14; and seawater crystal, 3×10^4 (corresponding to 3% salinity). Based on 3% salinity, the main components of the seawater crystal solution were as follows (mg/L): Na^+, 9880; Cl^-, 18025; Mg^{2+}, 950; SO_4^{2-}, 2500; K^+, 360; Ca^{2+}, 300; Zn^{2+}, 0.015; Mn^{2+}, 0.013; Fe^{2+}, 0.13; Co^{2+}, 3×10^{-4}; Mo^{6+}, 3×10^{-3}; I^-, 0.07; Sr^+, 7.5×10^{-3}; I^-, 70; Se^{6+}, 3.5×10^{-4}; Cu, 0.05; Al^{3+}, 0.01; PO_4^{3-}-P, 0.045; NH_4^+-N, 0.02; NO_2^--N, 0.01; and NO_3^--N, 0.3.

2.3. Analytical methods

The measurements of COD, NH_4^+-N, NO_2^--N, NO_3^--N, and MLSS were performed according to the Chinese NEPA standard methods (NEPA, 2002). The DO concentration in the reactor was measured with a dissolved oxygen meter (oxi 330i, WTW, Germany).

2.4. Determination of SOUR

A certain amount of activated sludge from the SBR was transferred into a pre-cleaned biochemical oxygen demand (BOD) bottle. The BOD bottle was fully filled with the pre-aerated synthetic wastewater with 3% salinity and then airproofed by the rubber stopper with an oxygen-sensing probe. The mixed liquor in the BOD bottle was mixed by a magnetic stirrer (RH basic 1, IKA, Germany) at room temperature. The DO concentration was recorded at an interval of 30 s until DO concentration below 1.0 mg/L. The MLSS in the BOD bottle was regarded as an invariant during the whole experiment due to the shorter operational time. The SOUR was calculated from DO-time curve based on the MLSS in the BOD bottle.

2.5. Determination of SAOR, SNOR and SNRR

The SAOR, SNOR and SNRR at different C/N ratios were determined immediately before the C/N ratio was changed to a new value. The tests of SAOR, SNOR and SNRR were performed in a 500-mL Erlenmeyer flask with 100 mL activated sludge from SBR and 350 mL synthetic saline wastewater, respectively. The sludge and the synthetic wastewater in the Erlenmeyer flask were mixed by a magnetic stirrer (RH basic 1, IKA, Germany). The nitrogen sources for the test of the SAOR, SNOR and SNRR were NH_4Cl, $NaNO_2$ and $NaNO_3$, respectively. The initial NH_4^+-N, NO_2^--N and NO_3^--N concentration were 120, 147 and 303 mg L^{-1}, respectively. The air was introduced into the Erlenmeyer flask by an air diffuser in the test of SAOR or SNOR, and the nitrogen gas was filled in the Erlenmeyer flask for the anaerobic condition in the SNNR test. The MLSS in the Erlenmeyer flask was regarded as an invariant during the test of SAOR, SNOR and SNRR due to the shorter operational time, respectively. The SAOR, SNOR and SNRR were calculated by

monitoring the decreased rate of NH_4^+-N, NO_2^--N and NO_3^--N versus time, respectively.

2.6. Polymerase chain reaction-denaturing gradient gel electrophoresis (PCR-DGGE) and sequence analysis

2.6.1. DNA extraction

The DNA was extracted from 0.25 g (dry weight) activated sludge using PowerSoil@ DNA Islation Kit (Anbisheng Inc., China) according to the manufacturer's protocol.

2.6.2. PCR amplification

PCR amplifications were carried out in an *i*Cycler Thermal Cycler PCR (Bio-Rad Co., Ltd., USA). The bacterial primer 101F with a GC clamp (5'- CGC CCG CCGCGCGCGGCGGGCGGGGCGGGGGCACGG GGGGTGGCGGACGGG TGA GTAA -3') and the universal primer 534 R (5'- ATT ACC GCG GCT GCT GG-3'), targeting the 16S rRNA V3 variable region, were used to amplify 16S rDNA (Muyzer et al., 1993). The 50 μL reaction mixture contained 2 μL extracted DNA template, 0.5 μL TaKaRa Taq™ DNA polymerase (5 U μL^{-1}, TaKaRa Biotechnology Dalian Co. Ltd., China), 5 μL 10×PCR buffer (Mg^{2+} plus, TaKaRa Biotechnology Dalian Co. Ltd., China), 4 μL dNTP (each 2.5 mmol/L), 1 μL each primer (each 20 μmol/L, TaKaRa Biotechnology Dalian Co. Ltd., China) and adjusted to a final volume of 50 μL with sterile deionized water. The PCR amplification of 16S rDNA was performed according to the Touchdown PCR program: initial denaturation at 95 ºC for 5 min, denaturation at 95 ºC for 30 s, annealing at 56 ºC for 30 s (the temperature was decreased by 0.5 ºC every cycle until the touchdown temperature of 51 ºC was reached), extension at 72 ºC for 45 s, denaturation at 95 ºC for 30 s after 10 cycles, annealing at 51 ºC for 30 s, extension at 72 ºC for 45 s followed by a final extension at 72 ºC for 10 min after 20 cycles and end at 4 ºC. The PCR products stained with ethidium bromide (EB) were electrophoresed in a 1.2 % (w/v) agarose gel at 120 V for 40 min, and quantified by comparison with a standard marker (DL 2000, TaKaRa Biotechnology Dalian Co. Ltd., China).

2.6.3. DGGE analysis

DGGE was performed using a DCode™ Universal Mutation Detection System (Bio-Rad Co., Ltd., USA). PCR samples containing 40 μL PCR amplification products and 8 μL 6×Loading buffer were loaded into each well of a 8% (w/v, g mL^{-1}) polyacrylamide (37.5 : 1, acrylamide : bisacrylamide) gel in 1×TAE buffer (Tris-acetate-EDTA buffer) using a denaturing gradient ranging from 30 % to 60% (30% denaturant agent contained 2.52 g urea, 2.4 mL deionized formamide, 4 mL 40% polyacrylamide, 0.4 mL 50×TAE buffer and adjusted to a final volume of 20 mL with deionized water; the content of each component for 60% denaturant agent was twice of the 30%). Electrophoreses were performed at 65 ºC and 150 V for 420 min. After electrophoresis, the gel was stained with 0.1% $AgNO_3$ for 25 min, and then rinsed with Milli-Q water. The gels were visualized under UV light with the Gel Doc XR System (Bio-Rad Co., Ltd., USA).

DGGE profiles were analyzed using the Quantity One software (version 4.6.2, Bio-Rad Co., Ltd., USA). Dendrograms relating band pattern similarities were automatically calculated using the unweighted pair group method with the arithmetic average (UPGMA) clustering algorithm, which was included in the Quantity One software. The diversity of microbial community was examined by the Shannon diversity index (*H*). *H* was calculated on the basis of the bands on the gel tracks that were applied for the generation of the dendrograms by using the intensities of the bands as judged by peak heights in the densitormetric curves. The equation for the Shannon diversity index is as follows (Eq. 1):

$$H = -\sum (n_i / N) \log(n_i / N) \quad (1)$$

where n_i is the height of the peak, *N* is the sum of all peak heights of the densitometric curve.

2.6.4. Sequence analysis

The DGGE bands were excised from the gel, re-amplified and electrophoresed again in DGGE gel to confirm the mobility of the bands. The new PCR products were cloned. The cloned products were sequenced by Shanghai Jinsirui Biological Science and Technology Co., Ltd. Bacteria serial numbers were obtained from ribosomal database project (http://rdp.cme.msu.edu/). Sequence comparisons were conducted with the BLAST search option in the NCBI nucleotide sequence database (http://www.ncbi.nlm.nih.gov/). A phylogenetic tree was constructed by using MEGA 4.0 (Kumar et al., 2004), and reference sequences used in tree construction were acquired from GenBank.

3. Results and discussion

3.1. Effect of COD/N ratio on COD removal

The variations of COD concentration in the influent and effluent at different COD/N ratios are shown in Fig. 1. When the influent COD concentration varied between 287 mg/L and 315 mg/L, the influent COD/N ratios at 20, 10, 6 and 30 were obtained by varying the influent NH_4^+-N concentration. The average COD removal efficiency at the steady state decreased from 87.46% to 64.87% with the decrease of influent COD/N ratio from 20 to 6. When the COD/N ratio suddenly increased from 6 to 30, the average COD removal efficiency at the steady state increased from 64.87% to 88.34%. The results in this study were conflict with the previous research for treating salt-free wastewater (Rene et al., 2008). As the decrease of influent COD/N ratio may

increase in the influent salinity, it can be postulated that the increase of salinity may inhibit heterotrophic activity. However, the intensive study is required to verify this presumption in the future.

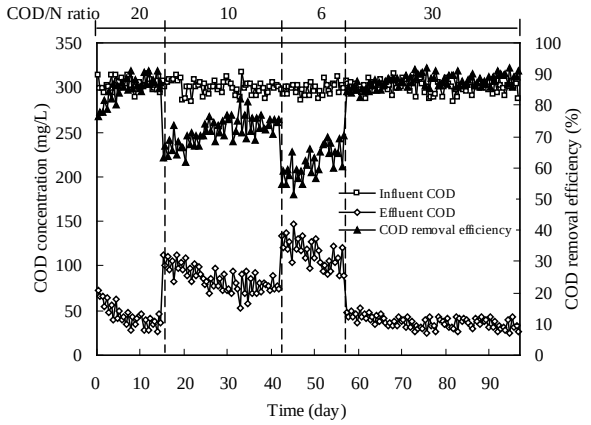


Fig. 1. Evolution of influent and effluent COD concentration at different COD/N ratios

3.2. Effect of COD/N ratio on nitrogen removal

The variations of NH_4^+-N, NO_2^--N and NO_3^--N in the influent and effluent at different COD/N ratios are shown in Fig. 2.

The average NH_4^+-N removal efficiency at steady state decreased from 79.74% to 64.55% with the decrease of the COD/N ratio from 20 to 6. Duk et al. (1997) reported that the NH_4^+-N removal efficiency decreased with the decrease of COD/N due to the increase of NH_4^+-N loading, which was consistent with the results in the present study. When the COD/N ratio increased from 6 to 30, the average NH_4^+-N removal efficiency at the steady state increased from 64.55% to 89.01%. No obvious accumulation of NO_2^--N and NO_3^--N in the effluent was found in the present study. The results could be explained that autotrophic denitrification accompanied with heterotrophic denitrification might simultaneously occur in the anoxic-aerobic SBR due to the lack of organic carbon source at low influent COD/N ratio.

3.3. Effect of COD/N ratio on SOUR

The sludge samples for SOUR analysis at the steady states were obtained from the anoxic-aerobic SBR on day 14, 35, 56 and 90, corresponding to COD/N ratio of 20, 10, 6 and 30, respectively. As shown in Fig. 3, the SOUR increased from 34.71 to 39.24 mg/(g MLSS h) with the decrease of COD/N ratio from 20 to 6. The results could be explained that the activity of nitrifying bacteria increased with the increase of influent NH_4^+-N and more oxygen was required for the oxidation of NH_4^+-N and NO_2^--N. In addition, the lower COD/N ratio could result in the increase of smaller microbial flocs.

The smaller microbial flocs provided a larger surface area for oxygen transfer, and they exhibited higher respirometric activity of microbes at lower COD/N ratio. Khan et al. (2013) reported the similar results that the SOUR at a C/N ratio of 10 was higher than that at a C/N ratio of 20 due to the present of smaller microbial-flocs at low C/N ratio. However, when the COD/N ratio suddenly increased from 6 to 30, the SOUR decreased from 39.24 to 31.68 mg/(g MLSS h) in the present study.

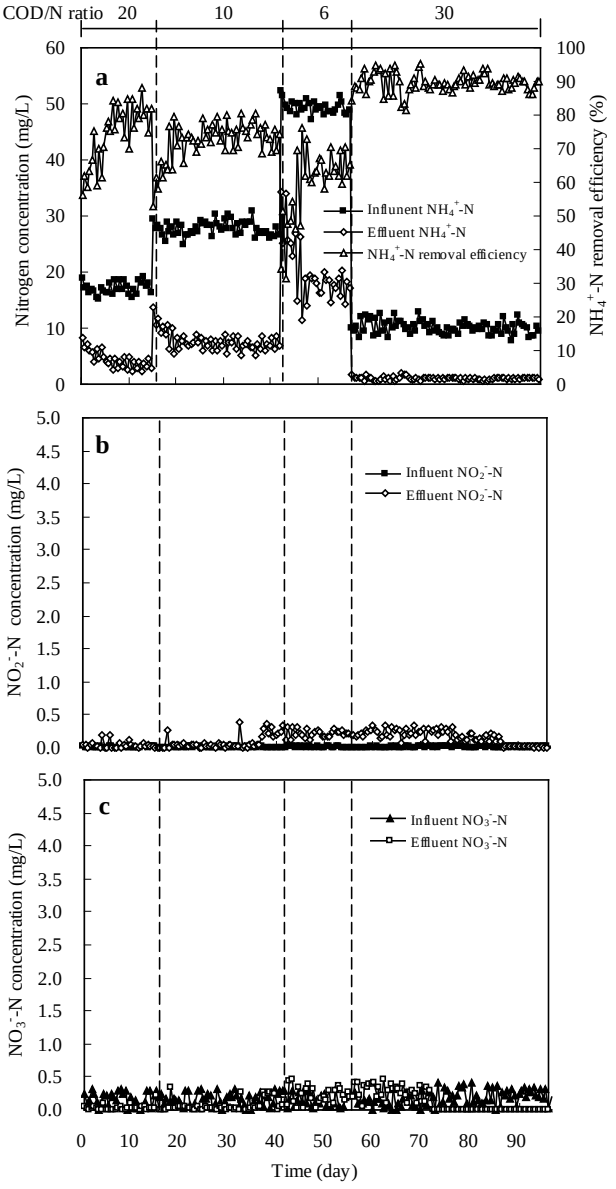


Fig. 2. Evolution of influent and effluent NH_4^+-N (a), NO_2^--N (b) and NO_3^--N (c) concentrations at different COD/N ratios

3.4. Effect of COD/N ratio on SAOR, SNOR and SNNR

The sludge samples for SAOR, SNOR and SNNR analysis were obtained from the anoxic-aerobic SBR on day 14, 35, 56 and 90, corresponding to COD/N ratio of 20, 10, 6 and 30, respectively (Fig. 4). As shown in Fig. 4a, the SAOR increased from 1.61 to 1.70 mg N/(g MLSS h) with the decrease of

COD/N ratio from 20 to 6, and the SNOR increased from 1.35 to 1.43 mg N/(g MLSS h).

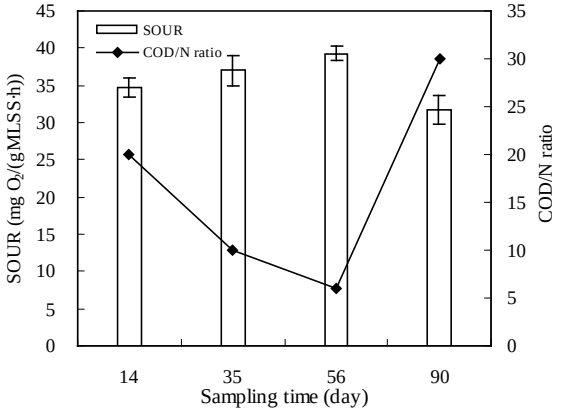


Fig. 3. Evolution of SOUR at different COD/N ratios

When the COD/N ratio suddenly increased from 6 to 30, the SAOR and SNOR decreased from 1.70 to 1.66 and from 1.43 to 1.27 mg N/(g MLSS h), respectively. Carrera et al. (2004) also reported that he nitrification rate decreased as the influent COD/N ratio increased from 0.71 to 3.4. Some researchers reported that the substrate concentration for NH_4^+-N and NO_2^--N oxidation bacteria increased with the decrease of COD/N ratio, which stimulated the growth of ammonia- oxidizing bacteria and nitrite-oxidizing bacteria (Fu et al., 2004; Harremoës and Sinkjaer, 1995). The variation of SNRR at different COD/N ratios is shown in Fig. 4b. The SNRR decreased from 27.65 to 17.68 mg N/(g MLSS h) with the decrease of COD/N ratio from 20 to 6. Choi et al. (2008) reported the similar result that the decrease of C/N ratio led to a lower denitrification rate and poorer assimilation of organic matter and nutrients. When the COD/N ratio increased from 6 to 30, the SNRR increased from 17.68 to 21.57 mg N/(g MLSS h). Liu et al. (2010) demonstrated that the fraction of denitrifying populations over nitrifying populations increased with the increase of COD/N ratio, and the denitrifying populations became the dominant at high COD/N ratio. On the contrary, the decrease of denitrifying bacterial populations at low C/N ratio could result in the decrease of SNRR.

3.5. Effect of COD/N ratio on microbial community

The microbial communities of the anoxic-aerobic SBR at different COD/N ratios were investigated through PCR-DGGE analysis (Fig. 5). As shown in Fig. 5a, the DGGE analysis showed that there were some changes in the band profiles at different C/N ratios as well as the variations in band intensity. Some bands (1-3, 8, 9, 12, 13, 15, 17, 18, and 20) were consistently present at all the influent COD/N ratios though their intensities varied in different periods. The other bands (4-7, 10, 11, 14, 16, and 19) appeared at some COD/N ratios and were not necessarily present throughout the whole operational period. It is evident that some micrograms adapting to the variation of COD/N ratio tend to become predominant bacteria, while others tend to deplete or weaken gradually.

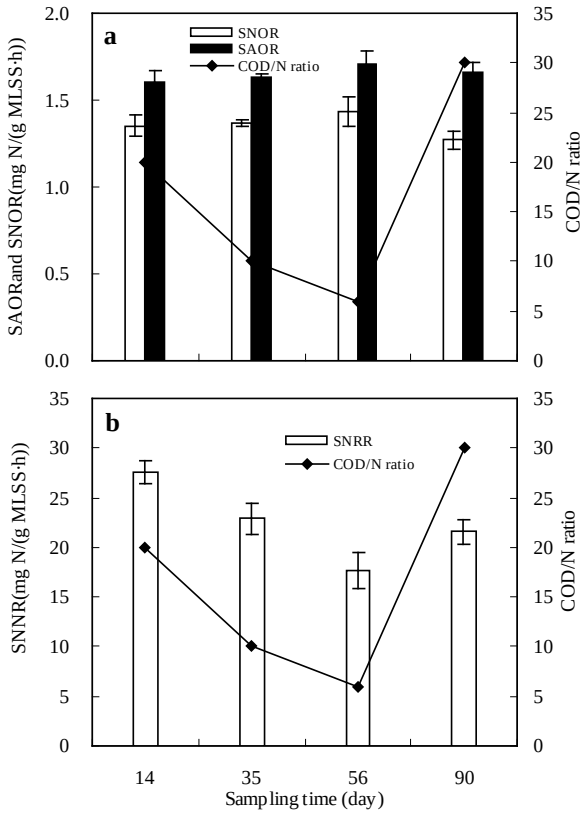


Fig. 4. Evolution of SAOR, SNOR (a) and SNRR (b) at different COD/N ratios

The UPGMA cluster analysis was used to analyze the microbial community similarities among different salinities. Fig. 5b shows that the microbial populations were categorized into two groups. The sludge sample at the COD/N ratio of 20 represented the first group, and the other sludge samples belonged to the second group. The similarity of microbial community between the first group and the second group was only 0.71, suggesting that the variation of COD/N ratio could affect the microbial community structure. The sludge samples at the COD/N ratio of 30 were categorized into the first sub-group in the second group, and those at the COD/N ratio of 10 and 6 belonged to the second sub-group. The similarity of microbial community in the sludge samples between the first sub-group and second sub-group was 0.79, and that was 0.88 between the COD/N ratio of 10 and 6. In addition, the diversity of microbial community in DGGE profile was evaluated by the Shannon diversity index (H). The Shannon diversity indices of microbial community were 2.19, 2.10, 2.17 and 2.07 at the C/N ratio of 20, 10, 6 and 30, respectively.

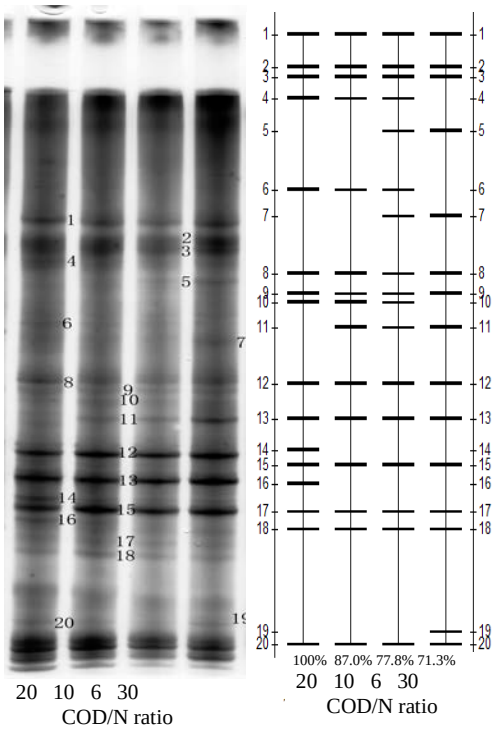
To gain further insight into the microbial community structure, twenty discernable bands were excised from the DGGE gel and sequenced. By using the BLAST program of Genbank, the detected

sequences were compared with sequences deposited in the database, and the most similar sequences of discernable DGGE bands are listed in Table 1.

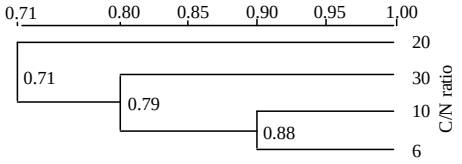
Zoogloea ramigera (band 14) and *Micropruina glycogenica* (16) were only at the COD/N ratio of 20, and it was not detected at the other COD/N ratios.

Some bacteria were found in the whole operational period, such as *Nitrosomonas* sp (band 1), *Ohtaekwangia kribbensis* (band 2), *Propionicimonas paludicola* (band 3), *Clostridium* sp. Z-0511 (band 8), *Aminomonas omnivores* (band 9), *Methylobacillus flagellatus* (band 12), *Azoarcus indigens* (band 13), *Hoeflea marina* (band 15), *Rhizobium giardinii* (band 17), *Phycisphaera mikurensis* (band 18) and *Azoarcus anaerobius* (band 20), suggesting these bacteria were capable of tolerating up to the shock of COD/N ratio. *Nitrosomonas* sp belonged to ammonia-oxidizing bacteria, and they could grow at the salinity of 3% (Nicolaisen and Ramsing, 2002).

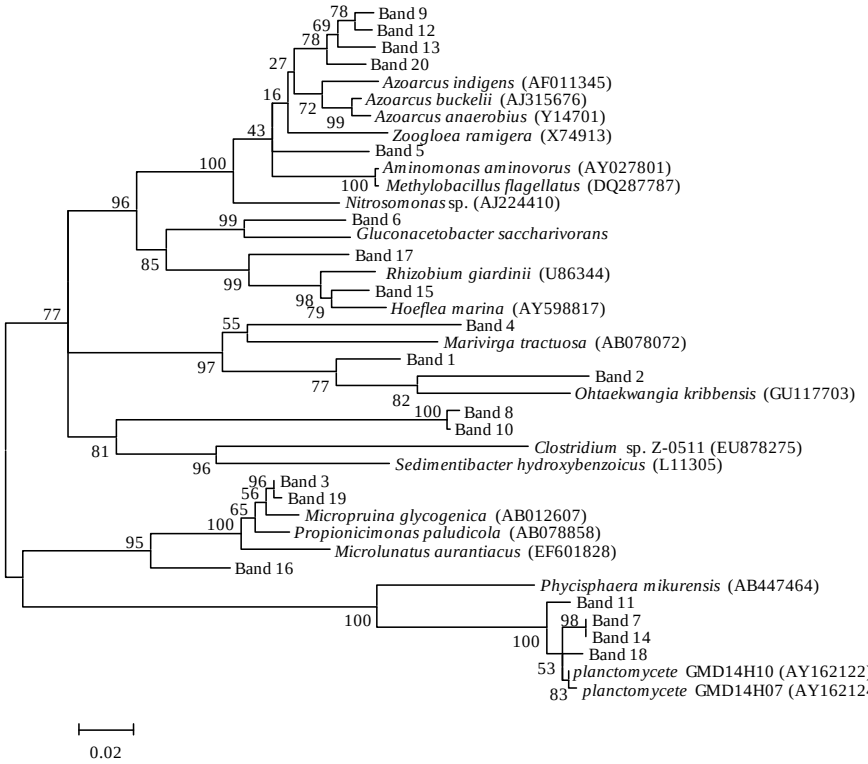
Rhizobium giardinii could utilize a wide range of carbohydrates as sole carbon sources for growth and a wide range of amino acids as sole nitrogen sources (Amarger et al., 1997). *Azoarcus anaerobius* was denitrifying bacterium, and it was observed in all samples at different COD/N ratios (Springer et al., 1998).



(a)



(b)



(c)

Fig. 5. DGGE gel banding profiles of microbial communities in the aerobic-anoxic SBR at different COD/N ratios (a), percent similarity analysis of lanes on the DGGE gel (b), and phylogenetic tree of bacteria based on the results of BLAST (c)

Table 1. Closest phylogenetic affiliations of sequences obtained from sludge samples at different COD/N ratios

Bands	***Closest related sequences***	***Accession number***	***Similarity (%)***	***Class containing related sequences***
1	*Nitrosomonas* sp.	AJ224410	95	β-proteobacteria
2	*Ohtaekwangia kribbensis*	GU117703	96	Bacteroidetes
3	*Propionicimonas paludicola*	AB078858	95	Actinobacteria
4	*Marivirga tractuosa*	AB078072	95	Sphingobacteria
5	*Azoarcus buckelii*	AJ315676	98	β-proteobacteria
6	*Gluconacetobacter saccharivorans*	AJ012466	100	α-proteobacteria
7	*planctomycete* GMD14H10	AY162122	98	Phycisphaerae
8	*Clostridium* sp. Z-0511	EU878275	88	δ-proteobacteria
9	*Aminomonas aminovorus*	AY027801	100	β-proteobacteria
10	*Sedimentibacter hydroxybenzoicus*	L11305	91	Clostridia
11	*planctomycete* GMD14H07	AY162124	97	Phycisphaerae
12	*Methylobacillus flagellatus*	DQ287787	98	β-proteobacteria
13	*Azoarcus indigens*	AF011345	98	β-proteobacteria
14	*Zoogloea ramigera*	X74913	90	β-proteobacteria
15	*Hoeflea marina*	AY598817	100	α-proteobacteria
16	*Micropruina glycogenica*	AB012607	95	Actinobacteria
17	*Rhizobium giardinii*	U86344	94	α-proteobacteria
18	*Phycisphaera mikurensis*	AB447464	94	Phycisphaerae
19	*Microlunatus aurantiacus*	EF601828	98	Actinobacteria
20	*Azoarcus anaerobius*	Y14701	100	β-proteobacteria

Although *Azoarcus buckelii* (band 5), *planctomycete* GMD14H10 (band 7), *planctomycete* GMD14H07 (band 11), and *Microlunatus aurantiacus* (19) were not detected at the COD/N ratio of 20, they become predominant bacteria at the COD/N ratio of 6 and 30. *planctomycete GMD14H10* and *planctomycete GMD14H07* belonged to autotrophic microorganisms, and they could oxidize ammonia-nitrogen (Pynaert et al., 2003). The existence of *planctomycete GMD14H10* and *planctomycete GMD14H07* at low COD/N ratio might be one of the reasons resulted in the increase of SAOR with the decrease of COD/N ratio. Although some bacteria were found at the C/N ratio of 20, such as *Marivirga tractuosa* (band 4), *Gluconacetobacter saccharivorans* (band 6), *Zoogloea ramigera* (band 14) and *Micropruina glycogenica* (band 16), they gradually depleted or weaken with the variation of C/N ratio in the influent. Fig. 5c showed the phylogenetic relationships among the DGGE fragments and 16S rDNA of isolated bacteria. The sequences belonging to phylum *Proteobacteria* were the most abundant with over 80% of the total bacterial sequences in all samples, and *β-proteobacteria* sequences were the most common class. Other minor lineage detected were the class *Actinobacteria*, *Bacteroidetes*, *Phycisphaerae*, *Sphingobacteria* and *Clostridia*.

4. Conclusions

The average removal efficiency of COD and NH_4^+-N at steady states decreased with the decrease of COD/N ratio in the SBR. No obvious variations of NO_2^--N and NO_3^--N in the effluent were found during the whole operational period. The SAOR, SNOR and SOUR increased and the SNRR decreased with the decrease of influent COD/N ratio from 20 to 6. The diversity indexes of microbial community were 2.19, 2.10, 2.17 and 2.07 at the COD/N ratio of 20, 10, 6 and 30, respectively. Some bacteria were consistently present at all the influent COD/N ratios though their intensities varied in different periods, the others appeared at some COD/N ratios and were not necessarily present throughout the whole operational period. It is evident that some micrograms adapting to the variation of COD/N ratio tend to become predominant bacteria, while others tend to deplete or weaken gradually.

Acknowledgements

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