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ORIGINAL PAPER

Effect of hydraulic retention time on performance of an anoxic–aerobic sequencing batch reactor treating saline wastewater

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Abstract The effects of hydraulic retention time on the performance and microbial community structure of an anoxic–aerobic sequencing batch reactor treating saline wastewater were investigated. The average removal efficiencies of chemical oxygen demand and ammonia nitrogen decreased from 90 and 85 % to 68 and 71 % with the decrease in hydraulic retention time from 17 to 9 h, respectively. No obvious accumulation of nitrate nitrogen and nitrite nitrogen in the effluent was found. The contents of polysaccharide and protein in extracellular polymeric substances increased with the decrease in hydraulic retention time. The polysaccharide/protein ratio decreased from 1.18 to 1.11 with the decrease in hydraulic retention time from 17 to 9 h. The increase in extracellular polymeric substances with the decrease in hydraulic retention time led to the increase in sludge volume index. The specific ammonium oxidation rate, specific nitrite oxidation rate, specific nitrate reduction rate, and specific oxygen uptake rate increased with the decrease in hydraulic retention time. The diversity indices of microbial community decreased from 2.69 to 2.39 with the decrease in hydraulic retention time from 17 to 9 h. The α-*proteobacteria* were the dominant groups under hydraulic retention time of 17, 14, 11, and 9 h, which constituted 46, 30, 40, and 40 % of the whole microbial community, respectively.

Keywords Hydraulic retention time · Sequencing batch reactor · Extracellular polymeric substances · Microbial activity · Microbial community structure

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Introduction

The saline wastewaters are mainly derived from seafood processing, mariculture, oil production, cheese manufacturing, seawater for toilet flushing and industrial water, pharmaceutical process, and chemical production, which is rich in organic matter and nitrogen compound. When these saline wastewaters are discharged into the environment without prior treatment, they can cause severe damage by the contamination of soil, surface, and groundwater. The saline wastewaters are usually treated through physico-chemical technology and biological treatment process. The physico-chemical technologies include evaporation, coagulation–flocculation, ion exchange, and membrane technology (Lefebvre and Moletta 2006). As the physico-chemical technologies are generally energy-consuming, their startup and running costs are relatively high (Jang et al. 2013). Biological treatment processes have advantages over physico-chemical technology due to their low cost and high efficiency, and they have been widely used in removal of organic matter and nitrogen compound (Lefebvre and Moletta 2006). As the high salinity in the saline wastewaters can cause the plasmolysis, dehydration, disintegration of bacteria cell, and loss of cell activity, it results in the low removal efficiencies of organic matter and nitrogen (Dan et al. 2003; Ng et al. 2005). However, some biological processes have been satisfactorily employed in the

treatment of saline wastewater by adapting non-halophilic microflora to hypersaline conditions (Panswad and Anan 1999; Campos et al. 2002; Gebauer 2004; Jang et al. 2013; Shi et al. 2012).

Sequencing batch reactor (SBR) is known to be particularly robust and to withstand extreme conditions, which incorporates alternating aerobic and anaerobic periods to achieve nitrification and denitrification, and it has often been employed to treat saline wastewater (Boopathy et al. 2007; Fontenot et al. 2007; Rene et al. 2008; Moussavi et al. 2010). The performance and microbial community of bioreactor can be affected by many influencing factors, such as nutrient content (Rene et al. 2008), anoxic/aerobic phase fraction (Hu et al. 2011), salinity (Fontenot et al. 2007), solid retention time (Moussa et al. 2005), and hydraulic retention time (HRT) (Kargi and Uygur 2003). Among the above-mentioned influencing factors, HRT is regarded as one of important operating parameters affecting the performance and microbial community of bioreactor (Wang et al. 2009). Li et al. (2013) investigated the effects of HRT on the nitrification activities and population dynamics of a conventional activated sludge system fed with synthetic inorganic wastewater, and they found that a short HRT strengthened the dominant position of the fast-growing *Nitrosomonas* sp., which was responsible for the increase in the specific ammonium oxidation rate with the decrease in HRT. Lefebvre et al. (2005) reported that the removal efficiencies of chemical oxygen demand (COD) and total nitrogen in a SBR treating tannery soak liquor decreased from 95 and 96 % to 92 and 54 % with the decrease in HRT from 5 to 3.3 days at 34 g NaCl/L, respectively. Durai et al. (2011) investigated the effect of HRT on the performance of SBR treating tannery wastewater by salt-tolerant bacterial strains, and they found that the COD removal efficiency significantly decreased with the decrease in HRT from 3 to 2 days. Muda et al. (2011) investigated the physical characteristics, microbial activities of the granular sludge from a SBR treating the synthetic textile wastewater under different HRTs, and they found that the HRT increase caused a decrease in biomass concentration, specific biomass growth rate, endogenous decay rate, and biomass yield. Zhang et al. (2006) evaluated the effects of HRT on the mixed anaerobic microbial community grown with glucose in a continuous stirred tank reactor. Han et al. (2010) reported the effects of HRT on the structure and function of sludge microbial community in a yeast-predominant activated sludge system for synthetic industrial wastewater. Although some researchers have reported the effect of HRT on the performance of some bioreactors, up to date and to our knowledge, little information has been found in investigating the effect of HRT on protein (PN) and polysaccharide (PS) in extracellular polymeric substances (EPS), microbial activity, and performance and microbial community of an anoxic–aerobic SBR treating saline wastewater.

The major objectives of this study were (a) to investigate the effect of HRT on the performance of anoxic–aerobic SBR treating saline wastewater; (b) to determine the effect of extracellular polymeric substances (EPS) on the sludge settleability under different HRTs; and (c) to analyze the effect of HRT on the microbial activity and community structure.

The study was carried out at the Key Lab of Marine Environmental Science and Ecology, Ministry of Education, Ocean University of China, Qingdao City, China, from September 2012 to January 2013.

Materials and methods

Reactor setup and operation

A lab-scale plexiglass anoxic–aerobic SBR with a working volume of 7.7 L was used under different HRTs. The SBR had an internal diameter of 14 cm and a total height of 55 cm. 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 every 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 the air was introduced at the aerobic stage by two air diffusers at the bottom of the reactor.

The SBR cycles during different phases of operation are shown in Table 1. The system was operated at room temperature (20–30 °C). The dissolved oxygen (DO) concentration at the aerobic stage was above 2.0 mg/L, and the DO concentration at the anoxic stage was below 0.5 mg/L.

Seed sludge and wastewater composition

The seed sludge was obtained from a domesticated system treating 3 % salinity wastewater in our laboratory. The initial mixed liquid suspended sludge (MLSS) in the SBR was 3500 mg/L. The composition of synthetic saline wastewater was as follows (mg/L): glucose, 384; NH_4Cl, 60; Na_2HPO_4, 14; and seawater crystal 3×10^4 (corresponding to the salinity of 3 %). According to the data provided by the sales company (Zhongyan Marine Science and Technology Co., Ltd. in Tianjin City, China), the main components of the seawater crystal solution at 3 % salinity were provided as follows (mg/L): Na^+, 9,880; Cl^-, 18,025; Mg^{2+}, 950; SO_4^{2-}, 2,500; K^+, 360; Ca^{2+}, 300; Zn^{2+}, 1.5×10^{-2}; Mn^{2+}, 1.3×10^{-2}; Fe^{2+}, 1.3×10^{-1}; 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}; and other inorganic ions.

Table 1 Operation conditions of the SBR

HRT (h)	Operational day (d)	Cycle (h)	Influent (h)	Aerobic stage (h)	Anoxic stage (h)	Settling (h)	Effluent withdrawal (h)
17	0–14	12	0.25	7	3	1.5	0.25
14	15–41	10	0.25	5.6	2.4	1.5	0.25
11	42–56	8	0.25	4.2	1.8	1.5	0.25
9	57–96	6	0.25	2.8	1.2	1.5	0.25

Analytical methods

The measurements of COD, ammonia nitrogen (NH_4^+-N), nitrite nitrogen (NO_2^--N), nitrate nitrogen (NO_3^--N), mixed liquor volatile suspended solids (MLVSS), mixed liquor suspended solids (MLSS), and sludge volume index (SVI) were carried out according to the Chinese NEPA standard methods (Chinese NEPA 2002). The dissolved oxygen (DO) concentration in the reactor was measured by a dissolved oxygen meter (oxi 330i, WTW, Germany). The EPS of the sludge samples were extracted by a thermal extraction (Chang and Lee 1998). The protein (PN) in the EPS extractions was measured by the method of Lowery et al. (1951) with bovine serum albumin as the standard. The polysaccharide (PS) in the EPS extractions was determined as glucose equivalents using the anthrone-sulfuric acid method (Dubois et al. 1956).

The determination of specific oxygen utilization rate (SOUR)

A certain amount of activated sludge from the SBR was transferred into a pre-cleaned BOD bottle. The BOD bottle was fully filled in with the pre-aerated mineral medium which had the same composition as synthetic wastewater. The mineral medium was pre-aerated in order to keep the DO concentration above 8.0 mg/L for 10 min. And then, the rubber stopper was firmly pressed down the BOD bottle. An oxygen-sensing probe passed through the rubber stopper was inserted into the BOD bottle, and the DO concentration was recorded at an interval of 30 s until the DO concentration below 1.0 mg/L. MLSS was determined at the end of batch experiment. The SOUR was calculated from DO–time curve based on the concentration of activated sludge in the BOD bottle. The mixed liquor in the BOD bottle was mixed by a magnetic stirrer (RH basic 1, IKA, Germany), and the SOUR test was conducted at room temperature.

The determinations of specific ammonium oxidation rate (SAOR), specific nitrite oxidation rate (SNOR), and specific nitrate reduction rate (SNRR)

The SAOR, SNOR, and SNRR were determined by batch experiments. In the SAOR (or SNOR) determination, batch experiment was performed in a 500-mL Erlenmeyer flask with 100 mL activated sludge from SBR and 400 mL mineral medium at room temperature. The mixed liquor in the Erlenmeyer flask was mixed by a magnetic stirrer (RH basic 1, IKA, Germany), and the air was introduced by an air diffuser (ACO-002, Zhejiang Sensen Industrial Co., Ltd., China). The composition of mineral medium for the determination of SAOR (or SNOR) was as follows (mg/L): NH_4Cl, 120 (or $NaNO_2$, 147); Na_2HPO_4, 14; and seawater crystal 3×10^4 (corresponding to the salinity 3 %). Sample was taken at an interval of 15 min to analyze the concentrations of NH_4^+-N (or NO_2^--N), and MLSS was determined at the end of batch experiment. The ammonium oxidation rate (or nitrite oxidation rate) was calculated from the slope of the linear progression curve of NH_4^+-N (or NO_2^--N) versus time, and the SAOR (or SNOR) was calculated from the ratio of ammonium oxidation rate (or nitrite oxidation rate) and MLSS.

In the SNRR determination, batch experiment was performed in a 500-mL Erlenmeyer flask with 100 mL activated sludge from the SBR and 400 mL mineral medium at room temperature. The mixed liquor in the Erlenmeyer flask was mixed by a magnetic stirrer (RH basic 1, IKA, Germany), and anaerobic environment was obtained by introducing nitrogen gas continuously through a gas diffuser. The composition of mineral medium for the determination of SNRR was as follows (mg/L): glucose, 640; $NaNO_3$, 303; Na_2HPO_4, 14; and seawater crystal 3×10^4 (corresponding to the salinity 3 %). Sample was taken at an interval of 15 min to analyze the concentrations of NO_3^--N, and MLSS was determined at the end of batch experiment. The nitrate reduction rate was calculated from the slope of the linear progression curve of NO_3^--N versus time, and the SNRR was calculated from the ratio of nitrate reduction rate and MLSS.

PCR-DGGE and sequence analysis

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.

PCR amplification

PCR amplifications were carried out in an iCycler Thermal Cycler PCR (Bio-Rad Co., Ltd., USA). The bacterial primer 101F with a GC clamp (5′-CGCCCGCCGCGCGCGGCG GGCGGGGCGGGGGCACGGGGGGTGGCGGACGGGT GAGTAA-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^{TM} DNA polymerase (5 U/μL, 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 (Muyzer et al. 1993): initial denaturation at 95 °C for 5 min, denaturation at 95 °C for 30 s, annealing at 56 °C for 30 s (the temperature was decreased by 0.5 °C every cycle until the touchdown temperature of 51 °C was reached), extension at 72 °C for 45 s, denaturation at 95 °C for 30 s after 10 cycles, annealing at 51 °C for 30 s, extension at 72 °C for 45 s followed by a final extension at 72 °C for 10 min after 20 cycles, and end at 4 °C. 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).

DGGE analysis

DGGE was performed using a DCode^{TM} 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) 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, 4.0 mL 40 % polyacrylamide, 0.4 mL 50 × TAE buffer and adjusted to a final volume of 20.0 mL with deionized water; the content of each component for 60 % denaturant agent was twice of the 30 %). Electrophoreses were performed at 65 °C 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) (Shannon and Weaver 1949). 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 densitometric curves. The equation for the Shannon diversity index is as follows:

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

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

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 using the neighbor-joining method by the software MEGA 4.0 (Saitou and Nei 1987; Kumar et al. 2004), and reference sequences used in tree construction were acquired from GenBank. The bootstrap analysis was performed on the basis of 1,000 bootstrap replications (Felsenstein 1985). The sequences obtained in this study were submitted to the

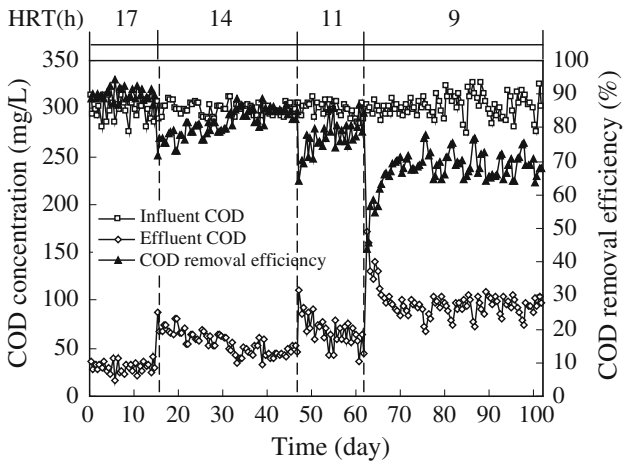


Fig. 1 Variations of influent and effluent COD concentrations under different HRTs

DDBJ database under accession numbers AB896738-AB896757.

Results and discussion

Effect of HRT on COD removal

The time courses of COD removal efficiency under different HRTs are shown in Fig. 1. The average COD removal efficiencies gradually decreased from 90 to 68 % with the decrease in HRT from 17 to 9 h at steady states. It is clear that the shorter HRTs operated the lower removal efficiencies of COD achieved. As the influent COD concentrations varied in the range of 274 and 327 mg/L during the whole operational period, the average organic loading rates (OLR) under HRTs of 17, 14, 11, and 9 h corresponded to 0.42, 0.51, 0.65, and 0.80 kg COD/(m^3 day), respectively. The decrease in COD removal efficiency could be related to the shorter contact time between the activated sludge and organic matter. Kapdan (2005) reported the similar results that the COD removal efficiency decreased with the increase in OLR in an anaerobic packed column reactor treating synthetic dyestuff wastewater. In addition, the decrease in COD removal efficiency might also be due to the fact that some bacteria were washed out from the SBR when the HRT was less than facultative bacteria generation time. Yang et al. (2006) illustrated that some bacteria were washed out of UASB while the HRT was less than 12 h, and the COD removal efficiency decreased with the decrease in HRT.

Effect of HRT on nitrogen removal

The time courses of NH_4^+-N, NO_3^--N, and NO_2^--N concentrations in the influent and effluent under different HRTs are shown in Fig. 2. The average NH_4^+-N removal efficiency at steady state decreased from 85 to 71 % with the decrease in HRT from 17 to 9 h. The average NH_4^+-N loading rate increased with the decrease in HRT, which lead to the decrease in NH_4^+-N removal efficiency in the aerobic–anoxic SBR treating saline wastewater. Compared to heterotrophic bacteria, the growth of ammonia-oxidizing bacteria was relatively slow (Isaka et al. 2013). The lower growth rate of ammonia-oxidizing bacteria led to the decrease in the proportion of ammonia-oxidizing bacteria among the total bacteria with the decrease in HRT (Li et al. 2013). The effluent concentrations of NO_2^--N and NO_3^--N were less than 0.4 mg/L during the whole operation time (Fig. 2b, c). No obvious variations of NO_2^--N and NO_3^--N in effluent were found under different HRT, which indicated that the denitrification process was not influenced by the decrease in HRT.

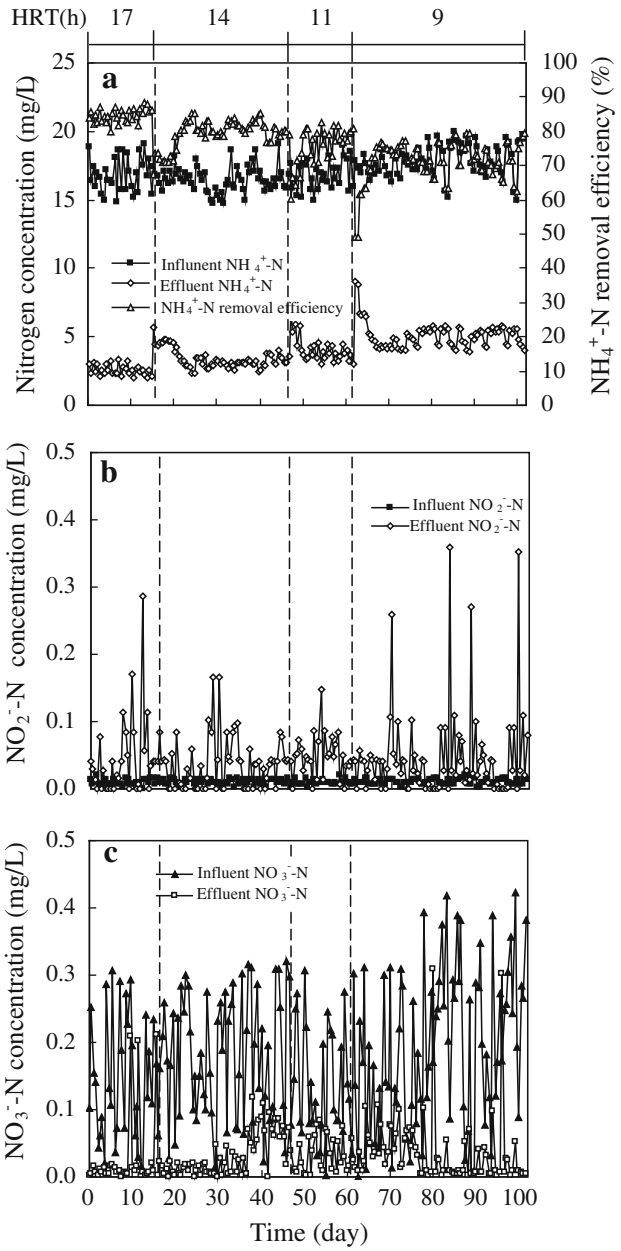


Fig. 2 Variations of influent and effluent concentrations of NH_4^+-N (**a**), NO_2^--N (**b**), and NO_3^--N (**c**) under different HRTs

Relationship between EPS and sludge settleability under different HRTs

EPS are secreted by microorganisms in biological wastewater treatment systems, and the variations of production and composition of EPS have significant influences on the physico-chemical properties of microbial aggregates (Li and Yang 2007). In order to characterize the relationship between EPS and sludge settleability, the sludge sample at steady state conditions were obtained from the anoxic–aerobic SBR on day 13, 40, 55, and 95, corresponding to

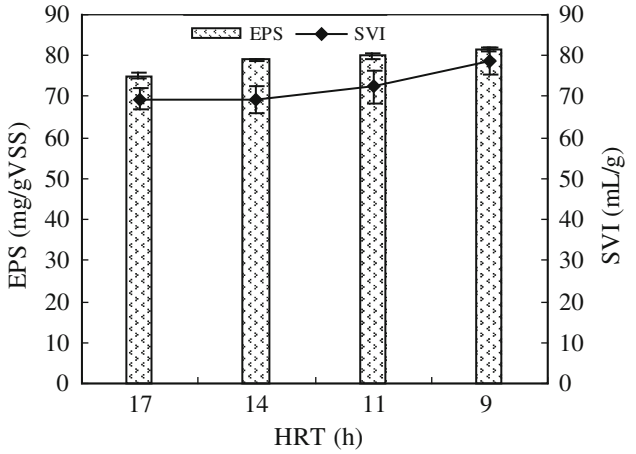


Fig. 3 Variations of EPS and SVI under different HRTs

HRT of 17, 14, 11, and 9 h, respectively. As shown in Fig. 3, the EPS content increased from 75.1 to 81.5 mg/g VSS with the decrease in HRT from 17 to 9 h. The contact time between the microbes and organic matter was relatively sufficient at the longer HRT, which can lead to the nutrient lack in the anoxic–aerobic SBR. Zhang and Bishop (2001) reported that the part of EPS could be utilized by the microbes as carbon or energy sources under the conditions of nutrient lack. Park et al. (2004) illustrated that the EPS content at the long HRT was less than that at the short HRT. Similarly, the SVI contents increased from 69 to 79 mL/g with the decrease in HRT from 17 to 9 h. As the EPS have significant influences on the physico-chemical properties of microbial aggregates in the bioreactor, the variations of SVI content under different HRTs are related to the variation of EPS in the SBR. The EPS exhibit a dynamic double-layered structure, and the outer layer contained a large amount of bound water (Zhang et al. 2011). The increase in EPS content resulted in the increase in bound water in activated sludge flocs, which decreased the performance of sludge sedimentation. Many previous researches reported that the increase in EPS content could lead to the increase in SVI (Li and Yang 2007; Park et al. 2004). In addition, as there are many charged functional groups in EPS, their content and composition influence the surface charge of microbial aggregates (Sheng et al. 2010), which also resulted in a decrease in the settleability of microbial aggregates.

The variations of PN and PS contents in the EPS under different HRTs are shown in Fig. 4. The PN and PS contents in EPS increased from 40.7 and 34.4 mg/g VSS on day 13 to 43.0 and 39.0 mg/g VSS on day 95 with the decrease in HRT from 17 to 9 h, respectively (Fig. 4a). The results suggested that the decrease in HRT stimulated the microorganisms to produce more PN and PS in the EPS, which was consistent with the previous research that PN and PS contents in the bound EPS at the long HRT were

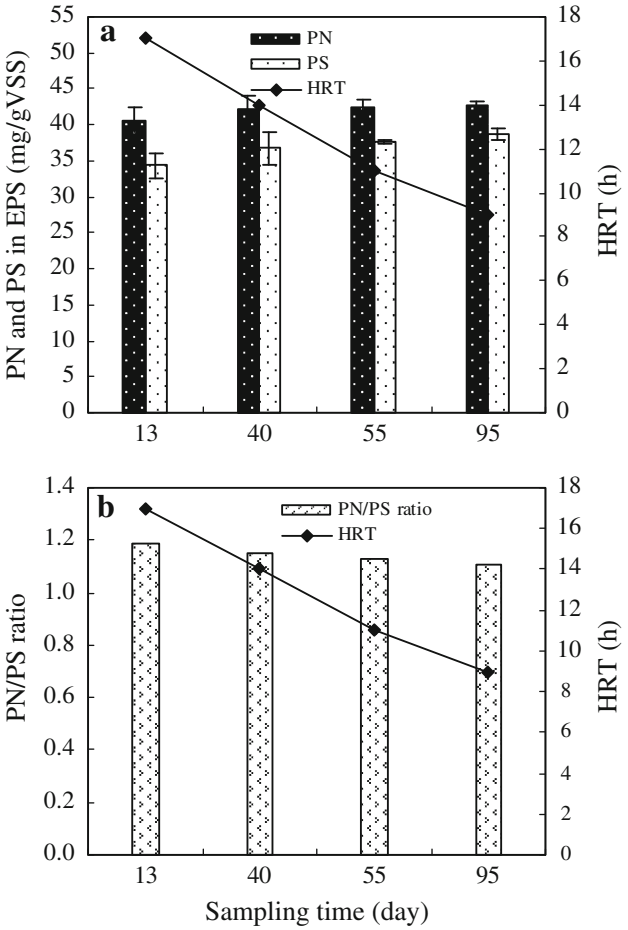


Fig. 4 Variations of production and composition in EPS under different HRTs. **a** PN and PS; **b** PN/PS ratio

less than those at the short HRT (Park et al. 2004). The PN and PS contents in the EPS were increased by 5 and 13 % with the decrease in HRT from 17 to 9 h, respectively, suggesting that PS in the EPS was more sensitive to the variation of HRT. The PN/PS ratios in the EPS gradually decreased from 1.18 to 1.11 with the decrease in HRT from 17 to 9 (Fig. 4b). As the PN in the EPS was hydrophobic, the low PN/PS in EPS reduced cell surface hydrophobicity, which decreased the flocculating ability of sludge. The low flocculating ability of sludge was not beneficial to the settling of sludge. Chen et al. (2010) reported similar results that lower PN/PS ratio in EPS could produce poor sludge settleability.

Effect of HRT on microbial activity

The SOUR of the sludge from the anoxic–aerobic SBR were investigated on day 13, 40, 55, and 95, corresponding to HRT of 17, 14, 11, and 9 h, respectively. As shown in Fig. 5a, the SOUR increased from 39 to 44 mg O_2/(g MLSS h) with the decrease in HRT from

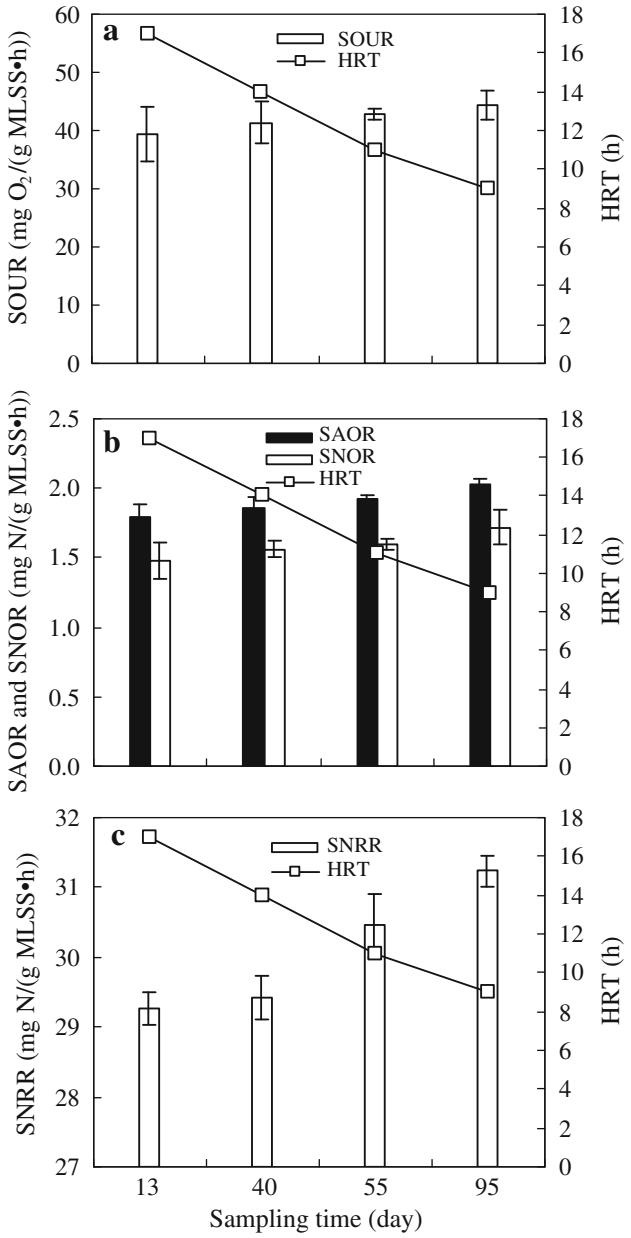


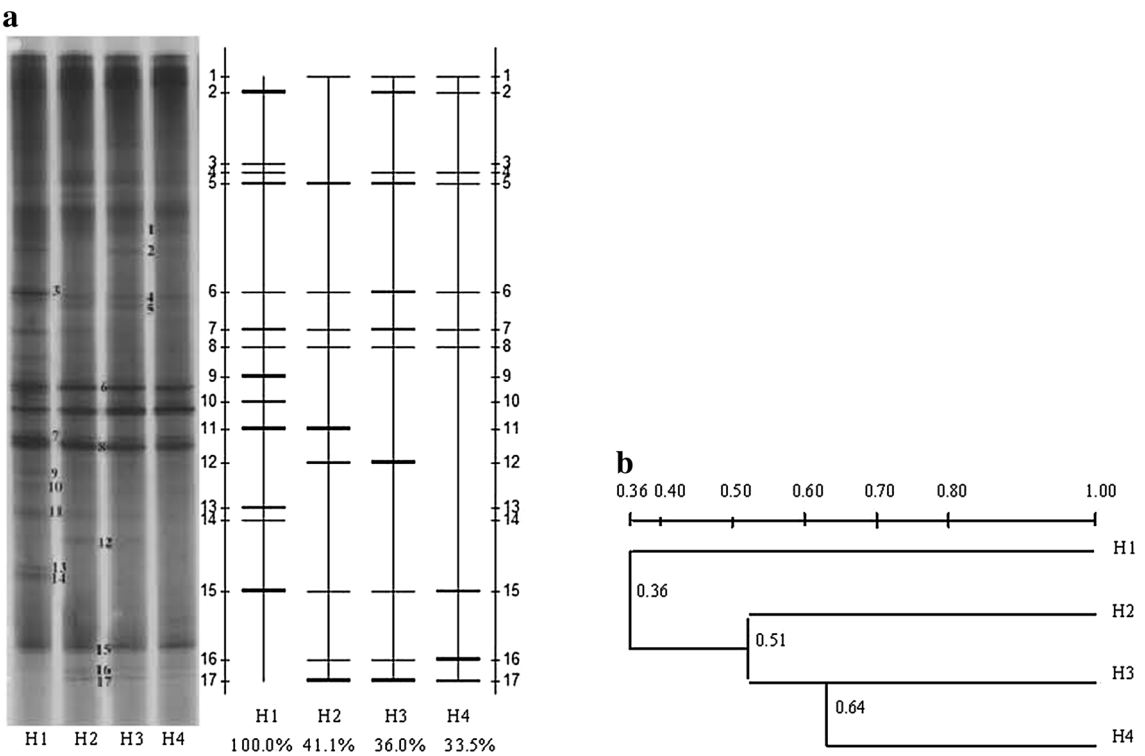
Fig. 5 Variations of SOUR (**a**), SAOR, SNOR (**b**), and SNRR (**c**) under different HRTs

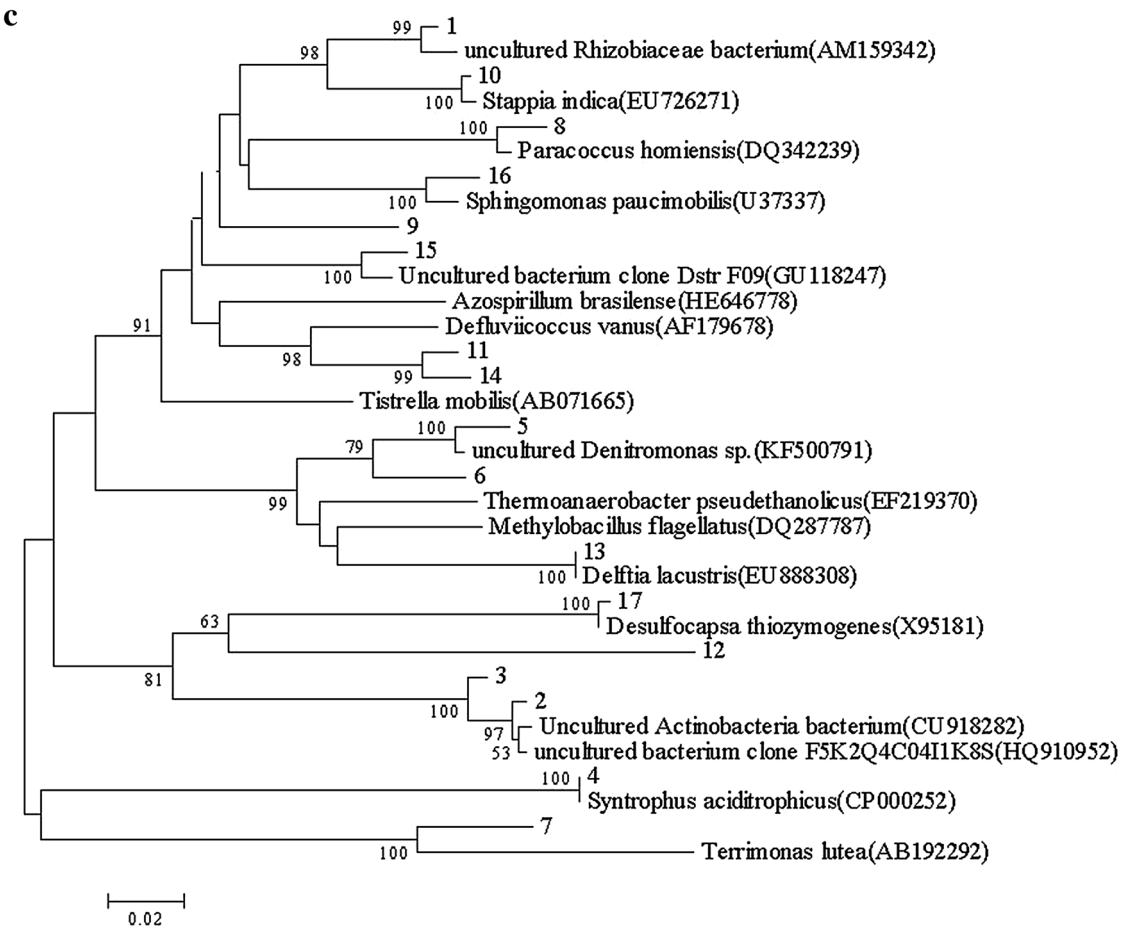
17 to 9 h, which can be explained that more carbonaceous substrate was provided to bacteria at short HRT and led to an increase in SOUR values. Barr et al. (1996) reported the similar results that the SOUR values increased with the decrease in HRT in an activated sludge reactor treating bleached kraft mill effluent. Fig. 5b shows the variations of SAOR and SNOR under different HRTs. The SAOR and SNOR increased from 1.79 and 1.48 mg N/(g MLSS h) to 2.0 and 1.7 mg N/(g MLSS h) with the decrease in HRT from 17 to 9 h. The NH_4^+-N loading rate in the SBR increased with the decrease in HRT, which resulted the increase in the SAOR and SNOR. Hwang et al. (2009) reported that the specific nitrification rate increased linearly with the specific NH_4^+-N loading rate in a SBR. As shown in Fig. 5c, the SNRR increased from 29.3 to 31.2 mg N/(g MLSS h) with the decrease in HRT from 17 to 9 h. Kim et al. (2008) found that average specific denitrification rate increased from 15.0 to 48.0 mg N/(g MLSS h) with the decrease in HRT from 24.9 to 7.9 h in the biological pre-denitrification process for the simultaneous removal of toxic pollutants from cokes wastewater.

Effect of HRT on microbial community structure

The microbial community structures of the anoxic–aerobic SBR under different HRTs were investigated through PCR-DGGE analysis. As shown in Fig. 6a, the DGGE analysis showed that there were some changes in the band profile with the decrease in the HRT from 17 to 9 h as well as the variations in band intensity. Some bands (5, 8–11, and 18) were observed in all the samples under different HRTs, and their variations were found in the band intensity. The other bands (1–4, 6, 7, 12–17, 19, and 20) appeared under different HRTs, and they were not necessarily present throughout the whole operational period. It is evident that some microbes adapting to HRT variation tend to become predominant bacteria, while others tend to deplete or gradually weaken. The UPGMA clustering analysis was used to analyze the microbial community similarities under different HRTs. Figure 6b shows that the microbial populations were categorized into separate groups. The first group represented the sludge sample under 17 h HRT, and the second group was sub-categorized into two groups. The similarity of microbial community between the first group and the second group was less than 30 %, which indicated that the decrease in HRT had a significant effect on the microbial community structure. The diversity indices of microbial community decreased from 2.62 to 2.29 with the decrease in hydraulic retention time from 17 to 9 h, indicating low microbial diversity in the aerobic–anoxic SBR treating saline wastewater at short HRT.

To gain further insight into the microbial community structure, twenty discernable bands were excised from the DGGE gel and sequenced, and the accurate sequencing of bands 6, 7, and 9 were not available. By using the BLAST program of GenBank, the detected sequences were compared with sequences deposited in the database. The most similar sequences of discernable DGGE bands are listed in Table 2. *Methylobacillus flagellatus* (band 8), *Terrimonas lutea* (band 10), *and Paracoccus homiensis* (band 11) were present in all the samples, which were proved that they could utilize


a
H1 H2 H3 H4
100.0% 41.1% 36.0% 33.5%
b
0.36 0.40 0.50 0.60 0.70 0.80 1.00
H1
H2
H3
H4
0.36
0.51
0.64


c
uncultured Rhizobiaceae bacterium(AM159342)
Stappia indica(EU726271)
Paracoccus homiensis(DQ342239)
Sphingomonas paucimobilis(U37337)
Uncultured bacterium clone Dstr F09(GU118247)
Azospirillum brasilense(HE646778)
Defluviicoccus vanus(AF179678)
Tistrella mobilis(AB071665)
uncultured Denitromonas sp.(KF500791)
Thermoanaerobacter pseudethanolicus(EF219370)
Methylobacillus flagellatus(DQ287787)
Delftia lacustris(EU888308)
Desulfocapsa thiozymogenes(X95181)
Uncultured Actinobacteria bacterium(CU918282)
uncultured bacterium clone F5K2Q4C04I1K8S(HQ910952)
Syntrophus aciditrophicus(CP000252)
Terrimonas lutea(AB192292)
0.02

◀**Fig. 6** Analysis of microbial populations under different HRTs. **a** DGGE gel banding profiles of microbial communities in the aerobic–anoxic SBR under different HRTs. H1: HRT = 17 h; H2: HRT = 14 h; H3: HRT = 11 h; H4: HRT = 9 h. **b** Percent similarity analysis of lanes on the DGGE gel. **c** Phylogenetic tree of bacteria based on the results of BLAST. The phylogenetic tree was constructed using the neighbor-joining method. The numbers at the nodes are bootstrap confidence values expressed as percentages of 1,000 bootstrap replications. Bootstrap values less than 50 % are not shown

glucose as organic substrate (Chistoserdova et al. 2007; Xie and Yokota 2006; Kim et al. 2006). The results suggest that these bacteria species could adapt to the HRT variation in the anoxic–aerobic SBR treating saline wastewater. The bands 12, 13, 16, and 17 were related to *Defluviicoccus vanus*, *Stappia indica, Delftia lacustris*, *and Azospirillum brasilense*, respectively, and they appeared at 17 h HRT and then disappeared with

Table 2 Closest phylogenetic affiliations of sequences obtained from sludge samples at different HRTs

Bands	Closest related sequences (Accession number[a])	Accession number[b]	Similarity (%)	Class containing related sequences
1	uncultured *Rhizobiaceae* bacterium AM159342	AB896738	97	α-proteobacteria
2	*Uncultured* Actinobacteria *bacterium* CU918282	AB896739	98	_
3	uncultured bacterium clone F5K2Q4C04I1K8S GU912285	AB896740	97	_
4	*Syntrophus aciditrophicus* CP000252	AB896741	98	δ-proteobacteria
5	uncultured *Denitromonas* sp. KF500791	AB896742	98	β-proteobacteria
8	*Methylobacillus flagellatus* DQ287787	AB896745	99	β-proteobacteria
10	*Terrimonas lutea* AB192292	AB896747	100	ε-proteobacteria
11	*Paracoccus homiensis* DQ342239	AB896748	98	α-proteobacteria
12	*Defluviicoccus vanus* AF179678	AB896749	99	α-proteobacteria
13	*Stappia indica* EU726271	AB896750	99	α-proteobacteria
14	*Tistrella mobilis* AB071665	AB896751	100	α-proteobacteria
15	*Thermoanaerobacter pseudethanolicus* EF219370	AB896752	100	Clostridia
16	*Delftia lacustris* EU888308	AB896753	100	β-proteobacteria
17	*Azospirillum brasilense* HE646778	AB896754	100	α-proteobacteria
18	Uncultured bacterium clone Dstr_F09 GU118247	AB896755	97	_
19	*Sphingomonas paucimobilis* U37337	AB896756	98	α-proteobacteria
20	*Desulfocapsa thiozymogenes* X95181	AB896757	98	δ-proteobacteria

Accurate sequencing of bands 6, 7, and 9 were not available

[a] The accession numbers were obtained from ribosomal database project

[b] The accession numbers were obtained from the DDBJ database

the decrease in HRT. *Defluviicoccus vanus* was able to reduce nitrate but most likely unable to reduce nitrite (Wang et al. 2008). *Stappia indica* was moderately halophilic bacteria and could reduce nitrate (Lai et al. 2010). *Delftia lacustris* could utilize D-glucose as energy and carbon sources, and reduce nitrate (Jørgensen et al. 2009). The sequence of band 17 was 100 % similar to *Azospirillum brasilense*, which was capable of removing ammonium and phosphorous ions in wastewater (de-Bashan et al. 2002). The disappearance of *Azospirillum brasilense* might result in the significant decrease in the removal efficiencies of NH_4^+-N with the reduction in HRT. As *Sphingomonas paucimobilis* (band 19) and *Desulfocapsa thiozymogenes* (band 20) did not appear at 17 h HRT, they appear between 14 and 9 h HRT. *Sphingomonas paucimobilis* was identified as denitrifying bacterium (Jørgensen and Pauli 1995), which could explain why no obvious accumulation of NO_2^--N and NO_3^--N was found during the whole operational period. *Syntrophus aciditrophicus* (band 4) appeared at the HRT of 17, 14, and 9 h, but they disappeared at the HRT of 11 h. *Tistrella mobilis* (band 14) was only found at the HRT of 17 and 11 h, and *Thermoanaerobacter pseudethanolicus* (band 15) appeared at the HRT of 14 and 11 h. *Tistrella mobilis* could reduce nitrate to nitrite but not to nitrogen (Shi et al. 2002). *Thermoanaerobacter pseudethanolicus* could have the capability to ferment glucose (Onyenwoke et al. 2007).

The phylogenic tree based on DGGE profiles is shown in Fig. 6c. The *α-proteobacteria* were the dominant groups under HRT of 17, 14, 11, and 9 h, which constituted 46, 30, 40, and 40 % of the whole microbial community, respectively. The *β-proteobacteria* were the next groups constituting 23, 20, 20, and 20 % under HRT of 17, 14, 11, and 9 h, respectively. Although the anoxic–aerobic SBR under different HRTs had the same dominant groups, such as *α-proteobacteria* and *β-proteobacteria*, the phylogenic analysis showed some significant difference in the presence of bacteria species. For example, *Tistrella mobilis* was detected at the HRT of 17 and 11 h, but it was not found in the HRT of 14 and 9 h.

Conclusion

The average removal efficiencies of COD and NH_4^+-N decreased with the decrease in HRT, and no obvious accumulations of NO_3^--N and NO_2^--N in the effluent were found during the whole operational period. The increase in EPS with the decrease in HRT led to the increase in sludge volume index (SVI). The PN and PS in the EPS increased with the decrease in HRT, respectively. The PN/PS ratio decreased from 1.18 to 1.11 with the decrease in HRT from 17 to 9 h. The SAOR, SNOR, SNNR, and SOUR increased with the decrease in HRT. The HRT variations have significant effect on the microbial community structures of the anoxic–aerobic SBR. The diversity indices of microbial community decreased from 2.69 to 2.39 with the decrease in HRT from 17 to 9 h. The *α-proteobacteria* were the dominant groups under HRT of 17, 14, 11, and 9 h, which constituted 46, 30, 40, and 40 % of the whole microbial community, respectively.

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