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Effect of salinity on removal performance and activated sludge characteristics in sequencing batch reactors

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ABSTRACT

The removal performance, activated sludge characteristics and microbial community in sequencing batch reactors (SBRs) were studied at salinity ranging from 0 to 20 g/L. Results showed that salinity deteriorated the removal performance. Removal rate of ammonium (NH_4^+-N), total phosphorus (TP) and chemical oxygen demand (COD) were gradually dropped from 95.34%, 93.58% and 94.88% (0 g/L) to 62.98%, 55.64% and 55.78% (20 g/L), respectively. The removals of NH_4^+-N and TP were mainly influenced during aerobic phase. Besides, salinity increased the extracellular polymeric substances (EPS) content of activated sludge, decreased the content of protein (PN) and loosely bound extracellular polymeric substances (LB-EPS) which led to better settleability of activated sludge. Moreover, salinity inhibited the dehydrogenase activity (DHA) of activated sludge. Sequence analysis illustrated *Zoogloea* and *Thioclava* were predominant at 0 and 20 g/L salinity, respectively. The difference of microbial community under high salinity was likely caused by the variation of richness.

1. Introduction

To offset the shortage of fresh water, the directly use of seawater as industrial water and domestic water is common in coastal regions (Jin et al., 2016a). At the same time, the discharge of salt-contained wastewater from industries including chemical industries, pharmaceutical factories and smelteries increases rapidly (He et al., 2017). Wastewater is usually defined as saline wastewater when the salinity is below 10 g/L, otherwise it is regarded as hypersaline wastewater or brines (Pernetti and Di, 2005). There are not only a large amount of inorganic salts in saline wastewater, but also organic matters and nitrogen (Lefebvre and Moletta, 2006). Therefore, performance especially nitrogen removal of wastewater treatment plants are affected considerably (Zhang et al., 2012), and it is usually more difficult to meet the discharge standards of the effluent.

Treatment processes for saline wastewater can be summarized as physicochemical and biological treatment methods (He et al., 2017). Physicochemical methods include electrolytic processes, membrane separation and flocculation, etc. (Salmanikhas et al., 2016). These methods usually lead to secondary pollutants and high treatment costs, therefore are only applied to remove salts and organic matters from hypersaline wastewater (Lefebvre and Moletta, 2006; Mirbolooki et al., 2017). While biological treatment processes are cost-effective and of low-risk for secondary pollution, consequently they are commonly used to treat saline wastewater (Ahmadi et al., 2017). Nevertheless, there usually are challenges in treatment of saline wastewater using biological treatment processes (Jin et al., 2016b). A large number of studies on biological treatment of saline wastewater proved that salinity could affect biological system performance negatively in a dramatic extent (Hong et al., 2013; Yan et al., 2017). For example, it may lead to the increase of suspended solid concentration in effluent, the decline of organic removal efficiency and the inhibition of bacterial metabolism (Bassin et al., 2011).

Recently, many investigations focused on developing different treatment methods to improve removal rates (Lefebvre and Moletta, 2006; He et al., 2017). For example, Jemli et al. (2015) added salt-tolerant bacteria to membrane bioreactor (MBR) and continuous stirred-tank reactor (CSTR), and found that the removal efficiency of total organic carbon in MBR was higher than that in CSTR when treating with hypersaline wastewater of 55 g/L salinity. Salmanikhas

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et al. (2016) combined fluidized bed with activated sludge process to build a hybrid treatment system, and reported the COD removal by the hybrid treatment system was obviously higher than that by an activated sludge system. Tan et al. (2017) used sea mud to cultivate marine activated sludge when treating saline phenolic wastewater, and found this kind of marine activated sludge showed a great performance on the removal of COD, ammonium (NH_4^+-N) and phenol, achieved at 80%, 68% and 99%, respectively. However, there are few studies on the effect of salinity on the removal efficiency of nitrogen and phosphorus in activated sludge systems. Moreover, the mechanisms on the influence of salinity on dehydrogenase activity (DHA), extracellular polymeric substances (EPS) and microbial community of activated sludge are also not available which could be important performance indexes to reflect microbial degrading capacity, physiochemical characters and dominant microbial populations of activated sludge, respectively (Yin et al., 2005; Sheng et al., 2010; Cheng et al., 2016).

Therefore, this study aimed to overcome these gaps. The effect of salinity on removal efficiency of NH_4^+-N, total phosphorus (TP) and COD was studied in SBR, and the influence on activated sludge characteristics including sedimentation performance, EPS and DHA was investigated. The influence on microbial community structure under different salinity was also examined. These results were supposed to be referred for better design and operation of biological treatment processes for saline wastewater.

2. Materials and methods

2.1. Activated sludge and wastewater

Activated sludge used in this study was obtained from Yuelu District wastewater treatment plant in Changsha, China. And the activated sludge was fed with synthetic wastewater in this study. The composition of the synthetic wastewater was as follows: 460 mg/L Glucose, 94.39 mg/L $(NH_4)_2SO_4$, 17.57 mg/L KH_2PO_4, 0.5 ml/L trace elements. The recipe of trace elements was in accordance with Wang et al. (2008), per liter trace elements contained: 1.5 g $FeCl_3{\cdot}6H_2O$, 0.18 g KI, 0.15 g $CoCl_2{\cdot}6H_2O$, 0.15 g H_3BO_3, 0.12 g $MnCl_2{\cdot}4H_2O$, 0.12 g $ZnSO_4{\cdot}7H_2O$, 0.06 g $Na_2MoO_4{\cdot}2H_2O$, 0.03 g $CuSO_4{\cdot}5H_2O$ and 10 g EDTA.

The saline wastewater was made by the synthetic wastewater containing certain dosage of sodium chloride in accordance with the demand during the experiments.

2.2. Configuration and operation of reactors

Two parallel lab-scale sequencing batch reactors (SBRs) with an effective volume of 2.5 L were used in the experiments. One of them named SBR1 was the control reactor, for which sodium chloride was not added in the influent stream. The other one named SBR2 was salt-adding reactor, for which salinity in the influent was gradually increased from 0 to 20 g/L. Both of them were inoculated by activated sludge, and the initial mixed liquor suspended solids (MLSS) concentration of reactors was approximately 4500 mg/L.

Three cycles per day were operated with the SBRs. Each cycle lasted 8 h, including feeding (0.05 h), standing (1 h), aeration (3.5 h), anoxia (2 h), settling (0.5 h), decanting (0.05 h) and idle period (0.9 h).

As shown in Fig. 1a, a magnetic stirrer (CJJ78-1, Dadi Automated Instrument Co., Ltd., China) was used to achieve anoxia during anoxia phase for the reactor. The influent and effluent streams were fed to and discharged from the reactor by peristaltic pumps (KDS-FB 2S17Y, Kamoer Fluid Technology Co., Ltd., China). Air was pumped to the reactor through aeration diffusers during aeration phase by an electromagnetic air compressor (ACO-005, Sunsun Industry Co., Ltd., China). The temperature of reactor was 20 °C and controlled by temperature conditioners (R-H008-025, Easy Aqua Co., Ltd., Japan). The SBR system was controlled by time controllers (ZYT16G, Shanghai Toone Electronics Co., Ltd., China).

2.3. Analytical methods

Removal performance was evaluated by the removals of NH_4^+-N, TP and COD. Every day, effluent samples were collected after the first operation cycle and then analyzed for NH_4^+-N and TP. When the removal rate of NH_4^+-N and TP were stable, the COD removal efficiency and the variation of NH_4^+-N, nitrate nitrogen (NO_3^--N), nitrite nitrogen (NO_2^--N) and TP during an operation cycle were measured. Before measured, all the effluent samples were filtrated by 0.45 μm fiberglass filter. And 20 mL sludge mixed liquor samples were collected every 0.5 h during an operation cycle and immediately centrifuged at 3500*g* for 5 min, the supernatants were collected for detecting the variation of NH_4^+-N, NO_3^--N, NO_2^--N and TP. The measurements of NH_4^+-N, NO_3^--N and NO_2^--N were according to Luo et al. (2016), Zhang et al. (2015) and Zhang et al. (2014), respectively. The measurement of COD was in accordance with Wen et al. (2016).

Activated sludge characteristics were estimated by measuring the sludge volume index (SVI), EPS and DHA, after the removal performance of SBR was steady at each salinity. The measurement of SVI was in accordance with Zhao et al. (2016). The extraction of tightly bound extracellular polymeric substances (TB-EPS) and loosely bound extracellular polymeric substances (LB-EPS) was in accordance with Li and Yang (2007). The protein (PN) and polysaccharides (PS) were measured using coomassie brilliant blue G-250 method (Hou et al., 2007) and anthrone-sulfuric acid method (Cheng et al., 2015) with BSA and glucose as standard solution, respectively. The measurement method of DHA was according to Yin et al. (2005).

In addition, when the removal performance of SBR was stable at the salinity of 0, 5, 10, 15 and 20 g/L, five activated sludge samples named C, S1, S2, S3 and S4 were collected for 16S rRNA sequencing analysis, respectively.

2.4. DNA extraction and PCR amplification

Total genome DNA of five activated sludge samples was extracted according to Cai et al. (2013). Two specific primers with barcodes, 515F (5′-GTGCCAGCMGCCGCGG-3′) and 806R (5′-GGACTACHVGGGTWTCTAAT-3′) were used to amplify the V4 region of 16S rRNA genes. During PCR reactions, Phusion® High-Fidelity PCR Master Mix (New England Biolabs, USA) was used to ensure the efficiency and veracity of amplification. PCR products were detected and electrophoresed on 2% agarose gel after mixed with same volume of 1× loading buffer. Products with bright main strip between 400 bp and 450 bp were purified with Qiagen Gel Extraction Kit (Qiagen, Germany).

2.5. Sequencing and data analysis

Sequencing library was built by using TruSeq® DNA PCR-Free Sample Preparation Kit (Illumina, USA) following manufacturer's recommendations. After quantified by Qubit@ 2.0 Fluorometer (Thermo Scientific, USA) and Agilent Bioanalyzer 2100 system (Agilent, USA), the library was 250 bp paired-end sequenced on Illumina HiSeq2500 platform. Paired-end reads were truncated through cutting off unique barcode and primer. Then merged the reads using FLASH (V1.2.7), filtrated merged tags using QIIME (V1.7.0), and removed chimera sequences in accordance with Haas et al. (2011).

The remaining sequences were clustered as Operational Taxonomic Units (OTUs) using Uparse (V7.0.1001) with 97% identity. Screened representative sequence for each OTU was annotated by RDP Classifier (V2.2) against GreenGene Database using confidence threshold of 80%. Then the normalization of OTUs abundance information was conducted by using the minimum amount of data in the sample as the standard. The following analysis was performed based on normalized data.

Alpha diversity was applied to analyze complexity of species diversity for each sample through 5 indices (Observed-species, Chao, Shannon, Simpson and Coverage) which were calculated with QIIME

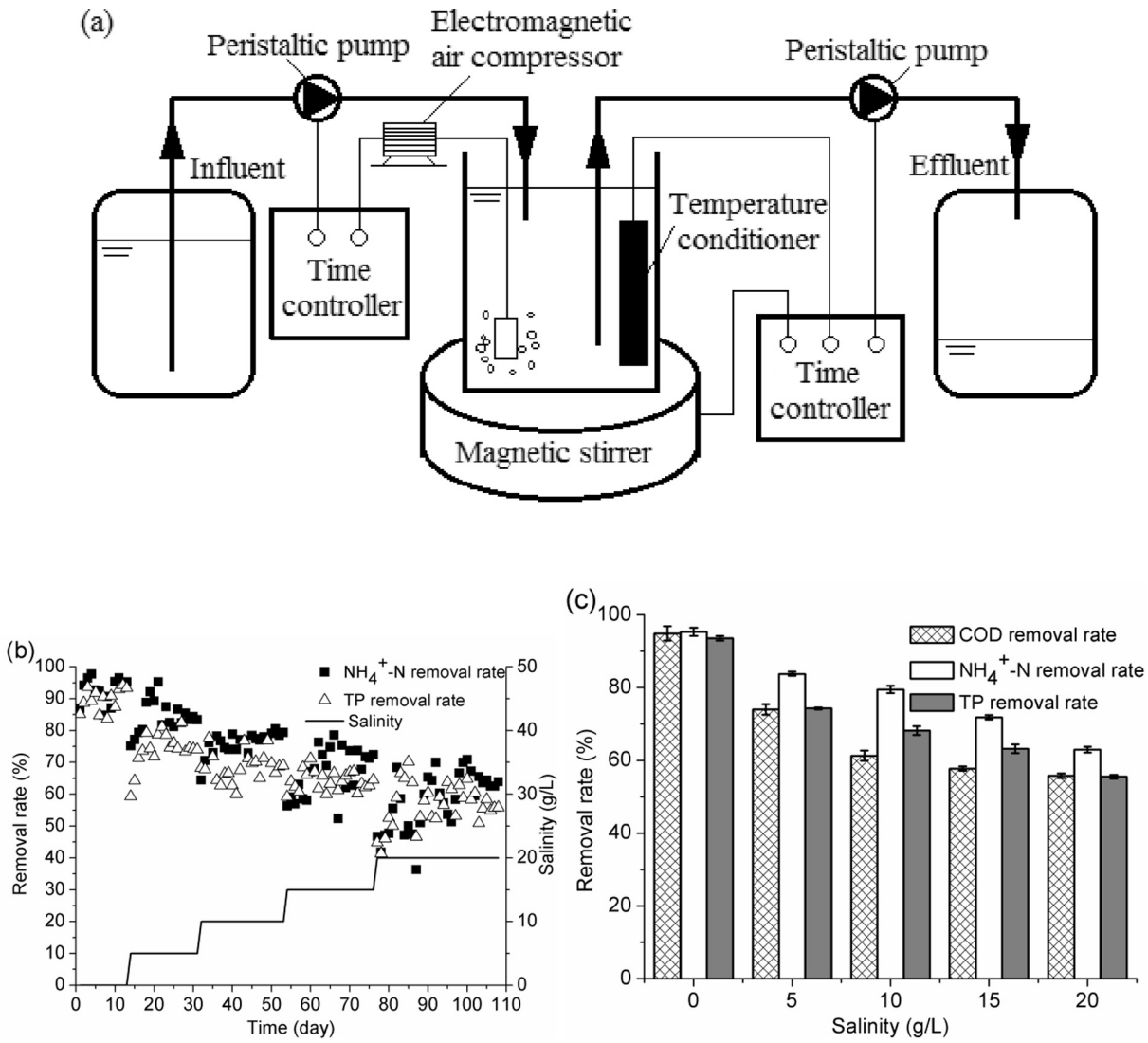

Fig. 1. Configuration of SBR (a) and the variation of removal efficiency in SBR with increased salinity (b-c): (b) removal rate of NH_4^+-N and TP in SBR over 108 days; (c) average removal rate of NH_4^+-N, TP and COD at stable state.

(V1.7.0) and displayed with R software (V2.15.3). Chao index was calculated to identify community richness, Shannon and Simpson indices were used to identify community diversity and Coverage index was used to evaluate the characterized sequencing depth. The Veen diagram was painted to display the OTUs difference of five samples intuitively.

Unweighted Pair-group Method with Arithmetic Mean (UPGMA) Clustering was used to analyze similarity of samples and calculated by QIIME (V1.7.0).

3. Results and discussion

3.1. Effect of salinity on removal performance

The removal efficiency of NH_4^+-N, TP and COD with the increase of salinity in SBR were shown in Fig. 1b and c. In the study, salinity gradually increased from 0 to 20 g/L, contained five saline stages (0, 5, 10, 15 and 20 g/L). For each salinity, it cost 13 days, 18 days, 22 days, 23 days and 32 days to reach the stable state for the SBR system, respectively. From Fig. 1b, the removal rate of NH_4^+-N and TP presented decreasing trend with the increase of salinity, although they were a little bit fluctuant during operation. The removal rate of COD was gradually declined from 94.88% to 55.77% as the salinity increased from 0 to 20 g/L (shown in Fig. 1c). And it should be noted that the COD removal efficiency was similar at the salinity of 15 and 20 g/L, they were 57.74% and 55.77%, respectively. The decreasing trends of NH_4^+-N, TP and COD removal efficiency were different from the results of Zhao et al. (2016). They reported that the removal efficiency of NH_4^+-N, TP and COD were not affected dramatically when the salinity was lower than 20 g/L. This difference may result from the diversity of treatment process and the microbial species in activated sludge seed.

As shown in Fig. 1c, the influence of salinity on the phosphorus removal was greater than that on the nitrogen removal. When the salinity increased to 5 g/L, the removal of TP was dramatically inhibited, the removal rate of TP decreased from 93.58% to 74.29%. However, the removal rate of NH_4^+-N only decreased from 95.34% to 83.79%. It could be inferred that compared with phosphorus-accumulating bacteria, nitrobacteria and denitrifying bacteria had better salt-tolerant ability. Besides, both denitrification and phosphorus removals were obviously shocked when the salinity was higher than 10 g/L. When the salinity increased to 20 g/L, the removal rate of NH_4^+-N and TP were dropped to 62.98% and 55.50%, respectively. It meant that when the salinity beyond their tolerance, both phosphorus-accumulating bacteria, nitrobacteria and denitrifying bacteria were affected.

For further studying the effect of salinity on nitrogen and phosphorus removal, the variation of NH_4^+-N, NO_3^--N, NO_2^--N and TP

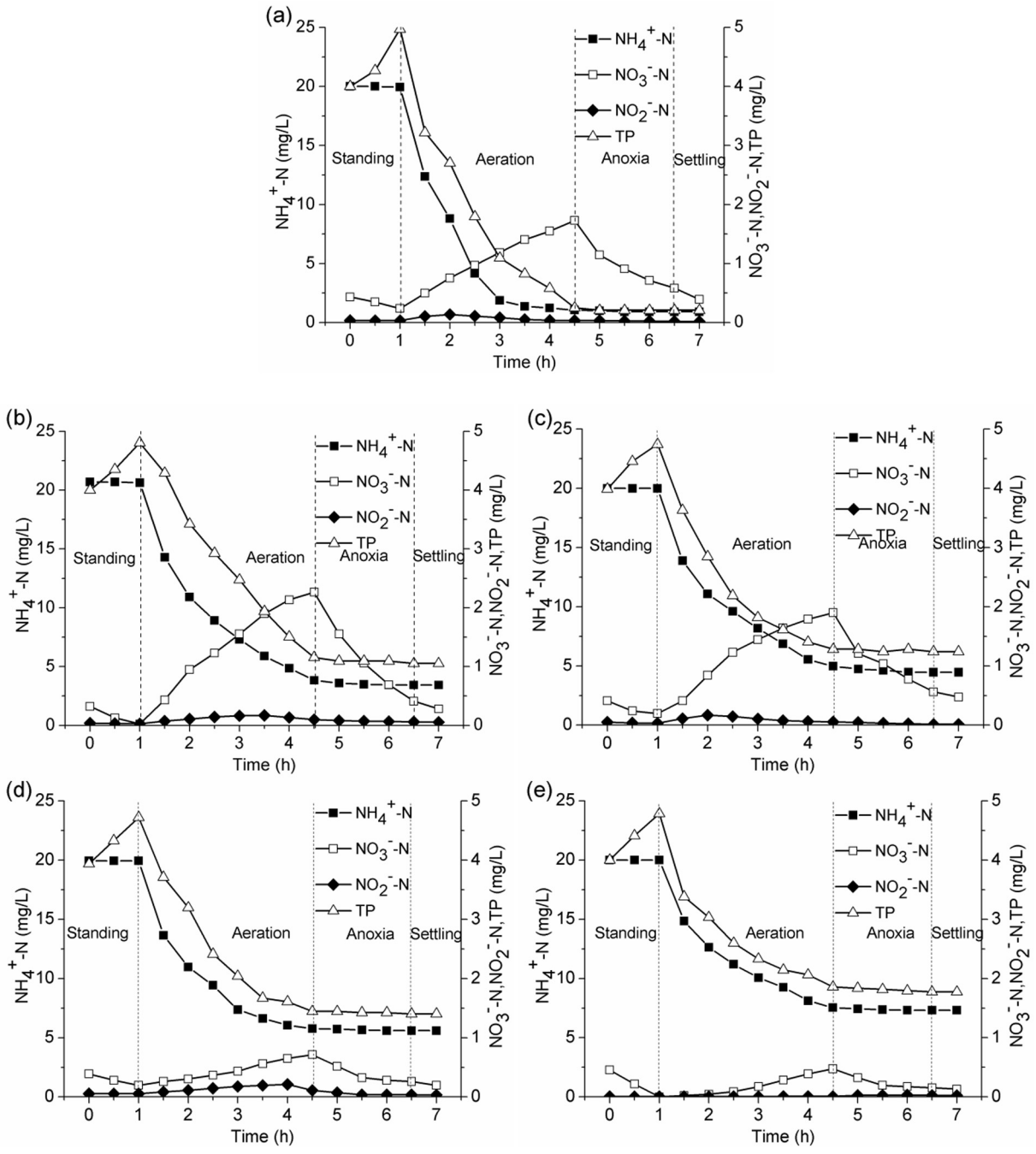

Fig. 2. The variation of NH_4^+-N, NO_3^--N, NO_2^--N and TP during an operation cycle under different salinity: (a) 0 g/L (b) 5 g/L (c) 10 g/L (d) 15 g/L (e) 20 g/L.

during an operation cycle was investigated. Fig. 2 displayed that during the aeration period the removal efficiency of nitrogen and phosphorus decreased with the increase of salinity. The result indicated the salinity seriously affected the activity of activated sludge during aeration period. Furthermore, at the salinity of 0, 5, 10, 15 and 20 g/L, the concentration of NO_3^--N was 1.73, 2.27, 1.90, 0.71 and 0.48 mg/L after aeration, respectively. However the concentration of NO_2^--N didn't show distinct variation. It meant that when the salinity was higher than 10 g/L, the nitrification was inhibited dramatically during aeration period. This phenomenon may caused by the salt in wastewater could affect the movement of oxygen to liquid phase directly and then influence the activity and metabolism of microorganism (Bassin et al., 2011; Rene et al., 2008). It could be inferred that when treating saline wastewater, increasing aeration rate or lengthening aeration time moderately can get good removal efficiency of nitrogen and phosphorus.

3.2. Influence of salinity on activated sludge performance

3.2.1. Effect of salinity on sedimentation of activated sludge

In this study, SVI was measured to express the sedimentation performance of SBR. As shown in Fig. 3a, the SVI value was 94.81 mL/g initially, which was between 50 and 120 mL/g and meant that activated sludge had good performance (Zhao et al., 2016). Then the SVI value decreased notably with the addition of inorganic salt. When the salinity increased to 10 g/L, the SVI value reduced to 52.00 mL/g and it indicated activated sludge was still showed a good performance. However, when the salinity was 15 and 20 g/L the SVI values were lower than 50 mL/g (37.12 mL/g and 19.53 mL/g, respectively) and it indicated the activity of activated sludge was affected (Zhao et al., 2016).

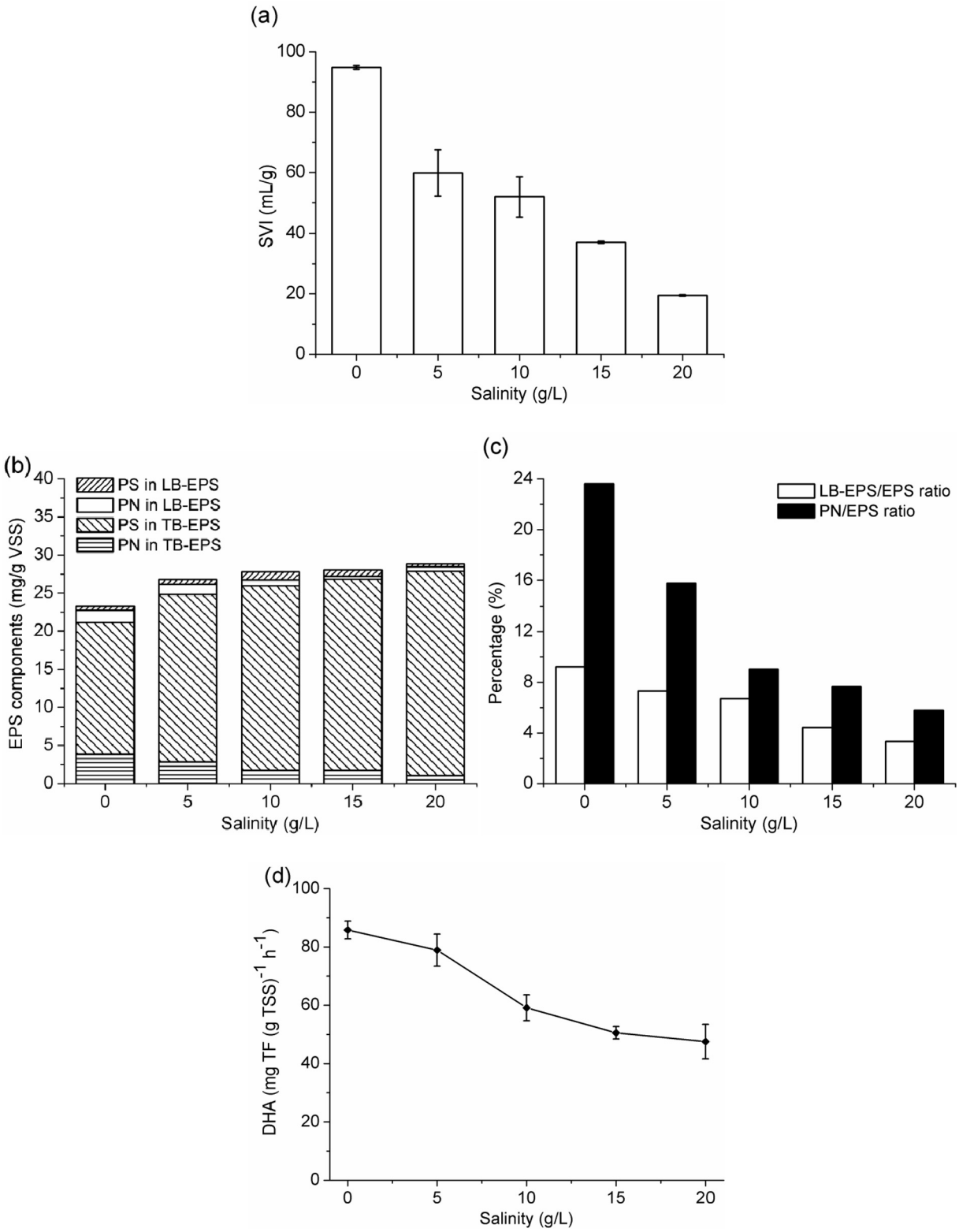

Fig. 3. The variation of activated sludge characteristics under different salinity: (a) SVI; (b) PN and PS contents in EPS; (c) LB-EPS/EPS ratio and PN/EPS ratio; (d) DHA.

This downtrend of SVI value was also found in the study of Bassin et al. (2012). As reported by Bassin et al. (2012), when the salinity increased from 0 to 20 g/L, the SVI value decreased from 110 to 60 mL/g.

This phenomenon indicated that with the increase of salinity in the SBR system, the sedimentation performance of activated sludge was better. The reason may be that the activated sludge flocs were smaller and closer in the condition of high salinity and then accelerates the settlement of activated sludge (Moussa et al., 2006; He et al., 2017). However, the increase of salinity had side effect on the activity of activated sludge. When the salinity was higher than 10 g/L, it showed obvious inhibition on activated sludge activity. It may result from microbes including filamentous bacteria were greatly inhibited at high salinity (Ng et al., 2005; Pronk et al., 2014), and the observed microbial species at different salinity showed a decreasing trend in this study

Table 1
Alpha indices of 5 activated samples.

Sample	Salinity (g/L)	Observed species	Shannon	Simpson	Chao1	Coverage
C	0	633	6.126	0.967	655.338	0.999
S1	5	604	4.526	0.865	657.01	0.998
S2	10	538	5.221	0.922	612.82	0.999
S3	15	545	5.458	0.947	601.875	0.998
S4	20	516	4.6	0.859	580.225	0.998

(shown in Table 1).

3.2.2. Effect of salinity on EPS

EPS is produced by microbes during metabolism (Yang et al., 2010), and the main compositions of it were protein and polysaccharides, accounting for 70–80%. For activated sludge, EPS plays an important role in physicochemical character, flocculation structure, surface charge, sedimentation and dewatering performance (Sheng et al., 2010).

In this study, PN and PS in EPS (including TB-EPS and LB-EPS) were measured. Fig. 3b showed the changes of PN and PS in EPS of activated sludge from SBR. From Fig. 3b, the total EPS content increased with the increase of salinity, from 23.30 mg/g VSS initially to 28.83 mg/g VSS at 20 g/L. This trend was in agreement with the research of Hong et al. (2013). And the increase of total EPS may result from EPS could increase the resistance of activated sludge to toxic substances, and protect the viability of cells in unfavorable condition such as high salinity environment (Laspidou et al., 2002). At the same time, the increase of EPS could help microorganisms adapt to saline environment through adjusting the osmotic pressure balance of cells (Johir et al., 2013).

Besides, from Fig. 3b, the TB-EPS content was higher than the LB-EPS content, and the PS content was higher than the PN content in total EPS at all salinities in this study. This phenomenon was different from the research of Wang et al. (2016). They used an anoxic-aerobic sequencing batch biofilm reactor to study the effect of salinity on EPS, and reported the main composition of EPS was PS rather than PN (Wang et al., 2016). The reason of this divergence may be the difference of activated sludge character and treatment operation.

Furthermore, as Fig. 3c displayed, the percentage of LB-EPS and PN in total EPS both decreased gradually with the increase of salinity. When salinity was 0 g/L, the content of LB-EPS and PN were 9.22% and 23.61%, respectively. As the salinity increased to 20 g/L, they dropped to 3.33% and 5.78%, respectively. The decrease of LB-EPS would contribute to the settlement of activated sludge, because LB-EPS was negative to sludge settlement (Li and Yang, 2007). This could explain the variation of activated sludge sedimentation performance at different salinity discussed before. In addition, from previous research PS played an important role in the protection of cells and it could relieve the sodion pressure on cells (Zhang et al., 2011; Artiga et al., 2008), therefore the PS content increased with salinity in this study. Oppositely, high concentration of sodion led to the decline of PN (Sheng et al., 2010). These may explain the decline of PN in total EPS with the increase of salinity.

3.2.3. Effect of salinity on DHA

Dehydrogenase is a kind of intracellular enzyme which related to oxidative phosphorylation of organics (Zhang et al., 2016). DHA is frequently measured due to it not only reveals microbial degrading ability of pollutant but also reflects the degradation of organics and the operation effect of treatment system (Yin et al., 2005). Therefore in order to evaluate the effect of salinity on activated sludge, DHA of activated sludge at different salinity was studied. Fig. 3d showed that the DHA of activated sludge decreased with the increase of salinity. DHA dropped sharply from 85.81 mg TF $(g\ TSS)^{-1}\ h^{-1}$ to 59.13 mg TF $(g\ TSS)^{-1}\ h^{-1}$ as the salinity increased from 0 to 10 g/L. When the salinity increased from 15 to 20 g/L, the DHA decreased slowly from 50.54 mg TF $(g\ TSS)^{-1}\ h^{-1}$ to 47.52 mg TF $(g\ TSS)^{-1}\ h^{-1}$.

This phenomenon may result from the addition of salt increased the osmotic pressure of SBR system. When the osmotic pressure above the tolerance of microorganisms, it restrained the normal metabolism and destroyed enzymes including dehydrogenase of microbes (He et al., 2017). Moreover, as seen in this study, the variation trends of DHA and COD removal efficiency were similar. They both dropped sharply at low salinity ($\leq$10 g/L) and then declined slowly at higher salinity ($\geq$15 g/L). This result indicated that the change of DHA of activated sludge may influence the removal efficiency of COD directly when treating saline wastewater.

3.3. Effect of salinity on microbial community

3.3.1. Microbial community structure analysis

Fig. 4a and b displayed 10 major kinds of activated sludge samples on different levels including phylum and class. On phylum level (Fig. 4a), the dominant phyla in all samples were *Proteobacteria*, *Bacteroidetes*, *Saccharibacteria*, *Actinobacteria* and *Firmicutes*. The result was similar to previous research of Zhang et al. (2016). They studied the effect of pressurized aeration on bacterial community of activated sludge when salt existed, and found *Proteobacteria*, *Bacteroidetes* and *Firmicutes* were dominant phyla. Furthermore, Fig. 4a showed that *Proteobacteria* was the most abundant phylum in all samples except S4 sample, because this kind of bacteria could use organic as nutrition. Ferrer-Polonio et al. (2015) also got the similar result in their study. When the salinity increased from 0 to 5 g/L, *Proteobacteria* increased from 67.32% (C) to 78.66% (S1) and then it displayed a decreasing trend from 78.66% (S1) to 32.81% (S4) as salinity increased from 5 to 20 g/L. Nevertheless, *Bacteroidetes* and *Saccharibacteria* increased remarkably from 12.43% and 6.64% (C) to 44.13% and 16.17% (S4), respectively. Moreover when the salinity reached to 20 g/L, *Bacteroidetes* replaced *Proteobacteria* as the most abundant phylum in the SBR sludge. It meant that compared with *Proteobacteria* phylum, *Bacteroidetes* phylum could survive easier under the salt stress. According to previous study that *Bacteroidetes* phylum was a kind of most abundant microbial communities in marine which played an important role in organic degradation (Díez-Vives et al., 2012). Because of this, for adapting the condition of inorganic salt environment, the *Bacteroidetes* survived, grew and flourished, and then quickly developed into the main phylum. Fig. 4a also revealed the increasing trend of *Firmicutes* which commonly existed in anaerobic digestion, and they could produce endospores to resist extreme conditions outside (Park et al., 2014). Based on this feature, *Firmicutes* could survive and flourish even the salinity in SBR increased to 20 g/L. Inversely it showed decreasing trends of *Acidobacteria*, *Verrucomicrobia*, *Planctomycetes*, *Nitrospirae* and *Chloroflexi*. It was noteworthy that *Nitrospirae* contained nitrite oxidizing bacteria which was related to the removal of nitrogen (Guadie et al., 2014), and *Chloroflexi* was regarded as predominant biomass that played an important role in the degradation of carbohydrates (Wang et al., 2015; Niu et al., 2016).

From Fig. 4b, the community composition on class level was further analyzed. As Fig. 4b displayed, *Betaproteobacteria* was the most dominant identified class in all samples, which was reported as one of the highly appeared bacteria in wastewater biological treatment and played important roles in denitrification (Hao et al., 2013). Other classes of *Proteobacteria* such as *Gammaproteobacteria*, *Alphaproteobacteria* and *Deltaproteobacteria* were also found in this study. It should be noted that most of *Proteobacteria* bacteria were decreased except *Alphaproteobacteria*, the abundance of *Alphaproteobacteria* increased gradually from 6.21% (C) to 11.20% (S4) and became the second dominant class when the salinity was 20 g/L. *Bacteroidetes* microorganisms such as *Sphingobacteriia*, *Bacteroidia*, *WCHB1-32* and *Cytophagia* existed in all samples. *Sphingobacteriia* dropped from initially 7.75% (C) to 4.15% (S1) as salinity increased from 0 to 5 g/L, and then gradually increased to 6.46% (S3), finally reduced to 2.71% (S4). As seen, the dynamic

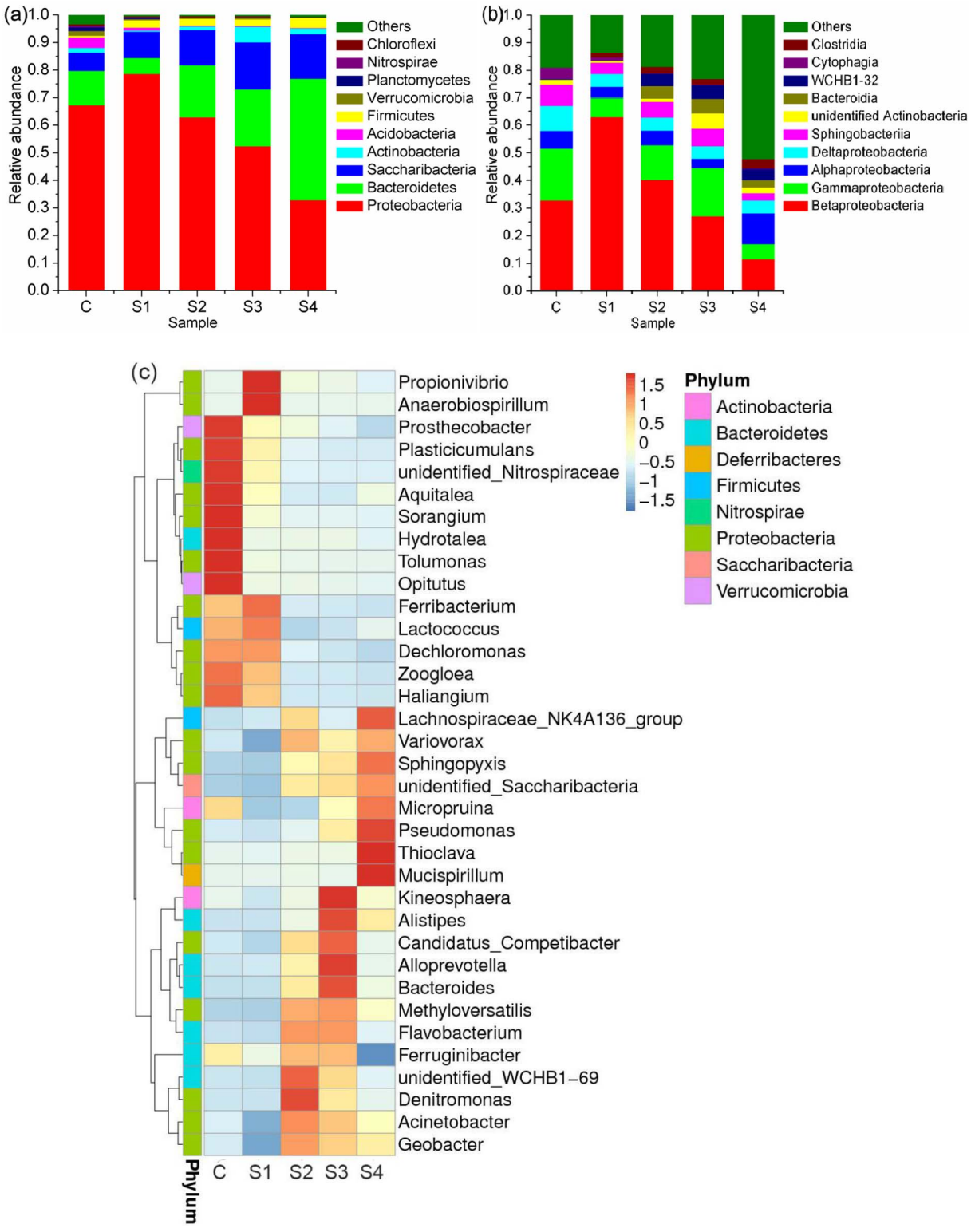

Fig. 4. Microbial community of 5 activated sludge samples on the phylum (a), class (b) and genus (c) levels.

variation of *Sphingobacteriia* was apparently influenced by salinity (Cortés-Lorenzo et al., 2014). *Bacteroidia* increased remarkably from 0.01% (C) to 5.25% (S3), and then reduced to 2.57% (S4). *WCHB1-32* increased from 0.25% (C) to 4.13% (S4). However, *Cytophagia* gradually reduced from 4.05% (C) to 0.11% (S4) with the salinity increased from 0 to 20 g/L. In addition, the abundance of *Clostridia* in *Firmicutes* increased dramatically from 0.04% (C) to 3.4% (S4), and it was reported as anaerobic bacteria (Fernándeznaveira et al., 2017).

In order to analysis bacteria community clearly on genus level, the 35 major genera belong to 8 different phyla (*Actinobacteria*, *Bacteroidetes*, *Deferribacteres*, *Firmicutes*, *Nitrospirae*, *Proteobacteria*, *Saccharibacteria* and *Verrucomicrobia*) were displayed in the relative abundance heat map whose values were standardized, shown in Fig. 4c. The C sample and S1 sample had some similarities compared to other samples (S2, S3 and S4). It indicated that the influence of salinity on bacteria was bearable at low salinity (< 10 g/L), however the microbial

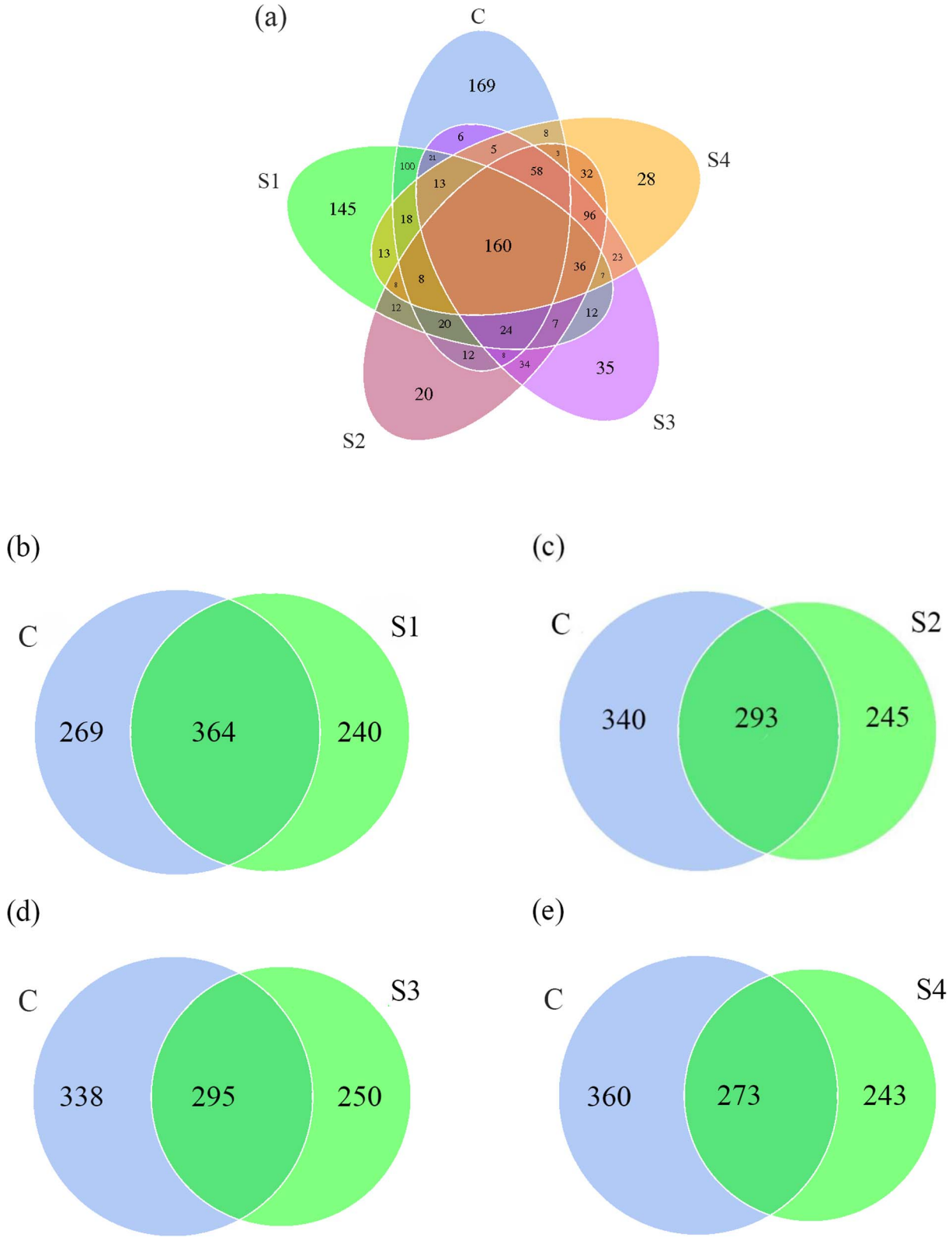

Fig. 5. The Venn diagrams of the microbial communities.

community was greatly shocked when the salinity was at least 10 g/L. *Propionivibrio*, *Ferribacterium*, *Dechloromonas*, *Zoogloea*, *Thioclava*, *Methyloversatilis* and *Denitromonas* were found in all samples, and they belong to *Rhodocyclaceae* which was reported as the predominant family responsible for denitrification (Zhang et al., 2016). Although all samples contained *Rhodocyclaceae* bacteria, the most abundant genus of them in each sample was different, *Zoogloea* and *Thioclava* had best performance at 0 and 20 g/L salinity, respectively. It meant that there was diversity of *Rhodocyclaceae* bacteria on salt-tolerant ability and *Thioclava* was salt-tolerant.

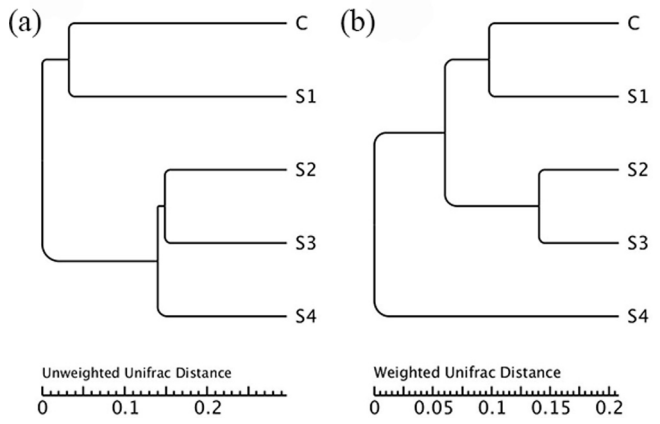

Fig. 6. UPGMA analysis based on unweighted unifrac distance (a) and weighted unifrac distance (b) of 5 activated sludge samples.

3.3.2. *Effect of salinity on microbial richness and diversity*

Alpha diversity could reflect abundance and diversity of microbial community. The result was shown in Table 1. The coverage of all samples were more than 99%, it reflected that the sequencing depth of each sample was adequate. The decrease of Chao1 value indicated the reduction of community richness with the increase of salinity. At the same time, the variation of Shannon and Simpson value revealed that the addition of inorganic salt led to the decrease of community diversity in the SBR system, especially at 20 g/L of salinity. Moreover, the observed species was reduced with the increase of salinity, dropped from 633 (C) to 516 (S4) when salinity increased from 0 to 20 g/L. It meant that the increase of salinity in SBR had side effect on microbial diversity. This phenomenon may caused by the death of no salt-tolerant bacteria in activated sludge, and the result was in agreement with the research by Zhao et al. (2016).

The Veen diagram between control status (C) and activated sludge at different salinity were shown in Fig. 5b–e. Shared species among all samples were 160 (shown in Fig. 5a). Furthermore, shared species between sample C and S1, S2, S3 and S4 were 364, 293, 295 and 273, respectively. It demonstrated that the side effect of inorganic salt on microbial community diversity of activated sludge in SBR was deeper with the increase of salinity.

The results of UPGMA analysis in phylum level were shown in Fig. 6. Fig. 6a was based on unweighted unifrac distance, which was used to compare the difference of samples on microbial diversity. Fig. 6b was based on weighted unifrac distance, which was used to compare the difference of samples on microbial richness and diversity. Fig. 6a showed that salinity affected the kinds of microbes. Moreover, C and S1 were similar, S2, S3 and S4 were similar. However, as shown in Fig. 6b, the similarity between S4 and S2/S3 were less. It could be inferred that the increase of salinity not only reduced the kind of bacteria but also affected the community richness of microbes. Furthermore, the major difference of activated sludge at high salinity (20 g/L) was more likely caused by the variation of richness rather than the diversity.

4. Conclusions

Salinity ranging from 0 to 20 g/L reduced the content of LB-EPS in EPS of activated sludge from SBR, and improved the sedimentation performance of activated sludge. However, increased salinity inhibited DHA of activated sludge, and reduced the richness and diversity of microbial community, which led to the deterioration of removal performance for nitrogen, COD and phosphorus. Furthermore, the removal of nitrogen and phosphorus was affected mainly during aerobic period. Therefore, increasing aeration rate, extending aeration duration moderately or adding halotolerant bacteria (such as *Thioclava*) could improve nitrogen and phosphorus removal for the treatment of saline wastewater.

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