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Performance and characterization of the microbial community structures in the activated sludge from wastewater treatment plant at high altitudes in Tibet of China

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ABSTRACT

The performance of wastewater treatment at high altitudes may differ from that at low altitudes. In this study, we investigated the performance of two wastewater treatment plants (WWTPs) at different altitudes based on the activated sludge by comparison of the sludge load, and found that the sludge load of sewage treatment plant in Lhasa (LS) (0.049 kg BOD_5/kg MLSS· d) was lower than that in Nan Jing (NJ) (0.1 kg BOD_5/kg MLSS· d), that's about half of NJ. Then we further investigated the influent quality and activated sludge properties of the two WWTPs at different altitudes, and found that the concentration of influent sewage in LS was low: BOD_5/COD < 0.4 and the BOD_5/TN < 2.5. The SV, MLSS, MLVSS and SVI in LS were less than that in NJ. The HRT of two WWTPs were the same. The SRT in LS (25 d) was longer than that in NJ (16 d). Next, we examined the microbial community structure of activated sludge of two WWTPs at different altitudes, and significant microbial changes were discovered, especially in the four seasons of 2015 between LS and NJ. The richness and evenness of community in LS were less than that in NJ, but the significant variables correlated with the variances of bacterial communities in NJ were more than that in LS. The results are of certain significance to understand the operations of WWTPs at high altitudes.

Keywords: High altitude; Performance; Influent quality; Activated sludge properties; Microbial community structures

1. Introduction

With the rapid social and economic development at high altitudes, the sewage discharge increases gradually in big cities of plateau regions. As the sewage treatment can decrease organic and inorganic pollutants, especially nutrients such as nitrogen and phosphorus, it is important the water environment in high altitude regions. Many full-scale wastewater treatment plants (WWTPs) above elevation of 3000 m in China, which represent biological wastewater treatment such as WWTPs in Lhasa, have been governed to purify the discharged wastewater. Moreover, because the operation of WWTPs at high latitudes faces several unusual obstacles raised by extreme climatic conditions in these regions, such as strong solar radiation, sharp/extreme/large variation in temperature between day and night, rarefied air, and low oxygen pressure [1], the stable operation of WWTPs by widely-used sewage treatment technology in plain area based on the activated sludge or biological process in plateau region is very important.

The sludge load (Ns) may externally reflects the performance and stable operation of WWTPs directly. The sludge load refers to the organic pollutants removal acti-

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vated sludge unit mass per unit time per unit volume, kg COD(BOD)/(kg MLSS · d). The formula of Ns is as following: Ns = F/M = QS/(VX). In the formula, Q is the influent flow per day, S is the concentration of COD (BOD), V is the volume of aeration tank, and X is the concentration of sludge. Ns mean the F/M ratio on microbial metabolism. It represents a balanced relationship between the microorganism quantity and the amount of food. It is related to the influent quality, the volume of aeration tank and the sludge properties which are related to microbial community directly. In the activated sludge process, the sludge properties are crucial to the effective control of the process performance [2]. The core of activated sludge process is the activated sludge and the microorganisms contained by it. The properties of activated sludge, which will affect the effluent treatment effect and the effluent quality, are related to microorganisms, so we have carried out numerous studies to investigate the sludge properties in phenol removal through biodegradation by the SBR [3–5].

The influent quality will affects the removal efficiency and the stable operation of WWTPs. If the influent ratio of C/ N is too high, the heterotrophic organisms will tend to out-compete the nitrifying organisms in the use of the available ammonia nitrogen [6]. Yun [7] showed that, with the addition of organics, the nitritation activity would fail in the reactors. Sharma [8] reported that, the nitrification rate would increase as the influent COD/TKN decreased. Luo [9] investigated the impact of influent ratio of COD/N in disintegration of aerobic granular sludge. As shown in the results, the decreased COD/N ratios of 2 and 1 strongly influenced the stability of aerobic granular sludge with regard to the physical properties and nitrification efficiency. And when the ratio was decreased to 1, it would lead to aerobic granular sludge disintegration.

A better understanding in the microbial community structure of activated sludge in WWTPs can be helpful to elucidate the mechanism of biological pollutant removal and improve the treatment performance and operational stability [10,11]. To the best of our knowledge, the bacterial community compositions, spatial (or temporal) dynamic and related influence variables in activated sludge have been characterized by the amounts in previous studies [12,13]. Although we have achieved some knowledge in the microbial community structures of activated sludge in a biological wastewater treatment system, most of that is concentrated in the WWTPs at low altitudes. Therefore, we need more information on the microbial community structures to understand the stable operation of WWTPs at high altitudes better.

The objective of this paper is to investigate the performance and stable operation of two wastewater treatment plants at different altitudes. The two plants have similar process in treating domestic wastewater by the Cyclic Activated Sludge System (CASS). The performance of activated sludge wastewater treatment is further researched based on three aspects, influent quality, activated sludge properties and microbial community structures. These results are expected to provide some practical guidance to the operation of WWTPs in treating domestic wastewater at high altitudes.

2. Materials and methods

2.1. Objectives of wastewater treatment plants

The two wastewater treatment plants (WWTPs) are located in different cities of China with different geographical conditions. The sewage treatment plant in Lhasa (LS), located at east longitude 91°07′, north latitude 29°39′, has an altitude of about 3600 m above sea level. And the sewage treatment plant in NanJing (NJ), located at east longitude 118°46′, north latitude 32°03′, has an altitude of about 60 m above sea level. The Cyclic Activated Sludge System (CASS) is used by the two WWTPs to treat domestic wastewater. Influent quality such as COD, BOD_5, NH_4-N^+ and SS are collected for the two WWTPs from daily monitoring of sewage treatment plants. Biomass properties, including settling velocity (SV), mixed liquor suspended solids (MLSS) and solid residence time (SRT), are collected for the two WWTPs from monitoring of sewage treatment plants. Meanwhile, sludge volume index (SVI) are calculated based on SV and MLSS, and mixed liquor volatile suspended solids (MLVSS) are calculated based on MLSS. Hydraulic residence time (HRT) is calculated based on HRT = $V/Q\ (h)$, in which V is the volume of aeration tank, while $Q\ (h)$ is the inflow per hour.

2.2. Collection of samples

The activated sludge samples were collected from the aeration tanks of each system in April, June, October and December 2015. Triplicate samples at least five meters apart from each other were collected into each tank. Each sample was mixed by five activated sludge replicates randomly collected within a circle with 5 m of diameter. And the samples were kept in a dry ice box immediately after collection for express and stored at –80°C in a lab for DNA extraction. After melting at room temperature, the sample was centrifuged at 14000 g for 8 min. Then the supernatant was decanted, and 5 g of the pellet was weighted out, for the use in the next step of DNA extraction.

2.3. DNA extraction and PCR amplification

The microbial DNA was extracted from AS samples using the E.Z.N.A.® Soil DNA Kit (Omega Bio-tek, Norcross, GA, U.S.) according to the manufacturer's protocol. The V4-V5 regions of the bacteria 16S ribosomal RNA gene were amplified by PCR (at 95°C for 5 min, followed by 27 cycles at 95°C for 30 s, 55°C for 30 s, 72°C for 45 s and a final extension at 72°C for 10 min) using primers 341F 5′-CCTAYGGGRBGCASCAG-3 and 806R 5′- GGACTACNNGGGTATCTAAT-3′, where the barcode is an eight-base sequence unique to each sample. The PCR reactions were performed in triplicate 20 µL mixture containing 4 µL of 5 × FastPfu Buffer, 2 µL of 2.5 mM dNTPs, 0.8 µL of each primer (5 µM), 0.4 µL of FastPfu Polymerase, and 10 ng of template DNA. The amplicons were extracted from 2% agarose gel, purified by use of the AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, U.S.) according to the manufacturer's instructions and quantified by use of QuantiFluor™ -ST (Promega, U.S.).

2.4. Library construction and sequencing

The purified PCR products were quantified by Qubit®3.0 (Life Invitrogen), and every 24 amplicons, whose barcodes were different were mixed equally. The pooled DNA product was used to construct a Illumina Pair-End library following Illumina's genomic DNA library preparation procedure. Then the amplicon library was sequenced in pairs (2 × 250) on an Illumina MiSeq platform (Shanghai BIOZERON Co., Ltd) according to the standard protocol.

2.5. Processing the sequencing data

Raw FASTQ files were demultiplexed, and filtered based on the quality by use of QIIME (version 1.17) with the following criteria: (i) The 250 bp reads should be truncated at any site, and an average quality score <20 over a 10 bp sliding window should be gotten by discarding the truncated reads which were shorter than 50 bp. (ii) Exact barcode matching. 2 nucleotide mismatches in primer matching and reads containing ambiguous characters should be removed. (iii) Only sequences which overlap longer than 10 bp could be assembled according to their overlapped sequences. Reads which could not be assembled should be discarded.

Operational Units (OTUs) were clustered with 97% similarity cutoff by use of UPARSE (Version 7.1 http://drive 5.com/uparse/), and the chimeric sequences were identified and removed by use of UCHIME. The phylogenetic affiliation of each 16S rRNA gene sequence was analyzed by RDP Classifier (http://rdp. cme. msu. edu/) against the silva 16S rRNA database by use of 70% of confidence threshold [14].

2.6. α- and β-diversity analyses

The bacterial community structure of each sample was detected by T-RFLP method. The α-diversity indices (Shannon-H index and Evenness index) for each sample were calculated by the Vegan 2.0 package in R. And the β-diversity analysis was performed by UniFrac [15] to compare the results of the principal component analysis (PCA) by the community ecology package, R-forge (Vegan 2.0 package was used to generate a PCA figure). The similarity analysis of the OTU compositions of samples was performed by clustering analysis based on the Bray-Curtis similarity by PAST 3.0 [16].

3. Results and discussion

3.1. Parameters

The food/microorganism ratio (F/M) is one of the most important factors in biological wastewater treatment systems. F/M in wastewater biological treatment reflects the performance of activated sludge wastewater treatment directly. As shown in Table 1, the performance of activated sludge wastewater treatment in LS (0.049 kg BOD_5/kg MLSS· d) was lower than that in NJ (0.1 kg BOD_5/kg MLSS · d). The low sludge load could not provide enough nutrients to ensure the normal growth of microorganisms, so the microbes entered into the endogenous respiration period. The F/M in LS was lower, which could make it more difficult for the bacteria with not enough acclimatization ability to exist in theory. Some previous studies confirmed that the F/M ratio shaped the microbial abundance and the composition in sludge bioreactors [17]. In order to solve the problem, the quantity of supplied organics should be adequate to maintain a higher F/M value. Otherwise, the low F/M value would lead to much consumption of organic substance and thus insufficient supply of carbon substrate for denitrification.

Table 1
The comparison of the values of parameters SV, MLSS, MLVSS, SVI, HRT and SRT between LS and NJ

	LS	NJ
SV (%)	25	30
MLSS (mg/L)	2500	4500
MLVSS (mg/L)	1875	3375
SVI (mL/g)	80	100
F/M (kg BOD_5/kg MLSS· d)	0.049	0.1
HRT (h)	4	4
SRT (d)	25	16

The comparison of the values of parameters SV, MLSS, MLVSS, SVI, HRT and SRT between LS and NJ is shown in Table 1. The SV, MLSS, MLVSS, SVI in LS were less than that in NJ. From Table 1 we can see that the activated sludge properties in LS were lower than that of the sewage treatment plant in NJ. However, the SV, MLSS, MLVSS, SVI values in LS were within normal range. The HRTs of two WWTPs were the same. And the SRT in LS was longer than that in NJ. The longer SRT implied that the bacteria in LS might take more time to adapt to the pollutants in the influent, and the bacteria with greater adaptation ability would be enriched. As autotrophic nitrifiers grew very slowly, a long SRT was required to maintain a certain amount of nitrifiers and ensure effective nitrification [18].

3.2. Influent sewage

The fluctuation of influent COD, BOD_5, NH_4-N^+ and SS values in LS in 2015 is shown in Fig. 1. The influent COD, BOD_5, NH_4-N^+ and SS in 2015 were 70.52–126.30 mg/L, 18.28 –31.88 mg/L, 8.18–21.50 mg/L, and 42.40–85.72 mg/L. Results showed that the concentration of influent COD, BOD_5, NH_3-N and SS in the sewage treatment plants in Lhasa, in which the sewage belonged to the urban sewage with low concentration. The reasons were as follows: Firstly, Lhasa was a city with small scale, so its underdeveloped economy and undeveloped township enterprises were relative to the big city in China; Secondly, because of the imperfect underground sewage pipe network and serious groundwater seepage, it would cause a certain degree of dilution in sewage; Thirdly, it would also cause a certain degree of dilution in sewage because the rainwater and sewage were collected together.

The comparison of removal rates of COD, BOD_5, NH_4-N^+ and SS between LS and NJ is shown in Table 2. It was found that the NH_4-N^+ removal efficiency in LS was lower than that in NJ. The removal efficiency of ammonium nitrogen was

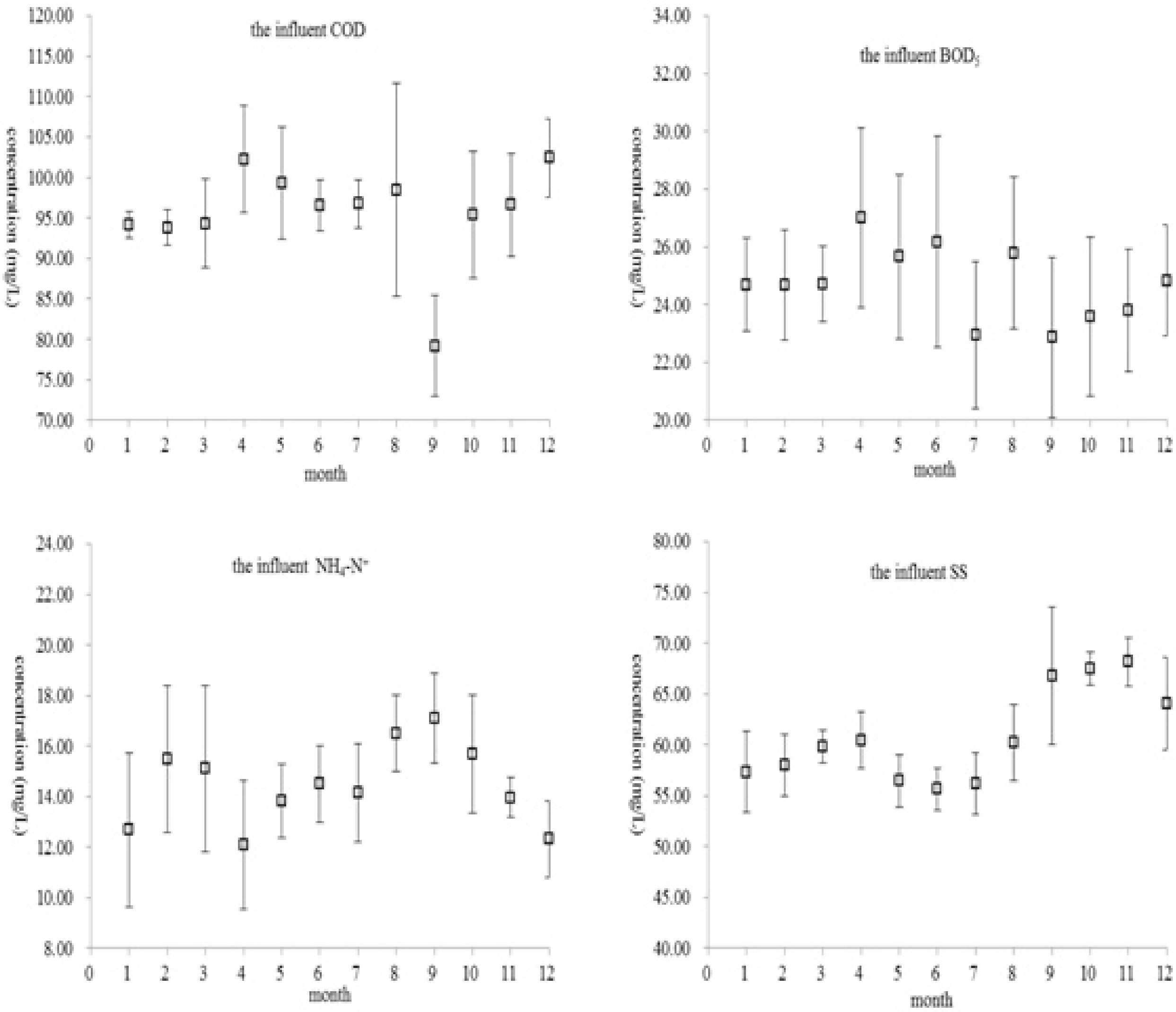

Fig. 1. The fluctuation of influent COD, BOD_5, NH_4-N^+ and SS values in LS in 2015.

Table 2
The comparison of removal rate of COD, BOD_5, NH_4-N^+ and SS between LS and NJ in 2015

Removal rate (%)	LS	NJ
COD	68.4–88.6	79.2–91.2
BOD_5	88.3–92.6	86.8–93.8
NH_4-N^+	57.8–67.9	78.9–93.7
SS	81.2–94.4	82.3–92.5

limited by the available BOD and COD in the influent. And the NH_4-N^+ removal in LS would be restricted by the low influent BOD_5 and COD concentration. The BOD_5/COD and BOD_5/TN ratios in LS in 2015 are shown in Fig. 2. In the figure, the BOD_5/COD ratio was less than 0.4, while the BOD_5/TN ratio was less than 2.5 in LS in 2015. BOD_5/COD was often used to predict the biodegradability of sewage. When BOD_5/COD ranged between 0.4 and 0.6, the biodegradability was considered to be fine. When BOD_5/COD ranged between 0.2 and 0.4, it would indicate that the sewage might contain refractory biodegradation [19]. Therefore, the BOD_5/COD ratio could indicate that the biodegradation in wastewater was poor. The main reason was that, the industrial wastewater would tend to be mixed in Lhasa sewage collection pipe network, which would result in domestic sewage consisting of different types of refractory organic pollutants [20].

The C/N ratio of domestic wastewaters is, however, often lower than these prescribed values, and the nitrogen removal is limited by the lack of available organic carbon source [21]. Meanwhile, the total rate of nitrogen removal can't keep it high, so the influent should contain BOD_5/TN. Usually, when the BOD_5/TKN in the denitrification reactor is more than 4 to 6, the carbon source may be considered to be adequate [20]. According to Fig. 2, the mean of BOD_5/TN is less than 2.5 in Lhasa sewage treatment plant, from which we can conclude that the plant lacks carbon source in influent denitrification, which means that the wastewater is sewage at low C/N ratio requiring con-

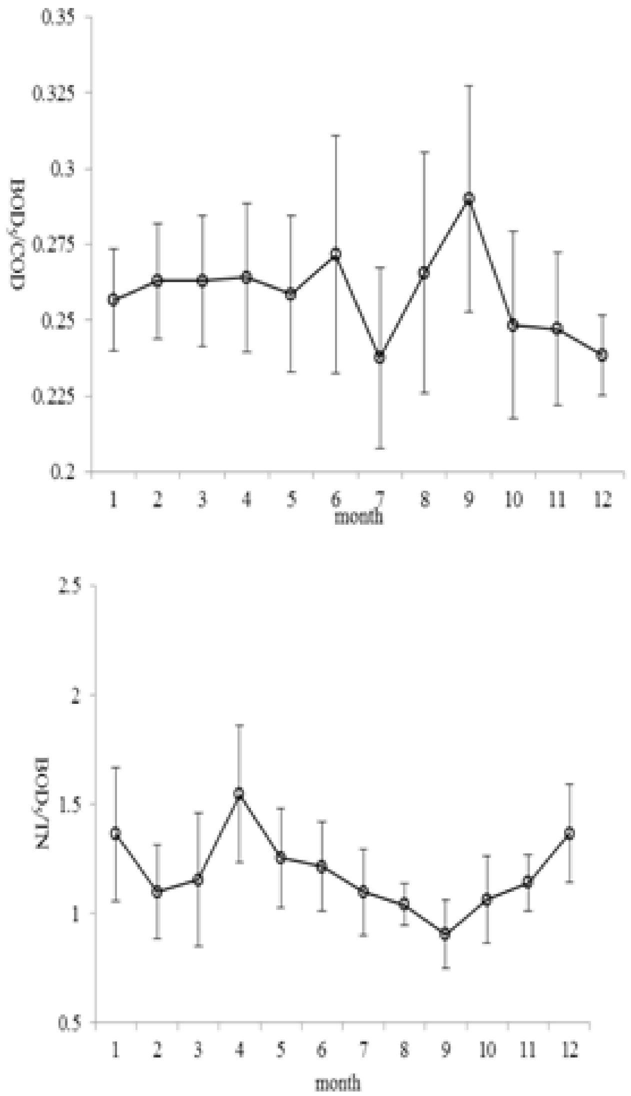

Fig. 2. The BOD_5/COD and BOD_5/TN ratios in LS in 2015.

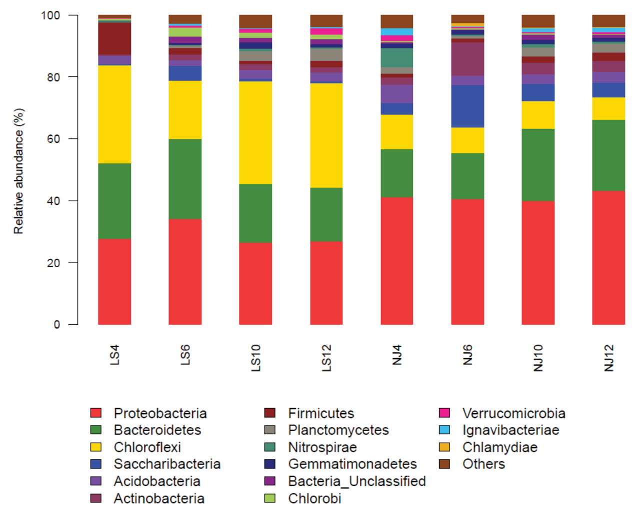

Fig. 3. Relative abundance of bacterial phyla of the four seasons between LS and NJ in 2015. Minor phylum refers to the phyla with maximum abundance of 1% in a sample.

trolling additional carbon source appropriately. It is still difficult to realize a satisfactory nitrogen removal when the influent COD/TN ratio is less than 4.0 [22–23], and the carbon shortage is unfavorable for denitrification [24]. Therefore, it is desired for many municipal WWTPs to look for practical and cost-effective alternative technologies in nitrogen removal. To get satisfactory nitrogen removal performance for wastewater with a C/N ratio lower than the critical value, one way is to introduce an innovative nitrogen removal pathway or a treatment process which can support nitrogen removal with a low or zero organic carbon demand, and the other way is to add external carbon for denitrification.

3.3. Microbial community structures

Fig. 3 summarizes the relative abundances of the bacterial community on the phylum level in LS and NJ. It showed that *proteobacteria* represented the most abundant phylum. *Bacteroidetes* was the second most abundant phylum, followed by *actinobacteria* and *chloroflexi* in NJ in April, June, October and December 2015. This result was similar to those observed in many previous studies focusing on municipal WWTPs [16,25]. As shown in Fig. 3, *proteobacteria* represented the most abundant phylum, and *bacteroidetes* was the second most abundant phylum, followed by *Chloroflexi* in LS in June 2015. *Chloroflexi* represented the most abundant phylum, and *proteobacteria* was the second most abundant phylum, followed by *Bacteroidetes* in LS in April, October and December 2015. As the structure of activated sludge bacteria could affect the effect of sewage treatment system, the SV and MLSS in LS in June were more than April, October and December. The activated sludge properties in LS in June were more than April, October and December. However, the SV and MLSS in LS in April, June, October and December were within normal range. Because the higher SVI of activated sludge was usually related to various types of filamentous organisms, *actinobacteria* and the main filamentous organisms were focused on subsequently. The relative abundances of *actinobacteria* in LS (0.31%, 1.78%, 1.92% and 1.72%) were lower than in NJ (2.21%, 10.94%, 3.88% and 3.37%) in April, June, October and December 2015 as shown in Fig 3. In addition, a large portion of the sequences was grouped into unclassified sequences in this study.

Fig. 4 summarizes the relative abundances of the bacterial community at the genus level in LS and NJ. It showed that *roseiflexus* represented the most abundant genus in LS in April, June, October and December 2015. As shown in Fig. 4, *bacteroidetes unclassifiedly* represented the most abundant genus in NJ in October and December 2015, *saprospiraceae unclassifiedly* represented the most abundant genus in NJ in April 2015, and *saccharibacteria norank* represented the most abundant genus in NJ in June 2015. In addition, a large portion of the sequences was grouped into unclassified sequences in this study.

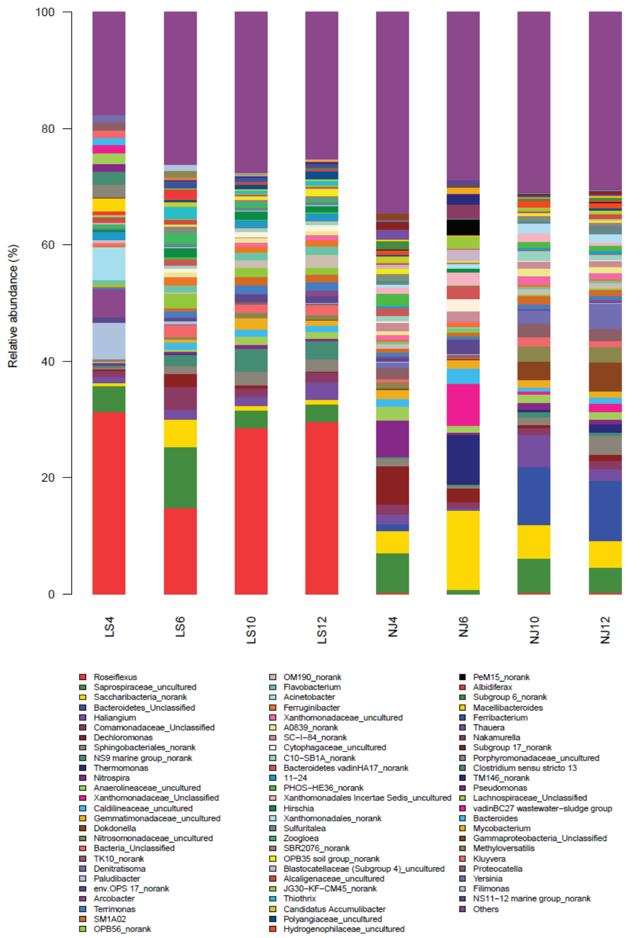

Fig. 4. Relative abundance of bacterial genus of the four seasons between LS and NJ in 2015. Minor genus refers to the genus with maximum abundance of 1% in a sample.

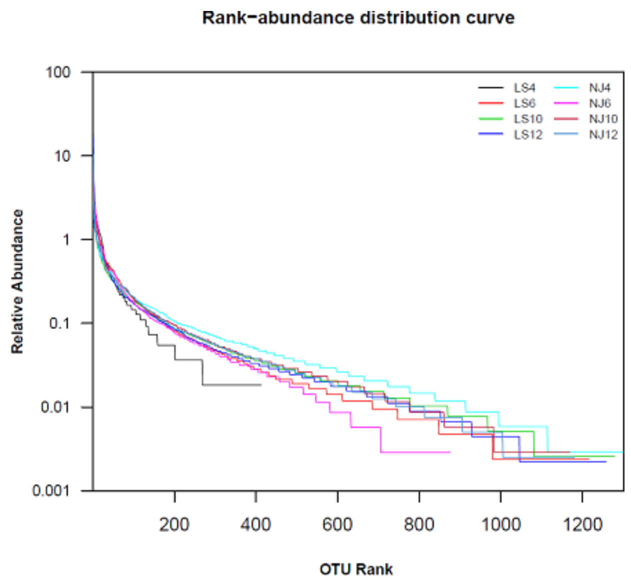

Fig. 5. Relative abundance of the OTU richness and evenness of bacterial communities of the four seasons between LS and NJ in 2015.

3.4. The relationship among sludge properties, treatment performance and microbial community structures

The community richness and evenness are two important factors which could influence the functional stability and general performances of WWTPs [26]. Wittebolle [27] demonstrated that communities with higher evenness could present more functional resistance to environmental stress. Werner [28] reported that the methanogenic activity and substrate removal efficiency could be correlated with community evenness in full-scale bioenergy systems. Johnson [26] demonstrated the significantly positive correlation between functional and taxonomic richness empirically. And Niu [29] the declining trend in evenness would be reflected by distinct trends of individual bacterial phyla in relative abundance. In conclusion, the richness and evenness of community could be important to determine the general performance of WWTPs.

The richness and evenness of WWTP community are related with elevational gradient. As reported by Niu [29], the variance partitioning analysis showed that elevation statistically accounted for approximately 8.1% of bacterial community variances at elevations higher than 1200 m. As shown in Fig. 5, the richness and evenness of WWTP community in LS were less than that in NJ. Niu [29] reported that a significant change in the evenness of bacterial community was observed at around 1,200 m of elevation with a linear decline towards the highest elevation 3,660 m. As mentioned above, the less richness and evenness at high elevations might imply the less functional richness and more susceptible functional stability of WWTP communities than those at low elevations. Furthermore, at high elevations, the low richness and evenness might imply a decreased functional resistance of WWTP communities to the extreme environmental stress on the Tibet plateau. Though the empirical test in functional richness and stability of WWTPs with high elevation was lacking, these speculations were partly supported the real performances of WWTPs at high elevations across the world. In Bolivia (above 4,000 m of elevation), many WWTPs were difficult to reach the required effluent quality for effluent BOD or COD and enteric virus according to Bolivian environmental law [30,31].

As shown in Fig. 6, the correlation analysis showed that there was positive correlation among COD, F/M, SS, MLSS and SV. There was negative correlation among COD, BOD_5 and NH_4-N^+. And there was negative correlation among water temperature, DO and height. Different response of bacterial community structures were observed at lower and higher elevations respectively. Different bacterial community structures were associated with the environment at different altitudes. The bacterial community structures in LS were the result of long-term domestication at low temperatures, higher elevations and in the influent sewage with low concentration. The significant variables were more correlated with the variances of bacterial communities in NJ more than that in LS. This result was similar to those observed in many previous studies focusing on municipal WWTPs [29].

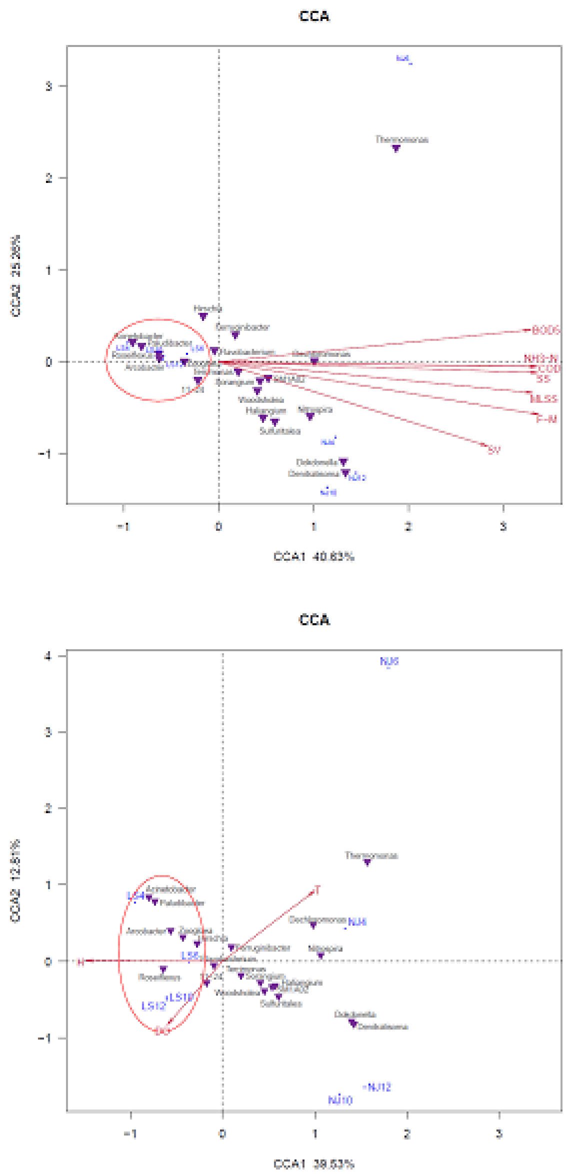

Fig. 6. The correlation analysis between bacterial communities and environmental factors. RDA ordinations corresponding to bacterial communities were used for the WWTPs. BOD_5, biological oxygen demand; COD, chemical oxygen demand; NH_3-N, ammonia nitrogen; SS, suspended solid; MLSS, mixed liquor suspended solid; F/M, the sludge load; SV, settling velocity; H, elevation; T, the water temperature; DO, dissolved oxygen.

4. Conclusions

The comparison of treatment performance indicated that NH_4-N^+ removal efficiency of in LS was lower than that in NJ. The average of influent BOD_5/COD in LS sewage treatment plant was lower than 0.4, so the biodegradability is poor. The averages of influent BOD_5/TN, as domestic sewage of low carbon and nitrogen ratio, were both lower than 4, which showed a serious shortage of carbon sources. The performance of activated sludge in LS was lower than that in NJ. The analysis on microbial community structures indicated the difference between the two WWTPs. The richness and evenness of WWTP community in LS were less than that in NJ. The significant variables were more correlated with the variances of bacterial communities at lower elevations than those at higher elevations. The analysis provided a better understanding in the correlation among sludge properties, treatment performance and microbial community structures in sewage treatment at high altitudes.

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Reference

[1] M. Juanico, H. Weinberg, N. Soto, Process design of waste stabilization ponds at high altitude in Bolivia, Water Sci. Technol., (2000) 307–313.
[2] R. Govoreanu, H. Saveyn, P. vander, Simultaneous determination of activated sludge floc size distribution by different techniques, Water Sci. Technol., 50 (2003) 39–46.
[3] E.T. Yoong, P.A. Lant, P.F. Greenfield, In situ respirometry in an SBR treating wastewater with high phenol concentrations, Water Res., 34 (1999) 239–245.
[4] A. Uygur, F. Kargi, Phenol inhibition of biological nutrient removal in a four-step sequencing batch reactor, Process Biochem., 39 (2004) 2123–2128.
[5] Y.-M. Chen, T.-F. Lin, C. Huang, J.-C. Lin, Cometabolic degradation kinetics of TCE and phenol by *Pseudomonas putida*, Chemosphere, 72 (2008) 1671–1680.
[6] E.A. Strauss, G.A. Lamberti, Regulation of nitrification in aquatic sediments by organic carbon, Limnol Oceanogr., 45 (2000) 1854–1859.
[7] Z. Yun, Y.H. Jung, B.R. Lim, The stability of nitrite nitrification with strong nitrogenous wastewater: effects of organic concentration and microbial diversity, Water Sci. Technol., 49 (2004) 5–6.
[8] R. Sharma, S.K. Gupta, Influence of chemical oxygen demand/total Kjeldahl nitrogen ratio and sludge age on nitrification of nitrogenous wastewater, Water Environ. Res., 76 (2004) 155–161.
[9] J.-H. Luo, T.-W. Hao, L. Wei, Impact of influent COD/N ratio on disintegration of aerobic granular sludge, Water Res., 62 (2014) 127–135.
[10] A. Briones, L. Raskin, Diversity and dynamics of microbial communities in engineered environments and their implications for process stability, Curr Opin. Biotech., 14 (2003) 270–276.
[11] B.E. Rittmann, M. Hausner, F. Loffler, A vista for microbial ecology and environmental biotechnology, Environ Sci. Technol., 40 (2006) 1096–1103.
[12] G.F. Wells, H.D. Park, B. Eggleston, C.A. Francis, C.S. Criddle, Fine-scale bacterial community dynamics and the taxa-time relationship within a full-scale activated sludge bioreactor, Water Res., 45 (2011) 5476–5488.
[13] K. Hashimoto, M. Matsuda, D. Inoue, M. Ike, Bacterial community dynamics in a full-scale municipal wastewater treatment plant employing conventional activated sludge process, J. Biosci. Bioeng., 118 (2014) 64–71.
[14] R.A. Katherine, J.Y. Carl, K. Angela, Habitat degradation impacts black howler monkey (Alouatta pigra) gastrointestinal microbiomes, ISME J., (2013) 1344–1353.
[15] C. Lozupone, M.E. Lladser, D. Knights, J. Stombaugh, R. Knight, UniFrac: an effective distance metric for microbial community comparison, ISME J., 5 (2011) 169.

[16] T. Zhang, M.-F. Shao, L. Ye, 454 pyro sequencing reveals bacterial diversity of activated sludge from 14 sewage treatment plants, ISME J., 6 (2012) 1137–1147.
[17] J. Cardinali-Rezende, J.C. Araujo, P.G. Almeida, C.A. Chernicharo, J.L. Sanz, E. Chartone-Souza, A.M. Nascimento, Organic loading rate and food-to-microorganism ratio shape prokaryotic diversity in a demo-scale up-flow anaerobic sludge blanket reactor treating domestic wastewater, Anton. Leeuw. INT. J. G., 104 (2013) 993–1003.
[18] B. Li, G.-X. Wu, Effects of sludge retention times on nutrient removal and nitrous oxide emission in biological nutrient removal processes, Inter. J. Env. Res. Pub. Heal., 11 (2014) 3553–3569.
[19] H.-Y. Hu, W.-Y. Zhao, Q.-Y. Wu, Industrial wastewater pollution control approach and technology research and development needs, Environ Sci. Res., 23 (2010) 861–868.
[20] X.-C. Zheng, Y.-X. Li, Phosphorus and Nitrogen Removal from Sewage, China Construction Industry Press, Beijing, BJ, 1998, p. 15.
[21] H.D. Ryu, S.I. Lee, Comparison of 4-stage biological aerated filter (BAF) with MLE process in nitrogen removal from low carbon-to-nitrogen wastewater, Environ. Eng. Sci., 26 (2009) 163–170.
[22] J.J. Her, J.-S. Huang, Influences of carbon source and C/N ratio on nitrate/nitrite denitrification and carbon breakthrough, Bioresour. Technol., 54 (1995) 45–51.
[23] H.-B. Liu, C.-Z. Yang, W.-H. Pu, Removal of nitrogen from wastewater for reusing to boiler feed-water by an anaerobic/aerobic/membrane bioreactor, Chem. Eng. J., 140 (2008) 122–129.
[24] P.-S. Sheng, P.N. Carles, M. Brian, Effective biological nitrogen removal treatment processes for domestic wastewaters with low C/N ratios: A review, Environ. Eng. Sci., 27 (2010) 111–126.
[25] S. Xia, L. Duan, Y. Song, J. Li, Y.M. Piceno, G.L. Andersen, L. Alvarez-Cohen, I. Moreno-Andrade, C.L. Huang, S.W. Hermanowicz, Bacterial community structure in geographically distributed biological wastewater treatment reactors, Environ. Sci. Technol., 44 (2010) 7391–7396.
[26] D.R. Johnson, T.K. Lee, J. Park, K. Fenner, D.E. Helbling, The functional and taxonomic richness of wastewater treatment plant microbial communities are associated with each other and with ambient nitrogen and carbon availability, Environ. Microbiol., 17 (2015) 4851–4860.
[27] L. Wittebolle, M. Marzorati, L. Clement, A. Balloi, D. Daffonchio, K. Heylen, P. De Vos, W. Verstraete, N. Boon, Initial community evenness favours functionality under selective stress, Nature, 458 (2009) 623–626.
[28] J.J. Werner, D. Knights, M.L. Garcia, NB. Scalfone, S. Smith, K. Yarasheski, T.A. Cummings, A.R. Beers, R. Knight, L.T. Angenent, Bacterial community structures are unique and resilient in full-scale bioenergy systems, Proc. Natl. Acad. Sci., U.S.A. 108 (2011) 4158–4163.
[29] L.-H. Niu, Y. Li, P.-F. Wang, Understanding the linkage between elevation and activated sludge bacterial community along a 3600 m elevational gradient in China, Appl. Environ. Microbiol., 7 (2015) 1–28.
[30] J. Zabalaga, G. Amy, M.E. Von, Evaluation of agricultural reuse practices and relevant guidelines for the Alba Rancho WWTP (primary and secondary facultative ponds) in Cochabamba, Bolivia, Water Sci. Technol., 55 (2007) 469–475.
[31] E.M. Symonds, M.E. Verbyla, J.O. Lukasik, R.C. Kafle, M. Breitbart, J.R. Mihelcic, A case study of enteric virus removal and insights into the associated risk of water reuse for two wastewater treatment pond systems in Bolivia, Water Res., 65 (2014) 257–270.