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Activated sludge culture domestication at high altitudes in Tibet of China

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ABSTRACT

The domestication activated sludge of cyclic activated sludge system reactor in Nyingtri of Tibet is the research object in this paper. The changes of pollutants, microbial community structure, and the species diversity during the activated sludge acclimation were investigated. It was found that the removal rate of chemical oxygen demand and total phosphorus increased gradually and became basically stable at the 45th day of the activated sludge domestication stage. The main microbial community structure tended to be stable after the 45th day of the domestication stage. The main fungal and archaeal community structure tended to be stable after the 55th day of the domestication stage. The relationship among the changes of pollutants, microbial community structure, and species diversity was further researched. The treatment performance could be explained by the relative operational unit (OUT) richness and evenness of microbial communities of activated sludge. The treatment performance gradually became basically stable at the 45th day because the relative OUT richness and evenness climbed gradually and peaked at the 45th day. The research results will provide certain theoretical foundation and scientific guidance for the cultivation and domestication of activated sludge on plateaus.

Keywords: Plateau; Sludge domestication; Microbial community structure; Diversity; Stability

1. Introduction

Sewage treatment plants are of great significance to improve environmental quality, consolidate pollution control and ecological protection, and ensure sustainable economic and social development in high-altitude regions [1]. However, the operation of wastewater treatment plants (WWTPs) at high altitudes is faced with several unusual obstacles due to the extreme climatic conditions of these regions [2]. The stable operation of WWTPs by widely used sewage treatment technology in plain area is very important. With the rapid social and economic development at high altitudes, the number of sewage treatment plants has been increasing gradually. By transforming organic pollutants into inorganic ones, the activated sludge cultivation and domestication process has contributed a lot to the improvement of the aquatic environment worldwide and is still the most widely used process for the treatment of municipal wastewater because of its low operation cost and high performance [3]. Plateau regions are no exception. There are two main WWTPs in Tibet named Lhasa and Chamdo, using activated sludge process, respectively, for cyclic activated sludge system (CASS) and anaerobic-anoxic-oxic.

Activated sludge, the core of a biological wastewater treatment system, generally contains many types of microorganisms, such as bacteria and fungi, while bacteria hold approximately 95% of the total microbial population [4] and play a key role in the purification of water. The domestication of activated sludge is the key link of activated sludge

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system. The micro-ecosystem of activated sludge suitable for different water quality and operating conditions was constructed through the selection of the conditions of domestication [5,6]. To the best of our knowledge, there is close relationship between activated sludge microfauna and stable operation of WWTPs. Activated sludge microfauna can be used as indicators of activated sludge wastewater treatment performance, so as to reflect the running state of the activated sludge system [7–12].

A better understanding of the microbial community structure of activated sludge in WWTPs can help elucidate the mechanisms of biological pollutant removal and improve the treatment performance and operational stability [13,14]. In a pilot-scale sequencing batch reactor activated sludge process, aerobic granular sludge was cultivated using soy protein wastewater as the external carbon source to alter the influent chemical oxygen demand (COD)/N ratio [15]. Ye and Zhang [16] demonstrated that 454 pyrosequencing was a powerful approach for identifying bacterial communities in activated sludge treatment plants. Winkler [17] showed that despite differences, general bacterial population of both systems has comparable average species richness, entropy, and evenness, suggesting that different bacteria share the same functionality. Ibarbalz [18] indicated that each industrial activated sludge system has its own unique bacterial community which was significantly different from the municipal plant. 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.

Many researches have been carried out in low-elevation WWTPs. More information on the activated sludge culture domestication is needed to better understand the stable operation of WWTPs at high altitude. In that case, some critical questions important to the rational design and advancement of wastewater treatment systems need to be further answered: How to change the relative operational unit (OUT) richness and the evenness of microbial communities (bacterial, fungal, archaeal) of the activated sludge under the different stages of cultivation and domestication at high altitudes? How to shift microbial community structures (bacterial, fungal, archaeal) of the activated sludge under the different stages of cultivation and domestication at high altitudes? However, the relationship among the changes of pollutants, microbial community structure, and species diversity under the different stages of cultivation and domestication in plateaus are still unknown.

The activated sludge cultivation and domestication of CASS reactor in Tibet Nyingtri is the research object in this paper. The objective of this paper is to investigate the changes of pollutants, microbial community structure, and species diversity during the activated sludge acclimation. The performance of activated sludge wastewater treatment is further researched based on three aspects, the changes of pollutants, microbial community structure, and species diversity under the different stages of cultivation and domestication at high altitudes. These results are expected to provide practical guidance to the activated sludge culture domestication and the operation of WWTPs in treating domestic wastewater at high altitudes.

2. Materials and methods

2.1. Reactor and operation

The CASS process unit was placed in the environmental science and engineering laboratory of the Tibet Agricultural and Animal Husbandry College in Nyingtri of Tibet. The CASS process unit is shown in Fig. 1. The main body of the reactor is made of organic glass. The reactor volume was 0.045 m^3. The activated sludge of Lhasa sewage treatment plant was used for the use of sludge. Initial sludge concentration was 2.5 g/L. The reactors ran continuously. The influent sewage and the aeration system of device were adjusted by the creep pump. The influent sewage pH was maintained between 7.2 and 8.4. The stable dissolved oxygen (DO) in water (5.0 ± 0.5 mg/L) was maintained during the test stages. The influent sewage was made by artificial water. The composition of synthetic sewage is shown in Table 1. The domestication activated sludge took about 65 d.

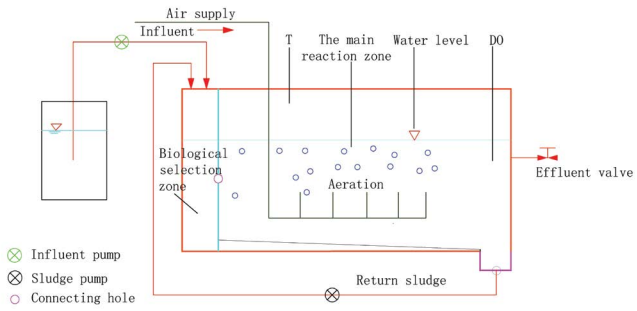

Fig. 1. The CASS process unit.

Table 1
The composition of synthetic sewage

Species	Composition	Concentration (mg/L)	Confect
Organics	COD	200	Glucose
Nitrogen compounds	NH_4-N^+	15 (the initial 30 d), 30 (after 35 d)	NH_4CI
Phosphorus compounds	TP	5	K_2HPO_4
Other additions and trace elements (mg/L)	$MgSO_4·7H_2O$ (12); $FeSO_4·7H_2O$ (10); $CaCl_2$ (30); $NaHCO_3$ (50); pH (7.2–8.4); H_3BO_3 (0.15); $CoCl_2·6H_2O$ (0.15); $CuSO_4·6H_2O$ (0.03); $FeCl_3·6H_2O$ (1.5); KI (0.03); $MnCl_2·2H_2O$ (0.12); $(NH_4)_2Mo_7O_{24}·2H_2O$ (0.06); $ZnSO_4·7H_2O$ (0.12)		

The CASS reactor ran for four cycles per day, with each cycle for 6 h, divided into two stages. The water aeration phase of reactor took 4 h. The precipitation drainage stage after the aeration completion of natural precipitation took 2 h. And then, the reactor went above the clear liquid through the outlet and ran back to the next cycle.

2.2. Sample collection and effluent quality analysis

The effluent quality parameters were measured per 2 d during the activated sludge acclimation. The effluent quality parameters such as COD, NH_4-N^+, NO_2^--N, NO_3^--N, and total phosphorus (TP) were measured according to Chinese standard methods. The water temperature and DO were measured by the portable DO meter (JPBJ-608). PH was measured by digital display (PHS-25).

The reactor began to be sampled every 10 d since the 5th day begins for microbiological analysis. The 10 mL mixed liquor suspended solids (MLSS) reaction mixture was extracted from the reactor. The 10 mL MLSS reaction mixture was kept in a dry ice box immediately after collection for stored and transported at −80°C in a laboratory for DNA extraction. After melting at room temperature, the sample was centrifuged at 14,000 g for 8 min. Then the supernatant was decanted, and 5 g of the pellet was weighted out, for use in the next step of DNA extraction.

2.3. DNA extraction and polymerase chain reaction amplification

The microbial DNA was extracted from activated sludge samples using the E.Z.N.A.® Soil DNA Kit (Omega Biotek, Norcross, GA, USA) according to the manufacturer's protocols. The V4–V5 regions of the bacterial 16S ribosomal ribonucleic acid (rRNA) gene were amplified by polymerase chain reaction (PCR) (at 95°C for 5 min, followed by 27 cycles at 95°C for 30 s, 55°C for 30 s, and 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 archaeal gene was amplified by PCR (95°C for 5 min, followed by 27 cycles at 95°C for 30 s, 55°C for 30 s, and 72°C for 45 s, and a final extension at 72°C for 10 min, 10°C until halted by user) using primers Arch519F 5′-CAGCCGCCGCGGTAA-3′ and Arch915R 5′-GTGCTCCCCCGCCAATTCCT-3′, where barcode is an eight-base sequence unique to each sample. The fungal gene was amplified by PCR (95°C for 5 min, followed by 27 cycles at 95°C for 30 s, 55°C for 30 s, and 72°C for 45 s, and a final extension at 72°C for 10 min, 10°C until halted by user) using primers ITS1F 5′-CTTGGTCATTTAGAGGAAGTAA-3′ and ITS2R 5′-GCTGCGTTCTTCATCGATGC-3′, where barcode is an eight-base sequence unique to each sample. The PCR reactions were performed in triplicate; 20 μL of mixture containing 4 μL of 5× FastPfu Buffer, 2 μL of 2.5 mM deoxyribonucleoside triphosphates, 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 and purified by using the AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, USA) according to the manufacturer's instructions and quantified by using QuantiFluor™-ST (Promega, USA).

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 an 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, Shanghai) according to the standard protocol.

2.5. Processing the sequencing data

Raw FASTQ files were demultiplexed and filtered based on the quality by using QIIME (version 1.17) with the following criteria: (1) The 250 bp reads should be truncated at any site, and an average quality score <20 over a 10 bp sliding window got by discarding the truncated reads which were shorter than 50 bp. (2) Exact barcode matching: 2 nucleotide mismatches in primer matching and reads containing ambiguous characters should be removed. (3) 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.

OUTs were clustered with 97% similarity cutoff by using UPARSE (Version 7.1 http://drive5.com/uparse/), and the chimeric sequences were identified and removed by using UCHIME. The phylogenetic affiliation of each 16S rRNA gene sequence was analyzed by ribosomal database project Classifier (http://rdp.cme.msu.edu/) against the silva 16S rRNA database by using 70% of confidence threshold [19].

2.6. Data processing and analysis

The rarefaction analysis based on Mothur v.1.21.1 [20] was conducted to reveal the diversity indices, including the Chao, ACE, and Shannon diversity indices. The similarity analysis of the OUT compositions of samples was performed by clustering analysis based on the Bray-Curtis similarity by PAST 3.0 [21]. Use AutoCAD2012, Origin8.0, and R language statistical analysis software to complete the chart production.

3. Results and discussion

3.1. Process performance

The removal result of carbon, nitrogen, and phosphorus is shown in Fig. 2. It shows that the COD and TP water concentration gradually decreased through the whole initial stage of cultivation and domestication. The influent NH_4-N^+ increased from 15 to 30 mg/L in the former 31 d; then it decreased gradually and became stable at the 55th day. The effluent NO_2^--N concentration increased gradually over the initial period, reaching to a relatively stable value around the 22th day. The average removal rate of COD and TP was 81.84% and 74.22%, respectively, during the period from 32th to 64th day. The removal rate of COD and TP increased gradually and became basically stable at the 45th day.

Simultaneous nitrification and denitrification may utilize the nitrite. The stable accumulation of nitrite was found in the reactor. The low DO and a certain level of free ammonia (FA) concentrations were believed to be two major factors

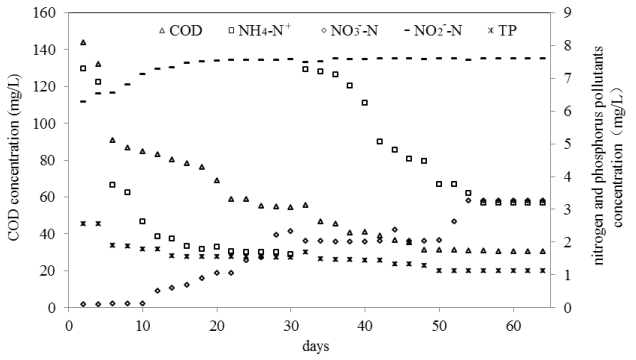

Fig. 2. The effluent parameter COD, NH_4-N^+, NO_3^--N, NO_2^--N, and TP values.

that contribute to nitrous acid accumulation. Some studies believed that the concentration of DO for nitrite accumulation should be lower than 0.5 mg/L [22]. Peng [23] reported that the activity of nitric acid oxidative bacteria will decrease by 50% when the FA concentration reaches to 0.7 mg/L. Chung [24] found that the concentration of FA required for the nitric accumulation in domesticated sludge is 5–10 mg/L. Cao [25] investigated the effect of pH on nitrite accumulation during wastewater denitrification. The results showed that the pH during denitrification can be kept constant using buffer solutions, and nitrite accumulation is faster at low pH than at high pH, regardless of whether the pH of the mixed liquor is stable during denitrification. In the operating cycle of this system, the DO concentration was basically higher than 4 mg/L. The influent sewage pH was maintained between 7.2 and 8.4. Therefore, the high concentration of FA in this test may be the main reason for the accumulation of nitrite.

3.2. The richness and evenness of microbial communities of activated sludge

The relative OUT richness and evenness of bacterial communities of activated sludge are shown in Fig. 3. It can be seen that the relative OUT richness and evenness topped at the 45th day of the initial stage.

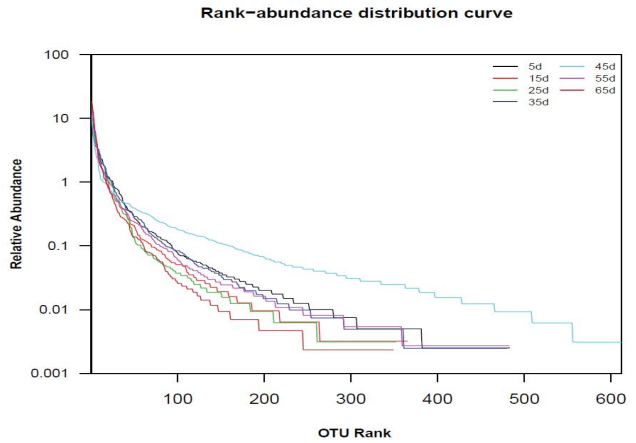

Fig. 3. Relative abundance of the OUT richness and evenness of bacterial communities of activated sludge.

The relative OUT richness and evenness of fungal communities of activated sludge are shown in Fig. 4. As it shows, the relative OUT richness and evenness of fungal communities of activated sludge peaked at the 45th day of the initial stage.

The relative OUT richness and evenness of archaeal communities of activated sludge are shown in Fig. 5. On the 5th day of the initial stage, the relative OUT richness and evenness of archaeal communities of activated sludge had their minimum values. After that they climbed gradually and peaked at the 45th day.

3.3. Microbial community structures

Fig. 6 summarizes the relative abundances of the bacterial community on the phylum level of activated sludge. It shows that *Bacteroidetes* represented the most abundant phylum of the bacterial community. *Proteobacteria* was the second most abundant phylum, followed by *Firmicutes*, *Saccharibacteria*, *Chloroflexi*, *Actinobacteria*, and *Acidobacteria* during the 5th day to the 15th day. The main bacterial community structure showed no obvious change during the 5th day to the 15th day. Then it changed most significantly from the 15th

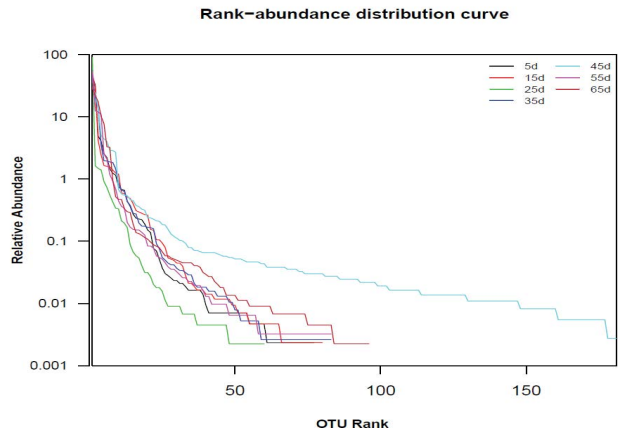

Fig. 4. Relative abundance of the OUT richness and evenness of fungal communities of activated sludge.

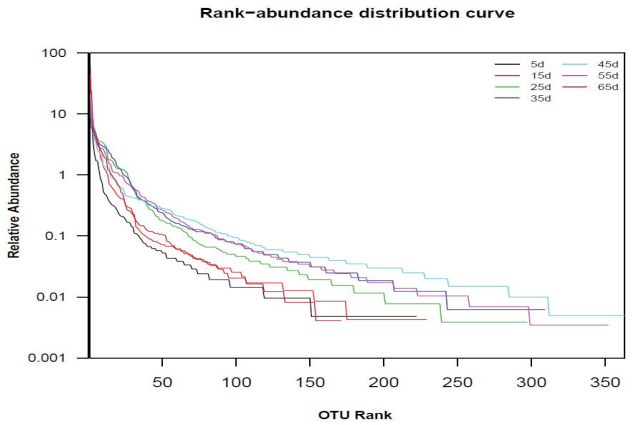

Fig. 5. Relative abundance of the OUT richness and evenness of archaeal communities of activated sludge.

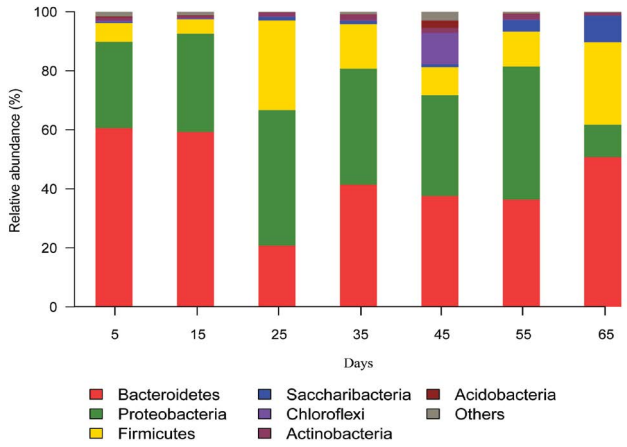

Fig. 6. Relative abundance of bacterial phyla of activated sludge.

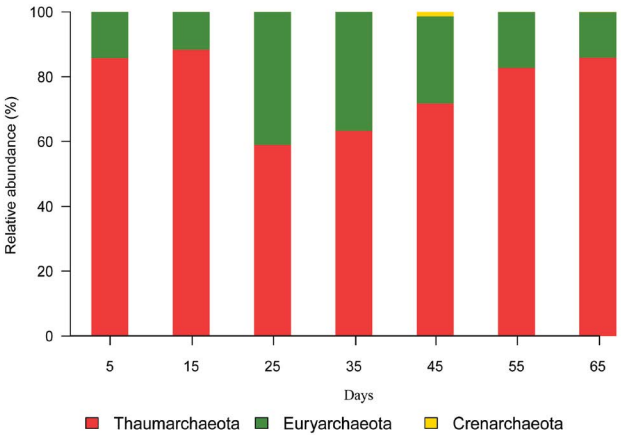

Fig. 8. Relative abundance of archaeal phyla of activated sludge.

day to the 25th day, followed by a stable state after the 45th day of the initial stage. *Bacteroidetes* decreased significantly in the domestication stage. *Proteobacteria* represented the most abundant phylum of the bacterial community.

Fig. 7 summarizes the relative abundances of the fungal community on the phylum level of activated sludge. It shows that *Ascomycota* represented the most abundant phylum of the fungal community. *Basidiomycota* was the second most abundant phylum, followed by *Fungi unclassified*. The main structure of fungal community experienced slight change from the 5th day to the 15th day, and then it went through most significant change from the 25th day to the 45th day and tended to be stable after the 55th day of the initial stage.

Fig. 8 summarizes the relative abundances of the archaeal community on the phylum level of activated sludge. It shows that *Thaumarchaeota* represented the most abundant phylum of the archaeal community. *Euryarchaeita* was the second most abundant phylum, followed by *Crenarchaeota*. The main structure of archaeal community had no obvious change from the 5th day to the 15th day, and then it changed most significantly from the 25th day to the 45th day and tended to be stable after the 55th day of the initial stage.

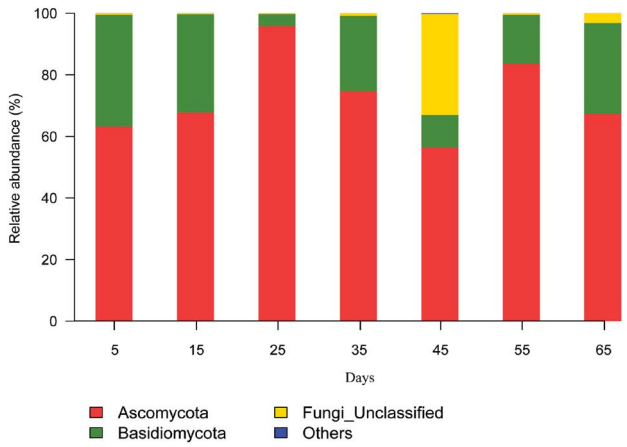

Fig. 7. Relative abundance of fungal phyla of activated sludge.

3.4. The relationship among the changes of pollutants, microbial community structure, and species diversity

The treatment performance could be explained by the relative OUT richness and evenness of microbial communities of activated sludge. 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. As shown in Figs. (3)–(5), on the 45th day of the initial stage, the relative abundance species evenness of microbial communities reached their peak values. As shown in Fig. 6, the main bacterial community structure followed a stable state after the 45th day of the initial stage. The removal rate of COD and TP increased gradually and became basically stable at the 45th day of the activated sludge domestication stage.

As shown in Fig. 8, the main structure of archaeal community changed most significantly from the 25th day to the 45th day and tended to be stable after the 55th day of the initial stage. The influent NH_4-N^+ increased from 15 to 30 mg/L in the former 31 d; then the effluent NH_4-N^+ decreased gradually and became stable at the 55th day. In conclusion, the richness and evenness of community could be important to determine the general performance of WWTPs. The treatment performance gradually became basically stable at the 45th day because the relative OUT richness and evenness climbed gradually and peaked at the 45th day. The cultivation of sludge was a sequencing process, which was due to the self-modulation of the microorganisms to the environmental changes in different periods of time, forming the community structure that was best for the CASS process in plateau areas.

4. Conclusions

This study explored the changes of pollutants during the cultivation and demonstration of activated sludge using CASS sludge reactor in plateaus. Microbial community

structure and its diversity were also analyzed. On the 5th day of the initial stage of activated sludge cultivation, it was found that the relative abundance and the species evenness of archaeal communities had minimum values. On the 45th day of the initial stage, the relative abundance and the species evenness of microbial communities reached their peak values. The main microbial community structure was not obviously changed during the 5th day to the 15th day; during the 15th day to the 25th day, the main bacterial community structure changed most significantly and tended to be stable after the 45th day of the initial stage. During 25th day to the 45th day, the main fungal and archaeal community structure changed most significantly and tended to be stable after the 55th day of the start-up phase. The treatment performance gradually became basically stable at the 45th day because the relative OUT richness and evenness climbed gradually and peaked at the 45th day of the activated sludge domestication stage. It is proved that the activated sludge cultivated and domesticated for 45 d using the seeding method can fully cultivate and domesticate the sludge in plateau areas. The analysis provides a better understanding in the activated sludge cultivation and domestication in plateau areas.

Acknowledgement

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