Efficient municipal wastewater treatment by oxidation ditch process at low temperature: Bacterial community structure in activated sludge

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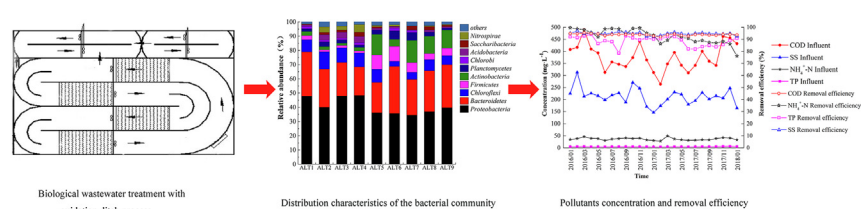[a] College of Resources and Environmental Science, Xinjiang University, Urumqi, Xinjiang, China
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HIGHLIGHTS

- Efficient low temperature pollutant removal and good sludge settleability achieved.
- The relative abundance of nitrifying bacteria was higher at lower temperatures.
- Ultra-low sludge load with high DO, effectively ensures good WWTP operation.

GRAPHICAL ABSTRACT

Biological wastewater treatment with oxidation ditch process

Distribution characteristics of the bacterial community

Pollutants concentration and removal efficiency

ARTICLE INFO

ABSTRACT

Temperature is a key element affecting the activity of microorganisms in activated sludge. Low water temperature generally leads to decreasing wastewater treatment efficiency and destroying sludge settleability. In this study, activated sludge samples from a municipal wastewater treatment plant (WWTP) implementing oxidation ditch process was used to investigate the bacterial community characteristics of a system that operates well in a cold region (Xinjiang, China) by high-throughput 16S rRNA gene sequencing. The results showed that the influent temperature was 7–12 °C in winter and 13–17 °C in summer, while the sludge volume index (SVI) of samples was between 51 and 74 mL/g. The average removal efficiencies for chemical oxygen demand (COD), biochemical oxygen demand (BOD$_5$), suspended solid (SS), ammonia nitrogen (NH$_4^+$-N), total nitrogen (TN), and total phosphorus (TP) were 94%, 95%, 95%, 91%, 73% and 89%, respectively. The bacteria were distributed in 32 phyla and 559 genera. The dominant phyla were *Proteobacteria* (28.85–48.45%), *Bacteroidetes* (20.00–31.22%), *Chloroflexi* (3.59–12.23%), *Actinobacteria* (1.58–15.54%) and *Firmicutes* (1.38–10.49%). The dominant genera were *Saprospiraceae_norank* (4.41–12.23%), *Comamonadaceae_unclassified* (3.82–8.83%), *Anaerolineaceae_norank* (1.39–9.35%), *Dokdonella* (1.13–11.26%), *Candidatus_Microthrix* (0.26–7.50%), *Flavobacterium* (0.32–8.14%), *Ferribacterium* (0.36–5.19%) and *Nitrospira* (0.084–5.37%), which were different from those found in warm-region WWTPs. Contrary to previous studies, the relative abundance of ammonia-oxidizing bacteria (AOB; *Nitrosomonas* and *Nitrosomonadaceae*) and nitrite-oxidizing bacteria (NOB; *Nitrospira*) increased when the temperature decreased. The successful operation of this WWTP suggests that cold-region WWTPs can achieve good pollutants removal efficiency by simultaneously maintaining an ultra-low sludge load and high dissolved oxygen concentration in the oxidation ditch. The findings of this study provide fundamental knowledge required for an efficient and stable operation of WWTPs in cold regions.

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1. Introduction

Oxidation ditch process is a modification of conventional activated sludge process using a closed ditch-like aeration tank, which can achieve simultaneous removal of organic carbon, nitrogen and phosphorus in alternating anoxic-oxic zones within the looped channel (Terashima et al., 2016; Han et al., 2018). Because of its high removal efficiency, low operational cost and lower management requirements, the oxidation ditch process is widely used in wastewater treatment plants (WWTPs) (Zhang et al., 2016). The microbial community in oxidation ditch systems determines the removal efficiency of pollutants (Miura et al., 2007). Many studies have confirmed that wastewater temperature is one of the most crucial factors influencing microbial growth rates, reproduction and metabolism (Nedwell, 1999; Morales et al., 2016). Cold regions experience low average annual temperatures (1–15 °C), including most of Canada, the northern United States, northern Europe, Russia and northern China (Wittgren and Mæhlum, 1997). Low temperatures have several negative effects on oxidation ditch process, with microbial activity and substrate utilization rates often declining, leading to a deterioration of wastewater treatment performance (Salvetti et al., 2006; Yang et al., 2013; Zhou et al., 2018). At the same time, low water temperatures decrease activated sludge floc and sludge settleability performance, resulting in sludge bulking (Krishna and Van Loosdrecht, 1999; Duan et al., 2016).

In cold regions, most WWTPs have difficulties with sludge bulking and exhibit poor treatment effects because of extreme cold and long winters. In northern Europe, the increase of sludge volume index (SVI) in WWTPs at low temperature in winter indicates that the deterioration of sludge settleability was high (Gurung et al., 2017). In northeast China, such as Shenyang, Harbin and Mudanjiang, the removal rate of ammonia nitrogen in WWTPs is significantly reduced due to low temperatures, causing unsatisfactory treatment performance (Cui et al., 2012; Liu et al., 2019). In Xinjiang of northwest China, inefficient treatment performance and sludge bulking in winter are widespread in WWTPs (Xu et al., 2018). However, a WWTP using oxidation ditch processes located in northernmost Xinjiang, which experiences arid and cold conditions, with winter lasting for half a year and the lowest temperature is below −50 °C, showed efficient removal of pollutants and good sludge settleability performance, despite its water temperature in winter and summer being 7–12 °C and 13–17 °C, respectively, and the annual water influent temperature being lower than 12 °C for 180 days. Currently there are few reports on the bacterial community structure of activated sludge in WWTPs that can not only maintain good sludge settleability performance but also efficient removal of pollutants at low temperatures.

Studies show that the proportion of culturable microorganisms in activated sludge is less than 1% of all microorganisms. Therefore, conventional culture-independent biological molecular techniques, such as denaturing gradient gel electrophoresis (DGGE) (Hesham et al., 2011), fluorescent in situ hybridization (FISH) (Luxmy et al., 2000) and restriction fragment length polymorphism (RFLP) (Eschenhagen et al., 2003), could not effectively detect the presence of many low-abundance microorganisms in activated sludge, so they could not fully reveal the microbial structure and composition of activated sludge (Xia et al., 2010; Wang et al., 2012). Due to its high capacity, low cost and accurate quantification, high-throughput sequencing techniques has gradually become a vital and highly efficient tool that provides abundant, authentic and reliable gene sequence information to reconstruct microbial communities (Salipante et al., 2014). In terms of wastewater treatment, many studies have been conducted to explore the bacterial community of activated sludge in WWTPs (Metch et al., 2019; Awolusi et al., 2018). From these studies it can be seen that

Proteobacteria, *Bacteroidetes*, *Chloroflexi* (Qin et al., 2018), *Saprospiraceae*, *Comamonadaceae*, *Anaerolineaceae*, *Nirrospira*, *Dechloromonas*, *Candidatus_ Accumulibacter* are most commonly identified as dominant bacteria and are functionally related to hydrolysis, nitrification, denitrification and biological phosphorus removal in activated sludge of WWTPs (Chen et al., 2016).

In this study, the bacterial community structure in activated sludge from the oxidation ditch of a WWTP located in Altay city (China) under low temperature was analysed by 16S rRNA gene high-throughput sequencing. The purpose of this study was to explore the characteristics of the microbial community in cold-region and establish the specific operating conditions to maintain a good settleability performance and treatment effects in a WWTP at low temperatures. The information collected in this study will provide a reference or theoretical basis on how to control sludge bulking in WWTPs in cold regions and maintain good sludge settleability performance, as well as improving the treatment effects and operational efficiency.

2. Materials and methods

2.1. Activated sludge samples collection

Activated sludge samples (n = 9) labeled ALT1-9 were collected from the aerobic section of the oxidation ditch of a WWTP located in Altay city, Xinjiang (China), between January 2016 and January 2018. In this study, the characteristic of the flow pattern in the oxidation ditch is pushing flow in partial zones, but it's completely mixed on the whole. Samples were made in two consecutive years and collected from the same site at the end of the aerobic section of oxidation ditch. Detailed information about the samples is given in Table 1. As shown in Table 1, most of samples were collected in the temperature between 8.5 and 11.5 °C, and the SVI values of all samples were between 51 and 74 mL/g. The designed scale of the WWTP was 1.5×10^4 m³/d. The main WWTP influent is municipal sewage and the effluent implements Class 1B of GB18918-2002 (Zhou et al., 2018). The mixed liquor suspended solids (MLSS) of this WWTP is 4800 mg/L and the sludge load is 0.077 kgBOD$_5$/kgMLSS·d. The activated sludge samples were stored at −80 °C in the dark until further use.

2.2. Analytical methods for water quality and sludge monitoring

The following items of chemical oxygen demand (COD), biochemical oxygen demand (BOD$_5$), suspended solid (SS), ammonia nitrogen (NH$_4^+$-N), total nitrogen (TN), total phosphorus (TP) and MLSS were measured according to the Standard Method. Specifically, COD was measured by the Open Reflux Method. BOD$_5$ was measured by 5-Day BOD Test, which consists of filling with samples, to overflowing, an airtight bottle of the specified size and incubating it at the specified temperature for 5 days, dissolved oxygen is measured initially and after incubation, and the BOD$_5$ is computed from

Table 1
Detailed information regarding samples made in this study.

Sample	Sampling date	Temperature (°C)	SVI (mL/g)
ALT1	01-13-2016	8.7	54
ALT2	10-09-2016	13.6	51
ALT3	12-14-2016	10.4	55
ALT4	01-06-2017	8.5	64
ALT5	05-03-2017	11.5	72
ALT6	07-11-2017	17.4	74
ALT7	09-21-2017	15.2	65
ALT8	11-18-2017	11.5	57
ALT9	01-03-2018	8.6	59

the difference between initial and final dissolved oxygen. SS and MLSS were measured by gravimetric method, samples are evaporated in a weighed dish and dried to constant weight in an oven at 103–105 °C, the increase in weight over that of the empty dish represents the values. NH_4^+-N was determined by Flow Injection Analysis. TN was measured by the In-Line UV/Persulfate Digestion and Oxidation with Flow Injection Analysis. TP was measured by In-line UV/Persulfate Digestion and Flow Injection Analysis (APHA, 2012). The dissolved oxygen (DO) concentration was measured with a portable DO analyzer (AZ8403) and water temperature was measured with a mercury thermometer. SVI values were determined by reading the percentage of sludge volume in the mixture of water and sludge after 30 min settling in a 1000 mL measuring cylinder and calculated from the dry weight in MLSS.

2.3. DNA extraction and PCR amplification

Activated sludge samples were sent to Majorbio Bio-Pharm Technology Co. Ltd. (Shanghai, China) for DNA extraction and PCR amplification. The E.Z.N.A soil Kit (Omega Bio-Tek, Norcross, GA, U.S.) was used for the microbial DNA extraction in sludge samples. The Nano-Drop 2000 UV–vis spectrophotometer (Thermo Scientific, Wilmington, USA) was used for measuring the final DNA concentration and purification, while the DNA quality was checked by 1% agarose gel electrophoresis. The V4-V5 hypervariable region of the bacteria 16S rRNA gene was amplified with primers 515F (5′-GTGCCAGCMGCCGCGG- 3′) and 907R (5′-CCGTCAATTCMTT TRAGTTT-3′) on a thermocycler PCR system (GeneAmp9700, ABI, USA). The PCRs were executed in triplicate 20 μL reaction mixtures, 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 AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, USA) was used together with quantification using the QuantiFluor-ST system (Promega, USA) for the first extraction of PCR products from a 2% agarose gel and for further purification.

2.4. Illumina sequencing and data analysis

Purified amplicons were pooled in equimolar concentrations and paired-end sequenced (2 × 300) on an Illumina MiSeq platform (Illumina, San Diego, USA) following the standard protocols of Majorbio Bio-Pharm Technology Co. Ltd. (Shanghai, China). The raw reads were uploaded into the NCBI Sequence Read Archive (SRA) database, recorded under accession number SRP198327. Data analysis was conducted using the i-Sanger platform (http://www.i-sanger.com/), which is provided by Majorbio Bio-Pharm Technology Co. Ltd. (Shanghai, China). The operational taxonomic units (OTUs) were clustered at a level of 97% similarity cutoff using UPARSE (v. 7.1) with a novel 'greedy' algorithm that simultaneously performs chimera filtering and OTU clustering. The taxonomy of each 16S rRNA gene sequence was analyzed with the RDP Classifier algorithm and then the 16S rRNA gene tags were aligned with SILVA (SSU123) 16S rRNA database using a confidence threshold of 70%. The Chao, Shannon, Heip and coverage percentage microbial diversity indices, were calculated using the Mothur program (v.1.30.1). The analysis of variance (ANOVA) of the Chao and Shannon diversity indices between samples were determined by Origin (v.9.0), and p values ⩽ 0.05 were considered significant.

3. Results and discussion

3.1. Pollutants removal efficiency

The influent and effluent in this WWTP was monitored during this study, and the hydraulic retention time of the oxidation ditch was 23.5 h and the average BOD$_5$/COD ratio value was 0.36. It was reported that the BOD$_5$/COD ratio should exceed 0.20 for bio-treatability in the activated sludge process (Laing, 1991) and therefore, the biochemical properties were considered to be good. The average BOD$_5$/TP and BOD$_5$/TN ratios in the WWTP influent were 28 and 3.70, respectively. It is generally accepted that when the BOD$_5$/TP ratio is >20, there is sufficient carbon source available for biological phosphorus removal and that a BOD$_5$/TN ratio of >2.86, allows complete denitrification of nitrate to be achieved (Gajewska et al., 2015). This indicates that the carbon source availability in the WWTP met the demand for nitrogen and phosphorus removal. The removal efficiencies for COD, BOD$_5$, SS, NH_4^+-N, TN and TP are shown in Fig. 1. The concentrations of COD, BOD$_5$, NH_4^+-N and TN in the influent varied seasonally and were obviously higher in winter than that in summer. Furthermore, the concentrations of COD and BOD$_5$ increased significantly in winter, reaching 2- to 3-fold more than the influent concentration in summer due to the decrease in water consumption in winter. However, the treatment effect was not affected and the average removal efficiencies of COD, BOD$_5$, NH_4^+-N and TN reached 94%, 95%, 91% and 73%, respectively. The concentrations of SS and TP in the influent were relatively stable and the average removal efficiencies were 95% and 89%, respectively. In winter the temperature was low, the concentrations of NH_4^+-N and TN increased slightly, the concentration of TP maintained relatively stable, but the concentration of COD increased in this WWTP. It is well known that microorganisms require 7–9 and 5–7 g COD to remove 1 g of nitrogen and phosphorus, respectively (Kujawa and Klapwijk, 1999; Henze et al., 2008). Thus, in winter the COD concentration in this WWTP could meet the demand of nitrogen and phosphorus removal. In addition, although low temperatures have negative effects on the microbial activity in activated sludge, it has been reported that ordinary heterotrophs can be more affected by low temperature compared with nitrifying bacteria and most of the phosphate accumulating organisms (Xie et al., 2013; Panswad et al., 2003). Therefore, more COD was utilized by bacteria related to nitrogen and phosphorus removal to achieve efficient nitrogen and phosphorus removal at low temperatures in this WWTP. Hence, the WWTP effluent met the emission standard limits.

3.2. Diversity of bacterial communities in activated sludge

Alpha diversity analysis reflects the species richness and diversity of the bacterial communities in the activated sludge. Illumina sequencing of all sludge samples resulted in 30,263–56,598 effective bacterial sequences, which were grouped into 804–1046 OTUs (Table 2). The coverage percentage of all samples was greater than 99%, showing that most bacterial communities in the sludge samples were detected and that sequencing could represent the real microbial community. The evenness of the microbial community in activated sludge was measured by the Heip index. It can be seen that Heip index was lower in the ALT1, ALT4 and ALT9 samples, which had temperatures below 10 °C, indicating that low temperature had a certain influence on the evenness of the bacterial communities. The temperature decrease during the winter may have led to the death of some bacteria, while some bacteria adapted to low temperature survived, explaining the deterioration of the evenness of the bacteria in the activated sludge. The Chao and Shannon diversity indices, which are used to estimate the amount of total species and microbial diversity, represent the species richness and the diversity of community, respectively (Zhang et al., 2017). ANOVA showed that there was no significant difference between the Chao and Shannon indices among these nine samples (p values > 0.05), which showed that the diversity and richness of the bacterial communities from this WWTP had little variations and that the community structure of the activated sludge was relatively stable.
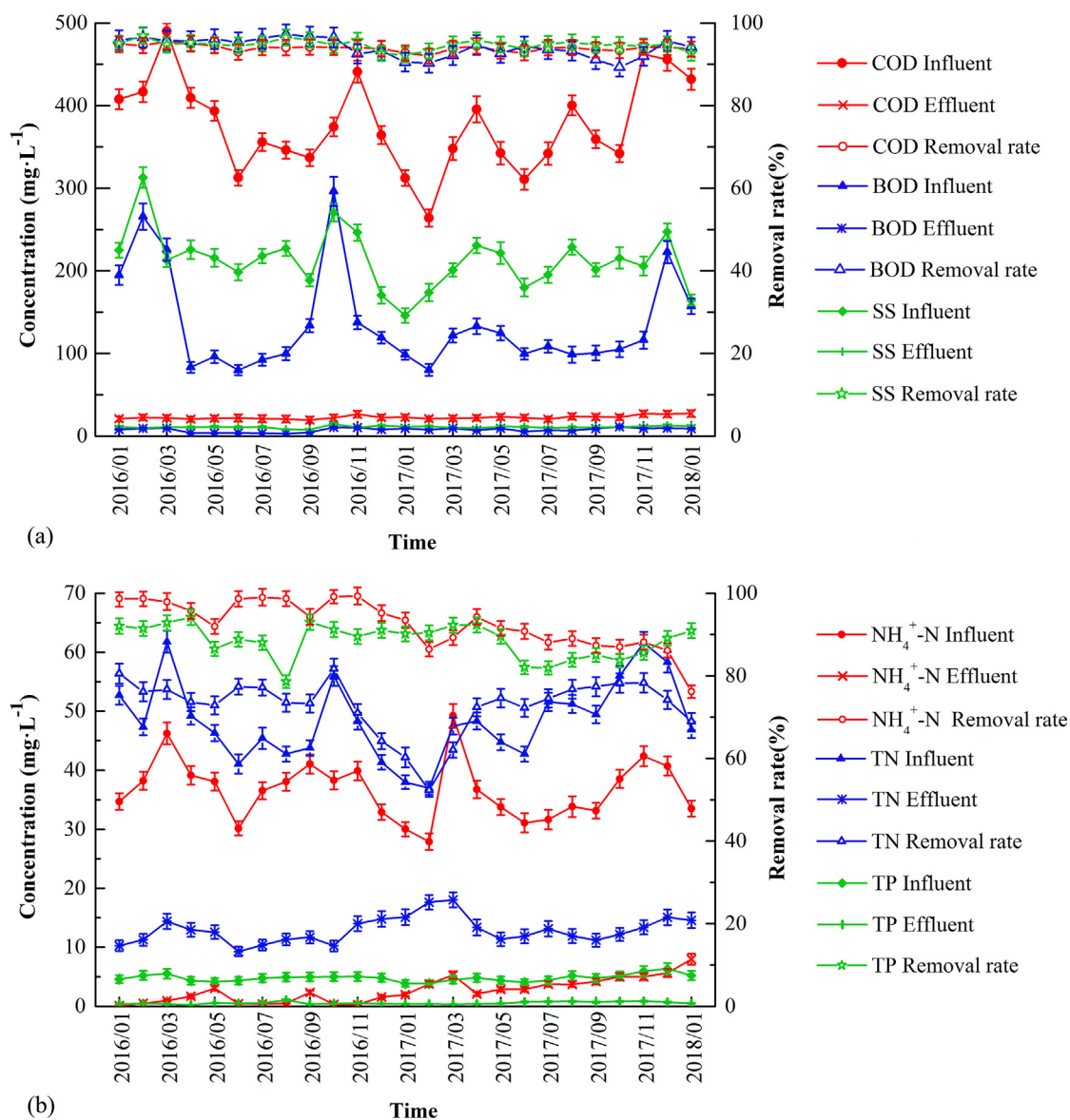
Fig. 1. Removal of COD, BOD$_5$ and SS (a); and NH$_4^+$-N, TN and TP (b) in the WWTP located in Altay, China, from January 2016 to January 2018. Error bars represent standard deviations of triplicate measurements.

Table 2
Richness and Diversity indices of the bacterial communities in activated sludge samples.

Sample	Reads	OTU	Chao	Shannon	Heip	Coverage
ALT1	56,598	969	1103	4.86	0.13	0.996
ALT2	31,717	945	1126	5.20	0.19	0.991
ALT3	32,390	943	1168	4.93	0.15	0.993
ALT4	38,394	804	1040	4.57	0.12	0.991
ALT5	30,263	999	1130	5.23	0.19	0.993
ALT6	34,838	902	1109	5.21	0.20	0.990
ALT7	40,817	983	1160	5.36	0.21	0.993
ALT8	43,747	970	1103	5.18	0.18	0.993
ALT9	45,596	1046	1207	5.28	0.14	0.992

3.3. Distribution characteristics of the bacterial communities

Analysis was performed at the phylum and genus levels to better understand the distribution of bacterial communities in the WWTP. In total, 32 phyla were detected in these activated sludge samples, with 22 phyla exhibiting a relative abundance greater than 0.1% in at least one sludge sample. The distribution of the top 10 phyla is shown in Fig. 2. Among all samples, *Proteobacteria* (28.85–48.45%) and *Bacteroidetes* (20.00–31.22%) were the most dominant bacteria (Fig. 2), overall accounting for 57.45–78.87% of the bacterial community. *Proteobacteria* play important roles in nitrogen and phosphorus removal and in the degradation of various pollutants (Cydzik-Kwiatkowska and Zielinska, 2016). While dealing with complex macromolecular organisms, *Bacteroidetes*
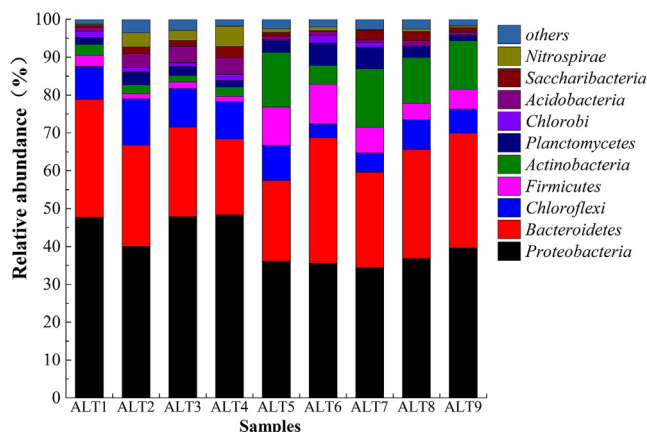
Fig. 2. Bacterial community structure (% relative abundance) at the phylum level of sludge samples. ALT1-9 represent the 9 activated sludge samples from the WWTP located in Altay, China.

has a strong metabolic capacity to decompose proteins, lipids and other macromolecules into simple compounds (Larsen et al., 2008). Other dominant bacteria in the sludge samples included *Chloroflexi* (3.59–12.23%), *Actinobacteria* (1.58–15.54%) and *Firmicutes* (1.38–10.49%). Previous studies have shown that *Chloroflexi* bacteria exist in the form of floc skeletons in sludge to promote sludge flocculation (Kragelund et al., 2007), *Actinobacteria* bacteria play a role in enhanced biological phosphorus removal systems (Seviour et al., 2008) and *Firmicutes* bacteria can consume nitrite resources and take part in denitrification (Liu et al., 2008). These results are similar to a previous study on cold-region WWTPs in Harbin (China), which showed that the dominant bacterial phyla of the activated sludge were *Proteobacteria*, *Bacteroidetes* and *Chloroflexi* (Wen et al., 2015; Cui et al., 2012). The relative abundance of the phylum *Nitrospirae* was 5.37% in ALT4, which had the lowest water

temperature (8.5 °C), while their abundance was only 0.96% in ALT6, which had the highest water temperature (17.1 °C) in the present study. Interestingly, the abundance of nitrifying bacteria increased with decreasing water temperature, which is contradictory to previous studies on *Nitrospirae* which generally suggest that low temperatures might inhibit the activity of nitrifying bacteria (Ducey et al., 2010; Zhou et al., 2018). Siripong and Rittmann (2007) have shown that bacterial genera belonging to the phylum *Nitrospirae*, such as *Nitrospira*, may be more diverse previously described and that low temperatures may enhance their diversity, resulting in an increased relative abundance of *Nitrospirae*.

At the genus level, a total of 559 bacterial genera were present in all sludge samples. Overall, 226 bacterial genera exhibited a relative abundance greater than 0.1% in at least one sludge sample and accounted for 95.23–97.83% of the overall bacterial community, which demonstrated that most bacterial genera in activated sludge accounted for an extremely low relative abundance at this WWTP. The top 50 most abundant bacterial genera are shown in Fig. 3. The dominant genera of the activated sludge from this WWTP were *Saprospiraceae_norank* (4.41–12.23%), *Comamonadaceae_unclassified* (3.82–8.83%), *Anaerolineaceae_norank* (1.39–9.35%) and *Dokdonella* (1.13–11.26%). Previous studies have confirmed that during wastewater treatment in activated sludge systems, *Saprospiraceae* can contribute to hydrolysis of certain proteins in wastewater (Xia et al., 2008); *Anaerolineaceae* is related to phosphorus removal (Yamada and Sekiguchi, 2009) and *Dokdonella* is involved in biological nitrogen removal (Han et al., 2016). A previous study on the treatment of groundwater contaminated by polycyclic aromatic hydrocarbons showed that *Comamonadaceae* was typical aromatic hydrocarbon decomposing bacteria typical (Hoeckenreiner et al., 2015). It was also reported by Das and Kazy (2014) that *Comamonadaceae* related to hydrocarbonoclastic was the dominant bacteria in the bioremediation of hydrocarbon contaminated oily sludge. In this study, *Comamonadaceae* was dominant and played a role in the degradation of aromatic compounds and denitrification (Xu et al., 2017). Other
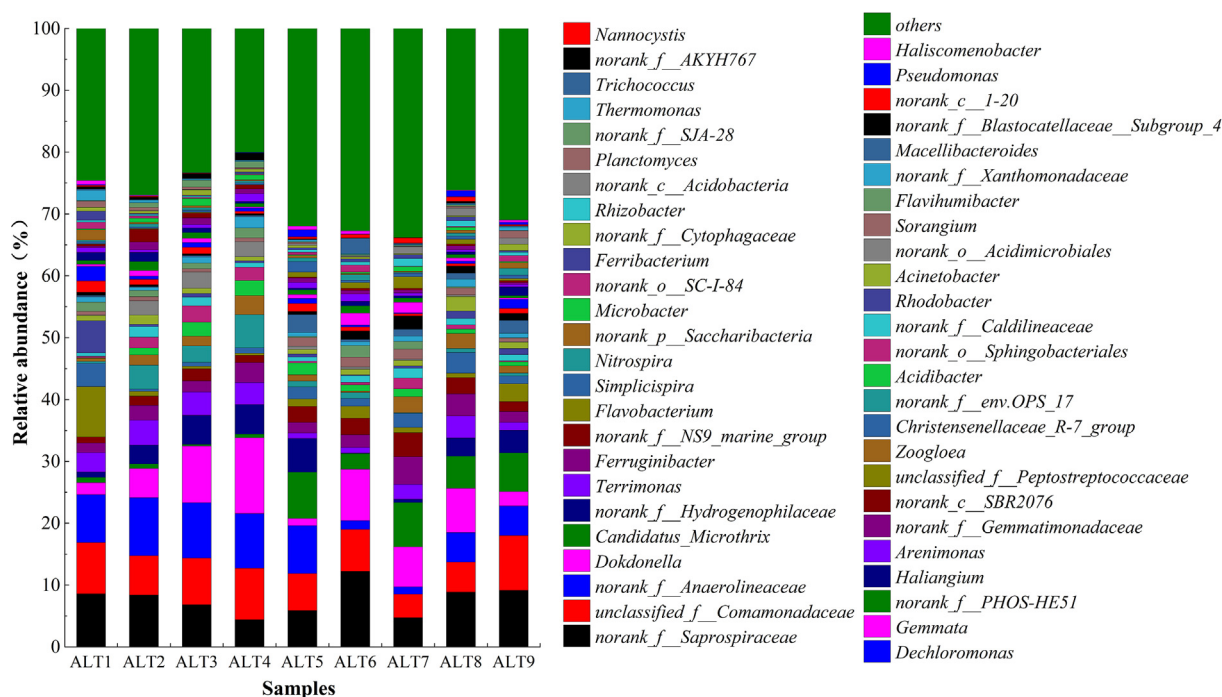

Fig. 3. Bacterial community structure (% relative abundance) at the genus level in the sludge samples. ALT1-9 represent the 9 activated sludge samples from the WWTP located in Altay, China.

bacterial genera with relatively high abundance were *Candidatus_Microthrix* (0.26–7.50%), *Flavobacterium* (0.32–8.14%), *Ferribacterium* (0.36–5.19%) and *Nitrospira* (0.08–5.37%). Previous research has shown that the dominant bacterial genera of activated sludge from warm-region municipal WWTPs with oxidation ditch process in Hangzhou, Shaoxing, Wuhan, and Shenzhen (China) were generally *Thauera, Desulfuromonas, unclassified genera from Comamonadacae, Xanthomonadacea, Anaerolinraceae* and *Rhodocyclaceae* (Qin et al., 2018; Zhang et al., 2017). As for the WWTPs with different process, *Rhizobiaceae, Rhodocyclaceae*, and *Pseudomonas* have been observed to be the dominant bacteria (Shen et al., 2018). In this study, *Desulfuromonas* and *Rhizobiaceae* were not detectable, and the relative abundance of *Thauera, Pseudomonas, Xanthomonadacea*, and *Rhodocyclaceae* were very low, accounting for only 0.15–0.67%, 0.03–1.18%, 0–1.65%, and 0–0.10%, respectively. These results are different from the dominant bacterial genera identified from the cold-region WWTP of this study, thereby implying that wastewater temperature is a vital environmental factor affecting the bacterial community structure of activated sludge. Although the genus *Legionella* and genus *Mycobacterium* were found to be the potentially pathogenic bacteria in WWTPs (Kulkarni et al., 2018), these bacteria were not detectable in the current study, indicating that the efficient treatment performance of this WWTP was environmental-friendly of public health.

Filamentous bacteria are functional bacteria with a similar morphology and play an important role in composing the skeleton structure of activated sludge (Gnida et al., 2018). Previous study indicated that filamentous bacteria mainly served as the skeleton of activated sludge floc, thereby facilitating the adhesion and growth of important functional populations when it had a moderate proportion in the activated sludge (Per Halkjaer et al., 2010). *Saprospiraceae_norank, Anaerolineaceae_norank, Flavobacterium* and *Candidatus_Microthrix* were the main filamentous bacteria detected in the sludge samples, with average relative abundances of 7.68%, 6.08%, 3.46% and 1.92%, respectively. The relative abundance of filamentous bacteria in the activated sludge was found to be sufficient in the present study, which was essential for pollutant removal and sludge settling performance (Wang et al., 2016).

Furthermore, psychrophiles at genus level, namely *Flavobacterium, Pseudomonas, Cyanobacteria, Halomonas* (Siddiqui et al., 2013), *Trichococcus* (Parshina et al., 2019) and *Acinetobacter*, were detected in this study. Of these, *Flavobacterium* (0.32–8.14%) and *Acinetobacter* (0.14–1.02%) had the highest relative abundance.

Relevant studies show that, under conditions of less than 15 °C, *Flavobacterium* could be used as the main denitrifying bacteria to reduce nitrate in wastewater (Broman et al., 2017). Additionally, at temperatures as low as 4 °C, cold-resistant *Acinetobacter* can quickly adapt, maintain growth and reproduction and become dominant in the removal of pollutants (Gonzalez-Martinez et al., 2018; Mortazavi et al., 2015). In this study, *Flavobacterium* and *Acinetobacter* were found with higher relative abundances in the lower water temperature samples, compared with the higher water temperature samples. These results conform to the growth regulation of psychrophiles, demonstrating that these psychrophiles grew well within the water temperature range and promoted the removal of pollutants.

According to the sequencing results, functional bacteria related to nitrogen and phosphorus removal were detected, including ammonia-oxidizing bacteria (AOB; *Nitrosomonas* (0.07–0.68%) and *Nitrosomonadaceae* (0.08–0.52%)), nitrite-oxidizing bacteria (NOB; *Nitrospira* (0.08–5.37%)) and phosphate accumulating organisms (PAOs; *Candidatus_ Accumulibacter* (0.01–0.55%) and *Tetrasphaera* (0.12–0.82%)). The abundances of these bacteria were compared using a heatmap (Fig. 4). A previous study showed that nitrifying bacteria were sensitive to temperature (Antoniou et al., 1990). When the temperature was lower than 15 °C, the activity of the nitrifying bacteria began to decrease along with the nitrification rate, and the nitrification rate decreased by 50% at temperature lower than 12 °C (Ducey et al., 2010). There was almost no nitrification observed when the temperature was lower than 5 °C, (Ducey et al., 2010). Nevertheless, in the present study, the relative abundance of AOB and NOB in lower temperature samples was generally higher than those in higher temperature samples. NOB were the most abundant (5.37%) in ALT4 (8.5 °C, the lowest sampling temperature) and the least abundant (0.46%) in ALT7 (17.4 °C, the highest sampling temperature). In contrast to previous studies, the low temperature had no adverse effect on the abundance and apparent activity of AOB and NOB in the activated sludge from this WWTP, resulting in complete nitrification within the aerobic zone of the oxidation ditch. This might be attributed to the low temperature enhancing the diversity of the NOB and increased their relative abundance (Siripong and Rittmann, 2007). In previous studies, most reported AOB and NOB are aerobic bacteria (Chain et al., 2003; Winkler et al., 2012). For example, Irvin et al. (2007) reported that when DO concentrations decreased from 5.0–5.5 mg/L to 1.0 mg/L, the sum of AOB and NOB populations
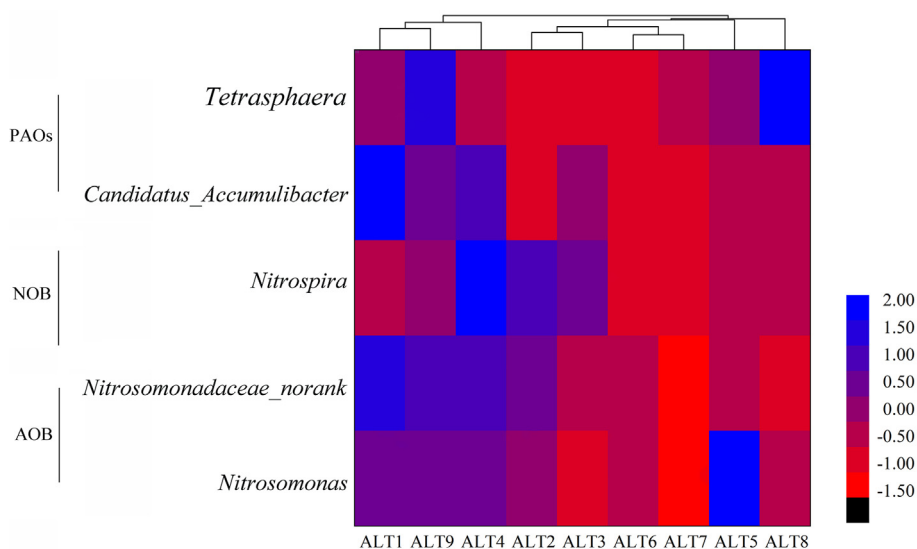

Fig. 4. Heatmap of AOB, NOB and PAOs in the activated sludge samples. ALT1-9 represent the 9 activated sludge samples from the WWTP located in Altay, China.

Table 3
Operation of some municipal wastewater treatment plants.

Process	Location	Temperature (°C)	Sludge load (kg/kgMLSS·d)	DO (mg/L)	SVI (mL/g)	References
CAS	Ontario, Canada	<20.0	0.10–0.43	1.4–4.0	60–350	Nakhla and Lugowski (2010)
A²/O	Beijing, China	15.0–15.5	0.036–0.044	1.0–2.0	135–164	Fan et al. (2018)
MBR	Mikkeli, Finland	7.0–20.0	0.02–0.05	–	100–180	Gurung et al. (2017)
IFAS	Trondheim, Norway	11.5–13.8	0.31–0.66	2.16–5.02	75–300	Di Trapani et al. (2013)
OD	Xinjiang, China	13.0	0.02	1.5	200–260	Xu et al. (2018)
OD	Tangshan, China	12.9–20.0	0.08–0.13	2.58–5.79	118–304	Xie et al. (2007)

CAS, Conventional Activated Sludge process; A²/O, Anaerobic/Anoxic/Aerobic process; MBR, Membrane Bioreactor; IFAS, Integrated Fixed-film Activated Sludge; OD, Oxidation Ditch.

decreased sharply from 8.6% ± 0.4 to 3.6% ± 0.4. The average DO concentration was 5.85 mg/L in this WWTP, which provided a good DO environment for the growth and reproduction of AOB and NOB. It may also explain why the relative abundance of AOB and NOB did not decreased at low temperatures in the present study. Bacterial community analysis of the WWTP in Liaoning Province (China), showed a similar phenomenon in that the relative abundance of *Nitrospira* increased during winter (Liu et al., 2019).

Similar to that of nitrifying bacteria, the relative abundance of PAOs in the activated sludge samples increased with the decrease of temperature. The relative abundance of *Candidatus_Accumulibacter* and *Tetrasphaera* were 0.21% and 0.35%, respectively, in ALT4 (8.5 °C), but only accounted for 0.12% and 0.01%, respectively, in ALT6 (17.4 °C). It is hypothesized that PAOs can change their metabolic pathways at low temperatures to enrich the total amount of bacteria, achieving good biological phosphorus removal (Erdal et al., 2003). Lower water temperatures were conducive to the growth of *Candidatus_Accumulibacter* (Tian et al., 2013) and *Tetrasphaera* (Welles et al., 2015), making them more competitive at low temperatures. The variation trend of PAOs in this WWTP was consistent with previous research results.

3.4. Engineering measures in WWTP

The processes, operational conditions and sludge statuses adopted by some WWTPs in cold regions are shown in Table 3. It is evident that no matter what process is adopted, the key factors affecting the treatment and sludge settleability performance of municipal WWTPs lie in their operational conditions. Low temperature, low sludge load and low dissolved oxygen (DO) concentration may lead to the poor operation of WWTPs. In order to adapt to a low temperature environment, municipal WWTPs in cold regions should take appropriate operational measures. A previous study showed that when the influent temperature was below 10 °C, the BOD_5 sludge load of a WWTP with a delayed aeration process should not exceed 0.08 kg/kgMLSS·d (Sayigh and Malina, 1978) and that the increase of the DO concentration could enhance the rate of nitrification (Pochana and Keller, 1999; Stenstrom and Poduska, 1980), which effectively inhibited the degree of sludge bulking.

The WWTP in this study was located in a cold region, with water temperatures below 10 °C during the winter time. It was operated with an average DO concentration of 5.85 mg/L in the oxidation ditch and a SVI value of 50–80 mL/g. Thus, these operational conditions can be considered as low temperature, ultra-low sludge load and high DO concentration. With annual average water temperatures in the WWTP of only 12 °C, the BOD_5 sludge load can be controlled at 0.077 kg BOD_5/kgMLSS·d to ensure the removal efficiency of COD and BOD_5 under continuous low temperature operational conditions. At the same time, it was observed that maintaining high DO concentrations of 5.85 mg/L was an effective measure to restrain sludge bulking under the conditions of low temperature and low sludge loads. Therefore, under these conditions, pollutants were effectively removed, the sludge settleability performance was good, and the annual effluent stability reached the standard. Therefore, in order to adapt to a low temperature environment, engineering measures targeting an ultra-low sludge load with high dissolved oxygen should be adopted to ensure the effective operation of cold-region WWTPs. These results show that WWTPs in cold regions can maintain good operation at low temperatures by adjusting their operational parameters. According to this study it seems that adopting an ultra-low sludge load with a high DO concentration would be an effective engineering measure that ensures efficient pollutant removal and controls the problem of sludge bulking at low temperatures.

4. Conclusion

The average removal efficiencies for COD, BOD_5, SS, NH_4^+-N, TN and TP were 94%, 95%, 95%, 91%, 73% and 89% in this WWTP, respectively. The bacterial community structure in the activated sludge of an oxidation ditch was stable. Under the low water temperature conditions during the winter, the evenness of the microbial community decreased while the richness and diversity remained relatively stable. The most dominant bacteria phyla were *Proteobacteria* and *Bacteroidetes* with a total relative abundance of 57.45–78.87%. *Saprospiraceae_norank* (4.41–12.23%), *Comamonadaceae_unclassified* (3.82–8.83%), *Anaerolineaceae_norank* (1.39–9.35%) and *Dokdonella* (1.13–11.26%) were the dominant genera. These dominant genera differed from those reported in warmregion municipal WWTPs. *Saprospiraceae_norank*, *Anaerolineaceae_norank*, *Candidatus_Microthrix* and *Flavobacterium* were the main filamentous bacteria in the activated sludge of this WWTP. *Flavobacterium*, *Trichococcus*, *Acinetobacter* and *Pseudomonas* grew well as psychrophiles and promoted the removal of pollutants. The low temperature in this WWTP had no adverse effect on AOB, NOB and PAOs, which were found at a higher abundance in lower temperature samples. This WWTP adopted an ultralow sludge load with high dissolved oxygen to maintain effective removal of pollutants and a good sludge settleability performance. Thereby, the operational experience gained in this WWTP scenario provides a reference for municipal WWTPs in cold regions, with ultra-low sludge load and high DO levels being an effective measure to help WWTPs maintain effective treatment performance and operational efficiency.

CRediT authorship contribution statement

Yuanshuang Luo: Investigation, Formal analysis, Writing - original draft, Writing - review & editing, Visualization. **Junqin Yao:** Conceptualization, Methodology, Validation, Resources, Data curation, Writing - review & editing, Project administration, Supervision, Funding acquisition. **Xiyuan Wang:** Formal analysis, Writing - review & editing. **Meiying Zheng:** Resources. **Deyong Guo:** Resources. **Yinguang Chen:** Validation, Data curation, Supervision, Project administration, Funding acquisition.

Declaration of Competing Interest

The authors declared that there is no conflict of interest.

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