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REVIEW ARTICLE

Current status of municipal wastewater treatment plants in North-east China: implications for reforming and upgrading

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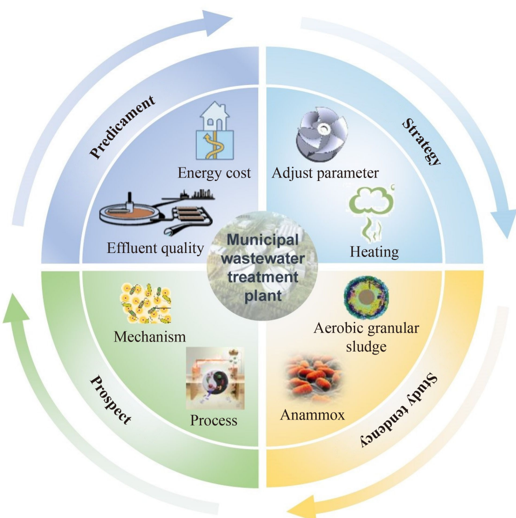
HIGHLIGHTS

- The performance and costs of 20 municipal WWTPs were analyzed.
- Effluent COD and NH_4^+-N effluent exceed the limits more frequently in winter.
- Nitrification and refractory pollutant removal are limited at low temperatures.
- To meet the national standards, electricity cost must increase by > 42% in winter.
- Anammox, granular sludge, and aerobic denitrification are promising technologies.

GRAPHIC ABSTRACT



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ABSTRACT

Climate affects the natural landscape, the economic productivity of societies, and the lifestyles of its inhabitants. It also influences municipal wastewater treatment. Biological processes are widely employed in municipal wastewater treatment plants (WWTPs), and the prolonged cold conditions brought by the winter months each year pose obstacles to meeting the national standards in relatively cold regions. Therefore, both a systematic analysis of existing technical bottlenecks as well as promising novel technologies are urgently needed for these cold regions. Taking North-east China as a case, this review studied and analyzed the main challenges affecting 20 municipal WWTPs. Moreover, we outlined the currently employed strategies and research issues pertaining to low temperature conditions. Low temperatures have been found to reduce the metabolism of microbes by 58% or more, thereby leading to chemical oxygen demand (COD) and NH_4^+-N levels that have frequently exceeded the national standard during the winter months. Furthermore, the extracellular matrix tends to lead to activated sludge bulking issues. Widely employed strategies to combat these issues include increasing the aeration intensity, reflux volume, and flocculant addition; however, these strategies increase electricity consumption by > 42% in the winter months. Internationally, the processes of anaerobic ammonium oxidation (anammox), granular sludge, and aerobic denitrification have become the focus of research for overcoming low temperature. These have inspired us to review and propose directions for the further development of novel technologies suitable for cold regions, thereby overcoming the issues inherent in traditional processes that have failed to meet the presently reformed WWTP requirements.

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1 Present status of cold regions: Case of North-east China

Cold climate regions are defined as those in which the coldest month is characterized by a mean temperature below −3 °C and the warmest above 10 °C (Xiao et al., 2022). According to this classification, cold regions include Alaska, Scandinavia, East Europe, Canada, Northern contiguous USA, Russia, and North-east China. North-east China is at 38°43′–53°33′ N and 115°50′–135°20′ E, covers an area of 1.24 × 10^6 km^2, and has a typical cold-temperate continental monsoon climate. Winters in North-east China usually last for 6 months from November to April and experience a minimum temperature of –40 °C and an average temperature of –5.4 °C (over the last two decades). North-east China is one of the largest heavy industrial bases in China and has been constructed since 1930 (Ren et al., 2020). It is also the most significant grain-producing region in China (Wang et al., 2018). The ratio of heavy industries to total industries within the region has been determined to be nearly 80%, which is 20% greater than the national average; meanwhile, rice production in this region accounts for about 16%–20% of China's total rice production.

The rapid development of the industrial and agricultural manufacturing industries has caused the ecological environment of North-east China to deteriorate rapidly. Interestingly, North-east China is located at a particular geographical position that is considered crucial for recovering China's ecological environment. Several main rivers originate from North-east China, including the Tumen River, Songhua River, Heilong River, Wusuli River, Nen River, Liao River, and Yalu River. Some of these represent important international rivers flowing out of the Chinese territory. For instance, the Songhua River is important as it functions as a source of drinking water for both Jilin City and Harbin City. However, almost 16% of the Songhua River's state-controlled sections in Jilin Province have been deemed unqualified for the standard, and the area of soil erosion in Heilongjiang Province was found to account for 17% of the province's area (Fu et al., 2019; Wang et al., 2019; Zhang et al., 2020). In 2003, the Intervention of the North-east China Revitalization Strategy was issued by the Chinese government (the State Council of the People's Republic of China) (Ren et al., 2020). North-east China is expected to experience an important period of transformation, whereby it will shift away from being an industrial civilization and move toward becoming an ecological civilization.

Municipal WWTP is one of the main links to realize ecological civilization, which connects industrial wastewater, agricultural wastewater, municipal wastewater, and rivers to construct a water circulation system. The tailwater of industrial wastewater is discharged to municipal WWTPs and mixed with municipal wastewater, while the effluent is discharged into rivers. In North-east China, 40% of agricultural irrigation water is surface water. Chemical fertilizers and pesticides used in agricultural production also enter the rivers. As a consequence, the rapid development of industrial and agricultural manufacturing industries has caused the ecological environment of North-east China to have deteriorated markedly.

Low temperatures have a negative effect on the attenuation of pollutants, which poses a serious challenge for the performance of municipal WWTPs. Therefore, there is a crucial need to optimize municipal wastewater treatment in such particularly cold regions. As shown in Fig. 1, this review analyzed the current challenges, including frequent effluent quality deterioration, sludge bulking, and high operational costs. This review also summarized the current strategies adopted by municipal WWTPs and analyzed the scientific research innovations employed in wastewater treatments functioning at low temperatures, including those involving anammox, aerobic denitrification, nitrification, and granular sludge. Finally, this review proposed suggestions for improving wastewater treatment conducted under low temperature conditions.

2 Overcoming the effects of low temperatures: strategies and challenges

2.1 Overview of municipal WWTPs

From 2015 to 2019, the municipal wastewater treatment volume (m^3/a) of Jilin, Liaoning, and Heilongjiang provinces had all increased steadily (Fig. 2(a)). According to the National List of Centralized Wastewater Treatment Facilities (China), there were 624 WWTPs in North-east China until the year 2020, and the total treatment volume reached 1.6 × 10^7 m^3/d.

Fig. 2(a) shows that WWTPs with a relatively low influent quantity (1–10 m^3/d) were dominant across all three provinces. Taking Jilin province as an example, more than 80% of WWTPs had a treatment capacity of less than 10^5 m^3/d, however, the percentage of WWTPs with a capacity > 5.0 × 10^5 m^3/d was only 0.7%. Furthermore, biological processes were employed by all the WWTPs in North-east China. As shown in Fig. 2(b), the classical anaerobic/anoxic/oxic (A/A/O) process was the most commonly employed, while the anoxic/oxic (A/O) process, cyclic activated sludge system (CASS), and cyclic activated sludge technology (CAST) were also widely adopted. The above processes are also well-established and widely used in other cities. Furthermore, the oxidation ditch (OD) process has been used in 21% of WWTPs in Southern China, which is significantly higher than that in North-east China (Zhang et al., 2016). Because the OD process covers a large area, pool surface

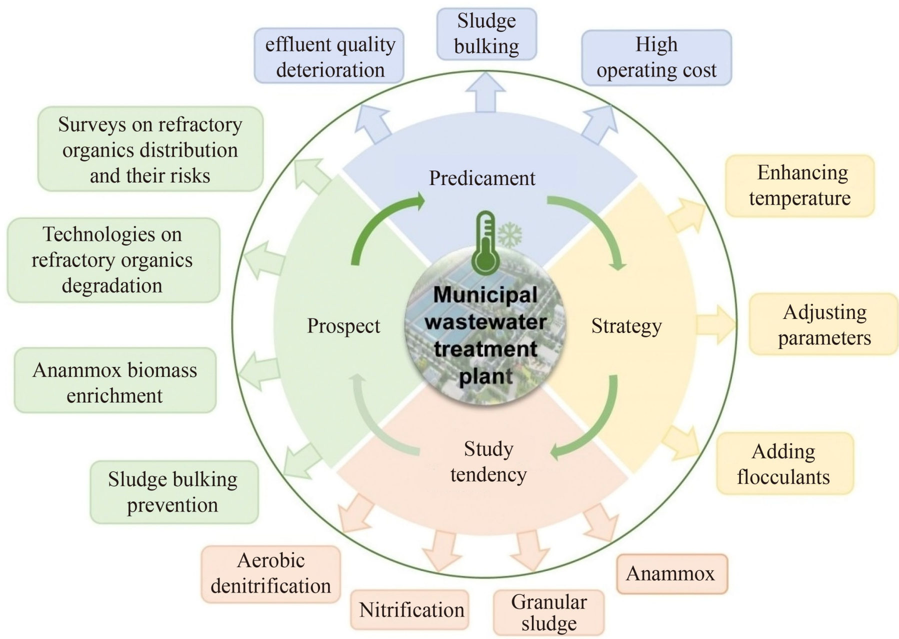

Fig. 1 Overview of the challenges, strategies, study hotspots, and prospects for municipal wastewater treatment at low temperatures.

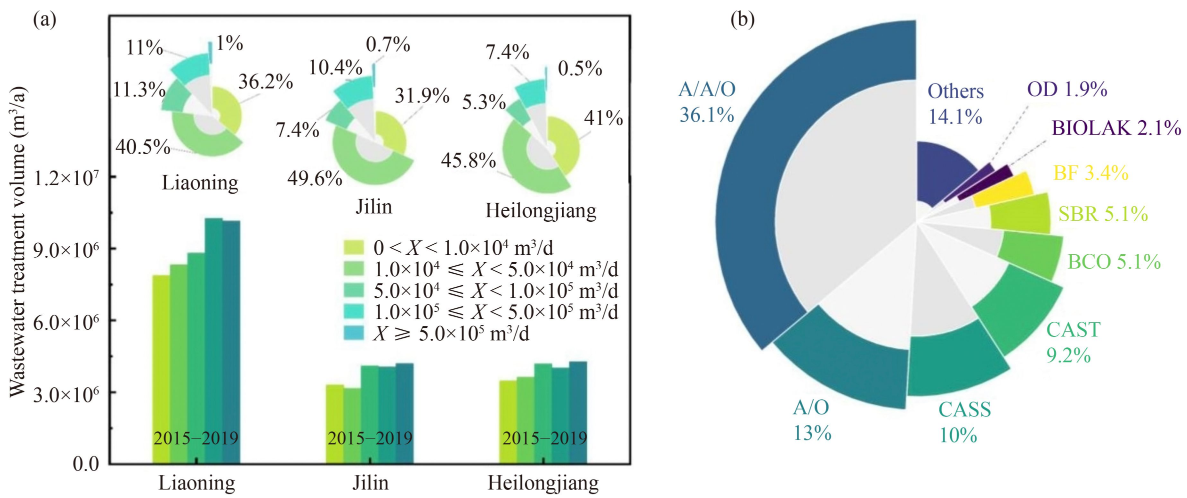

Fig. 2 Overview of municipal wastewater treatment in North-east China: (a) wastewater treatment capacity (column graph shows the annual wastewater treatment volume, pie graph shows the proportion of wastewater treatment plants with different treatment capacities), (b) wastewater treatment process composition (A/A/O: anaerobic/anoxic/oxic, A/O: anoxic/oxic, CASS: cyclic activated sludge system, CAST: cyclic activated sludge technology, BCO: biological contact oxidation, SBR: sequencing batch reactor, BF: biofilm, OD: oxidation ditch).

freezing occurs readily during winter, which is not conducive to microbial metabolism nor equipment maintenance in North-east China.

2.2 Increasingly strict discharging requirements

Many policies and regulations have been issued to limit the discharge of pollutants and alleviate water body pollution in recent years. During 2011–2015, the Chinese government proposed detailed rules for increasing the municipal wastewater treatment capacity, improving wastewater collection, and increasing treatment efficiencies (especially for organic carbon). During 2016–2020, stricter policies were implemented. The nitrogen and phosphorus removal process was required to be further enhanced. Many cities had regulated WWTP effluents to meet the first-level A standard set by the Discharge Standard of Pollutants for Municipal Wastewater Treatment Plants (GB 18918-2002, China), especially for those situated in areas in proximity to lakes, reservoirs, and inshore water catchments. During this term, the wastewater treatment ratio of cities and rural areas was predicted to reach 95% and 85%, respectively.

However, ambient temperature is a crucial parameter that determines microbial generation times, which metabolic pathways are employed by the microbes, and their activities (Jantarakasem et al., 2020). Thus, low temperatures can significantly affect the performances of WWTPs that harness biological processes as part of their core treatment strategies. Such impacted processes include organic degradation, nitrogen transformation, and sludge settleability (Zhou et al., 2016). Most functional flora that are found present in activated sludge are mesophilic microorganisms, and their microbial activity reduces by 50% when the ambient temperature declines to 10 °C. Low wastewater temperatures decrease the permeability of membrane lipid bilayers in microorganisms, thereby slowing down and even arresting the membrane proteins that normally pump ions, transfer nutrients, and conduct respiration (Dhaulaniya et al., 2019). Municipal WWTPs in North-east China have been placed under pressure to meet the increasingly stricter standards put forth by the government, especially as policies for reusing reclaimed water were issued in China at the beginning of 2021.

2.3 Challenge I: Effluent quality deterioration

2.3.1 General parameters

Our group surveyed 20 municipal WWTPs and their operations from 2011 to 2018, among which, 14 were situated in Heilongjiang province and 6 in Jilin province. Fig. 3 shows that, generally, the effluents met the national discharge standard of China (GB 18918-2002) in the summer months. The COD, BOD, and NH_4^+-N more frequently exceeded the discharge limit in the winter months as the bioactivities of microorganisms were sensitive to temperature. Total nitrogen (TN) in the effluents fluctuated along with NH_4^+-N, indicating that nitrification processes are sensitive to low temperatures. Phosphorus removal could be enhanced by flocculation, which was widely employed in winter, so there was no obvious discrepancy between the summer and winter months for this process; however, the winter months saw a marked compensatory increase in the cost of conducting this process.

2.3.2 Refractory organics

Agricultural and industrial practices cause the release of multiple refractory organics. As shown in Table 1, most of the refractory organics detected in the influents were able to be removed via conventional wastewater treatment processes performed under ambient temperatures of 19–21 °C, and notably, amoxicillin was almost completely removed. Comparatively, low temperatures lead to significantly reduced removal efficiencies. The removal rate for atenolol was determined to be less than 21% at 10 °C in municipal WWTPs. For the membrane bioreactor (MBR), when the temperature decreased from 28 °C to 15 °C, the trimethoprim removal efficiency dropped from 80% to less than 60% (Table 1). Even at mild temperatures, the degradation of both carbamazepine and trimethoprim have been found to be limited, thereby causing micropollutants to remain in the effluent. Such residual refractory organics may cause the diffusion of antibiotic resistance genes within microbial pathogens that cause human diseases (Zhang et al., 2022b). Additionally, nitrogen removal was found to be inhibited by refractory organics at low temperatures, i.e., the removal rate of ammonia declined from 73% to 45% when the ambient temperature decreased from 25–28 °C to 4–8 °C under the additional stressful condition of being exposed to a concentration of 0.2 μg/L of levofloxacin (Lu et al., 2021).

2.3.3 Nutrients

The traditional nitrogen removal process includes nitrification and denitrification stages in WWTPs. The nitrification process is conducted by ammonium oxidizing bacteria (AOB) and nitrite-oxidizing bacteria (NOB). In municipal WWTPs, the functional gene number of AOB and NOB is kept stable throughout the year, while conversely, their activities have been found to fluctuate greatly (Chen et al., 2018). The optimal temperature for both AOB and NOB is 35 °C, however, nitrification rates were shown to decline 58% when the temperature decreased from 20 to 10 °C. Furthermore, nitrification virtually terminates at temperatures $\leqslant$ 5 °C (Ducey et al., 2010). Likewise, the growth and metabolism of denitrifiers are markedly inhibited within the temperature range of 15–20 °C. The microbial community is also seasonally regulated; *Nitrosomonas aestuarli* has been found to occupy 51.3% of the community in summer, while *Nitrosomonas ureae* becomes the predominant genera in winter (Pan et al., 2018). Such reduced microbial abundance and activity can cause nitrogen removal performances to decline, and the evolution of dominant genera may also cause variations in nitrogen removal behavior, both of which are factors that could lead to the excessive nutrient discharge observed in municipal WWTPs in North-east China.

2.4 Sludge bulking

Sludge bulking is another issue that frequently occurs in cold winters, leading to poor settling ability and severe sludge loss (Liu et al., 2020). Ninety percent of sludge bulking is caused by the overgrowth of filamentous bacteria, while low temperatures usually induce non-

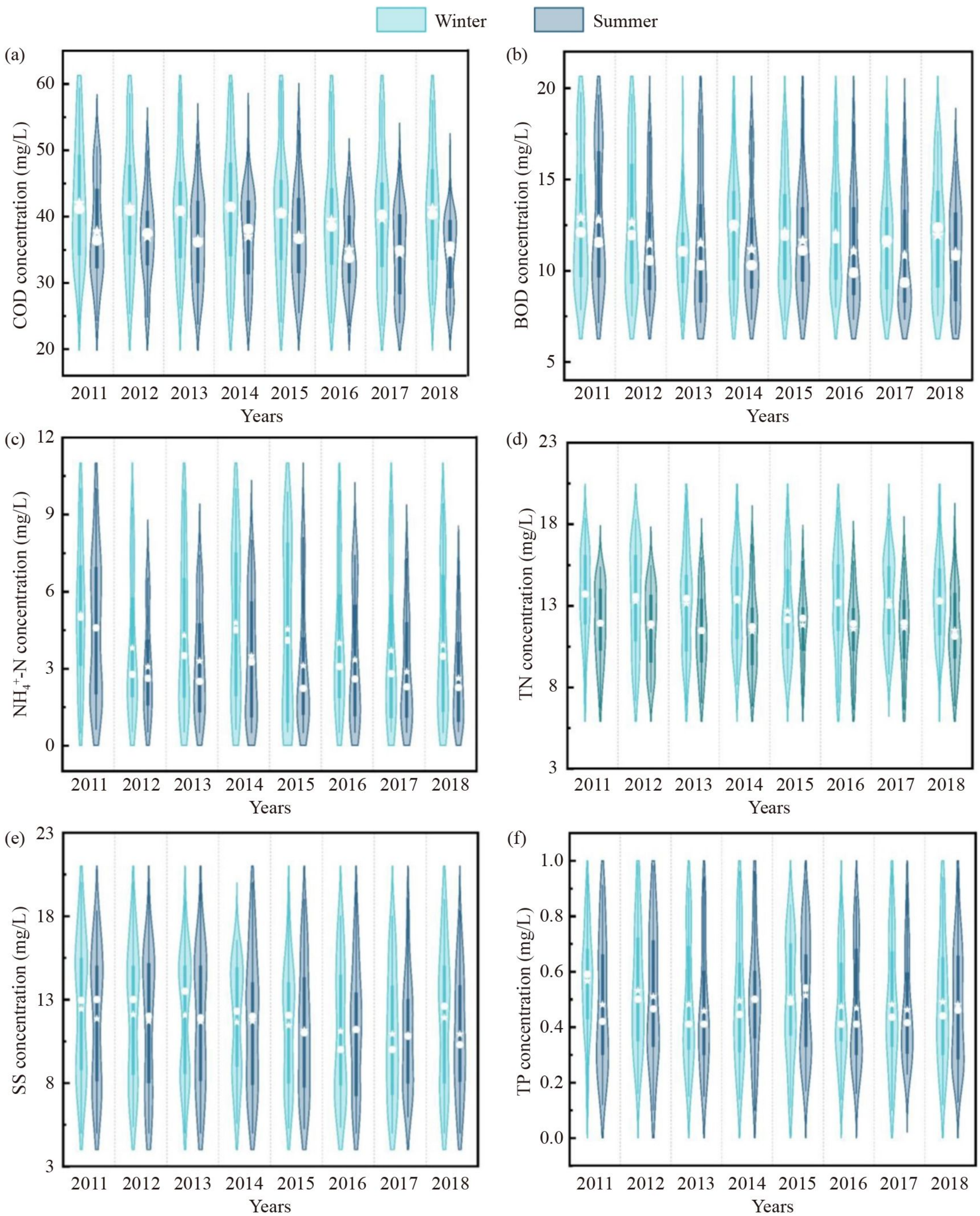

Fig. 3 Variations of the influent and effluent qualities of 20 WWTPs in North-east China from 2011 to 2018. (a) COD, (b) BOD, (c) NH_4^+-N, (d) TN, (e) SS, (f) TP. (Dec. to Feb. comprised the winter months with wastewater temperatures below 10 °C, while Jun. to Aug. comprised the summer months, which was a period distinguished by the highest wastewater temperature measured for the year).

filamentous sludge bulking (Wang et al., 2016; Li et al., 2020). In winter, organic matter can not be completely degraded, and so exists as an extracellular substrate with high viscosity that wraps the bacteria within it (Tandoi et al., 2006). Because this highly viscous molecule contains many hydroxyl groups, it is hydrophilic and hence has a strong binding force with water, which makes activated sludge difficult to separate from the water. The highly viscous metabolites can also absorb fine bubbles produced by aeration, thereby decreasing the density and worsening the sedimentation (Peng et al., 2003; Guo et al., 2014).

2.5 Challenge II: High operational costs

To promote biodegradation at low temperatures, munici-

Table 1 Effects of low temperatures on the removal efficiency of refractory organics

Treatment Process	Organics	Initial concentration	Removal efficiency(%)	Temperature(°C)	Reference
Activated sludge	Amoxicillin	–	100	19	Castiglioni et al. (2006)
		–	49	10	
Activated sludge	Atenolol	–	55	19	Castiglioni et al. (2006)
		–	< 21	10	
Activated sludge	Pharmaceuticals	–	88	21	Sui et al. (2011)
		–	61	1	
Activated sludge	Clarithromycin	1329 ng/L	66	20	Gnida et al. (2020)
		1836 ng/L	50	0	
Membrane bioreactor	Fenoprop	5 μg/L	32	35	Hai et al. (2011)
			13	10	
Membrane bioreactor	Carbamazepine	5 μg/L	33	20	Hai et al. (2011)
			19	10	
Membrane bioreactor	Trimethoprim	334 ng/L	> 80	28	Zheng et al. (2019)
		371 ng/L	< 60	15	
Biofilm	Iopromide	0.000069 mol/L	99	20	Piai et al. (2020)
			50	5	
Biological granular activated carbon	Iopromide	0.000069 mol/L	> 99	20	Piai et al. (2020)
			50	5	
Shallow open-water unit ponds	46 micropollutants	276 ng/L	70	24	Wang et al. (2021)
		1912 ng/L	27	4	

pal WWTPs commonly need to increase their sludge concentrations and enhance the strength of aeration in winter, both of which are measures that increase energy consumption (Kruglova et al., 2017; Petropoulos et al., 2017; Rodriguez-Sanchez et al., 2019). For the 20 municipal WWTPs considered in this review, their detailed wastewater treatment capacities and operating conditions are listed in Table S1. The electricity cost and electricity consumption data obtained for each month were collected from 2011 to 2018, and the differences of each WWTP between summer and winter were calculated using the analysis of variance (ANOVA). The electricity consumption was calculated according to the wastewater treatment capacity. Furthermore, the Pearson correlation between electricity consumption and temperature was fitted using Origin (v 2021).

As shown in Fig. 4(a), the electrical costs of the WWTPs in winter were significantly greater than those in summer ($P < 0.001$) based on the 468 samples obtained from the 20 WWTPs during both seasons. According to the electricity consumptions of the 20 municipal WWTPs, the average electricity consumption was 0.34 $kW\cdot h/m^3$ in winter and 0.24 $kW\cdot h/m^3$ in summer. The results indicate that the energy consumption could be significantly increased by 42% in winter (Fig. 4(b)). Based on the plots representing the electricity consumption changes parallel to the ambient temperature changes, the data of WWTP A (n = 245) and B (n = 310) both showed a good linear relationship (R = 0.64 and 0.61). This indicates that these WWTPs increased their incurred energy costs in compensation to meet the standards set for effluents.

3 Current strategies for overcoming the impacts of low temperatures

3.1 Promoting nitrogen removal

Nitrifier activity influences total nitrogen removal, and the optimal temperature for this process is 35 °C. Because of this, deliberately increasing the water temperature is one strategy that can be used to increase nitrifier performance. Insulation is often provided to prevent freezing and blockage, and steam heating of the aeration tank is also used to maintain warm temperatures (Zhou et al., 2018a). Enhancing the aeration volume of the blower has also been shown to effectively increase water temperatures in winter (Zhou et al., 2018a). Furthermore, when temperatures are reduced from 25 to 10 °C, the doubling time of nitrifiers is prolonged from 2–2.6 d to 4.5–5.5 d (Peng and Zhu, 2006). Thus, the reflux volume and reflux ratio are often regulated to ensure a reasonable SVI (sludge volume index), ratio of MLVSS (mixed liquid volatile suspended solids), and MLSS (mixed liquid suspended solids). When the temperature drops by more than 10 °C, the SRT (sludge retention time) must exceed 14 d (Kos, 1998). Regrettably, the above strategies lead to the higher energy consumption observed

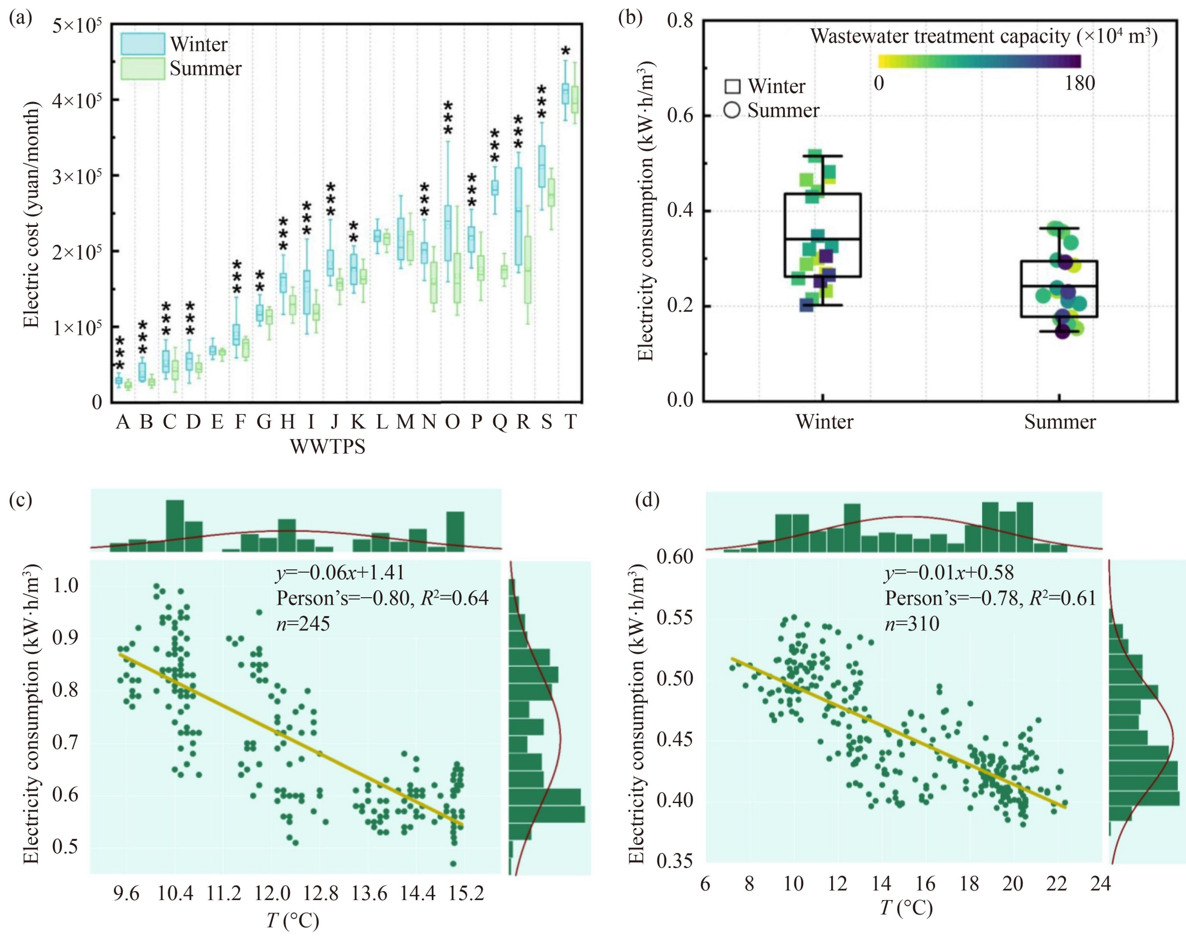

Fig. 4 Electricity consumption and costs of WWTPs in winter versus summer. (a) Electrical costs of 20 WWTPs in winter versus summer, (b) the electricity consumption of 20 WWTPs with different treatment capacities in winter versus summer, (c) relationship between the electricity consumption and temperature of Plant A, (d) relationship between the electricity consumption and temperature of Plant B.

in winter.

3.2 Improving sludge settleability

The addition of flocculants is one of the most common ways to inhibit sludge bulking (Guo et al., 2012). Inorganic polymer flocculants (including polyaluminum chloride and polymeric ferric sulfate) are widely used, and their use increases the cost of wastewater and sludge treatments (Zhang et al., 2021). Furthermore, the MLSS is advised to be kept below 4000 mg/L in winter to achieve a low mud level in the secondary settling tank. However, to ensure an excellent nitrogen removal performance at low temperatures, MLSS is often maintained above 6000 mg/L, which is contradictory to the conditions advised for controlling sludge bulking.

The essence of poor sludge settleability in winter is induced by overproduction of extracellular polymeric substances (EPSs). To solve this issue, the preferred method is targeted to inhibit over synthesis of EPSs. Recently, microbial extracellular substance synthesis is found to be regulated by the quorum sensing (QS) system, which involves the synthesis and recognition of signaling molecules. Degrading the relevant signaling molecules, inhibiting signal molecule production, and blocking signal molecule recognition, had been all confirmed to be efficient for inhibiting the synthesis of extracellular substance (Lee et al., 2016; Mukherjee et al., 2018). The feasibility of these strategies have been approved by the successful control of membrane fouling in MBRs (Lee et al., 2016). However, we still know little on whether improving sludge settleability could be realized by regulating the QS process. In the future, it is urgent to identify the signaling molecules that induce the secretion of extracellular substances in activated sludge systems, reveal their regulatory pathways, and block the secretion of extracellular substances by interfering the relevant process to inhibit sludge bulking.

3.3 Gaps for enhancing micropollutant removal

Micropollutants; including pharmaceuticals, personal care products, hormones, industrial chemicals, and pesticides; are frequently detected in municipal WWTPs (Faria et al., 2020; Pesqueira et al., 2020). However, COD is currently the sole index used as per the guidance of the Discharge Standard of Pollutants for Municipal Wastewater Treatment Plant (GB 18918-2002, China) to limit organic matter discharge, while there is currently no specific index to limit micropollutant discharging. Consequently, the remaining COD concentrations of municipal WWTP effluents tend to be refractory, even when not exceeding the limit.

Temperature influences the biodegradation of micropollutants and brings ecological risks in winter. When the temperature is lower than 5 °C, the process of biodegradation is terminated, and micropollutants accumulate as a consequence (Piai et al., 2020). It has been found that antibiotic resistance gene abundance and temperature have a negative correlation in WWTPs (Schages et al., 2020). Furthermore, micropollutants discharged into surface water are continuously accumulated in winter. During the thawing term in spring, the concentration of micropollutants in water increase sharply, thereby jeopardizing the safety of aquatic organisms. Therefore, it will be necessary to formulate strict micropollutant discharge standards for municipal WWTPs in North-east China.

Currently, advanced process applied in municipal WWTPs for refractory organics removal mainly include ozone oxidation, coagulation and sedimentation, and MBR. Researchers are trying to explore more systematic economic method to achieve the micropollutants elimination. For instance, co-substrate was a feasible strategy for refractory organics degradation, which could significantly improve the removal of refractories, including bensulfuron, trichloroethylene, and bisphenol A (Luo et al., 2008; Li et al., 2014; Heidari et al., 2017). Furthermore, the simultaneous combination of advanced oxidation and biodegradation (SCAOB) process also could remove various micropollutants, in which refractory organics is first oxidized by reactive radicals and produced intermediates are simultaneously degraded by microorganisms protected by porous carriers (Xiong et al., 2018; Song et al., 2019). Thus, co-substrate and SCAOB technologies have the potential to remove micropollutants from municipal wastewater, while their performances at low temperature still require exploration.

4 Bibliometric analysis of research

4.1 Overview of active research areas

We searched for publications on wastewater treatment conducted at low temperatures using the Science Citation Index Expanded: Thomson Reuters "Web of Science". The searched terms included "low temperature OR cold climatic region AND wastewater OR sewage". The publication time was restricted to 2010–2020. The keywords of the selected documents were assembled and then statistically analyzed using bibliometric methods.

According to the statistical results (Fig. 5), over the past decade, research on wastewater treatments at low temperatures has mainly focused on nitrogen removal, while anammox, anaerobic digestion, aerobic denitrification, granular sludge, activated sludge, and nitrification were also popular topics of study. Furthermore, microbial diversity, treatment processes, extracellular polymeric substrates, and sludge characteristics related to wastewater treatments conducted at low temperatures were also widely noted in the literature. However, the above-mentioned novel technologies have not yet been applied to municipal WWTPs in North-east China.

4.2 Popular technologies that are likely suitable for the treatment of wastewater under low temperature conditions

4.2.1 Anammox

Anammox bacteria can utilize NO_2^--N as an electron acceptor and oxidize NH_4^+-N into N_2 under anaerobic conditions (Kartal et al., 2011). Anammox activity has been detected in the sediments of the Songhua River (−30 °C) in North-east China (Zhu et al., 2015). Under low temperature conditions, anammox can synthesize chaperones of RNA, proteins, and chemicals, which then serve to preserve energy for the core metabolism of the microbes by reducing biosynthesis (Kouba et al., 2022). By this mechanism, anammox bacteria can achieve nitrogen removal at low temperatures. A previous study found that when influent NH_4^+-N was at the concentration of 21 mg/L and the ambient temperature was 15 °C, the total nitrogen content in the effluent was maintained below 10 mg/L via the partial nitrification/anammox (PNA) process in a moving bed biofilm reactor (MBBR) with limited oxygen concentrations (Laureni et al., 2016). Another study found that when the temperature dropped to 10 °C, the total nitrogen content in the effluent was still found to be as low as < 8 mg/L via the PNA process in a MBBR (Gilbert et al., 2014). Aside from the potential physiology of anammox bacteria, biofilms represent an advancement from the traditionally used activated sludge, with enough biomass immobilized in the system to allow the anammox bacteria to be retained. Additionally, oxygen gradients have been shown to induce an inter-anaerobic region, thereby causing NOB inhibition and simultaneously promoting the growth of AOB over NOB (Chen et al., 2019). On this basis, anammox activity involving the PNA process can be maintained at low temperatures.

Anammox bacteria are ubiquitous in wastewater

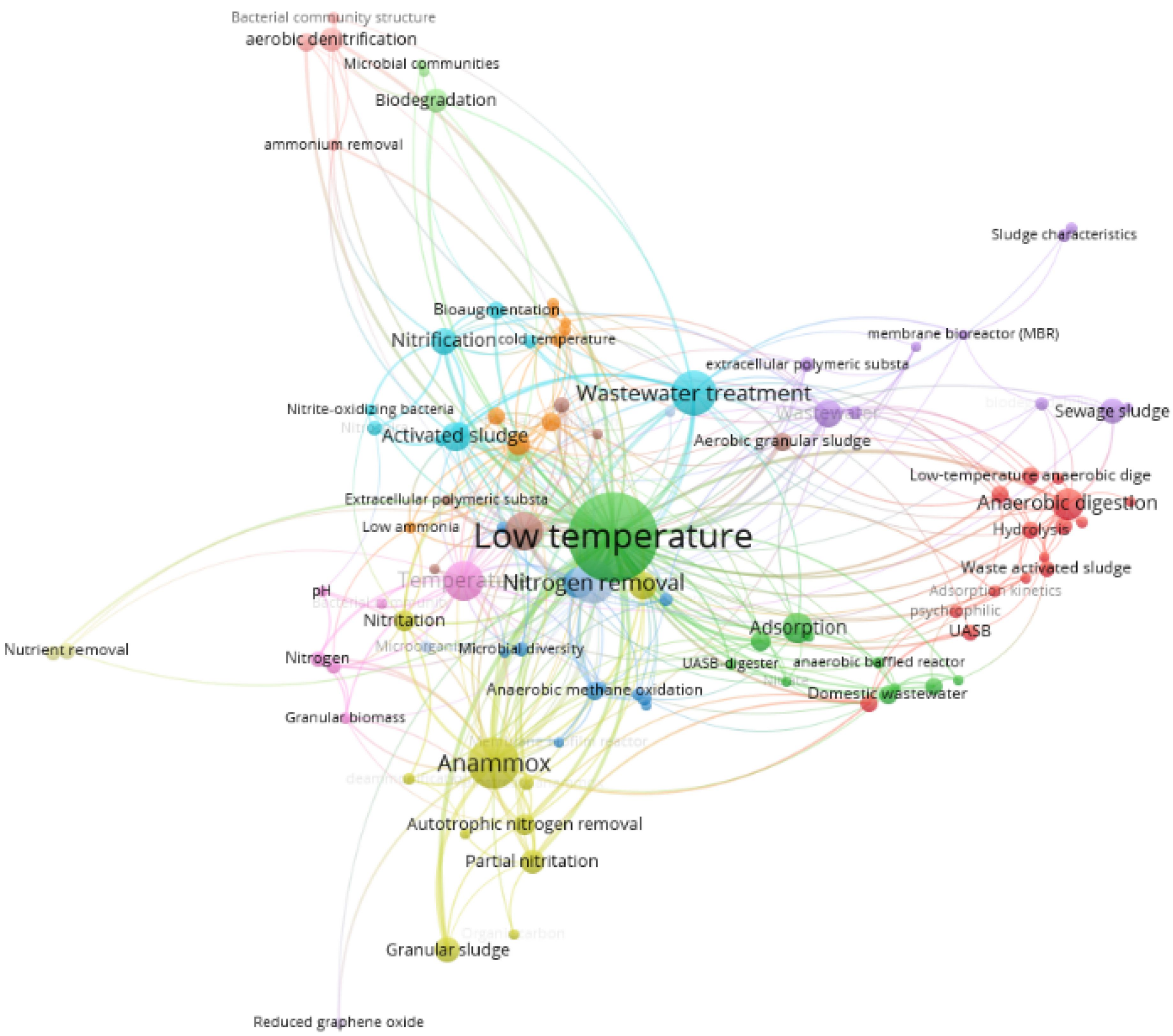

Fig. 5 Keyword cluster analysis of research hotspots in wastewater treatments conducted at low temperatures.

treatment process, but their abundance in such processes is magnitudes lower than that of a high-strength ammonium wastewater anammox treatment plant (Zhang et al., 2022a). Therefore, an urgent problem yet to be solved is realizing the in situ enrichment of anammox bacteria for the municipal wastewater treatment process. It has been found that the relative abundance of anammox bacteria in anoxic-carrier biofilms could reach 0.11%, which contributed to 15.9% of nitrogen loss via the partial denitrification-anammox pathway in a full-scale municipal WWTP (Li et al., 2019). Hence, adding carriers may be feasible, and other operating parameters and enrichment strategies at low temperatures still require exploration.

4.2.2 Granular sludge

Granular sludge is comprised of self-immobilized biofilms visible to the naked eye, with sizes up to several mm deep (Winkler et al., 2018). Aerobic granular sludge (AGS) has been shown to achieve high biomass concentrations, effective biomass-water separation, and harbor different microbial groups, such as AOB, NOB, anammox bacteria, polyphosphate-accumulating organisms (PAOs), and glycogen-accumulating organisms (GAOs). Granules are comprised of a consortium of microorganisms, including producers of cryoprotective EPS, thus protecting sensitive microorganisms from low temperature conditions (Reino et al., 2016). Therefore, the biological removal of nutrients and recalcitrant organic matter can be achieved in AGS at low temperatures. One study reported that when the temperature gradually declined from 20 to 8 °C, an AGS reactor achieved a nitrogen removal efficiency and phosphorus removal efficiency of 44% and 97%, respectively (de Kreuk et al., 2005). Another study found that the trimethoprim removal efficiency reached 50%–60% at both 26 °C and 7 °C during the AGS process.

Currently, AGS technology has mainly been achieved in SBR (de Kreuk et al., 2005; He et al., 2020). However, continuous processes, including A/A/O and A/O, are dominantly employed in North-east China, and the report on cultivating AGS in these two processes is lacking. Strategies for achieving sludge granulation in a continuous wastewater treatment process need to be explored. It has been widely reported that metal ions, organic matter, and bio-char benefit sludge granulation, while the mechanisms and factors underlying this effect are unclear (Han et al., 2022). Therefore, targeted carriers to promote granulation could be designed for continuous wastewater treatment processes, especially for granulation at low temperatures.

4.2.3 Enhancing nitrification

Reduced nitrification activity at low temperatures causes excessive NH_4^+-N and TN to be remained in the effluents of WWTPs. Because of this, parameter optimization and bioaugmentation strategies were investigated. The optimizing operation parameters investigated included improving the dissolved oxygen (DO) concentration, prolonging the hydraulic retention time (HRT), and prolonging the SRT (Zhou et al., 2018b; Cui et al., 2020). Bioaugmentation aims to promote the acclimatization of microorganisms to harsh conditions, mainly by supplying nutrients, supplying other acclimated microorganisms, microbial self-immobilization, and intermittent enrichment technologies (Kim et al., 2006; Ducey et al., 2010; Arévalo et al., 2014). In one study, when treating municipal wastewater at 10 °C, the ammonium conversion rate of the biofilm on carriers was found to be 4 times that of the suspended sludge (Gilbert et al., 2015). Compared with parameter optimization and process upgrading, bioaugmentation technology has garnered more attention, as it can be used to obtain the long generation times of microorganisms by artificial enrichment. The most popular systems harnessed for bioaugmentation are membrane bioreactors, MBBRs, and biofilters (Cui et al., 2019; Jin et al., 2021).

Recently, both MBRs and MBBRs have been widely applied for the upgradation of municipal WWTPs, and even a combined process utilizing both a MBBR and MBR has been used in North-east China. In the MBBR process, the fluidization of the carriers is achieved by aeration and stirring simultaneously, which significantly increases energy consumption. The question of how to maintain fluidization via energy-saving methods requires further exploration. Furthermore, how to alleviate the membrane clogging problem of MBRs at low temperature conditions also requires further research.

4.2.4 Aerobic denitrification

Aerobic denitrification refers to denitrification carried out by aerobic denitrifiers in the presence of oxygen, whereby oxygen and nitrate are simultaneously utilized as electron acceptors (Yang et al., 2020). Under low temperature conditions, aerobic denitrifiers exhibit relatively lower membrane stability and membrane transport efficiencies. Meanwhile, some ATP binding cassette (ABC) transporter genes and fatty acid desaturase genes in aerobic denitrifiers are upregulated, which is of great benefit to providing nutrients, protective substances, and metal ions. Fatty acid desaturase genes can increase membrane fluidity and improve the freezing resistance of organisms (Zhao et al., 2021). Therefore, these allow for excellent nitrogen removal capacities under low temperature conditions. As shown in Table 2, the denitrification activity of *Pseudomonas alcaligenes* could reach 15.33 mg N/(g·SS·h) under the ambient temperature of 5 °C (Wang et al., 2015), thereby indicating its great potential for usage in improving nitrogen removal in municipal WWTPs in North-east China.

Aerobic denitrifiers exhibit excellent nitrogen removal ability at low temperatures, but this feature has not been widely applied to wastewater treatment. This is because most aerobic denitrifiers are autotrophic, and so can be outcompeted by heterotrophic microorganisms over the course of long-term operation. Therefore, the enrichment and retention of aerobic denitrifiers in activated sludge requires further study. Additional research should also be conducted on how to transform aerobic denitrifiers into stable bacterial agents and strengthen their nitrogen removal performance at low temperatures.

Table 2 Species of aerobic denitrifiers and their nitrogen removal performances at low temperature

Mixed culture/Strains	Nitrogen removal activity	Temperature (°C)	Reference
Nitrosomonas sp., *Pseudomonas* sp. and *Rhodoferax ferrireducens*	53.11 mg N/(g·SS·h)	10	Zou et al. (2014)
Pseudomonas sp. and *Rhodoferax ferrireducens*	9.6 mg N/(g·SS·h)	10	Yao et al. (2013a)
Acinetobacter sp. HA2	1.88 mg N/(L·h)	10	Yao et al. (2013b)
Pseudomonas alcaligenes	15.33 mg N/(g·SS·h)	5	Wang et al. (2015)
Acinetobacter sp. TAC-1	3.5 mg N/(L·h)	5	Zhao et al. (2021)
Microbacterium sp. SFA13	0.11 mg N/(L·h)	5	Zhang et al. (2013)
Pseudomonas stutzeri YZN-001	0.3 mg N/(L·h)	4	Zhang et al. (2011)
Acinetobacter sp. Y16	0.092 mg N/(L·h)	2	Huang et al. (2013)

5 Conclusions and outlook

North-east China is a severely cold region. The total quantity of municipal wastewater reached 1.6×10^7 m^3/d in the year 2019. More than 80% of these WWTPs had a treatment capacity of less than 10^4 m^3/d, and the A/A/O and CASS processes were dominant. During the last decade, the discharge standard set for pollutants in municipal WWTPs has become increasingly stricter. However, due to the sensitivity of microorganism to ambient temperature, both COD and NH_4^+-N were found to have frequently exceeded the discharge limits in winter, according to data obtained from a survey of 20 municipal WWTPs. Low temperatures also cause refractory residual and sludge bulking issues.

Technologies including Anammox, granular sludge, and aerobic denitrification have advantages in enhancing microbial activity and have been extensively studied during the last decade. These technologies generally involve high biomass, long retention times, and strong resistance to extreme environments. By reviewing the currently functioning WWTPs and the present research on them, the following suggestions are given to solve the current issues affecting WWTPs.

1) *Surveys on the distribution of refractory organics and their risks*. Temperature influences the existence and biodegradation of refractory organics, and the chronic low temperatures that characterize winter aggravate the accumulation of pollutants in multi-interfaces. Therefore, the effects of low temperatures on the forms and distributions of organics in sewage require verification. The corresponding inhibitory mechanism and microbial behaviors also require exploration.

2) *Technologies for the degradation of refractory organics*. There is difficulty in removing refractory organics via microbial degradation at low temperatures. Co-substrate and SCAOB are feasible strategies to obtain metabolic energy and improve enzymatic activities of microorganisms. However, their performance for micropollutants removal at low temperature still require exploration. Furthermore, screening for functional microorganisms is also necessary, and this can allow for the exploration of strategies to enhance their enrichment and expression for use in municipal WWTPs.

3) *Anammox biomass enrichment*. Anammox bacteria are ubiquitous in WWTPs, and their activity is positively correlated with their abundance. Current research has primarily been conducted by inoculating anammox sludge, and there remains the challenge of quickly enriching anammox biomass. Therefore, it will be necessary to clarify the enrichment and growth mechanism of anammox bacteria from activated sludge, reveal the signaling pathway responsible for the expression of their functional genes, and identify their essential exogenous growth factors.

4) *Sludge bulking prevention*. Sludge bulking leads to the sludge loss. Therefore, it will be necessary to clarify the mechanism of more extracellular matrix being secreted under low temperature conditions, as well as to explore the pathway of inhibiting extracellular matrix synthesizing. Finally, efforts should be made to propose a targeted prevention strategy and to develop inactivation reagents or methods applied to inhibit sludge bulking.

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