Research advances of microbial denitrification and application in black and odorous water

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Abstract: High nitrogen content is considered to be one of the main reasons for the black and odor phenomenon in rivers. Microbial denitrification has been widely concerned because of its simple operation, high economic efficiency, short repair time and little impact on the surrounding environment. However, its denitrification process is also affected by environmental factors, pollutants and changes in microbial communities. In this paper, the main bacterium participating in sewage treatment of nitrification, denitrification and anaerobic ammonia oxidation were introduced, and then the adaptation situation and distribution of microbial community in each denitrification process were summarized. Finally, applications and prospects were objectively provided by microbial agents, constructed wetlands and ecological floating islands. According to the existing research results, it is believed that microbial remediation has a broad prospect in the treatment of urban black and odorous water bodies. However, it is difficult to maintain a stable bacterial community structure, denitrification activity and environmental adaptability of microbial remediation technology in river channels, which is the bottleneck of its application in the treatment of black and odorous water.

1. Introduction
At present, there is no clear definition of black and odorous water in the world. It is defined as "the generic term for water bodies showing unpleasant color and emitting unpleasant smell" in the Guidelines for the treatment of urban black and odorous water body by the Ministry of Housing and Urban-Rural Development (MOHURD) of the People's Republic of China. High nitrogen content is considered to be one of the main causes for black and odorous pollution in rivers, and the way of denitrogenation in urban rivers has received extensive attention in recent years[1]. Microorganisms exist widely in river ecosystems and play a key role in the geochemical process, especially in the transformation of nutrients (such as nitrogen, phosphorus and sulfur). It is found that the self-purification function of river ecosystems can remove nitrogen pollutants through the processes of ammoniation, nitrification, denitrification and anaerobic ammonia oxidation driven by microorganisms[2].

Microbial remediation is to achieve the purpose of water purification on the basis of the biological metabolism of pollutants by microorganisms. It has the advantages of simple operation, economic efficiency, short remediation time and little impact on the surrounding environment. However, the process of microbial denitrogenation is also affected by environmental factors, pollutant types and changes in microbial communities[2-3]. The flow velocity of urban rivers and the pollutants received will affect the oxygen demand of microorganisms in sediments, as well as the structure and integrity of eukaryotes and bacterial communities [4]. Therefore, unlike wastewater treatment plants, the microbial action of river ecosystems is affected by more factors. Researchers have studied the influencing factors...
of denitrification microorganisms in wastewater treatment from various aspects, and also had some practices in water ecological management. In this paper, the characteristics of nitrogen cycling microorganisms in sewage treatment, the selection effect of environmental factors on nitrogen cycling microorganisms, and the application of microorganisms in water environment treatment practice were summarized, hoping to provide reference for the treatment of black and odorous water by microbial remediation technology.

2. Influencing factors of microbial nitrogen cycling process
The common nitrogen cycling processes in water mainly include nitrification, denitrification and anaerobic ammonia oxidation (ANAMMOX). Among them, nitrification process includes aerobic ammonia oxidation and nitrite oxidation processes, and denitrification process and ANAMMOX process are collectively referred to as "nitrate dissimilation reduction process". The activity of microorganisms involved in nitrogen cycle is easily affected by environmental factors. At present, it is found that dissolved oxygen (DO), temperature, substrate concentration, pH and microbial community distribution are the main environmental factors affecting the activity of microorganisms in wastewater denitrification.

2.1. Nitrification process
Traditional biological nitrification is a two-stage reaction, in which ammonia-oxidizing bacteria (AOB) belonging to *Nitrosococcus*, *Nitrosomonas* and *Nitrosospira* complete the nitrosation process, while nitrite-oxidizing bacteria (NOB) belonging to *Nitrobacter*, *Nitrococcus*, *Nitrospina* and *Nitrospira* complete the nitrification process[5]. This is a key process in the nitrogen cycle whose production determines the path of the nitrogen cycle.

Both AOB and NOB are autotrophic microorganisms, but NOB is more heterogeneous than AOB and is composed of four groups of bacteria, namely *Nitrobacter*, *Nitrococcus*, *Nitrospina* and *Nitrospira*, which are quite different in evolution, while AOB all belong to *Betaproteobacteria*. *Nitrobacter*, *Nitrococcus* and *Nitrospina* belong to *Alphaproteobacteria*, *Gammaproteobacteria* and *Deltaproteobacteria* respectively, while *Nitrospira* belongs to a special microbial phylum, that is, *Nitrospira*.

At present, the control measures of environmental factors aimed at enhancing nitrification activity of AOB and NOB microbial community are to control the dissolved oxygen (DO), temperature, pH, free ammonia (FA), free nitrite acid (FNA), sludge retention time (SRT), etc.

2.1.1 DO
As one of the necessary substrates for nitrification, proper DO is an important condition to ensure the activity of nitrifying bacteria. Oxygen supply mode and DO concentration are the key parameters of wastewater treatment process control, which affect the development of microorganisms in nitrogen cycle. The affinity constants of AOB and NOB for oxygen are 0.3 mg/L and 1.1 mg/L, respectively[6], which are quite different. It is also found that AOB has strong tolerance to the change of DO, and the alternate aerobic and anaerobic environment is beneficial to the enrichment and growth of AOB[7]. Therefore, it is feasible to control the appropriate DO level by engineering means for strengthening ecological denitrogenation in river courses. In recent years, many scholars have studied the regulation of NOB growth by controlling DO concentration to maintain long-term stable operation of nitrification system.

In the wastewater treatment system, for the suspended sludge system, it is generally considered that short-cut nitrification may be realized when the environmental DO is less than 1.20 mg/L, but it is difficult to realize with high DO. According to Chen et al.[8], Xie Qinglin et al.[9], and Cui et al.[10], at the DO concentration of 0.30–0.50 mg/L, 0.80–1.20 mg/L and 0.10–0.60 mg/L respectively, high NH₄⁺-N removal rate and NO₂⁻-N accumulation in short-cut nitrification are all achieved. At the same time, Wu Chunlei et al.[11] also found that it was difficult to realize short-cut nitrification when DO concentration was 2.00–2.50 mg/L. For attached growing biofilm system, short-cut nitrification can be realized at a higher DO level.
There was still confusion in the literatures about the behavior of nitrification bacteria in different DO level. Oxygen supply mode and DO concentration affect the development of microorganisms in nitrogen cycle. Thus, the microbial community structure of AOB and NOB could be adjusted through selecting sludge system and oxygen supply mode to change the supply of O2.

2.1.2 Temperature
Temperature is one of the key factors for denitrogenation efficiency. AOB and NOB are mesophilic bacteria, and their optimum growth temperature is between 20°C and 30°C. Temperature that is too high or too low will have a huge effect on the nitrification rate of nitrifying bacteria. At different temperatures, the growth rates of AOB and NOB are different. It is generally considered that the higher temperature of 30~35°C is the optimum temperature for short-cut nitrification. However, some studies have found that NOB activity is severely inhibited when the temperature is lower than 15°C, while AOB is relatively less inhibited, so NO3--N can be accumulated at lower temperature[12].

In addition, different nitrifying bacteria strains are sensitive to temperature in different degree. Siripong et al.[13] monitored the proportion of AOB in wastewater treatment plants, and found that the proportion of AOB changed obviously with the seasons. When the water temperature was low in winter, the proportion of Nitrosospira increased obviously, while when the water temperature was high in summer, the AOB was mainly Nitrosomonas. When studying the proportion of NOB at different temperatures, Alawi et al.[14] found that Nitrospira could adapt to a wide range of temperature, and grew normally at 10~28°C; however, when the temperature was lower than 17°C, the growth of Nitrobacter would be limited. Lücker et al.[15] found that Nitrotoga had a higher proportion at low temperature and was the dominant microbial phyla of NOB.

2.1.3 pH
pH has effects on the population structure of nitrifying bacteria in the following two major aspects. Firstly, the optimum pH value of nitrifying bacteria is between 7 and 8, and the growth rate of nitrifying bacteria will decrease in acidic or alkaline environment. AOB is more sensitive to pH change. Thus, it is necessary to maintain proper pH during nitrification. Secondly, the change of pH will affect the existing forms of ammonia, and then affect the population structure of nitrifying bacteria. With the increase of pH, the proportion of free ammonia (FA) in water will increase, while with the decrease of pH, the corresponding concentration of free nitrite acid (FNA) will increase. Excessive FA and FNA will significantly inhibit the activity of nitrifying bacteria. AOB and NOB have different tolerances to FA, which will inhibit NOB at the concentration of 0.1~1.0 mg/L, but will only inhibit AOB at the concentration of 10~150 mg/L[16]. FNA is an effective bactericide, and its inhibitory effect on NOB is also higher than that on AOB. Studies showed[17] that FNA may inhibit the activity of NOB to be less than 4.8% at the concentration of 0.22~1.35 mg/L, and the activity of AOB is greater than 80% in such case.

To sum up, Table 1 summarizes the suitable environmental characteristics of nitrification process. The optimum environment for nitrification process is where the dissolved oxygen is sufficient, the temperature is 20~30°C and the pH value is 7~8. It shows that sufficient dissolve oxygen (DO) and suitable temperature can remove NH4+-N from the water in the river ecosystems. Obviously, the key substrate they are competed was oxygen.

| Microbial community | Phylum          | Genus     | Oxygen affinity constant (mg·L⁻¹) | DO (mg·L⁻¹) | Temperature | pH | FA | FNA |
|---------------------|-----------------|-----------|----------------------------------|-------------|-------------|----|----|-----|
| AOB                 | Betaproteobacteria | Nitrosomonas | 0.3                               | ≤1.2        | 7~8 optimum temperature of 20~30°C; | 10~150 | 1.35 mg·L⁻¹ |
Nitrosovibrio is inhibited less than NOB at less than 15°C and 30~35°C mg·L⁻¹

| NOB Alphaproteobacteria | Nitrobacter | Inhibition value of 0.1~1.0 mg·L⁻¹ |
|-------------------------|-------------|----------------------------------|
| Alphaproteobacteria     | 1.1         | Inhibition value of 0.22~1.35 mg·L⁻¹ |
| Nitrobacter             | >1.2        | 20~30°C optimum                   |

2.2. Denitrification process

The denitrification process is the main process by which nitrogen is separated from the water and it plays an important role in reducing the nitrogen load in black and odorous water. Studies showed that the denitrification of urban river sediments can remove nearly 20% of the nitrogen load from the river[18]. Jin et al.[19] found that heterotrophic nitrification is an important nitrification process for the sediments of the Yangtze River estuary. Denitrifying bacteria mainly consist of heterotrophic denitrifying bacteria and autotrophic denitrifying bacteria, and their reactivity is usually closely related to environmental factors such as DO, temperature, carbon source, pH and carbon nitrogen ratio (C/N). Oxygen content is one of the key factors in the denitrification process of sediments. Among denitrifying bacteria, an important microbial community of denitrifying microorganisms, there are more than 50 genera and more than 130 strains frequently reported. Among them, the relatively common heterotrophic denitrifying bacteria strains are Pseudomonas, Bacillus, Hyphomicrobium, Enterbacter, Micrococcus, Brevibacterium, Alcaligenes, Paracoccus, etc[20]. With further study on heterotrophic denitrification process, recent studies have also found that, in addition to anaerobic denitrification process, there is also aerobic denitrification process, and some aerobic denitrifying bacteria strains have been isolated, such as hiosphaera pantotropha, Alcaligenes faecalis, Pseudomonas putida, Pseudomonas stutzeri T13, Pseudomonas stutzeri YZN-001, Klebsiella pneumonia CF-S9 and Acinetobacter sp. SYF26.

Recently, researchers found that some microorganisms in the wastewater containing nitrogen with low C/N can use reductive sulfides (elemental sulfur, sulfides, thiosulfates, etc.) as electron donors for autotrophic denitrification. The microbial community in this process is widely distributed in sea, land and other natural environment and artificial environments, and the reaction is conducted by chemoautotrophic bacteria, mainly Thiobacillus denitrificans. At present, it is found that sulfur-based autotrophic denitrifying bacteria mainly belong to Proteobacteria and are classified into the following categories[21]:

1) Chemoautotrophic bacteria: mainly including Thiobacillus denitrificans, Thiomicrospira denitrificans, Thiobacillus thioparus, etc.
2) Facultative autotrophic bacteria: mainly including Thiobacillus delicatus, Thiosphaera pantotropha, Thiobacillus thysaniris, Paracoccus denitrificans;
3) Filamentous bacteria, such as Thioplaca and Beggiatoa;
4) Other strains capable of denitrification and denitrogenation, such as Bacillus, Rhodococcus, Pseudomonas and Ochrobactrum.

Table 2 summarizes the typical characteristics of sulfur-based autotrophic denitrification[22-26].

| Category                        | Representative microorganism | Species            | Temperature          | pH               | Carbon source                                | Oxidation environment                        |
|--------------------------------|------------------------------|--------------------|----------------------|------------------|---------------------------------------------|----------------------------------------------|
| Obligately chemoautotrophic     | Thiobacillus                 | Thiobacillus       | 10~37°C (28~30°C     | 4.0~9.5          | CO₂ or inorganic carbon source; It can grow under both aerobic and anaerobic conditions |
| denitrificans                   |                              | optimum)           | (6.5~7.0 optimum)    |                  |                                             |                                              |
### Facultative autotrophic bacteria

| **Thiobacillus thioparus** | **Thiobacillus** |
|---------------------------|------------------|
| 28°C optimum              | 4.5~7.8 (6.6~7.2 optimum) |
| CO₂ or inorganic carbon source | It can only reduce nitrate to nitrite |
| When NO₃⁻ is the electron acceptor, S⁰ and N₂ are the main end products |

| **Thiobacillus denitrificans** | **Thiobacillus** |
|-------------------------------|------------------|
| 22°C optimum                  | 7.0 optimum      |
| CO₂ or inorganic carbon source |                  |
| Under anaerobic conditions, the sulfur oxidation capacity will be lost or the growth efficiency will be very low, but denitrification can be realized in both aerobic and anaerobic conditions |

| **Paracoccus denitrificans** | **Paracoccus** |
|-----------------------------|----------------|
| 30°C optimum                | 7~9.5          |
| CO₂ or inorganic carbon source, complex organic compound | Filamentous sulfur-oxidizing bacteria can store a large amount of nitrate in vacuoles for anaerobic autotrophic oxidation of sulfides, and most of them exist in the sea. |
| Under anaerobic conditions, the sulfur oxidation capacity will be lost or the growth efficiency will be very low, but denitrification can be realized in both aerobic and anaerobic conditions |

| **Thiobacillus delicatus** | **Thiobacillus** |
|---------------------------|------------------|
| 28°C optimum              | 7~9.5            |
| CO₂ or inorganic carbon source |                  |
| Under anaerobic conditions, the sulfur oxidation capacity will be lost or the growth efficiency will be very low, but denitrification can be realized in both aerobic and anaerobic conditions |

| **Thiobacillus thyasiris** | **Thiobacillus** |
|---------------------------|------------------|
| 28°C optimum              | 7~9.5            |
| CO₂ or inorganic carbon source |                  |
| Under anaerobic conditions, the sulfur oxidation capacity will be lost or the growth efficiency will be very low, but denitrification can be realized in both aerobic and anaerobic conditions |

| **Thiosphaera pantotropha** | **Paracoccus** |
|-----------------------------|----------------|
| 28°C optimum                | 7~9.5          |
| CO₂ or inorganic carbon source, complex organic compound | Filamentous sulfur-oxidizing bacteria can store a large amount of nitrate in vacuoles for anaerobic autotrophic oxidation of sulfides, and most of them exist in the sea. |
| Under anaerobic conditions, the sulfur oxidation capacity will be lost or the growth efficiency will be very low, but denitrification can be realized in both aerobic and anaerobic conditions |

### Filamentous bacteria

| **Thioplaca** | **Thioploca** |
|---------------|--------------|
| Beggiaota     | Beggiaota    |
| Filamentous sulfur-oxidizing bacteria can store a large amount of nitrate in vacuoles for anaerobic autotrophic oxidation of sulfides, and most of them exist in the sea. |
| Under anaerobic conditions, the sulfur oxidation capacity will be lost or the growth efficiency will be very low, but denitrification can be realized in both aerobic and anaerobic conditions |

Besides being proved in laboratory, the process of sulfur-based autotrophic denitrification has also been tried in practice. Huang et al.[27] maintained the denitrogenation effect of the sulfur-based autotrophic denitrification constructed wetland by intermittent aeration in low temperature (-5~10°C). It found that suitable sulfide can promote denitrification of sediments. Ren et al.[28] successfully treated low-carbon wastewater through sulfur-based autotrophic denitrification constructed wetland, so that it meet the reuse water standard of iron and steel enterprises.

Therefore, the denitrification process involves a wide range of microbial community types, and screening different microbial community can meet the denitrogenation requirements of black and odorous water in different environment. It is a good way to remove nitrogen from water by cultivating specific microbial community according to the characteristics of target water and carrying out corresponding engineering strengthening measures.

### 2.3. ANAMMOX process

An aerobic ammonium oxidation (ANAMMOX) is a new microbial nitrogen transformation approach, with chemoautotrophic ANAMMOX bacteria as executive bacteria, where N₂ is generated by the direct interaction of NO₂⁻ and NH₄⁺ without organic carbon source and O₂. This process does not produce or rarely produces greenhouse gas N₂O. At present, five genera of Planctomycetales i.e., Brocadia, Kuenenia, Anammoxoglobus, Jetteni and Scalindua have been classified as ANAMMOX bacteria[29]. ANAMMOX bacteria are very sensitive to environmental factors, so their activity is easily affected by environmental factors. However, they are widely distributed in nature, even in extreme temperature and pH environment.

#### 2.3.1. Substrate concentration

The study on the NH₄⁺-N concentration of ANAMMOX substrate still focuses on the field of high NH₄⁺-N (>500 mgN/L) wastewater denitrogenation. Under the low concentration of NH₄⁺-N (<100 mgN/L), it is difficult to achieve sufficient and stable supply of key substrate nitrite nitrogen[30]. Therefore, when the NH₄⁺-N concentration of the influent is reduced to 100 mg/L, the NH₄⁺-N removal rate is reduced to about 40%, and the NH₄⁺-N removal load is reduced to about 0.5 kg N·m⁻³·d⁻¹, in a dramatic manner[31].

#### 2.3.2. Temperature

The optimum growth temperature range of ANAMMOX bacteria is 30~40°C. de Almeida Fernandes et al. found that the denitrogenation efficiency of ANAMMOX decreased with the temperature decreasing from 35°C to 20°C[32], so the temperature of most ANAMMOX reactors maintained above 30°C[33] to alleviate
the problem that the growth rate of anaerobic ammonoxidation bacteria decreased due to low temperature and the operation efficiency of ANAMMOX was low. However, Gilbert et al. [34] also achieved a low denitrogenation efficiency of about 40% in short-cut nitrification–ANAMMOX process at 13°C. Chen et al. [35] found that the activity of ANAMMOX bacteria could not be inhibited at 25°C.

2.3.3. **Dissolved oxygen (DO)**

DO should be strictly controlled in ANAMMOX reaction to avoid reversible or even irreversible inhibition. Egli et al. [36] observed reversible and irreversible ANAMMOX inhibition at DO concentrations of 0.08 mg/L and 1.44 mg/L, respectively. Irreversible inhibition was also observed under intermittent aeration (with DO concentration of 0.1~0.16 mg/L). Therefore, ANAMMOX bacteria need to survive under anaerobic conditions with low DO.

2.3.4. **pH**

A stable pH environment should be maintained for the stable metabolism of ANAMMOX. The pH value can directly affect the growth and enzyme activity of bacteria, and can also indirectly affect the activity of ANAMMOX by affecting the concentration of FA and FNA around the microbial community. The results showed that the optimum pH value for the growth and activity of ANAMMOX bacteria in wastewater treatment was between 7.2 and 7.6 [37], but its adaptable pH range was between 6.5 and 9.3. Because of its strong alkali resistance, it has higher activity against other microbial community in higher pH environment.

2.3.5. **Distribution of microbial community**

In recent years, there have been extensive studies on ANAMMOX in different spatial scales, including river sediments in region scale, paddy soils, river sediments in small area scale, wetland and lake sediments and even micro-scale in rhizosphere soil. It shows that ANAMMOX bacteria exist widely in different scales, especially in all sediments studied. Therefore, geographical distribution and community status of ANAMMOX bacteria can be used to effectively enhance the role of microorganisms in water nitrogen cycle. Sediment is considered to be the key part of denitrification and ANAMMOX in water system due to the existence of oxygen–anoxic interface, and the microorganisms at this interface have obvious denitrogenation activity. Some studies have observed that there is a high ANAMMOX rate at the upper limit of saturated soil, which indicates that the ANAMMOX process has significant activity at the water-land interface [38]. The results also showed that ANAMMOX was widespread in freshwater, and high abundance of ANAMMOX could be detected in dryland soil with high water content (>29%) [39]. Even some studies have shown that ANAMMOX bacteria could be reactivated by water after long-term dormancy in arid soil [40]. In addition, in the ocean, up to 50% of nitrogen cycle is caused by ANAMMOX process. Table 3 summarizes the common environments of ANAMMOX bacteria.

| Microbial community | Phylum       | Genus               | Common environment                  |
|---------------------|--------------|---------------------|-------------------------------------|
| ANAMMOX bacteria    | Planctomycetales | Brocadia         | Terrestrial soil, freshwater, wetland and freshwater extreme environment |
|                     |              | Kuenenia Anammoxoglobus | Artificial reactor                 |
|                     |              | Jetteni Scalindua | Soil, wetland and artificial reactor |
|                     |              |                     | Marine environment                 |

In recent years, a biochemical reaction with the characteristics of "ANAMMOX" and "iron reduction" has been discovered, which is called Feammox. According to the study of Huang et al. [41], when the culture system DO<0.02 mg/L, it was beneficial to ammonia oxidation. Compared with DO>0.02 mg/L, the abundance of Acidimicrobiaceae bacterium A6 capable of ammonia oxidation and iron reduction increased in total bacteria, while the abundance of other AOB and ANAMMOX bacteria decreased. This strain called A6 provides a microbial basis for Feammox.

Liu [42] considered that Feammox can adapt to acidic environment (pH 4–5) and keep strong activity at low temperature, and it can be combined with ANAMMOX. Huang et al. [41] found that 64.5% of ammonia nitrogen removal rate was achieved in the continuous flow anaerobic biofilm reactor with pH value of 4–5.
and NH$_4^+$-N of 60 mg/L.

It can be seen that ANAMMOX is suitable in the anaerobic weak alkaline environment, and ANAMMOX bacteria community is widely distributed in nature, which can be cultivated and enriched in most environments. In weak acidic environment, if suitable iron ions are provided as electron donors for its reaction, Feammox can occur, and nitrogen can also be removed, so it is widely used in denitrogenation of river ecosystems.

To sum up, DO concentration is very important for microbial nitrogen removal, and nitrification, denitrification and ANAMMOX have different requirements for it. Therefore, creating DO concentration gradient in river channel is the premise of microbial nitrogen removal. At present, anaerobic and aerobic alternate environment can be created in river channel through constructed wetland structure design, microbial embedding and biofilm filler. There are many environmental factors that affect the function of nitrogen cycling microorganisms.

Although the microbial community is widely distributed, the application of microbial nitrogen removal technology in black and odorous water still faces a series of difficulties. It is a feasible means to control and create suitable conditions for different nitrogen removal processes by engineering means. Therefore, the potential existence of the metabolic pathways proposed here may provide a new idea made with biofilm systems consisting of aerobic ammonia oxidizing bacteria in the outer layers of the biofilm, and anammox bacteria in the anaerobic core. In these biofilm systems, the community structure of AOB and NOB may play the key role who would be frequently encountered at the aerobic-anaerobic interface.

3. Application of microbial denitrogenation in black and odorous water

3.1. In-situ remediation through microbial agents

Because the natural purification speed of indigenous microorganisms in water environment is slow, in order to degrade pollutants as soon as possible and restore water ecology, people pay attention to applying microbial agents in pollutant removal from rivers. Due to strong target, high activity and relatively low cost, microbial agents have many experimental studies and practical applications in the treatment of black and odorous rivers.

Tang et al.[43] used denitrogenation bacteria agent and biological stimulants to remediate nitrogen contaminated sediments. After 115 days of treatment, the removal rate of TN in sediments was 14.7%, and the removal rate of nitrate nitrogen in the overlying water was also improved. It was found that the diversity of bacterial community in the sediments increased significantly, and the dominant bacterial phyla were Nitrospirae, Deferribacteres and Chloroflexi. Guo et al.[44] added Pseudomonas stutzeri strain T1 to Taihu Lake water samples. It was found that the removal rate of ammonium nitrogen and nitrate nitrogen by the strain reached 60% and 75% respectively, and the final sample water quality was improved from Class V to Class II. Wu et al.[45] put denitrogenation bacteria agent into a river in Xinbei District, Changzhou City, and found that the agent had certain purification effect on excessive NH$_2$-N, TN and TP in the river. The degradation efficiency for NH$_2$-N is higher but easy to rebound and rise. Wang[46] added compound microbial agent in the Qiongjiang River at Xiaodu section of Tongnan District for biological enhanced treatment of water. The removal rates of COD, NH$_3$-N and TP reached 51.72%, 41.44% and 52.38% respectively, which proved that biological enhanced technology can effectively treat river. Therefore, it can be found that suitable screened microbial agents have ideal denitrogenation capacity for black and odorous water. However, these remain to be studied about the impact of microbial agents on the environment and how to keep the permanent effect.

3.2. Constructed wetland treatment

Constructed wetland is called ecological wastewater treatment plant, which removes pollutants from wastewater by physical adsorption and biodegradation. Because of its simple operation and maintenance, low management cost, no secondary pollution and landscape value, constructed wetland has been widely used. However, the denitrification efficiency of constructed wetland depends on organic carbon source, and the lack of organic carbon is the primary obstacle to nitrate removal. And the biggest obstacle to the development of constructed wetlands is that the purification efficiency of it will be affected by seasonal low temperature.
Zheng et al. [47] used the combined constructed wetland for pollution treatment of Zaohe River in Xi'an. Using the combined process of horizontal subsurface flow and surface flow for constructed wetland, they construct a wetland with an area of 8000 m², and operated for two years with a treatment capacity of 362 m³/d. The average treatment efficiencies of COD, SS, NH₄⁺-N and TP were 74.5%, 92%, 57.5% and 69.2% respectively. Huang et al. [48] improved denitrogenation capacity and reduced greenhouse gas emissions by constructing a comprehensive vertical flow wetland, and found that ANAMMOX, denitrifying anaerobic methane oxidation (DAMO) and denitrification reactions can occur simultaneously in the wetland, and the denitrification process is stable in the system, which proved that the denitrification ability for polluted water can be enhanced by coupling various denitrogenation processes in the constructed wetland. By combining aerobic/anoxic process and Fe/C micro electrolysis technology with vertical flow constructed wetland, Deng et al. [49] established efficient water system composed of aerobic organisms (e.g. *Nitrosomonas*), anaerobic organisms (e.g. *Nitrospira*), autotrophic denitrifying bacteria (e.g. *Thiobacillus, Hydrogenophaga* and *Thiobacillus*), heterotrophic denitrifying bacteria (e.g. denitrifying bacteria) and Fe oxidizing bacteria (FOB) (e.g. *Acidithiobacillus ferrooxidans*). The removal rates of ammonia nitrogen, total nitrogen, total phosphorus and COD were 94.3%, 86.2%, 98.0% and 92.7% respectively in the low temperature environment of -11.5°C~8.0°C, achieving excellent effects of grey water treatment.

### 3.3. Purification through ecological floating bed

Under the principle of ecosystem, ecological floating bed (EFB) uses environment-friendly materials to build environment for aquatic plant culture and growth in water, integrates biological dynamics and soilless culture, and plants terrestrial or higher aquatic plants in polluted water, so as to purify water, provide beautiful environment and a place for birds and fish to perch and lay eggs. It is not affected by water depth and eutrophication degree, and can improve landscape and save construction cost. Olguín et al. [50] established and tested the EFBs in two artificial ponds, and found that the synergistic effect of microorganisms and plants can effectively remove nutrients, especially nitrate, increase DO in water to a certain extent, and remove 9%~86% of *Escherichia coli*. But the development of EFB is restricted by flood control and drainage requirements in most districts.

To sum up, because the input of exogenous nitrogen in urban rivers is complex and influenced by many environmental factors, the internal reaction of river sediments is more intense and the reaction mechanism is more complex than that of other sediments. The improvement of black and odorous water is a highly professional systematic project, and it is difficult to eliminate black and odorous water by using a single treatment technology. Therefore, in practical engineering, different technologies need to be combined and applied, and a long-term mechanism should be established for permanently clean water.

### 4. Conclusion

Microbial remediation technology is a simple, economical and efficient technology with short remediation time and little impact on the surrounding environment, which has broad application prospects in the field of urban black and odorous water treatment. There are a large number of microorganisms related to nitrogen cycle in riverbank sediments and bottom sediments, but the microbial community structure established under the traditional theory of biological denitrogenation from wastewater has high requirements for the environment and is difficult to support its enrichment and efficient denitrogenation in rivers. The microbial nitrogen cycle involves a wide range of microbial types, and screening different microbial community can meet the denitrogenation requirements of black and odorous water in different environment. Therefore, it is a good way to cultivate specific microbial community. In practice, microbial agents can effectively purify water in a short time, but the relatively simple microbial community structure is not suitable for maintaining the purification effect for a long time. At present, the purification efficiency of constructed wetlands is also affected by seasonal low temperature environment, and the development of EFB is restricted by flood control and drainage requirements. Therefore, the practical forms of microbial denitrification in black and odorous water need to be further explored. Due to the different requirements of DO and substrate in different processes of nitrogen cycle, reasonable microbial community structure of biofilm in natural environment is particularly important for its stability and adaptability of denitrogenation capacity. Therefore, further
study on the microbial community structure is necessary for the permanent clean rivers. Future studies under the field of urban black and odorous water treatment will need to clarify which conditions specifically build for ideal microbial community structure, and what their ecological niche is in these kinds of environments.

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