

Application of Microorganisms in Treating the Nitrogen-Containing Environment, Heavy Metal Water, and Sulfur-Containing Environment

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Abstract. Environmental problems including nitrogen pollution, heavy metal pollution, and the air's sulfur pollution are becoming increasingly serious nowadays, and pose a critical threat to people's health. Utilizing microorganisms to fight pollution on account of its resistance and high efficiency to the environment. By choosing to employ nitrifying bacteria to degrade nitrogen pollution, and meanwhile, applying fungus for heavy metals absorption and using SRB for desulfurization. According to literature research, it is found that nitrifying bacteria possess the ability to degrade fast. Fungus, affected by pH, adsorption time, initial concentration, and coexisting ion concentration, can efficiently adsorb heavy metal ions. SRB, influenced by different strains, pH, and temperature, embodies higher desulfurization efficiency compared with desulfurization technologies. This work provides a comprehensive understanding of these three types of pollution and suggests microorganisms as the most efficient solutions for reducing the threat of pollution to people in the future.

Keywords: Pollution, Nitrogen, Heavy metal, Sulphur, Microorganism.

1. Introduction

Water pollution caused by effluents, livestock culture, mining and domestic sewage discharge is the leading cause of generating N, heavy metal ion hazards. Nitrogen in the form of free ammonia and ammonium ions in water can be a significant pollutant. Its content has always been one of the essential indicators of water quality, and severe ammonia nitrogen pollution will endanger aquaculture and affect human health. Thus, nitrogen elements have a significant impact on aquaculture. It mainly comes from domestic sewage and livestock manure. An appropriate amount of ammonia is usually added to the water in the aquaculture process to remove bacteria and plankton attributed to its toxicity and to ensure the healthy growth of aquaculture organisms. For example, appropriate ammonia concentrations can be used to treat dactylodiasis during the aquaculture of *Japanese eel* [1]. Heavy metals that lead to environmental pollution mainly include mercury, cadmium, lead, arsenic and other elements with significant biological toxicity, zinc, copper, and cobalt with specific toxicity. They can be converted into toxic alkyl compounds, absorbed by plants and then accumulate in the human body through the food chain, affecting human health. The micro-element concentrations in river sediment compartments can be used to reveal the history and intensity of local and regional pollution [2].

In addition, air pollution is also a significant environmental pollution problem. In 2015, Nature published a report on biological death [3]. This report pointed out that the number of organisms killed by air pollution accounted for the most significant proportion of the total number of biological deaths globally, indicating that air pollution treatment was urgently needed. Sulfur-containing substances produced after the combustion of fossil fuels are one of the critical factors causing a series of environmental problems such as atmospheric pollution and acidic precipitation. Sulfur can be

removed from coal simultaneously when microorganisms are used for metabolism under suitable conditions. And two types of microorganisms can achieve the effect of desulphurization. Therefore, microorganisms have many application prospects in environmental pollution control. This paper will briefly introduce nitrogen pollution in water, heavy metal ion pollution in soil and water, and sulfur pollution in the atmosphere. And summarized the degradation effect of microorganisms such as bacteria and fungi.

2. Microbial Degradation Of Ammonia Nitrogen

2.1. N Pollution And Its Toxicity

Ammonia nitrogen can poison animals and even cause animal death in severe cases. Studies have shown that if aquatic animals live in low concentration ammonia nitrogen water for a long time, it is easy to produce bacterial infection, slow growth and development, and reduced tolerance to the external environment. The high concentration of ammonia nitrogen will affect the catalytic effect of enzymes and the stability of cell membranes in aquaculture animals and destroy their excretion system and osmotic balance. The acute toxicity of ammonia is primarily reflected in its effects on the central nervous system of vertebrates. Almost all vertebrates experience convulsions and death soon after ammonia poisoning. For instance, the present data suggest that an elevated concentration of ammonium ions will replace the action of potassium ions in the nervous system to depolarise neurons, leading to over activation of NMDA type glutamate receptors. Therefore, excessive calcium ion influx. Ultimately, it causes the central neuron system cells to die [4]. In addition, in converting ammonia nitrogen into nitrate nitrogen, oxygen in aquaculture water can be consumed (1 mg ammonia nitrogen requires 4.6mg dissolved O₂ to be oxidized into nitrate-nitrogen), which causes insufficient dissolved oxygen in the water. Besides, once the nitrate-nitrogen is formed, it can oxidize ferrous ions on hemoglobin in organisms. The protein loses the ability to carry oxygen molecules and eventually causes the death of organisms.

2.2. The Nature Of Microbial Degradation Of Ammonia Nitrogen

In nature, the nitrogen cycle (Figure.1) is an integral part of the global biogeochemical cycle. It mainly has the following several processes, including living organisms. Plants assimilate inorganic nitrogen into organic nitrogen. And then, organic nitrogen moves to the animal along the food chain. In the remains of animals and plants, organic N compounds in the discharge are decomposed by microorganisms to form ammonia, which in the soil is eventually oxidized to nitrate by the action of bacteria. Nitrate can also be reduced to nitrite and further reduced to nitrogen. The section involving microorganisms can also be subdivided into several specific steps. First, organic nitrogen in wastewater is converted to ammonia nitrogen. The process is called ammonification. The organisms involved in ammonification are ammonifying bacteria. Common ammonifying bacteria include *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas fluorescens*, and *Proteus*. They can secrete extracellular proteases to hydrolyse proteins into amino acids, subsequently denitrified to produce ammonia nitrogen. Later, ammonia nitrogen is sequentially converted into nitrite nitrogen and nitrate nitrogen by the action of nitrifying bacteria and ammonia-oxidizing bacteria. The process is called nitrification. Ammonia-oxidizing and nitric acid bacteria are called nitrifying bacteria and are chemoautotroph microbes. Generally, only inorganic carbon can be used instead of organic carbon to sustain its growth. Therefore, the growth cycle of nitrifying bacteria is prolonged. In addition, ammonia-oxidizing bacteria and nitric acid bacteria are strictly aerobic, so nitrification needs to be performed under oxygen-sufficient conditions.

Common ammonia-oxidizing bacteria can be divided into *Nitrosomonas*, *Nitrosospirillum*, and *Nitrosococcus*. Similarly, nitrifying bacteria can be divided into three groups. They are *Nitrobacter*, *Nitrospira*, and *Nitrococcus*, respectively. Because the whole nitrification process needs to be completed by two different chemical energy autotrophic bacteria together, nitrification is specifically divided into two main steps. First, ammonia nitrogen is converted to nitrite nitrogen by the action of

ammonia-oxidizing bacteria. This step determines the rate of nitrification. Subsequently, the nitrite nitrogen is converted to stable nitrate nitrogen. This is the end of the nitrification process. The last two steps of the entire biological denitrification process, the sequential conversion of nitrite nitrogen to nitrate nitrogen and nitrogen gas, are called denitrification. Microorganisms capable of denitrifying are called denitrifying bacteria. Common denitrifying bacteria include *Pseudomonas spp.*, *Alcaligenes spp.*, and *Arthrobacter spp.*

The most predominant application of nitrifying and denitrifying bacteria is the activated sludge process in secondary treatment in wastewater treatment. It has found mature large-scale applications (Figure.2).

The activated sludge process, using microorganisms as the main body, combines wastewater with sludge-like flocs (clumps aggregated by microorganisms) to stir and aerate, allowing the organic, inorganic contaminants in the wastewater to decompose, from which the biological sludge is subsequently isolated, and partial sludge can be refluxed into an aeration tank as required.

2.3. Other Bacteria That Degrade Ammonia-N Contamination

Many nitrifying bacteria do not belong to autotrophs, and denitrifying bacteria do not belong to anaerobes. They are heterotrophic nitrifiers and aerobic denitrifiers, respectively. Heterotrophic nitrifiers convert ammonium nitrogen to hydroxylamine, nitrite, and nitrate while using organic substrates for growth. Aerobic denitrifiers are a class of heterotrophic bacteria that perform denitrification under aerobic conditions with an organic carbon source as an energy source and are mainly found in the genera *Pseudomonas*, *Alcaligenes*, *Paracoccus*, and *Bacillus*. In 1983, Robertson first isolated the aerobic denitrifier *Thiosphaera pantotropa*, a strain that has both aerobic and heterotrophic denitrification capacities in a sulphur removal and denitrification treatment system. Therefore, it is also the first isolated heterotrophic nitrifying-aerobic denitrifier [5].

Many current studies on heterotrophic nitrifying aerobic denitrifiers are in progress, mainly for the following reasons. Although autotrophic nitrifying bacteria have high nitrification rates, many limiting factors exist. First, its growth is prolonged, and the bacterial concentration is difficult to maintain at a high level during the biological denitrification process. Second, autotrophic nitrifying bacteria have extremely demanding environmental requirements, with an optimal growth temperature of around 30 degrees C and thus a meagre nitrification rate in winter. And it requires higher dissolved oxygen concentration, neutral partial base condition, and a low C/N ratio (due to its growth on an inorganic carbon source) to grow and undergo nitrification.

Whereas heterotrophic nitrifiers have many more relaxed environmental requirements than autotrophic nitrifying bacteria, they are still better able to nitrify at low temperatures and high C/N ratios and do not require giant amounts of dissolved oxygen. Thus, they can reach higher bacterial concentration, thus making up for the disadvantage that their nitrification rate is lower than that of autotrophic nitrifying bacteria.

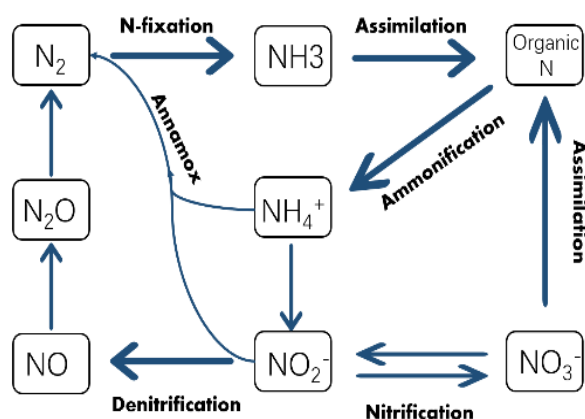


Figure 1 N-cycle in nature

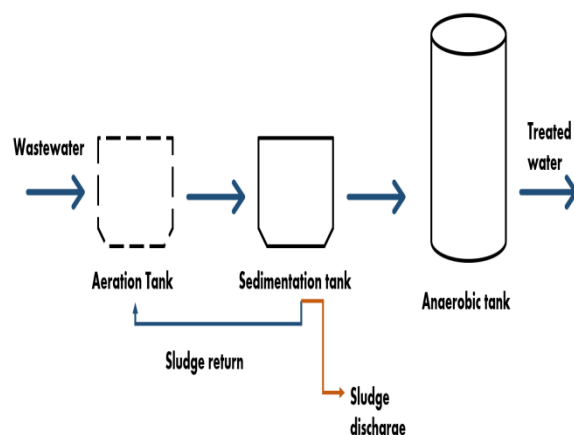


Figure 2 Activated sludge method

3. Microbial Treatment Of Heavy Metal Ions In Polluted Water

Microbial adsorption uses biological, chemical structure and composition characteristics to adsorb heavy metal ions in wastewater and through solid-liquid separation to remove heavy metal ions in wastewater. Many microorganisms can adsorb metal ions, such as algae, bacteria, and fungi, often chosen as adsorbents.

3.1. The Adsorption Process Of Heavy Metal Ions

Water polluted by heavy metals will change with time, and the concentration of heavy metal ions will be higher and higher. Heavy metal ions inhibit the metabolism and reproduction of microorganisms in these waters. These microorganisms make structural and metabolic changes to survive in heavy metal environments. In a rich metal environment, these microorganisms have strong resistance to the corresponding heavy metals and adsorption capacity.

The fungus is a eukaryotic microorganism with a large quantity and is widely distributed in nature. Most fungi exhibit filamentous or hyphal growth. The fungus can form luxuriant mycelium. The mycelium is coarser and more extended. The spread has no limitation and a fast growth speed. The fungus spores are tiny and few and have strong stress resistance. These characteristics lay a foundation for the fungi to adsorb heavy metal ions with high intensity. Fungus and yeast biomass from this process can be readily available as a source for metal removal processes [6].

According to Pedro's experimental design, making a medium that contains glucose, yeast extract, sterile vitamin solution, trace metal solution and so on to culture the fungus, then using the fungus to complete the adsorption [7]. In a recent study, the adsorption of metal ions by fungus is generally divided into two stages [8]. The first stage is the fast adsorption stage, which is passive. In this process, there are many polymers outside the cell, functional groups on the cell wall, and metal ions binding. It is characterized by fast, reversible, and independent of Energy Metabolism. The second stage is the slow adsorption stage, which is initiative and usually reaches the equilibrium adsorption capacity in a few minutes. At this stage, it often takes several hours to get equilibrium adsorption and then transfer heavy metal ions to the cell by intracellular metabolism and cell diffusion process control. The fungus has a large adsorption capacity for various metal ions, which is influenced by many factors, such as PH, adsorption time, initial concentration of metal ions, and coexisting ion concentration.

3.2. Influencing Factor Of Absorption

3.2.1. pH

The pH of the solution affects the activity of functional groups, the chemical action of metal ions and the complexing competition of ions at the complexing sites. In Yeti's study, When *Phanerochaete*

Chrysosporium adsorbed Pb^{2+} , the solubility of Pb^{2+} was higher when the pH value was lower, and the protonation of the cell wall surface was higher, which decreased the affinity between the cell wall and PB, resulting in a lower adsorption capacity [9].

3.2.2. Adsorption Time

The adsorption time is another essential factor which affects the adsorption capacity. The first stage of adsorption is the rapid adsorption stage. It intakes a few minutes to reach the balance of adsorption; the second stage is the slow adsorption stage; this stage often needs a few hours to achieve the balance of adsorption. According to Yeti, When *Phanerochaete Chrysosporium* adsorbed PB, the adsorption rate was faster in the first hour and reached equilibrium in two hours [9].

3.2.3. Initial Concentration Of Metal Ions

The initial concentration of heavy metal ions is also an essential factor affecting adsorption capacity. Increasing the initial concentration of metal ions can increase the initial adsorption rate of ions. The larger the ratio of heavy metal ions concentration to biomass, the larger the adsorption capacity of the unit adsorbent.

3.2.4. Coexisting Ion Concentration

Finally, the content of coexisting ions in the solution also influences the adsorption capacity. The number of metal-binding groups on the cell surface is limited, and the addition of coexisting ions leads to competition for these active groups, which reduces metal ion adsorption.

Table 1. The ability and condition of fungus to adsorb metals by Gao Wei [8]

Heavy metal ions	pH	Adsorption time(min)	Initial concentration(mg/L)	Adsorption capacity (m g/g)
Cu^{2+}	6.0	120	-	0.25
Cr^{3+}	4.0	30	3000	59.8
Pb^{2+}	4.0	180	10	80
Cd^{2+}	5.5	60	5	52.8
Cd^{2+}	5.5	1440	393.5	63
Zn^{2+}	7.5	240	100	80

3.3. Other Applications Of Adsorption Technology In Heavy Industry

The adsorption process can be used to treat oily industrial wastewater, such as seawater pollution caused by oil tanker spills, factory oily waste, etc. The adsorption method can also treat the wastewater in the textile industry. The rise of the printing and dyeing industry also brings about an increase in wastewater pollution. The chroma and COD in the wastewater are removed through solid adsorption capacity.

3.4. Limitations Of Fungus Adsorption

Although many studies have been carried out on the adsorption of heavy metals by fungus, few cases have been applied because of the high prices and the complex process. To realise the large-scale industrial application as soon as possible, it is necessary to do more detailed work on the cultivation of fungus, the research of adsorption mechanism, the optimisation of the adsorption model, gene technology, and immobilisation technology and so on.

4. Desulfurization By Microorganisms

4.1. Oxidizing Microorganisms

A typical example is the oxidizing microorganism, *Thiobacillus Ferrooxidans* (T.F) bacteria. Abu El-Eyuooun said that T. F bacteria is a Gram-negative bacterium, with the characteristics of aerobic,

chemoautotrophic, and acidophilic. It is suitable for the medium-temperature environment [10]. T. F bacteria widely exists in acidic mine water and an acidic environment containing iron or sulfur.

In the early, Braddock J F et al. had put forward the method of sulfur removal by oxidizing microorganisms and explained the principle [11]. The valence state of Sulfur in SO_2 is (+4), so its approach is to oxidize Sulfur in SO_2 to (+6), converting SO_2 in flue gas into SO_4^{2-} and enter the liquid phase, after that these substances carried out liquid phase desulfurization wastewater treatment. The article of Braddock J F revealed the metabolic pathway of T. F., and it could be deduced that the oxidation electron transport pathway of T. F. in the flue gas desulfurization process was shown in Figure 3[11].

As shown in Figure 3, T.F is divided into direct oxidation and indirect oxidation during desulfurization treatment. Direct oxidation refers to the direct capture of electrons from Sulfur (+4) in SO_3^{2-} by T.F and T.F convert them into SO_4^{2-} . Another oxidation means the product named Fe^{3+} , formed by oxidation of Fe^{2+} , continues to oxidize SO_3^{2-} in the liquid phase and forms the oxidized form of SO_4^{2-} .

Although T.F bacteria has a good effect on flue gas desulfurization, the wastewater generated in the desulfurization process still needs further treatment.

4.2. Sulfate-Reducing Microorganisms

For the sulfate-reducing microorganisms, Sulfate-reducing Bacteria (SRB) is a kind of anaerobic microorganisms that is suitable for the reducing method [12]. It widely exists in anoxic environments such as soil, seawater, river water, underground pipelines, and oil and gas wells.

The reducing desulfurization method is to reduce the valence of S in SO_2 (+4) to (-2), which means SO_2 is converted to sulfide by microbial action. Getting to this step, sulfide has a certain toxic effect. It is generally converted into elemental sulfur or metal sulfide precipitation and then used as a resource for the next step. Bradley et al. proposed that the reduction pathway of sulfate-reducing bacteria in the process of sulfate alienation was shown in Figure 4 [13].

Due to the low oxygen content in the flue gas, the form of SO_2 in the liquid phase may be dominated by sulfite [14]. For flue gas desulfurization by SRB, it reduces the step of converting SO_4^{2-} into Ammonium Persulfate (APS), so its reduction of sulfite will be more efficient and faster than sulfate reduction.

Badri et al. applied SRB to the study on SO_2 removal with good results in 1989 [15]. Jin et al. reported that the SO_2 removal rate could reach more than 95% and the operation cost could be greatly reduced by converting SO_2 into H_2S using sulfate-reducing bacteria and then converting H_2S to achieve the purpose of resource recovery and utilization in 2010 [16].

The Bio-FGD process which was developed by the companies in the Netherlands named HTSE&E and PAQUES converts SO_2 into SO_4^{2-} and SO_3^{2-} through an adsorber, and SRB is used in an anaerobic reactor to reduce SO_4^{2-} and SO_3^{2-} to sulfide, and the sulfide is converted into elemental sulfur for recycling in anaerobic reactors. The desulfurization rate of SO_2 is as high as 98%, and the purity of sulfur recovery is as high as 92%. It is pointed out that the technology has reached a practical technical level in the field of flue gas bio-desulfurization [17].

4.3. Comparison Of Desulfurization With Two Kinds Of Microorganisms

The oxidative microbial desulfurization technology represented by T.F. uses chemoautotrophic microorganisms which do not need to be supplemented with organic carbon sources. The reductive microbial desulfurization technology represented by SRB uses chemoheterotrophic microorganisms in itself, which needs additional nutrient sources such as carbon sources to maintain its activity. However, the former only transfers the sulfur oxides from the gas phase into the liquid phase, and the wastewater still needs secondary treatment; The latter reduces the sulfur oxides in the flue gas into sulfides by controlling the oxidation conditions and then produces by-products such as elemental sulfur. Therefore, the reductive microbial desulfurization technology is more suitable for future development.

4.4. SRB Biofiltration Mechanism

This is a biofilter model in figure 5. In the treatment of volatile organic compounds, contaminants flow with the gas stream into the biofilter as laminar flow [18]. When the gas passes through the filler, the pollutants are transferred from the gas phase to the liquid phase, and reach the surface of the biofilm through the liquid phase, after that they diffuse into the biofilm, and are captured and degraded by the microorganisms in the membrane.

As for the transfer between the phase states, the contaminants dissolved in the liquid membrane diffuse into the biofilm and are captured and degraded by the microorganisms under the push of the concentration gradient.

During the growth of biofilm, dissolved oxygen often becomes a limiting factor for the growth of biofilm due to the small solubility of oxygen in the water. In addition, nutrient concentration and substrate concentration are often limiting factors for biofilm growth.

4.5. Influencing Factors Of SRB On Sulfate Removal Efficiency

4.5.1. Effect Of Different Strains On Sulfate Removal

The ability of SRB to reduce sulfate partly reflects the desulfurization ability of the strain. Sun et al. isolated and purified nine SRB strains from environmental samples, and the strain SRB-12 (*Desulfovibrio oxamicus*) had the strongest reduction capacity, with the removal rate reaching 88.8% after 60h treatment with 1700mg/L sulfate at the initial mass concentration [19].

4.5.2. Effect Of Temperature On Desulfurization By SRB

SRB is generally divided into mesophilic SRB and thermophilic SRB, and the optimal growth temperature is 28~38°C and 54~70°C, respectively [20]. To investigate the optimal desulfurization temperature of SRB, Kong et al. comparatively analyzed the removal efficiency of sulfate by strain SRB-X2 under different temperature conditions. The results showed that the strain SRB-X2 could grow in the range of 15~55°C and the removal rate of sulfate was above 67%, and the removal rate at 30°C was the best.

4.5.3. Effect Of PH On Desulfurization By SRB

The pH directly affects the conformation, properties, and biological activity of the SRB enzyme system, and greatly affects the desulfurization performance of SRB. Yang et al. found that strain WJ1 could grow and produce H₂S at pH 5–10, and the optimal pH was about 8. Hardly generate H₂S when pH is less than 3 or higher than 11[20].

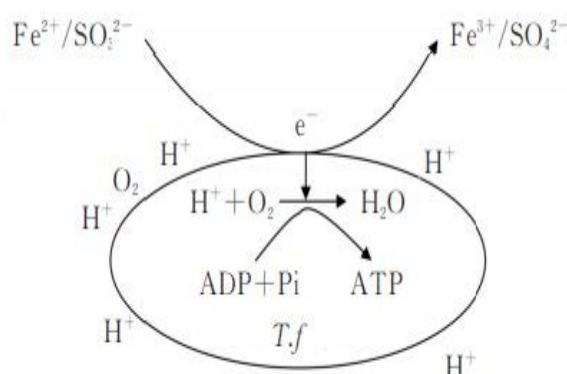


Figure 3. Electron transport chain of the oxygenation by *Thiobacillus ferrooxidans* [11]

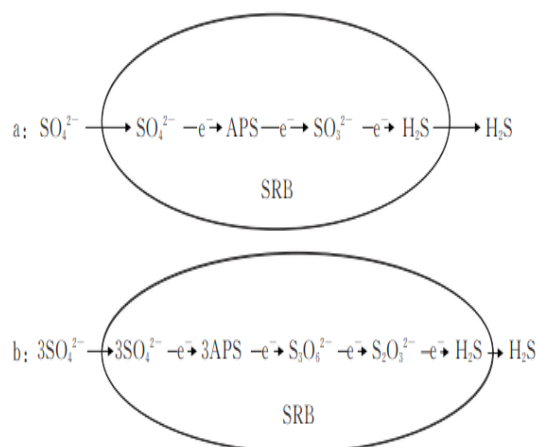


Figure 4. Two possible pathways of dissimilated sulfate reduction by SRB [13]

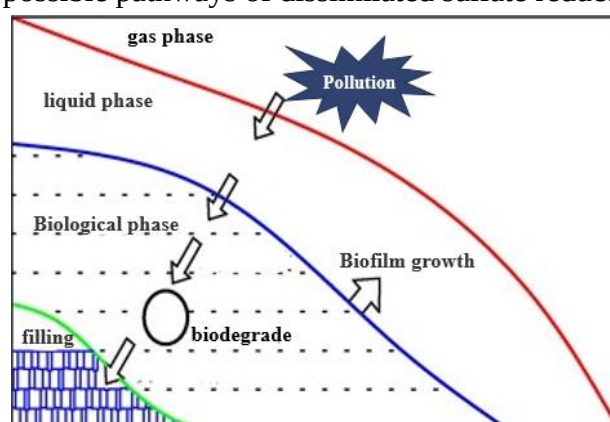


Figure 5. Schematic diagram of SRB biofiltration mechanism [18]

5. Conclusion

A series of recent studies have indicated that microorganisms are very effective in purifying the environment. This paper has addressed three environmental issues, including heavy metals and nitrogen pollution in water and sulfur pollution in the air. Nitrifiers could convert ammonium nitrogen to hydroxylamine, nitrite, and nitrate while absorbing nutrients from the organic substrate to grow. Fungi can use their adsorbability to treat heavy metals in wastewater. Sulfur-containing substances present in the air can enter Sulfate-reducing bacteria through a concentration gradient and be converted into several valuable substances. It all shows that Nitrogen pollution, pollution of heavy metals in water, and pollution of sulfur in the air could be dealt with effectively by microorganisms. The microbial environmental purification technology has a lot of advantages such as low energy consumption, simple equipment, and low operation cost so the technology would have great research value and practical application value in future life. However, this has been previously accessed only to a limited extent because these technologies have only succeeded in small experimental sites. People need to consider the high prices and the complex process in a large-scale industrial application. A new approach is therefore needed to effectively use microorganisms to harness the environment to realise the large-scale industrial application.

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