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Research and Application Progress of Immobilized Denitrifying Bacteria

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Abstract: *Immobilized microbial bacterial agent is an advanced ecological wastewater treatment technology, free microorganisms will be fixed on a specific carrier, and then slowly released, relying on the metabolic transformation of microorganisms to remove pollutants in the water, so as to achieve the purpose of purifying wastewater. This paper summarizes the strain selection, carrier optimization and fixation method of immobilized denitrifying bacteria technology, and summarizes the application of immobilized denitrifying bacteria agent in various types of wastewater treatment, and discusses the problems and improvement strategies of immobilized denitrifying bacteria agent in the practical application, so as to provide scientific reference for the development and application of immobilized bacteria agent technology.*

Keywords: *Immobilization techniques; Denitrification; Mycorrhizal agents; Ecological treatment*

1. Introduction

Immobilized denitrifying bacteria technology is the use of physical, chemical and other ways, the appropriate strains of bacteria with nitrogen removal ability fixed in a limited space, the use of immobilization technology, the denitrifying bacteria will be fixed in the carrier material, through the selection of strains and the optimization of the cultivation conditions, to maintain the activity of the strains, to build stable and efficient denitrifying system, through the metabolism of nitrogen by the bacteria to the nitrogen, the nitrogen-containing pollutants in the wastewater will be transformed. So as to purify the water body. Because the solid bacterial agent can make up for the short inventory of liquid bacterial agent, not easy to transport the defects, in recent years, attention has been paid. At present, immobilized microbial technology has been widely used in various types of wastewater purification, because the microorganisms are immobilized on specific carriers, which can enhance their stability and reduce the microbial loss rate, so as to improve the treatment efficiency and reduce the operating costs, which has a popularization value in the wastewater treatment industry. Fu Yutong et al. used ceramic grains as carriers to immobilize microorganisms in mariculture wastewater and successfully achieved the TN content of treated water to meet the secondary standard indicates that immobilized Vibrio alginolyticus YT-3 has considerable application potential in the field of denitrification of mariculture wastewater [1]; because immobilized bacterial agents have more advantages, they have been used in eutrophic water bodies, aquatic aquaculture, black-smelling water bodies, etc. It is especially suitable for those water treatment environments that require high system stability. At present, there are not many studies and practical applications on the in situ application of immobilized denitrifying bacteria, slow-release purification of eutrophication and aquaculture water body restoration, and they are still in the exploratory stage.

2. Characteristics and advantages of microbial fungicides

The way of growth of microbial bacterial agents directly affects the activity of the cells and the efficacy of water purification. The microorganisms are immobilized on top of certain solid carriers, such as activated carbon, resin, mineral carriers, biodegradable polymers, and so on, so that the activity and concentration of bacteria can be guaranteed, while liquid bacteriophages usually put microorganisms in a suspension or solution state, and they are different in terms of morphology, stability, application and efficiency. Liu Yuankun [2] and others found that the embedded immobilization technology can effectively improve the concentration and stability of advantageous microorganisms in the water body, and ensure the contact time and treatment efficiency of microorganisms and pollutants. Immobilized bacteriophages usually have strong resistance to shock loading due to their higher microbial concentration, and the former has better stability and tolerance. The activity and function of immobilized bacterial agents can be maintained for a longer period of time under long-term use and storage conditions. Compared with the suspension of bacterial growth system, immobilized bacteria can better resist the harsh external environment, compared with the ex situ treatment, immobilized bacterial agent in situ injection repair of water bodies has more obvious economic and environmental benefits. Liquid bacterial agent is mainly suitable for some short-term treatment, fast start-up and small-scale application of the environment. Yan Kechong [3] proposed the use of vapor-exploded corn stover powder immobilized indigenous composite microbial bacterial agent simultaneously as an additional carbon source to enhance the treatment of black-odor water bodies, and the results showed that the use of immobilized composite microbial bacterial agent can be more effective in removing pollutants, such as COD, NH3-N, and TP, from black-odor water bodies compared with ordinary free bacterial agent.

3. Types of immobilized denitrifying agents and denitrification mechanisms

Based on the source of the strain, bacteriophages can be broadly categorized into indigenous microbial bacteriophages and exotic microbial bacteriophages. The former is a highly efficient water purification microbial community selected through isolation and purification from polluted water or sediment samples. The latter is a preparation made by artificially screening and cultivating two or more different functional microorganisms with mutualistic or symbiotic effects, and compounding and cultivating them according to specific ratios. At present, functional microorganisms with nitrogen and phosphorus removal, aerobic denitrification and other specific degradation functions have been cultured and screened from aquaculture wastewater, domestic sewage, activated sludge and other environments. Shi Xiaotong et al [4] isolated and purified four strains of aerobic denitrifying bacteria from shrimp aquaculture pond substrate, and their total nitrogen removal rate was 71.4%, 51.25%, 55% and 60.7%. Tang Meizhen et al [5] screened a strain of cryobacterium Pseudomo-nas flava WD-3 from activated sludge substrate, and the results showed that the bacterium has high efficiency of nitrogen and phosphorus removal and degradation of organic pollutants. For compounding bacterial agents, specific effects can be achieved by mixing different strains or adding auxiliary substances, and the final selection and compounding process is optimized and adjusted according to specific needs as well as the actual situation.

In high nitrogen wastewater treatment, various types of microorganisms have a huge capacity to transform nitrogen, which can effectively reduce the total nitrogen in water, prevent nitrogen accumulation, and thus reduce eutrophication of water bodies. Ammonia-oxidizing bacteria and nitrite-oxidizing bacteria convert harmful ammonia into nitrite and nitrate by nitrification. Subsequently, denitrifying bacteria utilize these inorganic forms of nitrogen as electron acceptors in a reduction reaction that converts them to nitrogen gas, thereby removing nitrogen from the water column. Specifically functioning bacterial taxa such as synchronized nitrifying and denitrifying flora and anaerobic ammonia oxidizing bacteria have even more unique nitrogen removal pathways. The application of bacterial agents is aimed at using advantageous microbial communities to increase the biomass, density and biodiversity of functional microbial communities, thereby promoting the growth of beneficial microbial flora in the environment, while inhibiting the development of spoilage bacteria and pathogens, and realizing the decomposition of organic pollutants into harmless substances such as nitrification, oxidation, and denitrification through various microbial actions such as nitrification, oxidation and denitrification, thus achieving the goal of purifying water bodies by decomposing organic pollutants into harmless substances such as nitrogen, phosphate, and carbon dioxide. Thus, the purpose of purifying the water body is achieved.

4. Selection of immobilization carriers

Carriers are critical in microbial immobilization technology, directly affecting the activity of organisms, and their selection and application are essential for achieving efficient, stable and sustainable bioprocessing systems. High quality carrier materials provide sufficient surface area to facilitate microbial growth, enhance the structural stability of the system, and provide protection against the adverse effects of the external environment. Therefore, the selection of carriers for a specific application requires a combination of physicochemical properties, biocompatibility, and cost-benefit analysis to ensure optimal performance of immobilized microbial systems. Common immobilization carriers include

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paraffin particles, spherical porous ceramic particles, gel particles, polymer matrices, cellulose matrices, and silica gel matrices. According to their nature, they are divided into 3 categories: (1) Inorganic carriers, which are divided into natural inorganic carriers and synthetic carriers. These types of carriers have high chemical, physical and biological resistance. (2) Organic carriers are those that are filled with organic substances. Among them, natural organic carriers include two subclasses: polymer gel carriers and organic synthetic polymer gel carriers. (3) Composite carrier is a new type of carrier compounded by inorganic carrier and organic carrier, which can solve the problems faced by immobilized microbial technology in wastewater treatment, such as difficult to form balls, easy to break, and loss of activity. Composite carriers combine the advantages of inorganic and organic carriers, such as the mechanical strength and stability of inorganic carriers and the hydrophilicity, biodegradability, good biocompatibility and cheapness of organic carriers. Chen Feng [6] used sodium alginate, polyvinyl alcohol and activated carbon as immobilized carriers to immobilize the composite bacterial agent by adsorption-embedding method, and the results showed that the effect of immobilized composite bacterial agent for nitrogen and phosphorus removal was better than that of free composite bacterial agent. This new composite carrier is an important direction for the future research of immobilized microbial technology, which is expected to bring better results for the application of wastewater treatment and other fields. In the actual application process, the selection of the carrier must take into account the target microbial strains and their application requirements, and the selected carrier needs to ensure that the activity of the cells of the strain can be maintained for a long time, so as to realize a more stable and long-lasting biological treatment effect.

5. Immobilization technology method selection

Commonly used immobilization methods include adsorption method, embedding method and other new immobilization methods such as cross-linking method, covalent binding method and joint immobilization method. (1) Adsorption method is the most traditional immobilization method, and the carrier materials commonly used in the adsorption method are mostly naturally occurring porous inorganic materials. (2) Embedding method is the most widely used immobilization method, the use of polymers can form a gel characteristics of microorganisms embedded in the gel. The "adsorption-embedding" method is an emerging solid-state bacteriophage preparation technology in recent years. It has been widely used in atmospheric and water treatment because of its excellent product quality and shelf life. Wang Lingyun [7] explored the results of immobilized bacteriophage on shoreline oil spill pollution remediation showed that the immobilized bacteriophage obtained by SMZ-PVA adsorption-embedding immobilization method is simple, low-cost, and has good mass transfer performance. (3) Cross-linking method is a kind of carrier-free immobilization method, which mainly binds microorganisms to each other through covalent bonds produced by cross-linking agents. (4) Joint immobilization method is to use two or more immobilization methods to immobilize microorganisms, which makes up for the shortcomings of a single method, better preserves the activity of microorganisms, and improves the processing performance of microbial bacteriophage.

Table 1: Common microbial immobilization methods

Method	Principle	Advantage	Disadvantage
Adsorption	Microbial cells are adsorbed to surfaces in the presence of immobilized carriers	Easy to handle, maintains cellular activity, high utilization rate	Weak binding and rapid loss of activity
Embedding	Microbial cells are encapsulated by some polymers in a reticulated gel structure, and the substrate penetrates into the intermediate	Easy to operate	Fixed particles with high strength have poor mass transfer performance for substrates and oxygen in embedded materials
Crosslinking	Chemical agents react with surface groups of microbial cells to promote cross-linking between cells into a network structure	Tight connection between microorganisms and high stability performance	Difficult to operate and has a significant impact on microorganisms
Covalent binding	The surface active groups of the carrier react with the surface groups of microbial cells to form covalent bonds, enabling the carrier to bind with microorganisms	The carrier is tightly bound to microorganisms, with high stability performance	Difficult to operate

6. Application of immobilized nitrogen removal bacterial agents in wastewater treatment

Immobilized denitrifying bacterial agents have good application prospects, and the cases of their application in sewage and wastewater treatment include black smelly water, eutrophic water, aquaculture wastewater, industrial wastewater and urban wastewater, etc. Because of different biochemical characteristics of various types of wastewater, they have different requirements for the stability, durability and ability to deal with variable pollution loads of the immobilized bacterial agents. The following is a summary of application cases of immobilized bacterial agents in specific water bodies.

(1) Eutrophication of water bodies. It mainly focuses on degrading organic pollutants, removing nutrients such as nitrogen and phosphorus, and improving water quality. Immobilized denitrifying bacterial agents can improve the balance of nitrogen in the water body through metabolic transformation, and then effectively alleviate the degree of eutrophication. Guo Pengfei [8] explored the effect of immobilization of reed straw, an immobilized material, in various types of culture broth, and the results showed that reed straw could effectively improve the nitrogen pollution of eutrophic water bodies in culture broth with C/N of 8. Gao Yan [9] et al. used the national immobilization technique to deal with the eutrophication of spring polluted river and pond waters, and the study showed that the concentration indexes of ammonia nitrogen, total nitrogen, etc. were significantly reduced compared with the intake, and the overall decreasing trend was maintained in the case of rising nitrogen loads at the intake.

(2) Farming wastewater. Cai Xuyi [10] and others used immobilized bacterial agents to purify aquaculture water in situ, and the experimental results showed that the removal of CODMn and ammonia nitrogen in aquaculture ponds were significantly improved, and the addition of immobilized bacterial agents made the microbial diversity index and abundance index of the water increase, and the dominant microbial groups changed, and the number of certain bacterial groups, such as the phylum Ascomycetes, elevated helped to promote the transformation of ammonia nitrogen. Lei Mingda [11] studied the effect of a variety of microorganisms in the purification of water and the impact on the water environment, and clarified the preparation process of immobilized composite microorganisms water purification agent by determining the fermentation and culture conditions of composite microorganisms and studied the conditions of its use, which provided more options for dealing with the problem of ammonia and nitrogen of the water in the process of freshwater aquaculture.

(3) Industrial wastewater, for the application of wastewater containing high concentration of ammonia nitrogen or other nitrogen forms, Shao Yong [12] et al. used polyvinyl alcohol (PVA) and sodium alginate (SA) as the carriers for the encapsulation treatment and investigated the denitrification performance of the immobilized carriers on simulated industrial ammonia-nitrogen wastewater in a simulated three-phase fluidized bed SBR reactor. Cao Yiru [13] immobilized salt-tolerant aerobic bacteria and salt-tolerant denitrifying bacteria, which were cultured and enriched, into bacterial agents for deep denitrification of high-salt wastewater through biofortification technology, and experiments showed that this technology can effectively improve the biochemical denitrification effect of high-salt wastewater.

7. Conclusion

Immobilized denitrifying bacterial agents have unique advantages in improving the nutrient chemistry of water bodies and are suitable for the treatment of wastewater with low carbon to nitrogen ratios, and have been applied in the treatment of different types of water bodies. Future research should focus on the study and application of composite bacterial strains, mainly on what combination of methods and ratios has the best effect on pollutant removal, and research on inter-strain collaboration, competition, and pollutant removal mechanisms should be strengthened. In order to improve the treatment efficiency and application value, the technical customization and optimization of immobilized carrier materials, morphology and operating conditions to adapt to the environmental pressure of different water bodies and to maintain the long-term stability of the treatment effect are the key issues to be solved in the current research.

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