

The Degradation of Organic Pollutants in Chemical Wastewater by Functional Bacteria Immobilized on Agricultural Waste-Derived Biochar

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Abstract. Agricultural waste-based biochar has attracted significant attention in wastewater treatment due to its advantages such as low raw material cost, environmental friendliness, abundant active sites, and ease of modification. The combination of agricultural waste-based biochar and functional bacteria provides an optimized solution for the effective treatment of recalcitrant organic matter in chemical wastewater. As well as this paper systematically introduces the latest research progress in this technology, aiming to provide a comprehensive overview of the application of biochar-supported functional bacteria in the treatment of organic pollutants. This study analyzes the physicochemical properties of agricultural waste-based biochar and the degradation characteristics of functional bacteria, and summarizes the main immobilization methods and processes. Furthermore, it elucidates the immobilization and degradation mechanisms of biochar-supported functional bacteria and summarizes the key factors affecting degradation efficiency. In addition, it identifies problems and challenges such as biochar adsorption site saturation that urgently need to be addressed.

Keywords: Functional bacteria, biochar modification, immobilization, electron transfer.

1. Introduction

Chemical wastewater, as a key source of polluted water resources, contains a variety of recalcitrant pollutants, such as heavy metals, polycyclic aromatic hydrocarbons (PAHs), phenols, and antibiotics, which have been detected in large quantities of water bodies and soil [1-2], posing a threat to aquatic life and human health. In recent years, agricultural waste-based biochar has emerged as a novel environmentally friendly material, widely available, inexpensive, and easy to prepare [3]. Loading functional bacteria onto straw biochar can significantly improve the degradation rate and treatment efficiency, providing a green, economical, and efficient solution for wastewater treatment. Previous studies have shown that biochar, as a good carrier for functional bacteria substrates, has both adsorption and degradation effects. For example, in the study by Q. An et al., biochar showed good adsorption performance for pollutants such as heavy metals, nitrogen, phosphorus, and antibiotics. The study by X. Li et al. also found that by utilizing redox reactions, it can activate persulfate or hydrogen peroxide to generate reactive oxygen species (ROS), thereby decomposing antioxidants and other organic pollutants [3]. Functional bacteria exhibit low pollution, low cost, high efficiency, and easy attachment to the active sites of biochar, demonstrating high stability. Combining biochar with functional bacteria to construct biochar-supported functional bacteria composite materials can integrate the advantages of both achieving synergistic effects and more efficiently degrading recalcitrant organic matter in chemical wastewater. However, current research on biochar-supported functional bacteria mainly focuses on the degradation of soil pollutants, with relatively few reviews on the degradation of chemical liquid organic pollutants. Furthermore, there is a lack of summaries of factors influencing degradation for biochar-supported functional bacteria, helping future researchers obtain more optimized experimental designs directly.

This review will focus on the characteristics of biochar and functional bacteria, the specific application methods of biochar-supported functional bacteria, and the mechanism and influencing factors of this system's degradation of recalcitrant organic pollutants in chemical wastewater. This article aims to summarize and supplement the latest research progress in biochar-supported functional

bacteria technology for chemical wastewater treatment, providing a direct reference for subsequent research and applications.

2. Basic characteristics of agricultural waste-based biochar and functional bacteria

2.1. Preparation of agricultural waste-based biochar

Biochar is a solid product produced by pyrolyzing biomass at 300-800°C under anaerobic or hypoxic conditions after pretreatment processes such as dehydration/drying, crushing, and screening. For example, Ju F. F et al. used wheat straw as raw material, which was crushed, screened, and then rinsed to remove surface impurities. The treated biomass was then pyrolyzed in a nitrogen atmosphere [4].

2.2. Physicochemical properties of agricultural waste-based biochar

Biochar has high specific surface area, excellent exchange capacity and surface functional groups, which contribute to its chemical properties, enabling it to provide high-performance adsorption and certain degradation capabilities for organic, inorganic, and gaseous pollutants. Therefore, the physicochemical properties of biochar determine its adsorption performance and activation efficiency. Furthermore, the pyrolysis conditions during biochar production, surface charge, and free radical sites, from macroscopic to microscopic levels, all influence its physicochemical properties [4-6]. Therefore, these three factors are equally important for studying its physicochemical characteristics.

2.3. Degradation characteristics and adaptability of functional bacteria

Currently, commonly used biochar-immobilized functional bacteria include *Bacillus cereus*, *Bacillus subtilis* and *Citrobacter*. They mainly decompose organic pollutants through enzymes produced by microbial reproduction and metabolism. They gradually break down large organic molecules into small intermediate products, which are eventually mineralized into CO₂ and H₂O [7]. Since it is mainly catalyzed by enzymes, each enzyme targets a single substrate. Therefore, functional bacteria target and then degrade organic pollutants, showing strong specificity.

Functional bacteria and biochar are highly compatible. Biochar enhances the adaptability of functional bacteria in the environment, promotes the reproduction of microorganisms and enzyme activation; at the same time, biochar also helps functional bacteria transfer electrons and improve degradation efficiency.

3. Methods of using biochar-supported functional bacteria

3.1. Using biochar-supported functional bacteria

The adsorption method mainly relies on intermolecular forces or electrostatic forces for adsorption, which are achieved through van der Waals forces between microorganisms and biochar, hydrogen bonds, and weak interactions between organic pollutants and oxygen-containing functional groups on the surface of biochar, respectively. The order of the use of biochar and brine vary depending on the specific application scenario [8].

The cross-linking method utilizes a chemical reaction to cross-link microorganisms with the surface groups of biochar through chemical bonds. Microorganisms are added to brine, followed by biochar and stirring. Then, an appropriate amount of cross-linking agent is added to initiate the reaction.

The encapsulation method involves encapsulating microorganisms and biochar together in a polymer gel network. Pre-mixed sterilized biochar and microorganisms are added to sterile saline and stirred. Finally, a peristaltic pump is used to pump the mixture into a solution of specific metal particles such as Fe²⁺, Fe³⁺, Mg²⁺, etc. for modification, forming like black spherical beads [9].

3.2. Main application processes

Currently, the mainstream process method is adsorption culture. For example, XU X Y et al. pyrolyzed beer lees powder into biochar, and then added functional bacteria isolated from polycyclic aromatic hydrocarbon contaminated soil. It was mixed with magnesium chloride solution for modification, and then washed and dried [2]. JU F F. et al. added centrifugation of bacterial solution before the conventional procedures and after removing the upper bacterial solution, it was washed with sterile water and suspended. Finally, it was mixed with biochar for culture [5]. More uniquely, Zhang J Y et al. adopted a process combining embedding and cross-linking methods in their experiment. The mixed microorganisms loaded with alginate and biochar were stirred and mixed. The mixture was then pumped into calcium chloride solution using a peristaltic pump, and finally cross-linking solution was added for cross-linking fixation [9].

4. The core role of biochar-supported functional bacteria

4.1. Fixing and protecting

Immobilization technology allows functional bacteria to be immobilized on the surface or within the pores of biochar, forming a stable composite material. This technology improves the survival rate and stability of functional bacteria in the environment, prevents their loss during processing, and thus extends their service life. The porous structure and large specific surface area of biochar provide a favorable growth environment for functional bacteria, reducing the adverse effects of the external environment. Simultaneously, it reduces the direct damage of toxic substances to the functional bacteria, protecting them from environmental stress.

4.2. Enrichment and Degradation Enhancement

Biochar adsorbs and enriches organic pollutants on its surface through adsorption. This provides sufficient substrate for the degradation activities of functional bacteria, thereby improving the degradation efficiency of pollutants. In addition, the functional bacteria loaded on biochar are more conducive to their proliferation due to the more stable environment, which increases the density of functional bacteria and further improves the degradation rate. Xu W.J et al. investigated the effects of biochar on the metabolism of denitrifying *Paracoccus denitrificans*. The results showed that biochar immobilization enhanced the enzymatic detoxification and catalytic system of the strain. At the same time, the functional bacteria optimized the enzyme stress response and cell structure to improve the enrichment of pollutants and enhance the degradation capacity [10] [11].

5. Mechanisms of biochar-supported functional bacteria degrading organic pollutants

The structure of functional bacteria supported on biochar is extremely stable. The basic fixation of functional bacteria mainly includes physical adsorption, electrostatic attraction and pore filling. Among them, physical adsorption relies on intermolecular forces, including van der Waals forces, hydrogen bonds, and π - π interactions. Electrostatic adsorption is generated by the interpenetration of electron-deficient and electron-rich regions between oxygen-containing functional groups on the surface of biochar and organic matter [10]. Microorganisms gradually aggregate, and the extracellular matrix combines with the substrate to form a complete biofilm. Some nutrients on the surface of biochar promote the reproduction of microorganisms, while kinetic factors promote the diffusion of adherent microorganisms [8]. Under environmental influences, the biofilm may separate from the biochar, but it may still spread to other areas to continue adsorption and degradation, giving biochar-supported functional bacteria a stable and continuous degradation ability.

The main degradation mechanism of biochar-immobilized functional bacteria is dominated by enzymes produced by the metabolism of functional bacteria. Biochar can change the activity of

enzymes by adsorbing intermediate products in substrates and sediments, such as adsorbing nitrogen, phosphorus and potassium, thereby enhancing the efficiency of enzyme detoxification and enzyme metabolism. The excretion of some specific enzymes can also enhance the degradation efficiency of pollutants [7].

Electron transfer between biochar and functional bacteria is one of the core factors determining degradation efficiency. Electron transfer mainly relies on the structure of biochar and its active sites. Functional bacteria need electrons to carry out degradation work. Electron transfer on biochar can accelerate the metabolic rate of functional bacteria and break the ring bonds of organic matter more quickly. The extensive conjugation of sp^2 hybrid carbon in biochar endows it with excellent electrical conductivity, and its electron transfer capability can be enhanced through carbon structure. The sp^3 hybrid carbon acts as a donor to acceptor sp^2 hybrid carbon, which then migrates to the target pollutants. In this process, O-O or O-H bonds will break at the same time, generating reactive oxygen free radicals (ROS), which help improve degradation efficiency [12]. The redox enzymes produced by the metabolism of functional bacteria, while degrading organic matter, also reduce and modify the surface of biochar through metabolic activities, further improving conductivity [10].

Biochar-fixed microbial communities form a quorum sensing system (QS) in a stable microenvironment. Microorganisms communicate with each other by secreting signaling molecules, thereby regulating the growth, metabolism and enzyme expression of the microbial community and maintaining the structural stability of the degrading microbial community. At the same time, biochar can inhibit the excessive reproduction of miscellaneous bacteria and protect the ecological environment of functional bacteria [1]. Biochar can also upregulate the expression of degrading enzymes in the microbial community, induce enzyme stress response in the bacteria, enhance the antioxidant capacity of the microbial community, and strengthen ecological resilience. After functional bacteria colonize and form a biofilm, they secrete extracellular polymeric substance such as humic acid-like substances, thereby preventing functional bacteria from falling off and attracting other beneficial bacteria to colonize, thus constructing a synergistic network of the entire microbial community.

6. Key factors affecting degradation efficiency

6.1. Physical properties of biochar

The porous structure and large specific surface area of biochar provide physical attachment sites for functional bacteria. This can prevent functional bacteria from being damaged by factors such as mechanical collision. At the same time, functional bacteria are more likely to adsorb surrounding pollutants, thereby significantly increasing the contact opportunities and reaction time between microorganisms and pollutants [13]. In addition, functional bacteria can form multiple local high concentration sites on the carrier surface, which greatly improves the degradation efficiency.

6.2. Biochar modification methods

Appropriate modification methods can simultaneously optimize the physical structure and chemical properties of biochar, thereby greatly enhancing its ability to immobilize bacteria as a carrier [10]. For example, XU X Y et al. used $MgCl_2$ to modify biochar. The introduced metal ions such as Mg^{2+} and Fe^{2+} are precisely the nutrients required by microorganisms and can also stabilize enzyme activity for a long time, enhancing metabolic activity while playing a protective role. In addition, under the action of metal ions, the electron transfer performance and capacitance of biochar are significantly improved [10].

Specific modifications can enhance the conductivity of biochar, connect different microorganisms, efficiently transfer electrons, and achieve deep mineralization [12].

6.3. Surface chemical properties of biochar

The adsorption capacity of biochar for microorganisms is determined by surface functional groups. Oxygen-containing functional groups on the surface of biochar can enhance the immobilization capacity of functional bacteria through hydrogen bonding, electrostatic adsorption, and other forces, while also strengthening the adsorption of pollutants. XU WJ et al. studied the degradation of nitrate pollutants and tetracycline by functional bacteria and found that biochar stimulated the secretion of extracellular polymers, enhanced the adsorption of tetracycline, and promoted the reaction. Extracellular polymers are sticky and easy to attach to the surface of biochar, helping the bacteria to better adhere to the biochar and adsorb and enrich organic matter [11].

6.4. Surface degradation environment of biochar

The pH value of the degradation environment determines the degradation efficiency to a certain extent. A high pH value increases the negative charge on the surface of biochar and functional bacteria, resulting in stronger electrostatic repulsion, while the adhesion of functional bacteria will decrease, weakening the immobilization effect of biochar on functional bacteria. Interestingly, in addition to artificial regulation, the alkaline substances or ash present in biochar can play a buffering role, reducing the adverse effects of acidic conditions on microbial activity and maintaining the pH stability of the microenvironment [10].

Recalcitrant organic pollutants and heavy metals can cause oxidative damage to microorganisms. Biochar can activate the antioxidant defense system of microorganisms, maintain the activity of functional bacteria, and promote their reproduction and operation in polluted environments [11].

6.5. Metabolic regulation of functional bacteria

Functional bacteria have genes that encode specific degradation enzymes. Degradation capacity can be directly improved by enhancing the expression of these genes through genetic engineering [12]. Studies by XU W J et al. found that biochar can upregulate genes related to the tricarboxylic acid cycle and ATP-binding cassette transport proteins in bacteria. Biochar can be used as a signal to actively regulate the gene expression of functional bacteria, thereby enhancing the intercellular communication and cooperation capabilities [11]. In addition, by designing key enzymes, optimizing metabolic pathways, regulatory factors, and transgenic intermediates through genetic engineering, the bioremediation of recalcitrant organic pollutants can also be significantly enhanced [12].

7. Conclusions

This study systematically reviews the physicochemical properties of biochar, the degradation mechanism of functional bacteria, immobilization methods, mechanisms and key factors affecting degradation efficiency. Through a review of the latest literature, the study primarily investigates the latest production processes, fundamental principles and summaries of influencing factors, showcasing current cutting-edge research progress.

However, this technology still faces some challenges in practical applications. There are major challenges that the saturated sites of biochar adsorption and the decline in functional bacteria activity leads to the unsustainability of degradation. Future research could focus on the organic combination of electron transfer-driven composite catalytic oxidation and biochar-supported functional bacteria. Through the coupling of these two methods, free radicals can be continuously generated, overcoming the current situation where biochar active sites are supersaturated and unable to perform degradation.

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