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Development of Drought-Resistant Microbial Inoculants for Arid Mining Areas and Their Promoting Effect on Soil Aggregate Formation

Yaoyu Liu[1], Na Wang[2]

[1] Institute of Land Engineering and Technology, Shaanxi Provincial Land Engineering Construction Group Co., Ltd., Shaanxi 710075, China

[2] Key Laboratory of Degraded and Unused Land Consolidation Engineering, Ministry of Natural Resources, Shaanxi 710075, China

Abstract

The dilemma of soil degradation and vegetation restoration caused by the deterioration of the ecological environment in arid mining areas urgently requires innovative restoration technologies. In this paper, the research and development path and application value of drought-resistant microbial agents were systematically analyzed, and a mycorrhizal fungal symbiosis system was constructed to enhance environmental adaptability based on the screening of stress-tolerant strains such as bacillus and actinomycetes and the optimization of liquid/solid-state fermentation processes. It was clarified that microorganisms promoted the formation of soil aggregates by secreting extracellular polymers to form cementation networks and synergizing with the physical consolidation of hyphae, which significantly improved the average weight diameter (MWD) and erosion resistance. In view of the constraints such as soil texture heterogeneity, bacterial interaction effect and application methods, a strategy to enhance the function of microbial agents was proposed: molecular markers assisted in the screening of extreme environment tolerant strains, the development of multi-strain synergistic composite microbial agents, the combination of organic amendments to accurately regulate the soil microenvironment, and the establishment of a microbial agent-plant synergistic remediation model. The research results provide a theoretical basis for breaking through the technical bottleneck of soil structure improvement and ecological function reconstruction in arid mining areas, and promote the development of microbial remediation technology in the direction of standardization and engineering.

Keywords

Microbial Agents; Soil Aggregates; Ecological Restoration.

1. Introduction

With the large-scale development of mineral resources, arid mining areas are facing a series of ecological and environmental problems such as climate aridity, water shortage, and soil structure damage, which seriously restrict the regional ecological balance and sustainable development. Microbial remediation technology has become an important means of ecological restoration in arid mining areas due to its advantages of high efficiency, environmental protection and sustainability, and the research and development and application of drought-resistant microbial agents play a key role in improving soil quality and promoting vegetation restoration in mining areas. Scholars at home and abroad have carried out a lot of research on the screening of drought-resistant microbial strains, the preparation process of fungi and their application in arid mining areas, and have made some progress from the isolation and identification of dominant strains such as bacillus and actinomycetes to the optimization of

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preparation technologies such as liquid fermentation and solid-state fermentation. However, there are still deficiencies in the existing studies in terms of in-depth analysis of the mechanism of action of microbial agents and the differences in application effects under different environmental conditions. Therefore, this paper systematically reviews the research and development progress of drought-resistant microbial agents in arid mining areas, discusses their promoting effect on the formation of soil aggregates, and analyzes the factors affecting the effect of microbial agents and coping strategies, aiming to provide a theoretical basis and technical reference for ecological restoration in arid mining areas, and promote the further development and application of microbial agents in the field of ecological restoration in arid mining areas.

2. An Overview of Ecological and Environmental Problems and Microbial Remediation Technologies in Arid Mining Areas

2.1. Ecological and Environmental Characteristics of Arid Mining Areas.

Arid mining areas are generally distributed in arid and semi-arid areas with annual precipitation of less than 200mm and evaporation of more than 2000mm. At the same time, high-intensity coal mining activities have further exacerbated the deterioration of the ecological environment. On the one hand, a large amount of coal gangue, tailings and other wastes generated in the mining process are accumulated, which occupies a large amount of land resources and changes the original topography, resulting in the destruction of soil structure, the reduction of porosity, and the deterioration of soil aeration and permeability. On the other hand, heavy metals and organic pollutants produced by mining activities pollute soil and water bodies through rainwater leaching and surface runoff, resulting in a decline in soil fertility, difficulty in vegetation growth, and a serious weakening of the self-healing ability of the ecosystem in the mining area, resulting in a poor ecological environment with low vegetation coverage, serious soil erosion, and aggravated land desertification [1].

2.2. Principles and Advantages of Microbial Remediation Technology.

Microbial remediation technology is based on the metabolic activities of microorganisms, and realizes the degradation, fixation or transformation of pollutants through biodegradation, bioadsorption, biotransformation and other effects. Microorganisms can use their own secreted enzymes to decompose organic pollutants into harmless substances such as carbon dioxide and water. For heavy metal pollutants, microorganisms can reduce their bioavailability and toxicity through adsorption, precipitation, redox and other methods. Compared with traditional physicochemical remediation methods, microbial remediation technology has significant advantages. First of all, the technology is highly environmentally compatible, and microorganisms can grow and multiply in the natural environment without large-scale modification of the environment. Secondly, there will be no secondary pollution in the remediation process, and the remediation cost can be effectively reduced. In addition, microbial remediation technology is also sustainable, which can improve the structure of soil microbial community, enhance soil ecological function, and promote the self-healing and reconstruction of ecosystems during the remediation process.

2.3. Application Status of Microbial Remediation Technology in Arid Mining Areas.

In recent years, the application of microbial remediation technology in arid mining areas has gradually increased, and certain results have been achieved. In terms of soil remediation, inoculation of drought-tolerant and heavy metal-resistant microbial strains can effectively improve soil physical and chemical properties, increase the number and activity of beneficial microorganisms in the soil, promote the decomposition and transformation of organic matter

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in the soil, and increase soil fertility. In the field of vegetation restoration, the symbiotic characteristics of microbial agents and plants can improve the stress resistance and nutrient absorption capacity of plants, promote the growth of plant roots, and thus improve the survival rate and growth status of vegetation. However, there are still many challenges in the application of microbial remediation technology in arid mining areas. For example, the harsh environmental conditions in arid mining areas have a great impact on the survival and activity of microorganisms, resulting in unstable microbial remediation efficiency. The types of pollutants and environmental conditions in different mining areas are quite different, and the existing microbial agents lack universality, so it is necessary to screen and optimize the microbial agents for specific mining areas. In addition, the mechanism of action of microbial remediation technology is not fully understood, which limits its further promotion and application [2].

3. Screening of Drought-resistant Microbial Strains and Preparation Process of Microbial Agents

3.1. Screening of Drought-resistant Microbial Strains.

The screening of drought-resistant microbial strains should follow a multi-dimensional and systematic standard system. From the perspective of environmental adaptability, according to the international guidelines for microbial stress resistance, the target strain should have the ability to survive stably and maintain metabolic activity under extreme drought, high salt, high temperature and other stress environments. Extreme drought conditions are measured by water potential below -1.5 MPa, and high-salinity environments are defined as NaCl concentrations of 3% or more and high temperatures of more than 45°C, which are based on the stringent requirements for microbial tolerance in the special habitats of arid mining areas [3]. In terms of plant growth-promoting function, it is necessary to meet the core growth-promoting indicators specified in the Technical Regulations for the Production of Microbial Fertilizers, nitrogen-fixing bacteria should have high nitrogenase activity, phosphate-solubilizing bacteria should reach a certain amount of phosphorus-solubilization, and the strains should have the ability to synthesize plant hormones such as indoleacetic acid and cytokinin, so as to meet the dual needs of soil fertility improvement and plant growth promotion in vegetation restoration in mining areas. The screening process adopts a three-level screening system of "environment-directed acquisition-gradient enrichment culture-precise separation and identification" [4]. After collecting samples from soil, plant rhizosphere, rock fissures and other special habitats in arid mining areas, the improved Hoaglan culture medium was used for gradient drought stress enrichment culture, and the water potential of the culture medium was gradually improved, and the microbial communities with preliminary drought resistance were screened out. In the isolation process, drought-tolerant bacteria were isolated by selective medium such as mannitol as the only carbon source, and single colony purification was carried out by dilution coating plate method. Modern molecular biology techniques play a central role in the identification of strains. Based on 16S rRNA gene sequencing technology, BLAST comparison with authoritative databases such as NCBI and EzBioCloud was used to identify the genus and species of strains. Metagenomics analyzed the drought-resistant metabolic pathways of microbial communities at the level of functional genes, and detected the abundance of osmotic pressure regulator genes ompC and proU, antioxidant enzyme genes sod and cat, etc., to provide molecular biological evidence for the functional verification of bacterial species. The integration of traditional culture methods and molecular techniques has effectively improved the efficiency of strain screening and the accuracy of target strain identification [5].

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3.2. Common Strains of Drought-resistant Microorganisms.

As the core strain of drought-resistant microbial agents, Bacillus spp. has a unique spore structure that confers strong environmental tolerance. The spores form a dense cortex and spore shell structure in a dehydrated state, which can resist extreme environments such as high temperatures and ultraviolet radiation. Bacillus subtilis secretes extracellular enzymes such as β-1,3-glucanase and chitinase to degrade the cell wall of pathogenic bacteria, and synthesizes lipopeptide antibiotics such as surfactin to enhance plant stress resistance and inhibit the growth of pathogenic bacteria. Actinomycetes have shown unique advantages in ecological restoration in arid mining areas. Streptomyces can synthesize secondary metabolites such as polyketones and non-ribosomal peptides, improve the structure of soil aggregates, secrete plant hormones such as indole butyric acid and gibberellin, promote plant root meristem cell division, and significantly improve the absorption efficiency of water and nutrients by plants. The symbiotic system constructed by arbuscular mycorrhizal fungi and dark septum endophytes is an important way for plants to resist drought [6]. The mycelium of arbuscular mycorrhizal fungi can extend to the outside of the root to form a large hyphaal network, which can improve the efficiency of phosphorus absorption and the rate of water absorption by plants. Dark-colored septal endophytes regulate the abscisic acid signaling pathway and enhance the response ability of plants to drought stress by forming dark hyphae and microsclerotia structures in plant root cells. The synergy between the two plays an important role in vegetation restoration in arid mining areas.

3.3. Preparation Process of Microbial Inoculants.

Liquid fermentation technology is the mainstream process for the industrial production of microbial inoculants. Based on the fermentation kinetic model, the high-density fermentation of bacteria can be achieved by optimizing the parameters such as aeration volume, stirring speed, and temperature of the fermenter. In the fermentation process of Bacillus subtilis, the segmented dissolved oxygen control strategy can effectively improve the spore formation rate and shorten the fermentation cycle. Solid-state fermentation uses natural substrates such as bran and rice husk as carriers, which is more suitable for strains that are sensitive to fermentation conditions. In the solid state fermentation of Streptomyces, the "two-stage fermentation method" was adopted, in which aerobic fermentation in the early stage promoted the growth of mycelium, and anaerobic fermentation in the later stage induced the synthesis of secondary metabolites to increase the yield of antibiotics. The application of new fermentation technology has promoted the innovation of microbial preparation process. The mixed fermentation technology constructs a symbiotic fermentation system between bacillus and arbuscular mycorrhizal fungi, uses the polysaccharides secreted by bacillus to provide carbon source for arbuscular mycorrhizal fungi, and the growth factors produced by arbuscular mycorrhizal fungi promote the metabolic activity of bacillus and improve the comprehensive performance of bactericinal agents. Continuous fermentation technology improves production efficiency and reduces production costs by developing a new membrane separation-fermentation coupling system to achieve simultaneous cell retention and product separation.

The optimization of microbial agent formula should follow the design principle of "nutritional balance - protection and efficiency - stability and controllability". In terms of nutrient composition, the ratio of carbon and nitrogen sources was optimized by the response surface method to meet the needs of microbial growth and metabolism. The addition of protective agents such as trehalose and glycerin protects microbial cells from environmental stresses during the storage and application of microbial agents. The rational selection of synergists such as humic acid and chitosan can enhance the function of microbial agents, and humic acid can chelate heavy metal ions, adjust soil pH, and improve the physical and chemical properties of soil in mining areas. Chitosan can induce systemic resistance in plants and improve drought

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resistance. The Box-Behnken experimental design was used to systematically optimize the components in the formulation to improve the growth-promoting effect of the microbial agent [7].

3.4. Quality Control and Testing.

The quality control system covers three key links: raw materials, processes and finished products. Raw material testing uses PCR-DGGE technology to detect microbial contamination of media components, protective agents, etc., to ensure that the microbial load of raw materials is at a low level [8]. Process monitoring monitors the fermentation process in real time via an online biomass sensor, which automatically triggers a parameter adjustment mechanism when the cell concentration deviates from the set value. The finished product testing implements the standard of "Agricultural Microbial Agents" (GB 20287-2006). Effective viable count detection uses a combination of the most probable number method and real-time PCR to achieve a balance of accuracy and rapidity. The detection of miscellaneous bacteria rate adopts selective medium isolation and counting, and the miscellaneous bacteria rate is required to be controlled at a low standard. Through the establishment of a HACCP system, risk assessment and control of the whole production process are carried out to ensure that the qualification rate of microbial agents is at a high level, and to provide high-quality microbial agent products for ecological restoration in mining areas [9].

4. Promoting the Formation of Soil Aggregates by Microbial Agents

As the core unit of soil structure, the formation and stability of soil aggregates are very important for the maintenance of soil ecological functions. With their unique biological characteristics, microbial inoculants play a key role in the construction of soil aggregates and become an important biological means to improve soil quality.

4.1. Structure and Function of Soil Aggregates.

Soil aggregates are composite structures formed by the cementation of soil particles, organic matter, microorganisms and their metabolites through physical, chemical and biological interactions. According to the difference in particle size, it can be divided into large aggregates (>0.25 mm) and micro aggregates (<0.25 mm), which jointly construct the soil pore system. Large aggregates ensure soil aeration and water permeability, while micro-aggregates assume the function of water and fertilizer retention, which directly affects the aeration, water retention and nutrient transformation efficiency of soil, and is the basis for maintaining the health of soil ecosystem.

4.2. Mechanism of Microbial Agents Promoting the Formation of Soil Aggregates.

Extracellular polymers such as polysaccharides and proteins secreted by microorganisms during metabolism, like natural cementing agents, can adsorb soil particles, enhance the cohesion between particles through physical winding and chemical complexation, and directly promote the formation of aggregates. In addition, the microbial-driven decomposition and synthesis process of organic matter can produce organic colloids such as humus, which further enhances the agglomeration effect of soil particles. Microbial metabolic activities significantly changed the soil physical and chemical environment. The carbon dioxide and organic acids produced by its respiration can adjust the pH value of the soil, promote the release of cations such as calcium and iron, and participate in the cementation of aggregates. At the same time, microbial oxygen consumption changes the soil redox potential, affects the dissolution and precipitation process of minerals, and indirectly optimizes the agglomeration conditions of soil particles. The symbiotic system formed by mycorrhizal fungi and plant roots is of great

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significance for the construction of aggregates. Mycelium penetrates soil particles to form a physical network and enhance soil structure. Root exudates provide a carbon source for microorganisms, stimulate microbial metabolism, increase the secretion of extracellular polymers, and continuously promote the formation and stability of aggregates through the synergistic action of microorganisms, plants and soils.

4.3. Effect of Microbial Agents on the Stability of Soil Aggregates.

The stability of soil aggregates is often quantitatively evaluated by the average weight diameter (MWD) and geometric average diameter (GMD) measured by wet sieve method. High-stability aggregates can resist mechanical disturbances and water washout, maintaining soil structural integrity, and the application of microbial inoculants can significantly improve these indicators. Microbial agents enhance agglomerate stability through multiple pathways. On the one hand, it promotes the accumulation of organic matter and the synthesis of humus, forms organic and inorganic complexes, and strengthens the internal structure of aggregates. On the other hand, microbial activities improve soil pore distribution, optimize soil hydraulic properties, reduce the risk of aggregate disintegration, and thus improve the overall erosion resistance of soil.

5. Factors and Countermeasures Affecting the Effect of Microbial Agents

5.1. Factors Affecting the Effect of Microbial Agents.

The application effect of microbial agents in ecological restoration in arid mining areas is restricted by many factors. From the perspective of soil environment, soil texture has a significant effect on the activity of microbial agents, the large pores of sandy soil lead to the easy loss of microorganisms and the instability of the living environment, and the poor air permeability of clay restricts microbial respiration and metabolism. The imbalance of soil pH will also inhibit the function of microbial agents, and most beneficial microorganisms are suitable for survival in neutral to weakly acidic environments, and extreme pH values will destroy the cell structure of microorganisms and reduce enzyme activity. When soil nutrients are scarce, the growth and reproduction of microorganisms are restricted, and it is difficult to give full play to the role of promoting growth and restoration. In terms of microbial inoculants themselves, the characteristics of the strains determine their applicability, and some strains have insufficient stress resistance and low survival rate in harsh environments, and there may be antagonism between different strains, weakening the synergistic effect. At the same time, the quality of the microbial agent can not be ignored, the effective viable bacteria count is not up to standard, the inactivation of the bacteria caused by miscellaneous bacterial contamination or improper storage conditions will reduce the application effect. In addition, the application method directly affects the contact efficiency between the fungus agent and the soil and plants, and the seed dressing method may be interfered by the composition of the seed coating agent, and the uneven distribution of the fungus agent in the application of basal fertilizer, the insufficient or excessive application amount, and the application of the key period of plant growth are missed, which will affect the symbiotic efficiency of the fungus agent and the plant root.

5.2. Application Methods and Reasonable Countermeasures.

In order to improve the application effect of microbial inoculants in the ecological restoration of arid mining areas, reasonable application methods and countermeasures should be adopted. In terms of soil environment improvement, soil pH and texture can be adjusted by adding amendments, and organic fertilizer can be added to improve soil fertility to create a suitable living environment for microorganisms. For the microbial agent itself, the strains that are resistant to extreme environments should be screened, the compound microbial agent formula should be optimized to enhance the synergy between the strains, and the production process and quality inspection process should be strictly controlled to ensure the quality of the microbial agent. At the application level, it is necessary to select the most suitable application method according to the characteristics of soil and plants, accurately control the application amount and application time, and avoid waste of resources or poor effect caused by improper application. In addition, microbial agents can also be combined with other ecological restoration technologies, such as phytoremediation and physicochemical remediation, to give full play to their respective advantages, maximize the effect of ecological restoration in arid mining areas, and help the improvement and sustainable development of the ecological environment in arid mining areas.

6. Summary

In this paper, we systematically expound the research and development system of drought-resistant microbial agents and their ecological restoration mechanism in arid mining areas, and reveal the synergistic restoration principle of microbial metabolic activities by driving soil structure improvement and plant symbiotic effects. In view of the extreme environmental characteristics, the method of using molecular biology technology to deeply analyze the expression profiles of key drought resistance genes was introduced, with multi-dimensional stress resistance as the core screening criteria, so as to establish a functional microbiota system with environmental adaptability. In addition, by systematically optimizing the fermentation process parameters and innovating the stage control strategy, a new fermentation coupling system was successfully developed, which combined with the synergistic protection system to significantly enhance the survival of the microbial agent in the harsh environment. The study of mechanism of action showed that the active enzymes and characteristic metabolites secreted by microorganisms promoted the agglomeration of soil particles through the dual effects of physical adsorption and chemical complexation, and the mycelial bridging effect formed by the mycorrhizal symbiotic network could effectively integrate the soil-plant system, significantly improve the proportion of aggregates and structural stability, and comprehensively improve the ecological function of soil. It is worth noting that in practical application, it is necessary to focus on the establishment of an environmentally appropriate microbial agent formulation library and the development of targeted delivery technology, so as to break through the environmental limitations of microbial colonization through the integration of bacteria-plant synergistic strategies. Therefore, it is urgent to deepen the microbial community interaction mechanism and the dynamic model of environmental response, and systematically construct a multi-level verification system from gene expression regulation to ecosystem services, so as to promote the leapfrog development of microbial remediation technology in the direction of standardization and engineering.

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