

Original Research

Study of the Physiological Characteristics of *Microcoleus vaginatus* Combined with a Polymer Sand-Fixing Material Based on Attapulgit in the Laboratory

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Abstract

Comprehensive sand-fixation technology has potential application prospects for desertification control and restoration. The feasibility of combining biological and chemical sand-fixation technology was explored by studying the growth state and physiological characteristics of *Microcoleus vaginatus*. Dried *Microcoleus vaginatus* and a polymer sand-fixation material based on attapulgit modified by 4 M H₂SO₄ were combined as solids. The ratios of dried *Microcoleus vaginatus* to the polymer sand-fixation material by mass were 1:1, 1:2, 1:3 and 1:4. The amounts sprayed on the sand surface were 2 mg DW•cm⁻², 3 mg DW•cm⁻², 4 mg DW•cm⁻² and 5 mg DW•cm⁻². The results for chlorophyll a content, malondialdehyde content, soluble protein content and soluble sugar content showed that, under certain temperature and light conditions, the optimum environmental factors for the growth of *Microcoleus vaginatus* were a 1:3 ratio of dried *Microcoleus vaginatus* to the polymer sand-fixation material and a spraying rate of 3 mg DW•cm⁻². The results could provide a theoretical basis for a new sand fixation technology; the method presented here serves as another approach for combating desertification in arid and semiarid areas.

Keywords: *Microcoleus vaginatus*, attapulgit, physiological characteristics, sand-fixing material

Introduction

Desertification is one of the most severe ecological and environmental problems in the world; it will

bring major losses for society and the economy and is a serious threat to human survival and sustainable development. Due to the destruction of its ecological environment, China is one of the countries in the world that is suffering most seriously from desertification disasters [1]. Therefore, searching for an economical, environmental and effective sand-fixing material is of

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great importance and urgency for China in order to prevent and control desertification. It is well known that the growth of many types of vegetation is restricted by harsh and variable environments, such as very cold or hot and dry regions [2]; however, biological soil crusts (BSCs) can survive these conditions well because of their unique physiological and ecological properties. BSCs are organic complexes composed mainly of bacteria, fungi, algae, lichens, mosses, and other cryptogams and surface soil [3], and their existence and development have important value in improving the desert environment, such as effectively reducing soil erosion, changing water and nutrient cycling rates, maintaining soil moisture, improving soil nutrient levels and creating favourable conditions for the recovery of vegetation in desertification areas [4, 5]. As the colonizers and pioneers of BSCs, cyanobacteria plays an irreplaceable role in the formation of BSCs. Among the cyanobacteria, the dominant species *Microcoleus vaginatus* often accounts for most of the crust biomass [6]. *Microcoleus vaginatus* is also useful for environmental improvement in desert regions because it can bind sand particles together via its growth and secretion of exopolysaccharides, and it plays an important pioneer role in environmental regulation and in the ecological restoration of desert regions [7].

Attapulgit is a crystalline hydrated magnesium silicate with a fibrous morphology, a large specific surface area and a moderate cation exchange capacity, and it is beneficial for adsorption. Because of the special physical and chemical properties of attapulgit, it has been widely applied in many fields [8]. As attapulgit is modified by acids, there are many exchangeable cations and reactive $-OH$ groups on its surface [9]. It can add different polymers onto the chain of its natural polymers through graft copolymerization with certain functional groups [9], which provides attapulgit with better water absorption and water retention.

In desertification areas, the water content is very low. On the other hand, there are high diurnal temperature differences and a large range of temperature fluctuations that decrease the survival rate of BSCs and further influence the effect of sand-fixation materials in dry conditions. When *Microcoleus vaginatus* is mixed with a polymer sand-fixing material based on attapulgit at different ratios of mass, it may produce a new type of sand-fixation material that creates no secondary pollution and is a more nutrient-rich and economical sand-fixation material. However, there are presently few studies on this topic. Therefore, this study is intended to use an artificial culture of *Microcoleus vaginatus* with attapulgit modified by 4 M H_2SO_4 and polymer to find the optimal mass ratio and to further explore the bioactivity and self-reproduction of *Microcoleus vaginatus*. This study will provide an important theoretical basis for the preparation of a new type of sand-fixation material that creates no secondary pollution and has self-repairing functions; this study will also further encourage the

development and application of natural mineral material and biological resources.

Material and Methods

Cultivation of *Microcoleus Vaginatus*

Microcoleus vaginatus, a common dominant species in cyanobacterial soil crusts, was isolated from Shapotou, Zhongwei County, Ningxia Hui Autonomous Region, China (N 36°24'43.9", E 102°48'11.2") and was identified by microscopy. *Microcoleus vaginatus* was cultivated in BG11 medium at $120 \mu E \cdot m^{-2} \cdot s^{-1}$ light intensity and 25°C with a 12 h light/12 h dark cycle in a light incubator (HPG-280B, China). The sand material was purchased from a local market.

Preparation of The Polymer Sand-Fixing Material Based on Attapulgit

First, a four-necked flask equipped with a mechanical stirrer, a reflux condenser, a thermometer and a nitrogen line was purged with nitrogen to remove the dissolved oxygen from the system. Then, 7.1 g acrylamide (7.1 g, dissolved in 20 mL distilled water) was dispersed in a 250-mL four-necked flask. At the same time, 0.71 g attapulgit modified by 4 M H_2SO_4 was added, and the mixture was stirred continuously for 30 min. After 30 min, 0.09 mg potassium persulfate and 0.06 mg N,N'-methylene-bis-acrylamide were added, and the mixture solution was bathed at 50°C for 3 h to complete polymerization. Finally, 8 g of the polymer was ground and purged with 40 mL of 1.5 M NaOH and was gradually heated to 70°C and maintained for 2 h. The obtained products were washed with distilled water several times and dried in an oven at 70°C for 72 h.

Combination of *Microcoleus Vaginatus* and Polymer Sand-Fixation Material

Microcoleus vaginatus was placed in a drying oven at 30°C, and the drying degree was 85% water content. Dried *Microcoleus vaginatus* and the polymer sand-fixation material were combined as solids for the A1, A2, A3 and A4 treatments (A1 (the ratio of dried *Microcoleus vaginatus* to the polymer sand-fixation material was 1:1, the amounts sprayed on the sandy surface were $2 \text{ mg DW} \cdot \text{cm}^{-2}$, $3 \text{ mg DW} \cdot \text{cm}^{-2}$, $4 \text{ mg DW} \cdot \text{cm}^{-2}$ and $5 \text{ mg DW} \cdot \text{cm}^{-2}$; A2 (the ratio of solid masses was 1:2, the amounts sprayed were the same as in A1; A3 (the ratio of solid masses was 1:3, the amounts sprayed were the same as in A1); A4 (the ratio of solid masses was 1:4, the amounts sprayed were the same as in A1)). The mixture was sprayed as a dry powder on a Petri dish filled with sand. Tap water was sprayed on the Petri dish three times a day, and BG11 was sprayed once every seven days to replenish the nutrients. *Microcoleus vaginatus* was grown for 60 days

under conditions of 25°C and 120 $\mu\text{E}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ in a light incubator, and the best proportion of *Microcoleus vaginatus* to the polymer sand-fixation material was determined according to its growth and development characteristics. The dried *Microcoleus vaginatus* and the polymer sand-fixation material were combined for the A5 treatment (the ratio of solid masses was 1:3, the amount sprayed on the sandy surface was 3 mg DW $\cdot\text{cm}^{-2}$), and the growth time and environmental conditions were the same as those of the A1, A2, A3 and A4.

Chlorophyll A (Chl a) Measurements

To monitor the effects of the different spraying amounts on the growth of *Microcoleus vaginatus*, the content of Chl a was assayed. Chl a was extracted in 95% ethanol and characterized by the method of Lan [10]. Absorbance values for the supernatants were recorded at 649 nm and 665 nm for Chl a (UV-300, UK).

Estimation of Malondialdehyde (MDA)

MDA content was measured by the method of Xie [11], with some modifications, and the absorbance of the supernatant was measured at 450, 532 and 600 nm (UV-300, UK).

The Determination of Soluble Protein

The soluble protein assay was based on the method described by Bradford [12], and the absorbance

was recorded at 595 nm (UV-300, UK). Protein concentrations were quantified using the Coomassie Brilliant Blue G-250 method using bovine serum albumin (0-100 $\mu\text{g}\cdot\text{mL}^{-1}$) as the standard.

Measurements of Soluble Sugar

According to the methods of Li [13], with minor modifications, soluble sugar was determined, and the absorbance at 630 nm was measured with a spectrophotometer (UV-300, UK). The standard curve for soluble sugar was prepared by sucrose (0-100 $\mu\text{g}\cdot\text{mL}^{-1}$). Each experiment was performed at least in triplicate.

Statistical Analysis

Data were analysed using a GLM (General Linear Model) with the Statistical Package for the Social Science (SPSS) version 19.0 software. Pairwise comparisons were performed using the least significant difference (LSD) procedure at the 0.05 significance level.

Results and Discussion

Adding a BSC inoculum to the soil surface is the most effective approach for enhancing BSC recovery, especially in sandy soils [14, 15]. Recently, hydrophilic polymers have been widely used as soil fixing agents or

Table 1. Development characteristics of *Microcoleus vaginatus* (growth for 60 days).

The amounts sprayed (mgDWcm ⁻²)	The mixture ratio	Colour	Shape	Ph	Thickness (mm)	Dry weight (mg $\cdot\text{cm}^{-2}$)
A1	1:1	Grey	Flat	7.780 \pm 0.010	1.393 \pm 0.015	0.028 \pm 0.001
A1	1:2	Grey	Flat	7.663 \pm 0.0058	1.420 \pm 0.010	0.032 \pm 0.001
A1	1:3	Grey	Pinnacled	7.390 \pm 0.010	1.563 \pm 0.006	0.048 \pm 0.001
A1	1:4	Dark	Rolling	7.540 \pm 0.010	1.487 \pm 0.006	0.042 \pm 0.001
A2	1:1	Grey	Flat	7.810 \pm 0.010	1.387 \pm 0.006	0.034 \pm 0.001
A2	1:2	Grey	Flat	7.640 \pm 0.010	1.430 \pm 0.010	0.043 \pm 0.001
A2	1:3	Black	Rolling	7.190 \pm 0.010	1.790 \pm 0.010	0.069 \pm 0.001
A2	1:4	Dark	Rolling	7.587 \pm 0.0058	1.680 \pm 0.010	0.055 \pm 0.0005
A3	1:1	Grey	Flat	7.670 \pm 0.010	1.440 \pm 0.010	0.032 \pm 0.001
A3	1:2	Grey	Flat	7.660 \pm 0.010	1.470 \pm 0.010	0.037 \pm 0.001
A3	1:3	Dark	Pinnacled	7.087 \pm 0.0058	1.640 \pm 0.010	0.065 \pm 0.001
A3	1:4	Black	Pinnacled	7.310 \pm 0.010	1.503 \pm 0.006	0.042 \pm 0.001
A4	1:1	Grey	Flat	7.887 \pm 0.006	1.350 \pm 0.010	0.032 \pm 0.001
A4	1:2	Grey	Flat	7.740 \pm 0.010	1.410 \pm 0.010	0.036 \pm 0.0006
A4	1:3	Dark	Rolling	7.430 \pm 0.010	1.693 \pm 0.006	0.061 \pm 0.001
A4	1:4	Black	Rolling	7.790 \pm 0.010	1.570 \pm 0.010	0.049 \pm 0.001

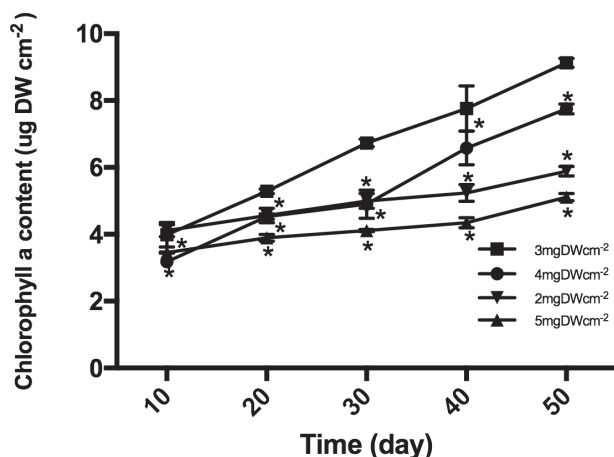


Fig. 1. Changes of chlorophyll a content from *Microcoleus vaginatus* combined with sand-fixation polymer material based on attapulgite, *compare to 3 mg DW•cm⁻², $p < 0.05$.

conditioners to combat soil erosion [16]. Attapulgite has a special chain layer crystal structure, as attapulgite is modified by acids and contains active hydroxyl groups to form polymers with specific functional groups. However, for combined applications with cyanobacteria, attapulgite and other polymers that have no harmful effects on cyanobacterial growth should be considered [17]. Therefore, in the present study, we attempted to identify whether *Microcoleus vaginatus* with modified attapulgite and polymers mixed at different ratios of mass and different spraying levels were harmful to cyanobacterial physiological characteristics and could fix sand particles.

Effects of different ratios of mass on biological activities were studied firstly, *Microcoleus vaginatus* grew for 60 days under the conditions of 25°C and 120 $\mu\text{E}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ in a light incubator. The results showed that there were significant differences in the pH, thickness and dry weight of *Microcoleus vaginatus*

under different ratios ($p < 0.05$). The optimum environment for the growth of *Microcoleus vaginatus* was a 1:3 ratio of solid masses (Table 1). Therefore, a 1:3 ratio of the solid mass of dried *Microcoleus vaginatus* to the polymer sand-fixation material was used for future experiments.

Biological soil crusts exhibit many adaptive strategies in response to abiotic environmental stresses. These stresses can influence developmental processes and lead to growth restriction, making adaptive responses a high priority for plants. These adaptive mechanisms include changes in physiological and biochemical processes [18]. The Chl a concentration is a useful quantitative indicator of the degree of cyanobacterial soil crust development in the study of plant physiology, especially photosynthesis. To some extent, the Chl a content reflects the growth activity and biomass of cyanobacterial soil crusts [19]. Chl a also provides an indirect measure of nutrient status [20]. In our study, under certain conditions of temperature and light, the sprayed amount significantly affected the Chl a content of *Microcoleus vaginatus* ($p < 0.05$). As time continued, the Chl a content gradually increased in the four groups. Of the four spraying levels, the Chl a content at 3 mg DW•cm⁻² was the highest (Fig. 1). At the 3 mg DW•cm⁻² spraying level, the Chl a content of *Microcoleus vaginatus* grown on the sand surface was lower than that of *Microcoleus vaginatus* combined with the polymer sand-fixation material (Table 2). The results suggested that *Microcoleus vaginatus* could adapt to these conditions and grow well. It was also evident that the sprayed amount was an important factor affecting the properties of *Microcoleus vaginatus* combined with the polymer sand-fixation material.

MDA is the final product of lipid peroxidation. MDA content is considered to be one of the most important manifestations of lipid peroxidation, and its concentration is related to the degree of membrane lipid

Table 2. The physiological characteristics of *Microcoleus vaginatus* under different the growth.

Day		Chlorophyll a content (μgDWcm^{-2})	MDA content ($\mu\text{molDWcm}^{-2}$)	Soluble protein content (mgDWcm^{-2})	Soluble sugar content (μgDWcm^{-2})
10	A3	3.987±0.367	0.146±0.032	0.748±0.007	9.342±0.105
	A5	4.000±0.147	0.207±0.013 [#]	0.680±0.006 [#]	6.636±0.015 [#]
20	A3	5.290±0.066	0.235±0.032	0.832±0.008	9.594±0.158
	A5	5.007±0.009 [#]	0.329±0.021 [#]	0.735±0.008 [#]	6.931±0.057 [#]
30	A3	6.733±0.112	0.254±0.032	0.893±0.013	9.779±0.198
	A5	6.186±0.218 [#]	0.366±0.025 [#]	0.780±0.015 [#]	7.498±0.154 [#]
40	A3	7.760±0.676	0.308±0.036	0.947±0.007	10.091±0.169
	A5	6.564±0.211 [#]	0.390±0.025 [#]	0.838±0.018 [#]	7.647±0.013 [#]
50	A3	9.130±0.132	0.346±0.029	0.976±0.020	10.437±0.166
	A5	8.807±0.079 [#]	0.517±0.025 [#]	0.892±0.005 [#]	8.477±0.307 [#]

[#]compare to A3, $p < 0.05$

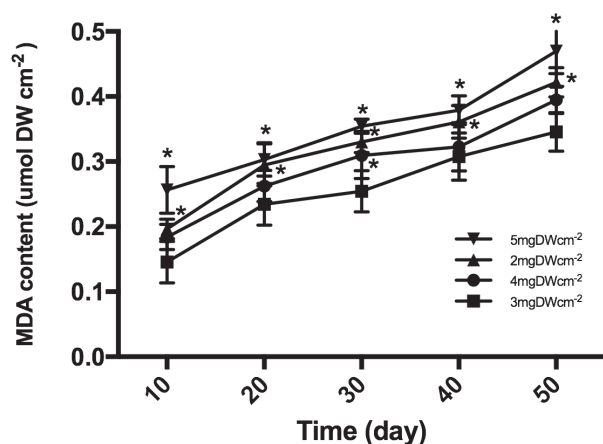


Fig. 2. Changes of MDA content from *Microcoleus vaginatus* combined with sand-fixation polymer material based on attapulgit. *compare to 3 mg DW·cm⁻², $p < 0.05$.

peroxidation [21]. In the present study, under certain conditions of temperature and light, the amount sprayed significantly affected the MDA content of *Microcoleus vaginatus* ($p < 0.05$). As the time increased, the MDA content gradually increased in the four groups. Of the four spraying levels, the MDA content at 3 mg DW·cm⁻² was the lowest (Fig. 2). At the 3 mg DW·cm⁻² spraying level, the MDA content of *Microcoleus vaginatus* grown on the sand surface was higher than that of *Microcoleus vaginatus* combined with polymer sand-fixation material (Table 2). The results showed that this spraying rate had little influence on *Microcoleus vaginatus* and was suitable for their growth and amplification. Research found that low MDA content indicated that plants were under less stress, and high MDA content indicated more serious oxidative damage to the plant cell membrane [22, 23], which was consistent with our results.

Soluble protein makes up various antioxidant enzymes, and changes in their content reflect protein

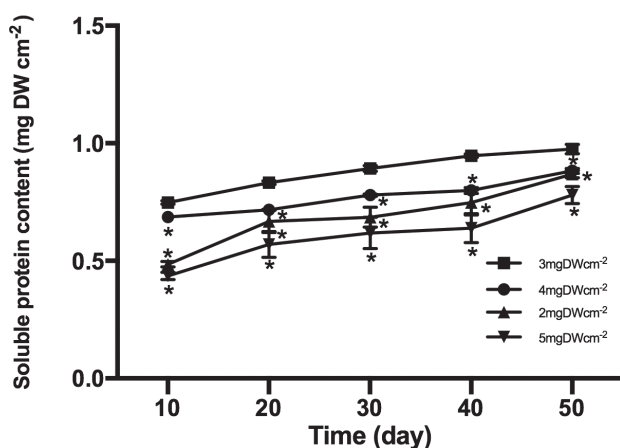


Fig. 3. Changes of soluble protein content from *Microcoleus vaginatus* combined with sand-fixation polymer material based on attapulgit. *compare to 3 mg DW·cm⁻², $p < 0.05$.

synthesis and the degradation of various information in the cell. Soluble protein content indirectly reflects the total metabolic status of plants [24]. In our study, under certain conditions of temperature and light, the amount sprayed significantly affected the soluble protein content of *Microcoleus vaginatus* ($p < 0.05$). With increasing time, the soluble protein content gradually increased in the four groups. Of the four spraying levels, the soluble protein content at 3 mg DW·cm⁻² was the highest (Fig. 3). At the 3 mg DW·cm⁻² spraying level, the soluble protein content of *Microcoleus vaginatus* grown on the sand surface was lower than that of *Microcoleus vaginatus* combined with the polymer sand-fixation material (Table 2). The results suggested that the number of functional proteins in cells was also increased, which was conducive to maintaining the osmotic pressure and the normal metabolism of cells, thereby enhancing the protective effect on *Microcoleus vaginatus* cells and improving the water retention ability of cells.

Soluble sugar is an important constituent and source of energy for living organisms [25]. As an intermediate or end product of metabolism, it participates in the metabolic regulation of various plants at different concentrations. Therefore, it plays an important role in regulating plant physiological processes such as growth, development and stress resistance [26, 27]. At the same time, soluble sugar is also thought to be a osmotic regulators in cells; It maintains the pressure potential of cells, reduces water loss, and helps to maintain the normal function of cells [28]. In our study, under certain conditions of temperature and light, the sprayed amount significantly affected the soluble protein content of *Microcoleus vaginatus* ($p < 0.05$). With increasing time, the soluble protein content gradually increased in the four groups. Of the four levels of spraying, the soluble protein content at 3 mg DW·cm⁻² was the highest (Fig. 4). At the 3 mg DW·cm⁻² spraying level, the soluble protein content of *Microcoleus vaginatus* grown on the sand surface was lower than that of *Microcoleus vaginatus* combined with the polymer sand-fixation

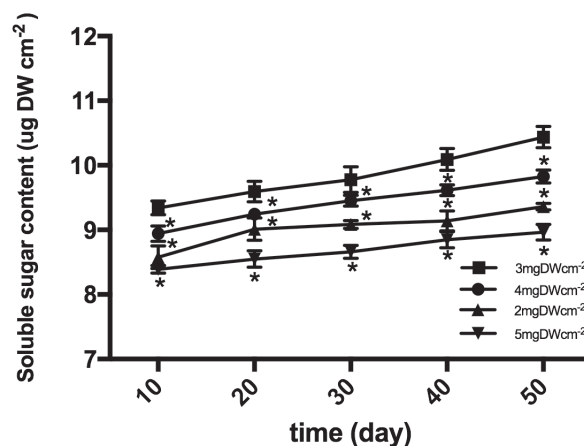


Fig. 4. Changes of soluble sugar content from *Microcoleus vaginatus* combined with sand-fixation polymer material based on attapulgit. *compare to 3 mg DW·cm⁻², $p < 0.05$.

material (Table 2). The results suggested that this spraying level was beneficial to *Microcoleus vaginatus* and made them grow well, which made them better able to resist stress and cope with environmental change. This further indicated that *Microcoleus vaginatus*, when combined with the polymer sand-fixing material, had good physiological characteristics and was more adaptable to environmental *cMicrocoleus vaginatus* combined with the polymer sand-fixing material based on attapulgite might be able to build a healthy, stable, efficient and productive ecosystem in deserts and to reflect their synergy because of the functions of *Microcoleus vaginatus*, such as strengthening the anti-erosion capacity of the sandy soil surface, maintaining the soil moisture and improving the nutrient condition of soil in desertification areas [29, 30]. After the attapulgite was modified by 4 M H₂SO₄, it had better water absorption and water retention capabilities and could slowly release water for the plant to absorb and to use. The modified attapulgite could not only decrease the deep leakage of water, decrease soil nutrient loss, improve water use efficiency and reduce soil erosion but also regulate soil water, temperature and aeration conditions and enhance the soil structure and soil fertility [31, 32] to promote the formation and development of *Microcoleus vaginatus*.

Conclusions

In summary, by studying the growth state and physiological characteristics of *Microcoleus vaginatus* mixed at different ratios and inoculum concentrations of a polymer sand-fixing material based on attapulgite, it was found that the optimum environmental factors for the growth of *Microcoleus vaginatus* combined with the polymer sand-fixation material were a 1:3 ratio of solid mass and a spraying amount of 3 mg DW•cm⁻². Under laboratory conditions, *Microcoleus vaginatus* grew rapidly and experienced minimal stress from the external environment. The physiological properties and biological activities of the inoculated *Microcoleus vaginatus* were stable during the study period, and the results could provide a theoretical basis for a new sand-fixation technology. The method presented here serves as another approach for combating desertification in arid and semiarid areas.

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Conflict of Interest

The authors declare no conflict of interest.

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