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Diffusion Limited Aggregation Model and *Microcoleus* Morphological and Growth Assessment on the Soil Surface

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ABSTRACT

The arid land cyanobacteria *Microcoleus* has specific features such as formation of filamentous network on the soil surface, producing photosynthetic based nutrients, releasing exopolysaccharides contribute to biofilm formation, etc., which makes it a promising candidate in accelerating biological soil crust restoration. An understanding with regards to its structural growth pattern in the soil would be useful in developing soil restoration technologies. To help this promising activity, the aims of this study was to present a 2D diffusion limited aggregation (DLA) model showing cyanobacteria growth in the soil surface. A common DLA model was developed followed by model simulations assessment on laboratory *Microcoleus* biofilm grown in Petri dishes. The soil covered particles by biofilm was determined using image processing method. Simplifications were assumed in the model development and DLA model was solved using NetLogo program. It was found that the morphology of *Microcoleus* grown in the soil can be modeled by DLA process. A good agreement was achieved between the results obtained by DLA model simulation and the experimental data obtained by the *Microcoleus* biofilm cultivation on the soil surface. The simulation approach of restoration process may be helpful in designing artificial biological soil crust restoration processes applicable in the arid and semi-arid areas to combat desertification.

KEYWORDS: Biofilm, biological soil crust, desertification, diffusion limited aggregation (DLA), growth patterns, soil restoration.

1. INTRODUCTION

Cyanobacteria *Microcoleus* is recognized as an effective constituent in the restoration of biological soil crust, functions in protecting the soil against erosive factors such as the wind, and improvement of the soil fertility by providing organic nutrients, etc. [1–4]. Numerous investigations have been conducted to evaluate the *Microcoleus* diversity and distribution in different parts of the world, especially in arid and semi-arid areas [5]. However, *Microcoleus* growth pattern has been rarely investigated [5,6]. *Microcoleus* may have various morphological and growth forms which depend on the environmental and nutritional conditions. Growing of *Microcoleus* on different agar concentrations was reported to be associated with various morphological forms. These results have indicated that morphological growth pattern of *Microcoleus* depends on the parameters such as the hardness of the solid agar culture medium and inoculation condition; the lesser dense colonies and longer filaments are obtained in lower agar concentrations [7].

From practical aspects and the technological perspectives, morphological structure of *Microcoleus* is essential in biological soil crust restoration process, which is a sustainable natural system to protect the soil from the wind erosion in the arid and semi-arid areas. However, there are problems that demand resolution such as experimental setups which depend on different variables and difficulty of performance in practice. Quantification of soil coverage by *Microcoleus* biofilm through simulation approaches supports and would be useful for developing accelerated biological soil crust restoration systems in the arid and semi-arid areas in order to cope against the rapid global desertification [8,9].

An *in silico* approach for prediction of the biofilm growth has been well documented and reviewed in the last few decades [10]. Recent approaches for studying morphological structure were mostly focused on the hybrid models that combine a model simulation and continuous measurement parts both at the same time [11]. In simulation approach, the mass balance based on diffusion-reaction model is a common conventional model for describing microbial biofilm growth [12]. It simulates the profile of substrate diffusion in the biofilm and substrate interaction with the cells within the biofilm [13]. However, it does not provide a 2D and 3D visualization display of the morphological growth structure. Since biofilm growth patterns have fractal nature, an effective visualization system

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is required for precise navigation to show how it fills space. The simultaneous simulation and representation of biofilm formation with computer programs are complex and depends on different variables. A common simple model which has been reported in the literature regarding biofilm formation by moss, lichen, and similar biological soil crust forming organisms is diffusion-limited aggregation [14]. However, a 2D and 3D visualization of *Microcoleus* biofilm growth have not been reported using DLA model. The features of DLA model could be used to produce the biofilm growth patterns developing over the time [15]. DLA model describes how a fractal is built from seed particles in low concentrations. The DLA model has been recognized suitable to reproduce the microbial biofilm coverage on the substratum [13,16].

In DLA model, to simulate the growth pattern and 2D visualization of DLA algorithm, the particles is released once at a time in a square boundary far enough from the fixed, single, or multiple seeds. The released particles then undergo a random walk in a pure stochastic way inside of the lattice. In the DLA models, the simulations are frequently performed on a finite, discrete lattice. The square lattice is defined by the user to determine the size of the output. Various regular lattice shapes of square, circle or triangular, etc. have been introduced as well. However for computational simplicity, commonly DLA is generated on a square lattice. A 2D simulation is defined by the x and y coordinations. By defining the total lattice the number of patches will be determined and the dividing of these two parameters will indicate the covered surface by the biofilm. The number of seed points in the whole lattice and the initial cell concentration (cell particles) are defined. The output of this step would be considered as the initial condition. The mechanisms of particle layouts in the lattice is determined by the Brownian motion, respectively [17].

The growth steps of DLA can be defined inductively as the following steps: the introduction of a randomly moving particle in a large distance from a particle aggregate in the lattice which adheres irreversibly at its first point of contact with the aggregate, thereby forming the next aggregate particle, and so on. To simulate shape with DLA, the above rule is modified such that a randomly moving particle irreversibly adheres to its point of the first contact with the aggregate only if the point of that contact overlaps with a simulated surface. An adherence probability coefficient which is defined between 0 and 1, determining the possibility of connection of the particle to aggregate by the hit. By definition, every particle at the time of connecting to the aggregate has hit value equal to 1. Otherwise, it is less than 1. Individually moving particles which contact the aggregate at the point not overlapping with the object are not connected. The probability coefficient of these particles is 0. In this case, a moving particle does not connect to aggregate and is deleted [18].

In brief, biofilm coverage grows to cover the surface, based on the seed positions and adherence probability of the scattered moving particles. The simulation algorithm will be stopped if the aggregation of the particles does not grow for 100 iterations hits. To ensure that there are enough moving particles to cover the surface, the area occupied by the particles is considered 6 times more as the area outlined by the analyzed object. The DLA algorithm may use in 2D and 3D forms to reproduce the biofilm growth shapes. Regarding 3D, particles move randomly on a 3D lattice instead of 2D. In DLA model, particles motion (i.e., diffusion) in random trajectories, adhere to one another (i.e., aggregates) to form fractal structures similar to the microbial biofilms, which have not regular form and growth patterns in the real culture conditions [17].

The purpose of this communication is to represent the *Microcoleus* growth pattern in the desert soil by reproducing morphological growth with diffusion limited aggregation model to quantify surface coverage. In addition, a comparison was performed with the results obtained for cultures in laboratory condition as well as model simulations.

2. MATERIALS AND METHODS

2.1 Assumptions and model settings

In this study, a desert soil surface was considered as substratum. *Microcoleus* filaments grow on it and a biofilm was formed. A uniform inoculation was implemented in the laboratory cultures and a random particle distribution on a simulation lattice. The inoculated filaments (seeds) are growing gradually and cover all the soil surface. To reduce the complexity of the modeling problem, the following assumptions were considered in developing the simulations with DLA model. The *Microcoleus* filamentous seed is uniformly distributed on the lattice and is considered as a single particle, the growth of initial filaments is in a random direction and fractal, according to the program specifications, different filaments have a uniform and similar growth pattern. The *Microcoleus* cell density is constant, the biofilm growth lattice is a square along x and y-axis with the diameter of -100 and 100 with the total surface of (dimensions of 100 x 100, totally 10000 patches), and the solution algorithm will stop in boundaries. The lattice particles are considered square in shape.

2.2 Model formulation and simulations

In the DLA model, a discrete version of the continuum diffusion equation (equation 1) is used. The numeric discrete form is shown in equation 2. As equation shows, the diffusion depends on the diffusivity parameter as well as time and distance. These parameters are specific to the microorganisms, and the problem initial and boundary conditions should be defined for solving partial differential equation (PDE). The first step in the *Microcoleus* biofilm formation in the model is the adherence of the inoculants filaments scattered on the soil surface. These initial conditions are defined with the number of seed particles (multiple starting points) randomly distributed on the whole grid. Here, the initial seed concentration (i.e., seed number) were considered as 10, 50, 100, and 200 for the simulation.

The second step in a succession of *Microcoleus* biofilm formation is characterized by an increase in the cell numbers resulting by consumption of the nutrient and cell division. The succession step is defined in the DLA model by the number of substrate particles scattered and random walk through the whole lattice. Here, the increasing numbers of 1 to 30000 were used to show the biofilm growth rate in the simulation.

In addition, all the substrate particle have no similar possibility to be consumed by living cells and conversion to biomass. To include the possibility of substrate conversion to the biomass, an adherence probability coefficient was selected. The adherence probability coefficient was selected to be 0.01, 0.1, 0.3, 0.5, 0.75, and 1.0 in the simulations.

The lattice was defined as square cells while other options are spherical or continuous biofilm. The equation 2 was solved by the program. In addition, the *Microcoleus* parameters including the maximum growth rate (μ_{max}) and the Michaelis - Menten constant were selected as 0.1 (h^{-1}) and 3.48E-4, respectively. The model simulation was performed in NetLogo software (5.3.1) [19].

$$\frac{dS}{dt} + D\frac{d^2S}{dz^2} = 0 \quad (1)$$

$$C(x, y, t+1) = C(x, y, t) + D\_CO2\_num*((C(x+1, y, t)-2*(C(x, y, t))+C(x-1, y, t)) + (C(x, y+1, t)-2*(C(x, y, t))+C(x, y-1, t))) \quad (2)$$

in which C is concentration, D_CO2_num is diffusivity.

2.3 *Microcoleus* growth condition

The cyanobacteria *Microcoleus* isolated from the biological soil crust of the semi-arid area has been used in the present experiments [7]. Cultivations were conducted in 80 mm Petri dishes. Desert soil particles (60 g) with the dimensions less than 2 mm were put in the Petri dishes. The prepared Petri dishes were inoculated with the *Microcoleus* suspension (0.8 g/L and 500 g/m^2) using the manual spraying method. The cultures were illuminated with the fluorescent lamp at the light intensity of 40 μE/m^2.s as measured by a light meter (TES 1332A Digital LUX Meter, Taiwan) from the top and kept at constant temperature of 25°C ± 2. The images of the biofilm growth on the soil surface were acquired applying a digital camera (Nikon 4500 Coolpix, Nikon, Japan) during cultivation period and were analyzed with image processing software of Adobe Photoshop CC 2014 developed in the previous study [20]. The results obtained through laboratory cultivation of the *Microcoleus* was used to compare and evaluate results achieved using the simulation displays and validation of the model, as well.

3. RESULTS

Microcoleus biofilm formed on the soil surface and was grown during laboratory solid state cultures in the Petri dishes. Figure 1 reflects the spatial biofilm structure of the *Microcoleus* in the 15th and 30th days of the cultivation as they appear by visual inspection. Moreover, each picture is shown along with its corresponding image generated by image processing software. As indicated, these visualization inspection pictures only show little information of the snapshot images and are not enough for the analyzing the structural features of the *Microcoleus* biofilms. The real condition for the biofilm formation is much more complex, as it depends on the characteristics of the organism, environmental condition, as well as their interactions. Therefore a DLA model was used for simple, easy, and quick simulation of surface coverage by *Microcoleus* biofilm.

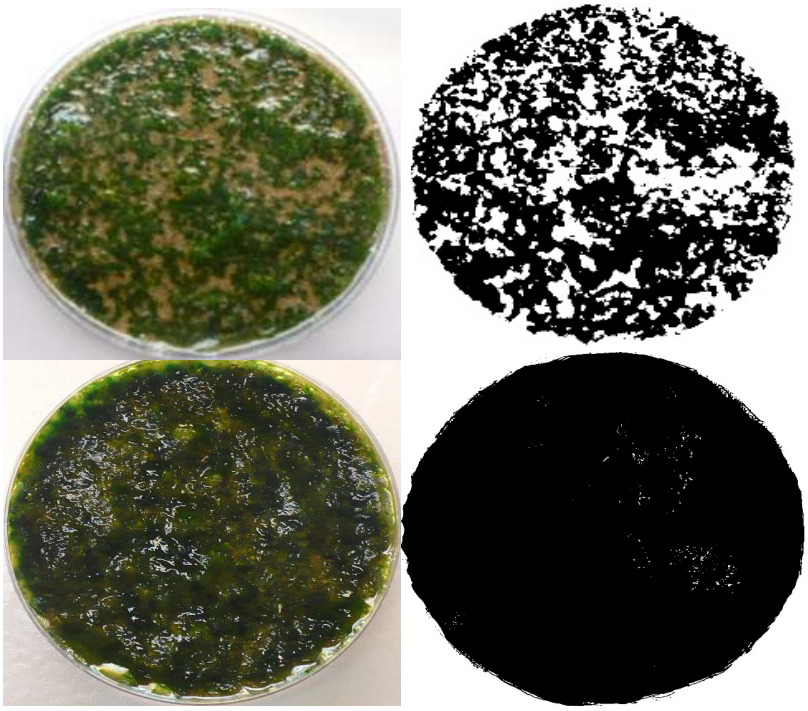

Figure 1. A snapshot of the *Microcoleus* cultures on the desert soil surface and a simulated image from a similar picture used for coverage measurement.

Figure 2 demonstrates a 2D representation of the diffusion limited aggregation simulation output. Different numbers of moving particles from 1 to 30000 were used in the simulation (1, 200, 500, 1000, 2000, 5000, 10000, 15000, 20000, 25000 and 30000). As it is shown, the surface coverage grows on the lattice gradually becomes similar to the experimental ones with an increasing number of particles. Here random walk propagation was considered to evaluate coverage of the surface. By time progress, the surface coverage grows along the lattice up to a 100%.

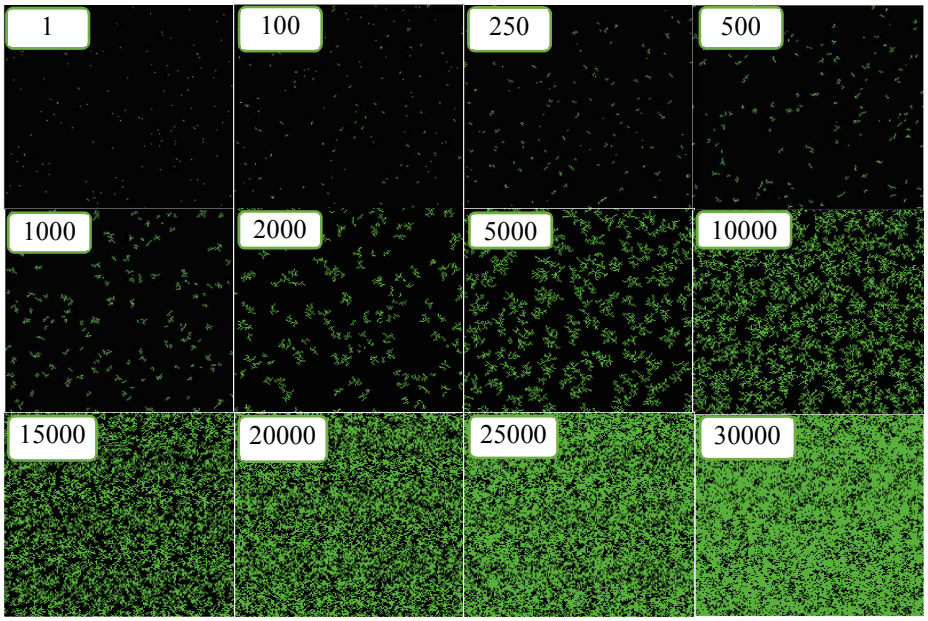

Figure 2. The simulation progress of soil surface coverage by *Microcoleus* modeled by NetLogo program. Particles = 1, 200, 500, 1000, 2000, 5000, 10000, 15000, 20000, 25000, and 30000. Probability adherence coefficient = 0.5, seed number = 150.

Figure 3 shows the relationship between particle numbers and the percent surface coverage. The presented model and other similar models have the potential to be used as an index for *Microcoleus* biofilm growth and other biological soil crust organisms after calibration with the practical data. Furthermore, the physical parameters such as biofilm density, morphological growth pattern, and initial concentration of inoculums are the major effective parameters in simulation outputs. The coverage surface was calculated by dividing the number of particles per total lattice grid area. A relatively linear relationship was obtained in practice. Similarly, for the calculation of the soil surface covered by the cultures in the experiment, the number of colored pixels was divided to the total pixels surface (as calculated by image processing software) [20].

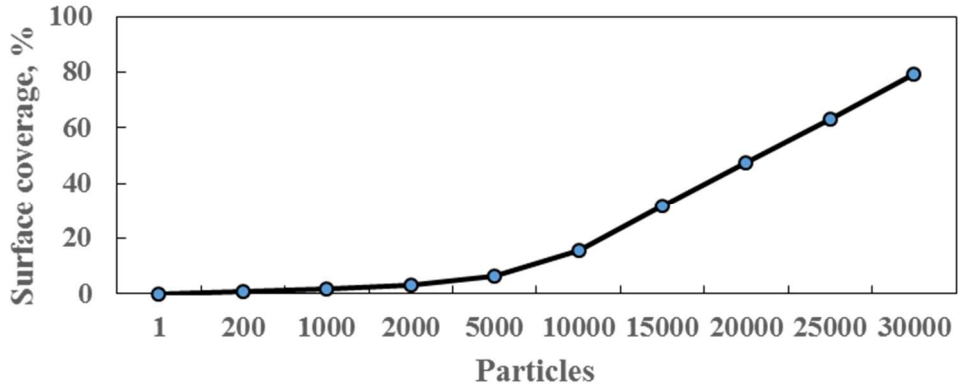

Figure 3. The relationship between particle numbers and coverage surface in the simulation model. The numbers were screened from 1 to 30000 and the corresponding surface coverage was from 0.01 to 95%

The main subject in the application of the model is the accurate model parameters usage which is commonly difficult to measure. Model parameters such as probability stickiness, patch size, and wiggle angel cab be selected by the user. These flexible conditions make the model powerful for future applications for the soil restoration practical operations. Figure 4. Represents the biofilm pattern with the various probabilities for the adherence of 0.01, 0.1, 0.3, 0.5, 0.75, and 1.0, respectively. The seed number was adjusted to the 150 and the number of particles at the 10000. As shown in figure 4, the various probability adherence coefficient results into different morphological forms output. Moreover, it causes different densities and surface coverage.

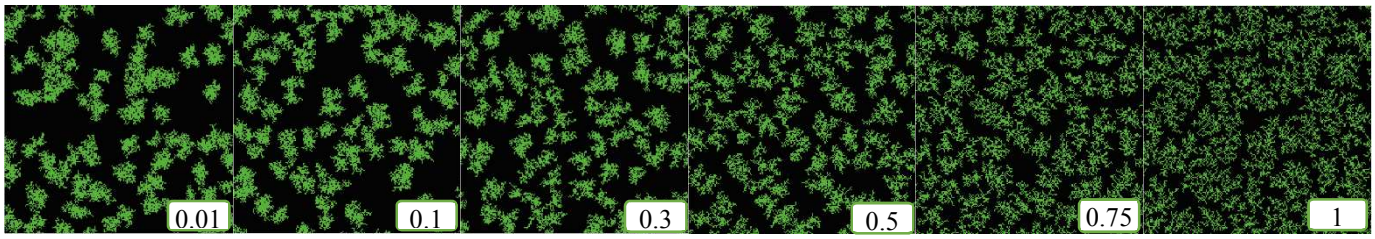

Figure 4. Simulation of the biofilm growth with various adherence probabilities of 0.01, 0.1, 0.3, 0.5, 0.75, and 1.0. Seed number = 150 and the number of particles = 10000.

Figure 5 displays simulation outputs by the 5 levels of initial seed numbers of 1, 50, 250, 500, and 1000. As figure 5 shows, more uniformly distributed results were obtained in the lattice by increased inoculated seeds as expected. In practice to have a more uniform inoculation, suspended seed spraying might be used.

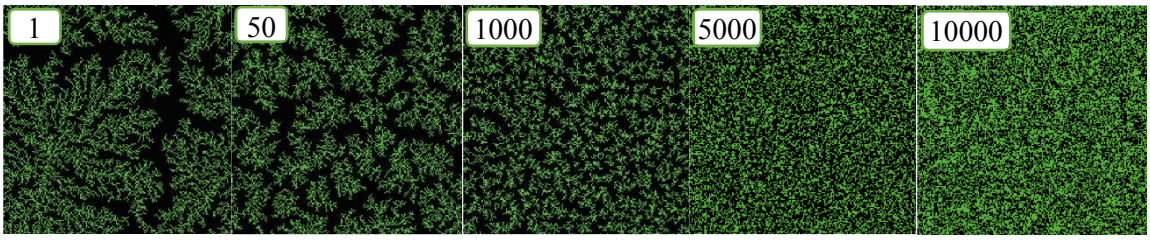

Figure 5. Simulation analysis of the biofilm growth versus a different number of seeds of 1, 50, 500, 1000, 5000, and 10000 seeds, respectively. Probability sticking coefficient = 1.0 and number of particles = 10000.

4. DISCUSSION

The main purpose of this study was to develop a diffusion limited aggregate (DLA) model of the *Microcoleus* biofilm in order to obtain insights into the morphology of this filamentous cyanobacterial growth on the soil surface when inoculated on the desert soil surface. To achieve this, a DLA algorithm was followed by the parameters corresponds to the *Microcoleus* and CO_2 as the limited substrate, then, the model's simulation results were compared with the experimental assessments.

The results have demonstrated that *Microcoleus* grows and covers both in laboratory cultures and in the simulation model outputs until all the surface is covered with these cyanobacteria. It starts with inoculation of *Microcoleus* suspension in Petri dishes and with a defined initial seed numbers in the model.

The pure *Microcoleus* biofilm growth on the soil surface has rarely been studied. The results of this study have provided the preliminary results of the further investigations. It shows that the biofilm is capable of covering soil surface completely and can be modeled by DLA algorithm for further practical applications. More detailed research are required regarding mesh coverage with the biofilm.

The photosynthetic microorganisms play an important role in soil porosity which affects final restoration systems. However, it is not clear how much the soil porosity affects subsequent soil function and requires modeling. Indeed, the model outputs have indicated that porosity of the coverage surface can be adjusted to what is in the real condition. As well, the variability of the porosity depends on the nutritional and environmental conditions. A higher cell density results to the less surface coverage and vice versa. Surface propagation happens following to the cell division and distribution of the proliferated cells on the surface. The propagation speed and coverage levels are dependent on microorganism physiological characteristics and the surrounding environmental conditions. This subject can be modeled in DLA algorithm by an adherence probability coefficient between 0 and 1. The lower coefficient is associated with more clogging and denser cell concentration compared to the lower density and higher surface coverage that is observed in the case of a higher coefficient. It has been reported that nutritional and environmental conditions affect the morphological structure and are important in the biofilm functions. Modeling of the morphological structure will provide insights for practical applications and designing of the artificial biological soil crust. In the proposed DLA model, the density and propagation will be defined only by the coefficient. However, in practice, it depends on more complex factors which need further investigation.

Both the laboratory and model simulation of the biofilm growth, illustrates the fractal propagation behavior on the soil surface. In the laboratory, biofilm growth happens with the increasing of cell numbers while, growth in DLA model will be done by increasing the number of moving particles. The fractal nature of the *Microcoleus*, as well as other biological soil crust (BSC) microorganisms, have various forms, therefore, additional studies are required to unravel the fractal growth style by the different strains. It should also be possible to reflect such diversity in the fractal model features either.

The simulation results show that it is possible to apply the model for evaluation of the surface coverage from close to zero up to 100% which is a range of coverage in most practical situations. Therefore, the model could possibly be used in almost all practical cases. The surface coverage was considered in some reports which describe surface coverage by microorganisms such as bacteria. From the practical viewpoint, it is an essential issue as in a non-fully covered soil the effects of the cell buried by the particle is one of the reasons for failure in the BSC recovery. The fast restoration processes would be more favorable in the field trials. In addition, the supplementary supporting methods such as shelters might also be useful, a feature that demands further investigations.

The simulation dimensions are defined by the user in the program which provides various scale options as required in the laboratory experiment and field trials. As the main hypothesis is the applicability of the model for the future large-scale BSC restoration processes, this specification is helpful for the laboratory simulation as well as field

trial. The present practical activities of the BSC restoration is limited because of technological and economic reasons. Modeling of the real conditions provides an easier insight, especially, for landscape applications.

The number of initial seed particles is adjustable by the user in the model for simulation. This condition provides situations to simulate the various initial inoculations. It also provides information regarding the output features. As inoculant production and cost are probably the major financial issues for the restoration activities in practice, the simulation model will provide a rough estimation about the required inoculants in the practical projects. It becomes more important if the restoration is due to be applied on a large scale or several inoculations are required for a special soil type or climatic conditions. This model provides the preliminary experimental results which should be validated by supplementary data in the field before the model can be applied in large scale. It also depends on the type and qualification of the used inoculant, which needs more investigations depending on the project location and characteristics.

In spite of the many aforementioned advantages, the proposed model has many limitations that should be considered for further investigations. A major limitation of the model is its two dimensional approaches. The real biofilm growth is three dimensional. Although various three dimensional models have been proposed in the literature, the adaptation of the models' parameters with *Microcoleus* biofilm has not been investigated. The three dimensional approach has more parameters, which makes it more complex for practical applications in large scale. Thus, BSC restoration requires simple 3D models for processes control and remains for further studies in the future.

One of the main challenges reported in the laboratory studies is the low adhesiveness of biofilm to the substratum, especially, after biofilm drying [21]. In the most of models this limitations has not been foreseen, an issue that needs more future studies to be included in the models. A new proposed approach in this regard might be consideration of a continuous multi-components biofilm including the living cells, released components, soil particles, as well as other factors. Taking different aspects into consideration makes the models more complex.

Time progress is a critical issue in the biofilm growth. The dynamics of the biofilm does not follow a steady state rule and change with the time. The geometry of the biofilm deformation and detachment happens mainly during biofilm growth and the following stationary growth phases [2,22]. These parameters are not constant and their modifications should be included in the suggested model.

In addition, another major factor in biological soil crust restoration process is the type and amount of adhering exopolysaccharides (EPS) released by *Microcoleus* cells that function as an adhesive compound to bind the surrounding soil particles. As the particles remain bonded to the filamentous sheath even after cell death [23]. The EPS release which is essential for the BSC function was not included in the presented DLA model and remains for further studies in the future.

By changing the environmental and nutritional conditions such as drought and UV irradiation to which the biofilm is exposed, the cell morphology and biofilm shape are modified by the bacterium in order to adapt new conditions. These different morphologies make irregularity and instability in the modeling parameters and simulation of the growth process. The model should be adapted to fit such variations. The effects of filamentous shape, density, porosity as well as other physical growth properties are major parameters that should be considered for further investigations [18,24–28]. These challenges are among the major subjects for further studies toward achievement of a comprehensive model applicable in the simulation and control of *Microcoleus* biofilm engineering.

5. CONCLUSIONS

The simulation of *Microcoleus* growth pattern is useful for the further studies of the biological soil crust restoration process technologies. Diffusion limited algorithm is a simple useful model to simulate the biofilm growth that was used in the present study for determining the morphology of *Microcoleus* biofilm. A good agreement was obtained between simulation and experimental assessments. Noticing to the fact that the present investigation still requires further research before attaining a conclusive outcome, further studies will definitely improve the robustness of the model and ability of which to simulate cyanobacteria growth pattern on the soil surface of the arid lands. In addition, further studies which will include the released adhesive EPS by the cells in the model would provide a more realistic model of the biological soil crust development technology through application of the filaments organisms for a number of applications such as dust emission reduction.

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