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A three-dimensional discrete lattice-based system for modeling the growth of aerial hyphae of filamentous fungi on solid surfaces: A tool for investigating micro-scale phenomena in solid-state fermentation

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ABSTRACT

In solid-state fermentation systems, the growth of aerial hyphae into the interparticle spaces increases the pressure drop through forcefully aerated beds. Aerial hyphae can also bind particles into agglomerates, restricting the transfer of O_2 to the particle surfaces. Despite these important effects, to date relatively little effort has been made to understand the growth patterns of these hyphae. In the current work we present a discrete lattice-based model that can be used to simulate the growth of the aerial hyphae of filamentous fungi. In the model, the elongation of hyphae involves the successive addition of 10 μm cubes, with random numbers being used to choose the direction of growth. The model was able to describe profiles available in the literature for the density of the aerial hyphae, as a function of height above the surface, for a situation in which the filamentous fungus *Rhizopus oligosporus* was grown on potato dextrose agar. The model can be modified to describe various different situations involving the growth of filamentous fungi in solid-state fermentation systems, such as the growth of penetrative hyphae and the growth of hyphae within the wet mycelial layer that often forms at the surfaces of particles. It therefore represents a useful tool for investigating phenomena that occur at the micro-scale in solid-state fermentation systems.

1. Introduction

Aerial hyphae play important roles in solid-state fermentation (SSF) processes that involve filamentous fungi. For example, it has been estimated that aerial hyphae are responsible for up to 75% of the overall O_2 uptake rate in SSF processes involving the fungus *Aspergillus oryzae* [1]. During periods of static operation of bioreactors, they can also form a mycelial network that binds substrate particles, thereby forming agglomerates. These agglomerates are problematic because they not only restrict the transport of O_2 to the surfaces of the particles within the agglomerates, but also favor the formation of channels within the substrate bed [2].

An understanding of the micro-scale phenomena that govern the growth of aerial hyphae would lay the basis for a better understanding of just how they influence the performance of SSF processes. In a related system, namely the growth of filamentous fungi in the form of pellets in submerged fermentation, various studies have characterized and modeled the spatial distribution of hyphae within the pellets and how this affects oxygen diffusion [3–7]. However, relatively little attention has been given to characterizing and modeling how aerial hyphae of filamentous fungi extend and branch to form a mycelial network within SSF systems [8,9].

In 1998, Nopharatana et al. [10] developed a mathematical model in which the growth of aerial hyphae was treated as a process involving the diffusion of hyphal tips in the space above the substrate surface. Later, confocal microscopy and image analysis algorithms were used to obtain experimental data for the density profile of biomass of *Rhizopus oligosporus* as a function of the height above the surface of a model solid substrate [11]. However, the results did not confirm the predictions of the earlier model: In the later periods of growth, the hyphal densities predicted by the model for heights above 1 mm were much greater than the experimental values.

The model of Nopharatana et al. [10] is a continuous model, in which discrete hyphal structures are not described; rather, the mycelium is treated as a continuous distribution of biomass [12]. However, as pointed out by Boswell [13], a discrete modeling

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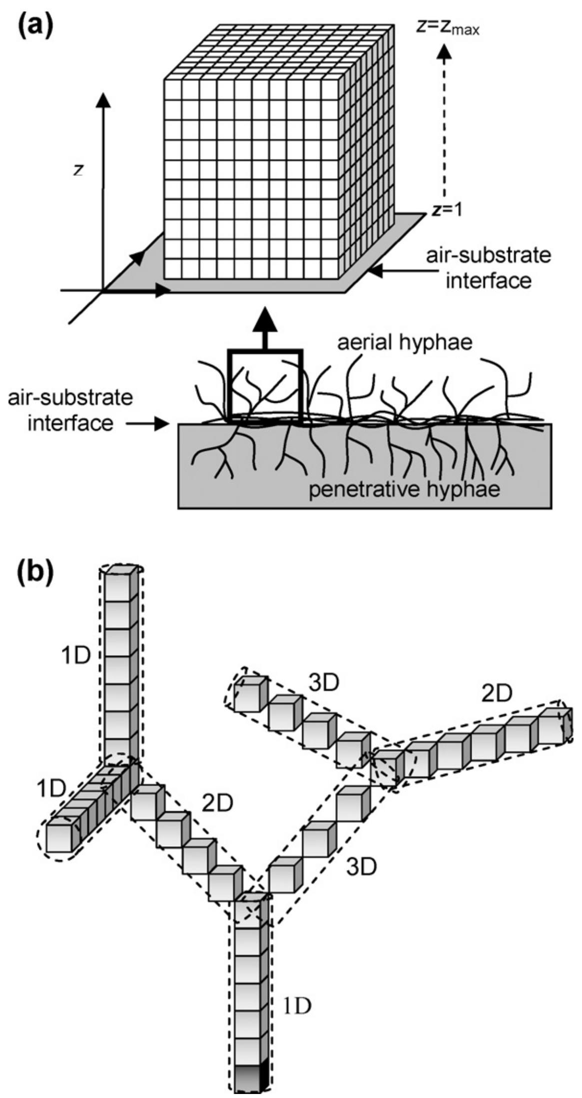

Fig. 1. Features of the system used to model the growth of aerial hyphae. (a) Division of the space above the surface into an array of cubes. The variable z, which denotes a horizontal plane of cubes, increases with height from plane at the bottom ($z = 1$) to the plane at the top of the available space ($z = z_{max}$). (b) Interconnected cubes represent a mycelial network originating from a spore (dark cube at the bottom). The dashed cylinders show how the various stretches between branch points represent hyphal segments. The labels 1D, 2D and 3D indicate the different orientations of the segments (see Section 2.3).

approach has a better chance of capturing micro-scale behavior, especially when growth occurs in heterogeneous environments. Therefore the aim of the current work was to develop a discrete approach that can be used to model growth of aerial hyphae in the heterogeneous environment that occurs in SSF systems. We demonstrate that the model developed is able to generate density profiles for aerial hyphae that are consistent with the experimental results of Nopharatana et al. [11]. It therefore represents a useful tool for investigating phenomena that occur at the micro-scale in solid-state fermentation systems.

2. Description of the discrete model

2.1. Basic features of the system

The space above the solid surface is divided into a 3-dimensional array of cubes (Fig. 1a). The side of the cube (of length $L = 10\ \mu m$) is chosen so as to give a cross-sectional area of the cube (L^2) similar to that of a fungal hypha. Each element of the array represents empty space, a hyphal tip or the fungal biomass of the hypha behind a tip. A hypha is formed by a sequence of adjacent cubes filled by biomass and may be branched (Fig. 1b).

The simulation involves successive iterations during which each hyphal tip chooses a particular direction and moves a certain number of cubes in that direction. As the tip moves through positions in the array it leaves behind a trail of biomass, which represents the hyphal tube behind the tip. New tips can be generated by branching events. Two types of hyphae with different branching patterns are recognized: vegetative hyphae and reproductive hyphae. The general sequence of events in the model is shown in Fig. 2. The rules applied in these events are outlined in the following subsections.

2.2. Assumptions

Several assumptions are made in this model. Firstly, hyphal biomass, once produced, does not disappear (i.e. the phenomenon of autolysis is not described). Secondly, hyphal biomass is immobile: once a cube is occupied by hyphal biomass, it remains occupied by that same hyphal biomass. The only movement in the system is the “laying down of new hyphal biomass” by the extending hyphal tips. Thirdly, nutrients and O_2 are available at the hyphal tips at concentrations that do not limit growth. Fourthly, only apical branching occurs (i.e. the phenomenon of lateral branching is not described). Fifthly, growth takes place in a closed box, since hyphae are not allowed to leave the system and do not enter the system from outside.

2.3. Direction taken and distance moved during iterations

At the beginning of each iteration, each hyphal tip needs to make a decision about the direction in which it will extend during that iteration. Each cube in the array that is occupied by a hyphal tip is surrounded by a probability field, superimposed on the 26 cubes adjacent to that of the tip (Fig. 3a). Each of these 26 cubes is assigned a probability, representing the probability that the tip will move in the direction of that cube during the next iteration. The sum of the probabilities of these 26 adjacent cubes is equal to unity. As explained in Section 2.5, steric considerations may mean that movement in certain directions is forbidden. In this case, the probabilities of choosing those directions are set to zero. The probabilities in all of the remaining available directions are normalized (i.e. such that they again sum to unity) by dividing by the sum of the probabilities of the available directions. A random number is then used to select which of the directions is taken by the tip.

Having chosen a direction, the hyphal tip extends a certain distance. The direction taken by the extending tip can have one of three orientations in relation to the axes of the grid (Fig. 1b): (i) it can be parallel to one of the axes of the grid and normal to the other two, in which case successive cubes within a hyphal segment will be joined by faces of the cube; (ii) it can be at a 45° angle to each of two of the axes of the grid and normal to the third axis, in which case successive cubes within a hyphal segment will be joined by an edge; (iii) it can be at a 45° angle to all three axes, in which case successive cubes within a hyphal segment will be joined by vertices. Growth in these three orientations will be referred to as 1D, 2D and 3D growth, respectively.

During a given time interval, a hypha extending in unrestricted space should extend by the same distance, regardless of its orientation. The number of cubes added to the hypha during an iteration therefore depends on the orientation of growth. Using Pythagoras' rule, if L is the length of the cube edge, then the length that one cube adds to the hypha, measured in the direction of growth, will be L, $L\sqrt{2}$ and $L\sqrt{3}$ for growth in 1D, 2D and 3D, respectively. If, during one iteration of unrestricted growth, $7n$ cubes are added for 1D