Application of Actinobacterial and Fungal Morphology on the Design of Operating Strategies in Bioprocess Development

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Abstract: Various options in biotechnology have been exercised to produce industrially important metabolites from filamentous organisms. To select a suitable production strategy, the direct and indirect roles of morphological parameters in controlling bioprocess variables are observed. Some progress has been made in quantitation of morphology.Useful correlations have been reported in the literature in this avenue. Of course, some of the models, with proper modification, can be used to explain the morphological phenomena. This review is concerned with the application of morphological variables to correlate with bioprocess parameters for explaining optimal production strategy.

INTRODUCTION

Fungi and actinobacteria are morphologically complex organisms differing in structure at different times in their life cycle, whether cultured in surface or in submerged growth. The morphology of these organisms plays a critical role in industrial biotechnology.

One of the most studied processes with filamentous microorganisms is penicillin G production by *Penicillium* sp. [1]. The complex models of cellular differentiation have limited potential for application in automatic control systems for industrial processes. For this purpose, the study of engineering aspects such as mixing, aeration, and reactor design in relation to growth, morphology, and productivity is necessary. Biochemical engineers are trying to elucidate the interdependence of rheological properties attributed to filamentous growth in fermented broths and transport phenomena related to bioreactor performance to optimize operating conditions in bioreactors for process improvement. Mycelial morphology, metabolite production, and consideration of morphological characteristics are significant avenues in bioprocess design [2].

The fungal fermentation is widely recognized as a complicated multiphase and multi-component process in which numerous problems can occur. Growth of the cultured organism and product formation are determined by a wide range of parameters, including the pH of the culture medium, the temperature of fermentation, the dissolved oxygen tension in the broth, the shear stress caused by the impeller, and the morphology. Thus, more knowledge on the effects of morphology and operational parameters is required to understand the fermentation process properly and effectively [3].

It is relevant to mention here that Pappagianni [4] reviews on understanding of growth mechanisms of fungal systems and the factors influencing fungal growth [5] suggested that the understanding of morphology is lagging behind in biopharmaceuticals producing filamentous organisms whereas Grimm *et al*. [6] hint at the relationship between morphology and productivity as a challenge.

However, the successful production of a fungal metabolite requires a detailed knowledge of the growth characteristics and the physiology of the fungus in question. Each fungus is unique in its anatomical, morphological, and physiological development. So there is a need for suitable correlation between the morphology, the overall growth of the organism, the relevant process variables, and the productivity. This review concentrates on quantitative approaches to morphological parameters and the process variables influencing them to suggest suitable operating strategies for bioprocess development.

QUANTITATION OF FILAMENTOUS ORGANISMS

Actinobacteria

Streptomycetes resemble fungi in their structure. Their branching and the filamentous arrangement of cells form a network called the mycelium. In addition to echoing fungi in their cellular structure, *Streptomycetes* resemble fungi in their elaborate life cycle. During the vegetative growth stage of development, DNA replication takes place without cellular division, creating the previously mentioned filamentous structure. *Streptomycetes* reproduce and disperse through the formation of spores, called conidia, which follows the period of vegetative growth. The spores are produced in aerial filaments (sporophores), which rise above the colony.The morphology of *Streptomyces olindensis* was differentiated into four classes: pellets, clumps, branched, and unbranched free filaments by image analysis [7].

Fungi

Fungi present many levels of epigenetic regulation that lead to morphological variations [8]. A comparative study

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reveals various methods of cell disintegration with reference to efficacy [9] as a wide range of morphological differences exists within the large group of filamentous fungi (Fig. **1**) to evaluate morphological parameters. To quantify such variation, one might look for the estimation of various parameters related to the morphology that might be correlated to process parameters.

PARAMETERS INVOLVED IN FUNGAL MORPHOLOGY

Morphology is quantified by the estimation of geometrical characteristics of individual filamentous mycelia represented as hyphae, and spherical colonies called pellets (cf. Table **1**). One needs to obtain the exact mechanism of pellet formatiom.

Hyphal geometry can be understood by the values of main hyphal length, equivalent hyphal length, mean hyphal growth unit, 'hair length', total hyphal length, hyphal fragmentation rate, average number of branch points, hyphal diameter, effective mean dimensionless length, branching frequency, total number of tips, number density formation, tip extension rate, hyphal polarity, ramification, angle of branching, and direction of branching. The derived parameters are morphology factor (length to diameter ratio of mycelial particles) and ratio of freely dispersed mycelia to pellets.

For pellets, number of pellets, pellets diameter, compactness of pellets, pellet volume fraction, average pellet mean size, roughness of elements, mean projected area of clumps, mean core convex area, mean core area, core circularity, and fullness of annular region are required as morphological parameters to understand the complex mycelial structure. However, direction of branching of freely suspended mycelia and compactness of the pellets are difficult to evaluate experimentally during production.

Hyphal Growth (Individual Filamentous Mycelia)

Hyphal growth rate, tip formation rate, tip extension rate, and branch formation rate were measured in *Mortierella*

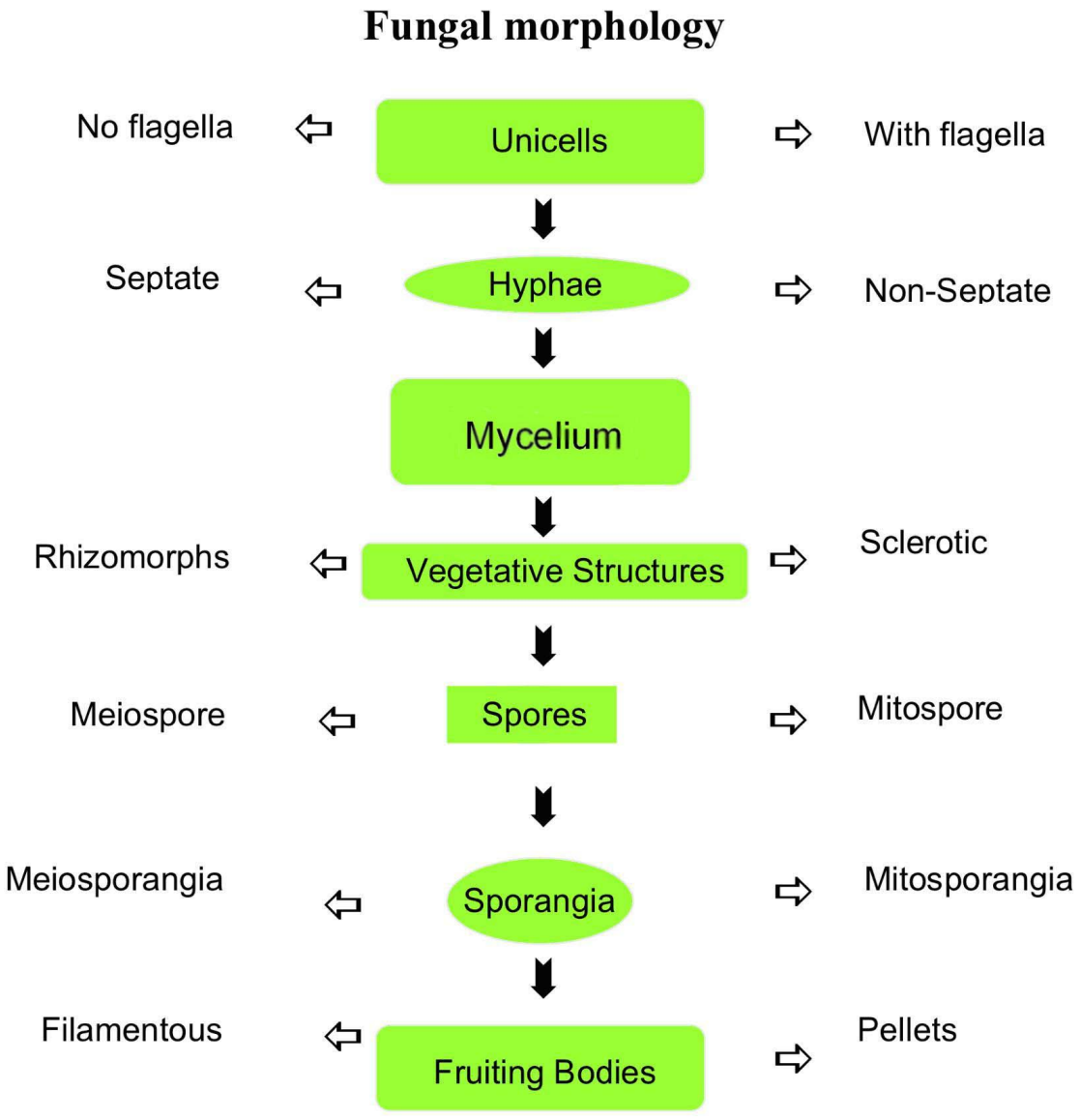

Fig. (1). Schematic representation of fungal morphology.

Table 1. Parameters Involved in Quantification of Actinobacterial and Fungal Morphology, Correlation to Process Variables and Probable Production Strategy

Organism	Fermenter Capacity and Mode of Operation	Parameter Studied	Correlation Between Mycelial Parameter and Process Parameter	Process Strategy	Reference*
Aspergillus alutaceus	Petri dish	Hyphal growth rate, hyphal length, branching pattern, hyphal tips, hyphal extension rate	Ochratoxin production positively correlated to branch density, μ unaltered , no mathematical relation	—	Blank *et al*., Lebensm-Weiss Technol, 31, 210 (1998)
Aspergillus awamori	3 L, Batch	Hairy length of pellets at different intensities, pellet size, pellet porosity	DOT no effect on mycelial fraction, porosity and pellet size inversely related to DOT Quantitative analysis, no mathematical relation to productivity	—	Cui *et al*., Biotechnol Bioeng, 57, 409 (1998)
a) *Aspergillus nidulans*	2 L, Erlenmeyer flask	Frequency of hyphae, % conidia with germ tubes	—	—	Rosenberger and Kessel, J Bacteriol, 94, 1464 (1967)
b)	Agar plates	Hyphal elongation	—	—	Riquelme *et al*., Protoplasma, 222, 211 (2003)
a) *Aspergillus niger*	6 L	Equivalent pellet diameter, clump roughness	Quantitative analysis	Dispersed mycelia for high productivity but causes poor mixing	Paul *et al*., Biochem Eng J, 3, 121 (1999)
b)	8 L, Batch, Fed batch,	% vacuolated vol. of filaments, mean diameter of filaments, mean length of filaments	No mathematical treatment	% vacuolated filaments more in fed batch at low reactant level lower portion of vacuoles posses higher μ and higher proportion of shorter filament having higher specific production rate in fed batch	Pappagianni, Process Biochem, 35, 359 (1999)
c)	6 L, Batch	Mean pellet convex area, Mean core convex area, mean pellet equivalent diameter, Mean core area, core circularity, fullness of annular region	—	Partial solution to scale up	Priede *et al*., Food Technol Biotechnol, 40, 57 (2002)
Aspergillus oryzae	6 L; Batch	Hyphal tip activity, mean total hyphal length, mean projected area, number of tip per hypha.	Quantitation	Agitation intensity to be manipulated to meet DOT, bulk mixing, broth rheology, mycelial morphology	Amanullah *et al*., Biotechnol Bioeng, 62, 434 (1999); 77, 815 (2002)
Aspergillus oryzae	80 m^3 batch	Equivalent hyphal length, total number of tips, average number of branch points, fragmentation rate	—	—	Li *et al*., Biotechnol Bioeng, 77, 60 (2002)

(Table 1) contd….

Organism	Fermenter Capacity and Mode of Operation	Parameter Studied	Correlation Between Mycelial Parameter and Process Parameter	Process Strategy	Reference*
Blakeslia trispora	250 mL Erlenmeyer flask	Microscopic view, no measurement	No	Dispersed growth in batch associated with increased β-carotene synthesis	Jeong *et al*., Current Microbiol 42, 225 (2001)
Cephalosporium acremonium	500 mL Erlenmeyer flask	Hyphae, orthospores, conidia, germlings	—	—	Nash and Huber, Appl Microbiol, 22, 6 (1971)
Fusarium moniliforme	6 L; Batch	Main Hyphal length, Total hyphal length, Hyphal width, hyphal growth unit, Hyphal growth volume, Mycelial clump perimeter, projected area, roughness	Quantitation	Generation of longer and thinner hyphae for better productivity	Priede *et al*., Biotechnol Bioeng, 48, 266 (1995)
a) *Neurospora crassa*	0.5 L; Batch	Ultrastructural studies of hyphae	—	—	Lowry *et al*., J Bacteriol, 94, 1757 (1967)
b)	Hollow glass tube for growth	Mycelial elongation, hyphal tip, nuclei	—	—	Selier *et al*., EMBO J 16, 3025 (1997)
a) *Penicillium chrysogenum*	0.5 L to 2 L; Batch	Pellets and filaments, hyphal and pellet diameter	—	—	Liu and Yu, Biotechnol Bioeng, 42, 777 (1993)
b)	2 L to 100 L; Batch	Effective hyphal length, hyphal growth unit, total hyphal length, number of branches	Apparent qualitative correlation exists between hyphal length and penicillin synthesis	—	Smith *et al*., Biotechnol Bioeng, 35, 1011 (1990)
c)	2 L to 75 L; Continuous, fed batch	Mean total hyphal length and mean projected area., P/V_L	—	—	Jüsten *et al*., Biotechnol Bioeng, 52, 672 (1996)
d)	Chemostat	Hyphal growth unit, hyphal branching frequency	Empirical relation between specific growth and hyphal branching		Morrison and Righelato, J Gen Microbiol, 81, 517 (1974)
Phytophthora parasitica, *Neurospora crassa*, *Schizophyllum commune*		Hyphal growth	—	—	Hunsley and Burnett, J Gen Microbiol, 62, 203 (1970)
Streptomyces griseus	6 L; Continuous	Mycelial clump area, perimeter, compactness, roughness	—	—	Obert *et al*., J Bacteriol, 172, 1180 (1990)
Streptomyces clavuligerus	7 L; Batch, fed batch	Main hyphal length, total hyphal length, number of tips, hyphal growth unit,	—	—	Belmar-Beiny and Thomas, Biotechnol Bioeng, 37, 456 (1991)
Thielaviopsis basicola	Batch	Hyphal length, diameter, degree of branching	—	—	Hood and Shew, Mycologia, 89, 793 (1997)
Trichoderma harzianum, *Fusarium culmorum*	Batch	Fragment length	—	—	Cheetham *et al*., J Microbiol Methods, 21, 113 (1995)

* For space limitation, details of references are not given in reference list.

alpina growing on 25 different combinations of carbon and nitrogen concentrations using a flow-through chamber. Experimental conditions were developed such as various nutrient concentrations having five defined C/N ratios to examine hyphal morphology. This system can be used to evaluate both morphological development and morphological parameters [10]. Chitinase and β-1,3-glucanase were used to study hyphal *in vitro* growth of *Glomus mosseae*. Chitinase applied to the hyphal tip inhibits hyphal extension and lysis of apex and alterations in the growth pattern. Chitinase or glucanase does not show any effect when applied to any part of the hyphae. The effect was similar to that of chitinase when both enzymes were applied to the hyphal tip [11]. Branching frequency in *Acremonium* sp. grown in batch culture may be shear-sensitive and branches more frequently as shear rate increases [12]. The synchrony of nuclear replication in individual and multinucleate hyphae of *Aspergillus nidulans* are reported. Protoplasmic organization of hyphal tips among fungi has been observed in vesicles and spitzenkörper.

The spitzenkörper is found in elongating hyphal tip of higher fungi. In this study, a possible relation between process of cell cycle and branching was studied by testing the effect of a nuclei distribution, mutation, cell cycle inhibitors, and conditional cell cycle mutations in combination with tip-growth inhibitors and varying substrate concentrations on branch initiation. Formation of branches was blocked after inhibition of nuclear division, which was not caused by a reduced growth rate [13]. The hyphal length of *Penicillium chrysogenum* during penicillin synthesis decreased at high agitation rate. Hyphal fragmentation occurred probably by both shaving-off the external clump hyphae and breakage of free hyphae. On the contrary, long and ramified hypha possess more interaction with substrate, causing higher enzyme yield [14]. Using a population model, the morphological data were analyzed in the literature. It appears that the fragmentation is the dominant process in fed-batch fermentations (Table **1**).

Pellets

The effect of fungal pellet size on the high yield of destruxin B by *Metarhizium anisopliae* led to a pellet of 2 mm diameter being the most suitable size for higher yield [15]. The influence of metal ions on pellet morphology and polygalacturonase synthesis by *Aspergillus niger* have been reported by Couri *et al*. [16]. The influence of modifications of the environmental conditions is observed on the growth of *A. niger* in terms of hyphal morphological patterns in pellets. A comparative study of the results obtained from shake-flask and bioreactor cultures showed that the intra- and extracellular catalase production was related neither to the fungal biomass nor to the size of the pellet. However, this production may be directly related to the external layer of the pellet and precisely to the morphology of the hyphae in this region [17]. The effect of the application of a pulsing flow to fluidized-bed bioreactors to control pellet morphology of *A. niger* and *Phanerochaete chrysosporium* are available in the literature [18]. In fermentation by periodical bleed feed operation, the decreased yield is most significant as biomass morphology changes from filamentous to pellet form [19]. The best maintenance of biotransformation capacity of pellets for a given media composition and cultivation conditions needs attention. During the change in morphology, when fluffy loose pellets were formed, the antibiotic production was high. This is contradictory to pectinase production [20]. The pellet morphology depends on inoculum level, genetic factors, medium composition, presence of surfactants, shear forces etc.

Studies on actinomycetes and fungi showed that the mass transfer, oxygen, nitrogen, phosphate limitations, and shear stresses are important process parameters during biosynthesis [21]. The control and regulation of hyphal growth and pellet size are of great importance [22]. Hence, a few important variables are referred here.

Important Process Parameters

Dissolved Oxygen

To study the effects of dissolved oxygen tension (DOT) on the morphology and growth kinetics of *A. nidulans*, a continuous-flow cultures has been used. In glucose limited continuous-flow cultures of *A. nidulans*, the mean length of hyphal segments and the degree of branching were reported to be independent of the DOT [23]. Study on the effect of low oxygen concentrations on growth and α-amylase production of *Aspergillus oryzae* in model solid state fermentations systems was reported by Rahardjo *et al*. [24]. During on-line rheology measurements and control in fungal fermentations, the viscosity of the broth was controlled by dilutions alone or dilutions in combination with growth control. Factors influencing the rheological properties of broth are biomass concentration, specific growth rate, mixing qualities (impeller speed and working volume), and the dissolved oxygen concentration [23]. Intensive agitation has altered the morphology of *Penicillium janthinellum* and an increase in oxygen concentration decreases activity of xylanase [25]. Pecinase from *Aspergillus sojae* and viscosity are higher at higher DOT with increased number of small-sized pellets [20].

Effect of Medium Composition

New versions of Monod-type growth relations were used in the morphometric evaluation of the specific growth rate of *A. niger* grown on agar plates at high glucose levels [26]. A qualitative method is available for comparative study between agar plate and solid-state culture to understand their physiological differences [26]. Studies in conventional batch culture have confirmed that the initial glucose concentration in the fermentation medium affects citric acid production by *A. niger* and the fungal morphology [27]. Time profiles of morphological parameters, viz., mean perimeter of clump, mean length of filament, mean diameter of filaments, and mean diameter of vacuoles, have been used to establish a relationship between vacuolation, fragmentation, and product formation under various agitation conditions and glucose levels [27]. The influence of carbon source and aeration rate on red pigment production in *Paecilomyces sinclairii* has been characterized with the aid of mycelial morphology and broth rheology [28]. The mycelial morphology of the *Ashbya gossypii* culture with mineral support is different from that of the cultures without support, which enables the accumulation of intracellular riboflavin. In the presence of mineral support, mycelia may be stable and the riboflavin production period may be extended [29].

Enzyme activity and fungal morphology were investigated using various concentrations of phosphate for the production of xylanase by *Aspergillus awamori* on synthetic medium in shake-flask cultures. Mycelia and small pellets produce higher enzyme productivity than large pellets.

Effect of Elicitors

The effects of elicitors on *P. chrysogenum* morphology was established with the help of image analysis. A considerable increase in spore numbers was observed in all mannan oligosaccharide-supplemented stirred-tank reactor cultures. When oligosaccharide elicitors have been added to liquid cultures of *P. chrysogenum*, an increase in tip numbers and clump size was observed. Probable understanding of the mechanism of elicitation will facilitate further application in different bio-industries [30].

Effect of pH

The assessment of microbody luminal pH (between 7 and 7.5) in *P. chrysogenum* is compatible with the enzymes localized in organelles involved in penicillin biosynthesis [31]. Pilot-scale studies were performed to determine the impact of dissolved oxygen, temperature, pH, and carbon dioxide on the process for the scale-up of fungal fermentation for the production of pneumocandins. The effect of substrate pH determines the growth rate of the fungus and proliferation of the fungus in medium. This gives vital information for determining the subculture frequency and for designing substrate parameters for nursery/plantation programs [32].

Effect of Agitation Intensity

Qualitative relationship between agitation levels and medium viscosity has been observed in phytase production and *A. niger* morphology in submerged and solid-state cultures. Fungal morphology was greatly influenced by agitation with the morphological forms of small pellets and entangled mycelia predominating at low agitation, while the free filamentous form was obtained at 300 rpm. The morphological change in pellets is a good indicator for identifying the cell activity for exo-biopolymer production under agitated conditions [10].

Much information is available on mean projected area, mean total hyphal length, number of tips, mean total length, and many facets of hyphal morphogenesis, but precise understanding of the structural, biochemical, and genetic basis of apical growth remain unanswered so far. There is no correlation between agitation intensity on mycelial morphology and recombinant protein production in chemostat and fedbatch cultures. The dependence of mycelial morphology on type of impeller (Rushton-type impellers and pipe spargers) and agitation intensity has been observed in a chemostat culture of *A. oryzae* (Table **1**). The mechanical properties of the filamentous fungi cell wall increased during the deceleration phase and resulted in higher resistance of the mycelia in the shear-dominant environment in the bioreactor. Higher agitation rates increase the oxygen transfer rate and bulk mixing without significant mechanical damage or loss of enzyme productivity. The mean convex perimeter of clumps should be within the threshold value to achieve increased production. The fragmentation of filamentous fungal hyphae depends on two phenomena: hydrodynamic stresses leading to hyphal breakage and hyphal tensile strength for resisting breakage. Agitation induced mycelial fragmentation of *A. oryzae* have been studied considering morphological (equivalent hyphal length) and hydrodynamic variables (specific power input and kinematic viscocity). Turbulent hydrodynamic theory was used for developing correlations (Table **1**) that allowed experimental data of morphology and hydrodynamics to be used to estimate relative tensile strength of filamentous fungi.

The overall economic decision on the use of correct forms of morphology may be constrained by the design of the fermenters [33]. Studies clearly demonstrate that morphology is an important factor in determining the overall performance of citric acid production processes [34]. A special bioreactor design is required to provide mycelial and other producers sensitive to deformation forces with mixed cultivation conditions. Axial mixing systems are promising in this context. This can be a tool for scale-up and scale-down in geometrically dissimilar systems [35].

The relation between morphological parameters and specific growth rate of organism is established for specific bioreactions [24]. The study was used to assess filamentous fungal morphology and fragmentation behavior in large-scale fedbatch fungal fermentations. To accomplish this, large-scale *A. oryzae* fermentations used for the production of a recombinant enzyme were carried out at two different impeller power levels. The efficient production of lactic acid by the filamentous fungus *Rhizopus arrhizus* in a stirred tank reactor requires a floating biomass with filamentous or pellet morphology [19].

Optimal conditions required for the mycelial growth of *Aspergillus cinnamonea* in a submerged shake flask cultures were determined to understand the relationship between parameters involved in enzyme production and fungal morphology and their dependence on cultivation conditions [36].

MODELING IN FUNGAL MORPHOLOGY

A mathematical model for apical growth, septation, and branching of mycelial microorganisms consists of two parts: the deterministic (hyphal tip growth and septation) and the stochastic (mycelial branching, direction of tip growth). These models describe morphological development of mycelia up to the formation of pellets. Two mathematical models presented simulation of the single microbial pellet growth of *P. chrysogenum* [37]. Various empirical correlations (Table **1**) from a single-hyphal model were employed to accomplish the highest possible correspondence to the layer model. Three-dimensional growth of a fungus has been simulated, based on a model for the evolution of microscopic morphology of *Trichoderma reesei.* From this simulation, it is possible to extend a model describing the kinetics of hyphal extension and stochastic branching to three dimensions. This model could be used to simulate the formation of a pellet from one or more spores [38]. A population model has been used to analyze single hyphal growth and fragmentation in submerged cultures. To find the solutions for population models, Monte Carlo simulation is compared with a discretization method and is observed to be faster than the Monte Carlo simulation method. A number of measurements of total hyphal length and the number of tips revealed that it is possible to distinguish the four models used in the analysis (cf. Table **2**). *Penicillium camembertii* cultivated on a solid

Table 2. Models for Mycelial Morphology

Organism	Expression for Growth Rate	Parameters	Reference
Aspergillus nidulans	$\mu_x = (2 \ln 2)\ \overline{u}_r / \overline{\gamma}\ L_{av}$, $\overline{\gamma} = \frac{\overline{N}_s}{\overline{N}_t}$ $\overline{u}_r$ = average velocity of hyphal tip extension $\overline{N}_s$ = average number of segments $\overline{N}_t$ = number of tips $\mu_x = \mu_{\max} \frac{(X + X_c)}{2X}\left[1 - \left(\frac{X}{X_m}\right)^n\right]$ X_m = max. biomass, X_c = critical biomass	Microscopic and Macroscopic parameters, no relation to productivity suggested, correlation between hyphal geometry and μ available	Viniegra- González Biotechnol Bioeng, 42, 1 (1993)
Aspergillus oryzae i)	$\sigma_h = \tau_{s\,s} \ln (F/q_{frag}) + \tau_s \ln k_2$ $k_{frag} = c\,(\rho / N_{web}\, d_0\, w)^{0.5} N^{1.75} D_i^{0.5} (\upsilon / \sigma_h^2)^{0.25}$	σ_h = Hyphal tensile strength Hyphal fragmentation rate with hydrodynamic variables	Li *et al*. Biotechnol Bioeng, 77, 60 (2002)
ii)	$L_{e,avg} = [(n_{avg} + 1)/2\, n_{avg}] / (L_{t,,avg} - L_{tip})$ $d L_{e,avg} / dt = k_{frag} (L_{e,avg} - L_{e,eq})$ $d A_{avg} / dt = k_{frag} (A_{avg} - A_{eq})$ $d L_{t,avg} / dt = n_{avg} q_{tip} - q_{frag} L_{t,avg}$ $d n_{avg} / dt = q_{bran} - q_{frag} n_{avg}$ $q_{frag} = (1/L_{e,avg})(d L_{e,avg} / dt)$ $q_{bran} = k_{bran} L_{t,,avg}$ $q_{tip} = (k_{tip} L_{t,,avg}) / (K_t + L_{t,,avg})$	$L_{e,avg}$ = equivalent hyphal length, L_{eq} = equilibrium hyphal length, A_{avg} = mean projected area, q_{frag} = avg. specific fragmentation rate, q_{bran} =hyphal branching rate, q_{tip} =hyphal tip extension rate	Li *et al*. Biotechnol Bioeng, 70, 300 (2000)
Fungi in general	$K_r = \left[k_2\left(\sqrt{S_0} - \sqrt{S_i}\right)\right]\sqrt{\sigma_m}$	Growth of surface colonies initially developed for bacteria extended to fungi	Pirt, Gen Microbiol, 47, 181 (1967)
	$L_{t,avg} = = [(\sqrt{k_{tip}}\, k_{bran}) / k_{frag}]$ $n_{avg} = N_t / e = k_{bran} / k_{frag}$ $l_{e,max} = k_{tensile}\, d^{3/8} (p_g / V)^{-1/4}$	Avg. total hyphal length Avg. number of tips Max. effective length No correlation on to productivity suggested	Krabben *et al*., Chem Eng Sci, 52, 2641 (1997)
Strepotomyces hygroscopicus, Penicillium chrysogenum	$\mu = (k_a Z_a + k_s S_s) \frac{S}{S + K_s}$	Metamorphosis and growth reactions, No correlation to productivity suggested	Nielsen, Biotechnol Bioeng, 41, 715 (1993)
Penicillium chrysogenum i)	$\sqrt{\tau} = \sqrt{\tau_0} + K_c \sqrt{\gamma}$ $\tau_0 = \delta C_x^{2.3 - 2.5}$	Significance of yield stress: yield stress = f (Morphology factor, mycelial concentration)	Liu and Yu, Biotechnol Bioeng, 42, 777 (1993)
ii)	$n_R = L_R / (h g u_R)$ $L_R(t) = L_R(t - \Delta t) + \Delta L_R^+(t) + \Delta L_R^-(t)$	Number of tips Mycelial length	Meyerhoff and Bellgardt, Bioprocess Eng, 12, 315 (1995)
Trichoderma ressei	$q^i_{tip} = k_{tip,1} + k_{tip,2} (l_{br} / l_{br} + K_t)$	Tip extension rate	Lejeune and Baron, Biotechnol Bioeng, 53, 139 (1997)

Note: For abbreviations and their units relevant reference may be considered.

medium has been analyzed for growth kinetics. The Verlhust or logistic model was tested and validated for fitting the substrate consumption (lactate and peptone), ammonia production, and proton transfer kinetics. The model is useful to analyze growth because it allows the characterization at the end of exponential phase and in the beginning of stationary phase [39]. Advances have been made in both empirical and mechanistic types of kinetic models. An insight of various kinetic and intraparticle phenomena represent within the system. Factors on modeling, basic kinetic equations, effect of environmental conditions on growth, death kinetics, and intraparticle phenomena in solid state fermentation have been reported by Mitchell *et al*. [40].

Gierz and Bartinicki-Garcia [41] described a three-dimensional model of fungal morphogenesis based on the vesicle supply center concept. The 'neighbour-sensing' model brings together the basic essentials of hyphal growth kinetics into a vector-based mathematical model in which the growth vector of each virtual hyphal tip was calculated by reference to the surrounding virtual mycelium. This model can simulate growth on semisolid substrata like agar or soil, which enables realistic simulation of mycelial colonies of filamentous fungi grown on Petri-dish-style experimental conditions. The biomechanical models of hyphal growth in actinomycetes were investigated. This model exhibits realistic hyphal shapes and indicates a self-similar tip growth mechanism consistent with that observed experimentally [42]. A population- based morphologically structured model for hyphal growth and product formation in streptomycin fermentation was successfully applied to batch fermentation process [43].

However, a relationship between the morphology and productivity is a complex phenomenon involving mass transport, molecular basis of morphological analysis, and flow behavior in mycelial structure [6].

A new technique based on a fractal model has been developed to quantify the macroscopic morphology of mycelia. Studies show that the mycelial mass in the bioreactors can be treated as a fractal structure, with its fractal dimension being a quantitative morphological index [44]. To understand the generation of various colony patterns of filamentous fungi, a colony model with single- and multi-mycelial layers that originated by consuming a limited amount of nutrient was developed [45]. A model for branch initiation in *A. nidulans* is used to measure growth parameters. Kim *et al.* [46] reported the kinetic model to describe the morphological differentiation and the celphalosporin C produced from *Cephalosporium acremonium*. The proposed model is based on the total cell mass consisting of hyphae. Swollen hyphal fragments and arthrospores can only produce celphalosporin C. Some approximation has been made by considering total cell mass to correlate with antibiotics production [47].

CONCLUSIONS

Powerful methods to characterize and estimate morphological parameters have generated important data. However, correlation of physical parameters (viz., rheology, agitation intensity, dissolved oxygen level, pH, and temperature) of biological reaction media, cell mass, morphology, and productivity is yet to be developed to obtain a moderately simple model based on a sound mechanistic background with proper physiology of morphogenesis of filamentous organism. The highly structured model of Megee *et al*. [48] using 44 parameters cannot be experimentally validated so far [49]. At present, it is difficult to obtain a direct link between morphology and productivity. However, different models developed for fungal morphology can be modified and manipulated to explain the experimental methods to a large extent. One might consider with present-day knowledge of quantitative morphology to improve the performance of the bioreactor.

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