A population-based morphologically structured model for hyphal growth and product formation in streptomycin fermentation

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Summary

A population model discriminating the hyphae according to the hyphal length and a morphologically structured model considering the specific function of different morphological forms of a hypha are combined together to describe mycelial growth, substrate consumption and secondary metabolite formation in streptomycin fermentation. In the population model, the growth modes of hyphae with different age or length are considered, while in the morphologically structured model, the morphological forms of hyphae and their functions in growth and metabolism are described. The population model and the morphologically structured model are interrelated by a branching function and a differentiation function. In the model, the growth rate of immature apical compartment is distinguished from those of matured ones, branching is proposed to occur only in the subapical region, and the hyphal compartment is assumed to synthesize secondary metabolites. The model is successfully applied to simulate the batch fermentation process of streptomycin production. The growth characteristics of filamentous microorganisms are also discussed using the model predictions.

Nomenclature

- \( A \) : Cross section area of hypha (\( \mu m^2 \))
- \( B(t) \) : Branching function (\( h^{-1} \))
- \( P(y, t) \) : Hyphal number function
- \( k_1 \) : Rate constant for hyphal tip extension (\( h^{-1} \))
- \( K_1 \) : Saturation constant for apical cell growth (\( g \cdot l^{-1} \))
- \( K_2 \) : Saturation constant for branch formation (\( g \cdot l^{-1} \))
- \( k_{D1} \) : Rate constant for hyphal differentiation (\( h^{-1} \))
- \( K_{D1} \) : Saturation constant for hyphal differentiation (\( g \cdot l^{-1} \))
- \( k_{D1} \) : Rate constant for hyphal deactivation (\( h^{-1} \))
- \( K_{D1} \) : Saturation constant for hyphal deactivation (\( g \cdot l^{-1} \))
- \( k_H \) : Rate constant for streptomycin deactivation (\( h^{-1} \))
- \( K_1 \) : Inhibition constant for streptomycin production (\( g \cdot l^{-1} \))
- \( k_P \) : Rate constant for streptomycin production (\( g \cdot l^{-1} \))
- \( K_P \) : Saturation constant for streptomycin production (\( g \cdot l^{-1} \))
- \( L_H \) : Length of hyphal compartment (\( \mu m \))
- \( P(y, t) \) : Hyphal number distribution function
- \( r_{NB} \) : Branch formation rate (\( h^{-1} \))
- \( r(y, t) \) : Hyphal differentiation function
- \( S \) : Substrate concentration (\( g \cdot l^{-1} \))
- \( y_A \) : Length of apical compartment in newly formed hypha (\( \mu m \))
- \( y_B \) : Length of subapical compartment in small hypha (\( \mu m \))
- \( y_{MA} \) : Length of matured apical compartment (\( \mu m \))
- \( y_{MB} \) : Length of matured subapical compartment (\( \mu m \))
- \( Y_{X/S} \) : Yield coefficient
- \( Y_{P/S} \) : Yield coefficient
- \( a \) : Morphological fraction of apical compartment
- \( \phi \) : Branch frequency
- \( \phi_0 \) : Maximum branch frequency
- \( \rho \) : Hyphal cell density (\( kg \cdot l^{-1} \))

Introduction

Filamentous microorganisms are widely applied in industrial fermentation processes for the production of...
antibiotics, enzymes, and organic acids etc. The fermentation technologies of such fermentation processes have been well developed, while study on the kinetics of mycelial growth and product formation in filamentous microorganisms is inadequate due to the complexity of mycelial growth and secondary metabolism. These microorganisms grow by hyphal tip extension and branching, prominently differing from unicellular microorganisms such as bacteria or yeasts. In addition, morphological differentiation usually occurs in filamentous microorganisms and is generally related with secondary metabolite formation. For example, cephalosporin C is only formed by swollen hyphae of Cephalosporium acremonium, which are generated from normal hyphae through differentiation in consequence of glucose depletion (Matsumura et al. 1981). Therefore, unstructured models are incapable of describing the growth and product formation in filamentous microorganisms, and more comprehensive models are needed for this kind of microorganism.

The concept of “morphology” in hyphal growth and differentiation was first proposed by Megee et al. (1970). They specified two differentiation forms in an actively growing hypha: an apical compartment (A) and a hyphal compartment (H). Compartment H was formed from compartment A through tip extension and differentiation, and compartment A was generated from compartment H through branching. Nielsen (1993) structured the hypha into apical, subapical and hyphal compartments. In their model assumptions, the apical compartment was responsible for hyphal extension, and the subapical compartment branched to form new apical compartment, which was a good approach to experimental observations, for branching seldom occurs in the rear part of the hypha. The Nielsen model was further applied in simulation of penicillin fermentation and re-tamycin fermentation with good agreement (Zangirolami et al. 1998; Birol et al. 2002; Giudici et al. 2004). Based on the quantitative measurement of morphological differentiation with an image analytical system, Paul & Thomas (1996) incorporated hyphal differentiation into the morphologically structured model for penicillin fermentation process. The model well described vacuole formation and glucose repression in penicillin production.

In the present work, coupled with the morphology description, a population balance (Ramkrishina 1977) is applied to distinguish the hyphae with different hyphal length. In the culture of filamentous microorganisms, there are several types of hyphae such as newly formed hyphae that contain the apical compartment only, small hyphae that contain apical compartment and the sub-apical compartment, large hyphae that contain the apical compartment, subapical compartment and hyphal compartment, old hyphae that only contain the hyphal compartment. The modes of growth and metabolism are different in these hyphae, e.g., newly formed hyphae grow exponentially and do not produce secondary metabolites, while large hyphae grow linearly with tip extension and produce secondary metabolites in their hyphal compartments. Therefore, application of population balance to describe the growth and metabolism of different hyphae discriminatingly might increase the accuracy of the morphologically structured model.

Model description

Population model

An actively growing mycelial element is schematically shown in Figure 1. In the mycelial element, hypha 1 is the initial hypha. When it grows, it branches in the subapical region to form hypha 2–5, and 7. Hypha 6 is the secondary branch formed by hypha 2. In the population model, each line in Figure 1 is considered as an individual hypha, and each newly formed branch is considered as a new hypha. Once a new branch is formed, it grows and metabolizes independently, that is, it is no longer related with the hypha from which it is generated. The growth and metabolic pattern of a new branch resembles a new hypha that germinates from a spore.

Denote \( F(y, t) \) as the number of actively growing hyphae with length shorter than \( y \) at time \( t \). Obviously, \( F(y, t) \geq 0 \), and \( F(y, t) \) is a monotonically increasing function of \( y \) at any time \( t \).

In bulky fermentation process, because the population of hyphae is huge, \( F(y, t) \) is continuous and differentiable, and its partial differentials, \( \frac{\partial F}{\partial y} \) and \( \frac{\partial F}{\partial t} \), are also continuous functions of \( y \) and \( t \). Let

\[
P(y, t) = \frac{\partial F}{\partial y}
\]

(1)

where \( P(y, t) \) is the distribution function of the hyphal population. Because \( F(y, t) \) is a monotonically increasing function of \( y \), we have

\[
P(y, t) \geq 0
\]

\[
P(y_m, t) = 0
\]