MODELING OF NUTRIENT MIST REACTOR TO GROW HAIRY ROOTS

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by

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Certificate

This is to certify that the thesis titled **"Modeling of Nutrient Mist Reactor to Grow Hairy Roots"** being submitted by **Mr. Sambasiva Rao Katuri** in the Department of Chemical Engineering, Indian Institute of Technology, Delhi, for the award of the degree of **Doctor of Philosophy**, is a record of bonafide research work carried out by him under my guidance and supervision. In my opinion, the thesis has reached the standards fulfilling the requirements of the regulations relating to the degree. The results contained in this thesis have not been submitted for the award of any other degree, diploma, associateship or similar title of any university or institution.

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New Delhi **Sambasiva Rao Katuri**

Abstract

Secondary metabolites extracted from hairy roots are extremely valuable due to their use in pharmaceuticals, dyes and so forth. Cultivation of hairy roots in bioreactor systems has emerged as an important route for the synthesis of secondary metabolites. The nutrient mist reactor offers numerous benefits over other bioreactor systems and is widely preferred for hairy root cultures. The present thesis focuses on developing mathematical models to study the growth of hairy roots in nutrient mist reactors.

A simple model termed as the 'Preliminary Growth Model' is proposed to study the growth of primary and secondary roots with culture time. The flow of nutrients through both the external mass transport and internal transport are considered for this model. The growth coefficients are estimated by fitting the model with the published experimental data. It is observed that growth of the primary root can decrease as much as to one-fifth of its initial growth during elongation-branching phase. Also, it is observed that the growth of primary root can decrease further during maturity phase when the absorbed nutrients are only used for its sustenance. Interestingly, the model results pointed out that the secondary roots grow at a faster rate than their primary root during both elongation-branching phase and maturity phase of the primary root. An unusual discontinuity in the growth curve has been reported at the maturity age which is a serious limitation for this model.

The 'Advanced Growth Model' is then proposed which requires prior knowledge of only primary root elongation phase growth for any hairy root. This data can be easily gathered from experiments. For this model, only one growth coefficient needs to be fitted from the available experimental data. An area fraction parameter has also been introduced to explain internal flow of nutrients mathematically between the primary root and its secondary roots. The advanced growth model produces smooth and continuous growth curves unlike previous models and also fits closely with the available experimental results. Good biomass yield is observed during simulations for short inter node distances, low nutrient-biomass ratios, lower maintenance rates and larger growth rates of primary roots. Both the models are successful in explaining the growth of hairy root cultures. The concepts of internal flow, nutrient consumption for maintenance and nutrient consumption for growth are described qualitatively in the preliminary growth model, but more quantitatively in the advanced growth model.

Another major contribution of the present thesis is to develop unique mathematical model for mist-ON cycle and mist-OFF cycle operations of nutrient mist reactors and its integration with the growth kinetics equations. The model is developed in terms of 'Liquid film thickness over the roots' and 'Nutrients concentration in the liquid film' which are the limiting conditions for the nutrient mist reactor operation. Extensive simulations are performed to understand the effect of duration for the mist-ON and mist-OFF cycles, the mist feed rates and the nutrients concentrations in the mist. The pros and cons of longer/shorter mist-ON and longer/shorter mist-OFF cycles are discussed for every permissible parameter that aid in better yields of biomass. The results for film thickness show the importance of intermittent operation instead of continuous operation of nutrient mist reactors. Further, formation of thick liquid films has to be avoided by keeping mist-ON cycles short and low mist flow rates, so that the diffusion of oxygen to the root culture never gets affected. The concentration of nutrients in the liquid film change dynamically during mist-ON cycle and mist-OFF cycles. Keeping mist cycles short allows frequent mixing of fresh mist and increases the film concentration. High concentrations in the film are also observed with high feed concentrations and short mist-OFF cycles. Further, low drainage rates keep adequate amount of nutrients in the film for longer times. It has been shown that high growth rates are possible whenever the mass transfer rate of nutrients across the root surface increases.

Nutrients in the liquid films get depleted when nutrient mist reactor operates for short mist-ON and long mist-OFF cycles. Nutrients in the film do not fall below critical concentration even with longer mist-OFF cycles if the mist feed concentration is kept high. Hence, reactor can be operated under short mist-ON and long mist-OFF cycles if the drainage rate is low as it saves mist requirement to the reactor. Conversely, long mist-ON cycles are allowed when the drainage rate is very high to supply adequate nutrients for sustained growth of hairy roots as film thickness buildup is slow. However, longer mist-ON cycle operation notably increases mist feed supply to the reactor. Moreover, the nutrient mist reactor gets completely filled up with liquid if long mist-ON cycle is used with low mist drainage from the roots. Therefore, change in mist duty cycles shows a remarkable effect on the performance of nutrient mist reactor.

The growth models presented in this thesis will provide a platform for the researchers to understand various aspects of hairy root cultures. The simulation of these unique reactor model equations provide valuable input to scale up of nutrient mist reactor for commercial exploitation of secondary metabolites.

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