Mathematical Modeling of Mist Bioreactor for the Growth of Hairy Roots

Sambasiva Rao Katuri[1] Ritu Ranjan[2] Rajesh Khanna[3]
Department of Chemical Engineering[1, 2, 3]
Indian Institute of Technology Delhi
New Delhi, India – 110016

ABSTRACT

Plant derived chemicals are valuable sources for a variety of pharmaceuticals, flavors, dyes, fragrance, stimulants, poisons. Hairy roots are induced in susceptible plants by transformation with *Agrobacterium rhizogenes*. In the present work, a strategy has been chosen to study the parameters for sustained growth of hairy roots in nutrient mist reactor. This study is based on the development of a mathematical model at root bed level per unit mass basis. Model equations were developed to study the operating parameters for constant specific growth rate processes. Important parameters, selected for this study are effective mist flow rate, nutrient concentration available to roots, duration of mist-ON/mist-OFF cycle and drainage characteristics of the root bed. A new method to describe the drainage characteristics of the hairy root bed is also shown in this study. It is clear from the results that the mist ON/mist-OFF cycles play an important role in an efficient operation of mist reactor and good growth rate can be achieved either by increasing nutrient concentration or by changing the mist duty cycles. This study will provide the much required engineering input to the hairy root technology.

Keywords
Mist-ON/mist-OFF cycle, Capture efficiency, Nutrient Depletion, Drainage rate, hairy root.

1. INTRODUCTION

Plants produce many useful and commercially interesting secondary metabolites and in vitro culture of transformed hairy roots has been proposed as a potential source of these important compounds [1]. Hairy roots are induced in susceptible plants by transformation with *Agrobacterium rhizogenes*. Hairy roots are less prone to genetic variation than callus or suspended cells [2]. These roots have rapid growth rate, high product yield, simple medium requirements and culture stability. One of the most important limitations for the commercial exploitation of hairy roots is the development of technologies for large scale culture. A variety of reactor configurations has been used to cultivate hairy roots, including stirred tank reactor[3], bubble column reactor, airlift reactor[4,5], trickle bed reactor and nutrient mist reactors[1]. In recent years, researchers have focused on mist reactors because these reactors have distinct advantages over liquid phase reactors including the ability to manipulate the gas composition, to allow effective gas exchange in a densely growing biomass, and to control secondary metabolite production. Roots growing in the highly aerated environment of a mist reactor do not exhibit O_2 limitation and stress. Nutrient metabolism in this environment is more efficient than in liquid culture.

2. NUTRIENT MIST REACTOR

In mist reactor, the plant organ culture is dispersed in an air phase by immobilizing on the mesh containing support and the liquid medium is introduced into the reactor as a mist of small micron sized droplets by an ultrasonic transducer. Currently an acoustic window consisting of a thin sheet is very much in use which can be incorporated into a reactor of almost any size or shape. The tissue is continuously bathed in nutrient mist, providing an environment for rapid replenishment of nutrients as well as removal of toxic byproducts. The nutrient mist gets dispersed homogeneously within the culture chamber, eliminating the need for mechanical agitation and thereby reducing the damaging shear.

It has been reported that the roots grow well when the mist is supplied on an intermittent mist-ON/mist-OFF basis instead of continuous misting operation. Mist deposition is a key step in the mass transfer of nutrients to the roots in mist bioreactors [7]. It is reported in literature that in a mist reactor, higher growth yields can be achieved with increased droplet deposition and by manipulating the mist-ON/mist-OFF cycle. While some deposition is required for providing nutrients to the growing roots, any excess deposition will lead to formation of a thick liquid layer along the root surface. This will impede gas transfer to the roots and the system will behave as if it is a liquid phase reactor. Thus mist-ON cycle has to be stopped before such a condition is reached. The deposited liquid will then reduce in volume through drainage and will result in the reduction of mass transfer resistance. As no fresh nutrients are being fed, the deposited liquid gets depleted in nutrient concentration resulting in reduced availability of liquid phase nutrients. Thus, the mist-OFF cycle also has to be stopped before the concentration in the liquid layer goes below the essential level required for the specified growth.

Our research is focused on the development of model equations for intermittent misting cycles to study the effect of design parameters on duration of misting period. The parameters studied in the present work are nutrient concentration, mist feed flow rate to the reactor and drainage rate. Based on the above considerations, model equations are expressed in terms of specific liquid hold up (H) and nutrient concentration (C) profiles.

3. MATHEMATICAL MODEL

The assumptions are, (a). Uniform and complete mixing in the held up liquid. (b). the consumption by the growing roots can be taken to be proportional to dry mass of the roots. (c). the structure of hairy root is assumed as a cylinder and whole root bed is treated as a sphere. (d). the distribution of liquid over root surface is uniform. (e). the specific growth rate (μ) is taken as constant. (f) Mist capture by the root bed is proportional to the volume of the root bed, i.e. volume controlled flow rate and (g). Linear drainage rate characteristics.

Development of mist-ON cycle Equations:

Basis for the model is unit mass of the root bed. When mist flow rate to the bed is proportional to the volume of the root bed then the mist flow rate to the bed at time t is $f = f_0$, where *f* is the mist flow rate to the bed per unit mass at time t and f_0 is initial mist flow rate per unit mass, felt by bed (mL/day/mg). The capture efficiency (η) is the ratio of the liquid volume captured by the hairy root bed to the total volume of mist fed per unit time and F_R is the mist flow rate to the reactor.

The specific growth rate is defined as $\mu = \frac{1}{M_R}\frac{dM_R}{dt}$, where M_R is the mass of root bed and M_{R0} is the initial mass of root bed. Rate of consumption of nutrients by roots = K_1 mass(nutrient)/mass-day. The drainage characteristics are assumed to be linear. H is the specific liquid hold up on root bed surface at time't'(mL/mg) and H_{sat} is the specific liquid hold up at saturation(mL/mg). The linear drainage rate is given as $K_2(H - H_{sat})$, where K_2 is the drainage rate constant (day^{-1}).

The model equations for mist-ON cycle are obtained after writing overall liquid balance and component balance,

$$\frac{dH}{dt} = \eta F_R - K_2(H - H_{sat}) \quad (1)$$

$$\frac{dC}{dt} = \frac{\eta F_R}{H}[C_F - C] - \frac{K_1}{H} \quad (2)$$

During mist-OFF cycle, the supply of the feed is stopped therefore flow rate of feed becomes zero. So the above equations will reduce in the following form -

$$\frac{dH}{dt} = -K_2(H - H_{sat}) \quad (3)$$

$$\frac{dC}{dt} = -\frac{K_1}{H} \quad (4)$$

Mist-OFF cycle was stopped for relative approach to the C_C as Y_{OFF} defined by $Y_{OFF} = \frac{C - C_{S-OFF}}{C_{S-OFF} - C_C}$, where, C_{S-OFF} is the concentration at the start of the mist-OFF cycle

If specific liquid hold up (H_E) is less than the critical specific liquid hold up (H_C), then the reactor can run for longer time without any mass transfer issues. If $H_E > H_C$, then the mist-ON cycle should be switched off at $H = H_C$. In other words, the concentration rises from C_C to C_E during mist-ON cycle, where as it drops from C_E to C_C during mist-OFF cycle. Where, H_E is specific liquid holdup at equilibrium in the bed (mg/mL), H_C is critical specific liquid hold up at any time in the bed (mL/mg), C_E is concentration of the nutrient in the bed at equilibrium and C_C is the critical concentration of the nutrient in the bed (mg/mL).

The physical parameters, used to simulate the results are : Nutrient concentration (Sucrose) in the feed (C_F) = 50 mg/mL,[6], Specific growth rate (μ) = 0.2 day-1, [6]; Feed flow rate (F_R) = 2.11 mL/day/mg. Droplet capture efficiency (η) = 0.1, [6]). Initial mass of the roots (M_{R0}) = 600 mg. Nutrient concentration (Sucrose) at equilibrium (C_E) = 10 mg/mL. Specific liquid holdup at saturation (H_S) = 0.01 mL/mg, [6]). Proportionality constant (K_1) for growth equation = 0.3 per day, ([6]).

Solutions for mist-ON and mist-OFF cycle were obtained by integrating the set of coupled Equations, in time as initial value problems by using NAG Library subroutine D02EJF. The subroutine D02EJF is a variable order and variable time-step method which uses GEARS Algorithm to integrate differential equations. It automatically chooses the required time steps and order. Simulations were performed for various levels of error tolerance till the solution did not change with changing tolerance. Repeated solutions for mist-ON/mist-OFF cycle were used to simulate for the complete reactor

4. RESULTS AND DISCUSSION

Figure 1 presents the variation of concentration of liquid nutrient held over the root surface with time for different feed concentrations. As C_F increases, the mist-ON cycle will increase and mist-OFF cycle duration increases faster as compared to mist-ON cycle time because concentration is the limiting parameter for mist-OFF cycle. Also it is clear from figure 1, the number of cycles are increasing with decrease in C_F. Higher C_F is recommended as high nutrient concentration results good growth of hairy roots. If C_F is very high, the diffusion rates of gas phase nutrients will decrease. Hence it will affect the growth. If reactor is operated with less concentrated nutrients, the intermittent operation will behave like a continuous misting cycles because there is continuous demand for nutrients.

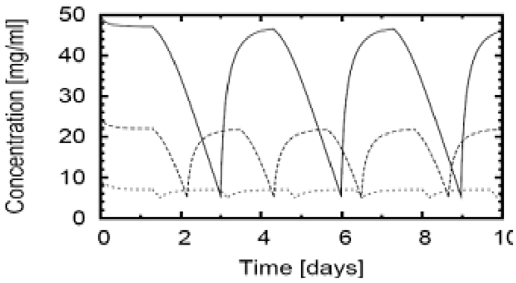

Figure 1: Variation of concentration with time for different values of feed concentrations (C_F). Solid lines to dotted lines represents results for C_F = 50, 25 and 10 mg/mL, respectively.

Figure 2 presents the variation of Specific liquid hold up with time for different feed concentrations. As C_F increases mist-OFF cycle duration increases faster as compared to mist-ON cycle duration and number of cycles decreasing. Figure 1 and 2 concludes that the feed concentration have significant effect on growth of hairy roots in mist reactor. It would be feasible to operate the reactor for moderate concentration of the nutrient medium. It is also concluded that at low C_F, reactor can also be operated with continuous mode or intermittently with large number of mist -ON/mist-OFF cycles and a stable specific liquid hold up profile can be observed during continuous misting operation.

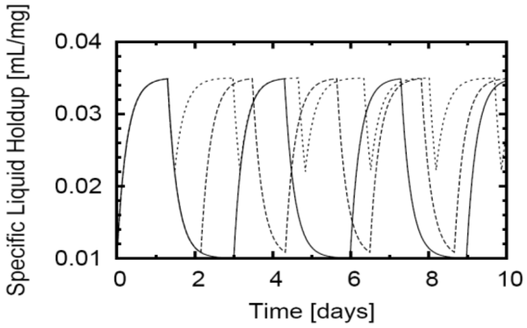

Figure 2: Variation of Specific liquid hold up (H) with time for different feed concentrations. Solid lines to dotted lines represents results for C_F = 50, 25 and 10 mg/Ml, respectively.

Figure 3 presents the variation of nutrient concentration with time for different feed flow rates. As the concentration is the limiting factor during mist-OFF cycle, concentration profile provides useful information for the duration of mist-OFF cycle. There is direct effect of Feed flow rates on the thickness of the held up liquid across the root surface. As concentration remains same, no change in mist-OFF cycle will be observed for different flow rates.

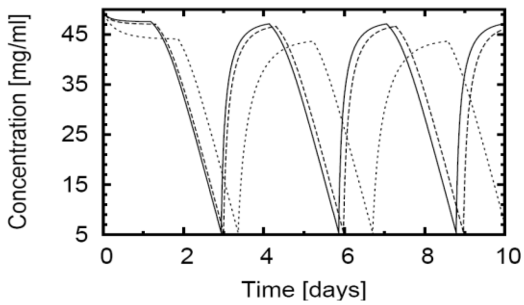

Figure 3: Variation of concentration with time for different values of effective mist flow rates (f_m). Solid lines to dotted lines represents results for f_m = 0.05,0.1 and 0.2 mL/mg-day, respectively.

Figure 4 presents the variation of Specific liquid hold up with time for different feed flow rates. As flow rate increases mist-ON cycle decreases but mist-OFF cycle duration remains same as compared to mist-ON cycle because concentration is the limiting parameter for mist-OFF cycle. Also, the number of cycles are increasing with decrease in flow rates. And if flow rate is very low then the mist-ON cycle duration will be very high which is not recommended for the efficient operation of nutrient mist reactor.

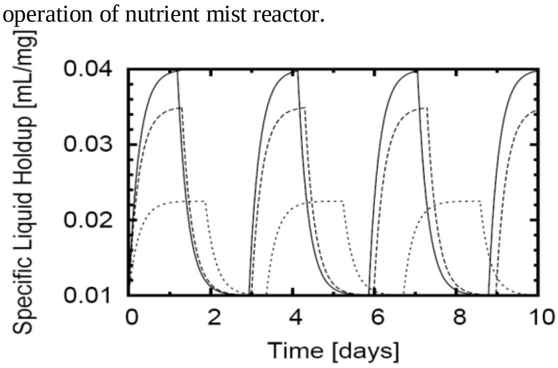

Figure 4: Variation of Specific liquid hold up with time for different effective mist flow rates. Solid lines to dotted lines represents results for f_m = 0.05, 0.1 and 0.2 mL/mg-day, c

Figure 5 presents the variation of concentration with time for different drainage rates. As drainage rate increases, the switching between mist-ON and mist-OFF cycles is faster. Hence, the total cycle duration decreases and the number of cycles increase for the specified period of operation. The curves shown that K_2 has no effect on C_E and C_C limits.

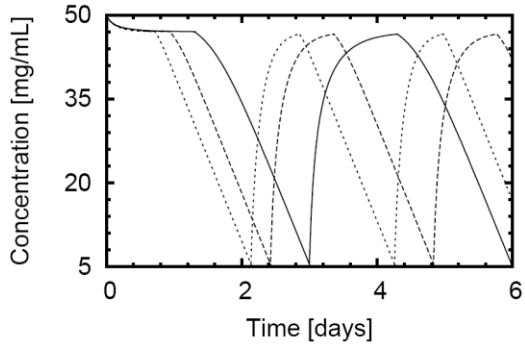

Figure 5: Variation of concentration with time during ON & OFF cycles for different values of K_2. Solid lines to dotted lines represents results for K_2 = 4, 8 and 16 day^{-1}.respectively

Figure 6 presents the variation of specific liquid hold up with drainage coefficient during mist-ON and mist-OFF cycles. The depletion of the equilibrium film thickness becomes faster for higher values of K_2 because drainage rate increases. As drainage rate increases, the mist-OFF cycle duration will decrease faster. Between the two cycles the gap showing in the graph is more for higher values of K_2. In this gap period, there is no drainage from the root bed and also no feed enters into the reactor. Because of this, the roots growth will be stopped and they may die because of non availability of nutrients. So higher values of K_2 are not good for the reactor operation. Hence the preferable value of K_2 would be which can be achieved for less mist-ON time period and more mist-OFF time period.

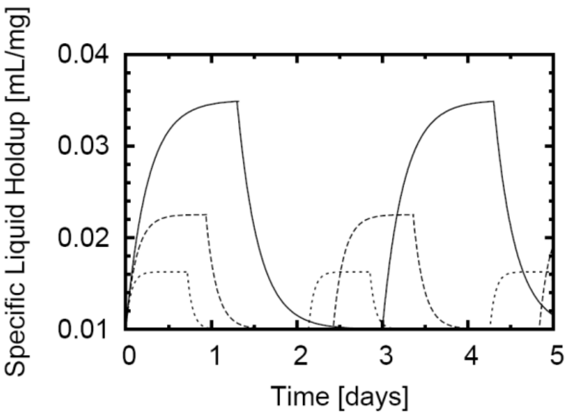

Figure 6: Variation of film thickness with time during ON & OFF cycles for different values of K_2. Solid lines to dotted lines represents results for K_2 = 4, 8 and 16 day^{-1}, respectively.

Figure 7 presents the mist-ON time fraction change with C_C value. As C_C increasing, the mist-ON cycle also increases for the given feed and K_2 value. When drainage rate increases, then the mist-ON time fraction also increases a little but not regular. This means that the change in K_2 value also effects mist-OFF duration. Since both the cycles are varying, the figure is not showing large variations for mist-ON cycle time with K_2 value.

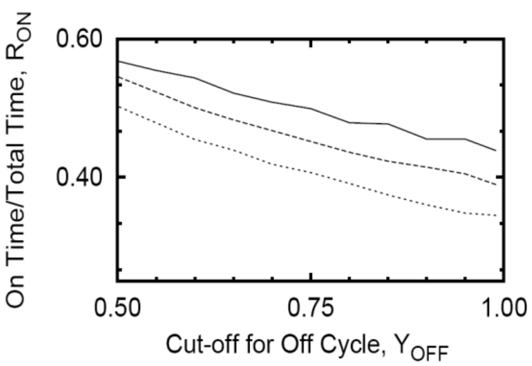

Figure 7: Variation of ON time with Y_{OFF} for different values of K_2. Solid lines to dotted lines represents results for K_2 = 4, 8 and 16 day^{-1}, respectively.

5. CONCLUSIONS

This work has been carried out with an aim to gain an understanding of the process conditions for sustained and efficient operation of nutrient mist reactor for the growth of *Agrobacterium Rhizogenes* mediated hairy roots.

The study is based on the development of a mathematical model at root bed level per unit mass basis. Model equations were developed to study the operating parameters for constant specific growth rate processes. Important parameters for the nutrient mist reactor operation- effective mist flow rate, nutrient concentration available to roots on duration of mist-ON/mist-OFF cycles and linear drainage characteristics of the root bed are studied. It is clear from the results, the mist ON/mist-OFF cycles play an important role in an efficient operation of mist reactor. It is observed that the good growth rate can be achieved either by increasing nutrient concentration or by changing the mist duty cycles. This study will provide the much required engineering input to the hairy root technology. Also it will initiate the process of commercialization of this very important and useful technology by leading to the important stages of pilot scale experiments.

6. REFERENCES

[1] Flores, H. E. and W. R. Curtis (1992) Approaches to understanding and manipulating the biosynthetic potential of plant roots. Ann. N Y Acad. Sci. 665: 188-209.

[2] Aird, E. L. H., J. D. Hamill, and M. J. C. Rhodes (1988) Cytogenetic analysis of hairy root cultures from a number of plant species transformed by Agrobacterium rhizogenes. Plant Cell Tissue Organ Cult. 15: 47-57.

[3] Hilton, M. G. and M. J. C. Rhodes (1990) Growth and hyoscyamine production of 'hairy root' cultures of Datura stramonium in a modified stirred tank reactor. Appl. Microbiol. Biotechnol. 33: 132-138.

[4] Sharp, J. M. and P. M. Doran (1990) Characteristics of growth and tropane alkaloid synthesis in Atropa belladonna roots transformed by Agrobacterium rhizogenes. J. Biotechnol. 16: 171-186.

[5] Taya, M., A. Yoyama, O. Kondo, T. Kobayashi, and C. Matsui (1989) Growth characteristics of plant hairy roots and their cultures in bioreactors. J. Chem. Eng. Jpn. 22: 84-89.

[6] Dilorio A.A., Cheetham R.D. and Weathers P.J. (1992) 'Growth of transformed roots in a nutrient mist bioreactor: Reactor performance and evalution', Appl. Microbiol. Biotechnol., Vol. 37, pp. 457-462.