MODELLING OF MIST REACTOR : EFFECT OF PACKING FRACTION AND FILM THICKNESS ON THE GROWTH OF HAIRY ROOTS

Manish Vashishtha* and Kumar Saurabh
Department of Chemical Engineering
Malaviya National Institute of Technology,
J.L.N Marg, Jaipur, India
*Corresponding E-mail: mvche.mnit@gmail.com

KEYWORDS

Nutrient Mist Reactor (NMR),differential equation, Hairy roots, Mist ON-OFF cycle, MATLAB.

ABSTRACT

Hairy roots have been successfully cultivated in a variety of reactor configurations. Nutrient mist reactors have been found specially suited to grow these roots because of its easy operation, high oxygen concentration, lack of shear, low pressure, ease in manipulating the gas composition, effective gas exchange in a densely growing biomass and ease of scaling up. In present work, a mathematical model has been developed to study the effect of variation of packing fraction and liquid film thickness on growth rate, liquid hold up and held up liquid concentration of nutrients. The simulation of developed model equations for the nutrient mist reactor is done with the help of MATLAB software.

INTRODUCTION

Higher plants are the source of varieties of biochemicals, which are produced from both primary and secondary metabolism. The metabolites produced from secondary metabolism are of immense importance because of their various important biological activities like antibiotic, insecticidal, hormonal properties, and valuable pharmacological and pharmaceutical activities [1]. In addition many of the secondary metabolites are also used as flavours, fragrances and agrochemicals. Various culture systems have been adopted for the production of important secondary metabolites from the plants. Although there are some commercialized, industrial scale plant cell cultures [2], the biggest challenge of producing secondary metabolites from plant cell cultures is that secondary metabolites are generally produced by specialized cells and/or distinct developmental stages [3]. Also, many products of interest are synthesized in organized tissues (roots), but not formed in suspension or callus culture (shoots, leaves). Due to this, a great deal of attention has been focussed on the root culture for the production of secondary metabolites.

HAIRY ROOT CULTURES

Hairy roots can be induced in susceptible plants by transformation with *Agrobacterium rhizogenes,* resulting in so called hairy root disease. These roots can be grown indefinitely with rapid growth rate and are less prone to genetic variation than callus or suspended cells. Therefore, hairy roots can offer a valuable root derived secondary metabolites that are useful in pharmaceuticals, cosmetics and food additives.

The cultivation of hairy roots in the bioreactors faces several challenges. When bioreactors are designed for mass cultivation of hairy roots at production scales, the physiology and morphology of hairy roots and their unusual rheological properties should be taken into consideration. One of them is to ensure sufficient mass transfer in the culture. Non-uniform distribution of biomass in the medium (and culture vessel) causes different technological problems. In addition to the hairy root growth restriction, the densely packed mass of roots causes nutrient and especially oxygen limitations, this leads to the reduction in secondary metabolite production or even to cell necrosis and autolysis.

Bioreactor’s Design For Hairy Root Cultivation

For cultivation of hairy roots, variety of reactor configurations has been used by different workers [4, 5, 6]. Based on the continuous phase, reactors used to culture hairy roots can be roughly divided into three types: liquid phase, gas phase and hybrid reactors.

In the liquid phase reactors (e.g. Stirred tank reactors, bubble column reactor, airlift reactor and submerged convective flow reactors) roots are submerged in the medium in liquid phase reactors so the term ‘submerged reactors’ are also used for this type of reactors. The main drawbacks in cultivation of hairy roots using liquid phase reactors were damaging of roots resulting in callus formation and ultimately poor biomass production [7]. Also the oxygen deficiency due to mass transport limitation is a growth limiting factor in these liquid phase reactors.

In gas phase reactors (e.g. Trickle bed reactor, droplet phase and nutrient mist reactors (NMR)), the roots are exposed to air or other gas mixtures. Nutrients (medium)

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are usually delivered to roots as droplets. However, there is considerable variation in size of droplets used in different gas phase bioreactors. For NMR, using ultrasonic transducers, the droplet size is usually micron scale (0.5- 30 μm) [8]. For trickle bed or other gas phase reactors, using spray nozzles, the size of droplets may be much larger [9]. Since the continuous phase is gas, the roots must be immobilized in the reactors. The disadvantage of gas phase reactors is that there is no way to uniformly distribute the roots in the growth chamber without manual loading.

The problems associated with gas phase reactors can be overcome by using hybrid reactors. Here, the reactors are firstly run as a bubble column reactor in order to suspend, distribute and attach roots to the packing rings in the reactors. After two weeks of growth, root's clumps were dense and the reactor was switched to a trickle bed operation, thus exposing roots to a gas environment [10].

Nutrient Mist Reactor

Nutrient mist reactors (NMR) are basically a gas phase reactor. It consists of a mist generating system, a culture medium reservoir, a peristaltic liquid pump, a filtered air supply, a gas flow meter, a timer to regulate the misting time and a culture chamber. Some supporting means are also required by many of the NMR to support the suspended roots in the growth chamber. The successful use of various design of mist reactors have been reported by many scientists [8, 11]. In NMR, the hairy root culture is dispersed in an air phase by immobilizing on the mesh support and the liquid medium is introduced in the reactor in the form of mist of very small droplet size (0.5-30 μm). The very small size mist droplets are generated by an ultrasonic transducer and these mist droplets are carried to the bed by air as opposed to being dropped onto the bed. Because of such arrangement, there is an even distribution of liquid media (nutrients) throughout the root bed and there is less liquid hold up. However there is considerable variation in the size of droplets.

While comparing the performance and operation of NMR (for the cultivation of hairy roots) with other possible reactor's designs, it was found that they offer definite advantage over other reactor configurations such as ease of operation, high dissolved oxygen tension present in the mist and ease of scale up.

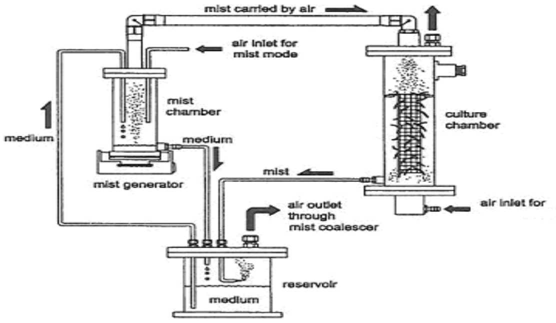

Figure 1: Flow Diagram Of Mist Reactor [11]

The comparison between NMR with various bioreactors for the cultivation of hairy roots was initially done by P. J. Whitney [12]. The tissue in NMR is continuously bathed in nutrient mist, providing an environment for rapid replenishment of nutrients as well as removal of toxic by-products. Also the nutrient mist can be dispersed homogeneously within the culture chamber, eliminating the need of mechanical agitation and thereby reducing the shear stress. Also the carrier gas for nutrient mist can consist of any gas mixture, enabling physiological studies of gaseous environments to be performed.

MATHEMATICAL MODEL

The various factors affecting the performance of NMR were firstly described by Wyslouzil et al. [13]. The inherent assumptions of filter models were valid in the bioreactors for the description of particle capture efficiency (η) by the root bed. The particle capture efficiency (η), which is the ratio of the liquid volume captured by the hairy root bed to the total volume of mist fed per unit time, represents the overall effect of the following three contributions (i) diffusion (capture due to the random motion of the droplets), (ii) interception (capture due to the particle's size), and (iii) impaction (capture due to the particle's inertia). The capture efficiency of root bed generally increases with an increase in droplet diameter.

THEORY AND CONCEPTS

An important controlling parameter for the operation of NMR is drainage rate. The effect of drainage rate using logarithmic and linear drainage models have been studied by Ranjan et al. [14]. In the linear model, the drainage rate is proportional to the difference between the specific liquid holdup at any time and the liquid holdup at saturation ($Y - Y_{sat}$). In the logarithmic model, it is proportional to the logarithmic difference between the liquid holdup at any time and the liquid accumulated at saturation [$\ln(Y) - \ln(Y_{sat})$]. The proportionality constant in either case has to be determined by fitting the model to data gathered from actual drainage experiments performed on similar beds. Another important parameter that affects the NMR performance is packing fraction (P_f) of root bed. Roots in NMR are often too sparsely packed to capture mist particles efficiently and therefore, cannot meet the nutrient demands required to maintain high growth rates. With increase in initial packing fraction, growth rates of A. annua hairy roots increased significantly [15] as shown in **Figure 2**.

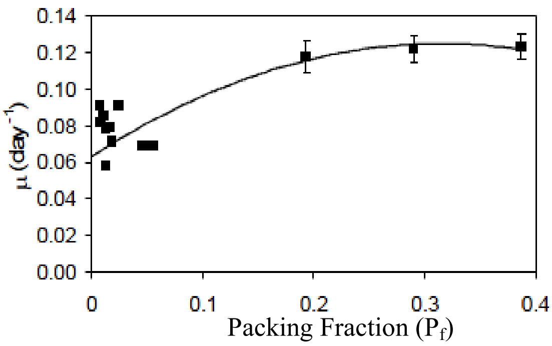

Figure 2: Effect Of Initial Packing Fraction On The Growth Rate Of Transformed Roots Of A. Annua Grown In The Small Mist Reactor [15].

Assumptions

The following assumptions have been made for the development of model equations used in present analysis of NMR.

1. There is complete mixing in the held up liquid.
2. The distribution of liquid over the root surface is uniform.
3. The structure of hairy root is assumed as cylinder and the whole root bed is also treated as cylinder.
4. The nutrient consumption by the growing roots can be taken to be proportional to dry mass of the root.
5. There is a constant capture efficiency of nutrients by the roots.
6. Linear drainage rate characteristics.
7. The combined effect of nutrient mass transfer and resistance provided by the growing film thickness with increased feed flow rate is taken to be negligible.

Development Of Mathematical Model

Model equations for a NMR can be developed by applying the mass and component balance across the root bed for mist-ON and mist-OFF cycles. Also the equation for the growth rate of roots can also be developed by considering Monad equation. Mist deposition is controlled by the capture of the liquid droplets by the root bed and determined by the capture efficiency. The capture efficiency (η) can be defined as the volume of the liquid captured by the root bed to the total volume of the mist per unit time.

Various data from different researches suggest that there should be an optimum misting cycle to achieve maximum growth of roots in NMR. Mist is deposited during the ON cycle of the reactor and the deposited liquid is then drained during the OFF cycle. During ON cycle, the liquid holdup will be distributed as layers of liquid over the roots. The nutrients in this liquid layer, such as sugar, are never depleted in the liquid phase, due to continuous fresh supply from the incoming mist. Thus, in the ON cycle, growth is most likely to be arrested by the mass transfer limitations of the gas phase nutrients through the liquid layer. During OFF cycle, the liquid layer gets continuously thinner (due to drainage) and depleted (due to uptake of nutrients by the roots). The ON and OFF cycles are schematically shown in **Figure 3 and 4** respectively.

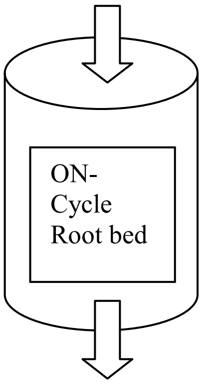

Figure 3

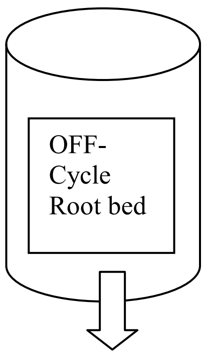

Figure 4

Figure 3 And 4: Schematic Diagram For The Mass Balance Across The Root Bed During ON Cycle And OFF Cycle Respectively.

The input and output terms for the mist in an NMR can be mathematically expressed as

Specific flow rate of mist into root bed

$$= \eta Q_0 \left(\frac{m_t}{m_0} \right)^{\alpha} \qquad \text{..... (1)}$$

The linear drainage rate of mist

$$= K_2 \, (Y - Y_{sat}) \qquad \text{...... (2)}$$

The value of exponent α for cylindrical root bed for the two extremes cases of size of liquid droplets i.e. for small droplets and large droplets are calculated and found to be **0** and **-1/2** respectively.

The rate of nutrient consumption by the roots can be derived by developing the equation for diffusion of mist per unit time per unit mass of the roots. Thus,

Rate of nutrient consumption by the roots

$$= \left(\frac{4D}{L_f d\rho} \right) (C - C_m) z \; .. \qquad \text{.... (3)}$$

The equation for growth rate of roots growing inside the NMR is based on Monad model and can be represented by first order kinetics,

Growth rate of roots

$$\frac{dm_t}{dt} = \mu m_t \qquad \text{..... (4)}$$

Growth rate of roots per unit mass of the roots:

$$\mu = \frac{1}{m_t}\frac{dm_t}{dt} \quad \text{...... (5)}$$

There is only a partial intake of nutrients by roots. Let this fraction of diffused nutrients be K_1. Therefore, combining equations 3-5 yield

$$\frac{dm_t}{dt} = \left(\frac{4DK_1}{L_f d\rho}\right)(C - C_m)m_t \quad \text{........(6)}$$

It is clear from Eq. 6 that the specific growth rate is dependent on diffusivity and concentration of nutrients and root bed parameters.

From the experimental findings of Towler et al. (2007), it was clear that the growth rate of hairy roots in the NMR is a strong function of packing fraction (P_f) of root bed. The experimental data showing variation of growth rate with varying packing fraction of root bed is tabulated below.

Table 1: Experimental Data Showing Variation Of Growth Rate With Packing Fraction [15]

Packing fraction (P_f)	0.01	0.12	0.2	0.3	0.39
Growth rate (μ)	0.07	0.098	0.108	0.12	0.119

The derivation of mathematical equation showing relation between P_f and μ is done by method of best fitting of curve. The following two equations were obtained. One is logarithmic and other is linear equation.

Logarithmic Equation

$\mu = 0.0137(\ln P_f) + 0.1318$ **......**(7)

Linear Equation

$\mu = 0.128P_f + 0.0767$ **......**(8)

The mathematical relation between growth rate of roots and liquid film thickness (L_f) can be obtained by suitable manipulation of equation 4 and 6, which gives

$$\mu \propto \frac{4DC}{Lf\rho d} \quad \text{...... (9)}$$

From the above equation, it is clear that the growth rate of roots varies with varying thickness of liquid film.

The equation for the ON cycle in an NMR can be derived by consideringoverall liquid balance per unit mass of the root bed which can be written as

Rate of mist retained in the reactor = Input – Drainage

Putting values from Equation 1 and 2 in above equation gives

$$\frac{dY}{dt} = \eta Q_0\left(\frac{m_t}{m_0}\right)^{\alpha} - K_2(Y - Y_{sat}) \quad \text{.......(10)}$$

The component balance when applied for the unit mass of root yields

$$\frac{d(YC)}{dt} = \eta Q_0\left(\frac{m_t}{m_0}\right)^{\alpha} C_0 - K_2(Y - Y_{sat})C - \left(\frac{4D}{L_f d\rho}\right)(C - C_m) \quad \text{.....(11)}$$

Further solving the above equation and putting the value from Eq. 10 gives

$$\frac{dC}{dt} = \frac{\eta Q_0\left(\frac{m_t}{m_0}\right)^{\alpha}}{Y}(C_0 - C) - \frac{1}{Y}\left(\frac{4D}{L_f d\rho}\right)(C - C_m) \quad \text{.....(12)}$$

Eq. 10 and Eq. 12 represents the specific liquid hold up profile and concentration profile at time t for mist-ON cycle respectively.

Similarly for the OFF cycle the equation can be derived, since the flow rate of feed during the OFF cycle becomes zero as the feed supply is stopped, hence Eq. 10 and Eq. 12 becomes;

$$\frac{dY}{dt} = -K_2(Y - Y_{sat}) \quad \text{........(13)}$$

$$\frac{dC}{dt} = -\frac{1}{Y}\left(\frac{4D}{L_f d\rho}\right)(C - C_m) \quad \text{.......(14)}$$

Eq. 13 and Eq. 14 represents the specific liquid hold up profile and concentration profile at time t for mist-OFF cycle respectively.

Numerical Simulation And Values Of Parameters

Solutions for mist ON - OFF cycles were obtained by integrating the set of coupled ordinary differential equations (ODEs) by ODE45 (an inbuilt ode solver) using MATLAB (version 7.12, Release name R2011a). The ode45 solver uses a variable step Runge-Kutta procedure. The numerical solver ODE45 combines a fourth order method and fifth order method, both of which are similar to the classical fourth order Runge-Kutta (RK) method. The modified RK method varies the step size, choosing the step size at each step in an attempt to achieve the desired accuracy.For the evaluation of the performance of NMR, numerical values for model parameters were taken from the literature [13, 15, 16]. Numerical values were chosen within a practical range for the root culture system. The chosen parameter values are Q_0 = 2.11 ml/day-mg, C_0 = 50 mg/ml, m_0 = 600 mg, μ = 0.2 per day, K_2 = 4 per day, Y_{Sat} = 0.01ml/mg, C_m = 10 mg/ml, D = 0.72 mm^2/day, η = 0.1, α = -0.5, ρ = 1000 mg/ml, d = 1 mm and L_f = 0.1 mm.

DISCUSSION OF RESULTS

The numerical results of the modelled equation for growth rate of roots inside the NMR is presented in **Figure 5** which shows the growth rate of roots with time. It was found that there is an exponential growth of roots with time.

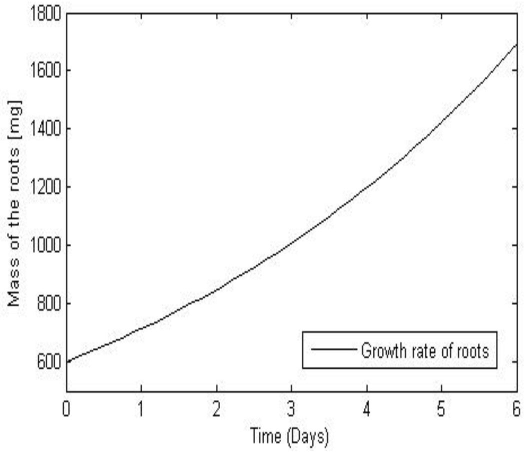

Figure 5: Variation Of Mass Of Root With Time In Mist Reactor

The experimental findings from the published literature of Towler et al. have been shown in **Figure 6**. By comparing figure 5 and 6, it can be seen that there is a close resemblance of the present model with the experimental data of *A. Annua* rootgrowth in the NMR (Towler et al.). This resemblance confirms the practical applicability of the present model.

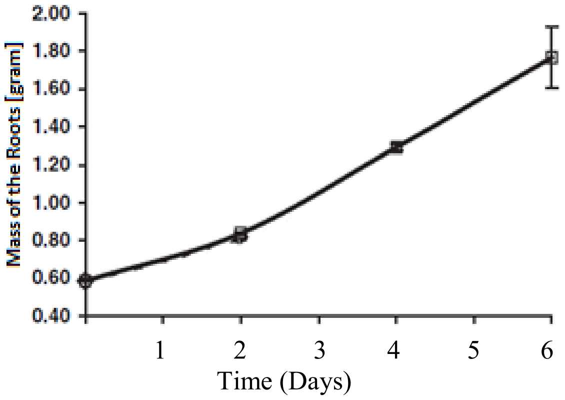

Figure 6: Experimental Finding For Growth Rate Of A. Annua In NMR From Literature [15].

Study Of Effect Of Packing Fraction (P_f) On Nmr Performance

The effect of P_f on growth rate is shown in **Figure 7** which presents the linear variation of mass of roots (growth of roots) with time for different packing fraction of root bed. Here, top to bottom lines correspond to P_f = 0.71, 0.60, 0.45 and 0.25 respectively. The value of other parameters are Q_0 = 2.11 ml/day-mg, C_0 = 50 mg/mldensity =1000mg/ml, m_0 = 600 mg, μ = 0.2 per day, K_1 = 0.3 per day, C_m = 10 mg/ml, D= 0.72 mm^2/day,L_f = 0.2 mm and d = 1mm. From this figure it is clear that with increasing value of packing fraction of root bed, the growth rate increases.

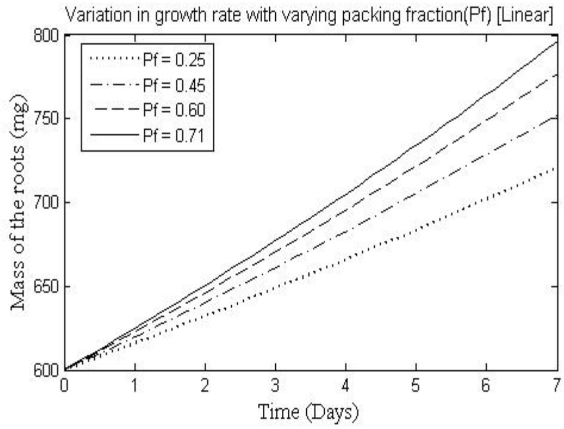

Figure 7: Variation Of Mass Of Roots With Time For Different Packing Fraction (P_f) .Top To Bottom Line Correspond To P_f = 0.71, 0.60, 0.45 And 0.25 Respectively.

This is due to the fact that the particle capture efficiency of root bed increases with increasing packing fraction of bed. Due to this, the availability of nutrients for the growing roots gets increased. This manifest itself in an increased growth rate at higher packing fractions. This result also supports the experimental findings of Towler et al. and thereby providing further support to the validity of present model.

The effect of P_f on liquid holdup is shown in **Figure 8.** The variation of liquid holdup with time for different packing fractions of root bed is shown. Here, top to bottom lines correspond to P_f = 0.25, 0.45, 0.60 and 0.71respectively. The other parameters remain same. It can be seen that liquid holdup decreases with higher packing fraction. This can be attributed to the fact that a higher packing fraction implies for higher growth rate which occurs at the expense of a greater consumption of nutrients, thereby leading to a lower liquid hold up.

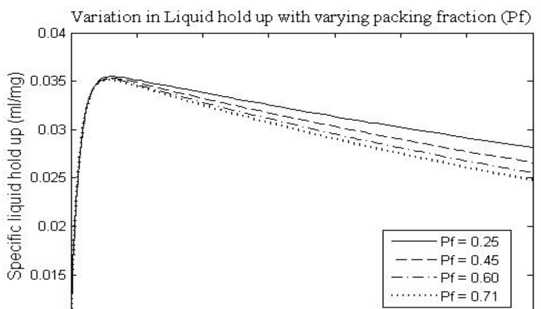

Figure 8: Variation Of Liquid Holdup With Time For Different Packing Fraction Of Bed (P_f). Top To Bottom Line Corresponds To P_f= 0.25, 0.45, 0.60 And 0.71 Respectively.

Figure 9 represents the variation of held up liquid concentration with time for different packing fractions. Following the same trend of above figure 8, the top to bottom lines correspond to P_f = 0.25, 0.45, 0.60 and 0.71respectively. The value of other parameters remains same. It can be observed from the figure that the decrease in concentration of nutrients is faster for more densely packed beds. The faster decrease in concentration is due to the fact that higher growth rate is observed at higher packing fraction and so more nutrient is consumed.

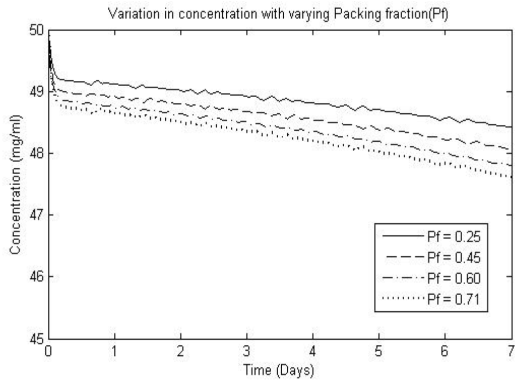

Figure 9: Variation Of Held Up Liquid Concentration With Time For Different Packing Fraction (P_f). Top To Bottom Line Corresponds To $P_{f\,=}$ 0.25, 0.45, 0.60 And 0.71 Respectively.

Study Of Effect Of Liquid Film Thickness (L_f) On Nmr Performance

Figure 10 presents the variation of mass of roots with time at different liquid film thickness. In this figure, top to bottom line correspond to L_f = 0.1 mm, 0.2 mm and 0.3 mm respectively. The value of other parameters are Q_0 = 2.11 ml/day-mg, C_0 = 50 mg/ml,density =1000mg/ml, m_0 = 600 mg, μ = 0.2 per day, K_1 = 0.3 per day, C_m = 10 mg/ml, D= 0.72 mm^2/day and d = 1mm. An increase in the film thickness leads to a decrease in growth rate. This increase in film thickness engenders higher mass transfer resistance for the transfer of nutrient from mist to root bed resulting in a decreased growth of roots.

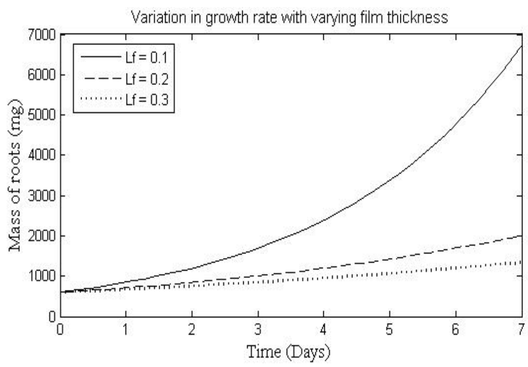

Figure 10: Variation Of Mass Of Roots With Time At Different Liquid Film Thickness (L_f). Top To Bottom Line Correspond To L_f = 0.1mm, 0.2 Mm And 0.3 Mm Respectively.

The variation of liquid holdup with time for different liquid film thicknessis shown in **Figure 11** where top to bottom lines correspond to L_f = 0.4 mm, 0.3 mm and 0.2 mmrespectively. The other parameters remain same. It is observed that liquid holdup increases with increasing liquid film thickness. These results are in accordance with theoretical expectation because the mass transfer resistance increases with increasing liquid film thickness and this will lead to a higher amount of liquid holdup inside the mist reactor.

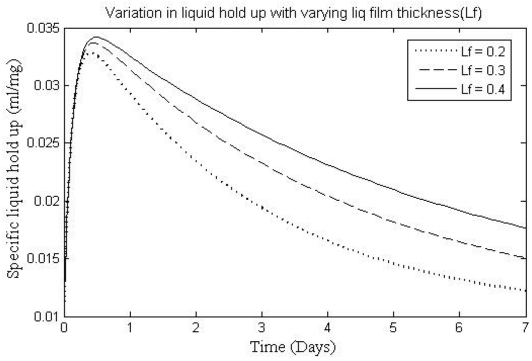

Figure 11: Variation Of Liquid Holdup With Time At Different Liquid Film Thickness (L_f).Top to bottom line corresponds to L_f = 0.4, 0.3 and 0.2 respectively.

Figure 12 represents the variation of held up liquid concentration with time for varying liquid film thickness and top to bottom lines correspond to L_f = 0.3 mm, 0.2 mm and 0.1 mmrespectively. The value of other parameters is kept same. These results are in tune with theoretical concept because the decrease in concentration is faster with thinner liquid films since with increased thinning of films, more nutrients will be transferred to the bed and get consumed.

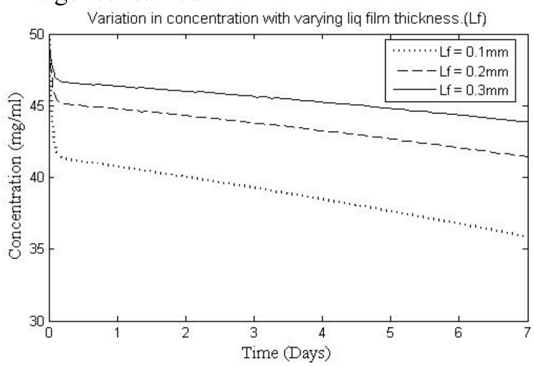

Figure 12: Variation Of Held Up Liquid Concentration With Time For Different Liquid Film Thickness (L_f). Top To Bottom Line Corresponds To L_f = 0.30mm, 0.20mm And 0.1mm Respectively.

CONCLUSION

For enhanced production of various useful secondary metabolites by plant culture system, different design and configurations of reactors have been studied for the production of secondary metabolites and it was found that NMR clearly offer some significant benefits for culturing hairy roots, including lack of oxygen stress, rapid growth, and production of high yields. The effect of packing fraction of root bed and liquid film thickness on growth of roots, liquid hold up and also on held up liquid concentration is studied with simulation of a simplified mathematical model for a cylindrical root bed.

In the present work, it is found that the growth rate of root increases while liquid holdup and held up liquid concentration decreases with increasing values of packing fraction of root bed. On the other hand, the growth rate of root decreases while liquid holdup and held up liquid concentration increases with increasing liquid film thickness. However, optimization of packing fraction and thickness of liquid film over the roots is still under ambit of active research on NMR performance analysis.

NOMENCLATURE

C : Nutrient concentration in the feed [mg/ml]
C_m : Minimum concentration of the nutrients at the root surface [mg/ml]
C_0 : Initial concentration of the nutrient in the feed [mg/ml]
D : Diffusivity coefficient of liquid nutrient [mm^2/day]
d : Root fibre diameter [mm]
K_1 : Proportionality constant for growth equation [day^{-1}]
K_2 : Proportionality constant for drainage equation [day^{-1}]
L_f : Liquid film thickness [mm].
m_0 : Mass of the root at time t = 0[mg].
m_t : Mass of the root at time 't'[mg].
P_f : Packing fraction of root bed.
Q_0 : Feed (Mist) flow rate at the start (t = 0) [ml/day]
Q_t : Feed (Mist) flow rate at any given time 't' [ml/day]
t : Time [day]
Y : Current specific liquid hold up [ml/mg]
Y_{Sat}: Specific liquid hold up at saturation [ml/mg]

GREEK LETTERS

α : Dimensionless exponent for effective flow rate
μ : Specific growth rate of the roots [per day]
ρ : Density of liquid [mg/ml]
η : Particle capture efficiency

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AUTHOR'S BIOGRAPHY

Manish Vashishthawas born in Karauli (Rajasthan, India) and obtained his Bachelor of Engineering (with Honours) in Chemical Engineering, from Malaviya National Institute of Technology (MNIT), Jaipur (India) and Master of Technology (M.Tech.) and Doctor of Philosophy (Ph.D) degrees in Chemical Engineering, from Indian Institute of Technology (IIT), Delhi, New Delhi (India). He is working as Assistant Professor in Department of Chemical Engineering, at MNIT, Jaipur. His areas of research include Interfacial Engineering, Thin liquid films, Modelling and Simulation, , Particle Science and Thermodynamics. He has published around 40 research papers in various Journals and conferences including some in high impact factor journals like Physical review E, Physical Chemistry Chemical Physics, Journal of Physical Chemistry B, Particuology etc. . He is life member of Institution of Engineers (India), Indian society for Technical Education and Indian Institute of Chemical Engineers.
His e-mail address is *mvche.mnit@gmail.com*