Abstract

A mathematical model has been developed to study the ON/OFF mist duty cycle to achieve specified growth of hairy roots. The availability and rate of transport of nutrients to the roots have been taken to be important parameters in design and operation of the reactor. Major mass transfer resistance is provided by the thin liquid film over the root surface which keeps building up during the ON cycle. The same film also acts as a finite reservoir of nutrients in the absence of any replenishment during the OFF cycle. This reservoir gets depleted as growing roots continue to consume the nutrients. The depletion limits the duration of the OFF cycle as it leads to the lowering of nutrient concentration below the critical value which is required for the specified growth rate. The depleted reservoir is replenished to an extent which depends on feed concentration and duration of next ON cycle. It is found that an increasing feed concentration fed-batch mode of operation leads to a better growth of the roots in the reactor. Interestingly, it also leads to an efficient operation of the reactor whereby the reactor operates at slightly above the required concentration and close to minimum mass transfer resistance.

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

Introduction

Bioreactor is a vessel, which is used to carry out one or more biochemical reactions to convert raw materials to products through the action of biocatalyst, enzyme microorganisms, cells of animal or plants.

Mist Bioreactors are bio reactors which is suitable for hairy root cultivation of plant cells. Static root mass is contained in a chamber that is mostly empty. Nutrients are supplied as mist of fine droplets suspended in circulating air currents that penetrates the spaces between the roots.

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 by-products. The nutrient mist gets dispersed homogenously within the culture chamber, eliminating the need for mechanical agitation and thereby reducing the damaging shear. Finally, the tissue can be effectively immobilized on a nylon support, which allows for batch or continuous operation. The designs of a mist reactor configuration have evolved as the applications of these systems have become more varied.

Mist deposition depends upon capture efficiency of mist droplets by the root bed. It has been found that good growth can be achieved by manipulating ON/OFF mist duty cycle on intermittent basis. Thus duration of ON/OFF cycle has become very important criteria for optimization of hairy root growth in the reactor chamber. The availability and rate of transport of nutrients to the roots have been taken to be important parameters in design and operation of the reactor.

Bioreactor is a vessel, which is used to carry out one or more biochemical reactions to convert raw materials to products through the action of biocatalyst, enzyme microorganisms, cells of animal or plants. The raw material could be an organic compound e.g. sugar or an inorganic chemical e.g., CO2 or even complex material such as meat, animal manure or waste stream. The product of conversion may be biomass (e.g., Bakers yeast growth associated primary metabolites (e.g., Ethanol, Citric acid) or non growth associated metabolites (Antibiotics, bioactive compounds for plants) etc. These products may be extra cellular or intracellular as well. A large number of bioreactor designs are therefore needed to accommodate great diversity of substrate product and biocatalyst. Cultivation of the cells or biocatalyst is done in a
perfectly mixed (submerged) mode or non mixed mode (surface) or via solid state cultivation.

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. Hairy roots are induced in susceptible plants by transformation with Agrobacterium rhizogenes. Hairy roots are less prone to genetic variation then callus or suspended cells. 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, bubble column reactor, airlift reactor, trickle bed reactor and nutrient mist reactors. 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 oxygen limitation and stress. Nutrient metabolism in this environment is more efficient than in liquid culture.

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Mist deposition depends upon capture efficiency of mist droplets by the root bed. It has been found that good growth can be achieved by manipulating ON/OFF mist duty cycle on intermittent basis. Thus duration of ON/OFF cycle has become very important criteria for optimization of hairy root growth in the reactor chamber. The availability and rate of transport of nutrients to the roots have been taken to be important parameters in design and operation of the reactor.

It has been reported that the roots grow well when the mist is supplied on an intermittent ON/OFF basis. Mist deposition is a key step in the mass transfer of nutrients to the roots in mist bioreactors. It is reported in literature that in a mist reactor, higher growth yields can be achieved with increased droplet deposition and by manipulating the ON/OFF cycle period. 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, the 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 OFF cycle also has to be stopped before the concentration in the liquid layer goes below the essential level required for the specified growth. This paper presents a mathematical model to optimize the duration of ON/OFF mist cycle based on the above considerations. Simulations were performed to arrive at possible values of these durations which would support the specified/required growth of hairy roots in mist bioreactors.

**Theory and concepts**

On increasing the duty cycle at fixed nutrient concentration from 5 min ON /20 min OFF cycle to 5 min ON /5 min OFF cycle increases root growth. Similar studies with Catharanthus tinctorius however, showed that on increasing the duty cycle further, from 5 min ON /6 min OFF to 5 min ON /2 min OFF, decreases root growth. These data suggest that there is an optimum misting cycle. The very existence of an optimum duration of duty cycles indicates deprivation of nutrients. It is expected that in conditions of sufficient availability of nutrients, the roots should grow at their maximum intrinsic growth rate as they do in shake flask experiments. Any growth rate in which is less than maximum growth rate automatically points to limitation of some kind. Mist is deposited during ON cycle of the reactor and the liquid thus deposited drains out during the OFF cycle. In the ON cycle, the liquid hold up will be distributed as layers of liquid over the roots. Thickness of the liquid layer, which is responsible for the mass transfer resistance and ensuing limitation on growth, can be estimated by
distributing the total hold up of liquid evenly along the length of the roots. This liquid layer is never depleted in the liquid phase nutrients such as sugar, due to continuous fresh supply from the incoming mist. Thus, in the ON cycle the growth is likely to be arrested by mass transfer limitation of the gas phase nutrients through the liquid layer. The drainage rate looks like another important controlling parameter for the operation of mist reactor. we have tried to include the effect of drainage rate by using logarithmic and linear drainage models. In the linear model the drainage rate is proportional to the difference of the volume of the liquid holdup at any time and the liquid hold up at saturation \( (H - H_s) \). In logarithmic model, it is proportional to the logarithmic difference of the liquid holdup at any time and the liquid accumulated at saturation \( \ln (H) - \ln (H_s) \). The proportionality constant in either case has to be found by fitting the model to data gathered from actual drainage experiments performed on similar beds.

**Mathematical model**

Based on the above considerations a mathematical model which describes the evolution of specific liquid hold up and the liquid layer Concentration is developed. It assumes uniform and complete mixing in the held up liquid. It also assumes constant capture efficiency for the sake of simplicity.

Specific quantities

1. Mass of the root at time \( t = M_R \)
2. Mass of the root initially = \( M_{RO} \)
3. Volume of the liquid attached to the roots = \( H = L \)
4. Volume of the liquid attached to the roots per unit mass of the roots = \( H_m = \frac{L}{M_R} \)
5. Concentration of the nutrients in the liquid = \( C \).
6. Growth rate of the roots = \( \frac{dM_R}{dt} \)
7. Growth rate of the roots per unit mass of the roots = \( \frac{d}{dt} \)
8. Volume of roots = \( \frac{M_R}{\rho_w} \)

Basis: unit mass of the root bed

Mist flow rate at any time \( t \) is: \( F = F_o \left( \frac{M_R}{M_{RO}} \right) \)

\( M_R \) is mass of the bed at time \( t \)
\( M_{RO} \) is initial mass of the bed
\( \alpha \) is exponent for effective feed rate

Let \( f_o \) and \( f \) denote the effective feed flow rate at the start \( t=0 \) and at any given time \( t \), respectively the corresponding specific effective flow rate will be given by \( F_o = f_o/M_{RO} \) and \( F = f /M_R \). If the density of the root bed remains constant then the volume dependent case is characterized by \( F = F_o \) as

\[
\frac{f}{M_R} = \frac{f_o}{M_{RO}} = F_o
\]

\( F \) keep on decreasing steadily for the area dependent case as

\[
\frac{f}{M_R} = \frac{f_o}{M_{RO}} \left( \frac{M_R}{M_{RO}} \right)^{2/3} = F_o \left( \frac{M_R}{M_{RO}} \right)^{-1/3}
\]

\[
\frac{f}{M_R} = \frac{f_o}{M_{RO}} \left( \frac{M_R}{M_{RO}} \right)^{-1/3} = F_o \left( \frac{M_R}{M_{RO}} \right)^{-1/3}
\]

Where \( A_o, V_o \) and \( A, V \) represents area and volume of the root bed at \( t = 0 \) and \( t=t \) respectively. In general the specific feed flow rate can be written as

\[
F = F_o \left( \frac{M_R}{M_{RO}} \right)\frac{\alpha}{\alpha}
\]

The two extreme cases of the volume dependent (small drops) and area dependent (large drops) are represented by 0 and \(-1/3\) respectively.

Specific flow rate of mist into the root bed=

\[
\eta F \left( \frac{M_R}{M_{RO}} \right)\frac{\alpha}{\alpha}
\]

(4.1)

Now, linear drainage rate of mist \( K_3(H_m - H_e) \)

(4.2)

The nutrient mass transfer taking place in the roots depends upon the diffusivity of the liquid thus the Mass transfer Coefficient, MTC, is directly proportional to the diffusivity per unit length of roots.

Therefore the rate of nutrient consumption by roots =
\[ ( \frac{4 \, k_1}{L_f \rho d_L} ) \left( C - C_m \right) \] (4.3)

Where \( k_1 \) = coefficient of diffusion in liquid.

\[ C = \text{Concentration of nutrient in feed} \]
\[ C_m = \text{minimum concentration of nutrient in feed} \]
\[ L_f = \text{Thickness of the liquid film} \]
\[ d = \text{Diameter of root} \]

The equation for the growth rate of root growing inside the NMR is based on monod model and can be represented by first order kinetics.

\[ \frac{dM_R}{dt} = \mu (M_R) \] (4.4)
\[ \mu = \frac{1}{M_R} \left( \frac{dM_R}{dt} \right) \] (4.5)

There is only a partial intake of nutrients by roots let this fraction of diffused nutrients be \( K_2 \) Therefore, combining equation (4.3) and (4.5) gives

\[ \frac{1}{M_R} \left( \frac{dM_R}{dt} \right) = \frac{4 \, k_1 \, k_2 \left( C - C_m \right)}{L_f \rho d_L} \]
\[ \frac{dM_R}{dt} = \left( \frac{4 \, k_1 \, k_2 \left( C - C_m \right)}{L_f \rho d_L} \right) M_R \] (4.6)

From this equation it is clear that specific growth rate is dependent on diffusivity and concentration of nutrients and root bed parameter. The equation for the ON cycle in an NMR by overall liquid balance per unit mass of root bed.

Rate of mist retained and reactor = input – drainage

Putting the value of equation (4.1) and (4.2) mentioned above

\[ \frac{dH_m}{dt} = \eta \, F_{RO} \left( \frac{M_R}{M_{RO}} \right)^{\alpha} - K_3 (H_m - H_e) \] (4.7)

Where, \( \eta \) = fraction of mist deposited on the roots.
Doing the component balance on the roots.

\[ F_{RO} \left( \frac{M_R}{M_{RO}} \right)^{\alpha} C_0 - k_3 (H_m - H_e) C - \left( \frac{4 \, k_1}{L_f \rho d_L} \right) (C - C_m) = \frac{d(H_m \, C)}{dt} \] (4.8)

On solving the above equation we get

\[ \frac{dC}{dt} = \eta \, F_{RO} \left( \frac{M_R}{M_{RO}} \right)^{\alpha} (C_0 - C) - \frac{4 \, k_1 (C - C_m)}{H_m L_f \rho d_L} \] (4.9)

Equation (4.7) and (4.9) represents specific liquid holdup profile and concentration at time \( t \) of mist ON cycle. similarly for OFF cycle the equation can be derived since the flow rate of the feed during off cycle become zero at the feed supply hence equation (4.7) and (4.9) becomes

\[ \frac{dH_m}{dt} = -K_3 (H_m - H_e) \] (4.10)
\[ \frac{dC}{dt} = -4 \, K_3 \frac{(C - C_m)}{H_m L_f \rho d_L} \] (4.11)

Equation (4.10) and (4.11) represents the specific liquid hold-up profile and concentration profile at time \( t \) of mist OFF cycle.

These equation obtained were simulated and the profiles were obtained for the dependence of the concentration of the nutrient in the roots, the liquid hold-up and the mass of the roots as a function of time. The effect of the change of parameters like the capture efficiency, diffusivity constant, drainage constant and feed concentration were studied in these profiles.

**Numerical solution and parameter values**

Solution of the ON cycle was 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 set up method which uses GEARS algorithm to integrate the differential equations. It automatically chooses the time required, steps and order. Simulations were performed for various levels of error tolerance till the solution did not change with changing tolerance. Similarly the solution for the OFF cycle was obtained by integrating the set of coupled equations. Repeated solutions for the ON cycle followed by the OFF cycle were used to
simulate for the complete reactor.
The mist flow rate is taken to be 1ml per mg-per day and the saturation liquid hold up (Hs) has been taken to be 0.01 ml/mg. Critical concentration of the nutrient required to maintain specific growth rate is taken to be 50mg/ml. Simulations have been performed for a total of 200 ON/OFF cycles. The check for depletion of concentration has been made at every cycle. The constant of proportionality in the drainage rate equation, K3 has been taken as 4, 8, 16 and 32 for the linear model. The concentration of the starting feed value CF has been varied between 60mg/ml to 100mg/ml. The increament in the feed concentration has been varied between 10 and 20mg/ml. HON and HOFF values are taken as 0.99, 0.75, 0.5 etc.

**Result**

The following are the variations of the concentration of nutrients in the roots, mass of the roots and liquid holdup of the roots with a change of the capture efficiency of the roots. = 0.01, 0.05, 0.1, 0.2

![Graph showing variation of concentration with time](image)

Figure 6.1: Variation of concentration with time

As stated earlier the capture efficiency is the fraction of the incoming mist in the feed that the roots capture. The results show that as the value of capture efficiency increases the concentration decreases. The topmost line refers for the value of = 0.2 and the bottom most for =0.01.
It can be seen that the slope becomes steep for higher values of capture efficiency and the mass of the roots gains the highest value for capture efficiency = 0.2. The capture efficiency = 0.01 the graph is almost a straight line with a constant slope in contrast with the parabolic nature for higher values.

For higher values of capture efficiency the amount of liquid holdup increases. Now as the hold up increases above a critical value it starts offering resistance to the mass transfer. This model will be optimized. It can be observed that the holdup oscillates around a constant value which is the equilibrium holdup value it switches from the OFF cycle to the ON cycle when the critical value of the holdup is reached.

2. The following are the variations with a change of K3 which is the proportionality constant for drainage rate of the mist from the reactor. K3=4, 8, 16, 32.
This graph shows that the concentration of the nutrients in the roots is independent of the drainage rate. It will be shown in the other graphs that it depends on the feed concentration, capture efficiency coefficient of diffusion in the liquid.

Figure 6.4: variation of concentration with time

The value of K3. For very high values of K3 the value of holdup is almost constant as the amount of mist coming in almost equals the amount of mist going out so the holdup neither increases nor decreases then. For lower values however a variation can be seen which revolves around the equilibrium liquid hold up.

Figure 6.5: variation of specific liquid holdup (ml/mg) as a function of time
Mass of the roots is also shown to be independent of the drainage rate constant. This parameter also depends predominantly on the capture efficiency and feed concentration.

3. The following are the variations with a change of $K_1$ which is the coefficient of diffusion in liquid. $K_1=0.50, 0.65, 0.75$

![Graph showing variation of mass with time](image)

**Figure 6.6: variation of mass with time**

For lower values on diffusivity the slope is almost constant and for higher values of diffusivity the graph is parabolic. As the value of $K_1$ increases the maximum mass attained by the roots increases. This implies that the growth rate of the roots is better for higher values of $K_1$.

![Graph showing variations with $K_1$](image)

**Figure 6.7: variation of mass with time**
For higher values of the diffusion coefficient lower values of concentration of the nutrients in the roots is obtained because more nutrients are being diffused into the roots so the concentration decreases at a higher rate.

The following are the variations with a change in the feed concentration Cf. Cf=50, 30, 20 (mg/ml)

The liquid holdup is independent of the change in feed concentration. This can be explained with the fact the amount of liquid that the roots hold depends on their capture efficiency and the drainage rate constant. The change in feed concentration shall majorly affect the mass and concentration of the nutrients in the roots.
It can be seen that as the feed as the Cf increases the mass of the roots increases. The maximum value of roots is however obtained for a higher value of the diffusion constant which implies that a change in the value of $K_1$ affects the mass more than a change in value of Cf.

The concentration achieved is maximum for Cf=50 mg/ml. this result is in agreement with the fact that as the concentration of the nutrients in the feed is increased the concentration of the nutrients in the liquid holdup of the roots increases.

Conclusion
A study of the effect of the governing parameters on the growth of the roots and the operating conditions of the nutrient mist reactor is studied. A mathematical model has been developed for the depletion of the nutrient during ON/OFF mist reactor. It is shown that on increasing feed concentration for fed-batch mode of operation can maintain a specified growth rate in the reactor. Also on increasing the capture efficiency of the roots better growth of the roots is obtained. The drainage rate constant has no effect on the mass and concentration in the hold up and it affects only the hold up of the roots.
Abbreviations
1. C = Concentration of the nutrients in the liquid
2. C_m = minimum concentration of nutrient in feed
3. C_f = Concentration of nutrient in feed
4. d = Diameter of root
5. dM/dt = Growth rate of the roots
6. f = feed flow rate per unit mass at time t
7. f_0 = initial feed flow rate per unit mass
8. H = L = Volume of the liquid attached to the roots
9. H_m = L/M_0 = Volume of the liquid attached to the roots per unit mass of the roots
10. k_1 = coefficient of diffusion in liquid
11. k_2 = fraction of the diffused nutrient in the growth of root
12. k_3 = Drainage rate constant
13. L_f = Thickness of the liquid film
14. M_0 = Mass of the root at time t
15. M_80 = Mass of the root initially
16. M_0/M_0 = Volume of roots
17. M_b = mass of the bed at time t
18. M_0 = initial mass of the bed
19. (dM_b/dt)/M_b = Growth rate of the roots per unit mass of the roots
20. α = exponent for effective feed rate
21. η = fraction of mist deposited on the roots.

References


