ISSN: 2277-9655
Scientific Journal Impact Factor: 3.449
(ISRA), Impact Factor: 1.852

IJESRT
INTERNATIONAL JOURNAL OF ENGINEERING SCIENCES & RESEARCH TECHNOLOGY

Modelling of Nutrient Mist Reactor for ON/OFF Duty Cycle

Shweta Bajpai and Rajesh Kumar Kaushal
Department of Chemical Engineering, Institute of Engineering & Science, IPS Academy, Indore [M.P.], India
ms.shwetabajpai@gmail.com

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 resis¬tance 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 ab¬sence 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 a bio reactor which is suitable for This 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 02 limitation and stress. Nutrient metabolism in this environment is more efficient than in liquid culture.

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.

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 - Hs). In logarithmic model, it is proportional to the logarithmic difference of the liquid holdup at any time and the liquid accumulated at saturation (In (H) - In (Hs)). 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 = $H_m = \frac{L}{M_R}$ 1
5. Concentration of the nutrients in the liquid = C.
6. Growth rate of the roots = $\frac{dMR}{dt}$
7. Growth rate of the roots per unit mass of the roots = $\frac{\left(\frac{dMR}{dt}\right)}{M_R}$
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
α 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

$$f = f_o\left(\frac{v}{v_o}\right) = f_o\frac{M_R}{M_{RO}}$$

$$F = \frac{f}{M_R} = \frac{f_o}{M_{RO}} = F_o$$

F keep on decreasing steadily for the area dependent case as

$$f = f_o\left(\frac{A}{A_o}\right) = f_o\left(\frac{M_R}{M_{RO}}\right)^{2/3}$$

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

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

$$F = F_o\left(\frac{M_R}{M_{RO}}\right)^{\alpha}$$

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

Specific flow rate of mist into the root bed=

$$\eta F\left(\frac{M_R}{M_{RO}}\right)^{\alpha} \quad (4.1)$$

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 =

$$\left(\frac{4\,k_1}{(L_f\,\rho d)}\right)(C - C_m) \qquad (4.3)$$

Where k_1 = coefficient of diffusion in liquid.

C= Concentration of nutrient in feed

C_m = minimum concentration of nutrient in feed

L_f = Thickness of the liquid film

d = 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.

$$\frac{dM_R}{dt} = \mu\,(M_R) \qquad (4.4)$$

$$\mu = \frac{1}{M_R}\left(\frac{dM_R}{dt}\right) \qquad (4.5)$$

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

$$\frac{1}{M_R}\left(\frac{dM_R}{dt}\right) = \frac{4k_1\,k_2\,(C - C_M)}{L_f\rho d}$$

$$\frac{dM_R}{dt} = \left\{\frac{4k_1\,k_2\,(C - C_M)}{L_f\rho d}\right\} M_R \qquad (4.6)$$

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

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

Where, η= fraction of mist deposited on the roots.
Doing the component balance on the roots.

$$\eta\,F_{RO}\left(\frac{M_R}{M_{RO}}\right)^{\alpha} \mathrm{C_o} - \mathrm{k_3}(H_m - H_e)C - \left(\frac{4k_1}{L_f\rho d}\right)(C - C_m) = \frac{d(H_m\,C)}{dt} \qquad (4.8)$$

On solving the above equation we get

$$\frac{dC}{dt} = \frac{\eta\,F_{RO}\left(\frac{M_R}{M_{RO}}\right)^{\alpha}(C_o - C)}{H_m} - \frac{4K_1(C - C_m)}{H_m L_f \rho d} \qquad (4.9)$$

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

$$\frac{dH_m}{dt} = -K_3(H_m - H_e) \qquad (4.10)$$

$$\frac{dC}{dt} = -4K_1\frac{(C - C_M)}{H_m\,L_f\rho d} \qquad (4.11)$$

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 increment 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

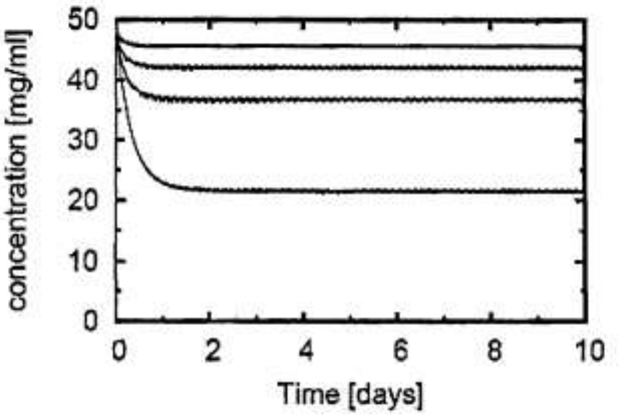

Figure 6.1: Variation of concentration with time

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.

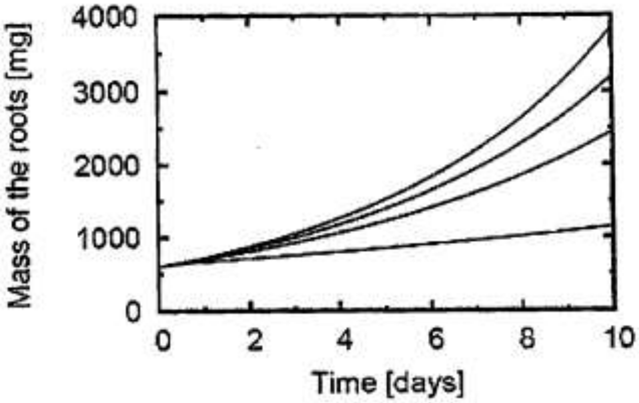

Figure 6.2: variation of mass of the roots with time

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.

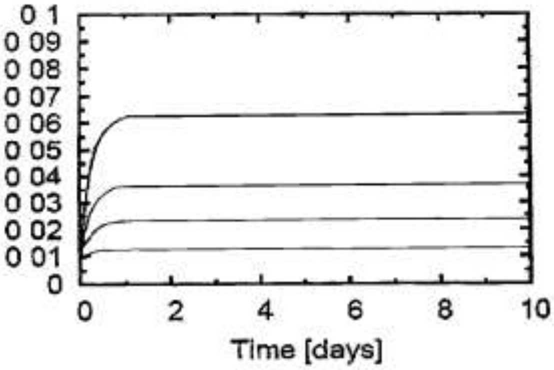

Figure 6.3: Specific liquid holdup (ml/mg) as a function of time

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.

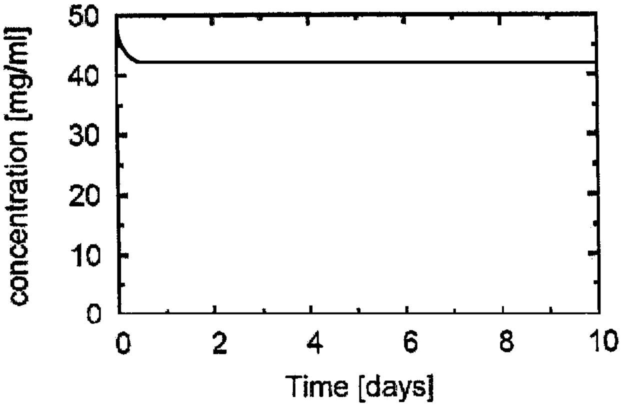

Figure 6.4: **variation of concentration with time**

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.

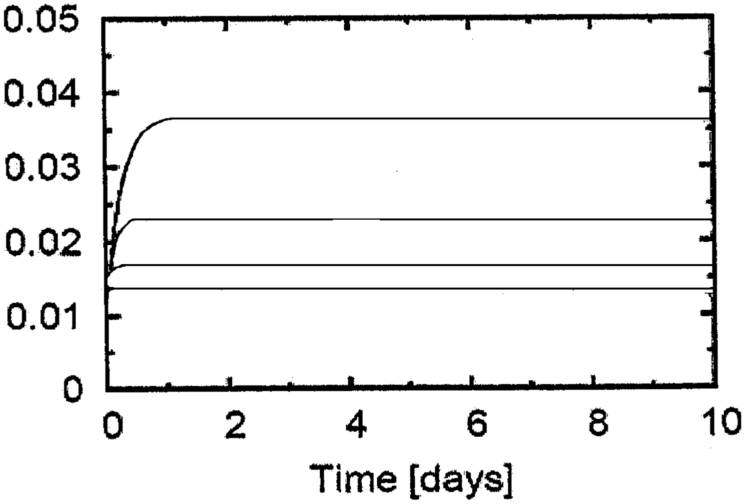

Figure 6.5: **variation of specific liquid holdup (ml/mg) as a function of time**

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.

ISSN: 2277-9655
Scientific Journal Impact Factor: 3.449
(ISRA), Impact Factor: 1.852

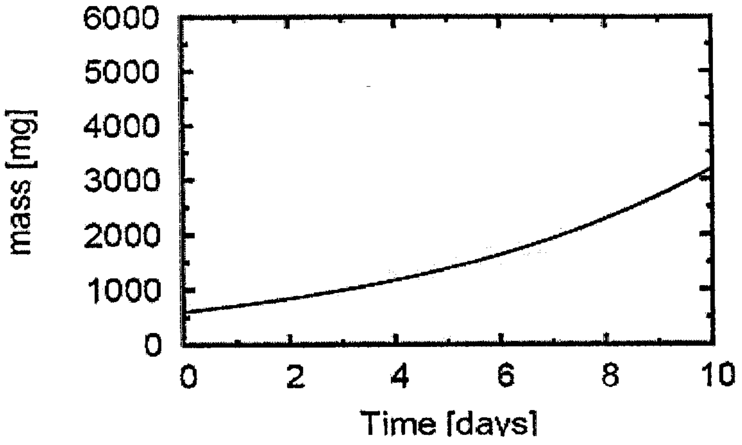

Figure 6.6: **variation of mass with time**

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 K1 which is the coefficient of diffusion in liquid. K1=0.50, 0.65, 0.75

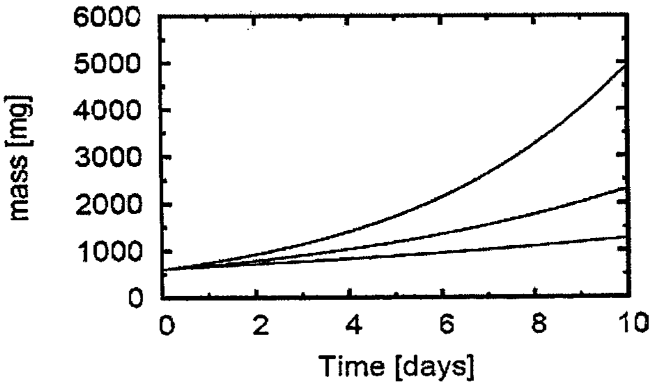

Figure 6.7: **variation of mass with time**

For lower values on diffusivity the slope is almost constant and for higher values of diffusivity the graph is parabolic. As the value of K1 increases the maximum mass attained by the roots increases. This implies that the growth rate of the roots is better for higher values of K1.

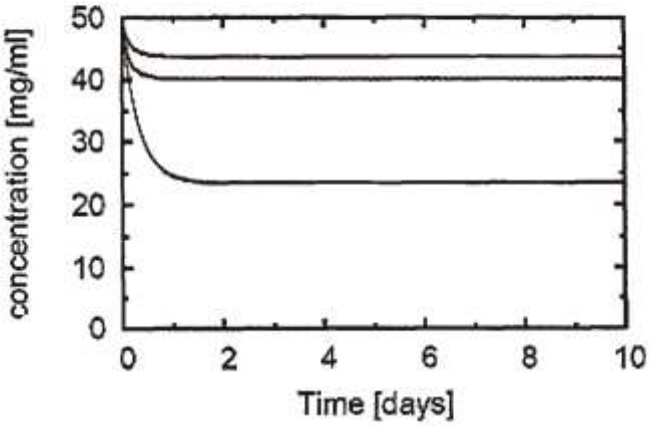

Figure 6.8: **variation of concentration with time**

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)

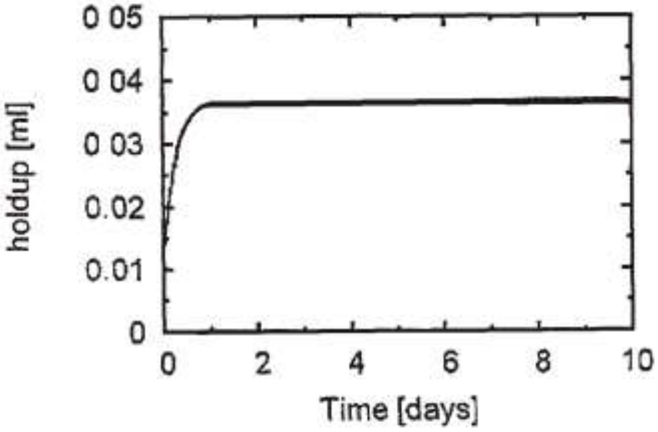

Figure 6.9: **variation of liquid holdup with time**

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.

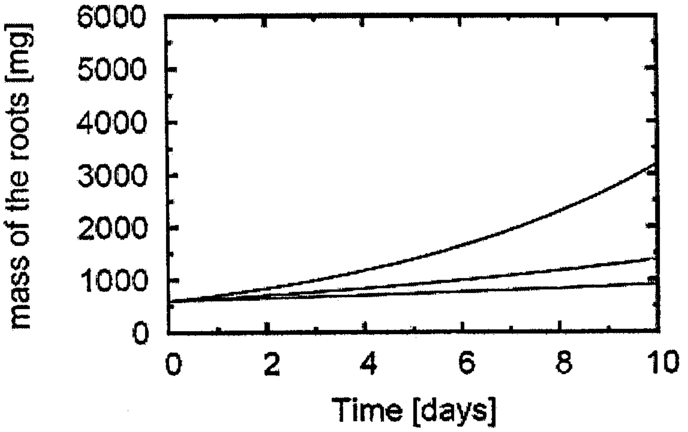

Figure 6.10: **variation of mass of the roots with time**

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 K1 affects the mass more than a change in value of Cf

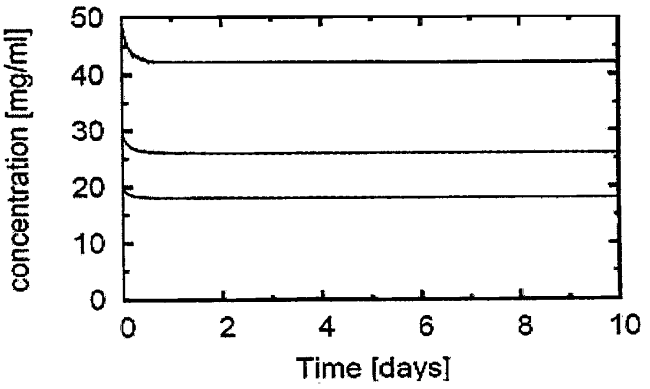

Figure 6.11: **variation of concentration with time**

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. $d M_R/ d t$ =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. $H_m = L/M_R1$=Volume of the liquid attached to the roots per unit mass of the roots
10. k1 = coefficient of diffusion in liquid.
11. k2=fraction of the diffused nutrient in the growth of root
12. k3 =Drainage rate constant
13. L_f = Thickness of the liquid film
14. M_R =Mass of the root at time t
15. M_{RO} =Mass of the root initially
16. M_R/ρ_w =Volume of roots
17. M_R = mass of the bed at time t
18. M_{RO} = initial mass of the bed
19. $(d M_R/d t)/M_R$ = 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

1. Aird, E.L.H, Hamill J.D., and Rhodes M.J.C. (1988) "*Cytogenetic analysis of hairy root cultures from a number of plant species transformed by Agrobacterium rhizogenes*". Plant Cell Tiss. Organ. ChIt. 15: 47-57.
2. Banerjee, S., Rahman, L., Uniyal, G.C. and Ahuja, P.S. (1998) "*Enhanced production of valepotriates by Agrobacterium rhizogenes induced hairy root cultures of Valeriana wallichii DC*". Plant Sci. 131: 203 - 208.
3. Correll MJ, Wu Y, Weathers PJ (2001) "*Controlling hyperhydration of carnations (Dianthus caryophyllus L.) grown in a mist reactor*". Biotechnol Bioeng 74:307-314
4. Caspeta L., Rodolfo Q., and Villarreal M. L. (2005) "*Novel Airlift Reactor Fitting for Hairy Root Cultures*" Developmental and Performance Studies. Biotechnol. 21 : 735-740.
5. Dilorio A. A, Cheetham R. D., and. Weathers P. J (1992) "*Growth of transformed roots in a nutrient mist bioreactor: reactor performance and evaluation*". Appl. Microbiol. Biotechnol. 37: 457-462.
6. Friberg, J.A., Weathers, PJ. and Gibson, D.G. (1992) "*Culture of amebocytes in a nutrient mist bioreactor"*. Vitro Cell. Dev. BioI. 28A: 215-217.
7. Kim YJ, Weathers PJ, Wyslouzil BE (2002) "*Growth of Artemisia annua hairy roots in liquid and gas-phase reactors*". Biotechnol Bioeng 80:454-464
8. Kim YJ, Wyslouzil B, Weathers J (2002) "*Secondary metabolism of hairy roots in bioreactors*". In Vitro Cell Develop Biol Plant 38:1-10
9. Khwaja Osama, Somvanshi Pallavi , Pandey Asheesh Kumar , Mishra Bhartendu Nath , (2013)"*Modelling of Nutrient Mist Reactor for Hairy Root Growth using Artificial Neural Network*" European Journal of Scientific Research . 97(4):516-526
10. Liu CZ, Wang YC, Zhao B, Guo C, Ouyang F, Ye HC, Li GF (1999) "*Development of a nutrient mist bioreactor for growth of hairy roots*". Vitro Cell Dev Biol Plant 35:271-274
11. Mallol A, Cusido RM, Palazon J, Bonfill M, Morales C, Pinol MT. (2001)"*Ginsenoside production in different phenotypes of Panax ginseng transformed roots"*. Phytochemistry.57(3):365-71.
12. Souret FF, KimYJ, Wyslouzil BE, Wobbe KK, Weathers PJ (2003) "*Scale-up of Artemisia annua L. hairy roots cultures produces complex patterns of terpenoid gene expression*". Biotechnol Bioeng 83: 653-667
13. Towler MJ, Wyslouzil BE, Weathers PJ (2007) "*Using an aerosol deposition model to increase hairy root growth in a mist reactor*". Biotechnol Bioeng 96:881-891

14. Ranjan Ritu , Ahmed Naseem , Khanna Rajesh and Mishra B. N. (2009) "*Design of an ON/OFF mist duty cycle in mist bioreactors for the growth of hairy roots*". Biotechnology and Bioprocess EngineeringFebruary Volume 14, Issue 1, pp 38-45
15. Ranjan Ritu, Khanna Rajesh, Sambasiva and Rao Katuri (2010) "*Development of a mathematical model for sustained operation of nutrient mist reactor to grow hairy roots.*"Research Article, Biotechnol. Bioinf. Bioeng. 1(4):465-472
16. Sivakumar G, Liu C, Towler MJ, Weathers PJ.(2010) "*Biomass production of hairy roots of Artemisia annua and Arachis hypogaea in a scaled-up mist bioreactor*". Biotechnol Bioeng. Dec 1;107(5):802-13. doi: 10.1002/bit.22892.
17. Wyslouzil, B.E., Whipple M., Chatterjee C., Walcerz D.B.,. Weat.hers P.J and Hart D.P. (1997) "*Mist deposition onto hairy root cultures Aerosol modeling and experiments"*. Biotechnol. Prog. 13: 185-194.
18. Weathers PJ, Bunk G, McCoy M (2005) "*The effect of phytohormones on growth and artemisinin production in Artemisia annua hairy roots*". Vitro Cell Devel Biol Plant41:47-53
19. Wyslouzil BE, Waterbury RG, Weathers PJ (2000) "*The growth of single roots of Artemisia annua in nutrient mist bioreactors*". Biotechnol Bioeng 70:143-150