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A strategy to choose process parameters for sustained operation of nutrient mist reactor to grow hairy roots

Ritu Ranjan[1], Sambashiva Katuri Rao[2], Rajesh Khanna[3]
[1,2,3]*Department of Chemical Engineering, Indian Institute of Technology Delhi (I.I.T.Delhi), New Delhi - 110016, India*

Abstract: *Feed concentration and feed flow rate along with the relative durations of the mist-ON/ mist-OFF cycles are the important process parameters for the growth of hairy roots in a nutrient mist reactor. The paper presents guidelines for choosing these parameters for an efficient operation of the reactor with intermittent mist-ON/mist-OFF cycles. It is based on a theoretical model of nutrient mist reactor at the root bed level and its numerical simulation. This model describes the evolution of liquid holdup and concentration present in the root bed. One can also run the reactor with intermittent mist-ON and mist-OF cycles, at very low cumulative mist-ON duration for any given total run time to prevent any nutrient deficiency and water logging in the bed. It is found that in some conditions mist has to be switched on for only about 10% of the total runtime while the mist can be switched off for 90% of the total run time. The duration of both mist-ON / mist-OFF cycles are effected by the process parameters and thus, the ratio of mist-ON time to total run time, may not follow any fixed trend in some cases. The guidelines presented here will help in efficient operation and design of nutrient mist reactors.*

Keywords: *Drainage rate, hairy root, mist deposition, mist-ON cycle/mist-OFF cycle, saturation.*

I. Introduction

Hairy root culture are of particular interest for the production of high-value plant secondary metabolites because these cultures are genetically stable, grow rapidly, and produce a spectrum and quantity of secondary metabolites similar to that of the parent plant (Aird, 1988). Hairy roots produced by genetic transformation with *Agrobacterium rhizogenes* are a potential means for manufacture of plant secondary metabolites (Verma and Singh, 2008; Ferreira, 2005; and Kim, 2002). These can be generated from a large variety of species (Guillon, 2006) and a source of many important phytochemicals (Wyslouzil, 19997; Carvalho, 1998; Kim, 2001; Sudha, 2003; Weathers, 2005; and Lorence, 2004). Hairy root cultures have gained importance because of their stability and ability to grow in a hormone free medium (Souret, 2003; Shanks, 1999; Sevon, 2002; and Parr, 1988). Their root growth is very complicated due to its dense root network and, branching processes (Sharp, 1990).

The unique growth pattern and fragile nature of hairy roots have led to many different reactor configurations to avoid injuring the roots while optimizing the supply of nutrients and gases (Ranjan, 2008; Srivastava, 2007; Sivakumar, 2006; Eibl, 2006; McAlister, 2005; Palazon, 2003; Pavlov, 2005; Pavlov, 2006; Hilton, 1990; Taya, 1989; and Sharp, 1990). One of the successful reactors is nutrient mist reactor (NMR) which is simple in design and economical for cultivation of hairy roots (Flores, 1992; Williams, 2000; Ramakrishnan, 2004; and Wilson, 1990). This is a gas phase reactor in which the liquid medium is introduced into the bioreactor as a mist phase with very small droplets in the size range of 0.5 µm - 30 µm. NMR has definite advantages such as easy operation, presence of gas phase, lack of shear, low pressure, ease in manipulating the gas composition and effective gas exchange in a densely growing biomass and ease of scaling up (Towler, 2007; and Liu, 1999). The mass transfer limitation of oxygen can be eliminated by using gas phase reactors. These are unique because the gas composition in the reactor nvironment can be closely controlled; oxygen is not a limiting factor and pressure drops and shear rates are both low (Weathers, 1999; and Williams, 1999). Industrial production of secondary metabolites from hairy roots remains very challenging due to the complex fibrous structure and dense root growth network (Srivastava, 2007). Furthermore, root growth is not homogeneous. Growth of these valuable roots can be expressed in terms of increase in mass, number of tips and length. Also the growth of hairy roots and the production of secondary metabolite depend on the availability and composition of liquid media and gases at the root surface.

The availability of sufficient nutrients and oxygen to the root surface is very important for healthy growth of root mass (Dilorio, 1992b; Dilorio, 1992b; Flores, 1992). Also, the amount of available nutrients in this reactor is dependent upon the volume of mist which can be captured by the roots. Mist deposition is a key step in the mass transfer of nutrients to the roots in mist bioreactors (Wyslouzil, 1997). It is important to mention that the mist-ON/mist-OFF cycles determines the delivery of nutrients to the roots and also controls the gas exchange in the growth chamber, and thus this parameter is critical for ensuring root tissue viability and development. It is reported in literature that intermittent misting cycle helps in uniformly wetting the root surface and the short intermittent misting cycle prevents severe clogging of the bed by previously captured particles and allows time for liquid to drain from the roots between applications of the mist. The roots grow well when the mist is supplied on an intermittent mist-ON/mist-OFF basis. Higher growth yields can be achieved with increased droplet deposition and by manipulating the mist-ON/mist-OFF cycles (Towler, 2007).

It is also possible to sustain the performance by a continuous mist-ON cycle rather than an intermittent mist-ON/mist-OFF cycles by choosing the processes conditions carefully. Once the root species is chosen then the two major process conditions to be chosen are feed flow rate (F) and concentration of nutrients (Sucrose) in the feed, (C_F). For a continuous mist-ON cycle, the liquid holdup (H) and nutrient concentration in liquid layer (C) reach its static values, which depend on the values of C_F and F. For values of equilibrium specific liquid holdup (H_E) less than critical specific liquid holdup (H_C) and equilibrium feed concentration (C_E) more than critical feed concentration (C_C), the reactor can run indefinitely in the mist-ON cycle mode. H_C and C_C are the maximum value of H and minimum value of C which are required to run the system in safe mode without any mass transfer limitations of liquid and gas phase nutrients. While the reactor can now be operated without any botheration of mist-ON/mist-OFF cycle, one misses out on the increased energy savings provided by periods of mist-OFF cycle. In any case, intermittent cycles have to be analyzed to handle any unscheduled disruption in the mist-ON cycle. Any estimation of the maximum cushion of down time before the mist-ON cycle must be started is important in sustained production of hairy roots. An analysis of the intermittent mode of operation of NMR is presented in the present contribution. It is based on numerical simulations of a mathematical model which describes the evolution of liquid holdup and concentration.

II. Model Development And Numerical Simulations

Development of the mathematical model for an efficient operation of NMR has been shown in the following steps and a schematic diagram of this reactor and root bed for intermittent mist ON/OFF cycle is also shown in Fig. 1.

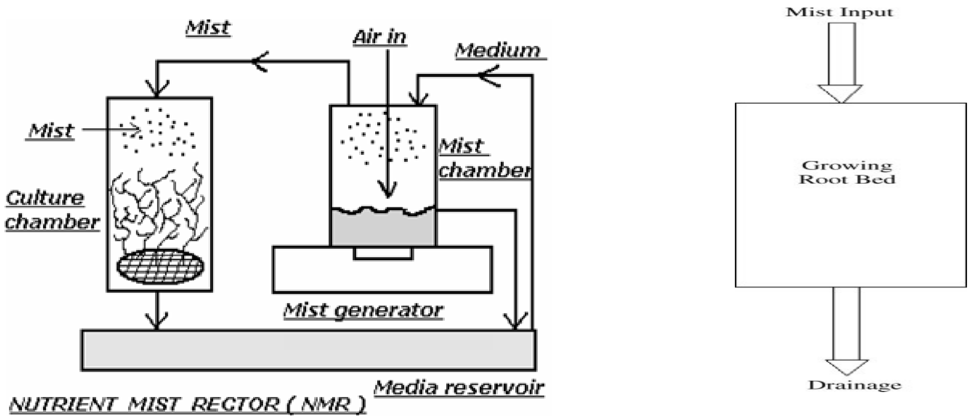

Fig. 1 Schematic diagrams of nutrient mist reactor and hairy root bed for intermittent mist ON/OFF cycle, respectively

A mathematical model for the evolution of liquid holdup and feed (nutrient) concentration is developed under the assumptions of uniform and complete mixing in the held up liquid. While, some deposition is required for providing nutrients to the growing roots, any excess deposition will lead to increased holdup in the form 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 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. The drainage rate has been modeled as proportional to difference between the specific dynamic liquid holdup, volume of held up liquid divided by mass of the root bed (H), and its value at saturation (H_S). The value of proportionality constant (K_2) for drainage have been taken for the literature (Raynor and Leith, 2000).

The effective feed flow rate per unit mass (F), is estimated based on the following argument. In a typical reactor configuration, the root bed may occupy only a small fraction of the reactor volume, at least in the early stages. The mist input flow rate at the inlet of the chamber may be held constant but the effective flow rate into the bed may change as the root bed expands and come in contact with more mist. The effective flow rate to the bed is likely to be governed by the relative dimensions of the bed and the reactor chamber. With the dimensions of the reactor chamber remaining constant this translates to relative dimensions of the bed at different times. One can intuitively see two extreme cases depending on whether the effective rate depends on the relative surface area or relative volumes. If the droplet size is comparable to the void spacing between the root hairs then most of them will deposit on the outer surface of the growing root bed. This will lead to relative surface area dependence of the effective feed flow rate. On the other hand if the droplets are much smaller then they will penetrate inside the bed and deposit there also, leading to a volume dependent effective feed flow rate. Let 'f_0' 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 rates will be given by $F_0 = f_0/M_{R0}$ and $F = f / M_R$, where 'M_{R0}', and 'M_R' are the mass of the root bed at the start and at the given time t, respectively. If the density of the root bed remains constant ($F = F_0$), then the volume dependent case is characterized by

$$f = f_0\left(\frac{V}{V_0}\right) = f_0\left(\frac{M_R}{M_{R0}}\right) \quad ; \quad F = \frac{f}{M_R} = \frac{f_0}{M_{R0}} = F_0 \tag{1}$$

F keeps on decreasing steadily for the area dependent case and expressed by

$$f = f_0\left(\frac{A}{A_0}\right) = f_0\left(\frac{M_R}{M_{R0}}\right)^{2/3} \quad ; \quad F = \frac{f}{M_R} = \frac{f_0}{M_{R0}}\left(\frac{M_R}{M_{R0}}\right)^{-1/3} = F_0\left(\frac{M_R}{M_{R0}}\right)^{-1/3} \tag{2}$$

Where M_{R0}, and M_R are the mass of the root bed at the start and at given time t, respectively. A_0, V_0 and A, V represents area and volume of the root bed at start and at given time t, respectively. f_0 and f denote the effective feed flow rate at start (t = 0) and at any given time t, respectively. In general, specific feed flow rate is expressed by

$$F = F_0\left(\frac{M_R}{M_{R0}}\right)^{\alpha} \tag{3}$$

Where α is an exponent for effective feed flow rate, whose value depends on the size of droplets. The root growth for first order kinetics is expressed by

$$\frac{dM_R}{dt} = \mu M_R \tag{4}$$

Where μ = Specific growth rate of the roots and is assumed to be constant.

2.1 Development of equations for mist-ON cycle

Taking into account all the assumptions made above, a mass balance for liquid and nutrients across the root bed after time t, is expressed by

$$\frac{dH}{dt} = F_0 \exp(\mu\alpha t) - K_2(H - H_S) \tag{5}$$

$$\frac{dC}{dt} = \left(\frac{F_0}{H}\right)\exp(\mu\alpha t)[C_F - C] - \frac{K_1}{H} \tag{6}$$

Where K_1 is rate of consumption by the roots per unit mass of the roots and K_2 is proportionality constant for drainage equation.

Analytical solution of the above set of Eqs. (4, 5 and 6) for the case of α = 0 and constant μ is expressed by

$$M_R = M_{R0}\exp(\mu t) \tag{7}$$

$$H = H_0 + \frac{F_0}{K_2}[1 - \exp(-K_2 t)] \tag{8}$$

$$\ln\left[\frac{F_0(C_F - C) - K_1}{F_0(C_F - C_0) - K_1}\right] = -\int_0^t \frac{F_0\,dt}{H_0 + \frac{F_0}{K_2}(1 - \exp(-K_2 t))} \tag{9}$$

Where M_{R0}, H_0 and C_0 are the values at the start or at t = 0.

If one wants to operate at feed flow rates which are higher than the upper limit of H then one has to stop the mist-ON cycle before H reaches to Hc. One can also estimate the maximum duration of mist-ON time for continuous operation of the reactor (putting $H = H_C$ in Eq. (8) as expressed by

$$T_C = \frac{1}{K_2}\left(\ln\left(1-\frac{K_2}{F_0}\right)(H_C - H_S)\right)^{-1} \tag{10}$$

Where T_C is the maximum duration of mist-ON cycle.

The mist has to be started again when C goes down to C_C. The maximum duration of the subsequent mist-ON cycles will depend on H value at the end of the preceding mist-OFF cycle. The lower limit of H is H_S. Thus, the subsequent mist-ON cycles have to be stopped earlier than T_C. As a result, T_C serves as an upper limit for durations of all mist-ON cycles.

2.2 Development of equations for mist-OFF cycle

During mist-OFF cycle, the supply of the feed is stopped therefore feed flow rate becomes zero. So the Eqs. (5) and (6) can be expressed by

$$\frac{dH}{dt} = -K_2(H - H_S) \tag{12}$$

$$\frac{dC}{dt} = -\frac{K_1}{H} \tag{13}$$

Specific liquid hold up across the root bed after time t for mist-OFF cycle is expressed by

$$H = H_S + (H_C - H_S)\exp(-K_2 t) \tag{14}$$

Nutrient concentration presents in the liquid at the end of mist-OFF cycle is expressed by

$$C = C_E - \frac{K_1 t}{H_S} - \frac{K_1}{K_2 H_S}\left[\ln\left(H_S + (H_C - H_S)\exp(-K_2 t)\right) - H_S \ln(H\ )\right] \tag{15}$$

Hence the maximum duration of mist-OFF cycle for an intermittent mist-ON/mist-OFF cycle (putting $t = T_M$ and $C = C_C$ in Eq. (14) is expressed by

$$T_M = \frac{H_S}{K_1}\left[C_E - C_C - \frac{K_1}{K_2 H_S}\left[\ln\left(H_S + (H_C - H_S)\exp(-K_2 T_M)\right) - H_S \ln(H\ )\right]\right] \tag{16}$$

Where T_M is the maximum duration of mist-OFF cycle.

Solutions for mist-ON/mist-OFF cycles were obtained by integrating the set of coupled Eqs. (5), (6), (12) and (13), respectively 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 cycle followed by mist-OFF cycle were used to simulate for the complete reactor. As both H and C rise to steady state values during a continuous mist-ON cycle, the ON cycle was terminated for given values of relative approach to steady state value of concentration (Y_{ON}), which is expressed by

$$Y_{ON} = \frac{C - C_N}{C_E - C_N} \tag{17}$$

Where, C_N is concentration at the start of mist-ON cycle. Similarly, mist-OFF cycle was stopped for relative approach to the C_C as Y_{OFF} and expressed by

$$Y_{OFF} = \frac{C - C_O}{C_O - C_C} \tag{18}$$

Where, Co is now the concentration at the start of the mist-OFF cycle rather than that of the mist-ON cycle.

The numerical values for model parameters were estimated from the literature. Since a wide range of such values has been reported, a suitable set is difficult to select. Consequently, parameter values were chosen or estimated such that they were within the practical ranges for the root culture system. The values of parameters, used to simulate the results are shown in Table 1. Simulations were performed with a large number of parameter values with in the range of reported/practical values. The specified growth rate has to be decided upon first. The growth rate achieved in the shake flask experiments can be a good guideline for the reasonable values.

Table 1. Physical parameters used in the model

Parameters	Values	References
Nutrient concentration (Sucrose) in feed, C_F	50 g/L	(Kim, 2001) (Towler, 2007)
Specific growth rate, μ	0.2 day^{-1}	(Kim, 2002) (Towler, 2007)
Feed (Mist) flow rate, F_0	0.1 mL/day/mg	(Towler, 2007)
Mist droplet capture efficiency, η	0.1	(Wyslouzil, 1997)
Initial root mass, M_{R0}	100 mg	(Towler, 2007)
Density of the roots, ρ_R	1000 mg/mL	(Towler, 2007)
Diameter of root, D_f	1 mm	(Wyslouzil, 1997)
Diffusivity Coefficient in liquids, D	0.72 mm^2/day	(Raynor, 2000)
Liquid film thickness, L_F	0.1 mm	(Raynor, 2000)
Critical concentration , C_C	5 mg/mL	Calculated
Specific liquid holdup at saturation, H_S	0.01 mL/mg	Towler (2007)
Proportionality constant for growth equation, K_1	0.3 day^{-1}	(Kim, 2002)
Proportionality constant for drainage rate, K_2	4 day^{-1}	Calculated
Critical liquid holdup, Hc	0.04 mL/mg	Calculated

III. Results And Discussions

The objective of the present work is to study process parameters (duration of mist-ON/ mist-OFF cycles, feed flow rate and feed concentration) for an efficient operation of NMR. The following results are discussed for the case when mist is supplied on intermittent mist-ON/mist-OFF basis. Fig. 1 presents the evolution of C and H during repeated mist-ON/mist-OFF cycles for different Y_{OFF}. Other parameter values are F_0 = 0.1 mL/day/mg, C_F = 50 mg/mL, K_1 = 0.3 per day, K_2 = 4 per day, H_S = 0.01 mL/mg, H_C = 0.04 mL/mg, C_C = 5 mg/mL and Y_{ON} = 0.99. The evolution settles into a steady almost periodic behavior very quickly. Both C and H rise steadily towards C_E and H_E during the mist-ON cycle. The rate decreases as they reach close the equilibrium values. The rise is arrested as mist-ON cycle reaches at a value, Y_{OFF} = 0.99. Both C and H start decreasing as the mist-OFF cycle starts. Different cutoffs for mist-OFF cycle shows that the duration for mist-OFF cycle is different in each case with larger cutoffs resulting in larger durations.

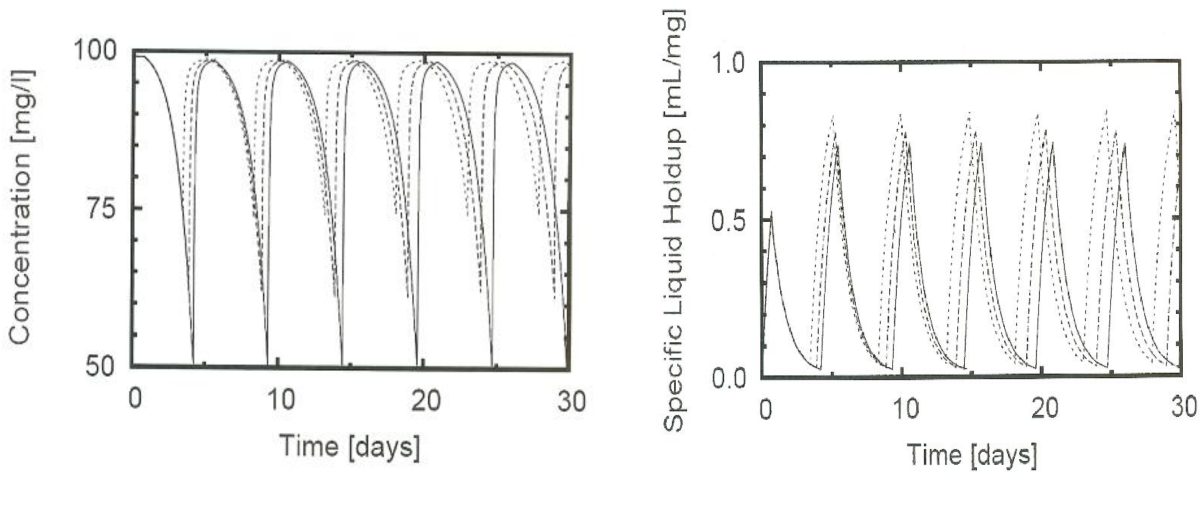

Fig. 1 The evolution of concentration and specific liquid holdup during repeated mist-ON / mist-OFF cycles for different Y_{OFF} . Solid lines, dashed and dotted lines correspond to Y_{OFF} = 0.99, 0.75 and 0.5, respectively.

Fig. 2 presents results for the same system for a constant Y_{OFF} and variable $Y_{ON.}$ Other parameter values are F_0 = 0.1mL/day/mg, C_F = 50 mg/mL, K_1 = 0.3 per day, K_2 = 4 per day, H_S = 0.01 mL/mg, H_C = 0.04 mL/mg, C_C = 5 mg/mL, Y_{OFF} = 0.99. Once again the evolution looks periodic. It is interesting to see that the corresponding cutoff for H is again much different from that of the C. It is observed that the evolution of C is mostly controlled by K_1 and less and indirectly controlled by K_2 through H. The evolution of H on the other hand, is governed by K_2. For this particular choice of K_1 and K_2, H evolves faster than C. For some other combination of K_1 and K_2, H may evolve at a slower rate. It is also not feasible to choose very low value of mist-ON cycle because the system will operate closer to C_C. Hence it is always feasible to operate the reactor slightly above this value.

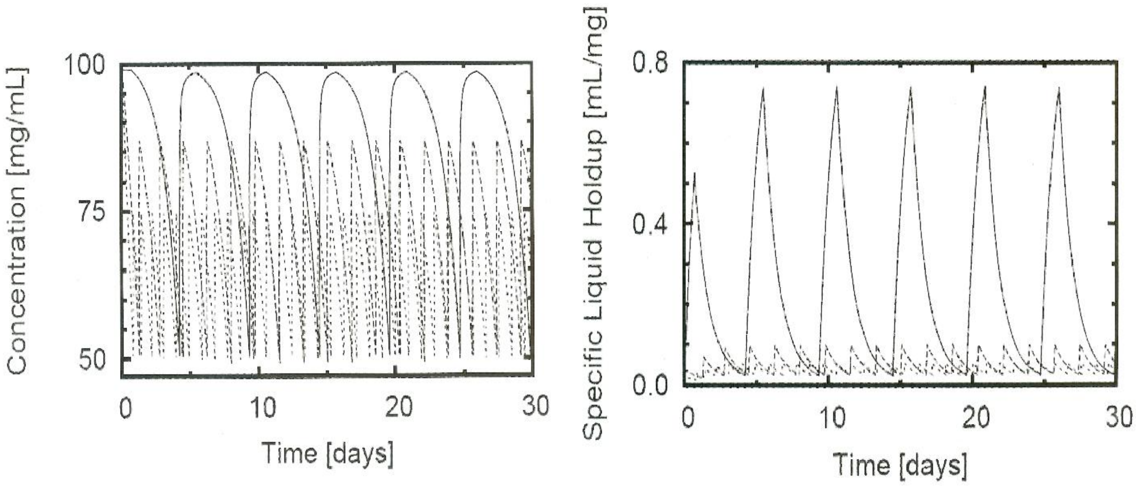

Fig. 2 Variation of concentration and specific liquid holdup with time for a constant Y_{OFF} and variable Y_{ON}. Solid lines, dashed and dotted lines correspond to Y_{ON} = 0.99, 0.75 and 0.5, respectively.

Fig. 3 presents the variation of ratio of cumulative duration of mist-ON cycles and the total running time (R_{ON}) with fractional value of Y_{OFF} for different process conditions. Other parameter values are F_0 = 0.1mL/day/ mg, C_F = 50 mg/mL, K_1 = 0.3 per day, K_2 = 4 per day, H_S = 0.01 mL/mg, H_C = 0.04 mL/mg, C_C = 5 mg/mL, Y_{OFF} = 0.99-0.05, Y_{ON} = 0.99. An intermittent operation is preferred over a continuous one, so that the reactor can be operated in an economical (power saving) and feasible manner. The values of H_S, H_C, C_C, K_1, K_2 have been kept constant whereas C_F and F have been varied. An increase in Y_{OFF} results a decrease in R_{ON} which also shows the increase in duration of mist-OFF cycle and corresponding decrease in mist-ON cycle. An increase in C_F leads to decrease in R_{ON} for longer duration of mist-OFF cycle. C_E increases with C_F and for a constant C_C, the permissible value of the concentration decay during the mist-OFF cycle is higher. It is also observed that an increase in F_o, leads to decrease in R_{ON}. Also, higher value of F leads to a shorter duration of mist-On cycles as the required buildup of the concentration is achieved at a faster rate. It does not effect mist-OFF cycle duration as C_E , C_C and the decay of concentration during mist-OFF cycle remains unchanged. It is also observed that there is no influence of C_F on the decay rate.

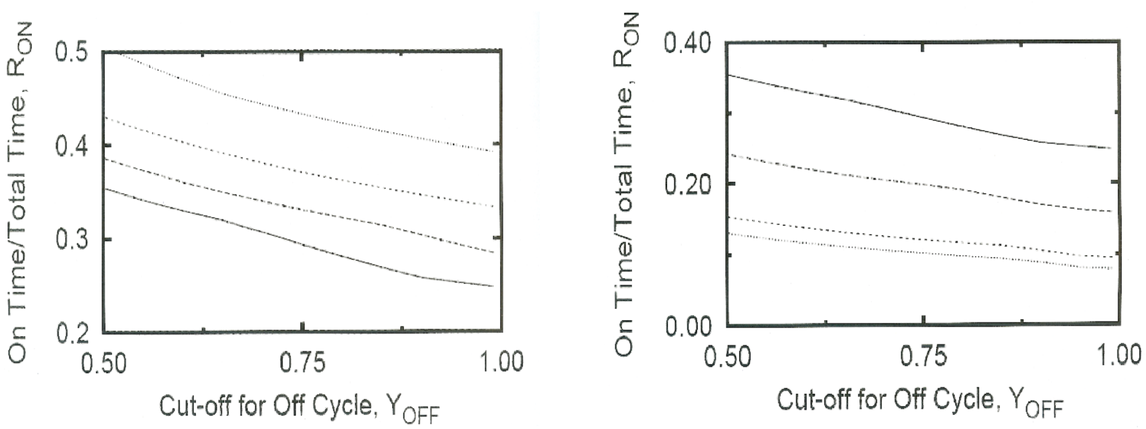

Fig. 4 Variation of ratio of cumulative duration of mist-ON cycles and the total time (R_{ON}) with fractional value of Y_{OFF} for different values of C_F and F, respectively. Solid lines, dashed, small dash and dotted lines correspond to C_F = 60, 50, 25 and 10 mg/mL, F = 0.05, 0.1, 0.2, 0.3 mL/day/mg, respectively.

Fig. 5 and Fig. 6 present the effect of the drainage constant K_2. Other parameter values are F_0 = 0.1mL/day/mg, C_F = 50 mg/mL, K_1 = 0.3 per day, H_S = 0.01 mL/mg, H_C = 0.04 mL/mg, C_C = 5 mg/mL, Y_{OFF} = 0.99, Y_{ON} = 0.99. The results for K_2 = 1 per day have already been presented in previous graphs. The evolutions of C and H are presented in Fig. 5. The variation of ratio R_{ON} with Y_{OFF} is presented in Fig. 6. It is observed that increasing the value of K_2 or drainage will result decrease in liquid holdup (Eq. (5)). During this period, H_E is also reduced (Eq. (6)), whereas C_E is not affected at all (Eq. (7)). This is corroborated by the results shown in Fig. 5 where solid, dashed, short dashed and dotted lines show progressively smaller slopes for both in the buildup and decay branches. The corresponding mist cycle durations are also reduced. The overall change in concentration remains constant in each cycle as the cutoff are in terms of equilibrium and critical concentrations which are not affected by change in K_2. Thus, faster rates translate to faster cycle durations. The rate of change of liquid holdup decreases as holdup reaches the saturation value. The dotted line in Fig. 5 for K_2 = 16 per day shows that most of the down cycle is carried out at liquid holdups close to H_S which is resulting in higher rate of change in concentration value. The duration of both the cycles are affected by the drainage rates. Depending on which cycle is affected more, the ratio R_{ON} will change likewise. The somewhat erratic behavior (Fig. 6) of R_{ON}, where it first increases and then decreases with K_2, proves this point and emphasizes the necessity of numerical simulation.

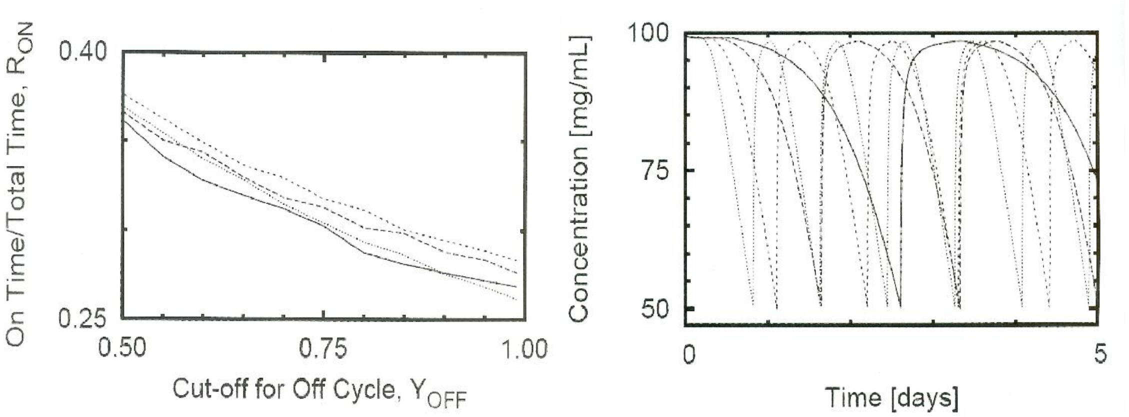

Fig. **5** Variation of concentration and specific liquid holdup with time for a constant Y_{OFF} and variable K_2. Solid lines, dashed, small dashed and dotted lines correspond to K_2 = 2, 4, 8, 16 per day, respectively.

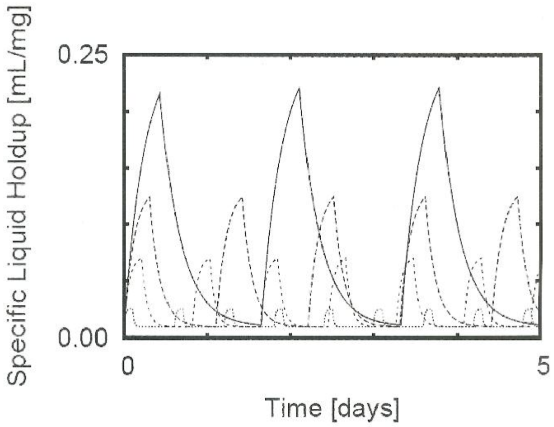

Fig. 6 Variation of ratio of cumulative duration of mist-ON cycles and the total running time (R_{ON}) with fractional value of Y_{OFF} for different value of K_2 . Solid lines, dashed, small dash and dotted lines correspond to K_2 = 2, 4, 8, 16 per day, respectively.

IV. Conclusions

A mathematical model has been developed to study the process conditions for an efficient operation of NMR for the growth of hairy roots. It is based on a theoretical model of NMR at the root bed level and its numerical simulation. This model describes the evolution of liquid holdup and concentration present in the root bed. This analytical solution to the model can lead to run the reactor with intermittent mist-ON and mist-OF cycles, at very low cumulative mist-ON duration for any given total run time to prevent any nutrient deficiency and water logging in the bed. Higher flow rates will lead to water logging and lower flow rates can lead to unacceptable high feed concentrations. Lower mist concentrations lead to requirement of high flow rates which may again lead to water logging. Thus, mist concentration has to be more than a critical value and flow rate has to lie within a range for operating a reactor in continuous mist-ON mode. Numerical simulations are used to understand the reactor performance for intermittent mist-ON and mist-OFF mode. A reasonable cut-off criterion for switching between ON-OFF cycles can be in terms of fractional approach to the end concentration for each cycle. The end concentration for mist-ON cycle is the equilibrium concentration and that for the mist-OFF cycle is the critical concentration. The gross efficiency of the reactor operation can be given in terms of the ratio of cumulative durations of the mist-ON cycles to total runtime, $R_{ON.}$ In some cases, it assumes very low value of 10% meaning that the mist can be switched off for 90% of the time. The duration of both the mist-ON and mist-OFF cycles are effected by the process parameters and thus, the ratio R_{ON}, may not follow any fixed trend in some cases. It is expected that these guidelines presented here will help in efficient operation and design of NMRs. More experimental data is needed to study the engineering aspect of hairy root technology in detail in future.

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