

**MODELING OF NUTRIENT MIST REACTOR TO GROW
HAIRY ROOTS**

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**DEPARTMENT OF CHEMICAL ENGINEERING
INDIAN INSTITUTE OF TECHNOLOGY DELHI**

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**MODELING OF NUTRIENT MIST REACTOR TO GROW
HAIRY ROOTS**

by

SAMBASIVA RAO KATURI

Department of Chemical Engineering

Submitted

in fulfillment of the requirements of the degree of Doctor of Philosophy

to the



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Certificate

This is to certify that the thesis titled “**Modeling of Nutrient Mist Reactor to Grow Hairy Roots**” being submitted by **Mr. Sambasiva Rao Katuri** in the Department of Chemical Engineering, Indian Institute of Technology, Delhi, for the award of the degree of **Doctor of Philosophy**, is a record of bonafide research work carried out by him under my guidance and supervision. In my opinion, the thesis has reached the standards fulfilling the requirements of the regulations relating to the degree. The results contained in this thesis have not been submitted for the award of any other degree, diploma, associateship or similar title of any university or institution.

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New Delhi

Sambasiva Rao Katuri

Abstract

Secondary metabolites extracted from hairy roots are extremely valuable due to their use in pharmaceuticals, dyes and so forth. Cultivation of hairy roots in bioreactor systems has emerged as an important route for the synthesis of secondary metabolites. The nutrient mist reactor offers numerous benefits over other bioreactor systems and is widely preferred for hairy root cultures. The present thesis focuses on developing mathematical models to study the growth of hairy roots in nutrient mist reactors.

A simple model termed as the ‘Preliminary Growth Model’ is proposed to study the growth of primary and secondary roots with culture time. The flow of nutrients through both the external mass transport and internal transport are considered for this model. The growth coefficients are estimated by fitting the model with the published experimental data. It is observed that growth of the primary root can decrease as much as to one-fifth of its initial growth during elongation-branching phase. Also, it is observed that the growth of primary root can decrease further during maturity phase when the absorbed nutrients are only used for its sustenance. Interestingly, the model results pointed out that the secondary roots grow at a faster rate than their primary root during both elongation-branching phase and maturity phase of the primary root. An unusual discontinuity in the growth curve has been reported at the maturity age which is a serious limitation for this model.

The ‘Advanced Growth Model’ is then proposed which requires prior knowledge of only primary root elongation phase growth for any hairy root. This data can be easily gathered from experiments. For this model, only one growth coefficient needs to be fitted from the available experimental data. An area fraction parameter has

also been introduced to explain internal flow of nutrients mathematically between the primary root and its secondary roots. The advanced growth model produces smooth and continuous growth curves unlike previous models and also fits closely with the available experimental results. Good biomass yield is observed during simulations for short inter node distances, low nutrient-biomass ratios, lower maintenance rates and larger growth rates of primary roots. Both the models are successful in explaining the growth of hairy root cultures. The concepts of internal flow, nutrient consumption for maintenance and nutrient consumption for growth are described qualitatively in the preliminary growth model, but more quantitatively in the advanced growth model.

Another major contribution of the present thesis is to develop unique mathematical model for mist-ON cycle and mist-OFF cycle operations of nutrient mist reactors and its integration with the growth kinetics equations. The model is developed in terms of 'Liquid film thickness over the roots' and 'Nutrients concentration in the liquid film' which are the limiting conditions for the nutrient mist reactor operation. Extensive simulations are performed to understand the effect of duration for the mist-ON and mist-OFF cycles, the mist feed rates and the nutrients concentrations in the mist. The pros and cons of longer/shorter mist-ON and longer/shorter mist-OFF cycles are discussed for every permissible parameter that aid in better yields of biomass. The results for film thickness show the importance of intermittent operation instead of continuous operation of nutrient mist reactors. Further, formation of thick liquid films has to be avoided by keeping mist-ON cycles short and low mist flow rates, so that the diffusion of oxygen to the root culture never gets affected. The concentration of nutrients in the liquid film change dynamically during mist-ON cycle and mist-OFF cycles. Keeping mist cycles short allows frequent mixing of fresh mist and increases the film concentration. High concentrations in the film are also observed with high feed concentrations and short mist-OFF cycles. Further, low drainage rates keep adequate amount of nutrients in the film for longer times. It has been shown that high growth rates are possible whenever the mass transfer rate of nutrients across the root surface increases.

Nutrients in the liquid films get depleted when nutrient mist reactor operates for short mist-ON and long mist-OFF cycles. Nutrients in the film do not fall below critical concentration even with longer mist-OFF cycles if the mist feed concentration is kept high. Hence, reactor can be operated under short mist-ON and long mist-OFF cycles if the drainage rate is low as it saves mist requirement to the reactor. Conversely, long mist-ON cycles are allowed when the drainage rate is very high to supply adequate nutrients for sustained growth of hairy roots as film thickness buildup is slow. However, longer mist-ON cycle operation notably increases mist feed supply to the reactor. Moreover, the nutrient mist reactor gets completely filled up with liquid if long mist-ON cycle is used with low mist drainage from the roots. Therefore, change in mist duty cycles shows a remarkable effect on the performance of nutrient mist reactor.

The growth models presented in this thesis will provide a platform for the researchers to understand various aspects of hairy root cultures. The simulation of these unique reactor model equations provide valuable input to scale up of nutrient mist reactor for commercial exploitation of secondary metabolites.

Contents

Certificate	i
Acknowledgements	iii
Abstract	v
Contents	ix
List of Figures	xiii
List of Tables	xxv
1 INTRODUCTION	1
1.1 What is Hairy Root?	5
1.1.1 Characteristics of Hairy Root Cultures	5
1.2 Bioreactors for Hairy Root Cultures	7
1.2.1 Liquid Phase Bioreactors	7
1.2.2 Gas Phase Bioreactors	14
1.3 Nutrient Mist Reactor(NMR)	18
1.3.1 Advantages of NMR	21
1.3.2 Operation of NMR	21
1.3.3 Problems Associated with Hairy Root Cultures in NMR	28
1.4 Scope and Objectives of the Work	29
1.4.1 Objectives	29

1.5	Methodology	30
1.6	Organization of Thesis	31
2	PRELIMINARY GROWTH MODEL FOR HAIRY ROOT CULTURES	33
2.1	External and Internal Nutrient Transport	34
2.2	Assumptions	35
2.3	Model Development	36
2.4	Numerical Solution of Model Equations	39
2.5	Results and Discussion	41
2.5.1	Bifurcation Analysis of the Model	49
2.5.2	Effect of Parameters	51
2.5.3	Effect of Initial Length of Primary Root	60
3	ADVANCED GROWTH MODEL FOR HAIRY ROOT CULTURES	65
3.1	Assumptions	66
3.2	Model Development	66
3.2.1	Dimensionless Quantities	69
3.2.2	Applicability of the model for different growth phases	71
3.2.3	Preliminary Growth Model versus Advanced Growth Model	72
3.3	Results and Discussions	74
3.3.1	Numerical Technique and Simulation Data	74
3.3.2	Validation of Model	76
3.3.3	Parametric study	83
4	MODELING OF MIST-ON/MIST-OFF CYCLES NUTRIENT MIST REACTOR	97
4.1	mist-ON and mist-OFF Cycles	97
4.2	Model Development	99
4.2.1	Assumptions	99
4.2.2	Model Equations for Mist-ON Cycle	102

4.2.3	Modeling the Mist-OFF Cycle for a NMR	103
4.3	Numerical Method	105
4.4	Simulation Methodology	107
4.5	Results and Discussion	110
4.5.1	Parameter Study	112
4.5.2	Effect of Mist Flow Rate	113
4.5.3	Effect of Feed Concentration	119
4.5.4	Effect of mist Drainage Rate	126
4.5.5	Effect of mist-ON/mist-OFF Cycle Durations	132
4.5.6	Effect of Inter Node Distance	136
4.5.7	Effect of Nutrient to Biomass Ratio	136
4.5.8	Effect of Sustenance Coefficient	141
4.5.9	Effect of Water Uptake Rate	144
4.6	Operational Strategies:Single Parameter	147
4.6.1	Strategy I: Mist-ON cycle duration	148
4.6.2	Strategy II: Mist-OFF cycle duration	148
4.6.3	Strategy III: Mist flow rate	150
4.6.4	Strategy IV: Mist feed concentration	151
4.7	Operating Strategy:Multiple parameters	152
4.7.1	Simulation-1: For mist-ON duration: 5(1-20):15(21-60):	153
4.7.2	Simulation-2: For mist-ON duration: 15(1-20):5(21-60):	154
4.7.3	Simulation-3: For mist-OFF duration: 5(1-20):15(21-60):	156
4.7.4	Simulation-4: For mist-OFF duration: 15(1-20):5(21-60):	158
4.7.5	Simulation-5: For mist flow: 15(1-20):20(21-60):	159
4.7.6	Simulation-6: For mist flow: 20(1-20):15(21-60):	161
4.7.7	Simulation-7: For mist feed concentration: 30(1-20):50(21-60):	162
4.7.8	Simulation-8: For mist feed concentration: 50(1-20):30(21-60):	163
5	NMR PERFORMANCE UNDER CRTITICAL CONDITIONS	167
5.1	Continuous Operation of NMR	168

5.2	Failure to Switch From OFF Cycle Mode to ON Cycle Mode	172
5.3	Phase Diagram for Successful NMR Operation	174
6	CONCLUSIONS	177
6.1	Summary and Conclusion	177
6.2	Recommendations for Future Work	185
	Bibliography	187
	List of Publications	199
	Bio-data	201

List of Figures

1.1	Schematic of a typical Nutrient Mist Reactor(NMR)	20
1.2	Schematic of Branching Phenomena	25
2.1	Schematic of nutrient uptake by primary and secondary roots. A: oxygen transfer from bulk air to bulk liquid film, B: oxygen transfer from bulk liquid to surface of the root, C: oxygen diffusion across root surface, 1: transfer of liquid nutrient from bulk phase to surface of root, and 2: flow of liquid nutrient across root surface.	35
2.2	The experimental data for a single <i>Artemisia annua</i> hairy root versus simulation results. Total length of roots and number of tips as function of culture time obtained from shake flask experiment with l_{p0} :2.0 cm, l_{pb} :2.2 cm, nd :0.05 cm, k_{p0} :0.096 d ⁻¹	43
2.3	The experimental data for a single <i>Artemisia annua</i> hairy root versus simulation results. Total length of roots and number of tips as function of culture time obtained from 1 min ON/15 min OFF NMR with l_{p0} :1.3 cm, l_{pb} :2.86 cm and k_{p0} :0.1577 d ⁻¹	45
2.4	The experimental data for a single <i>Artemisia annua</i> h hairy root versus simulation results. Total length of roots and number of tips as function of culture time obtained from 1 min ON/15 min OFF NMR with l_{p0} :1.5 cm and k_{p0} :0.15 d ⁻¹	46

2.5	The growth of a single <i>Artemisia annua</i> hairy root in a 1 min ON/15 min OFF NMR experiment with conditioned medium. The primary root length and total length with time from simulation versus the published experimental results.	47
2.6	The primary root length and total length of <i>Atropa belladonna</i> hairy roots with time from simulation versus the published experimental results from Shake flask. The inset picture presents primary root length	48
2.7	Nodal length versus Primary root growth coefficient during elongation only phase, as function of <i>rkpb</i>	50
2.8	Growth of primary root length with culture time for different growth rate of primary root during elongation phase, (k_{p0}): 0.024, 0.12 and 0.24 d ⁻¹	52
2.9	Number of secondary roots with culture time for different growth rate of primary root during elongation phase (k_{p0}): 0.024, 0.12 and 0.24 d ⁻¹	53
2.10	Total length of roots with culture time for different growth rate of primary root during elongation phase (k_{p0}): 0.024, 0.12 and 0.24 d ⁻¹ .	54
2.11	Primary root length with culture time for different internal nutrient sharing parameter (<i>rkpb</i>): 0.25, 0.50 and 0.75	55
2.12	Number of secondary roots produced with culture time for different internal sharing parameter (<i>rkpb</i>): 0.25, 0.50 and 0.75	56
2.13	Total length of roots with culture time for different internal nutrient sharing parameter (<i>rkpb</i>): 0.25, 0.50 and 0.75	56
2.14	Primary root length with culture time for different inter node distance (<i>nd</i>): 0.01, 0.05 and 0.10 cm	57
2.15	Total length of roots with culture time for different inter node distance(<i>nd</i>): 0.01, 0.05 and 0.10 cm	58
2.16	Number of secondary roots produced with culture time for different inter node distance (<i>nd</i>): 0.01, 0.05 and 0.10 cm	59

2.17	Primary root length with culture time for different branches growth rate relative to primary root growth during elongation phase (<i>rksb</i>): 0.5, 2.5 and 5.0	59
2.18	Growth of total length of roots with culture time for different branches growth rate relative to primary root growth during elongation phase (<i>rksb</i>): 0.5, 2.5 and 5.0	60
2.19	Number of secondary roots produced with culture time for different branches growth rate relative to primary root growth during elongation phase (<i>rksb</i>): 0.5, 2.5 and 5.0	61
2.20	Total length of roots with culture time for different initial lengths of primary root (l_{p0}): 1.0, 1.5 and 2.0 cm	61
2.21	Primary root length with culture time for different initial lengths of primary root (l_{p0}): 1.0, 1.5 and 2.0 cm	62
2.22	Number of secondary roots produced with culture time for different initial length of primary root(l_{p0}): 1.0, 1.5 and 2.0 cm	62
3.1	Estimation of k_p through fitting primary root length during elongation only phase for species <i>Atropa belladonna</i> (A), <i>Hyoscyamus muticus</i> (B), <i>Hyoscyamus niger</i> (C) and <i>Solanum aviculare</i> (D) for shake flask culture experiments. Solid and dotted lines represent simulation results. Experiment data shown for species A(\circ), B(Δ), C(\square), and D(\bullet).	77
3.2	Comparison of the predicted and experimental total length distribution for shake flask culture of <i>A. belladonna</i> . The length of primary root with culture time shown in inset picture.	79
3.3	Comparison of the predicted and experimental total length distribution for shake flask culture of <i>H.muticus</i> . The length of primary root with culture time shown in inset picture.	80
3.4	Comparison of the predicted and experimental total length distribution for shake flask culture of <i>H.niger</i> . The length of primary root with culture time shown in inset picture.	81

3.5	Comparison of the predicted and experimental total length distribution for shake flask culture of <i>S. aviculare</i> . The length of primary root with culture time shown in inside picture.	82
3.6	Comparison of the predicted and experimental final to initial dry weight ratio for shake flask culture of <i>A. hypogaea</i>	83
3.7	Comparison of the predicted and experimental final to initial dry weight ratio for shake flask culture of <i>Nicotiana tabacum</i>	84
3.8	Effect of primary root growth coefficient on total length of roots as function of culture time. The values of k_p : 0.1 d^{-1} , 0.15 d^{-1} and 0.2 d^{-1} , respectively. The initial length of roots is 2.0 cm and branching length 3.5 cm. The maturity of primary root shown in inset picture. .	86
3.9	Variation of number of tips with culture time for different values of k_p : 0.1 d^{-1} , 0.15 d^{-1} and 0.30 d^{-1}	87
3.10	Variation of total length of roots with culture time for different values of f_{nb} : $1.0 \text{ g} \cdot \text{g}^{-1}$, $3.0 \text{ g} \cdot \text{g}^{-1}$ and $5.0 \text{ g} \cdot \text{g}^{-1}$. The maturity of primary root with culture time shown in inset picture.	88
3.11	Variation of the number of tips with culture time for different values of f_{nb} : $1.0 \text{ g} \cdot \text{g}^{-1}$, $3.0 \text{ g} \cdot \text{g}^{-1}$ and $5.0 \text{ g} \cdot \text{g}^{-1}$	88
3.12	Effect of inter node distance on total length of roots with culture time for different nodal distances 0.20 cm, 0.40 cm and 0.60 cm. The primary root length with culture time shown in inset picture	90
3.13	Variation of the number of tips with culture time for different values of inter node distance : 0.20 cm, 0.40 cm and 0.60 cm	91
3.14	Effect of sustenance coefficient on total length of roots as function of culture time. The values of sustenance coefficient used are $0.05 \text{ g} \cdot \text{g}^{-1} \cdot \text{d}^{-1}$, $0.075 \text{ g} \cdot \text{g}^{-1} \cdot \text{d}^{-1}$ and $0.10 \text{ g} \cdot \text{g}^{-1} \cdot \text{d}^{-1}$, respectively. The change in length of primary root with culture time showing maturity phase for different values of sustenance coefficients shown in inset picture.	92

3.15	Effect of sustenance coefficient on number of tips produced with culture time. The values of sustenance coefficients used are $0.05 \text{ g} \cdot \text{g}^{-1} \cdot \text{d}^{-1}$, $0.075 \text{ g} \cdot \text{g}^{-1} \cdot \text{d}^{-1}$ and $0.10 \text{ g} \cdot \text{g}^{-1} \cdot \text{d}^{-1}$	93
3.16	Length of primary root with culture time for different radius ratio of secondary to primary roots(R_r): 1.0, 1/3 and 1/6.	93
3.17	Total length of roots with culture time for different radius ratio of secondary to primary roots(R_r): 1.0, 1/3 and 1/6.	94
3.18	Number of tips produced with culture time for different radius ratio of secondary to primary roots(R_r): 1.0, 1/3 and 1/6.	94
4.1	Schematic of mist-ON Cycle NMR Operation.	98
4.2	Schematic of mist-OFF Cycle NMR Operation.	98
4.3	Length of primary root with culture time for <i>Artemisia annua</i> hairy roots grown in NMR with conditioned B5 medium. Solid line represents model result and points represent experiments data.	110
4.4	Growth of <i>A.hypogaea</i> hairy roots in 1 L mist reactor. Lines represent simulation results and points represent experiments data.	111
4.5	Growth of <i>A. annua</i> hairy roots in 1 L mist reactor. Lines represent simulation result and points represent experiments data.	111
4.6	Liquid film thickness with time for different effective mist flow rate $1.0 \text{ l} \cdot \text{m}^{-2} \cdot \text{h}^{-1}$, $5.0 \text{ l} \cdot \text{m}^{-2} \cdot \text{h}^{-1}$, and $10.0 \text{ l} \cdot \text{m}^{-2} \cdot \text{h}^{-1}$ with mist flow coefficient K_1 values 1.0 m^{-2} , 5.0 m^{-2} and 10.0 m^{-2} , respectively. (F_0): $24.0 \text{ l} \cdot \text{d}^{-1}$, K_2 : 8.0 h^{-1} and misting cycles 5 min mist-ON/5 min mist-OFF.	113
4.7	Liquid film thickness with time for different effective mist flow rate $1.0 \text{ l} \cdot \text{m}^{-2} \cdot \text{h}^{-1}$, $5.0 \text{ l} \cdot \text{m}^{-2} \cdot \text{h}^{-1}$, and $10.0 \text{ l} \cdot \text{m}^{-2} \cdot \text{h}^{-1}$ with mist flow coefficient K_1 values 1.0 m^{-2} , 5.0 m^{-2} and 10.0 m^{-2} respectively. (F_0): $24.0 \text{ l} \cdot \text{d}^{-1}$, K_2 : 8.0 h^{-1} and misting cycles 15 min mist-ON/15 min mist-OFF.	114

4.8	Liquid film concentration with time for different effective mist flow rate $1.0 \text{ l} \cdot \text{m}^{-2} \cdot \text{h}^{-1}$, $5.0 \text{ l} \cdot \text{m}^{-2} \cdot \text{h}^{-1}$, and $10.0 \text{ l} \cdot \text{m}^{-2} \cdot \text{h}^{-1}$ with mist flow coefficient K_1 values 1.0 m^{-2} , 5.0 m^{-2} and 10.0 m^{-2} respectively. (F_0) : $24.0 \text{ l} \cdot \text{d}^{-1}$, K_2 : 8.0h^{-1} and misting cycles 5 min mist-ON/5 min mist-OFF.	115
4.9	Liquid film concentration with time for different effective mist flow rate $1.0 \text{ l} \cdot \text{m}^{-2} \cdot \text{h}^{-1}$, $5.0 \text{ l} \cdot \text{m}^{-2} \cdot \text{h}^{-1}$, and $10.0 \text{ l} \cdot \text{m}^{-2} \cdot \text{h}^{-1}$ with mist flow coefficient K_1 values 1.0 m^{-2} , 5.0 m^{-2} and 10.0 m^{-2} , respectively. (F_0) : $24.0 \text{ l} \cdot \text{d}^{-1}$, K_2 : 8.0h^{-1} and misting cycles 15 min mist-ON/15 min mist-OFF.	116
4.10	Length of primary root with time for different effective mist flow rate $1.0 \text{ l} \cdot \text{m}^{-2} \cdot \text{h}^{-1}$, $5.0 \text{ l} \cdot \text{m}^{-2} \cdot \text{h}^{-1}$, and $10.0 \text{ l} \cdot \text{m}^{-2} \cdot \text{h}^{-1}$ with mist flow coefficient K_1 values 1.0 m^{-2} , 5.0 m^{-2} and 10.0 m^{-2} , respectively. (F_0) : $24.0 \text{ l} \cdot \text{d}^{-1}$, K_2 : 8.0h^{-1} and misting cycles 5 min mist-ON/5 min mist-OFF.	117
4.11	Length of primary root with time for different effective mist flow rate $1.0 \text{ l} \cdot \text{m}^{-2} \cdot \text{h}^{-1}$, $5.0 \text{ l} \cdot \text{m}^{-2} \cdot \text{h}^{-1}$, and $10.0 \text{ l} \cdot \text{m}^{-2} \cdot \text{h}^{-1}$ with mist flow coefficient K_1 values 1.0 m^{-2} , 5.0 m^{-2} and 10.0 m^{-2} respectively. (F_0) : $24.0 \text{ l} \cdot \text{d}^{-1}$, K_2 : 8.0h^{-1} and misting cycles 15 min mist-ON/15 min mist-OFF.	118
4.12	Number of tips with time for different effective mist flow rate $1.0 \text{ l} \cdot \text{m}^{-2} \cdot \text{h}^{-1}$, $5.0 \text{ l} \cdot \text{m}^{-2} \cdot \text{h}^{-1}$, and $10.0 \text{ l} \cdot \text{m}^{-2} \cdot \text{h}^{-1}$ with mist flow coefficient K_1 values 1.0 m^{-2} , 5.0 m^{-2} and 10.0 m^{-2} respectively. (F_0) : $24.0 \text{ l} \cdot \text{d}^{-1}$, K_2 : 8.0h^{-1} , $nd = 0.10\text{cm}$ and misting cycles 5 min mist-ON/5 min mist-OFF.	119
4.13	Number of tips with time for different effective mist flow rate $1.0 \text{ l} \cdot \text{m}^{-2} \cdot \text{h}^{-1}$, $5.0 \text{ l} \cdot \text{m}^{-2} \cdot \text{h}^{-1}$, and $10.0 \text{ l} \cdot \text{m}^{-2} \cdot \text{h}^{-1}$ with mist flow coefficient K_1 values 1.0 m^{-2} , 5.0 m^{-2} and 10.0 m^{-2} respectively. (F_0) : $24.0 \text{ l} \cdot \text{d}^{-1}$, K_2 : 8.0h^{-1} , $nd = 0.10\text{cm}$ and misting cycles 15 min mist-ON/15 min mist-OFF.	120

4.14	Liquid film thickness with culture time for feed concentrations $50\text{g}\cdot\text{l}^{-1}$, $40\text{g}\cdot\text{l}^{-1}$ and $30\text{g}\cdot\text{l}^{-1}$ for 5min-ON/5min-OFF cycle NMR operation with $F_0: 24.0\text{ l}\cdot\text{d}^{-1}$, $K_2: 8.0\text{ h}^{-1}$	121
4.15	Liquid film thickness with culture time for feed concentrations $50\text{g}\cdot\text{l}^{-1}$, $40\text{g}\cdot\text{l}^{-1}$ and $30\text{g}\cdot\text{l}^{-1}$ for 15min-ON/15min-OFF cycle NMR operation with $F_0:24.0\text{ l}\cdot\text{d}^{-1}$, $K_2:8.0\text{ h}^{-1}$	121
4.16	Nutrient concentration with culture time for feed concentrations $50\text{g}\cdot\text{l}^{-1}$, $40\text{g}\cdot\text{l}^{-1}$ and $30\text{g}\cdot\text{l}^{-1}$ for 5min-ON/5min-OFF cycle NMR operation with $F_0:24.0\text{ l}\cdot\text{d}^{-1}$, $K_2:8.0\text{ h}^{-1}$	122
4.17	Nutrient concentration with culture time for feed concentrations $50\text{g}\cdot\text{l}^{-1}$, $40\text{g}\cdot\text{l}^{-1}$ and $30\text{g}\cdot\text{l}^{-1}$ for 15min-ON/15min-OFF cycle NMR operation with $F_0:4.0\text{ l}\cdot\text{d}^{-1}$, $K_2:8.0\text{ h}^{-1}$	122
4.18	Length of primary root with culture time for feed concentrations $50\text{g}\cdot\text{l}^{-1}$, $40\text{g}\cdot\text{l}^{-1}$ and $30\text{g}\cdot\text{l}^{-1}$ for 5min-ON/5min-OFF cycle NMR operation with $F_0:24.0\text{ l}\cdot\text{d}^{-1}$, $K_2:8.0\text{ h}^{-1}$	123
4.19	Length of primary root with culture time for feed concentrations $50\text{g}\cdot\text{l}^{-1}$, $40\text{g}\cdot\text{l}^{-1}$ and $30\text{g}\cdot\text{l}^{-1}$ for 15min-ON/15min-OFF cycle NMR operation with $F_0:24.0\text{ l}\cdot\text{d}^{-1}$, $K_2: 8.0\text{ h}^{-1}$	124
4.20	Number of tips with culture time for feed concentrations $50\text{g}\cdot\text{l}^{-1}$, $40\text{g}\cdot\text{l}^{-1}$ and $30\text{g}\cdot\text{l}^{-1}$ for 5min-ON/5min-OFF cycle NMR operation with $F_0:24.0\text{ l}\cdot\text{d}^{-1}$, $K_2:8.0\text{ h}^{-1}$ and $nd: 0.10\text{cm}$	125
4.21	Number of tips with culture time for feed concentrations $50\text{g}\cdot\text{l}^{-1}$, $40\text{g}\cdot\text{l}^{-1}$ and $30\text{g}\cdot\text{l}^{-1}$ for 15min-ON/15min-OFF cycle NMR operation with $F_0:24.0\text{ l}\cdot\text{d}^{-1}$, $K_2:8.0\text{ h}^{-1}$ and $nd: 0.10\text{cm}$	125
4.22	Liquid film thickness with culture time for different drainage coefficients 4.0h^{-1} , 8.0h^{-1} and 16.0 h^{-1} for 5min-ON/5min-OFF cycle NMR operation with $F_0:24\text{ l}\cdot\text{d}^{-1}$ and $C_F:50\text{g}\cdot\text{l}^{-1}$	126

4.23	liquid film thickness with culture time for different drainage coefficients 4.0h ⁻¹ , 8.0h ⁻¹ and 16.0 h ⁻¹ for 15min-ON/15min-OFF cycle NMR operation with $F_0:24.0l \cdot d^{-1}$ and $C_F:50.0g \cdot l^{-1}$	127
4.24	Concentration with culture time for different drainage coefficients 4.0h ⁻¹ , 8.0h ⁻¹ and 16.0 h ⁻¹ for 5min-ON/5min-OFF cycle NMR operation with $F_0:24.0l \cdot d^{-1}$ and $C_F:50.0g \cdot l^{-1}$	127
4.25	Concentration with culture time for different drainage coefficients 4.0h ⁻¹ , 8.0h ⁻¹ and 16.0 h ⁻¹ for 15min-ON/15min-OFF cycle NMR operation with $F_0:24.0l \cdot d^{-1}$ and $C_F:50.0g \cdot l^{-1}$	128
4.26	Length of primary root with culture time for different drainage coeffi- cients 4.0h ⁻¹ , 8.0h ⁻¹ and 16.0 h ⁻¹ for 5min-ON/5min-OFF cycle NMR operation with $F_0:24.0l \cdot d^{-1}$ and $C_F:50.0g \cdot l^{-1}$	128
4.27	Length of primary root with culture time for different drainage coef- ficients 4.0h ⁻¹ , 8.0h ⁻¹ and 16.0 h ⁻¹ for 15min-ON/15min-OFF cycle NMR operation with $F_0:24.0l \cdot d^{-1}$ and $C_F:50.0g \cdot l^{-1}$	129
4.28	Number of tips with culture time for different drainage coefficients 4.0h ⁻¹ , 8.0h ⁻¹ and 16.0h ⁻¹ for 5min-ON/5min-OFF cycle NMR op- eration with $F_0:24.0l \cdot d^{-1}$ and $C_F:50.0g \cdot l^{-1}$	129
4.29	Number of tips with culture time for different drainage coefficients 4.0h ⁻¹ , 8.0h ⁻¹ and 16.0h ⁻¹ for 15min-ON/15min-OFF cycle NMR op- eration with $F_0:24.0l \cdot d^{-1}$ and $C_F:50.0g \cdot l^{-1}$	130
4.30	Liquid film thickness with culture time for different mist-ON/mist-OFF cycles 5 min/15 min, 15 min/5 min, 5 min/5 min and 15 min/15 min with $F_0:24.0l \cdot d^{-1}$, $K_2:8.0h^{-1}$, and $50.0g \cdot l^{-1}$	132
4.31	concentration with culture time for different mist-ON/mist-OFF cycles 5 min/15 min, 15 min/5 min, 5 min/5 min and 15 min/15 min with $F_0:24.0l \cdot d^{-1}$, $K_2:8.0h^{-1}$ and $C_F:50.0g \cdot l^{-1}$	133

4.32	Length of primary root with culture time for different mist-ON/mist-OFF cycles 5 min/15 min, 15 min/5 min, 5 min/5 min and 15 min/15 min with $F_0:24.0l \cdot d^{-1}$, $K_2:8.0h^{-1}$ and $C_F:50.0g \cdot l^{-1}$	135
4.33	Number of tips with culture time for different mist-ON/mist-OFF cycles 5 min/15 min, 15 min/5 min, 5 min/5 min and 15 min/15 min with $F_0:24.0l \cdot d^{-1}$, $K_2:8.0h^{-1}$ and $C_F:50.0g \cdot l^{-1}$	135
4.34	Liquid film thickness with culture time for different inter node distances (nd) 0.05cm, 0.10cm, and 0.20cm with mist-ON/mist-OFF cycles 5min/5min, $F_0:24.0l \cdot d^{-1}$, $K_2:8.0h^{-1}$ and $C_F:50.0g \cdot l^{-1}$	137
4.35	Concentration with culture time for different inter node distances(nd) 0.05cm, 0.10cm and 0.20cm with mist-ON/mist-OFF cycles 5min/5min, $F_0:24.0l \cdot d^{-1}$, $K_2:8.0h^{-1}$ and $C_F:50.0g \cdot l^{-1}$	137
4.36	Length of primary root with culture time for different inter node distances (nd) 0.05cm, 0.10cm and 0.20cm with mist-ON/mist-OFF cycles 5min/5min, $F_0:24.0l \cdot d^{-1}$, $K_2:8.0h^{-1}$, and $C_F:50.0g \cdot l^{-1}$	138
4.37	Number of tips with culture time for different inter node distances (nd) 0.05cm, 0.10cm and 0.20cm with mist-ON/mist-OFF cycles 5min/5min.	138
4.38	Liquid film thickness with culture time for different nutrient to biomass ratio (f_{nb}) $2.5g \cdot g^{-1}$ and $5.0g \cdot g^{-1}$ with mist-ON/mist-OFF cycles 5min/5min.	139
4.39	concentration with culture time for different nutrient to biomass ratio (f_{nb}) $2.5g \cdot g^{-1}$ and $5.0g \cdot g^{-1}$ with mist-ON/mist-OFF cycles 5min/5min.	139
4.40	Length of primary root with culture time for different nutrient to biomass ratio (f_{nb}) $2.5g \cdot g^{-1}$ and $5.0g \cdot g^{-1}$ with mist-ON/mist-OFF cycles 5min/5min.	140
4.41	Number of tips with culture time for different nutrient to biomass ratio (f_{nb}) $2.5g \cdot g^{-1}$ and $5.0g \cdot g^{-1}$ with mist-ON/mist-OFF cycles 5min/5min.	140

4.42	Liquid film thickness with culture time for different sustenance coefficients (f_s) $0.10\text{g} \cdot \text{g}^{-1} \cdot \text{d}^{-1}$, $0.15\text{g} \cdot \text{g}^{-1} \cdot \text{d}^{-1}$, and $0.20\text{g} \cdot \text{g}^{-1} \cdot \text{d}^{-1}$ with mist-ON/mist-OFF cycles 5 min/5 min.	141
4.43	concentration with culture time for different sustenance coefficients (f_s) $0.10\text{g} \cdot \text{g}^{-1} \cdot \text{d}^{-1}$, $0.15\text{g} \cdot \text{g}^{-1} \cdot \text{d}^{-1}$, and $0.20\text{g} \cdot \text{g}^{-1} \cdot \text{d}^{-1}$ with mist-ON/mist-OFF cycles 5 min/5 min.	142
4.44	Length of primary root with culture time for different sustenance coefficients (f_s) $0.10\text{g} \cdot \text{g}^{-1} \cdot \text{d}^{-1}$, $0.15\text{g} \cdot \text{g}^{-1} \cdot \text{d}^{-1}$, and $0.20\text{g} \cdot \text{g}^{-1} \cdot \text{d}^{-1}$ with mist-ON/mist-OFF cycles 5 min/5 min.	143
4.45	Number of tips with culture time for different sustenance coefficients (f_s) $0.10\text{g} \cdot \text{g}^{-1} \cdot \text{d}^{-1}$, $0.15\text{g} \cdot \text{g}^{-1} \cdot \text{d}^{-1}$, and $0.20\text{g} \cdot \text{g}^{-1} \cdot \text{d}^{-1}$ with mist-ON/mist-OFF cycles 5 min/5 min.	143
4.46	Liquid film thickness with culture time for different water uptake rate coefficients (K_3) $0.20\text{l} \cdot \text{l}^{-1} \cdot \text{d}^{-1}$, $0.50\text{l} \cdot \text{l}^{-1} \cdot \text{d}^{-1}$ and $0.80\text{l} \cdot \text{l}^{-1} \cdot \text{d}^{-1}$ mist-ON/mist-OFF cycles 5min/5min.	144
4.47	concentration with culture time for different water uptake rate coefficients (K_3) $0.20\text{l} \cdot \text{l}^{-1} \cdot \text{d}^{-1}$, $0.50\text{l} \cdot \text{l}^{-1} \cdot \text{d}^{-1}$ and $0.80\text{l} \cdot \text{l}^{-1} \cdot \text{d}^{-1}$ mist-ON/mist-OFF cycles 5min/5min.	145
4.48	Length of Primary root with culture time for different water uptake rate coefficient (K_3) $0.20\text{l} \cdot \text{l}^{-1} \cdot \text{d}^{-1}$, $0.50\text{l} \cdot \text{l}^{-1} \cdot \text{d}^{-1}$ and $0.80\text{l} \cdot \text{l}^{-1} \cdot \text{d}^{-1}$ mist-ON/mist-OFF cycles 5min/5min.	145
4.49	Number of tips with culture time for different water uptake rate coefficients (K_3) $0.20\text{l} \cdot \text{l}^{-1} \cdot \text{d}^{-1}$, $0.50\text{l} \cdot \text{l}^{-1} \cdot \text{d}^{-1}$ and $0.80\text{l} \cdot \text{l}^{-1} \cdot \text{d}^{-1}$ mist-ON/mist-OFF cycles 5min/5min.	146
4.50	Root growth with culture time. Solid line represents mist-ON cycle:5min(cycle 1-20):15min(cycle 21-60), and dotted line represents mist-ON cycle: 15min(cycle 1-20):5min(cycle 21-60), 5min OFF(1-60). . .	149

4.51	Root growth with culture time. Solid line represents mist-OFF cycle:5min(cycle 1-20):15min(cycle 21-60), and dotted line represents mist-OFF cycle: 15min(cycle 1-20):5min(cycle 21-60), 5min ON(1-60).	150
4.52	Root growth with culture time. Solid line represents mist flow:15l·m ⁻² ·h ⁻¹ (cycle 1-20):20 l·m ⁻² ·h ⁻¹ (cycle 21-60), and dotted line represents mist flow: 20l·m ⁻² ·h ⁻¹ (cycle 1-20):15l·m ⁻² ·h ⁻¹ (cycle 21-60), 5min ON/5min OFF(1-60).	151
4.53	Root growth with culture time. Solid line represents CF:30g·l ⁻¹ (cycle 1-20):50 g·l ⁻¹ (cycle 21-60), and dotted line represents CF: 50g·l ⁻¹ (cycle 1-20):30g·l ⁻¹ (cycle 21-60), 5min ON/5min OFF(1-60).	152
4.54	Hairy Root Growth after 60 cycles for mist-ON: 5min(cycle 1-20) and 15min(cycle 21-60) with different operating strategies	154
4.55	Hairy Root Growth after 60 cycles for mist-ON: 15min(cycle 1-20) and 5min(cycle 21-60) with different operating strategies	156
4.56	Hairy Root Growth after 60 cycles for mist-OFF: 5min(cycle 1-20) and 15min(cycle 21-60) with different operating strategies.	157
4.57	Hairy Root Growth after 60 cycles for mist-OFF: 5min(cycle 1-20) and 15min(cycle 21-60) with different strategies.	159
4.58	Hairy Root Growth after 60 cycles for mist flow: 15l·m ⁻² ·h ⁻¹ (cycle 1-20) and 20l·m ⁻² ·h ⁻¹ (cycle 21-60) with different operating strategies.	160
4.59	Hairy Root Growth after 60 cycles for mist flow: 20l·m ⁻² ·h ⁻¹ (cycle 1-20) and 15l·m ⁻² ·h ⁻¹ (cycle 21-60) with different operating strategies.	162
4.60	Hairy Root Growth after 60 cycles for CF: 30g·l ⁻¹ (cycle 1-20) and 50g·l ⁻¹ (cycle 21-60) with different operating strategies.	163
4.61	Hairy Root Growth after 60 cycles for CF: 50g·l ⁻¹ (cycle 1-20) and 30g·l ⁻¹ (cycle 21-60) with different operating strategies	164
5.1	Liquid film thickness with time for different feed flow rates.	168
5.2	Liquid film thickness with time for different feed flow rates.	169

5.3	Nutrient concentration in the liquid film thickness with time for different feed flow rates.	170
5.4	Liquid film thickness with time for different drainage rate coefficients (K_2) 12, 24, 96 hr^{-1}	171
5.5	Liquid film thickness with time for different drainage rate coefficients (K_2): 12, 24 and 96 hr^{-1}	171
5.6	Maximum allowable OFF cycle time when mist flow is stopped-5min mist-ON/5min mist-OFF NMR Operation.	173
5.7	Maximum allowable OFF cycle time when mist flow is stopped-15min mist-ON/15min mist-OFF NMR Operation.	173
5.8	Phase diagram: Effective mist flow versus drainage rate coefficient for 5min ON/5min OFF Cycles and 15min ON/15min OFF Cycles NMR Operation	174

List of Tables

1.1	Important Plant Derived Secondary Metabolites and Utility	2
1.2	Bioactive Compounds Obtained From Plant Cell and Organ Cultures	3
1.3	Growth of Hairy Roots in Stirred Tank Reactors (STR)	10
1.4	Hairy Roots Growth in Bubble Column Reactors (BCR)	12
1.5	Hairy Roots in Rotating Drum Reactors (RDR)	14
1.6	Hairy Roots Growth in Air Lift Reactors (ALR)	16
1.7	Hairy Roots in Trickle Bed Reactors (TBR)	17
1.8	Hairy Roots Growth in Various Nutrient Mist Reactors(NMR)	18
1.9	Overview of Growth Models for Hairy Root Cultures	27
2.1	Fitted Growth Coefficient and Other Parameters for Preliminary Growth Model	42
3.1	Estimated k_p Values Through Fitting for Various Species	76
3.2	Fitted growth coefficient and other parameters for advanced growth model	85