CHEMICAL ENGINEERING TRANSACTIONS, Volume 14, 2008
Edited by G. Marino, E. Bardone, A. Viglia
 ISBN 978-88-95608-02-0

Mathematical Modelling Aspects of *Trichoderma reesei* System :A Review

Nitin Verma*,M.C.Bansal.,Vivek Kumar
Department of Paper Technology,Indian Institute of Technology,Roorkee,Saharanpur Campus,Saharanpur,247001,India.
*Corresponding author:nitiniit2004@rediffmail.com

Modelling the growth and product formation characteristics of various microorganisms is a very challenging task.There are many different approaches to modeling the microbial kinetics .Simplistic unstructured model do not offer much in terms of elucidating the exact nature of these processes.However more structured model often involve introducing process variables that can not be estimated reliably.Several studies dealing with modeling the growth and morphology of complex microorganisms such as filamentous fungi have been published in recent years.Complete quantitative description of its behaviour would lead to large sets of mathematical expressions describing the time evolution of a large number of variables.Futheremore ,these equations would contain a large number of parameters which would be difficult to obtain experimentally.Optimization of the productivity of biochemical processes by the optimal control approach is in general a difficult task.Accomplishement of such a task depends on the complexicity of model describing the biochemical process. The value of mathematical models to organize data, to consider interactions in complex systems in a rational way ,to correct the conventional wisdom and to understand essential qualitative features of biological system has been clearly documented in prior research.Impact of this research in biotechnology discovery has so for been limited ,but this will change in the future if we are adept in recognizing emerging opportunities and in integrating new concepts and tools into our research. In this review paper we are discussed the various model describing fungal growth system and also emphasized on *Trichodermma* system .

Introduction:

By definition ,biochemical engineers are concerned with biochemical system,often with systems employing growing cells.Even the simplest living cells is a system of such for bidding complexity that any mathematical description of it is an extremely modest approximation.The use of mathematics to provide a rigrous ,systematic and quantitative linkage between molecular and microscopic phenomenon on one hand and macroscopic process performance on the other hand(Baily J.E.1998).The study of filamentous fungi can be difficult through experimental means alone due to the complex city of their natural growth habitats and the microscopic scale of growth (eg Tip vesicle translocation and hyphal tip extension,mycelial growth)Mathematical modeling provides a complimentary ,powerful and efficient method of investigation.The aim of mathematical modeling is not to

form an extremely complex system of equation in an attempt to mirror reality instead the aim to reduce a complex (biological) system into a simple (mathematical system)that can be analyzed in for more details and from which key properties can be identified .Thus the aim of mathematical modeling is not about what to include ,but instead what can be omitted (Boswell G.P2003)(Melatiadis J et al 2001).Models can be categorized into probability or kinetic models (measuring growth rate or generation time) according to the mathematical approaches used.In case of filamentous fungi ,estimation of growth rate is more complicated owing to the formation of surface colonies and also of hyphae throughout the carbon source,a cell count is not appropriate.Empericals models simply describes the conditions underwhich a experiment was performed,that is the effect on microbial growth of the physical and chemical components of the carbon source.Such models are usually polynomials.(Gibson M.A and Hocking D.A. 1997)(Bail S and Klis F.M1999). Mathematical modeling of microbial growth has been used to estimate parameters (specific growth rate and lag time) required to study growth under different physical and chemical conditions.Mathematical models can be either empirical or mechanistic .Mechanistic models are preferred because they are derived to represent the biochemical processes controlling microbial growth.However,if the mechanism governing the process is unknown,mathematical fractions have to be used empirically.(Lopez S etal 2004)(Anseley M et al 1990).In this paper we are much emphasized various model on fungal growth system in generalized way and also on *Trichoderma* growth system

The various models describing fungal growth system:

Although a large body of work on the mathematical modeling of fungi is now established,many vital queries still remain unanswered and relevant and important problems un addressed. Predictive modeling of filamentous fungus growth has not received the same level of attention as that of bacterial growth.This may well be because of the inherent complexities associated with the quantification of fungal growth.Measurment of hyphal extension rates usually reported as radial growth rate ,is probably the simplest and most direct measures but it does not necessarily represents the true nature of fungal growth .Fungal hyphae can penetrate the physical 3-dimensions matrix of carbon souces.There are various models of fungal growth system have been studied.few are explained here.

Model of fungal mycelial growth:In general ,attempts at the mathematical modeling of fungal growth have either focused on mycelial level ,using quanitities such as biomass yield or have focusssed on growth on hyphal level such as hyphal tip growth ,branching.In the former spatial proportions are generally ignored ,while the latter temporal effects are often neglected The fungal mycelium is modeled as a distribution consisting of three components : active hyphae(Corresponding to those hyphae involved in the translocation of internal metabolites), Inactive hyphae(Denoting those hyphae not involved in translocation or growth.eg hyphal tips) An important distinction is made nutrients located within the fungus(internal) and those free in the outside environments(external).Intarnally located materials is used for metabolism and biosynthesis eg in the extension of hyphal tips

(creating new hyphae)branching (creating new hyphal tips)maintain and the uptake of external nutrient resources.This model is based on the physiology and growth characteristics of fungus(Boswell G.P.etal 2003)
In terms of the five variables (active hyphae,inactive hyphae,hyphal tips,internal substrate and internal substrate)The model has the following structure:
Change in active hyphae in a given area = New hyphae (laid down by moving tips)+reactivation of inactive hyphae-inactivation of active hyphae.
Change in inactive hyphae in a given area= Inactivation of active hyphae- reactivation of inactive hyphae-degradation of inactive hyphae.
Changes in hyphal tips in given area= Tip movement out of /into area+ branching from active hyphae-anaostomosis of tips into hyphae.
Changes in the internal substrate in the given area= Translocation (active and passive mechanisms)+ uptake into the fungus from external sources –maintanence cost of hyphae-growth costs of hyphal tips-active translocation costs
Change in external substrate in the given area= Diffusion of external substrare out of /into area –uptake by fungus.
The interactions between the components listed above satisfy the following system equations which are obtained from standerd conservation laws:

$\delta m/\delta t = f_m(m,m',p,s_i,s_e)$ = new hyphae- inactive hyphae

$\delta m'/\delta t = f_{m'}(m,m',p,s_i,s_e)$ = Inactivated hyphae – degradation

$\delta p/\delta t = -\delta/\delta_x J_p(m,m',p,s_i,s_e)+fp\ (m,m',p,s_i,s_e)$ =(tip migration)+(branching – anastomosis)

$\delta si/\delta t$ =[$-\delta/\delta x\ Ji^{pas}(m,m',p,s_i,s_e)+j_i^{act}(m,m',p,s_i,s_e)]+fi(m,m',p,s_i,s_e)$ = [translocation(passive andactive)]+[(uptakegrowthtrans.costs)]

$\delta s_e/\delta t = -\ \delta/\delta x\ J_e(m,m',p,s_i,s_e) + fe\ (m,m',p,s_i,s_e)$ = diffusion + (- uptake)

Where J and f denotes the flux (migration) and reaction (creation /loss) terms respectively,for each quantity.m(x,t)the active hyphal density at time t where x denotes spatial position;m'(x,t),the inactive hyphal density;p(x,t),the tip density;$s_i(x,t)$,the internal substrate concentration;and $s_e(x,t)$,the external substrate concentration. (Boswell G.P et al 2002) (Boswell G.P et al 2003)
Model of Fungal spore germination:Bizukojk M and Ledkowitz(2006) reported spore germination kinetics, according to this spores may be divided into 3 phases:Spore swelling germ tube emergence and germ tube elongation takes place in the first phase,The spores begins to swell increasing its dormant diameter significantly until a germ tube emerges in the second phase.In the third phase the elongation of germ tube is observed and its growth is usually exponential.

Kinetic model for the process of spore germination:The biomass content in the presented system is indirectly estimated as mean hyphal area because for the early stages of growth of any filamentous fungus.

$dA/dt = k_{sw} A$.This equation is valid if $t<t_e$,where t_e is the time when the tubes start to emerge.For t> te the following system of ordinary differential equation to be proposed.

$dcs/dt = - Y_{SA} ks.K_{I,INT}.K_{IA}.A/.K_{I,INT} + c_{INT}. K_{IA} + A$

$dc_N/dt = -Y_{NA} k_N K_{I,INT}.K_{IA}.A/.K_{I,INT} + c_{INT}. K_{IA} + A.$

$dc_{INT}/dt = - k_{INT}.c_{INT}/c_{INT} + K_{INT} - \mu_{INT}$

$dA/dt = \mu A$

Where μ is the specific growth rate of biomass expressed as the sum of the growth due to each substrate:

$\mu = ks.K_{I,INT}.K_{IA}.A/.K_{I,INT} + c_{INT}. K_{IA} + A + k_N K_{I,INT}.K_{IA}.A/.K_{I,INT} + c_{INT}. K_{IA} + A + k_{INT}.c_{INT}/c_{INT} + K_{INT}.$

WhereA,mean protected area of hyphae; c_{INT},Concentration of internal storage compounds;c_N,concentration of nitrogen source;c_S,concentration of carbon source;C,Circularity index;k_{INT},Internal storage compound uptake constant rate;k_N,N-source uptake constant rate,k_S,C-source uptake constant rate,k_{SW},spore swelling constant rate;$K_{I,A}$,Inhibition constant due to dimension of hyphae;$K_{I,INT}$,inhibition constant due to internal storage compound;K_{INT},saturation constant;L,mean hyphal length;te,spore swelling time,Y_{NA},yield of N source to hyphae;Y_{SA},yield of C source to hyphae, μ,specific growth rate of biomass.

Model of fungal hyphal tip growth: Tindemas S.H et al (2006) have been investigated the fungal tip growth and proposed vesicle supply centre(VSC) and diffuse vesicle supply centre model. According to VSC model vesicles mediated cell growth are created in the golgi bodies and first transported to the Spitzenkorper which act as an organizing center .From their the vesicles are released to ultimately fuse with the plasma membrane .The mathematical abstraction of the Spitzenkorper is a point like object called the vesicle supply center.These vesicles released randomly in all directions and move in a straight lines from the VSC to cell envelop.Once a vesicles hits the plasma membrane ,it fuses with the membranes and externalizes its contents causing a local expansion of the cell envelop .The model assumes the amount of wall material delivered to the cell envelop to be the same for any solid angle,as seen from the VSC.To this end the simplest explanation for an isotropic motion of the vesicles through the cytoplasm and instantaneous exocytosis at the cell wall.Diffusion allows for a finite vesicle exocytosis rate to be incorporated into the model in

a natural way,through the boundry conditions.The effective diffusion constant of the vesicles can be estimated from the Einstein relation $D=k_B T/6\mu\eta a$ assuming the vesicle size a to be roughly 50 nm and the viscosity η equals to that of water yields a diffusion constant of about $4\mu m^2/s$ taking into account the fact that cytoplasm is more viscous than water then D~ 1 $\mu m^2/s$.This implies that vesicles will take only a few secods to travel the VSC to the cell wall,making diffusion a viable method of vesicle delivery within the hyphal tip.

Models of *Trichoderma* growth system:

The *Trichodermma* system (like any other biological system)is of a complex nature.Complete quantitative description of its behaviour would leads to large sets of mathematical expressions describing the time evolution of a large number of variables.Furthermore ,these equations would contains a large number of parameters which would be difficult to obtain experimentally.The models describing biomass and product formation are listed as follows:

Biomass related models:

Modified Monod model: $dx_1/dt = \mu\ x_1\ (1 - 1/1\text{-}a\ (x_1\text{-}x_f)$, Where $a = So/K_s\ (x_f\text{-}x_o)$

Polynomial model: $dx_1/dt = \mu\ x_1[1 - (x_1/x_f)^q$

Humphery's model: $dx_1/dt = \mu\ x_1 - \delta\ x_1$Where $\mu = \mu_m\ .S/Ks+s$ and $\delta = \delta_m\ (1\text{-}S/K'_s + s)$

The generalized logistic model: $dx_1/dt = -\ x_1\ [1 - X_1/K_1]\{a_1 + 2a_2\ t + 3a_3\ t^2\}$

Whereas the enzyme production model are as follows:

The enzyme decay model: $dx_2/dt = b_1\ x_1 - b_2x_2$

Polynomial model: $dx_2/dt = b_1\ x_1 - (b_2x_2)^{b3}$

The generalized logistic model: $dx_2/dt = -\ X_2(1\text{-}X_2/K_2)\ \{a_4 + 2a_5t + 3a_6t^2\}$

Where a_1,a_2,a_3 are the coefficients of the polynomial in the generalized logistic growth model;a_4,a_5,a_6 are the coefficients of the polynomial in the generalized logistic product model;b_1 is the enzyme synthesis rate constant;b_2 is the enzyme decay rate constant;b_3 is the power coefficient in the polynomial model for enzyme synthesis;K_1 is the limiting cell mass concentration in biomass logistic model;K_s is the saturation constant,K'_s is the saturation death rate constant;q is the power coefficient in polynomial model;S is the substrate concentration;t is the fermentation time;x_0 is the intial biomass concentration;x_1 is the biomass concentration at time t;x_2 is the enzyme activity at time t;x_f is the final biomass concentration;δ is the specific death rate; μ is the specific growth rate.

Modified Monod model and Polynomial model for biomass are the simplified versions of the monod equation and do not require substrate concentration for determination.However they could not account for the death phase of the system.The model suggested by

Humpery's on the other hand assumes the growth rate to be the net effect of actual growth and death and depended on the concentration of limiting substrate present (Rakshit S.K and Sahai V 1991).

Thouldur A et al (1999) described structured and unstructured growth kinetic models

Unstructured kinetic model:.The simplest kinetic model assumes that sugars are converted into mycelial cell biomass ,which produce enzymes.

$dL/dt = - \mu_L \ X/Y_L$, $dZ/dt = - \mu_Z \ X/Y_Z$

$dX/dt = \mu_L X + \mu_Z \ X - k_d X$, $dP/dt = r_P.X$

Where L,Z,X and P are the lactose ,xylose ,cell mass and protein concentration respectively.The specific growth rates on lactose and xylose are μ_L and μ_Z respectively.Y_L and Y_Z are the corresponding cellmass yield.k_d is an endogenous growth terms ,r_P is the specific protein production rate.When both lactose and xylose present in the medium ,xylose is preferentially taken up.The following forms are postulated for the specific growth rate and protein production:

$\mu_L = (\mu_{maxL} .L/K_{S,L}+L)(K_{I,L}/K_{I,L}+Z), \mu_{L=\mu maxz} .Z/K_{S,z}+Z,$

$r_P = \alpha \mu_L+\beta \qquad t \geq 24h, rP = 0 \qquad t < 24h$

Structured Kinetic model:The structured kinetic model incorporates a limited amount of structure in the cell mass component.Cell mass divided into three categories – Primary mycelia,Secondary mycelia,Spores than the equations

$dL/dt = - \mu_L \ X_P/Y_L$, $dZ/dt = - \mu_Z \ X_P/Y_Z$, $dX_P/dt = (\mu_L + \mu_Z - k_1 - k_{d1})$, $dX_s/dt = k_1 X_P-(k_2+k_{d2})X_s$, $dX_0 = k_2 X_s$ Than

$dX/dt = dX_P/dt + dX_s/dt + dX_0 = (\mu_L + \mu_Z) X_P - k_{d1} X_P - k_{d2} X_s$

$dP/dt = r_P X_s$,

Where L,Z,X and P are the lactose ,xylose,cell mass and protein concentration,respectievely and X_P, X_s and X_0 are the cell mass contribution from primary mycelia,secondary mycelia and spores.The specific growth rates on lactose and xylose are μ_L and μ_Z respectively;Y_L and Y_Z are the corresponding cell mass yields.k_1 and k_2 are the constant rate terms for the conversion of primary mycelia to secondary mycelia and for the conversion of secondary mycelia to spores,respectively.k_{d1} and k_{d2} are endogenous death term and r_P is the specific protein production rate(Thouldar A Wet al 1999).

Velkovaska et al (1997) pointed out the concept of primary and secondary mycelium of *T.reesei* growth in terms of product formation.Cellulase (as a product) production by

Trichodermma can be defined as a Gaden III Type process.During this process two phase are noticeable.Primary and Secondary.In the primary phase ,biomass accumulation and normal metabolic activities reach their maximum then in secondary,later phase ,product accumulation and formation rate reach their maximum values.Microscopic observation shows that our young cells of *Trichoderma reesei* in the initial period of growth (the first several days) are not very active for production of cellulase enzyme.After the fast initial formation of primary mycelium ,microscopic analysis showed that mycelium structure starts to changeafter approximetaly 2-3 days and the eventual appearance of chlamydospores which is an important organs of asexual survivals and usually appears as a result of substrate exhaustation.Substrate exhaustation triggers a physiological transformations(appearance of secondary mycelium) in the cells to survive in medium with cellulose as the only substrate.More concentrated microsomes are found into young growing tips of secondary mycelia,these newer microsomes have a higher capacity to carry out protein synthesisand responsible for product formation.

The *Trichoderma* system is very complex .For development of model equations existance of structured biomass as primary and secondary mycelium are taken into account.

$dX_p/dt = \mu_m S X_p/K_s + S - k_1.X_p$ For primary mycelium

$dX_s/dt + k_1 X_p - k_d X_s$ For secondary mycelium

$dX/dt = \mu_m S X_p/K_s + S - k_d X_s$ For total biomass concentration

X in the medium is the sum of both mycelia types. The other constants are described earlier.

References :

Aynsley M.,A.C.Wood and A.R.Wright,1990,A mathematical model for the growth of mycelial fungi in submerged culture.Biotechnol Bioeng ,35,820-830.

Bail S and F.M.Klis,1999,Mechanistic and mathematical inactivation studies of food spoilage fungi.Fun Gen Biol,27,199-208.

Baily JE.,1998,Mathematical modeling and analysis in biochemical engineering : Past accomplishment and future oppurtunites.Biotechnol.Bioengg,14,8-20.

Bizukojk M and S.A.Ledakomicz,2006,Kinetic model for predict biomass content for *Aspergillus niger* germinating spores in the submerged culture.Process Biochem ,41,1063-171.

Boswell GP,H.Jacobs,F.A.Davidson,G.M.Gadd and K.Ritz,2002,Funnctional consequences of nutrient translocation in mycelial fungi,J .Theor Biol,217,459-477.

Boswell GP.,H.Jacobs ,G.M.Gadd,K.Ritz and F.A.Davidson,2003,A mathematical approach to studying fungal mycelia,Mycologist ,17,165-171.

Dahtigry P.,S.Marin ,M.Beyer and N.Morgan,2007,Mould germination :Data treatment and modeling .Int J food Microbiol,114,17-24.

Gibson AM and A.D.Hocking,1997,Advances in the predictive modeling of fungal growth in food .Trends Food Sci Technol,8,353-358.

Lopez S.,M.Prieto MS.Dijkstra and J.France,2004,Statistical evaluation of mathematical models for microbial growth.Int J food Microbiol,96,289-300.

MeletiadisJ.,J.F.G.MMeis,J.W.Mounton,andE.U.Palul.2001,Analysis of growth characteristics of filamentous fungi in different nutrient media .J Clin Microbiol,39,478-484.

Rakshit SK. and V.Sahai,1991,Optimal control strategy for the ethanol production of cellulase enzyme using the new mutant *Trichoderma reese*i E-12.Bioprocess Engg ,6,101-107.

Thouldar A,W.F.Ramirez and J.D.McMillan,1999,Mathematical modeling and optimization of cellulase proteins production using *Trichiodermma reesei* RL-P37,Biotechnol Bioengg ,66,1-15.

Tindermans SH.,N.Kern and B.M.Mulder,2006,The diffusive vesicle supply centre model for tip growth in fungal hyphae.J Theor Biol,238,937-948

Velkovska S.,M.R.Morten and D.F.Ollis,1997,Kinetic model for batch cellulase production by *Trichodermma reesei* RUT C30 ,J Biotechnol,54,83-94.