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# Mathematical models as devices to optimize microbial pectinase for chemical and pharmaceutical applications

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**ABSTRACT**

Pectinases are complex cocktail enzyme that degrades pectin protein present in higher plant tissues. These enzymes have enormous applications in pharmaceutical, chemical and other industrial sectors. About quarter sales of food enzymes mainly rely on microbial pectinases. Apart from other industrial sector, pectinase plays a key role in drug delivery studies. Mathematical modeling is a cost-effective tool for the control of production operations and optimization of process parameters which makes them as vital components of fermentation processes related to production of microbial pectinase. The merge of mathematical modeling with data obtained from experimental results gives interpretation of characteristic features of microorganisms. These models were designed in particular manner to attain maximal production of focused bio desired products having vital role in pharma based industries. Mathematical models discussed in this article are of diverse degrees of mathematical difficulty. The mathematical models which are used as devices to design for microbial growth and optimization like Gompertz, Baranyi, logistic model, response surface methodology, artificial neural network (ANN) were discussed. And also, kinetic models utilized for enzyme production like Monod, Michaelis-Menten, cybernetic and dynamic model their strength, weakness and the circumstances for the usage of these models has been discussed in this communication. These models will be alternate tools for analyzing and optimizing microbial pectinase production which ultimately increases its application in pharmaceutical, chemical and other industrial arena.

**KEY WORDS:** Enzyme kinetics, Mathematical modeling, Microbial growth, Pectinase, cock tail enzyme.

## 1. INTRODUCTION

Pectinases are group of inducible enzymes that degrade or disrupt the chemical bonds present in the pectin or pectic substances which are present in plant tissues. They are extensively distributed in microorganisms which include fungi, bacteria and higher plants. The production of pectinase is influenced by minimal concentrations of galacturonic acid. The maximum concentration (5%) of the galacturonic acid reveals self catabolite repression which ultimately affects the pectinase production in stationary phase (Jayani, 2005).This enzyme catalyzes pectin molecules by various mechanisms eliminative depolymerization (pectin lyases), hydrolytic depolymerases (Polygalacturonases) and esterases (Pectin esterase) (Tariq and Latif, 2012). Pectinase have wide range of applications in industrial sector that includes clarification of fruit juice, pulp and paper industry, retting of fibers, pectic waste water treatment and oil extraction etc., (Kohli and Gupta, 2015). Apart from industrial applications, pectinase plays very critical role in drug delivery studies. Implementation of mathematical modeling can be found in ancient civilizations which aim to understand and study the world around them (Wade, 2016). Mathematical models are indispensible for control and optimization of production operations which makes them as vital constituents of the fermentation process. The complex nature of the living systems and lifecycle of the microorganisms made bioengineering mathematical treatments more difficult. The merging of mathematical models with experimental data gives logical interpretations of experimental results and can be necessary for the investigation of new characteristics of microorganism. Moreover, these mathematical models can be utilized specifically for designing experiments to achieve maximum yields of the focused products (Thilakavathi, 2007). Predictive microbiology which includes mathematical equations is an essential device in food industrial sector to forecast the microbial behaviors (Cosano, 2006). Many mathematical models and equations have been developed in predictive microbiology for the interpretation of microbial growth in culture and food (Fujikawa and Morozumi, 2006). The fundamental tool to predict the shelf-life as well as food safety depends on the modeling of microbial growth (Buzrul, 2009). Microbial growth can be analyzed by the primary and secondary model based on the fitting and dynamic conditions. Primary models include Gompertz, logistic and Baranyi model. Various types of secondary models utilized by previous researchers such as response surface model (RSM) and artificial neural networks (ANN). Formulation of model, identification and assessment of parameters and solutions of equations make the designing of enzyme production kinetics in every stages of model development (Coppella and Dhurjati, 1989). Mathematical models of single and cell populations are very essential in systemizing data in a related description and also in optimizing and controlling production processes (Curien, 2003). This attempt was made to overview about the important mathematical models utilized for the microbial growth (primary and secondary models) and kinetic models for microbial pectinase production and their future prospective.

**Gompertz Model:** The Gompertz model best suited model which was most and broadly used mathematical models which were introduced to describe the human's mortality rate (Gompertz, 1825). It is a variety of mathematical model

for a time series, where microbial growth is slowest during the start and end of a time period which is in contrast to logistic model. Later, modified Gompertz model was utilized to better fit the microbial growth (Gibson, 1987). This model is one of the recommended models to describe microbial growth which helps in monitoring of microbial growth phase for enhanced production of pectinase.

$$x(t) = C + Ae^{-e-B^{(t-D)}}$$

Where $x(t)$ is $log_{10}\,(^{CFU}/_{ml})$ of cell concentration at time t, C is value of lower asymptome in units of $log_{10}\,(^{CFU}/_{ml})$; A is equal to $log_{10}(\frac{x_{max}}{x_0})$ ; $x_0$ is the initial population density; B is maximum relative growth rate at D in $\frac{1}{h}$ ; D is time at which absolute growth rate is maximum in h. (Jovanovic and Krstic, 2014).

**Baranyi Model:** Among the various growth models (Baranyi and Roberts, 1994) proposed a growth model in which the single variable is used to represent the physiological state of the cell. In this model, duration of lag is determined by the value of that variable at pre-inoculation and post-inoculation environment.

The explicit form of the model is the following:

$$Y(t) = Y_0 + \mu_{max} + \frac{1}{\mu_{max}} \ln e^{-vt} + e^{-h_0} - e^{-v-t.h_0} - \frac{1}{m} \ln(1 + e^{m\mu_{max}t} + \frac{1}{\mu_{max}} \ln\left(e^{-v.t} + e^{h_0} - e^{-v.t-h_0}\right) - 1)/e^{m\,y_{max}-y_0}$$

Where, $Y(t) = \ln\big(x(t)\big)$with $x(t)$ is the cell concentration($^{CFU}/_{ml}$); $Y_0 = \ln(x_0)$ , $Y_{max} = (x_{max})$, $x_0$ being the initial and $x_{max}$ is the asymptotic cell concentration; $\mu_{max}$ is the maximum growth at ($^1/_h$); $m$ and $v$ is a curvature parameters to characterize the transition from and to the exponential phase; $h_0$ is the dimensionless parameter quantifying the initial physiological state of the cells. From, lag time λ(h)can be calculated as$^{h_0}/_{\mu_{max}}$. Baranyi (1997), Suggests $v = \mu_{max}$ and $m = 1$ for the parameters of curvature. This ultimately decreases the no. of parameters by two, so the model has four parameters: $\mu_{max}$, $h_0$ and $Y_{max}$.

Baranyi and Roberts (1995), suggested that $h_0$, dimensionless parameter could be suitable indicator of the microorganism population to the normal environment. In a standardized experimental procedure, this indicator will be close to constant value which is equivalent to the assumption that the lag and $\mu_{max}$are inversely proportional. This model significantly helps in assessment of specific growth stage of microbe for pectinase production.

**Logistic Model:** The logistic growth model was proposed which can be utilized as a ubiquitous model for the population growth. This Logistic growth model can be a best model for yeast, bacterial or other microorganisms which were grown in constant and controlled conditions (Krebs, 1978).

The equation for the logistic model can be as follows:

$$\frac{dN}{dt} + rN(1 - \frac{N}{N_{max}})$$

Where $N$ the number of microbial population at time $t$. $r$ is the maximum growth rate or rate constant. $N_{max}$ refers to the maximum population (Stationary phase), which is often adaptability to the environment. Here, $N_{max}$ is an asymptote. This model includes the term,$\frac{-N}{N_{max}}$, which helps to inhibit the microbial growth at high population. When $N$ is very negligible (at lag phase), the value of the term is almost one which ultimately does not affect the growth rate. As $N$ increases to approach $N_{max}$, the value becomes zero thus make the rate of microbial growth to zero (during the stationary phase).

Thus, Logistic model cannot able to generate a sigmoid curve on a semi-logarithmic plot. The model generates a convex curve which shows monotonous increasing portion and stabilized one (without lag phase) at initial period (Vadasz, 2001). This model helps to observe the growth phase of microbe for the pectinase production in optimized conditions.

**Response Surface model (RSM):** Response Surface Methodology is a combination of statistical and mathematical methods which are most important for the modeling and problem analysis in which a response is influenced by more process variables. The most extensive application of this model can be seen in industrial field in situations where more input variable affect some performance measures called response in such problems can be made easier as well as feasible to depict with difficult mathematical formulation (Kumar, 2007). This model is mostly utilized for the optimization of media for the microbial pectinase production. This model has been enormously utilized to optimize biochemical and chemical processes, such as enzyme production (Barbosa, 2010), media optimization (Kunamneni and Singh, 2005), conditions of enzymatic hydrolysis (Shieh and Lai, 2000), parameters for processing of food (Ozer, 2004).

**Artificial neural network (ANN):** Artificial neural network (ANN) is a simplified and meticulous model of the structure of a biological network. A neural network is a tremendous parallel-distributed processor for accumulating the knowledge of experimental data and makes it accessible for use which also has natural propensity. The capacity of the ANNs is to make an efficient complex system by recognizing and reproducing the cause-effect relationships through trials for the multiple input-output systems.

**Model: Enzyme production kinetics**

**Monod model:** The Monod model is chief unstructured mathematical models for the growth of microbes (Burhan, 2005). Monod model is applied for the growth at specific rate, biomass yield based on oxygen consumption and maintenance coefficient for the production of microbial pectinase (Fernandez, 2011).

$$\mu = \frac{1}{X} \times \frac{dX}{dt}$$

**Michaelis- Menten model:** Michealis -Menten is a best and structured mathematical model of enzyme kinetics. This model describes the enzymatic reaction rate by relating it with the concentration of a substrate. Biochemical reactions that take place in a single substrate always assumed to follow this model. It is used to determine the maximum reaction rate of free and immobilized pectinase utilizing different concentration of substrate (Rehman, 2013; Vargas and Oliveira, 2015).

$$V_0 = \frac{V_{max}[S]}{K_m\,[S]}$$

**Cybernetic model:** The cybernetic model is generally based on the most favorable nature for the regulatory processes of microorganisms. The microbial behavior which is responsible for the metabolic regulation follows the principle of cybernetic framework which makes it apart from other kinetic models. This model is equally significant for modeling of both biphasic and diauxic growth phenomena. As, this model has more advantages over control theory of metabolites and has vast simulation based attempts regarded as significant model compared to others. The model which is developed by (Kompala, 1986) is based on the identification of favorable microbial growth nature on mixed substrates which gives structural representation of cell that controls significant biochemical processes. Cybernetic models are designed on the conceptualization of expressing the specific growth rate by utilizing effective concentrations of enzyme on single substrate but it was developed for the growth of microbes in multiple substrates. This model has the ability to forecast growth profiles of biomass, substrates utilization and kinetics of microbial pectinase production at different situations (Gadgil and Venkatesh, 1997).

**Dynamic model:** Process optimization and simulation tools become an essential tool in bioprocess. This dynamic model has the capacity to imitate the altering responses and conditions of the culture all through the fermentation process. This kind of models may be unstructured or structured models. The dynamic model is the best unstructured model which represents logistic and linear phases of both biomass growth and production of pectinase from microbes in a diauxic process (Miron, 2002).

**Table.1. Shows strength and weakness of growth and kinetic models**

| Model | Strength | Weakness |
|---|---|---|
| **Primary models** | | |
| Gompertz model | Significant for human mortality rate and growth of tumor cells. | Less advantageous over enzyme production as it only describes about lag and death phase of microbe. |
| Baranyi model | Dynamic model with good predictive capabilities, it can deal with time varying environmental conditions.<br><br>Indispensable model for prediction of shelf-life of foods and quantitative risk analysis of food production cycles (Foegeding, 1997). | The adjustment function will resulted in recursive formulae.<br><br>Relative complexity<br><br>Lack of mechanistic interpretation due to the change of environment dynamically during lag phase which makes the model dubious to apply. |
| Logistic model | Compares the growth of microbe in continuous and discrete fashion. | Applicable only for small populations and growth rate is fast as the growth is totally dependent on resources. |
| **Secondary models** | | |
| Response surface model | Essential to determine and simultaneously resolve multivariate equations.<br>Great advantage of implementing statistical techniques which focus at executing experimental design, build-up of empirical models and evaluating | Used only for the quadratic expressions. |

| | | |
|---|---|---|
| | independent variables on desired variable responses. | |
| Artificial neural network | No requirement of any preliminary specification of suitable fitting function.<br><br>It has a universal approximation capacity to approximate more or less all kinds of non-linear functions, inclusive of quadratic functions. | Does not assure the global optimal solution.<br>It also less-time consuming and cost-effective technique (Kundu, 2015)<br>The restrains of ANN have prompted researchers to generate ideas of hybridizing or merging ANN with other approaches in search for greater performance. Among available schemes, Genetic algorithm (GA) solves the optimization difficulties by imitating the principle of biological evolution. |
| **Models: Enzyme kinetics** | | |
| Monod model | Explains even a small range of binding properties that leads to enormous increase in the molecules of R-state leading to maximum attachment of ligand to the protein.<br>Utilized in enzyme production and successful application in hemoglobin regulation. | Mainly applicable for the proteins and ligand interactions. |
| Michaelis - menten model | Applied and standardized model for all biochemical reactions.<br>Applied for the enzymatic applications in industrial sector for better activity. | The substrate is used in both axes leads to errors and lower precision of estimates of $K_m$ and $V_{max}$. |
| Cybernetic model | Great advantageous over biphasic and diauxic growth and also it can be more significant for the action of enzymes on multiple substrates. | Less significant model for normal sigmoid growth and in use of single substrate reactions. |
| Dynamic model | Best model for the enzyme reactions as it deals with enzyme and the intermediate product simultaneously. | Model can distinguish the growth curve into two phases because of increase in biomass. |

## 2. METHODS & MATERIALS

**Softwares used for the construction of mathematical models:** The (Magellan) software was used for the analysis of microbial growth and end point assays. The softwares namely (Design expert 10.0, Minitab 14.0) were used for the construction of mathematical models for the optimization of media components, dependent and independent variables for the pectinase production.

## 3. RESULTS

The primary mathematical models were used mostly for the determination of microbial growth rate and different phases of growth in which microbe can produce primary and secondary products at specific time interval. On the other hand, secondary models were used for the bioprocess, optimization studies; to alter the parameters for maximal production of microbial pectinase. Compared to classical method of media optimization, the mathematical models gives accurate result for maximal pectinase production and also it reduces the factors which ultimately inhibit the growth and yield.

## 4. CONCLUSION

This attempt gives the clear idea about the various classifications of mathematical modeling that can be incorporated for the microbial growth and kinetics for enzyme production. All models have unique strength which plays an effective role in the production of enzyme and its applications. More attention needs to be regulated for the production of microbial pectinase on the utilization of these models in future will propagate the yield and applications of this enzyme in chemical, pharmaceutical and other industrial field.

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## REFERENCES

Baranyi J, Commentary, Simple is good as long as it is enough, Food Microbiology, 14, 1997, 189-192.

Baranyi J, Roberts TA, A dynamic approach to predicting bacterial growth in food, International Journal of Food Microbiology, 23, 1994, 277-294.

Baranyi J, Roberts TA, Mathematics of predictive food microbiology, International Journal of food Microbiology, 26, 1995, 199-218.

Barbosa AM, Giese EC, Dekker RFH, Borsato D, Perez AIB, Iranzo JFU, Extracellular β-glucosidase production by the yeast *Debaryomyces pseudopolymorphus* UCLM-NS7A, optimization using response surface methodology, New Biotechnology, 27, 2010, 374-381.

Burhan N, Sapundzhiev N, Beschkov V, Mathematical modeling of cyclodextrin-glucano transferase production by batch cultivation, Biochemical Engineering Journal, 24, 2005, 73-77.

Burzrul S, A suitable model of microbial growth, African Journal of Microbiology Research, 3, 2009, 468-474.

Coppella SJ, Dhurjati P, A detailed analysis of *Saccharomyces cerevisiae* growth kinetics in batch, fed-batch and hollow-fiber bioreactors, Chemical Engineering Journal, 41, 1989, B27-B35.

Cosano RMZ, Gimeno GG, Perez RR, Martinez HC, Performance of response surface model for prediction of *leuconostoc mesenteroides* growth parameters under different experimental conditions, Food control, 17, 2006, 429-438.

Curien G, Ravanel S, Dumas R, A kinetic model of the branch-point between the methionine and threonine biosynthesis pathways in *Arabidopsis thaliana*, European Journal of Biochemistry, 270, 2003, 4615-4627.

Fernandez DER, Leon JAR, Carvalho JCD, Sturn W, Soccol CR, The behavior of kinetic parameters in production of pectinase and xylanase by solid-state fermentation, Bioresource Technology, 102, 2011, 10657-10662.

Foegeding PM, Driving predictive modeling on a risk assessment path for enhanced food safety, International Journal of Food Microbiology, 36, 1997, 87-95.

Fujikawa H, Morozumi S, Modeling *Staphylococcus aureus* growth and enterotoxin production in milk, Food Microbiology, 23, 2006, 260-267.

Gadgil CJ, Venkatesh KV, Structured model for batch culture growth of *Lactobacillus bulgaricus*, Journal of Chemical Technology and Biotechnology, 68, 1997, 89-93.

Gibson AM, Bratchell N, Roberts TA, Predicting microbial growth, growth responses of *Salmonella* in a laboratory medium as affected by pH, sodium chloride and storage temperature, International Journal of Food Microbiology, 6, 1987, 155-178.

Gompertz B, On the nature of the function expressive of the law of human mortality and on a new mode of determining the value of life contingencies, Philosophical Transactions of the Royal Society London, 115, 1825, 513-585.

Jayani RS, Saxena S, Gupta R, Microbial pectinolytic enzymes, A review, Process Biochemistry, 40, 2005, 2931-2944.

Jovanovic M, Krstic M, Analysis of non-autonomous stochastic Gompertz model with delay, Applied Mathematics and Computation, 242, 2014, 101-108.

Kohli P, Gupta R, Alkaline pectinases, A review, Biocatalysis and Agricultural Biotechnology, 4, 2015, 279-285.

Kompala DS, Ramakrishna D, Jansen NB, Tsao GT, Investigation of bacterial growth on mixed substrates, experimental evaluation of cybernetic models, Biotechnology and Bioengineering, 28, 1986, 1044-1055.

Krebs CJ, Ecology. In, The Experimental Analysis of Distribution and Abudance, 2nd edn. Harper & Row Publishers, New York, 1978.

Kumar A, Prasad B, Mishra IM, Process parametric study for ethane carboxylic acid removal onto powder activated carbon using Box-Behnken design, Chemical Engineering and Technology, 30, 2007, 932-937.

Kunamneni S, Singh R, Response surface optimization of enzymatic hydrolysis of maize starch for higher glucose production, Biochemical Engineering Journal, 27, 2005, 179-190.

Kundu P, Paul V, Kumar V, Mishra IM, Formulation development, modeling and optimization of emulsification process using evolving RSM coupled hybrid ANN-GA framework, Chemical Engineering Research and Design, 104, 2015, 773-790.

Miron J, Gonyalez MP, Pastrana L, Murado MA, Diauxic production of glucose oxidase by *Aspergillus niger* in a submerged culture- a dynamic model, Enzyme and Microbial Technology, 31, 2002, 615-620.

Ozer EA, Ibanoglu S, Ainsworth P, Yagmur C, Expansion characteristics of a nutritious extruded snack food using response surface methodology, European Food Research and Technology, 218, 2004, 474-479.

Rehman HU, Aman A, Silipo A, Qader SAU, Molinaro A, Ansari A, Degradation of complex carbohydrate, Immobilization of pectinase from *Bacillus licheniformis* KIBGE-IB21 using calcium alginate as a support, Food Chemistry, 139, 2013, 1081-1086.

Shieh CJ, Lai YF, Application of response surface methodology to the study of methyl glucoside polyester synthesis parameters in a solvent-free system, Journal of Agricultural and Food Chemistry, 48, 2000, 1124-1128.

Tariq A, Latif Z, Isolation and biochemical characterization of bacterial isolates producing different levels of polygalacturonases from various sources, African Journal of Microbiology Research, 6, 2012, 7259-7264.

Thilakavathi M, Basak T, Panda T, Modeling of enzyme production kinetics, Applied Microbiology and Biotechnology, 73, 2007, 991-1007.

Vadasz AS, Vadasz P, Abashar ME, Gupthar AS, Recovery of an oscillatory mode of batch yeast growth in water for a pure culture, International Journal of Food Microbiology, 71, 2001, 219-234.

Vargas CEB, Oliveira, *In situ* immobilization of commercial pectinase in rigid polyurethane foam and application in the hydrolysis of pectic oligosaccharides, Journal of Molecular Catalysis B, Enzymatic, 122, 2015, 35-43.

Wade MJ, Harmand J, Perspectives in mathematical modeling for microbial ecology, Ecological Modelling, 321, 2016, 64-74.