Modeling of Esterase Production from *Saccharomyces cerevisiae*

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A suitable simple model tested by experiments is required to address complex biological reactions like esterase synthesis by *Saccharomyces cerevisiae*. Such an approach might be the answer to a proper bioprocessing strategy. In this regard, a logistic model for esterase production from *Saccharomyces cerevisiae* has been developed, which predicts well the cell mass, the carbon source (glucose) consumption, and the esterase activity. The accuracy of the model has been statistically examined by using the Student's t-test. The parameter sensitivity analysis showed that all five parameters (μ, K, X, Y, and Y) have significant influence on the predicted values of esterase activity.

Keywords: Esterase, logistic model, parameter sensitivity, *Saccharomyces cerevisiae*

Esterase splits esters into an acid and an alcohol in a chemical reaction with water during hydrolysis. Wide ranges of different esterases exist that differ in substrate specificity, protein structure, and biological function [13, 18, 20]. Esterase is an important biocatalyst for the industrial production of chiral intermediates. In general, esterases are easy to handle. The enzymes are quite stable, which is important for industrial processes. Enzymes, particularly esterases and lipases, are recognized as functional catalysts for asymmetric synthesis, and in several situations, their stereochemical control cannot be equaled by nonenzymatic catalysis [26]. Additionally, esterase is characterized by a large range of substrate selectivity combined with high stereoselectivity. In contrast to lipases, esterases are more specific to short-chain fatty acids and are used in aqueous or two-phase systems. Esterases are also used for the optical resolution of racemic mixtures. There are a few esterases that also hydrolyze tertiary alcohols. These enzymes have also been used to separate endo-/exo-

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kinetics facilitate data analysis and may provide a strategy for solving problems encountered in industrial fermentation processes [15, 25, 30].

**Materials and Methods**

**Microorganism**

*Saccharomyces cerevisiae* MTCC 36 was obtained from the Microbial Type Culture Collection, Institute of Microbial Technology, Chandigarh, India. The organism was maintained on yeast extract-peptone-dextrose-malt extract agar slants containing (g/l): yeast extract, 2.926; peptone, 7.109; dextrose, 13.765; malt extract, 3.028 [19].

**Experimental Method**

The 150 ml of medium was placed in a 500-ml Erlenmeyer flask. After sterilization of the medium, approximately 1×10⁶ cells were inoculated into the medium. The culture was incubated on a rotary shaker maintained at 180 rpm at 30°C. Samples were collected at regular intervals and were analyzed for glucose, enzyme, and cell mass. Glucose was estimated by the dinitrosalicylic acid method [17]. Cell mass was measured by the method suggested by Panda and Govindaraju [19]. An aliquot of 1 ml of sample from the fermentation broth was centrifuged at 4,000 rpm for 10 min. After centrifugation, the supernatant was decanted and again centrifuged with water for washing the cells. Cells were then transferred to a preweighted aluminum foil and kept in a drier at 60°C for 12 h or to a time until constant weight was reached. After drying, the weight of the aluminum foil with cells was measured. The amount of cell mass was calculated from the difference between the weight of foil with cells to the weight of the empty foil.

**Enzyme Assay**

Esterase was assayed by using p-nitrophenylacetate as the substrate. A reaction mixture containing 0.5 ml of enzyme solution, 1 ml of 0.15 M sodium phosphate buffer (pH 7.0), 2.5 ml of double-distilled water, and 1 ml of 1 mM substrate solution was incubated at 30°C for 30 min. A standard plot of p-nitrophenol vs. absorbance at 400 nm was used to find the concentration of liberated p-nitrophenol in the reaction mixture [28]. The enzyme activity was expressed in terms of Unit (U). One unit of esterase activity is defined as the amount of enzyme producing 1 μmol of the p-nitrophenol per minute under the standard assay conditions. Specific esterase activity is defined as the unit of esterase activity (U) per gram of dry cell mass equivalent (i.e., U/g dry cell mass).

**Theory**

The mathematical model consists of a set of ordinary differential equations taking into account the microbial growth, the substrate consumption, and the product formation with time. In the logistic model, the rate of cell mass increase may be limited by cell density [5]. Parameter $X_e$ is the upper limit of cell growth and it is called carrying capacity. If cell mass exceeds $X_e$, the cell growth rate becomes negative and cell numbers decline. The model is based on several assumptions. The specific growth rate depends on substrate concentration. Autoinhibition is assumed to occur within the cell itself. Substrate consumption is not related to the product formation (i.e., intracellular enzyme) and the product formation rate can be associated with the growth of cells. The rates are given below:

$$\frac{dX}{dt} = \mu X \left( 1 - \frac{X}{X_e} \right)$$  \hspace{1cm} (1)

$$\frac{dS}{dt} = -\frac{1}{Y} \frac{dX}{dt}$$  \hspace{1cm} (2)

$$\frac{dP}{dt} = V_{p\text{max}} \frac{dX}{dt}$$  \hspace{1cm} (3)

The expression (1−$X/X_e$) is employed in cell growth rate to describe the autoinhibition of the cell itself [31]. The parameters needed to be evaluated from the model using experimental data are the maximum specific growth rate ($\mu_{\text{max}}$), Monod's constant ($K_s$), maximum cell mass ($X_e$), yield coefficient of cell with respect to substrate ($Y_{X/S}$), and the yield coefficient of product with respect to cell ($Y_{P/X}$).

The model is supplemented with the data of three independent runs. The ordinary differential Eqs. (1)−(3) are solved simultaneously by using the fourth-order Runge-Kutta method, coupled with the optimization procedure called simulated annealing. The initial conditions for solving the ordinary differential equations are $X_0=0.1$ g/l, $P_0=0$, and substrate concentration differs for various concentrations of glucose. The algorithm for parameter estimation procedure is given in Appendix I. The objective function is to minimize the sum of the errors between experimental data and estimated values at all times. The parameters varied one at a time randomly to get a new set of parameters for the algorithm. With each set of parameters, the objective function is determined and the difference in the objective function ($\Delta$) with old and new sets of parameters is calculated. If the new set of values improves the objective function, the move is accepted. Otherwise, the move is accepted with a probability of $\exp(-\Delta/T)$, where $T$ is simulated annealing temperature, a dummy variable that is used to control the acceptance of uphill moves. Initially, $T$ is fixed at a higher value and is periodically annealed by proportional cooling schedule in the outer loop. At any specific temperature, the parameters are randomly varied number of times in the inner loop. Thus, the optimum parameter values are obtained after $T$ reaches a desired lower value [14]. The algorithm for simulated annealing procedure is given in Appendix II.

**Parameter Sensitivity Analysis**

The aim of the sensitivity analysis is to estimate a rate of change in the output of the model with respect to changes in model input. This is important for evaluating the applicability of the model, determining parameters, which is important to have more accurate results, and understanding the behavior of the system being modeled. The normalized sensitivity coefficients represent a percentage change in the predicted values due to a percentage change in the parameter values [22].

**Results and Discussion**

**Discussion on Experimental Results**

To develop a suitable model, it is necessary that the model should be tested with experimental results. In this regard,