Kinetic Studies of Alkaline Protease from *Bacillus licheniformis* NCIM-2042

Bhunia, Biswanath¹, Bikram Basak¹, Pinaki Bhattacharya², and Apurba Dey¹*

¹Department of Biotechnology, National Institute of Technology, Durgapur, Mahatma Gandhi Avenue, Durgapur-713209, India  
²Department of Chemical Engineering, Heritage Institute of Technology, Kolkata-700107, India

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An extensive investigation was carried out to describe the kinetics of cell growth, substrate consumption, and product formation in the batch fermentation using starch as substrate. Evaluation of intrinsic kinetic parameters was carried out using a best-fit unstructured model. A nonlinear regression technique was applied for computational purpose. The Andrew’s model showed a comparatively better $R^2$ value among all tested models. The values of specific growth rate ($\mu_{\text{max}}$), saturation constant ($K_S$), inhibition constant ($K_I$), and $Y_{X/S}$ were found to be 0.109 h⁻¹, 11.1 g/l, 0.012 g/l, and 1.003, respectively. The Leudeking–Piret model was used to study the product formation kinetics and the process was found to be growth-associated. The growth-associated constant ($\alpha$) for protease production was sensitive to substrate concentration. Its value was fairly constant up to a substrate concentration of 30.8 g/l, and then decreased.

**Keywords:** Cell growth, fermentation, kinetics, modeling, simulation

Proteases are obtained through a wide variety of sources such as plants, animals, and microorganisms. Alkaline protease is one of the most important groups of industrial enzymes, being extensively used in leather, food, pharmaceutical, textile, organic chemical synthesis, waste water treatment, and other industries [4, 29]. They hold a major share of the enzyme market, with two-third share alone in the detergent industry [2, 6, 14]. Exploitation of such microbial metabolism by regulating the critical fermentation parameters helps in commercial economic production of the required enzyme [32]. Hence, careful kinetic studies are required to monitor the growth of microorganisms on various levels of substrates and their role in the overall productivity in the fermentation process. A kinetic study provides huge quantitative information regarding the behavior of a system, which is essential for study of the fermentation process. The kinetic study also describes the biological significance of each parameter and their levels with statistical reliability [11]. In general, structured or unstructured models are used in a fermentation process. Structured models are used in intracellular metabolic pathways and unstructured models are most frequently employed for modeling microbial systems based on the simplicity and technical robustness. Both models are equally useful in bioprocesses [18, 34]. In most of the biotechnological processes, high concentration of substrates or products often lead to inhibitory effects. So substrate utilization reduces decreasing both the product yields and fermentation rates [17].

In this work, various unstructured kinetic models were used to characterize the fermentation process using *Bacillus*
*licheniformis* NCIM-2042. The Andrew-incorporated Leudeking–Piret model can be effectively used to explain the relationship between microbial growth, substrate utilization, and alkaline protease production. The Arrhenius model was used to evaluate the importance of temperature on the growth and alkaline protease production.

**Materials and Methods**

**Chemicals and Analysis**
Glucose (Sigma, USA), starch (Sigma, USA), amyloglucosidase (Sigma, USA), glucose oxidase and peroxidase (SRL, India), o-dianisidine dihydrochloride (Sigma, USA), Bradford reagent (Sigma, USA), bovine serum albumin (BSA; Himedia, India), trichloroacetic acid (Merck, India), and casein (Himedia, India) were used in this study. All other chemicals used were of analytical grade commercially available in India.

For model parameters estimation, a differential equation of the model was solved using Xpp out software based on the Runge–Kutta method. GraphPad Prism 5 software was used for nonlinear regression analysis.

**Microorganism and Seed Culture**
Protease-producing *Bacillus licheniformis* NCIM-2042 was procured from National Chemical Laboratory, Pune, India. The microorganism was grown on nutrient agar slants at 37°C at pH 7.4. It was maintained by subculturing on nutrient agar slants kept at pH 7.4. For production experiments, the culture was revived by adding a loopful of pure culture into 50 ml of sterile production media (pH 7.4).

**Protease Production in Bioreactor**
The experiments were carried out in a lab-scale 2.2 L bioreactor (New Brunswick, USA) with a 2 L working volume. A 2% fresh culture (OD<sub>600</sub> ≈ 0.2) was inoculated in the fermentor, containing an optimized medium (g/l); starch, 30.8; soybean meal, 78.89; MgSO<sub>4</sub>, 0.5; and NaCl, 5.3 at pH 7.4. The impeller speed was adjusted to 180 rpm and compressed sterile air was sparged at 3 vvm, cultivating for 4 days at 37°C. The culture was centrifuged at 10,000 × g for 10 min at 4°C. The supernatant was mixed with 1 ml of 4 M KOH in a test tube. One milliliter from each tube was then added to 11 ml of 0.5 M acetic acid and mixed. Three milliliter of amyloglucosidase (50 units/ml) was added and the tubes were placed in a water bath (70°C) for 30 min. The tubes were then boiled for 10 min and allowed to equilibrate at room temperature. The pH was adjusted by adding 0.4 ml of 6 M KOH and the tubes mixed and centrifuged (1,500 × g) to obtain a clear supernatant. The amount of glucose in the supernatant was then assayed using the glucose oxidase and peroxidase method [3].

**Biomass Estimation**
For dry cell weight (DCW) estimation, the cell pellet was washed twice with distilled water and then dried in a hot air oven. All experiments were done in triplicate.

**Mathematical Modeling**

**Cell growth kinetics.**

**Monod model**
The Monod model describes the relationship between the specific growth rate (µ) and the concentration of the limiting substrate [20]. The specific growth rate in the exponential phase was calculated using the following equation

\[
\frac{dX}{dt} = \mu X
\]

Equation (1) on integration gives

\[
X(t) = X_0 e^{\mu t}
\]

GraphPad Prism 5 software was used to find the kinetic parameters from the Monod equation.

\[
\mu = \frac{\mu_{\text{max}} S}{K_s + S}
\]

where \(\mu_{\text{max}}\) is the maximum specific growth rate, \(K_s\) is the saturation constant in the Monod equation, \(S\) is the substrate concentration, and \(\mu\) is the specific growth rate.

After evaluating several substrate inhibition kinetic models, the Andrew’s model [1] was considered for the substrate inhibition in Eq. (4).

\[
\mu = \frac{\mu_{\text{max}} S}{K_s + S + K_I S}
\]

where \(K_I\) is the inhibition constant in Andrew’s model.

**Logistics model**
The logistics model characterizes growth in terms of carrying capacity. The microbial growth is governed by a hyperbolic relationship and there is a limit to attain the maximum cell mass, which is described by the logistic equation [30].

\[
\frac{dX}{dt} = k X \left(1 - \frac{X}{X_m}\right)
\]

where \(k\) denotes the carrying capacity (h<sup>-1</sup>) and \(X_m\) is the maximum cell mass concentration (g/l). Equation (4) was integrated and the cell mass was found to be...
The growth phase was considered up to $t = t_\text{c}$, which is the time at which the cell reaches a critical cell concentration ($X_c$) beyond which exponential growth was not possible. Equation (9) on integration gives

$$X(t) = X_c e^{\mu t}$$

Equation (6) on integration gives

$$X(t) = \frac{X_c e^{\mu t}}{1 - (X/X_0)(1 - e^{-\frac{t}{t_\text{c}}})}$$

The carrying capacity ($k$) was determined by GraphPad Prism software from Eq. (6) by nonlinear regression analysis.

**Modified logistics model**

The modified logistics model is a modified form of the logistic equation by introducing an index of the inhibitory effect “$r$”. This model describes the deviation of growth from the exponential relationship [25].

$$\frac{dX}{dt} = kX[1 - (X/X_0)]$$

Equation (6) moves toward Eq. (1) (i.e., the bacterial growth will follow the exponential pattern). When the value of $r^2$ is zero, it indicates a complete inhibition of bacterial growth. On the other hand, for a one value for “$r$” ($r = 1$), Eq. (7) comes up to Eq. (5). When the value of $r$ is between 0 and 1, Eq. (6) shows a higher degree of inhibition in comparison with the logistic growth. The modified logistics model is a modified form of the logistic equation by introducing an index of the inhibitory effect “$r$”. This modified logistics model is a modified form of the logistic equation by introducing an index of the inhibitory effect “$r$”. This

For the large value of “$r$” Eq. (7) moves toward Eq. (1) (i.e., the bacterial growth will follow the exponential pattern). When the value of $r^2$ is zero, it indicates a complete inhibition of bacterial growth. On the other hand, for a one value for “$r$” ($r = 1$), Eq. (7) comes up to Eq. (5). When the value of $r$ is between 0 and 1, Eq. (6) shows a higher degree of inhibition in comparison with the logistic growth. The modified logistics model is a modified form of the logistic equation by introducing an index of the inhibitory effect “$r$”. This

The differential equation of the model was solved, and the simulated value for cell mass concentration, $X$, was calculated from $X_{\text{sim}}$ software based on the Runge-Kutta method. The simultaneously simulated substrate profile with $t$ was determined using Xpp out software.

**Kono and Asai model**

Kono and Asai [16] proposed a model where cells grow through a lag phase, transition phase, exponential phase, post-exponential phase, stationary phase, and death phase. However, the growth of *Bacillus licheniformis* NCIM-2042 was divided into two phases: the exponential and stationary growth phases. For the exponential growth phase

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

The exponential growth phase was considered up to $t_\text{c}$, which is the time at which the cell reaches a critical cell concentration ($X_c$) beyond which exponential growth was not possible. Equation (9) on integration gives

$$X(t) = X_c e^{\mu t}$$

The kinetic parameters were determined by GraphPad Prism software using nonlinear regression analysis.

**Simulation of biomass and substrate profile**

The substrate utilization kinetics is given by Eq. (11). A carbon substrate such as starch is used to form cell material and metabolic products as well as for maintenance of the cell.

$$\frac{dS}{dt} = \frac{1}{Y_{\text{SS}}} \frac{dX}{dt} Y_{\text{SS}} - MX$$

where $Y_{\text{SS}}$ and $Y_{\text{SM}}$ are yields of cell mass and product with respect to substrate, and $M$ is the maintenance coefficient for cells. The starch consumption equation is modified and given in Eq. (12), in which the amount of carbon substrate used for the product formation is assumed to be negligible.

$$\frac{dS}{dt} = \frac{1}{Y_{\text{SS}}} \frac{dX}{dt} MX$$

**Monod model**

At the stationary phase, $dX/dt$ is zero and $X$ is $X_m$. Therefore, $M$ can be obtained using the following equation:

$$M = \frac{[-(dS/dt)]}{X_m}$$

The carbon substrate (i.e., starch) used for cell growth was computed after deduction of starch used for maintenance of the cell from the experimental residual starch.

Now, $Y_{\text{SS}}$ is the ratio of cell mass growth and mass of substrate used for cell growth. $Y_{\text{SS}}$ can be expressed as

$$Y_{\text{SS}} = \frac{dX}{dS}$$

We assumed that $Y_{\text{SS}}$ is constant throughout the fermentation and the rate of starch utilization can be expressed as

$$\frac{dS}{dt} = \left(\frac{1}{Y_{\text{SS}}} \left(\frac{\mu_{\text{SS}} S}{K_S + S}\right) + M\right)X$$

$Y_{\text{SS}}$ is calculated from experimental data.

$$Y_{\text{SS}} = \frac{X - X_0}{S - S_0}$$

$X_0$ and $S_0$ are the initial biomass and substrate concentration, respectively.

The value of $X$ is replaced by Eq. (15) and we get

$$\frac{dS}{dt} = \left(\frac{1}{Y_{\text{SS}}} \left(\frac{\mu_{\text{SS}} S}{K_S + S}\right) + M\right)Y_{\text{SS}}(S - S_0) + X_c$$

For the Andrew model

$$\frac{dS}{dt} = \frac{1}{Y_{\text{SS}}} \frac{\mu_{\text{SS}} S}{K_S + S + K_S} X$$

The differential equation of the model was solved, and the simulated substrate profile with $t$ was determined using Xpp out software based on the Runge-Kutta method. The simultaneously simulated value for cell mass concentration, $X$, was calculated from Eq. (12). After integration of Eq. (12), we get

$$-(S - S_c) = 1/Y_{\text{SS}}[X_c(X - X_c) + M \int X_c e^{\mu x} dt]$$

Finally, we get

$$-(S - S_c) = 1/Y_{\text{SS}}[X_c(X - X_c) + \frac{M X_c e^{\mu x}}{\mu}]$$

**Logistics model and modified logistic model**

After integrating Eq. (12), for the logistic model we get

$$-(S - S_c) = 1/Y_{\text{SS}}\left[\frac{X_c e^{\mu x}}{1 - (X/X_0)(1 - e^{-\frac{t}{t_\text{c}}})} - X_c\right] + M/(X_c/k)\ln[1 - (X/X_0)(1 - e^{-\frac{t}{t_\text{c}}})]$$

For the modified logistic model

$$-(S - S_c) = 1/Y_{\text{SS}}\left[\frac{X_c e^{\mu x}}{1 - (X/X_0)(1 - e^{-\frac{t}{t_\text{c}}})} - X_c\right] + M/\mu \ln[1 - (X/X_0)(1 - e^{-\frac{t}{t_\text{c}}})]$$

From experimental data, $Y_{\text{SS}}$ and $M$ were determined by GraphPad Prism software from Eq. (21) and Eq. (22) by nonlinear regression analysis.
The simulated biomass profile with t was determined using Xpp out software. The simulated substrate profile with t was determined using Xpp out software. Kono and Asai model Equation (14) was rearranged and the simulated substrate profile with t was determined using Xpp out software.

Protease production kinetics.
Monod and Andrew model
Alkaline protease production kinetics was done using the Leudeking–Piret model. According to this model, the product formation rate depends on both the instantaneous biomass concentration (X) and the growth rate, in a linear manner.

\[ \frac{dP}{dt} = \alpha \frac{dX}{dt} + \beta X \]  

or

\[ r_n = \alpha r + \beta X \]

where \( \alpha \) and \( \beta \) are the product formation constants, which may vary with fermentation conditions. Dividing both sides by \( X \), we get the following equation:

\[ \frac{1}{X} r_n = \frac{1}{X} \alpha + \frac{\beta}{X} \]

or

\[ v = \alpha \mu + \beta \]

Regression analysis was used for best fit of the straight line on plot of \( v \) and \( \mu \) for finding the parameters \( \alpha \), \( \beta \).

Logistics model and modified logistics model
Integration of Eq. (24), using Eq. (5) (logistic incorporated Leudeking–Piret equation), resulted in

\[ (P - P_0) = \alpha \left[ \frac{e^{\mu t}}{1 - (X/X_m)(1 - e^{\mu t})} - 1 \right] X \]

\[ \beta \cdot (X/X_m) \ln \left[ 1 - (X/X_m)(1 - e^{\mu t}) \right] \]

Integration of Eq. (24), using Eq. (7) (modified logistic incorporated Luedeking-Piret equation), gives

\[ (P - P_0) = \alpha \left[ \frac{X_m e^{\mu t}}{1 - (X/X_m)(1 - e^{\mu t})} - X_m \right] + \]

\[ \beta \cdot 1/e^{\mu t} \cdot (X/X_m) \ln \left[ 1 - (X/X_m)(1 - e^{\mu t}) \right] \]

From experimental data, \( \alpha \) and \( \beta \) were determined by GraphPad Prism 5 software from Eq. (28) and (29) by nonlinear regression analysis.

Kono and Asai model
In the exponential phase of the Kono–Asai model, the rate of formation of product is given by

\[ \frac{dP}{dt} = q_{p1} X \]

Substituting Eq. (9) in Eq. (30) gives

\[ \frac{dP}{dt} = \frac{q_{p1}}{\mu} \frac{dX}{dt} \]

Integrating Eq. (31), we get

\[ (P - P_0) = \frac{q_{p1} X_m}{\mu} (e^{\mu t} - 1) \]

Till \( t = t_c \)

RESULTS AND DISCUSSION

Cell Growth and Substrate Utilization Kinetics
Protease production was done using Bacillus sp. in batch culture with the optimized medium. Graph Pad Prism 5 software was used to find the kinetic parameters using the Monod equation as shown in Fig. 1A. The values of kinetic parameters such as \( \mu_{max} \) and \( K_s \) were found to be equal to 0.075 h\(^{-1}\) and 5.784 g/l respectively. The correlation coefficient (\( R^2 \)) and Pearson correlation coefficient (r) were found to be 0.9691 and 0.9849, respectively. The lower correlation coefficient value of the Monod model by using experimental data might be due to either product or substrate inhibition. From the experiment, we found a typical sigmoid growth trend involving a lag phase, an exponential phase, and a stationary phase, which is similar...
to previous observations on microbial growth [24, 26, 32]. Analysis of the growth curve under different substrate concentration conditions suggested that starch concentration regulates the growth pattern. The specific growth rate increased up to 30.8 g/l of starch concentration. At higher starch concentrations, substrate inhibition of microbial growth was found. For protease production, no report of product inhibition was found. Thus, the effect of substrate inhibition was only considered for modeling.

Several substrate inhibition kinetic models were examined and compared in this work (Table 1). The Andrew’s model for substrate inhibition of microbial growth is more suitable for this process (Fig. 1B). The values of kinetic parameters such as $\mu_{max}$, $K_s$, and $K_i$ were found to be equal to 0.109 h$^{-1}$, 11.1 g/l, and 0.012 l/g, respectively. The correlation coefficient ($R^2$) and Pearson correlation coefficient ($r$) were found to be 0.9908 and 0.9954, respectively. The higher correlation coefficient value of Andrew’s model indicates that it is a better model compared with the Monod and other substrate inhibition models in this case.

In protease fermentation, the increase in biomass concentration was accompanied by a decrease of starch concentration. We considered that starch is consumed for cell growth and cell maintenance. $Y_{X/S}$ was calculated from Eq. (16) using experimental data, by averaging values of $Y_{X/S}$ obtained at different data points. Its value (0.979–1.003) was fairly constant up to a substrate concentration of 30.8 g/l, and then decreased.

**Protease Production Kinetics**

The protease production started when the cells entered the exponential phase. The maximum rate of protease and biomass production was found at about 72 h of incubation. This means that protease production was associated with cell growth, similar to other enzymes produced by microbial strains [13, 23, 27]. A plot of biomass growth ($X$) versus protease production ($U$) gave a straight line in Fig. 2 as well. Protease production increased progressively with an increase in starch concentration up to 30.8 g/l, but further increase in substrate concentration led to a decrease in

![Fig. 2. Relationship between substrate concentration and alkaline protease production.](image)

![Fig. 3. Plot of specific alkaline protease production rate vs. specific growth rate using the Leudeking–Piret model.](image)
Fig. 4. Time profile of biomass, substrate, and protease production in batch fermentation using different starch concentrations of 2.5 g/l (A), 5 g/l (B), 10 g/l (C), 15 g/l (D), 20 g/l (E), 25 g/l (F), 30.8 g/l (G), 35 g/l (H), and 40 g/l (I).
enzyme production, which indicates that protease production is carbon-source-mediated. The same finding was observed in other microbial strains [27, 28, 31, 32].

The Leudeking–Piret model was considered to find the mode of alkaline protease production. The plot of specific alkaline protease production rate vs. specific growth rate is shown in Fig. 3. The value of growth-associated constant ($\alpha$) was found to be 2.39. Since protease production is associated with cell growth, the value of the non-growth-associated parameter ($\beta$) was considered as zero. The Andrew’s model was used in Eq. (20) and (21) to find the simulated substrate utilization and biomass formation profile, respectively. Using the Leudeking–Piret model, a simulated protease production profile was obtained. Time-dependent biomass formation, substrate utilization, and product formation profiles are shown in Fig. 4, using different starch concentrations (2.5 to 40 g/l). A production medium with the same composition and various initial starch concentrations (2.5 to 40 g/l) was used to evaluate the effect of the growth-associated constant ($\alpha$) on substrate concentration. Fig. 5 showed that the growth-associated constant for protease production ($\alpha$) was sensitive to the substrate concentration. The value of coefficient ($\alpha$) in the growth phase was found to be maximum (2.47) at 10 g/l starch concentration. The value of coefficient was approximately the same up to the substrate concentration of 30.8 g/l. At higher starch concentrations, the value decreased. This might be due to catabolic repression on protease synthesis observed at higher concentrations of substrate [15, 21, 22].

Different Unstructured Model
Several unstructured kinetic models were tested and compared in this work. The models used in this study are Monod, Andrew, logistics, modified logistics and Kono and Asai. The Leudeking–Piret model was used to find the mode of alkaline protease production for all models except the Kono and Asai model. The simulated biomass, substrate, and product formation profiles are shown in Fig. 6A, 6B, and 6C, respectively, using different unstructured models. The initial starch concentration was taken as 30.8 g/l ($S_0$) in simulated media. Table 2 shows the estimated model parameters of the various unstructured models for microbial growth, substrate utilization, and product formation.

The correlation coefficient ($R^2$) for biomass, substrate, and product formation are shown in Table 3. The Andrew’s model showed a higher correlation coefficient value for biomass formation (0.9986), substrate utilization (0.991), and product formation (0.9875). The $R^2$ of the experimental and the predicted values was analyzed to find out the best-fit model for this enzyme production process. Among the various unstructured kinetic models tested, the Andrew’s model for microbial growth, and substrate utilization, and the Andrew-incorporated Luedeking–Piret model for protease production provided an accurate approximation of
the fermentation kinetics. The Andrew’s model showed comparatively better $R^2$ values among all the tested models for all kinetics. Thus it shows good resemblance of simulated substrate utilization, cell mass formation, and protease production values with the experimental findings. The little deviation of simulated and experimental curves for substrate utilization may be due to the assumption that $Y_{X/S}$ remained constant over the system, irrespective of time of fermentation [17].

In the present investigation, various unstructured models were tested to predict the biomass formation, substrate consumption, and product formation during the fermentation process. Table 3 gives a quantitative comparison between the suitability of the various unstructured models in the present system. It may be concluded that the Andrew’s model is the most suitable one in the present case for prediction of biomass formation and substrate consumption. As regard the product formation, the Leudeking–Piret model gives almost identical data for the Andrew and Monod models (Table 3). However, since the Andrew’s model has already been established as a better one over the Monod model, it may be concluded that, in totality, the Andrew’s model is the most suitable one to predict product distribution in the present case. It is further noticed that as in Fig. 5, alkaline protease production is growth-associated, and the growth-associated constant ($\alpha$) depends on substrate concentration beyond a concentration of 30.8 g/l.

### Nomenclature

- $K_i$: Inhibition constant (g/l)
- $K_s$: Saturation constant (g/l)
- $r_{ip}$: Rate of protease production (U/h)
- $r_{ix}$: Rate of cell mass production (g/l)
- $S$: Starch concentration (g/l)
- $S_i$: Initial starch concentration (g/l)
- $t$: Time of fermentation (h)
- $X$: Cell mass concentration at any time of fermentation (g/l)
- $X_0$: Initial cell mass concentration (g/l)
- $Y_{X/S}$: Yield coefficient
- $\alpha$: Growth-associated coefficient (h$^{-1}$)
- $\beta$: Non-growth-associated coefficient (h$^{-1}$)
- $\mu$: Specific growth rate (h$^{-1}$)
- $\nu$: Specific production rate (U/h)

### Table 2. Estimated kinetics model parameters for batch protease production.

<table>
<thead>
<tr>
<th>Kinetic Model</th>
<th>Growth kinetic model</th>
<th>Product formation model</th>
<th>Substrate utilization</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
<td>B</td>
<td>C</td>
</tr>
<tr>
<td>$\mu_{max}$</td>
<td>0.075</td>
<td>0.1089</td>
<td>-</td>
</tr>
<tr>
<td>$K$</td>
<td>-</td>
<td>-</td>
<td>0.0793</td>
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<tr>
<td>$X_m$</td>
<td>5.784</td>
<td>11.1</td>
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</tr>
<tr>
<td>$K_i$</td>
<td>-</td>
<td>0.012</td>
<td>-</td>
</tr>
<tr>
<td>$X_0$</td>
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<td>0.306</td>
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<td>-</td>
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</tr>
<tr>
<td>$\beta$</td>
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<td>-</td>
</tr>
<tr>
<td>$Y_{X/S}$</td>
<td>-</td>
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</tr>
<tr>
<td>$M$</td>
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<tr>
<td>$X_c$</td>
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<td>$T_c$</td>
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<td>$\mu$</td>
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<td>$P_t$</td>
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<tr>
<td>$P_{pit}$</td>
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### Table 3. Statistical analysis for evaluation of the different unstructured models.

<table>
<thead>
<tr>
<th>Different models</th>
<th>$R^2$ value for biomass</th>
<th>$R^2$ value for substrate</th>
<th>$R^2$ value for product</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monod</td>
<td>0.9985</td>
<td>0.9869</td>
<td>0.9875</td>
</tr>
<tr>
<td>Andrew</td>
<td>0.9986</td>
<td>0.991</td>
<td>0.9875</td>
</tr>
<tr>
<td>Logistics</td>
<td>0.9796</td>
<td>0.9752</td>
<td>0.9482</td>
</tr>
<tr>
<td>Modified logistics</td>
<td>0.9915</td>
<td>0.9664</td>
<td>0.9649</td>
</tr>
<tr>
<td>Kono and Asai</td>
<td>0.9765</td>
<td>0.9246</td>
<td>0.9604</td>
</tr>
</tbody>
</table>
\[ \frac{dX}{dt} \] Growth rate (g/l·h)

**REFERENCES**


