CHAPTER 4

FERMENTATION GROWTH KINETIC MODELING OF COQ10 PRODUCTION

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4.4 REFERENCES
4.1 INTRODUCTION

CoenzymeQ10 is a natural compound which consists of benzaquinone head joint together with the isoprene decaprenyl diphosphate side chain. Coenzyme Q10 play a very important role in the respiratory chain reaction in the inner mitochondrial membrane in human and in the plasma membrane of some prokaryotes. CoenzymeQ10 is also present in most active metabolisms organelles such as endoplasmic reticulum Golgi apparatus, lysosomes, peroxisomes etc. [1,2] It has antioxidant and anti-aging properties for which it is used as a pharmaceutical and nutraceutical compounds to prevent DNA damage due to oxidative damage stress .it also prevents the cardiovascular disease lipid per oxidation and protein oxidation.[3,4,5] Keeping in view of its valuable uses many efforts has been made to produce CoQ10 by chemical synthesis and by microbial production. Chemical synthesis is a complicated and costly process. Microbial production is better approach to produce CoQ10. [6] Many scientists have been working to develop a suitable fermentation from various numbers of bacteria such as Rhodobacter spheroids, Paracoccus denitrificans, agrobacterium, cryptococcus laurentii and tricosporan sporobolamycs etc. Many strategies have been applied to improve the production of CoQ10 such as strain improvement by Chemical and physical mutagenesis as well as by optimization of culture media and culture conditions for fermentation process. [7, 8, 9] Growth kinetics models of fermentation processes are also developed for better understanding and control and optimization of fermentation process and to know accurate and easy description of the process. [10, 11, 12, 13, 14] In this research a mathematical model of fermentation growth kinetics was developed of Paracoccus denitrificans for the production of CoQ10 based on calculated kinetic parameters and characteristics of substrate consumption and product formation for the improvement in CoQ10 production in batch fermentor.
4.2 MATERIALS AND METHODS

4.2.1 Medium components

The medium ingredients used for the growth of bacteria were sucrose 20g l\(^{-1}\), ammonium chloride 1g l\(^{-1}\), potassium di-hydrogen phosphate 2.3g l\(^{-1}\), di-sodium hydrogen phosphate 2.9g l\(^{-1}\), magnesium sulphate 0.5g l\(^{-1}\), sodium bicarbonate 0.5g l\(^{-1}\), calcium chloride 0.01 g l\(^{-1}\), ferrous ammonium citrate 0.05g l\(^{-1}\) (autoclaved separately), sodium succinate 6.75g l\(^{-1}\), yeast extract 10g l\(^{-1}\) and trace elements solution 1 ml l\(^{-1}\) were purchased from Qualigens (Mumbai, India). Organic solvents and reagents used for extraction, separation, product isolation and purification were obtained from Qualigens (Mumbai, India). All glass wares and materials used for this study were sterilized in autoclave (NSW Mumbai, INDIA) twice at 121\(^{\circ}\)C for 15 minutes to prevent any influences by other microorganisms.

4.2.2 Microorganisms and culture conditions

The bacterial strain used for this research was developed and procured from Advanced Centre for Biotechnology named as *Paracoccus denitrificans* ACBT-M4. Initially, the culture conditions were maintained for the growth, as LB media, pH 6.8 and 28°C incubation temperature. DCW was calculated with the standard curve (Annexure II).

4.2.3 Fermentation conditions

The size of fermentor (Sartorius) used for this study was 60 L having 30 liter working volume. The culture medium for fermentation was same as shake flask level. The fermentation medium having pH 6.8, adjusted with 25 % KOH and 20 % HCl solutions, and temperature 28°C. The aeration level, back pressure, and agitation speed were 0.5-0.7 vvm, 7.5 psi, 250 rpm respectively and maintained constant during the process run. The culture samples were collected at various time intervals to test the residual sugar and CoQ10 content. The packed cell volume (PCV) was measured by centrifuging 10 ml sample at 3000 rpm to measure the biomass content. The supernatant was analyzed for residual sugar. The residual sugar conc. analysis was done by di-nitro salicylic acid (DNSA) calorimetric methods [Annexure II].
4.2.4 Extraction process

50 ml broth was taken from exponential growth phase in conical flask, and added 10 g l\(^{-1}\) of glass beads, 1 ml l\(^{-1}\) triton X 114, 0.5 g l\(^{-1}\) SDS, the mixture of n-propanol and n-hexane in the ratio 1:2. The mixture was put on shaker at 32°C with 250 rpm for 2 h to extract CoQ10 from the bacterial cells. Organic layer is separated and analyzed by HPLC.

4.2.5 Analytical method

The HPLC system (Shimadzu, Class-VP-10) equipped with a 4.0 mm id X 250 mm Lichrospher RP-18 column LiChroCART 5 µ Merck was run at room temperature. The reverse phase HPLC method developed and validated for determination of CoQ10. The isocratic elution with ethanol and methanol in ratio 4:1 and 5.6 g l\(^{-1}\) sodium perclorate was used, at the flow rate of 1.0 ml/min. The injection volume was 20 µl and detected at the wavelength 275nm. The amount of CoQ10 was estimated from calibration curve obtained with a standard CoQ10.

4.3 RESULTS AND DISCUSSIONS

4.3.1 Model development

In this research fermentation growth kinetics model have been developed and subdivided in to three parts growth model, substrate utilization model, and product formation model and separately three equations are derived for the growth of Paracoccus denitrificans cells, sugar utilization and CoQ10 production. Previously scientists have used a Monod type of model for batch fermentation of low cell concentration fermentation processes. Recently some workers have developed a new modified model which is known as sigmoid shape model it is applied widely and described well the growth of many microorganisms. This model is used to show the self regulation made by the high cell concentration in fermentation.

In this research the equations have been developed for the product formation kinetics combined with growth associated and non growth associated product formation.

For cell concentration, X, the logistic model was derived as follows:
\[
\frac{dx}{dt} = \mu_m X \left(1 - \frac{X}{X_m}\right) \tag{1}
\]

where \(\mu_m\) is the maximum specific growth rate with respect to the fermentation conditions. With the following initial conditions:

\[X(t) = X_0, \quad S(t) = S_0, \quad P(1) = P_0 \text{ when } t = 0\]

By integration of Eq. (1), the kinetic model can be formulated. The biomass production rate yields the following equation (the logistic equation):

\[
X(t) = \frac{X_0 X_m e^{\mu_m t}}{X_m - X_0 + X_0 e^{\mu_m t}} \tag{2}
\]

This equation shows the relationship of biomass and the fermentation time, which is used to fit the experimental data of biomass concentration.

As observed in the present experiment (Figure 1), CoQ10 accumulation was associated not only with the cell growth rate but also with the cell concentration, therefore a product formation kinetic combined non-growth-associated and growth-associated contributions could be considered in this study. The following mixed growth-associated model can be adopted:

\[
\frac{dp}{dt} = \alpha \frac{dx}{dt} + \beta X = \alpha \mu X + \beta X = Y_{p,x} \frac{dX}{dt} + \beta X \tag{3}
\]

This equation can be integrated and described by the Eq. (4)

\[
P(t) = P_0 - Y_{p,x} X_0 + Y_{p,x} \frac{X_0 X_m e^{\mu_m t}}{X_m - X_0 + X_0 e^{\mu_m t}}
+ \frac{\beta X_m}{\mu_m} \ln \frac{X_m - X_0 + X_0 e^{\mu_m t}}{X_m} \tag{4}
\]

Finally, substrate utilization kinetic usually may be expressed by the following equation which consist both substrate consumption for maintenance and substrate conversion [19, 20].

\[
\frac{ds}{dt} = \frac{1}{Y_{s,x}} \frac{dX}{dt} + \frac{1}{Y_{p,x}} \frac{dP}{dt} mX \tag{5}
\]

Actually, CoQ10 is a component of the biomass [1,21,22], the second term of this equation may be neglected. So the substrate utilization rate can be written as
Growth kinetics of coenzyme Q10 production in 60 L batch fermentor

- coenzyme Q10
- Residual sugar
- DCW in gm/L (Biomass)

Figure 1 Fermentation profiles of *Paracoccus denitrificans* ACBT-M4 in 60 L fermentor
\[
\frac{ds}{dt} = \frac{1}{Y_{x/s}} \frac{dX}{dt} mX
\]  
(6)

The sugar utilization equation can be obtained as Eq. (7) by integrating Eq. (6)

\[
S(t) = S_0 + \frac{X_0}{Y_{x/s}} - \frac{X_0 X_m e^{\mu_m t}}{Y_{x/s} (X_m - X_0 + X_0 e^{\mu_m t})} \\
+ \frac{mX_m}{\mu_m} \ln \left( \frac{X_m - X_0 + X_0 e^{\mu_m t}}{X_m} \right)
\]  
(7)

4.3.2. Solving and analysis of the model

The model contains a total of nine kinetics parameters that must be determined in order to simulate the process, these values were evaluated by fitting the mathematical models to the experimental data, using software package Minitab 15© (trial version) (Levenberg Marquandt optimization), so as to minimize the sum of squares of the deviations between the experimental measurements and the model predications. Table 1 presents the values of the nine parameters obtained for modeling this fermentation process.

4.3.3. Paracoccus denitrificans ACBT-M4 growth kinetics

Biomass concentrations were fitted by Eq. (2) in Figure 2(a), using the estimated parameters. As can be observed from the figure, the model fits well to the experimental results, with high significance for model fitting (all the Pr< 0.002), and the correlation coefficients, r², of model fitting is 0.942. The maximum specific growth rate, \( \mu_m \), was 0.9416 h⁻¹.

4.3.4. Substrate consumption kinetics

The predicted evaluations of the sugar by Eq. (7) during the fermentation process are shown in Fig 2(b), together with the experimental data. This figure indicates that the prediction of the model agreed well with the experimental in this case. The model was verified with high significance (all the Pr< 0.002), and r² of model fitting was 0.942. The bacterial yield, \( Y_{x/s} \), was 19.67. The maintenance coefficient, \( m \), had a value, 0.0053.
Figure 2 Comparisons of experimental data and kinetic model predictions of Paracoccus denitrificans ACBT-M4 CoQ10 batch production in repeated batch fermentation (a) Bacterial growth (b) Sucrose utilization (c) CoQ10 production in 60 l Fermenter.
Table 1 Parameters of kinetic models for a batch culture of CoQ10 production

<table>
<thead>
<tr>
<th>Parameters</th>
<th>$X_0$ (g l$^{-1}$)</th>
<th>$X_m$ (g l$^{-1}$)</th>
<th>$\mu_m$ (h$^{-1}$)</th>
<th>$P_0$ (mg l$^{-1}$)</th>
<th>$Y_{p/x}$ (mg g$^{-1}$)</th>
<th>$\beta$ (mg l$^{-1}$)</th>
<th>$S_0$ (g l$^{-1}$)</th>
<th>$Y_{x/y}$ (mg g$^{-1}$)</th>
<th>$M$ (g g$^{-1}$ h$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Value</td>
<td>0.852</td>
<td>56.88</td>
<td>0.94</td>
<td>5.54</td>
<td>6.87</td>
<td>0.106</td>
<td>20.7</td>
<td>19.67</td>
<td>0.0053</td>
</tr>
<tr>
<td>SD</td>
<td>0.043</td>
<td>2.825</td>
<td>0.05</td>
<td>0.28</td>
<td>0.34</td>
<td>0.005</td>
<td>1.02</td>
<td>0.984</td>
<td>0.0003</td>
</tr>
</tbody>
</table>

Parameters were evaluated by the non-linear least squares method of Levenberg-Marquardt optimization, which was used to minimize the residual sum of squares, using batch experimental data. Values SD denote the approximal standard errors of the estimated parameters.

4.3.5. CoQ10 production kinetics

Experimental data and the predicated, by the kinetic model for CoQ10 accumulation are shown in Figure 2(c). A high significance for model fitting was verified (all the $Pr<0.002$), and $r^2$ is 0.950. There was little difference between the experimental data and the prediction results, and data predictions obtained from the model were reasonable and in some cases very accurate.

4.3.6 Model-batch CoQ10 fermentation

Based on estimated kinetic parameters and characteristics of product formation and substrate utilization, a strategy of model-batch operation was adopted in order to obtain improved CoQ10 accumulation. Other details are same as described in materials and methods. The results were shown in Figure 3. From this figure we can see that the production of CoQ10 reached 371.5 mg l$^{-1}$ in 72 h of incubation corresponding to 7.5 % more than that of batch operation, and the biomass climbed up to 64 g l$^{-1}$. The results suggested that high production of CoQ10 might be obtained if this model-batch strategy system can be employed.
Figure 2 (C) CoQ10 Production

Growth kinetics according to model simulation

Figure 3 Fermentation profile of CoQ10 production from Paracoccus denitrificans ACBT-M4 according to the model simulation in 60 l fermenter.
4.4 REFERENCES


