Model-based fed-batch cultivation of *R. eutropha* for enhanced biopolymer production

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Abstract

Batch kinetics of the PHB synthesis was established in a bioreactor under controlled environmental conditions of pH and dissolved oxygen. The data of the batch kinetics was used to develop and identify a mathematical model. The nutrient feeding strategies in the fed-batch cultivation were developed aiming at maintenance of limiting nutrients for either growth enhancement or increased intracellular product accumulation by offline computer simulation of mathematical model. The selected fed-batch fermentation strategies, using the predicted feeding profiles, were experimentally implemented to improve the growth/product formation/productivity and establish the validity of the developed model. The model predictions were very close to the observed experimental data points.

Keywords: PHB; Modelling; Model-based fed-batch cultivation

1. Introduction

Plastic materials have become an integral part of contemporary life because of many desirable properties including durability and resistance to degradation. These non-degradable plastics accumulate in the environment at a rate of more than 25 million tonnes per year [1]. Recently, the problems concerning the global environment and solid waste management have created significant interest in the development of biodegradable plastics, which must still retain the desired physical and chemical properties of conventional synthetic plastics.

Some of the biodegradable plastic materials under development include polyhydroxyalkanoates (PHAs), polyesters, aliphatic polyesters, polyacrylates, and copolymers and blends of starch and polypropylene can be regarded only as semi-biodegradable while PHA is 100% biodegradable.

Polyhydroxybutyrate (PHB) belongs to the class of biodegradable plastics, polyhydroxyalkanoates. These polymers are polyesters of various hydroxyalkanoates and are synthesized by numerous microorganisms as energy reserve materials under unbalanced cultivation conditions, i.e. by limitation of some essential nutrient and under excess available carbon source [2]. They play the same role in bacteria that fat does in humans or starch in plants.

PHB was first among the family of PHAs to be detected by Lemoigne in 1936 as a constituent of bacterium *Bacillus megaterium* [3]. Since then PHB has been shown to occur in a variety of taxonomically different groups. Most of the organisms (e.g. *Azotobacter, Bacillus, Pseudomonas, Rhizobium, Methylophilus, etc.* ) are capable of accumulating PHB up to 30–80% of their cellular dry weight [4].

PHB is a highly crystalline polymer and its melting point is 175 °C. It decomposes at 200 °C. Its mechanical properties like flexural modulus and tensile strength are similar to polypropylene. It is 100% biodegradable [5].

It is used in packaging films, bags, containers, disposable items like cups and diapers. It is also used as biodegradable carrier for long-term dosage of drugs and insecticides/pesticides [1].

PHB production by different microorganisms has been attempted but still there is a need to address the problem of improvement of yield and productivity of PHB production so that it can economically compete with the production cost of conventional plastic material. The applicability of model-based design of nutrient strategies for fed-batch cultivation for improved PHB production has been demonstrated.
2. Materials and Methods

2.1. Maintenance of Culture

The bacterial strain used in the present study was *Rhodobacter sphaeroides* NBR1 14690. It was maintained on Luria Agar slants at 4°C. It was subcultured every 15 days to maintain its viability.

2.2. Dry cell weight

Optical density (OD) of the suitably diluted cell suspension was measured at 600 nm against a medium blank in a spectrophotometer (Spekol 1200, Analytik Jena, Germany). Biomass was estimated from an OD versus concentration (g/l) correlation, 1 OD₆₀₀ = 0.446 g/l of biomass.

2.3. Fructose concentration

Fructose concentration was determined by the DNS method [6]. A standard plot of OD (540 nm) versus fructose concentration was used to get the correlation between optical density and concentration of fructose (1 OD₅₄₀ = 0.860 g/l fructose.

For the determination of residual fructose in the samples, the samples from fermenter were centrifuged at 10,000 rpm and the supernatant was suitably diluted to bring the fructose concentration in range of 0.1–1.0 g/l in order to use above correlation.

2.4. Nitrogen concentration

Ammonium sulphate concentration was estimated by Berthelot reaction [7,8]. Standard plot of OD at 630 nm versus ammonium sulphate concentration was used to get the correlation between optical density and concentration of ammonium sulphate. The correlation was obtained as, 1 OD₆₃₀ = 6.79 g/l ammonium sulphate.

For the determination of residual ammonium sulphate in the samples, the samples from fermenter were centrifuged at 10,000 rpm and suitably diluted to bring the concentration in range of 1–4 g/l. The supernatant was analysed for nitrogen using the correlation obtained from the standard plot.

2.5. PHB concentration

2.5.1. Gas chromatography (GC) [9]

An amount of 200 mg of PHB was dissolved by heating in 10 ml of dichloromethane (DCM). After cooling to room temperature, the mixture was made up to 10 ml. A total of 200, 400, 600, 800, 1000 µl of this solution were taken in tightly scalable bottles. A 2 ml of DCE, 2 ml of acidified propanol (1:4, hydrochloric acid:1-propanol) and 200 µl of internal standard (40 g/l benzoic acid prepared in 1-propanol) were added. The bottles were sealed with rubber seal and crimped by aluminium cap and kept in incubator at 100°C for 2 h. The mixture was shaken at the beginning and also during incubation from time to time. PHB was thus converted to propyl ester of hydroxylbutyric acid (HBA). After cooling to room temperature, 4 ml of water was added and mixture shaken for 20–30 s. The heavier phase (DCE-propanol) was injected into the gas chromatograph column.

The gas chromatography conditions were as follows: glass column packed with 2% Reoplex on Chromosorb W, oven
temperature, 130 °C; detector temperature, 170 °C; injector temperature, 160 °C; injection volume, 1 μl.

Ester of HBA eluted at 2.2 min while internal standard eluted at 5.9 min. A standard curve of peak area quotient Q (area of ester of HBA/area of ester of benzoic acid) versus PHB was plotted. The correlation obtained was, 1 unit (Q = 8.98 mg PHB).

For determination of PHB in cells, 50 ml of broth was centrifuged at 10,000 rpm. The cell pelleted was washed and resuspended in distilled water and then kept in oven at 50 °C for drying. A 40 mg of dried cells were treated as above. An 1 μl of the sample was injected into GC. The areas of ester of HBA and ester of benzoic acid obtained from GC graph were used to calculate Q. The amount of PHB in the cells was determined from the standard plot.

2.6. Media

The media for R. eutropha cultivation as reported in the literature was used in this investigation [10]. In all cases, fructose and rest of the media components were sterilized separately and mixed aseptically before inoculation.

2.7. Seed inoculum and culture conditions

2.7.1. Inoculum development

2.7.1.1. Preinculture 1. Media containing 10 g/l fructose and the other nutrients as described in [10] was used for inoculum development. A 50 ml of media was taken in a 250 ml flask and inoculated with two loopful of culture grown on Luria Agar slant. The flask was kept in the shaker at 150 rpm and 30 °C. The initial pH of the media was adjusted to 7 with 2N sodium hydroxide. When OD_{600} of the media in the flask reached 0.6 (10 times diluted), the required amount of inoculum was transferred to the next stage.

2.7.1.2. Preinculture 2. An 100 ml of media containing 40 g/l fructose and the other nutrients as described in [10] was taken in a 500 ml flask and inoculated with 5 ml of inoculum from the earlier stage. The flask was kept in the shaker at 150 rpm and temperature was maintained at 30 °C. The initial pH of the media was adjusted to 7 with 2N sodium hydroxide. When OD_{600} of the media in the flask reached 0.6 (10 times diluted), the required amount of inoculum was transferred to the next stage.

2.7.2. Batch fermentor studies

Development of inoculum was done in two stages as mentioned earlier. When OD_{600} of the media in the flask reached 0.6 (10 times diluted), the required amount of inoculum was transferred to the fermentor.

Fermentor studies were carried out in a biocengineering fermentor. Initial volume of the media taken was 1.5 l. The fermentor was then sterilized at 121 °C for 20 min in autoclave, cooled and then inoculated with 5% inoculum (75 ml for initial volume of 1.5 l). Temperature was maintained at 30 °C. pH was maintained at 7 using acid/dilute addition (2N sodium hydroxide and 2N hydrochloric acid). Oxygen was supplied by sparging sterile air at flow rate of 4 l/min. Dissolved oxygen concentration was maintained at 30% saturation value by manually adjusting the speed of the agitator. 2.8. Fed-batch fermentor studies

Development of inoculum was done in two stages as mentioned earlier. When OD_{600} of the media in the flask reached 0.6 (10 times diluted), the required amount of inoculum was transferred to the next stage (fermentor).

Fermentor studies were carried out in a biocengineering fermentor. Initial volume of the media taken was 1.5 l for 3 l reactor. The reactor was then sterilized at 121 °C for 20 min in autoclave, cooled and then inoculated with 5% inoculum (75 ml for initial volume of 1.5 l). Temperature was maintained at 30 °C. pH was maintained at 7 using acid/dilute addition (2N sodium hydroxide and 2N hydrochloric acid). Oxygen was supplied by sparging sterile air at flow rate of 4 l/min. Dissolved oxygen concentration was maintained at 30% saturation value by manually adjusting the speed of the agitator. The fermentor was run as batch till 17 h after which the feeding of nitrogen/fructose was started as per the various nutrient feeding strategies predicted by the model.

3. Experimental results

3.1. Cultivation of R. eutropha in bioreactor

For batch cultivation of R. eutropha, media and inoculum were used as described earlier. R. eutropha was grown at 30 °C, pH was maintained at 7 with acid/dilute addition (2N sodium hydroxide and 2N hydrochloric acid). Dissolved oxygen concentration was maintained at 30% of saturated value.

Three batch fermentor experiments were done. The average values of biomass, PHB and residual nutrients are plotted in Fig. 1. The culture entered the exponential phase

![Graph showing fermentation kinetics by R. eutropha. Comparison of the experimental data and model-based simulation results.](image-url)
after a lag of 4–5 h. Ammonium sulphate was completely consumed in 20 h. Intracellular PHB accumulation was induced from 20 h. Maximum biomass of 14 g/l was obtained at 60 h. The residual fructose concentration dropped from 40 to 8.5 g/l with 31.5 g/l of consumption. Maximum PHB concentration of 6.3 g/l was obtained at 60 h as shown in Fig. 1. Biomass yield to fructose, Y_{X/F} was 0.44, the product yield to fructose, Y_{P/F} was 0.19 and maximum specific growth rate was 0.28 h^{-1}. PHB content of the cells was 43.6%.

3.2. Modelling

Basic assumptions of the model were:

1. Biomass (\(X\)) is structured as having two components:
   (a) The catalytically active component consisting of proteins and nucleic acids (\(R\)).
   (b) The inert component which is the product PHB (\(P\)).
2. Nitrogen is the limiting substrate affecting the growth kinetics.

3.3. Batch model

\[ X = R + P \]

Rate of formation of \(R\) is given as

\[ \frac{dR}{dt} = \mu_R R \]  

(2)

The specific growth rate equation featured nutrient limitation by both macromoly and sigmoidal growth terms. The inhibition due to N/C ratio (studied by Mulchandani et al. [11]) was also incorporated in the model structure:

\[ \mu_R = \frac{\mu_{\text{max}}}{K_\mu + [\text{SN}] + \mu_{\text{max}}^* (\text{SN} / K_{\text{SN}})^n} \times \left( 1 - \left( \frac{[\text{SN}] / K_{\text{SN}}}{[\text{SN}] / K_{\text{SN}}} \right)^n \right) \]  

(3)

The rate of formation of product (\(P\)) was assumed to have growth and non-growth associated components, and inhibition due to high product concentration:

\[ \frac{dP}{dt} = K_1 (R - R_{\text{min}}) - K_2 P \frac{K_3 + [\text{SN}] + [\text{SN}] / K_{\text{SN}}}{K_4 + [\text{SN}] / K_{\text{SN}}} \]  

(4)

The fructose consumption rate was assumed to be due to growth of \(R\), formation of \(P\) and maintenance (proportional to \(R\)):

\[ \frac{dS_F}{dt} = -\frac{dR}{dt} - \frac{dP}{dt} + b \frac{dP}{dt} - c \frac{dR}{dt} \]  

(5)

Nitrogen was consumed due to the growth of the catalytically active biomass (\(R\)):

\[ \frac{dSN}{dt} = -f \frac{dR}{dt} \]  

(6)

Three batch experiments were done in 31 reactor. Average values of different process variables (biomass, nitrogen, PHB and fructose) at different time points were calculated and the experimental kinetic data was used for identification of model parameters. For optimal estimation of model parameters, a non-linear regression technique assisted by a computer program [12–14] was used to minimize the deviations between the model predictions and the experimental batch results. For the calculation of the model predictions, the system of differential equations of the model was solved using an integration program based on the Runge-Kutta method of fourth order. The optimization program for the direct search of the minimum of the multivariable function was based on the original method of Rosenbrock [15]. The minimization criteria used in the program were as follows:

\[ SSWR = \sum_{i=1}^{n} \sum_{j=1}^{m} w_i^2 \frac{\Delta P_{ij}^2}{w_j^2} \]

where SSWR represents sum of the square of weighted residuals, \(i\) and \(j\) the number of experimental data points and number of variables, respectively, \(w_j\) the weight of each variable (usually the maximum value of each variable) and \(\Delta P_{ij}\) denotes the difference between the model and experimental value.

The values of the optimized parameters were found out (Table 1). The relative parameter sensitivities as suggested by Volesky and Votava [14] was also conducted to establish the relative significance of each model parameter in the overall model simulation (based on high RPS values). Parameter \(K_1\) (indicating product accumulation rate) was identified to be the most sensitive model parameter indicating that the minor variation in this parameter will lead to significant deviation between model simulation and experimental observation.

The model equations described above in Eqs. (1)–(6) were simulated on the computer using the optimal values of the model parameters. A comparison of the model simulation (smooth line) and experimental data (points) is shown in.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>APS</th>
<th>RPS</th>
</tr>
</thead>
<tbody>
<tr>
<td>(r_{\text{act}} ) (( \text{h}^{-1} ))</td>
<td>0.2920</td>
<td>-0.4538</td>
<td>0.2655</td>
</tr>
<tr>
<td>( K_{\text{SN}} ) (( \mu ))</td>
<td>0.7480</td>
<td>-0.0452</td>
<td>0.0743</td>
</tr>
<tr>
<td>( \mu_{\text{max}} ) (( \mu ))</td>
<td>0.5780</td>
<td>-0.0206</td>
<td>0.0261</td>
</tr>
<tr>
<td>( K_{\text{SN}} ) (( \mu ))</td>
<td>5.5556</td>
<td>-0.0222</td>
<td>0.0263</td>
</tr>
<tr>
<td>( n ) (exponent)</td>
<td>2.0790</td>
<td>-0.0055</td>
<td>0.0253</td>
</tr>
<tr>
<td>( \lambda ) (g nitrogen/g biomass)</td>
<td>3.1400</td>
<td>0.00</td>
<td>0.01</td>
</tr>
<tr>
<td>( v ) (exponent)</td>
<td>3.1150</td>
<td>0.00</td>
<td>0.01</td>
</tr>
<tr>
<td>( J_0 ) (g nitrogen/g biomass)</td>
<td>0.3570</td>
<td>0.337</td>
<td>0.421</td>
</tr>
<tr>
<td>( K_{\text{SN}} ) (( \mu ))</td>
<td>0.1130</td>
<td>5.81</td>
<td>0.586</td>
</tr>
<tr>
<td>( J_0 ) (g biomass)</td>
<td>0.0384</td>
<td>-0.0057</td>
<td>0.1233</td>
</tr>
<tr>
<td>( K_{\text{SN}} ) (( \mu ))</td>
<td>0.0835</td>
<td>-2.460</td>
<td>0.478</td>
</tr>
<tr>
<td>( v ) (g PHB/g biomass)</td>
<td>0.1460</td>
<td>0.0199</td>
<td>0.0099</td>
</tr>
<tr>
<td>( v ) (g fructose/g PHB)</td>
<td>1.7640</td>
<td>-0.0015</td>
<td>0.2376</td>
</tr>
<tr>
<td>( v ) (g fructose/g biomass)</td>
<td>0.0240</td>
<td>-2.115</td>
<td>0.1116</td>
</tr>
</tbody>
</table>

APS: absolute parameter sensitivity; RPS: relative parameter sensitivity.
Fig. 1. The agreement between the model simulation and the experimental data points is clearly reflected.

To further evaluate the degree of reliability of the model, a method recommended by Bard [12] was used to test the hypothesis of a zero mean deviation of the model and experimental data for batch kinetics. The mean residual of each variable $\Delta_j$ was calculated as follows:

$$\Delta_j = \frac{1}{m} \sum_{i=1}^{n} \Delta_{ij},$$

where $n$ is the total number of experimental data points and $\Delta_{ij}$ is the difference between the experimental value of a variable and its corresponding model simulation value. The variance of the error of a residual ($S_j$) was then estimated as follows:

$$S_j = \frac{1}{n-1} \sum_{i=1}^{n} (\Delta_{ij} - \Delta_j)^2, \quad j = 1, m$$

where $m$ is the number of variables. The value of the statistics defined as

$$\lambda = \frac{(n-m)n}{(n-1)m} \sum_{j=1}^{m} \frac{\Delta_j^2}{S_j}$$

was calculated. The statistics $\lambda$ has the $F_{(n-m, n-m)}$ distribution. Its value was calculated as 0.110, which was less than the $F_{(4,12)}$ value obtained from $F$ tables for 99% confidence for the whole experimental set. This made it possible to accept the hypothesis of zero mean deviation between experimental data and the model. This established the validity of the model.

There have been previous studies on modelling of PHB fermentation. Some of the initial work on modelling of lithotrophic fermentation of R. eutropho was done by Sonnleitner et al. [16] wherein it was identified that with the exhaustion of the nitrogen in the media, the $N$ component of the biomass becomes constant and the biomass increases with $P$. The fermentation was identified to be product inhibited and PHB accumulation was found to be both growth associated and non-growth associated. Further work by Henschke and Lafferty [17] confirmed these findings. They also proposed a structural model to account for these findings. In their work the $R$ component of the cell was also assumed to be growing by a combination of moosed and sigmoidal kinetics. However, this model did not incorporate inhibition due to higher N/C content as indicated by the work of Mucuchandani et al. [11]. Inhibition studies of N/C on culture growth were done by Mucuchandani and a term accounting for N/C inhibition was incorporated. However, the model structure neglected the product inhibition term (−$k_3P$) and the term ($K_s/(K_s + S_i)$) which acts as a nitrogen switch for the production of PHB which was incorporated by Raje and Srivastava [10]. The model, structurally similar to the model by Raje and Srivastava [10] was used in the present study and the model parameters were identified by the observed batch kinetic data. The model was able to describe the different aspects of the batch culture metabolism of R. eutropho.

3.4. Feed-batch model

The batch model was extrapolated to fed-batch cultivation by incorporating the dilution factors. The fed-batch model equations are given below:

$$\frac{dV}{dt} = F_1 + F_2 = F, \quad \frac{dR}{dt} = RR - \frac{F \cdot R}{V}$$

$$\frac{dP}{dt} = RP - \frac{F \cdot P}{V} + \frac{dS_N}{dt} = \frac{F_1 \cdot S_N}{V} - \frac{F_2 \cdot S_N}{V}$$

$$\frac{dS_N}{dt} = RSF + \frac{F_2 \cdot S_N}{V} - \frac{F_2 \cdot S_N}{V}$$

Offline computer simulations of mathematical model for fed-batch were done to develop nutrient feeding strategies for limiting nutrients (fructose/nitrogen). Feed strategies showing growth enhancement and improved intracellular product accumulation were selected and experimentally implemented.

3.5. Feed-batch studies (constant nitrogen strategy)

A strategy was designed to maintain a constant low concentration of nitrogen in the fermentation broth, so that biomass increases and PHB accumulation can be induced in elevated levels of biomass. This was done by equating the rate (dS_N/dt) equal to zero and back calculating the nitrogen feeding rate for constant maintenance of nitrogen concentration in the bioreactor. It was observed from the simulation of polymer PHB production rate equation, that significantly high amount of polymer was accumulated under such conditions.

The feed rate of nitrogen was so designed such that pseudo steady state of low nitrogen concentration was maintained in the fermentation broth at a concentration available at 17 h, i.e. 0.4 g/L. This was done by simulating the feeding profile for which its first derivative was zero (dS_N/dt = 0). The feeding profile was reasonably complex (Table 2) to maintain pseudo steady state of nitrogen in the bioreactor. During computer simulation it was observed in this case that fructose was also completely exhausted at 36 h. Therefore, fructose feeding of 500 g/L at a constant rate of 0.025 g/L was also initiated at 29 h till the end of fermentation to ensure the availability of limiting nutrient (fructose).

<table>
<thead>
<tr>
<th>Table 2 Feeding rates for constant nitrogen strategy</th>
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<tbody>
<tr>
<td>Time (h)</td>
</tr>
<tr>
<td>17</td>
</tr>
<tr>
<td>29</td>
</tr>
<tr>
<td>33</td>
</tr>
<tr>
<td>39</td>
</tr>
</tbody>
</table>
and low nitrogen concentration. From computer simulation, it was observed that fermentation finished earlier than batch fermentor with significantly high biomass and PHB concentrations. Therefore, this strategy was experimentally implemented. Maximum biomass obtained was 41 g/l with PHB accumulation of 7 g/l at the end of 44 h as compared to model predicted values of 44 and 5.94 g/l, respectively. The productivity of 0.14 g/l/h was obtained. The profiles for biomass, PHB and residual nutrients are given in Fig. 2.

3.6 Constant feeding of nitrogen and fructose starting at the same time

The constant nitrogen strategy was difficult to implement since the flow rate of nitrogen was variable with respect to time. Hence a constant feeding strategy was designed with constant feeding of both nitrogen and fructose starting at 17 h when the nitrogen concentration in the reactor approached zero. Computer simulation of the model demonstrated PHB accumulation of 7.5 g/l in 48 h that was a significant improvement as compared to batch fermentation, therefore, this strategy was experimentally implemented.

Maximum biomass and PHB of 25.8 and 6.1 g/l was obtained at 48 h as compared to model predicted values of 32.5 and 7.5 g/l at 48 h. The profiles for biomass, PHB and residual nutrients are given in Fig. 3. Productivity of 0.13 g/l/h was obtained.

4. Discussion

Batch fermentation using fructose as a carbon source and ammonium sulphate as nitrogen source was studied by Mulchandani et al. [11]. Biomass concentration of 6.58 g/l and PHB accumulation of 3.78 g/l was obtained at 48 h, with productivity of 0.078 g/l/h. In the present study, batch fermentation of R. eutropha NGR B14690 resulted in biomass and PHB concentration of 14 and 6 g/l but the time of fermentation was 60 h leading to a productivity of 0.091 g/l/h.

There have been previous studies on modelling of PHB fermentation. Initial work was done on lithoautotrophic fermentation of R. eutropha [16,17] and PHB synthesis by methanotrophs [18]. Later, other researchers [11] studied substrate inhibition kinetics of R. eutropha grown in heterotrophic culture using fructose as a carbon source and proposed a model based on the experimental data which was further improved upon by Raje and Srivastava [19]. A model structure similar to that proposed by Raje and Srivastava was used in present study to describe growth/PHB synthesis by R. eutropha. The experimental batch data was used for evaluation of model parameters. A good agreement between the model simulation results and experimental data was observed.

Fed-batch fermentation for PHB production has been studied by various researchers. Fed-batch fermentation using nitrogen limitation [19] yielded biomass and PHB concentration of 164 and 121 g/l, respectively, after 50 h leading to a productivity of 2.42 g/l/h. Here, glucose concentration was maintained between 0 and 20 g/l using on-line enzymatic glucose analyzer. But particularly in a large-scale cultivation it is difficult to use such enzymatic sensors for control of substrate concentration.

Fed-batch studies on R. eutropha NCIMB 11599 were also carried out with phosphate limitation [20]. DO concentration was used as a feed back parameter for glucose feeding. Final biomass concentration of 281 g/l, PHB concentration of 232 g/l and productivity of 3.14 g/l/h were obtained.

A two-stage cultivation method was used to produce PHB using R. eutropha ATCC 17697 (equivalent to NGR B14690 used in the present study). In the first stage culture was grown on 10 g/l fructose as carbon source followed by autotrophic culture (where a mixture of CO₂, H₂ and O₂ was used for PHB accumulation [21]. PHB concentration of 21.6 g/l was obtained. Use of acetic acid instead of fructose in the two-stage method was also investigated. Acetic acid concentration was maintained at 1g/l by pH-stat feeding of acetic acid. Biomass and PHB accumulation of 22.9
and 12.6 g/l were obtained in 83 h leading to productivity of 0.15 g/l/h. But in autotrophic fermentations there was a possibility of spontaneous denaturation. To prevent this oxygen concentration in gas phase had to be kept below 6.5% v/v, which resulted in very low oxygen transfer rate.

In fermentation processes where cell growth/product formation is inhibited by high substrate concentrations, fed-batch fermentation is very helpful in removing the substrate inhibition and improving the productivity. The substrate concentration is maintained below a critical level by a substrate feeding in order to reduce the inhibitory effect of substrate. All the fed-batch studies mentioned above focused on maintenance of either pH, DO or substrate concentration, which were used a feedback parameter for deciding the feeding rate of the substrate. NCC concentration plays a significant role in overall PHB accumulation and this can be controlled near optimum values by model-based cultivation only.

Hence, in the present study, a different approach was used to optimize the feeding rates of limiting nutrients. The proposed batch model was extrapolated to fed-batch by incorporating dilution terms arising due to feeding of ammonium sulphate and fructose. Computer simulations of fed-batch model were used to predict the feeding strategies and feeding rates of fructose and ammonium sulphate. Simulation strategies featuring better productivity as compared to batch study were experimentally implemented. Ideally on-line measurement of process variables and feedback control is desirable for better results from model-based process optimization but this is cumbersome as it requires measurement of substrate and product concentrations. Measurement of product concentration is rather difficult when product is intracellular in nature. Therefore, in the absence of appropriate sensors in process industries, model-based offline and feed forward optimizations are rather simple and very useful in improving the process productivity. Model-based constant nitrogen strategy gave a productivity of 0.14 g/l/h while constant feeding strategy for nitrogen and fructose at 17 h gave productivity of 0.13 g/l/h. These values are comparable to those obtained by Sugimoto et al. [21] for autotrophic cultivation.

5. Conclusions

Batch kinetics of the PHB synthesis was established in a bioreactor under controlled conditions of pH and dissolved oxygen. A proposed model for the synthesis of PHB by R. eutrophus was used and model parameters were identified using the batch experimental results. The model successfully simulated the observed batch kinetics. The applicability of the developed mathematical model was demonstrated for the computer simulation of nutrient feed strategies for enhanced PHB production and accumulation in the cell population. The different fed-batch studies included constant nitrogen and constant feed of nitrogen. The constant nitrogen strategy gave a better value of productivity but it was difficult to implement due to the variable feed rates of nutrient in the actual fermentation conditions. The constant feed strategy was found to be simpler and achieved significant improvement in productivity as compared to batch. Model-based optimization is particularly suitable for provision of complex limiting nutrient conditions for desired product (PHB) formation.

References

