THE PREDICTION OF THE CELL AND SUBSTRATE IN A FED-BATCH FERMENTATION SYSTEM BY MODIFICATION OF MODELS

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ABSTRACT

This objective of this study is to predict cell masses and substrate consumption in a fed-batch system after switching from a batch mode. A 2.0 litre reactor vessel was used to monitor the growth characteristics of Candida utilis in a bioreactor with the aid of a computer. The study enabled the development of two major mathematical models and their respective modified models for the prediction of the cell and substrate throughout the fermentation cycle. In the first model, the overall yield coefficient, $Y_{x/s}$, obtained by multiple linear regressions relating yield to the input variables for the batch model was assumed to be constant throughout the fed-batch mode. With this yield, the modified Bergter and Knorre model is applied to determine the specific growth rate over the entire fed-batch fermentation cycle to predict both the cell and substrate concentrations. The second model assumes that the fed batch mode operates at maximum specific growth rate, μ_{max}, after switching from batch mode. The value was obtained by using multiple linear regressions to relate the maximum specific growth rate, μ_{max} to the input variables from the batch mode. Both the models were modified and have obtained reasonably good results when compared with the experimental data.

Keywords: *Bergter and Knorre Model, Cell and Substrate Prediction, Fed Batch System, Maximum Specific Growth Rate, Monod Equation, Overall Yield Coefficient*

1.0 INTRODUCTION

A fermentation model is an abstracted and generalised description of the relevant aspects of a fermentation process. With the introduction of mathematical modelling to the microbial growth kinetics, the behaviour of the fermentation system under all possible conditions can be investigated and the effects of changes of scales or conditions can also be predicted [1,2]. Several workers have applied mathematical and modelling techniques for describing batch as well as fed-batch processes. In most cases the use and the applicability of their models were restricted by imposing severe restrictions on the behaviour of the system under consideration [3-6] and in many cases, the studies were confined to theoretical considerations with little or no experimental data. As a result, it is unable to check the validity of the models and to get an accurate insight into the growth phenomena under these conditions[7].

In this study, simple approach by initiating a batch model whereby Monod model was used to predict the specific growth rates, μ. The work was further extended to cover fed-batch fermentation system. Curve fitting of the cell concentration at different times was applied in order to determine the specific growth rate. The predicted values were compared to the experimental findings which prompted the use of the Bergter and Knorre model which incorporated the empirical structure to describe lag phase after inoculation. To make the model agrees well with the experimental findings, an attempt was carried out to modify it. As a result a developed equation emerged which has a very good cell concentration prediction potential.

Ahmad's work [8] involving the overall yield coefficient which formulated by multiple linear regression was introduced to determine substrate concentration at given cell concentration in Model 1. A similar equation for the determination of the maximum specific growth rate was also formulated to be used in the fed-batch modelling in Model 2.

To further improve the developed models, modifications were made to account for the accelerator factor, A, and the cell maintenance, ω. The comparisons of the cell mass and the substrate consumption prediction with the experimental findings were made.

2.0 THEORY

2.1. Specific Growth Rate, μ, Determination

Experimentally, the specific growth rate was determined from the slope of the regression line from a semi logarithmic plot of the total cell mass against time.

$$1n \frac{x}{x_0} = \mu t \quad (1)$$

During the course of fermentation, the biomass growth is dependent on nutrient availability. The measurement of the change in the sugar concentration was used to determine the specific growth rate based on the Monod model.

2.2. MONOD EQUATION

The Monod Equation [9] states that:

$$\mu = \frac{\mu_{max} S}{K_s + S} \quad (2)$$

It is a functional relationship between the specific growth rate, μ, and the substrate concentration, *S*.

2.3. Bergter and Knorre Equation

This model is the extension of Monod equation which incorporated the initial lag phase after inoculation [10]:

$$\mu = \frac{\mu_{max}(1-e^{-t/T_{lag}})}{(K_s+S)} \quad (3)$$

Where:

μ_{max} = maximum specific growth rate;
T_{lag} = length of lag phase;
t = time elapsed from inoculation

2.4. μ_{max} and $Y_{x/s}$ Determination

There are more than two variables involved in the experimental data; the data thus can assume various forms, *i.e.* linear, polynomial, and logarithmic, *etc* by plotting the relation between values of μ_{max} and $Y_{x/s}$ against operating conditions. Two equations based on multiple regression technique were obtained to predict the rate of growth and the ultimate yield of the biomass for a range of values of inputs. The variables were normalised and a FORTRAN computer program was used to calculate the correlation coefficients.

μ_{max} = 0.0565 (agitation speed ratio) + 0.0287 (air flow rate ratio) + 0.3420 (temperature ratio) + 0.1426 (initial inoculum dosage ratio) – 0.0326 (initial sugar concentration ratio) + 0.0915 (4)

And

$Y_{x/s}$ = 0.3817 (agitation speed ratio) + 0.1896 (air flow rate ratio) + 0.1170 (temperature ratio) – 0.0723 (inoculum dosage ratio) – 0.2983 (initial sugar concentration ratio) – 0.0516 (5)

Where:

μ_{max} = maximum specific growth rate;
$Y_{x/s}$ = yield coefficient;
agitation speed ratio = agitation speed (rpm)/700 (rpm);
air flow rate ratio = air flow rate (l/min)/1.26 (l/min);
temperature ratio = temperature (ºC)/34 (ºC);
inoculum dosage ratio = inoculum dosage (%v/v)/5.0 (% v/v);
initial sugar ratio = initial sugar concentration (%w/v)/4.0 (%w/v).

3.0. METHODOLOGY

3.1. Model 1

Having incorporated lag phase into the model, Bergter and Knorre model has shown some improvement over the Monod model when compared against the experimental results. However the specific growth rate appears to follow a type of exponential increase to its maximum, before dropping off slightly at the end of the run, this has prompted the development of a new equation to describe the system more adequately by plotting various functions of the time delay function, maximum specific growth rate as well as the substrate concentration is as shown:

$$\mu = \frac{\mu_{max} MZ}{G^{0.25}} \quad (6)$$

Where:

M = time delay function $(1-e^{-t/T_{lag}})$;
Z = function of $[A + (X_{n-1}/X_n)]$,
A = accelerator factor *i.e.* 1.1; 1.4; 1.8;
G = function of substrate concentration *i.e.* $1 + e^{-s}$.

The developed Equation (6) has its limitations when dealing with systems in which the death rates are high i.e. constant fed-batch fermentations as the model does not consider the large death rate when the system utilises all its batch substrate, and is solely relying on the feed substrate for growth.

The cell and substrate concentration at any time after switching *i.e.* at the 5th hour, can be obtained from:

$$X_n = X_{n-1}\, e^{\mu(t-5)} \quad (7)$$

$$S_n = S_{n-1} + S_A - \Delta S \quad (8)$$

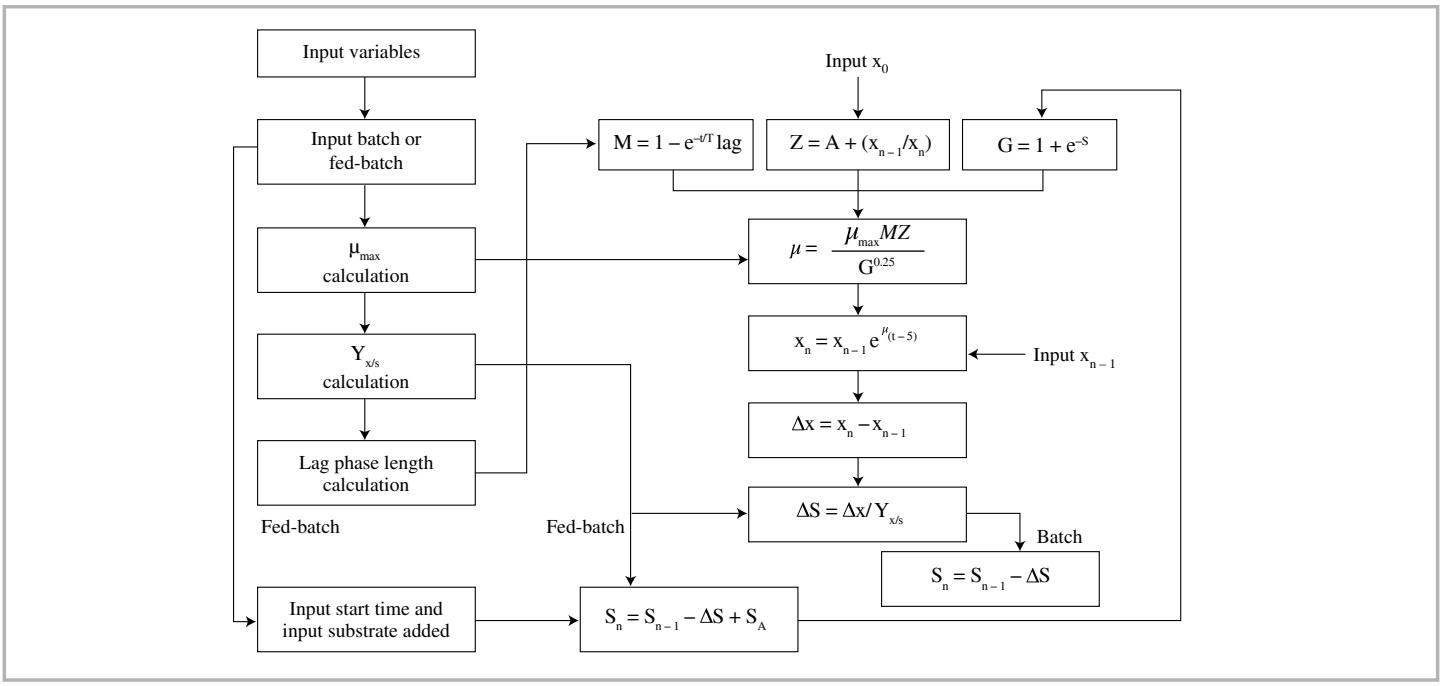

Figure 1: Simplified flow diagram for Model 1

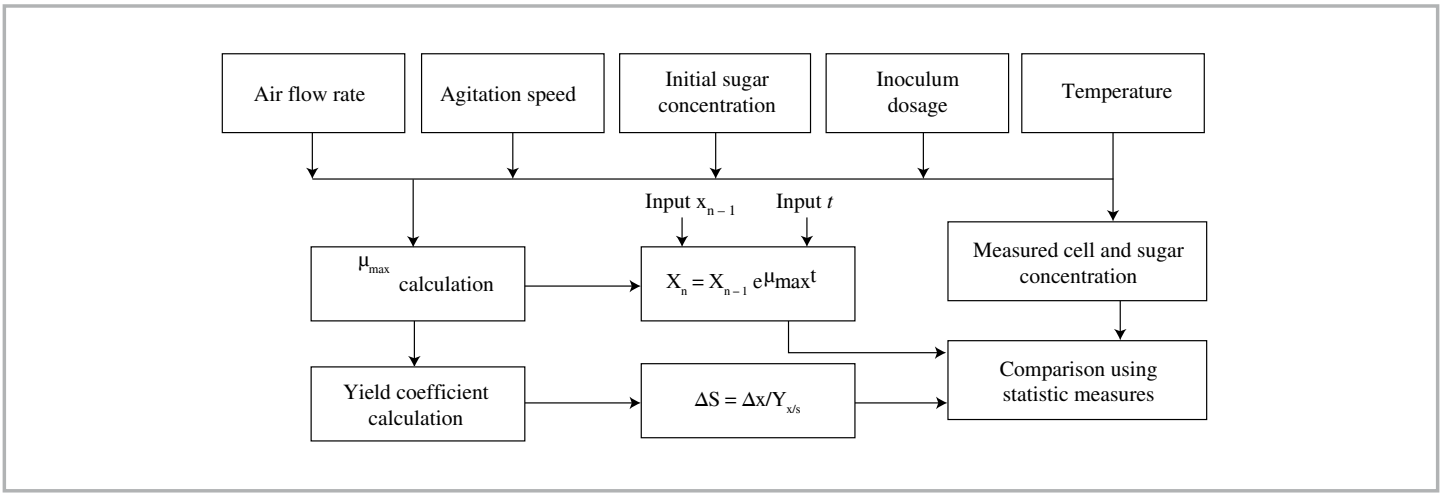

Figure 2: The structure of the predictive mathematical Model 2

Where:
X_n = cell concentration at any time, t;
X_{n-1} = cell concentration at time of switching;
S_n = substrate concentration at any time, t;
S_{n-1} = substrate concentration at time, t_{-1};
S_A = total amount of substrate added at time t.

3.2. MODIFIED MODEL 1: Acceleration Factor (A value)

From both the cell and substrate models, only cell model has strong agreement with the experimental findings. The *A* values which depends on the initial substrate composition were therefore applied to improve the model. The effect of different *A* values on the calculated μ values for fed batch system was studied in an attempt to meet the experimental conditions. In Model 1, the discrepancy can be discounted by manipulating *A* value as it is an acceleration factor. It was shown that a large value of A causes a faster increase in the values of the calculated specific growth rate.

3.3. MODEL 2

Model 2 is shown in Figure 2, the cell growth is assumed at μ_{max} after switching from a batch mode. Cell and substrate concentration can be predicted according to:

$$X_m = X_{m-1} e^{\mu_{max} t} \quad (9)$$

$$S_m = X_m (e^{\mu_{max}} - 1) Y_{x/s} \quad (10)$$

Where:
X_m = cell concentration at any time t;
X_{m-1} = cell concentration at the switching point;
μ_{max} = maximum specific growth rate;
t = time elapsed after switching point;
S_m = substrate concentration calculated;
$Y_{x/s}$ = yield coefficient.

3.4. MAINTENANCE COEFFICIENT (ω)

In Model 2, the micro-organisms are assumed to be growing at maximum specific growth rate. One significant concern with the Model 2 is that the model concern does not take into account the maintenance terms. Since the requirement of cells for maintenance energy affects the relation between growth rate and concentration of energy substrate, it should be considered in the model. The relationship between the overall yield, $Y_{x/s}$, the growth yield, Y_g and the maintenance is:

$$\frac{1}{Y_{x/s}} = \frac{1}{Y_g} + \frac{\omega}{\mu} \quad (11)$$

By definition, $q_E = \frac{\mu}{Y_{x/s}}$ (12)

At any instant of time, in a fed-batch system, the micro-organisms are assumed to be growing accordingly to the Monod kinetics and the metabolic quotient, q_E, is given by:

$$q_E = \frac{q_{max} S}{K_s + S} \quad (13)$$

From the growth kinetics, S>>Ks where $\mu_{max} = q_{max} Y_g$, μ can be simplified into:

$$\mu = \mu_{max} - \omega Y_g \quad (14)$$

Modifying Model 2 to include the maintenance term, ω, both the modified cell prediction Model and substrate consumption prediction Model become:

$$X_m = X_{m-1} e^{(\mu_{max} - \omega Yg)t} \quad (15)$$

$$\Delta S_m = (X_m - X_{m-1}) Y_g \quad (16)$$

Where:
X_m = cell concentration at any time t after switching point;
X_{m-1} = cell concentration at the time of switching;
μ_{max} = maximum specific growth rate;
t = time elapsed after switching point;
ΔS_m = substrate consumption by modified Model 2;
Y_g = true growth yield.

4.0 RESULTS AND DISCUSSIONS

The values obtained from the experimental growth rates and the predicted values from Monod were plotted against substrate concentration and time as shown in Figures 3 and 4.

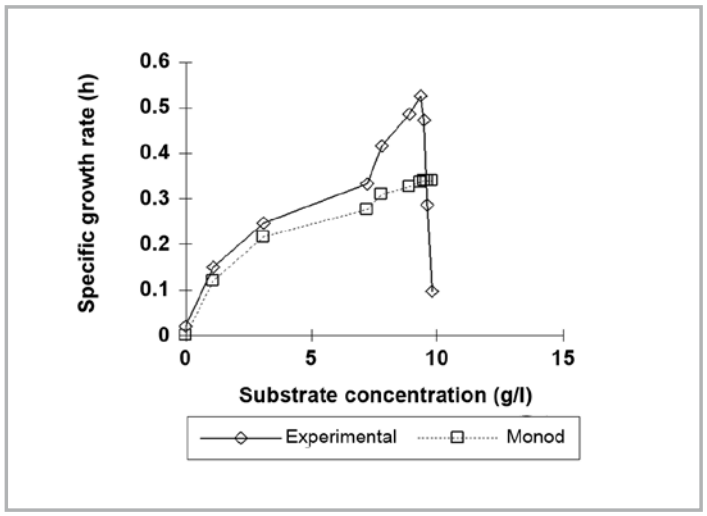

Figure 3: Comparison of μ between Monod Model and the experimental findings for different substrate concentrations

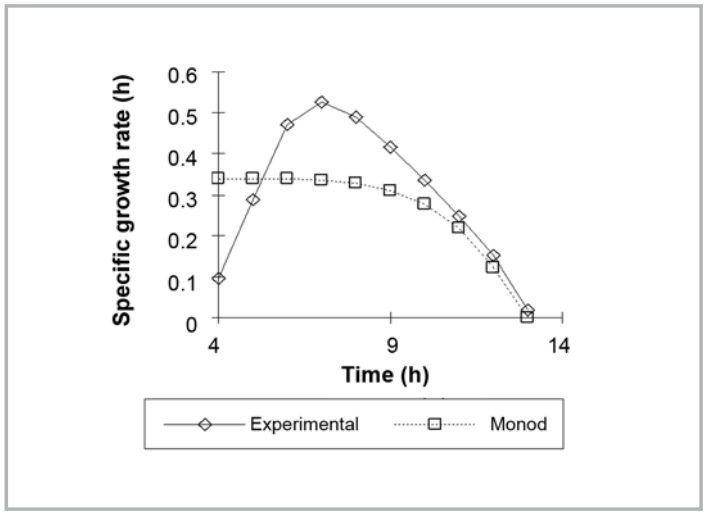

Figure 4: Comparison of μ between Monod Model and the experimental findings

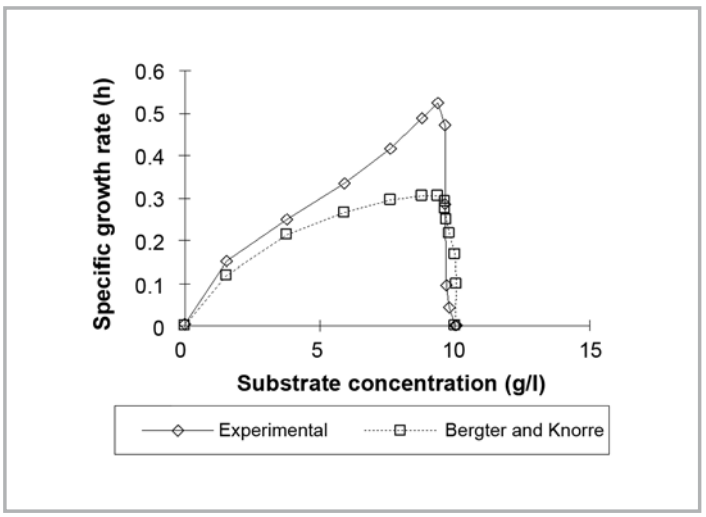

Figure 5: Comparison of μ between Bergter and Knorre Model and the experimental findings for different substrate concentrations

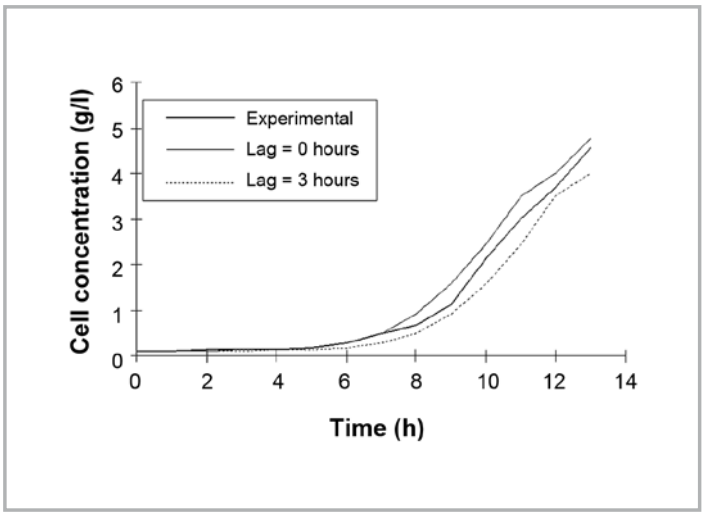

Figure 6: Calculated cell concentration with an inbuilt lag into Equation ($\mu = \mu_{max} MY/G^{0.25}$)

The predicted values from the Monod models for the cell concentration is not accurate enough as the initial lag phase was not taken into consideration. Thus, The Bergter and Knorre Model which incorporating lag phase and time delay function, function of cell and substrate concentration respectively was tested with the experimental data and has shown great improvement over the Monod Equation, the result is as shown in Figure 5. The Bergter and Knorre Equation is developed based on the prediction for the specific growth rate on the living cell concentration. It can be used to predict the maximum specific growth rate to be used in fed batch systems.

The cell concentration can be calculated for the entire length of the run, when the initial cell concentration is known and the specific growth rate is considered linear between the 0 to 3 hours lag phase. An inbuilt lag into the developed Equation 6 appears to reduce the necessity for the time delay function term as shown in Figure 6. However, since the rate of death cells was not considered, Equation 6 has its limitations when dealing with systems in which the death rates are high as in constant fed-batch system when the system utilises all its batch substrate and is solely dependent on the feed substrate for growth.

The modification of model 1 was made and the effect of different A values on the calculated specific growth rate for the fed-batch system is shown in Figure 7. It can be seen from the figure that an A value as high as 1.8 is required to meet the experimental conditions in a fed-batch system.

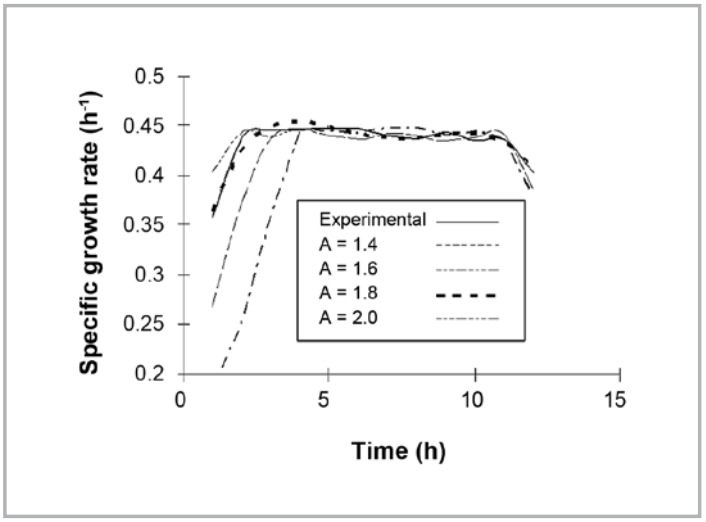

Figure 7: Calculated specific growth rates by using different acceleration factor values in a fed-batch system

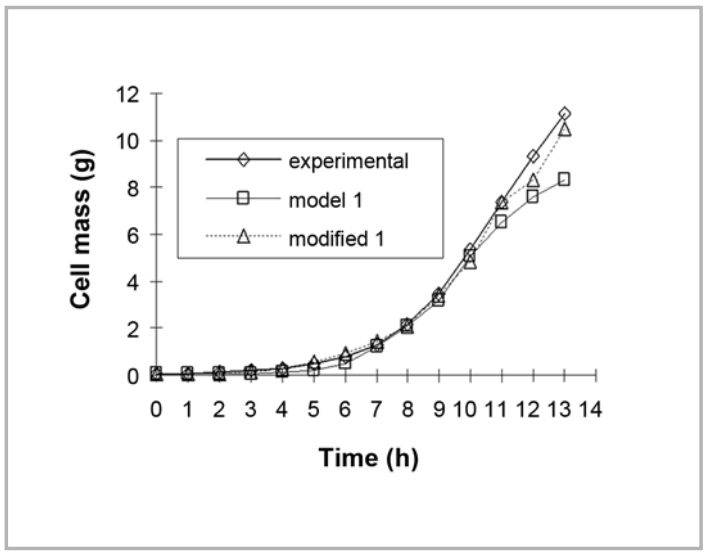

Figure 8: Comparison of cell masses between Model 1 with experimental findings

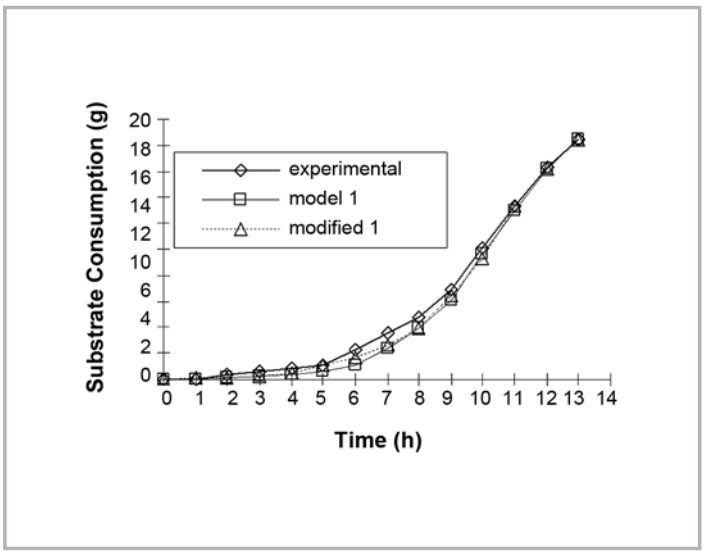

Figure 9: Comparison of substrate consumption between Model 1 with experimental findings

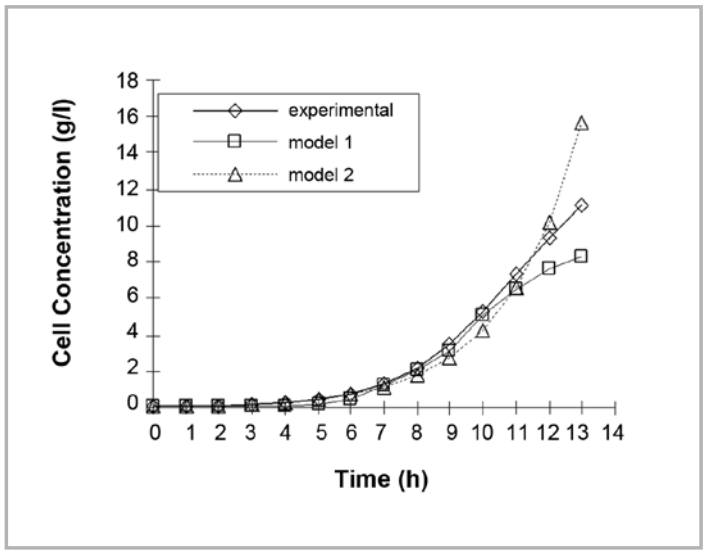

Figure 10: Comparison of the cell concentrations between Models and the experimental values

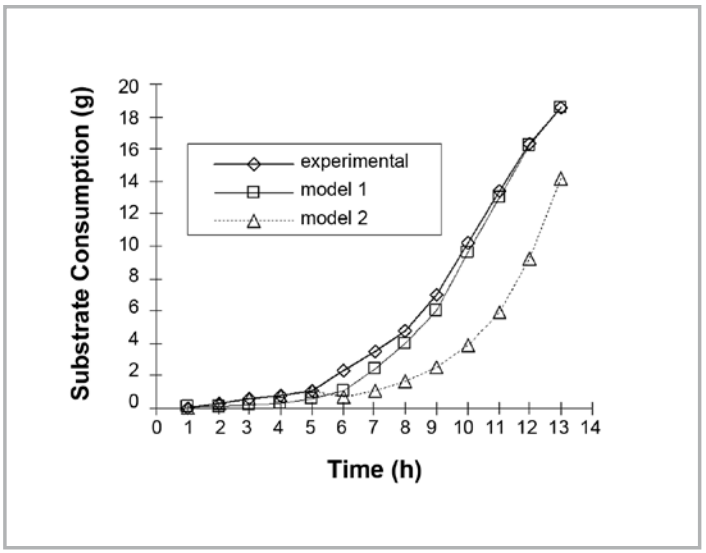

Figure 11: Comparison of substrate consumptions between models and the experimental values

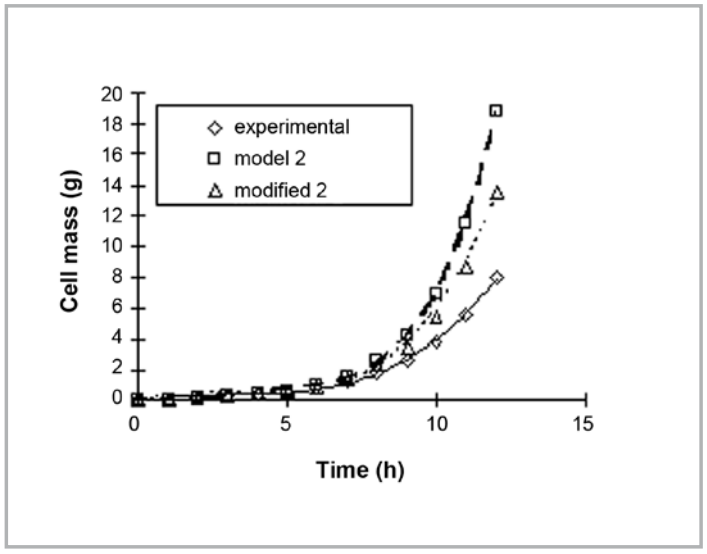

Figure 12: Comparison of cell masses between models 2 and experimental values

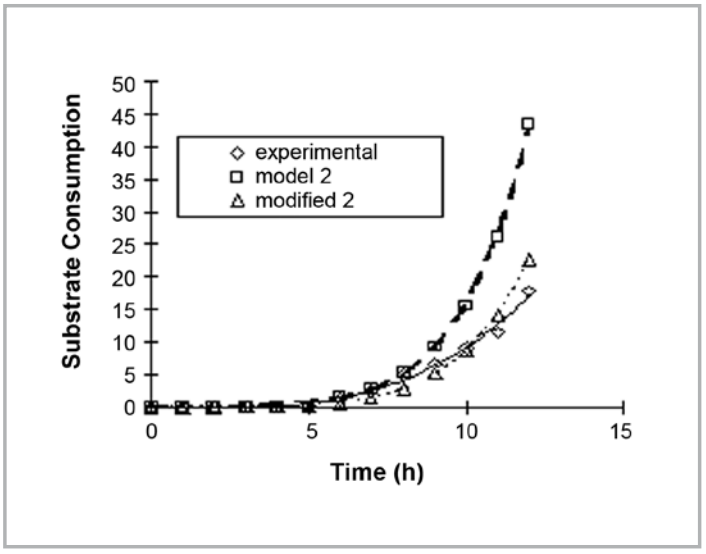

Figure 13: Comparison of substrate consumption between Models 2 and experimental values

Generally, Model 1 are in good agreement with the experimental findings and the comparisons between them are shown in Figures 8 and 9 for both cell and substrate prediction.

In Model 2, cell growth was assumed at μ_{max} after switching to a fed batch mode. The cell and substrate concentrations predicted by the Model 2 were compared with Model 1 and the experimental findings are as shown in Figure 10. From the comparison, the cell models once again have shown strong agreement with the experimental findings.

However, Figure 11 indicates that the substrate consumption predicted by Model 2 in comparison with the experimental values is not as agreeable as in the case for the prediction of cell concentration. This is largely due to the fact that the substrate is not only consumed to provide the necessary nutrient for cell growth but also for the maintenance of cell viability *i.e.* cell repair mechanism, transport process, *etc*. Therefore, the maintenance term and the true growth yield are both incorporated into Model 2, in an attempt to improve the prediction of the models. The modified cell and substrate prediction models are then compared with the experimental data as shown in Figures12 and 13. The predictive models were slightly overestimated by assuming in which the system operated at maximum growth rate and maximum yield in the fed-batch model. Possibly the maintenance demands are greater at lower specific growth rate despite that the cell growth inhibition and substrate inhibition could have occurred at maximum growth rate.

5.0 CONCLUSION

The cell and substrate prediction for fed-batch systems can be modelled fairly effectively based on the maximum specific growth rate and the yield coefficient developed by the multiple linear regression technique. Model 1 and the modified Model 1 with manipulation of acceleration factor have better prediction ability than the Model 2 regardless the incorporation of maintenance term and the true growth yield into the models concerned. Slight improvement was obtained in predicted cell and substrate concentrations.

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