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A Simple Model of Growth and Product Formation in Cell Suspensions of *Catharanthus roseus* G. Don

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ABSTRACT

This paper describes the application of a simple log-linear model to suspension cultures of the species *Catharanthus roseus* G. Don. Data obtained experimentally have been interpolated by a cubic spline to give data points of regular time period. A logarithmic transformation has been applied and a linear model fitted to these transformed variables. The results show that there is a reasonable fit to the experimental data cited.

Using this model a one-step ahead prediction is made for a series of untried experimental variables, and the results generated are compared with the experimental data subsequently obtained.

Index Entries: Log-linear model, for plant cell cultures; suspension culture, of plant cells; *Catharanthus roseus* growth, model for; product formation, serpentine; cell culture, log-linear model for plants; plant cell cultures, log-linear model for growth of.

INTRODUCTION

Plant cell suspension culture is becoming increasingly accepted as an alternative source of biochemicals (*1–3*). However, before this can be con-

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sidered as a commercially viable route, operational criteria will need to be defined in order to enable translation of results from bench-scale operations to industrial plant size. Like most aerobic cell cultures, plant cells require a balanced medium that includes sources of carbon, phosphate, nitrogen, hormones, and trace elements. Depending on the balance of these nutrients, growth and product synthesis will be regulated. In particular, sucrose has been shown to be a major nutritional factor that controls the biomass yield in addition to the growth rate of the culture (*4*) and the yield of secondary products (*5*).

Other factors that may play a significant role in the synthesis of secondary products include: light intensity/wavelength, ventilation, pH and temperature, although in the present study, these are held constant and may therefore be ignored.

Plant cells usually follow a similar sigmoidal growth pattern to that exhibited by other cell suspensions (*6*), although the mathematical description of the kinetics may vary from those proposed for microbial populations.

Recently it has been shown (*7*) that biomass production and carbon uptake in apple cultures could be described by kinetic equations previously proposed by Contois (*8*) and Aiba et al. (*9*). However, this paid no attention to other potential growth-limiting nutrients, or to the synthesis of secondary products.

This paper reports a simple mathematical system that attempts to predict theoretical results on the basis of limited experimental data. Attention is given both to limiting nutrients, and to the synthesis of secondary products.

MATERIALS AND METHODS

Cell cultures (line no. C87C) of *Catharanthus roseus* G. Don were established from leaf explants on a modification of Murashige and Skoog's medium (*10*). A full formulation of this medium is given elsewhere (*11*).

Experiments were performed using media containing 20, 50, and 80 g/L sucrose.

The medium pH was 5.8 prior to sterilization by autoclaving at 121°C 20 psi for 15 min.

Suspension cultures were routinely grown at 25°C in diffuse light in 250 mL Erlenmeyer flasks containing 100 mL of medium, and were shaken at 150 rpm.

Biomass was measured as fresh and dry weight (*12*). Residual carbohydrate in the spent medium was estimated with the anthrone (total carbohydrate) method (*13*).

Nitrate and ammonia levels in the medium were monitored using an Orion nitrate ion electrode (Model No. 93-07) and an EIL ammonia electrode (Model No. 8002-2), respectively.

Serpentine product contained within the cells was estimated by reverse-phase ion pair HPLC on a microbondapak C18 column, using methanol:water:*n*-heptane sulfanate (0.05*M*), with gradient elution (Morris and Woodhead, unpublished).

Suspension cultures of *Catharanthus roseus* G. Don were grown in shaken Erlenmeyer flasks at 2, 5, and 8% sucrose concentrations. Data from both the 2 and 8% experiments were used to produce a log-linear model. The model was then compared with experimentally determined data for the 5% case.

In addition, a one-step-ahead prediction has been generated for the same data in an attempt to illustrate how such a model could be used to optimize an operating process.

MODELING

Linear modeling describes processes under the assumption that the value of the variables at any time is equal to a weighted sum of these variables at previous times. If it is assumed that these variables are measured and that they are a function only of values recorded at the previous N observation times, then the model will contain Nn^2 parameters. In this paper N has been restricted to 1, thus minimizing the number of parameters.

In practical terms, linear models are often used when knowledge of the system dynamics is lacking, and provide a first approximation to a complex process.

Generation of the Linear Model

The linear model in discrete time form is as follows:

$$s\,(k+1) = a_{11}s(k) + a_{12}s_1(k) + a_{13}s_2(k) + a_{14}x_1(k) + a_{15}x(k) + a_{16}p(k)$$
$$s_1(k+1) = a_{21}s(k) + a_{22}s_1(k) + a_{23}s_2(k) + a_{24}x_1(k) + a_{25}x(k) + a_{26}p(k)$$
$$s_2(k+1) = a_{31}s(k) + a_{32}s_1(k) + a_{33}s_2(k) + a_{34}x_1(k) + a_{35}x(k) + a_{36}p(k)$$
$$x_1(k+1) = a_{41}s(k) + a_{42}\,s_1(k) + a_{43}s_2(k) + a_{44}x_1(k) + a_{45}x(k) + a_{46}p(k)$$
$$x\,(k+1) = a_{51}s(k) + a_{52}s + r_1(k) + a_{53}s_2(k) + a_{54}x_1(k) + a_{55}x(k) + a_{56}p(k)$$
$$p\,(k+1) = a_{61}s(k) + a_{62}s_1(k) + a_{63}s_2(k) + a_{64}x_1(k) + a_{65}x(k) + a_{66}p(k)$$

where a_{11}–a_{66} are constants

x = biomass concentration as dry weight
x_1 = biomass concentration as fresh weight
s = substrate concentration as carbohydrate source
s_1 = substrate concentration as nitrate source
s_2 = substrate concentration as ammonia source
p = product concentration as serpentine

Such a system has been used by Karim et al. (*14*) for a *Zymomonas mobilis* fermentation for ethanol production.

Although the above is equivalent to "established models" of Monod, Briggs/Haldane, and Luedeking-Piret at substrate concentrations much greater than the Michaelis-Menten constant, K_s, it is the nature of fermentation processes that they continue until the concentration has dropped well below this value. For these conditions, growth dynamics are then a function of substrate concentration and the linearized model cannot be considered a good approximation. In view of this, a revised logarithmic model has been generated with a view to improving this discrepancy.

The Log-Linear Model

The log-linear model is of the form:

$$s(k+1) = s(k)^{\alpha_{11}} \cdot s_1(k)^{\alpha_{12}} \cdot s_2(k)^{\alpha_{13}} \cdot x_1(k)^{\alpha_{14}} \cdot x(k)^{\alpha_{15}} \cdot p(k)^{\alpha_{16}} \cdot 10^{\alpha_{17}}$$

$$s_1(k+1) = s(k)^{\alpha_{21}} \cdot s_1(k)^{\alpha_{22}} \cdot s_2(k)^{\alpha_{23}} \cdot x_1(k)^{\alpha_{24}} \cdot x(k)^{\alpha_{25}} \cdot p(k)^{\alpha_{26}} \cdot 10^{\alpha_{27}}$$

$$s_2(k+1) = s(k)^{\alpha_{31}} \cdot s_1(k)^{\alpha_{32}} \cdot s_2(k)^{\alpha_{33}} \cdot x_1(k)^{\alpha_{34}} \cdot x(k)^{\alpha_{35}} \cdot p(k)^{\alpha_{36}} \cdot 10^{\alpha_{37}}$$

$$x_1(k+1) = s(k)^{\alpha_{41}} \cdot s_1(k)^{\alpha_{42}} \cdot s_2(k)^{\alpha_{43}} \cdot x_1(k)^{\alpha_{44}} \cdot x(k)^{\alpha_{45}} \cdot p(k)^{\alpha_{46}} \cdot 10^{\alpha_{47}}$$

$$x(k+1) = s(k)^{\alpha_{51}} \cdot s_1(k)^{\alpha_{52}} \cdot s_2(k)^{\alpha_{53}} \cdot x_1(k)^{\alpha_{54}} \cdot x(k)^{\alpha_{55}} \cdot p(k)^{\alpha_{56}} \cdot 10^{\alpha_{57}}$$

$$p(k+1) = s(k)^{\alpha_{61}} \cdot s_1(k)^{\alpha_{62}} \cdot s_2(k)^{\alpha_{63}} \cdot x_1(k)^{\alpha_{64}} \cdot x(k)^{\alpha_{65}} \cdot p(k)^{\alpha_{66}} \cdot 10^{\alpha_{67}}$$

where the constantc $10^{\alpha_{17}}$ to $10^{\alpha_{67}}$ are used to introduce a mean value for the measured variables.

It is of interest to note that the right hand sides of the above equations are typical of the forms used in the mass balance representation of chemical reactions.

A linear model can now be obtained by a logarithmic transformation of these equations.

NUMERICAL METHODS

The shake flask data produced were interpolated by a cubic spline (*15*) to give data with a fixed time interval of 6 d. A discrete time single-step log-linear model was then fitted to the six measured variables using the method of least squares (*16*). As a comparison, the original experimental data is given in Tables 1–3.

With the variables ordered carbohydrate, nitrate, ammonia, fresh weight, dry weight, serpentine, a constant (= 10), the transition matrix of the log-linear model, derived from the experiments at 2% and 8% was:

TABLE 1
Effect of 2% Sucrose Concentration on the Cultivation of Cells of *Catharanthus roseus* G. Don

Time interval, d	Fresh weight, gL^{-1}	Dry weight, gL^{-1}	Carbohydrate in medium, gL^{-1}	Serpentine yield, mg g^{-1} dry weight	Nitrate, m*M*	Ammonia, m*M*
0	48.9 ± 8.8	3.03 ± 0.45	21.2 ± 0.95	2.69 ± 0.2	38.7 ± 4.7	19.0 ± 0.1
6	163.4 ± 17.7	12.14 ± 0.01	2.9 ± 0.11	1.69 ± 0.1	12.2 ± 2.2	3.2 ± 0.6
10	247.2 ± 16.0	11.6 ± 0.4	0.28 ± 0.11	3.74 ± 0.4	6.1 ± 1.0	4.0 ± 0.2
16	237.0 ± 22.1	9.76 ± 0.36	0.16 ± 0.01	3.78 ± 0.3	4.3 ± 1.6	3.4 ± 0.2
21	211.4 ± 8.0	8.34 ± 0.34	0.51 ± 0.2	4.17 ± 0.6	11.4 ± 3.1	9.8 ± 2.8
27	160.0 ± 5.2	5.22 ± 0.36	0.22 ± 0.01	0.57 ± 0.06	18.7 ± 2.0	29.3 ± 2.9
31	146.4 ± 9.8	4.80 ± 0.4	0.25 ± 0.03	0.49 ± 0.02	22.3 ± 1.2	35.3 ± 5.5
37	121.9 ± 11.8	5.44 ± 0.15	0.21 ± 0.01	0.51 ± 0.03	21.7 ± 2.3	45.3 ± 9.3
42	142.2 ± 4.6	4.90 ± 0.2	0.21 ± 0.04	0.51 ± 0.12	17.0 ± 2.5	40.7 ± 10.0
48	130.5 ± 18.2	4.01 ± 0.5	0.28 ± 0.06	0.48 ± 0.05	11.7 ± 5.6	30.0 ± 8.1
52	104.7 ± 9.7	4.30 ± 0.5	0.17 ± 0.01	0.67 ± 0.03	20.0 ± 6.0	52.0 ± 6.0

TABLE 2
Effect of 5% Sucrose Concentration on the Cultivation of Cells of *Catharanthus roseus* G. Don

Time interval, d	Fresh weight, gL^{-1}	Dry weight, gL^{-1}	Carbohydrate in medium, gL^{-1}	Serpentine yield, mg g^{-1} dry weight	Nitrate, m*M*	Ammonia, m*M*
0	47.9 ± 2.9	4.66 ± 0.15	51.2 ± 1.2	2.2 ± 0.3	41.3 ± 0.3	15.0 ± 2.5
6	168.9 ± 6.8	27.9 ± 1.6	18.9 ± 2.0	1.58 ± 0.5	11.7 ± 0.3	2.4 ± 0.3
10	359.1 ± 64.3	31.1 ± 0.75	11.7 ± 1.5	3.03 ± 0.11	2.6 ± 0.3	2.4 ± 0.4
16	383.2 ± 17.6	26.6 ± 0.38	1.7 ± 0.5	5.94 ± 0.47	2.6 ± 0.1	1.6 ± 0.1
21	408.8 ± 14.1	22.7 ± 0.8	0.2 ± 0.06	6.83 ± 0.3	2.6 ± 0.2	3.8 ± 1.5
27	331.2 ± 15.6	18.6 ± 1.0	0.29 ± 0.11	9.24 ± 1.0	3.9 ± 0.9	3.7 ± 1.5
31	336.2 ± 27.1	16.4 ± 0.9	0.27 ± 0.03	12.87 ± 1.3	3.4 ± 1.2	3.9 ± 0.9
37	354.1 ± 26.1	13.9 ± 0.39	0.14 ± 0.07	14.34 ± 1.7	3.5 ± 1.2	6.0 ± 1.1
42	326.5 ± 50.3	12.8 ± 2.0	0.42 ± 0.04	12.3 ± 0.3	7.2 ± 1.2	11.1 ± 3.2
48	251.0 ± 24.8	9.9 ± 0.1	0.59 ± 0.2	3.4 ± 1.6	7.0 ± 1.8	14.9 ± 9.1
52	146.7 ± 7.6	6.3 ± 0.3	0.84 ± 0.06	1.7 ± 1.5	4.3 ± 0.3	23.5 ± 0.5

TABLE 3
Effect of 8% Sucrose Concentration on the Cultivation of Cells of *Catharanthus roseus* G. Don

Time interval, d	Fresh weight, gL^{-1}	Dry weight, gL^{-1}	Carbohydrate in medium, gL^{-1}	Serpentine yield, mg g^{-1} dry weight	Nitrate, m*M*	Ammonia, m*M*
0	44.0 ± 1.9	5.5 ± 0.15	80.8 ± 0.6	2.8 ± 0.4	40.7 ± 0.3	17.5 ± 0.5
6	124.5 ± 4.2	25.4 ± 2.0	50.8 ± 2.9	0.52 ± 0.13	13.7 ± 0.9	3.1 ± 0.97
10	224.7 ± 3.0	36.4 ± 0.25	26.7 ± 0.6	3.04 ± 0.15	5.5 ± 0.5	1.7 ± 0.2
16	247.7 ± 24.9	39.2 ± 2.1	9.7 ± 1.3	6.74 ± 0.28	2.0 ± 0.1	1.7 ± 0.2
21	337.7 ± 31.6	42.9 ± 1.2	2.4 ± 0.2	8.91 ± 0.4	1.9 ± 0.07	2.5 ± 0.2
27	405.4 ± 37.2	31.9 ± 1.7	0.4 ± 0.23	11.30 ± 0.23	2.4 ± 0.13	2.2 ± 0.4
31	344.3 ± 40.6	30.0 ± 2.6	0.23 ± 0.03	12.79 ± 1.1	1.9 ± 0.09	3.8 ± 0.65
37	421.5 ± 22.6	27.4 ± 1.9	0.13 ± 0.03	15.71 ± 0.55	1.9 ± 0.05	3.8 ± 1.21
42	451.7 ± 36.6	25.2 ± 1.0	0.43 ± 0.12	17.18 ± 1.2	1.4 ± 0.02	1.7 ± 0.6
48	401.0 ± 6.7	18.1 ± 1.6	0.29 ± 0.02	20.42 ± 0.89	1.5 ± 0.12	2.1 ± 0.6
52	304.4 ± 8.7	15.1 ± 1.6	0.69 ± 0.12	20.13 ± 0.16	2.8 ± 1.1	2.2 ± 0.4

$$\begin{bmatrix} 0.51 & -0.21 & 0.33 & -1.43 & 0.79 & 0.10 & 2.02 \\ 0.04 & 0.45 & 0.03 & 0.71 & -0.86 & -0.08 & -0.28 \\ 0.13 & 0.31 & 0.64 & 2.29 & -0.91 & -0.26 & -4.11 \\ -0.10 & -0.12 & -0.12 & -0.49 & 0.42 & 0.07 & 3.19 \\ 0.01 & 0.01 & 0.07 & -1.08 & 1.24 & 0.23 & 2.05 \\ -0.25 & -0.58 & -0.66 & -1.57 & 0.81 & 0.13 & 4.15 \\ 0.00 & 0.01 & -0.01 & 0.00 & 0.00 & 0.00 & 1.00 \end{bmatrix}$$

As a comparison and as an indication of parameter variability the transition matrix was reestimated using data from all three experiments.

$$\begin{bmatrix} 0.45 & 0.60 & 0.39 & -1.07 & 1.30 & 0.40 & -0.23 \\ 0.06 & 0.23 & 0.09 & 0.69 & -1.04 & -0.09 & 0.09 \\ -0.06 & 0.43 & 0.53 & 1.57 & -0.54 & -0.20 & -2.93 \\ -0.02 & 0.05 & -0.11 & 0.00 & 0.33 & 0.11 & 2.00 \\ 0.07 & -0.16 & 0.08 & -0.95 & 1.00 & 0.21 & 2.18 \\ -0.18 & -0.12 & -0.53 & -0.77 & 0.73 & 0.41 & 1.82 \\ 0.00 & 0.00 & 0.00 & 0.00 & 0.00 & 0.00 & 1.00 \end{bmatrix}$$

The quality of the model should be assessed according to how it performs the given task. Often, a model that is able to predict well over a short time span may give poor results for a longer prediction. The converse is also true.

In relation to the current study, two tests of quality are applied:

(a) The model is used to predict the state trajectory from $t = 0$ (i.e., from the setting up of the fermenter vessel). These results are illustrated in Fig. 1. This test is useful for deciding the optimum medium constituents.
(b) The model is used to predict the states at one measurement time from their values at the previous measurement time. These results are illustrated in Fig. 2. This test is useful for determining whether a substrate needs replenishing or when a particular product is at its peak prior to harvesting.

RESULTS AND DISCUSSION

In terms of its experimental validity, the model simulation generally gives excellent correlation of nutrient depletion (Figs. 1a, 1c, and 1e) and a reasonable fit with the increase in biomass, as represented in terms of fresh and dry weight (Figs. 1b and 1f). However, the agreement falls down when serpentine biosynthesis is predicted (Fig. 1d). A similar situation is also true for the one-step-ahead prediction (Fig. 2a–f). However, this might have been expected because of some of the limiting factors of the model, and the extra complexities involved in understanding secondary biosynthesis.

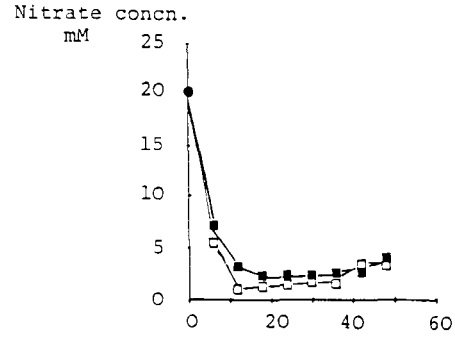


Fig. 1a.

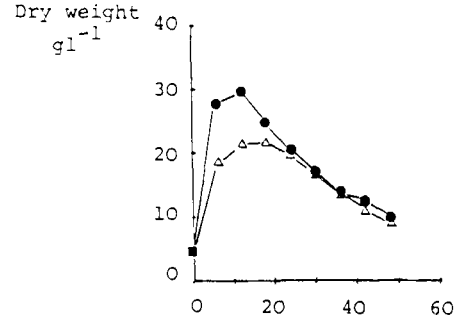


Fig. 1b.

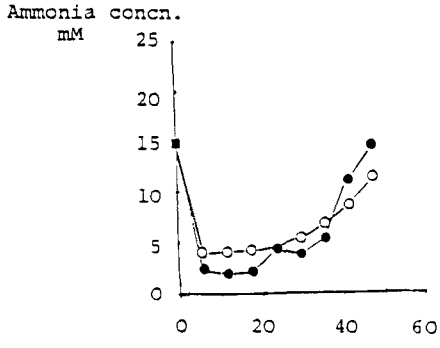


Fig. 1c.

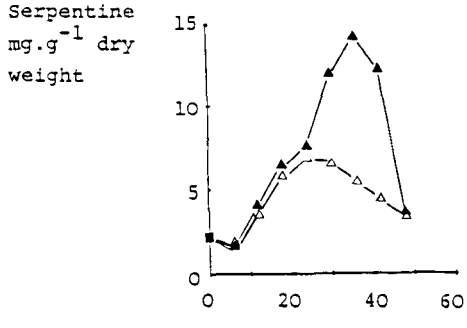
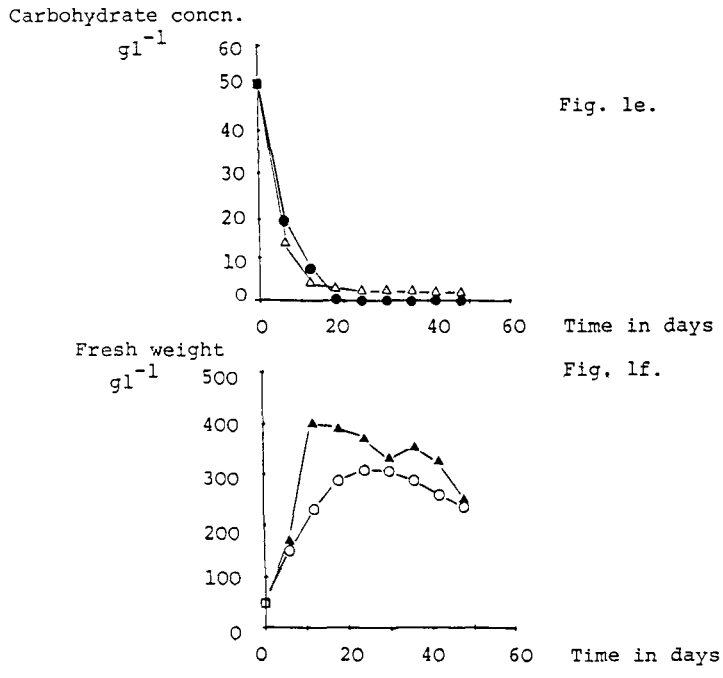


Fig. 1d.

Fig. 1a–f. Comparison of experimentally determined data (filled symbols) with that generated using the log-linear model.

Fig. 1. (*continued*)



A mathematical model of a process is essential if that process is to be operated efficiently and at maximum profit. Linear modeling is widely used for process control in physical systems because of the simplicity of the resulting equations. However, because of the nonlinearity of biologi-

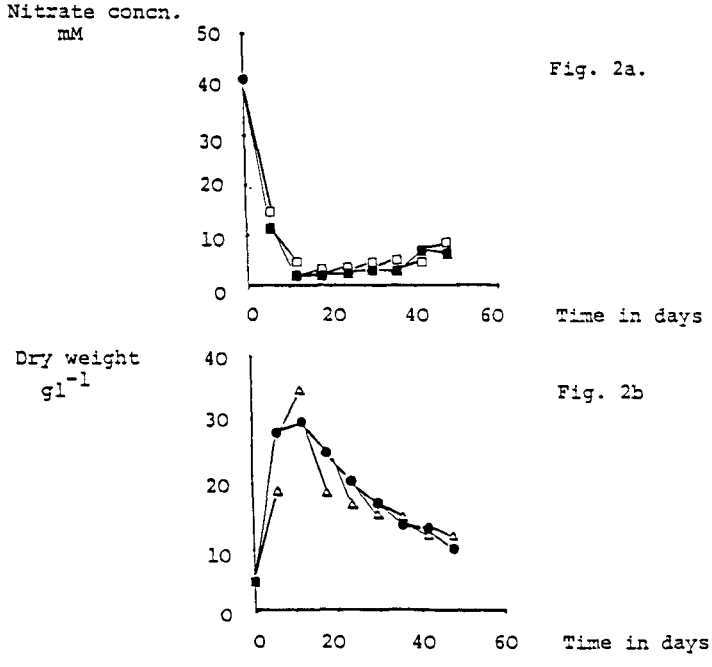
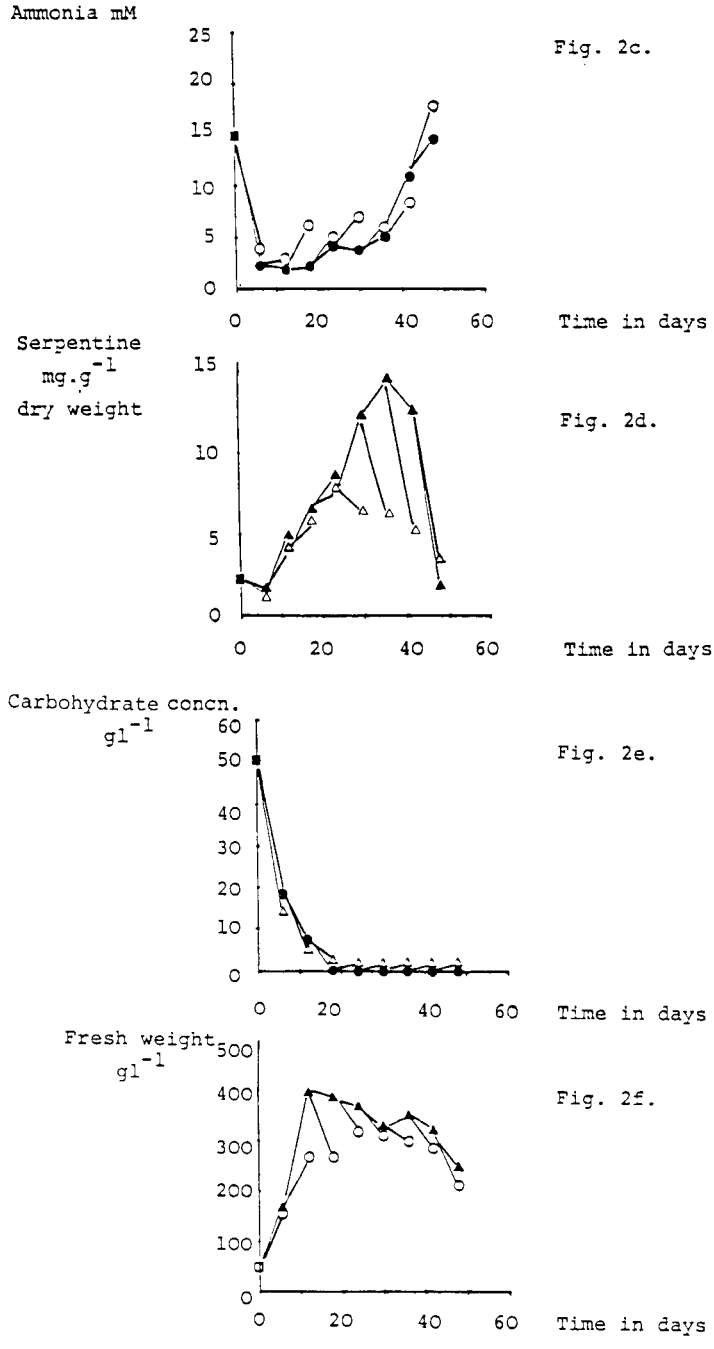


Fig. 2a–f. Comparison of experimentally determined data (filled symbols) with that generated using the one-step-ahead prediction model.

Fig. 2. (*continued*)



cal systems, these can often be useless unless modified in some way to take this into account. Nevertheles, they may still be of use in systems where little is known about the control variables, or where there is limited data available.

Briefly, the advantages and disadvantages of the formulation may be listed as follows:

Advantages

(1) It retains the advantages associated with a linear model, in that standard linear control techniques can be applied.
(2) The model parameters are fitted to minimize the sum of squares of the difference in logs of the observed and predicted measurements. This is desirable since many measuring devices have an accuracy expressed as a percentage of the reading, rather than as an absolute value.
(3) The variables are unable to change signs, thus the fitted model does not predict negative values of mass or density.
(4) The model formulation approximates the Monod factor $s/(s + K_s)$, over a range of s, by the expression $\log (10^{\epsilon} \cdot x^{\zeta} \cdot s^{\eta})$. Since the model formulation allows the product of several of these terms to be taken, the model is able to cater for multi-substrate kinetics.

Disadvantages

(1) The yield is no longer a constant; that is the decrease of substrate is no longer a constant multiple of the increase in biomass.
(2) It is not possible to express a change in a variable as the sum of two or more terms; however, one term will frequently swamp the others allowing the approximation to stand.

One useful feature of the model is that it seems to be unaffected by the change over in nutrient limitation of the culture, from being carbon-limited at 2% sucrose and nitrogen-limited at 8% sucrose.

In conclusion, the value of the system must therefore be seen in terms of it setting a basis for further development that will take into account some of the more empirical assumptions associated with the present model.

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