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Power-Law Approach to Modeling Biological Systems II. Application to Ethanol Production

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The use of the power-law formalism is illustrated by modeling yeast ethanol production in batch culture at high cell densities. Parameter values are estimated from experimental data. The results suggest that ethanol killing of viable cells and lysis of nonviable cells are major determinants of system behavior, whereas catabolism of ethanol and inhibition of cell growth by ethanol appear to be insignificant under these experimental conditions.

In recent years interest in fermentation-derived fuels and chemicals has increased sharply.[1] Among the fermentation products of significant economic concern is ethanol.[2] As a result, there is considerable research interest in improving ethanol production; one experimental approach that appears promising is yeast fermentation at high cell densities with on-line extraction.[3] We have chosen to model experiments related to this system as an illustration of the power-law formalism presented in the previous paper[4] because of the abundance of data obtained under well-controlled conditions.[5]

We begin with a simple unstructured model of yeast ethanol fermentation in a batch culture at high cell density. We refine this model by introducing in successive stages additional parameters to reflect relevant phenomena. At each stage the parameter values of the model are estimated from the experimental data.

This preliminary modeling of ethanol production (a) illustrates the use of the power-law formalism[4] and (b) demonstrates that even without detailed knowledge of the underlying mechanisms one can formulate reasonable first-order mathematical models. In these models ethanol killing of cells and lysis of non-viable cells are important determinants of the behavior of the total system, whereas ethanol catabolism and inhibition of cell growth by ethanol appear to be insignificant.

Simple Model

We begin with a simple model as shown in Fig. 1. We assume that the substrate glucose (X_3) is utilized in the production of ethanol (X_4), a process catalyzed by cellular enzymes that are functional in viable cells (X_1) but not in nonviable cells (X_2). Glucose also is used for the growth of viable cells, and ethanol activates the conversion of viable to nonviable cells.

The power-law equations describing this model are the following:

$$\begin{aligned}\dot{X}_1 &= \alpha_1 X_1^{g_{11}} X_3^{g_{13}} - \beta_1 X_1^{h_{11}} X_4^{h_{14}} \\ \dot{X}_2 &= \beta_1 X_1^{h_{11}} X_4^{h_{14}} \\ \dot{X}_3 &= -\beta_3 X_1^{h_{31}} X_3^{h_{33}} \\ \dot{X}_4 &= \alpha_4 X_1^{g_{41}} X_3^{g_{43}}\end{aligned} \qquad (1)$$

There is one equation for each variable. The change in each variable with time is the difference between its net rate of synthesis and its net rate of degradation. Each net rate term is a product of power functions, one for each reactant or modifier that affects that net rate. The α and β coefficients are apparent rate constants that are positive

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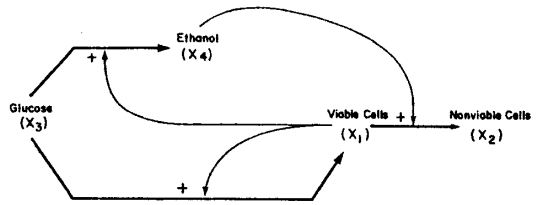

Fig. 1. Simple model of ethanol production by yeast. X_1 is the concentration of viable yeast cells; X_2 is the concentration of nonviable yeast cells; X_3 is the concentration of the substrate glucose; and X_4 is the concentration of ethanol. See text for further discussion.

real by definition. The g and h exponents are apparent kinetic orders that are positive real because there are no inhibitions in this model. An inhibitory interaction would be represented by a negative exponent associated with the concentration of the inhibitory metabolite.

The parameter values in these equations have been estimated from the data of Wang *et al.*[3,5] by using a computer algorithm[6] based on the Gauss-Newton method of interation. The results are shown in Fig. 2.

There is a good fit to the data for glucose, ethanol, and viable-cell mass. There is clearly an overestimate of nonviable- and total-cell mass, particularly at later times. These results suggest an obvious modification in the simple model of Fig. 1. By allowing for the possibility that nonviable cells may lyse, we lower the estimate for nonviable- and total-cell mass.

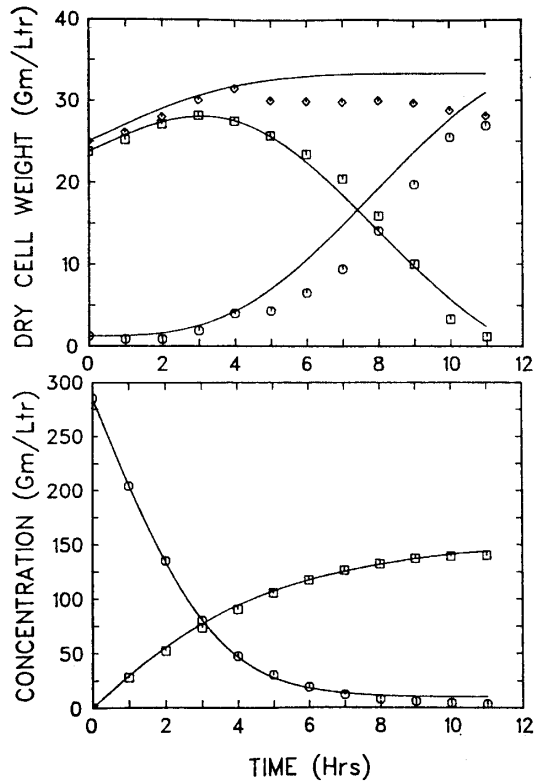

Fig. 2. Parameter estimation for the simple model of ethanol production by yeast. The experimental data of Wang *et al.*[3,5] are: upper panel, (□) viable cells (calculated from measurements of total dry weight and viability), (○) nonviable cells (calculated from measurements of total dry weight and viability), and (◇) total dry weight; bottom panel, (○) glucose, and (□) ethanol. The smooth curves in each case were drawn by computer for the model in Fig. 1 with parameter values that minimize the squared deviations from the experimental data. These values are: $\alpha_1=0.0000140$, $g_{11}=2.63$, $g_{13}=0.631$, $\beta_1=0.00000484$, $h_{11}=0.306$, $h_{14}=2.65$, $\beta_3=0.000125$, $h_{31}=2.68$, $h_{33}=0.867$, $\alpha_4=0.547$, $g_{41}=0.493$, $g_{43}=0.436$.

Simple Model Plus Lysis

Lysis of nonviable cells is indicated in the model of Fig. 3, everything else remains unchanged from Fig. 1. The corresponding equations in the power-law formalism are

$$\begin{aligned}\dot{X}_1&=\alpha_1 X_1^{g_{11}} X_3^{g_{13}}-\beta_1 X_1^{h_{11}} X_4^{h_{14}}\\ \dot{X}_2&=\beta_1 X_1^{h_{11}} X_4^{h_{14}}-\beta_2 X_2^{h_{22}}\\ \dot{X}_3&=-\beta_3 X_1^{h_{31}} X_3^{h_{33}}\\ \dot{X}_4&=\alpha_4 X_1^{g_{41}} X_3^{g_{43}}\end{aligned} \qquad (2)$$

where β_2 and h_{22} also have positive real values.

The results for this model with optimal parameter values are shown in Fig. 4. Again, there is a good fit to the data for glucose, ethanol, and viable cell mass, but in this case the fit for nonviable- and total-cell mass is considerably improved. The fit to the experimental data is statistically better than that in the previous case, even though two additional parameters are required.

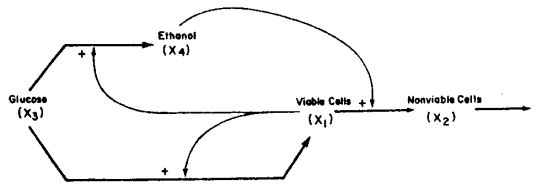

Fig. 3. Simple model of ethanol production by yeast with cell lysis. See legend for Fig. 1 and text for further discussion.

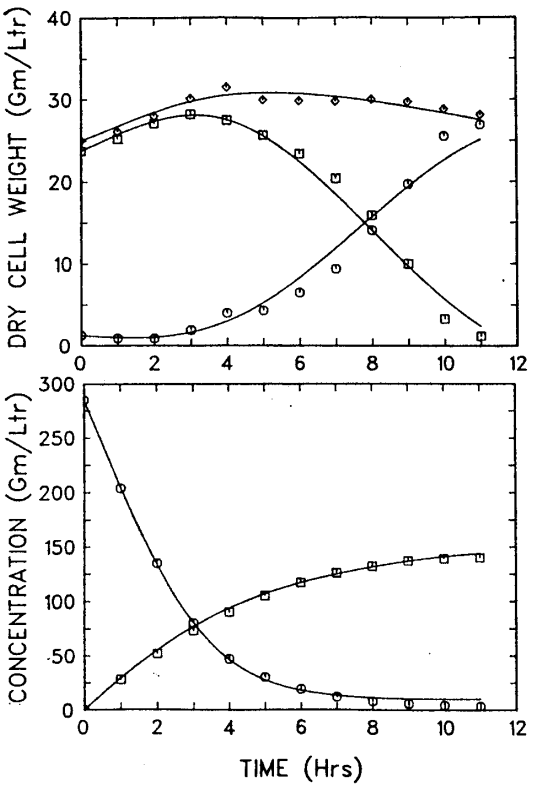

Fig. 4. Parameter estimation for the simple model of ethanol production by yeast with cell lysis. The optimal parameter values are: $\alpha_1=0.0000140$, $g_{11}=2.63$, $g_{13}=0.631$, $\beta_1=0.00000484$, $h_{11}=0.306$, $h_{14}=2.65$, $\beta_2=0.281$, $h_{22}=0.341$, $\beta_3=0.000125$, $h_{31}=2.68$, $h_{33}=0.867$, $\alpha_4=0.547$, $g_{41}=0.493$, $g_{43}=0.436$. See legend for Fig. 2 and text for further discussion.

Simple Model Plus Lysis Plus Ethanol Catabolism

Since there is evidence for ethanol catabolism,[7-10] we have asked whether this phenomenon is significant under experimental conditions with high initial cell densities. A model to represent this additional feature is shown in Fig. 5, everything else remains unchanged from Fig. 3. The corresponding

$$\begin{aligned}\dot{X}_1&=\alpha_1 X_1^{g_{11}} X_3^{g_{13}} X_4^{g_{14}}-\beta_1 X_1^{h_{11}} X_4^{h_{14}}\\ \dot{X}_2&=\beta_1 X_1^{h_{11}} X_4^{h_{14}}-\beta_2 X_2^{h_{22}}\\ \dot{X}_3&=-\beta_3 X_1^{h_{31}} X_3^{h_{33}}\\ \dot{X}_4&=\alpha_4 X_1^{g_{41}} X_3^{g_{43}}-\beta_4 X_1^{h_{41}} X_4^{h_{44}}\end{aligned} \tag{3}$$

where the extra parameters g_{14}, β_4, h_{41} and h_{44} have positive real values.

The results for this model with optimal

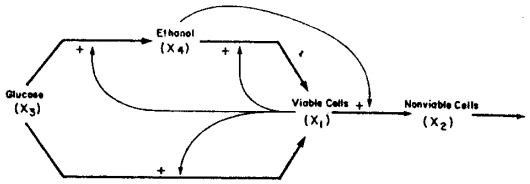

Fig. 5. Simple model of ethanol production by yeast with cell lysis and catabolism of ethanol. See legend for Fig. 1 and text for further discussion.

equations in the power-law formalism are parameter values are shown in Fig. 6. There has been a slight improvement in the fit to the data for glucose, ethanol, and viable cell mass. The fit for nonviable- and total-cell mass is also quite good. However, this improvement over the previous case requires four additional parameters and is only marginally significant.

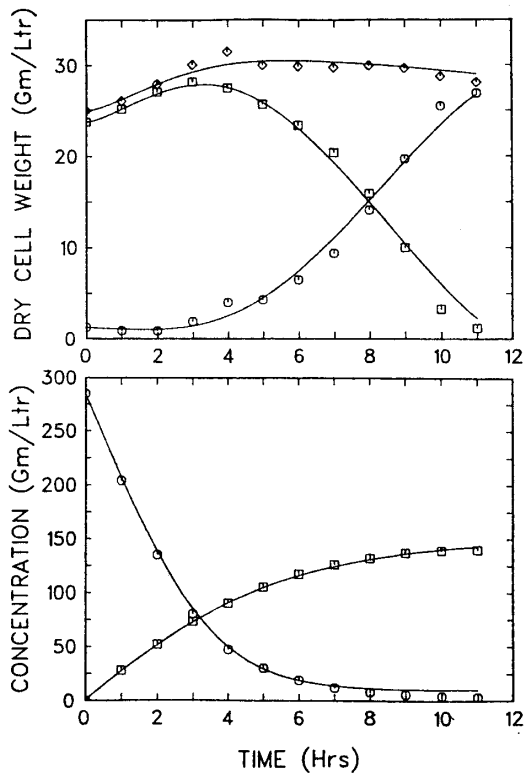

Fig. 6. Parameter estimation for the simple model of ethanol production by yeast with cell lysis and catabolism of ethanol. The optimal parameter values are: $\alpha_1=0.0000430$, $g_{11}=1.00$, $g_{13}=1.09$, $g_{14}=0.495$, $\beta_1=0.000000487$, $h_{11}=0.336$, $h_{14}=3.11$, $\beta_2=0.172$, $h_{22}=0.293$, $\beta_3=0.000125$, $h_{31}=2.68$, $h_{33}=0.867$, $\alpha_4=4.23$, $g_{41}=0.611$, $g_{43}=0.104$, $\beta_4=2.79$, $h_{41}=0.628$, $h_{44}=0.0796$. See legend for Fig. 2 and text for further discussion.

Discussion

The simplest model (Fig. 1) produces a reasonably good fit to the experimental data (Fig. 2), indicating that ethanol killing of viable cells is a major feature of the fermentation under these experimental conditions. The simple model plus lysis of non-viable cells (Fig. 3) produces a significantly better fit to the data (Fig. 4), indicating that lysis is also a major feature of the fermentation under these experimental conditions.

In the course of this analysis we augmented the model in Fig. 3 to include inhibition of cell growth by ethanol[11,12] without ethanol catabolism. An attempt to estimate the parameters of this augmented model showed that such inhibition was inconsistent with the experimental data; g_{14} and h_{34} were estimated to be positive, indicating *activation* rather than inhibition of cell growth and glucose utilization (data not shown). Furthermore, the fit to the experimental data was not significantly improved by such an activation.

We also augmented the model in Fig. 5 to include ethanol inhibition of cell growth. Again, activation rather than inhibition was indicated, and the fit to the experimental data was not significantly improved (data not shown).

Thus, for this series of models the best fit to the experimental data is provided by the model that includes killing of viable cells by ethanol and lysis of nonviable cells (Fig. 3). The other additions to this basic model that we tried produced questionable improvements, and when the increased number of parameters is taken into account the differences are not statistically significant.

These simple models are obviously not intended to be the final word on ethanol production. Their use here is primarily to illustrate the potential of the power-law formalism. The point is that one can use these same methods to develop more detailed and realistic models, as circumstances require.

Nomenclature

α_i apparent rate constant for the net synthesis of the ith element of the system

β_i apparent rate constant for the net utilization of the ith element of the system

g_{ij} apparent kinetic order for the net synthesis of the ith element with respect to the jth element

h_{ij} apparent kinetic order for the net utilization of the i-th element with respect to the j-th element

X_i concentration of a biological (biochemical) element within a spatially homogeneous compartment, subscript signifies both the name (type) and location (compartment) of the element (mg/l)

Acknowledgments

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