BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS **227,** 41–46 (1996)
ARTICLE NO. 1464

Kinetic Analysis and Comparison of Models of Xylose Metabolism by *Klebsiella planticola*

Simone Bastianoni,* Amalia Gastaldelli,† Claudia Bonechi,*
Chiara Mocenni,* and Claudio Rossi*,[1]

**Department of Chemical and Biosystem Sciences, University of Siena, Pian dei Mantellini 44, 53100 Siena, Italy; and †Institute of Clinical Physiology—Metabolism Unit, National Center of Research, Via Savi 8, 56100 Pisa, Italy*

Received August 6, 1996

A model for the degradation of xylose and ethanol production by *Klebsiella planticola* is proposed and compared with the exponential and Michaelis–Menten approaches. This model is based on the energy system diagrams and it is a simplified version of a previous model developed for the glucose and ethanol kinetics of the yeast *Saccharomices cerevisiae.* In this model the dynamics of the substrate and of the final product are strictly related by means of the cellular activity. This model shows superior performances with respect to the two alternatives, behaving better along the whole dynamics.

Interpretation of experimental results obtained by organized complex systems like cell organisms requires an appropriate approach. In the past, theoretical models have been proposed (1-3) and used for the elucidation of metabolic steps and for the calculation of kinetic parameters. Considering the cellular metabolic reactions resulting from activation, inhibition and feedback activities, the development of new theoretical models is of great importance for the biomolecular sciences. Such models must deal with a large number of interactions and must be flexible enough to adapt to existing approaches and experimental results. When a comparison between different approaches is required, the following properties must be analyzed and considered:

(i) the model should require the lowest number of parameters to be calculated;
(ii) the difference between the theoretical and the experimental behavior (residuals) should be reduced at the minimum value at any stage of the process. Residuals must be randomly distributed around zero. Regions where great discrepancy between the theoretical and experimental values must be avoided;
(iii) the model must be flexible enough to allow the addition or elimination of individual parts in order to fit specific metabolic activities;
(iv) the model should refer to parameters having precise biological meanings.

In order to evaluate which could be the best available model in the interpretation of biological events, a careful comparison of theoretical approaches is required.

The aim of this paper is to compare three different approaches utilized for the analysis of the metabolism of sugar by a bacterium cell culture. Our purpose is to identify a model which allows the best fit with the experimental results and considers a limited number of parameters all related to an identified biological function. Three different models were compared: a model based on a pure exponential behavior (4), a model which considers the metabolization process based on the classical Michaelis-Menten kinetic analysis (5-6) and finally a model previously

[1] To whom correspondence should be addressed. Fax: (39) 577 298004. E-mail: rossi@unisi.it.

0006-291X/96 $18.00

developed for sugar metabolization analysis (7). The three theoretical models were used to fit a set of experimental results related to the xylose metabolization by a selected *Klebsiella planticola* G11 strain (8). The experimental substrate consumption and the end-product formation were detected by ''in-vivo'' NMR spectroscopy using selective carbon-13 enrichment of the xylose substrate.

MATERIALS AND METHODS

Klebsiella planticola, G11, previously isolated and identified (8) were grown at 35 °C under nitrogen atmosphere in a culture medium containing 5.25 g/l KH_2PO_4, 6.85g/l K_2HPO_4, 5.0 g/l $NaHCO_3$, 0.1 g/l $MgSO_4$, 0.1 g/l NaCl, 0.2 g/l $(NH_4)_2SO_4$, 0.3 g/L urea, 0.02 g/l $CaCl_2$, 0.2 g/l yeast extract. Inocula for ''in vivo'' microbatch ^{13}C-NMR experiments were obtained by growing a single agar colony overnight in the medium with 10 g/l xylose added. The cells were then collected by centrifugation and used as inoculum for NMR measurements. The initial O.D. value was 0.5.

(1-^{13}C), xylose, obtained from Cambridge Isotope Laboratories, was used to obtained 10 g/l xylose substrate concentration. ^{13}C-NMR spectra were collected by a Varian XL-200 spectrometer operating at 200 and 50.29 MHz for proton and carbon nuclei respectively. Carbon spectra were recorded under continuous broad-band proton decoupling conditions. The microbatch was obtained in a coaxial tube containing 100% D_2O as NMR lock signal in the external section. All the NMR signals were referred to tetramethylsilane. The substrate and end-products concentrations were calculated from the intensity of the NMR lines through an appropriate calibration. The error in the calculated concentration was ± 3%.

RESULTS AND DISCUSSION

We utilized as a first model, one based on an exponential fitting with governing equations for the xylose (X(t)) and ethanol (E(t)) in the form:

$$X(t) = XMAX \cdot \exp\{-KX_1 \cdot t\} + XMIN \quad [1]$$

$$E(t) = EMAX \cdot (1 - \exp\{-KE_1 \cdot t\}) \quad [2]$$

where XMAX is the maximum value in the concentration of xylose; XMIN is the concentration at which the degradation of xylose is restricted; EMAX is the plateau concentration of ethanol; KX_1 and KE_1 are the exponents of the xylose and ethanol exponential dynamics. Two versions of the model (equation [1] above) are considered, containing either five or four parameters. In the second case, the fit was carried out constraining XMIN to a zero value; the exponentials and ethanol plateau were then estimated. In Figure 1 the results of the fitting procedure in the two cases are shown. The model with four parameters is not able to follow the experimental curve; the introduction of the parameter XMIN makes the value of R^2 rise until 0.996 with a Residual Sum of Squares (RSS) from 5.02 to 1.09 (Fig.1).

The Michaelis-Menten model was used in its differential version (5) to study the degradation dynamics of the sugar while the ethanol dynamics were fitted with the traditional Michaelis-Menten curve (growth and saturation):

$$\frac{dX}{dt} = -VXmax \frac{X}{KX_2 + X} \quad [3]$$

$$E(t) = VEmax \frac{t}{KE_2 + t} \quad [4]$$

where VEmax is the plateau level of the ethanol, VXmax is the initial degradation rate, and KX_2 and KE_2 are the constant of the Michaelis-Menten dynamics. This model (see Fig. 2) has four parameters and gives results that are markedly inferior to the exponential model. The use of the best fitting procedure is difficult as a result of the distance between the theoretical curve

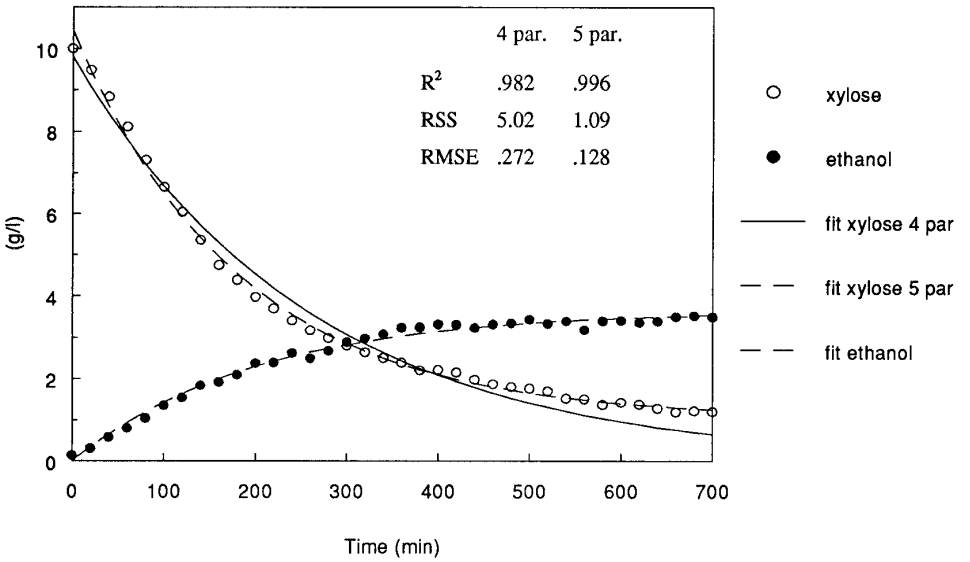

FIG. 1. The results of the fitting procedure in the case of the exponential model (four and five parameters). Data for xylose and ethanol were collected in an NMR experiment where initial concentration of xylose was 10 g/l, as described in Materials and Methods. Values of R^2, residual sum of squares (RSS), and root-mean-square error (RMSE) for these two models are used to compare the results.

and the experimental data with regards to the xylose dynamics. The obtained parameters have an error with a coefficient of variation of nearly 100%. The description of the ethanol production data is slightly better even if R^2 has smaller and RSS has greater values than the ones obtained using the first model.

The third model is based upon the energy system diagrams introduced by H. T. Odum in

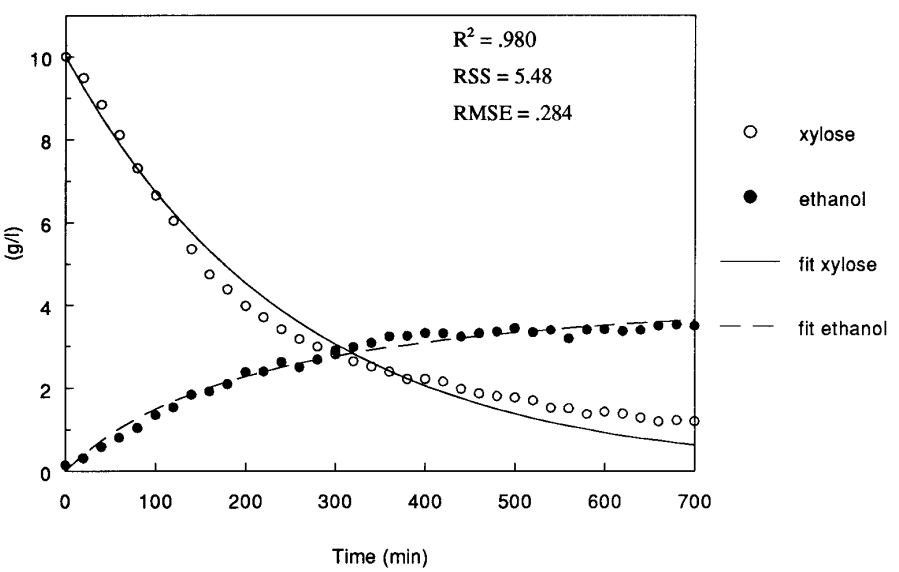

FIG. 2. The Michaelis–Menten model compared with the data set reported in Figure 1.

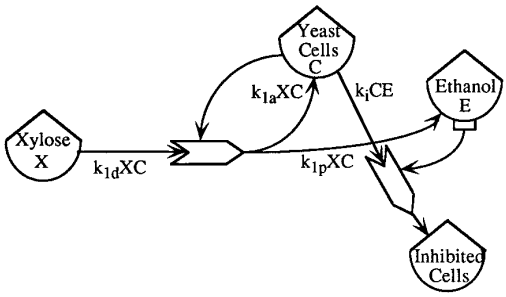

FIG. 3. The energy system diagram of the model in Equations 5–7, where xylose, active cells, ethanol, and inhibited cells are present.

the 70's to describe energy fluxes that pass through complex systems, both biological and ecological (9-10). This type of model could be described as compartmental where the fundamental components are identified and their relationships modeled using kinetic equations that are usually non-linear.

The general model (7) contains four *storages* (xylose, cells of *Klebsiella planticola,* ethanol, and bacteria cells inhibited by the end-product). The dynamic is described as follows: the sugar metabolization is defined (7) as the result of two simultaneous processes, an autocatalytic step and one which only depends on the number of active cells. The model takes account of the fact that the presence of sugar substrates promotes an energy flow from the sugar to the active cells. Part of the energy flow is used for the survival, maintenance and respiration processes of the microorganisms (''required minimum'') and the remainder to increase the concentration of active cells (i.e. cells involved in reproduction and activation processes). Ethanol acts as a controller of the number of active cells, depicted as an outflow from the active to the inhibited cells tank.

In the present case the xylose degradation rate slows when the sugar concentration approaches low values (1 g/l). Thus the equations can be simplified because there is no need to utilize the parts of the model that are referred to as ''required minimum''. Therefore the part of the model which considers the xylose consumption independent of the sugar concentration of the system can be neglected. The resulting model is represented in Figure 3. The systems can be described by the following equations, in which sugar (X(t)) degradation, ethanol (E(t)) production and cell (C(t)) activation are linked together as follow:

$$\frac{dX}{dt} = -k_{1d} \cdot X \cdot C \qquad [5]$$

$$\frac{dC}{dt} = k_{1a} \cdot X \cdot C - k_i \cdot C \cdot E \qquad [6]$$

$$\frac{dE}{dt} = k_{1p} \cdot X \cdot C \qquad [7]$$

where the four parameters that are estimated are the coefficients relating the concentration of xylose, cells and ethanol to the degradation of sugar (k_{1d}), activation (k_{1a}) and inhibition (k_i) of cells and production of ethanol (k_{1p}). The estimated values of these parameters corresponding to a minimum of RSS, together with their coefficient of variation (CV) are shown in Table 1.

The results of the fitting procedure are shown in Figure 4. This model with its four parameters

TABLE 1
Values of the Kinetic Parameters and of the Coefficient of Variation (CV) Calculated Using the Model in Figure 3

Parameter	Value	CV (%)
k_{1d}	0.09145	3.2
k_{1a}	0.03294	10.1
k_i	0.20422	6.8
k_{1p}	0.01829	3.5

can now be compared to those preceding. Such a comparison demonstrates that the compartmental model fit is always superior to the other two, both in terms of precision of parameter estimates and RSS. Our compartmental model behaves better across the complete degradation dynamics. Moreover our model describes the interaction between cells and substrate and cells and end-product, while in the other cases the dynamics of xylose and ethanol are separately described.

In conclusion, it appears that the Michaelis-Menten dynamic model is not adequate for the description of this system, even if its parameters have a biological significance. The exponential model with five parameters gives a good response but its use is purely mathematically descriptive. Our model shows a superior performance from the modellistic view and contains parameters that have a precise biological significance. Additionally, the degradation dynamics of the substrate, the activation and inhibition processes and the ethanol production are strictly coupled. This model allows us to evaluate the real level of the cellular activity, whose value is not correlated only to the concentration of biomass and is difficult to measure directly. Moreover the fitting procedure verified the validity of assuming negligible the part of the kinetics we referred to as ''required minimum''.

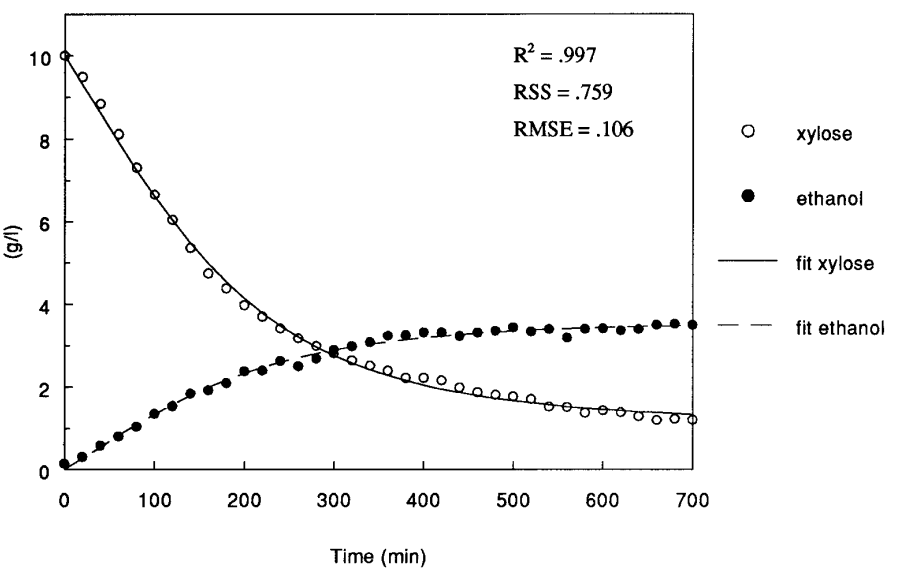

FIG. 4. The result of the fitting procedure of the model reported in Figure 3.

REFERENCES

1. Magar, M. E. (1972) Data Analysis in Biochemistry and Biophysics, Academic Press, New York.
2. Beechem, J. M. (1992) *in* Methods in Enzimology (Brand, L., and Johnson, M. J., Eds.), Vol. 210, pp. 37–54, Academic Press, San Diego.
3. Nielsen, J., and Villadsen, J. (1992) *Chem. Eng. Sci.* **47,** 4225–4270.
4. Halvorson, H. R. (1992) *in* Methods in Enzimology (Brand, L., and Johnson, M. J., Eds.), Vol. 210, pp. 54–67, Academic Press, San Diego.
5. den Hollander, J. A., Brown, T. R., Ugurbil, K., and Shulman, R. G., (1979) *Proc. Natl. Acad. Sci.* **205,** 6096–6100.
6. Shulman, R. G., Brown, T. R., Ugurbil, K., Ogawa, S., Cohen, S. N., and den Hollander, J. A. (1979) *Science* **205,** 160–176.
7. Bastianoni, S., Donati, A., Gastaldelli, A., Marchettini, N., Renzoni, D., and Rossi, C. (1996) *Biochem. Biophys. Res. Commun.* (submitted).
8. Rossi, C., Donati, A., Medaglini, D., Valassina, M., Bastianoni, S., and Cresta, E. (1995) *Biomass and Bioenergy* **8,** 197–202.
9. Odum, H. T. (1972) *in* Systems Analysis and Simulation in Ecology, Vol. II (Patten, B. C., Ed.), pp. 139–211, Academic Press, New York.
10. Odum, H. T. (1991) *in* Ecological Physical Chemistry (Rossi, C., and Tiezzi, E., Eds.), pp. 25–56, Elsevier, Amsterdam.