A modelling approach for the analysis of xylose–ethanol bioconversion

Claudio Rossi *, Marcello Porcelli, Chiara Mocenni, Nadia Marchettini, Steven Loiselle, Simone Bastianoni

Department of Chemical and Biosystem Sciences, University of Siena, Pian dei Mantellini, 44-53100 Siena, Italy

Abstract

A model for the degradation of xylose and ethanol production by Klebsiella planticola is proposed and compared with the exponential (E) and Michaelis–Menten (MM) approaches. This model follows an ecological approach, being based on H.T. Odum’s energy system diagrams and it is a simplified version 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 (E and MM), behaving better along the whole dynamics. Two different strains of K. planticola are analyzed to compare their performances from this viewpoint. © 1998 Elsevier Science B.V. All rights reserved.

Keywords: Modelling; Klebsiella planticola; Xylose–ethanol conversion

1. Introduction

Interpretation of experimental results obtained by organized complex systems like cell organisms requires an appropriate approach. In the past, theoretical models have been proposed and used for the elucidation of metabolic steps and for the calculation of kinetic parameters (Jacquez, 1996). Considering the cellular metabolic reactions resulting from activation, inhibition and feed-back 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:

1. The model should require the lowest number of parameters to be calculated.
2. The difference between the theoretical and the experimental behavior (residuals) should be reduced to a minimum at all stages of the

* Corresponding author. Tel.: +39 0577 298003; fax: +39 0577 298004
process. Residuals must be randomly distributed around zero. Regions where great discrepancy between the theoretical and experimental values must be avoided.

3. The model must be flexible enough to allow the addition or elimination of individual parts in order to fit specific metabolic activities.

4. 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 a well identified biological function.

Three different models were compared: a model that we originally developed for sugar metabolism by a yeast (Bastianoni et al., 1996a) and then we tested on a strain of *Klebsiella planticola* (Bastianoni et al., 1996b); a model based on a pure exponential behavior (Keller and Zellner, 1996) and a model which considers the metabolization process based on the classical Michaelis–Menten (MM) kinetic analysis (Bergmeyer, 1974). The three theoretical models were used to fit a set of experimental results related to the xylose metabolization by a selected *K. planticola* ATCC 33531.

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 (Shulman et al., 1979).

2. Materials and methods

The microorganism *K. planticola* ATCC 33531, previously isolated and identified (Ørskov, 1984) is grown at 35°C under nitrogen atmosphere in a culture containing 5.25 g/l KH₂PO₄, 6.85 g/l K₂HPO₄, 5.0 g/l NaHCO₃, 0.1 g/l MgSO₄, 0.1 g/l NaCl, 0.2 g/l (NH₄)₂SO₄, 0.3 g/l urea, 0.02 g/l CaCl₂ and 0.2 g/l yeast extract. The following trace elements were also present: Fe, Cu, Co, Mo, Mn.

The pH of the medium was adjusted to 7.5. Growth was followed by spectrophotometric optical density (OD) measurements at 660 nm. A unitary value of OD was verified corresponding to 0.53 g/l of dry weight of the biomass. Inocula for in vivo microbatch ¹³C-NMR experiments were prepared by growing a single agar colony overnight in the medium with 10 g/l of D-xylose added to accustom the microorganism to degrade this substrate. A fraction of this culture was diluted 2:100 in the medium for 2–3 further duplication cycles. The cells were then collected by centrifugation and used as the inoculum for NMR measurements.

¹³C-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. 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 (Rossi et al., 1992, 1995). The error in the calculated concentration was ±3%.

The mathematical program used to calculate the optimal parameters of the model is MLAB (Modelling LABoratory) (Bunow and Knott, 1992). This program, originally developed at the National Institute of Health, have shown very good flexibility and adaptability in solving simulation and modeling problems.

2.1. The model

The model discussed here uses the ‘language’ of the energy system diagrams introduced by H.T. Odum in the 70’s (Odum, 1971, 1991) to summarize the behavior of systems and the relations among their components. This kind of models has been developed to describe energy fluxes that pass through complex systems, from an ecological holistic viewpoint, “combining kinetics, energetics and economics” (Odum, 1983).

Our model (Bastianoni et al., 1996b) has successfully been used for the description of the
metabolism of another strain of K. planticola, the ‘wild’ G11 and showed very good correspondence with experimental data. We are now testing its ability in following the metabolism of xylose by a very selected one, the ATCC 33531. The model is composed by four storages (see Fig. 1): xylose, that is the (limited) source of energy; active cells of K. planticola; ethanol, that is the main product of the process and bacteria cells, that are inhibited by the end-product.

The dynamics of the sugar metabolism is assumed to be the result of an autocatalytic process that depends on the concentration of substrate and 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. The energy flow is used for increasing the concentration of active cells. Ethanol acts as a controller of the quantity of active cells, shown in Fig. 1 as an outflow from the active to the inhibited cells tank. The interaction between cells and ethanol does not imply an outflow of ethanol and this is graphically expressed by the box under the storage of ethanol.

In the present case the xylose degradation rate becomes irrelevant when the sugar concentration approaches low values (1 g/l). The resulting model is represented in Fig. 1. 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:

\[
\frac{dX}{dt} = -k_{1d} \cdot [X] \cdot [C] \\
\frac{dC}{dt} = k_{1a} \cdot [X] \cdot [C] - k_i \cdot [C] \cdot [E] \\
\frac{dE}{dt} = k_{1p} \cdot [X] \cdot [C]
\]

where the four parameters are the coefficients relating the concentration of xylose (X), cells (C) and ethanol (E) to the degradation of sugar (k_{1d}), activation (k_{1a}) and inhibition (k_i) of cells and production of ethanol (k_{1p}). These parameters have dimensions: for k_{1d} and k_{1p} min^{-1}; for k_{1a} and k_i g^{-1}·min^{-1}. A fourth equation, the one relative to inhibited cells (I) has to be written, even if it is not important for the simulation:

\[
\frac{dI}{dt} = k_i \cdot [C] \cdot [E]
\]

### 3. Results and discussion

The estimated values of the parameters as well as their corresponding minimum of the residual sum of squares (RSS) and their coefficient of variation (CV) are shown in the first two columns of Table 1. The results of the MLAB fitting procedure are shown in Fig. 2. The $R^2$ value is 0.9998; the RSS is 0.0468 (Porcelli, 1997).

![Diagram of the conversion of xylose to ethanol by K. planticola.](image)

**Table 1**

<table>
<thead>
<tr>
<th>Kinetic constants</th>
<th>Optimal values (G11)</th>
<th>CV</th>
<th>Optimal values (ATCC 33531)</th>
<th>CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>$k_{1d}$</td>
<td>$9.145 \cdot 10^{-4}$</td>
<td>3%</td>
<td>$1.083 \cdot 10^{-3}$</td>
<td>2%</td>
</tr>
<tr>
<td>$k_{1p}$</td>
<td>$1.829 \cdot 10^{-4}$</td>
<td>3%</td>
<td>$1.964 \cdot 10^{-4}$</td>
<td>2%</td>
</tr>
<tr>
<td>$k_{1a}$</td>
<td>$3.294 \cdot 10^{-4}$</td>
<td>10%</td>
<td>$4.399 \cdot 10^{-4}$</td>
<td>6%</td>
</tr>
<tr>
<td>$k_i$</td>
<td>$2.042 \cdot 10^{-3}$</td>
<td>7%</td>
<td>$1.551 \cdot 10^{-3}$</td>
<td>11%</td>
</tr>
</tbody>
</table>
We compared the performances of our model with two alternatives, taken from those most cited in literature. We utilized as a first comparison an exponential fitting with governing equations for the xylose \((X(t))\) and ethanol \((E(t))\) in the form:

\[
X(t) = X_{\text{max}} \cdot \exp\{ -KX_1 \cdot t \} \tag{4}
\]
\[
E(t) = E_{\text{max}} \cdot (1 - \exp\{ -KE_1 \cdot t \}) \tag{5}
\]

where \(X_{\text{max}}\) is the maximum value in the concentration of xylose; \(E_{\text{max}}\) is the plateau concentration of ethanol; \(KX_1\) and \(KE_1\) are the exponents of the xylose and ethanol exponential dynamics. In Fig. 3 the results of the fitting procedure are shown for the two cases. This model is less able to follow the experimental curve with an \(R^2\) value of 0.9981 and RSS of 0.4622 (Fig. 3).

The MM model was used in its differential version (den Hollander et al., 1979) to study the degradation dynamics of the sugar while the ethanol dynamics were fitted with the traditional MM curve (growth and saturation):

\[
\frac{dX}{dt} = -VX_{\text{max}} \frac{X}{KK_2 + X} \tag{6}
\]
\[
E(t) = VE_{\text{max}} \frac{t}{KE_2 + t} \tag{7}
\]

where \(VE_{\text{max}}\) is the plateau level of the ethanol, \(VX_{\text{max}}\) is the initial degradation rate and \(KK_2\) and \(KE_2\) are the constant of the MM dynamics. This model (see Fig. 4 for the plot of the best fit) has four parameters and gives results that are more representative than those of the exponential.
coefficients of variation are presented in Table 1. We obtained very similar values for each of the parameters and always with a low estimation error. Nonetheless, we can say that the strain ATCC 33531 has a more active metabolic process, having a higher rate of degradation of xylose ($k_{d1}$), a higher level of activation of the cells ($k_{1a}$) and rate of production of ethanol ($k_{1p}$), while the parameter that accounts for the inhibition ($k_i$) is lower.

In conclusion, it appears that our model shows a superior performance from the modellistic view and contains parameters that have a precise biological significance. The MM dynamic model and the exponential one show worse results for the description of this system and their parameters have less biological meaning. In our model the processes of degradation of the substrate, activation and inhibition of the cells 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. The parameters of the model have been used as a tool for a comparison between microorganism performances. In this case we have compared two strains of Klebsiella planticola. The values of the parameters suggest that the strain ATCC 33531 has a more efficient transformation model but less than the model here proposed, with an $R^2$ value of 0.9991 and RSS of 0.220.

Our four parameters model can now be compared with the earlier models examined. Such a comparison demonstrates that the proposed model’s 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.

The analysis of the residues shows that for the dynamics of xylose degradation the model we have proposed has the best performances and the residues are randomly distributed, while in the other two cases a trend in the distribution of residues (and of higher value) is shown (Fig. 5). The fit of the ethanol is slightly worse than in the case of the exponential and MM models, but the residues have very low values in all cases, strictly within the range of the error in the measurements (Fig. 6).

From the viewpoint of our model we can now compare the performances of the two strain of Klebsiella planticola, the wild G11 and the selected ATCC 33531. The values of the parameters after the best fit procedure and the respective coefficients of variation are presented in Table 1. We obtained very similar values for each of the parameters and always with a low estimation error. Nonetheless, we can say that the strain ATCC 33531 has a more active metabolic process, having a higher rate of degradation of xylose ($k_{d1}$), a higher level of activation of the cells ($k_{1a}$) and rate of production of ethanol ($k_{1p}$), while the parameter that accounts for the inhibition ($k_i$) is lower.

In conclusion, it appears that our model shows a superior performance from the modellistic view and contains parameters that have a precise biological significance. The MM dynamic model and the exponential one show worse results for the description of this system and their parameters have less biological meaning. In our model the processes of degradation of the substrate, activation and inhibition of the cells 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. The parameters of the model have been used as a tool for a comparison between microorganism performances. In this case we have compared two strains of Klebsiella planticola. The values of the parameters suggest that the strain ATCC 33531 has a more efficient transformation model but less than the model here proposed, with an $R^2$ value of 0.9991 and RSS of 0.220.

Our four parameters model can now be compared with the earlier models examined. Such a comparison demonstrates that the proposed model’s 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.

The analysis of the residues shows that for the dynamics of xylose degradation the model we have proposed has the best performances and the residues are randomly distributed, while in the other two cases a trend in the distribution of residues (and of higher value) is shown (Fig. 5). The fit of the ethanol is slightly worse than in the case of the exponential and MM models, but the residues have very low values in all cases, strictly within the range of the error in the measurements (Fig. 6).

From the viewpoint of our model we can now compare the performances of the two strain of Klebsiella planticola, the wild G11 and the selected ATCC 33531. The values of the parameters after the best fit procedure and the respective coefficients of variation are presented in Table 1. We obtained very similar values for each of the parameters and always with a low estimation error. Nonetheless, we can say that the strain ATCC 33531 has a more active metabolic process, having a higher rate of degradation of xylose ($k_{d1}$), a higher level of activation of the cells ($k_{1a}$) and rate of production of ethanol ($k_{1p}$), while the parameter that accounts for the inhibition ($k_i$) is lower.

In conclusion, it appears that our model shows a superior performance from the modellistic view and contains parameters that have a precise biological significance. The MM dynamic model and the exponential one show worse results for the description of this system and their parameters have less biological meaning. In our model the processes of degradation of the substrate, activation and inhibition of the cells 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. The parameters of the model have been used as a tool for a comparison between microorganism performances. In this case we have compared two strains of Klebsiella planticola. The values of the parameters suggest that the strain ATCC 33531 has a more efficient transformation model but less than the model here proposed, with an $R^2$ value of 0.9991 and RSS of 0.220.
process in all the aspects of the dynamics respect to the wild strain G11. We believe that this model can constitute a basis for a deeper understanding of microorganism dynamics, permitting to identify variations in individual aspects of the degradation processes. This modeling approach has allowed us to reveal a more comprehensive comparison of two microorganisms important in the sugar to ethanol transformation.

Acknowledgements

We specially thank Dr H.T. Odum and Dr M.T. Brown for their help in developing the first steps of this model.

References


