UDC 579.24:57.037:582.28
ISSN 1330-9862

original scientific paper

(FTB-1203)

A Model for Converting Solid State Fermentation Growth Profiles Between Absolute and Relative Measurement Bases

Graciele Viccini[1], *David A. Mitchell*[1*] *and Nadia Krieger*[2]

[1]Departamento de Bioquímica e Biologia Molecular, Universidade Federal do Paraná, Cx. P. 19046, Centro Politécnico, Curitiba 81531-990, Paraná, Brazil

[2]Departamento de Química, Universidade Federal do Paraná, Cx.P. 19081, Curitiba 81531-990, Paraná, Brazil

Received: November 25, 2002
Revised version: April 29, 2003
Accepted: June 9, 2003

Summary

A mathematical model is developed for converting between the two measurement bases commonly used in the construction of growth profiles in solid-state fermentation, namely absolute mass ratio m(dry biomass)/m(initial dry matter) and relative mass ratio m(dry biomass)/m(dry matter). These are not equivalent, due to the loss of dry matter as CO_2 during the fermentation. The model is equally applicable to any biomass component used in indirect measurements of growth, such as protein. Use of the model to convert absolute mass ratio of the biomass profiles for the growth of *Rhizopus oligosporus* to a relative basis gave profiles that agreed well with the experimentally determined relative biomass profiles. This agreement was obtained for three different fermentations using the same set of parameter values in the model, namely a yield coefficient of m(protein)/m(dry substrate) = 0.2 g/g and a maintenance coefficient of zero, giving confidence in the reliability of the model. The model was then used to show that the measurement basis used can affect the form of the curve and therefore can also affect the conclusion drawn about the type of kinetics shown by the organism, with the extent of this effect depending on the length of time that growth occurs and the values of the yield and maintenance coefficients. This work shows that great care must be taken in drawing conclusions about growth kinetics in solid-state fermentation.

Key words: solid-state fermentation, microbial growth kinetics, logistic equation, exponential growth kinetics, linear growth kinetics

Introduction

This paper develops a mathematical model that can be used to translate between the two biomass measurement bases that are commonly used in the construction of growth profiles in solid-state fermentation (SSF). This fermentation technique involves the growth of microorganisms in beds of moist solid substrate particles, in which interparticle spaces are filled with air, containing little or no free water. It has received renewed interest over the last two decades for the production of a range of biotechnological products (*1*). The solid nature of the culture medium makes SSF systems more difficult to study than the more traditional submerged liquid fer-

* Corresponding author; E-mail: davidmitchell@ufpr.br

mentation (SLF). Various of these difficulties, which interfere with our understanding of growth kinetics in SSF, are discussed in the following paragraphs.

In the majority of SLF processes, growth profiles can readily be constructed on the basis of optical densities, or dry weights of biomass obtained after the filtration of samples, whereas in SSF it is commonly impossible to obtain direct measurements of biomass. Kinetic studies must either be done in artificial systems that mimic SSF while enabling biomass measurement or be based on indirect methods of biomass estimation, such as the measurement of components of the biomass or either O_2 consumption or CO_2 formation.

Further, it is relatively straightforward to mix liquid broths, and as a result, in the construction of models for SLF, it is a simple matter of modelling the rate of growth as depending on the limiting nutrient concentration according to the Monod equation, since the substrate concentration is uniform throughout the medium. In contrast, the supply of nutrients in SSF is the result of a complex series of steps. If the carbon source is a polymer, this will include the release of hydrolytic enzymes and their diffusion into the substrate particle, reaction within the substrate particle to liberate soluble hydrolysis products, diffusion of these hydrolysis products within the solid matrix and their uptake by the biomass (*2*). A model that relates the growth rate to the nutrient concentration experienced by the biomass will therefore be highly complex, including partial differential equations with both time and space as independent variables. Given the fact that in the majority of bioreactors there are significant gradients at the macroscale, and therefore partial differential equations will already be necessary to describe the macroscale events, most researchers prefer to avoid modeling the microscale events in a mechanistic manner, and use simple empirical equations to describe the growth kinetics (*3-5*). The various empirical equations used to describe SSF growth profiles include the logistic, exponential, linear and deceleration equations (*5–6*), which are shown in Table 1.

Even with the use of simple empirical equations, there is a potential difficulty in interpreting growth profiles due to the fact that the dry matter of the substrate bed decreases during the fermentation due to the release of CO_2. Mass ratio of the biomass in SSF is expressed as m(dry biomass)/m(dry matter), and not as m(dry biomass)/V(dry matter), which is typically used in SLF studies. The problem is that two slightly different systems of units can be used to express the biomass content in the construction of kinetic profiles: m(dry biomass)/m(initial dry matter)(IDM) and m(dry biomass)/m(dry matter)(DM). The first of these, which will be referred to as the »absolute mass ratio«, is easy to measure in the experimental strategy in which a large number of identical flasks are prepared and whole flasks are sacrificed at different time points during the fermentation, a strategy that is widely used in laboratory SSF studies. In this case, even though the mass of dry solids decreases during the fermentation, the initial dry mass added to the flask is known. The second of these, which will be referred to as the »relative mass ratio«, is more usual when samples are removed from a substrate bed in a bioreactor. In this case it is difficult to calculate the initial dry mass to which the sample corresponds, unless the total mass of solids within the bioreactor and the water content are measured at each sampling time, but this is usually not done. As a simple example of the difference between the two measurement systems, for a microorganism that is not growing but is metabolizing substrate to maintain itself, the absolute mass ratio will remain constant while the relative mass ratio will increase due to the conversion of dry substrate matter into CO_2. Conversion between the two measurement systems is not possible with a direct conversion factor.

In the analyses of kinetics of growth in SSF systems, little attention has been paid to the measurement system used. The exponential, linear and logistic equations have been applied to profiles obtained in both measurement systems (*5*). The current paper develops a model that shows how conversions can be made between the two measurement systems, validates this model with experimental data and shows how this model can be used to explore the question of whether a conclusion about the type of kinetics drawn from the analysis of a growth profile expressed in one system of units would also have been drawn if the growth profile had been expressed on the basis of the other system of units.

Model Development

For illustrative purposes the symbol X used in the model is taken to represent the biomass. However, it can also be applied to kinetic profiles obtained on the basis of the measurement of components of the biomass, such as glucosamine, ergosterol or protein. In this case, the only change will be in the units used for X and in the values and units used for the yield and maintenance coefficients.

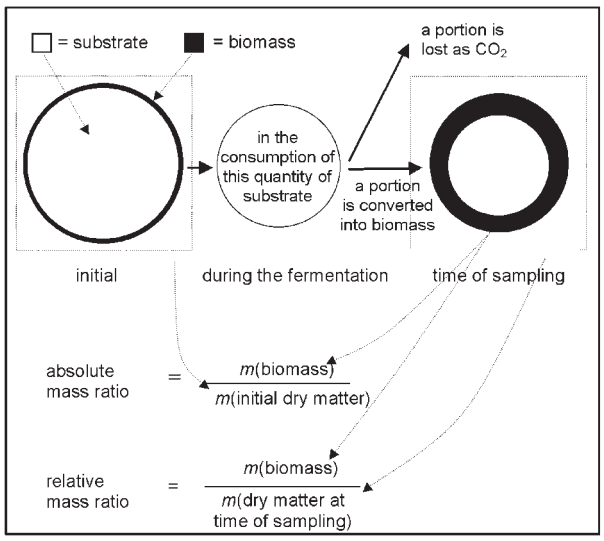

Fig. 1. Representation of the system, showing how the loss of dry matter as CO_2 during the fermentation means that the denominator differs for the calculation of the absolute and relative mass ratios

General equations

The system modeled is represented schematically in Fig. 1. The total amount of dry material at any time (D) is given by:

$$D = X + S \qquad /1/$$

where X is the dry biomass at that time (g) and S is the residual dry substrate at that time (g). Two mass ratios of the biomass are defined. The absolute mass ratio (C_A, m(dry biomass)/m(IDM)) at a given time is given by:

$$C_A = \frac{X}{D_0} \qquad /2/$$

where D_0 is the total amount of dry matter in the bioreactor at time zero, including both substrate (S_o) and inoculum (X_0). Relative mass ratio (C_R, m(dry biomass)/m(DM)) at a given time is given by:

$$C_R = \frac{X}{D} \qquad /3/$$

As a result of these definitions, the total amount of biomass can be expressed in either of two ways:

$$X = C_A D_o = C_R D \qquad /4/$$

The growth rate, in terms of m(dry biomass)/t (in g/h) can therefore be written as:

$$\frac{dX}{dt} = \frac{dC_R \cdot D}{dt} = D\frac{dC_R}{dt} + C_R\frac{dD}{dt} \qquad /5/$$

where t is the time (h). Substituting X with $C_A D_o$ and then dividing the whole equation by D_o gives:

$$\frac{dC_A}{dt} = \frac{1}{D_0}\left(D\frac{dC_R}{dt} + C_R\frac{dD}{dt}\right) \qquad /6/$$

Eq. /6/ can be rearranged to be explicit in dC_R/dt:

$$\frac{dC_R}{dt} = \frac{D_0}{D}\frac{dC_A}{dt} - \frac{C_R}{D}\frac{dD}{dt} \qquad /7/$$

Eq. /7/ says that the change in relative mass ratio during growth occurs due to an increase in the amount of biomass, as described by the first term on the right hand side, and due to the overall decrease in dry matter, as described by the second term on the right hand side.

The rate of change in total dry matter is given by the sum of the rates of changes in the biomass and substrate:

$$\frac{dD}{dt} = \frac{dX}{dt} + \frac{dS}{dt} \qquad /8/$$

Assuming that substrate is consumed for growth and maintenance, X and S are related by the following equation:

$$\frac{dS}{dt} = -\frac{1}{Y_{XS}}\frac{dX}{dt} - m_S X \qquad /9/$$

where Y_{XS} is the true growth yield m(dry biomass)/m(dry substrate) and m_S is the maintenance coefficient m(dry substrate)/m(dry biomass)/t (in h).

Substituting Eq. /9/ into Eq. /8/ and using the distributive law to separate out dX/dt gives:

$$\frac{dD}{dt} = \left(1 - \frac{1}{Y_{XS}}\right)\frac{dX}{dt} - m_S X \qquad /10/$$

or, substituting $C_A D_o$ for X:

$$\frac{dD}{dt} = \left(1 - \frac{1}{Y_{XS}}\right)\frac{dC_A D_0}{dt} - m_S C_A D_0 = D_0\left(\left(1 - \frac{1}{Y_{XS}}\right)\frac{dC_A}{dt} - m_S C_A\right) \qquad /11/$$

Model A→R: Conversion of an absolute profile into a relative profile

Given a growth profile plotted in terms of an absolute mass ratio of the biomass that is described by one of the empirical equations in Table 1 (*i.e.* one of the Eqs. /12/ to /15/), the graph that would have been obtained if the biomass had been measured in terms of relative mass ratio of the biomass (C_R) is plotted by integrating a model composed of the corresponding differential form of the equation (*i.e.* one of the Eqs. /16/ to /19/) in conjunction with Eqs. /7/ and /11/. In these equations C_{Amax} is the maximum absolute mass ratio of the biomass obtained and C_{Ao} is the initial absolute mass ratio of the biomass.

Model R→A: Conversion of a relative profile into an absolute profile

Given a kinetic profile plotted in terms of the relative mass ratio of the biomass that is described by one of the empirical equations in Table 1 (*i.e.* one of the Eqs. /20/ to /23/), the graph that would have been obtained if the biomass had been measured in terms of the absolute mass ratio of the biomass (C_A) is plotted by integrating a model composed of the corresponding differential form of the equation (*i.e.* one of the Eqs. /24/ to /27/) in conjunction with the Eqs. /6/ and /11/. In these equations C_{Rmax} is the maximum relative mass ratio of the biomass obtained and C_{Ro} is the initial relative mass ratio of the biomass.

Estimation of model parameters and method of solution

Where experimental growth profiles were available, least squares regression of the appropriate equation (*i.e.* one of the Eqs. /12/ to /15/ for profiles in terms of absolute concentration, one of the Eqs. /20/ to /23/ for profiles in terms of relative mass ratio) against the experimental data was used to obtain appropriate values for the parameters of the equation. The appropriate differential equation sets were solved using FORTRAN program subroutine DRKGS (*7*).

Materials and Methods

Microorganism and growth medium

Rhizopus oligosporus ACM145F was maintained on slopes containing (per 100 mL) cassava starch 1 g, $(NH_4)_2SO_4$ 1 g, K_2HPO_4 0.1 g, KH_2PO_4 0.1 g and agar 2 g. Slopes were incubated at 37 °C for 3 to 5 days to allow sporulation. A suspension containing N(spores)/V =

Table 1. Integrated and differentiated forms of the various empirical kinetic equations applied to SSF systems, showing the form of the equation for use with either absolute biomass measurements or relative biomass measurements

	Integrated kinetic equation		Differential form	
ABSOLUTE BASIS			(used in model A→R)	
Linear	$C_A=Kt+C_{Ao}$	/12/	$\frac{dC_A}{dt}=K$	/16/
Exponential	$C_A=C_{Ao}e^{-\mu}$	/13/	$\frac{dC_A}{dt}=\mu C_A$	/17/
Logistic	$C_A=\frac{C_{A_{max}}}{1+\left(\frac{C_{A_{max}}}{C_{A_0}}-1\right)e^{-\mu t}}$	/14/	$\frac{dC_A}{dt}=\mu C_A\left(1-\frac{C_A}{C_{A_{max}}}\right)$	/18/
Deceleration (*6*)	$C_A=C_{A_0}\exp\left[\frac{\mu}{k}(1-e^{-kt})\right]$	/15/	$\frac{dC_A}{dt}=\mu e^{-kt}C_A$	/19/
RELATIVE BASIS			(used in model R→A)	
Linear	$C_R=Kt+C_{Ro}$	/20/	$\frac{dC_R}{dt}=K$	/24/
Exponential	$C_R=C_{Ro}e^{-\mu t}$	/21/	$\frac{dC_R}{dt}=\mu C_R$	/25/
Logistic	$C_R=\frac{C_{R_{max}}}{1+\left(\frac{C_{R_{max}}}{C_{R_0}}-1\right)e^{-\mu t}}$	/22/	$\frac{dC_R}{dt}=\mu C_{RA}\left(1-\frac{C_R}{C_{R_{max}}}\right)$	/26/
Deceleration (*6*)	$C_R=C_{R_0}\exp\left[\frac{\mu}{k}(1-e^{-kt})\right]$	/23/	$\frac{dC_R}{dt}=\mu e^{-kt}C_R$	/27/

10^7/mL was prepared by adding germination solution, which contained (per 100 mL of distilled water) cassava starch 0.1 g, $(NH_4)_2SO_4$ 0.05 g, K_2HPO_4 0.1 g, KH_2PO_4 0.1 g and Tween-80 0.05 mL. Spores were pre-germinated at 40 °C on a rotary shaker at 300 rpm for 10 to 12 h.

The gel-based substrate consisted (per 100 mL of distilled water) of the appropriate amount of cassava starch, κ-carrageenan 4 g, $(NH_4)_2SO_4$ 1.5 g, urea 0.5 g, K_2HPO_4 0.1 g, KH_2PO_4 0.1 g. The pH was adjusted to 7.0. Two different gel-based substrates were used, one with cassava starch 5 g and one with cassava starch 25 g in the above mixture. The solution was stirred constantly at 100 °C for 15 min to gelatinize the starch, then pressed between glass plates to give a slab 6 mm thick, which was then cut into 6 mm cubes.

For the preparation of cassava substrate, frozen cassava tubers were thawed and cut into 1 cm thick slices. Each slice was cut radially to give chips with a thick end of 5 mm. A solution, whose volume in mL corresponded to half the mass of the substrate (in g) and which contained $(NH_4)_2SO_4$ 4.5 g, urea 1.5 g, K_2HPO_4 0.3 g, KH_2PO_4 0.3 g all per 100 mL of distilled water, was adjusted to pH=7.0 and added to the substrate. The mixture was kept at 100 °C until the liquid was absorbed and the starch was gelatinized.

Fermentation procedure and analyses

A mass of 5 g of cubes or chips were placed in sterile pre-weighed 50-mL conical flasks with loose-fitting aluminium caps. Pre-germinated spore suspension (0.5 mL) was added to each flask, which was re-weighed. Flasks were incubated at 37 °C. Six flasks were removed and weighed at each sampling time. Three flasks were used for determination of protein. The contents of these flasks were homogenized with 50 mL of water and protein was determined by the Folin reaction after solubilization with NaOH (*8*). Three flasks were used for determination of total dry matter, by drying to constant weight at 60 °C. The mass of dry matter in the flasks used for protein determination was calculated by assuming equal moisture contents for all flasks removed at a particular sampling time.

Results

The two conversion models are used to investigate the effect of substrate loss on the apparent kinetics. This is done by analyzing kinetic profiles that were obtained using one measurement basis and exploring what kinetics would have been observed if the growth profile had been determined using the other measurement basis. However, before doing this it is necessary to validate the model experimentally using data obtained in our own experiments and in one literature source in which biomass was determined using both measurement bases.

Validation against experimental growth profiles

Mass ratio profiles of the biomass, using protein as an indicator of growth, were collected for the growth of *Rhizopus oligosporus* on cassava and gel-based solid substrates, using both the relative and absolute measurement techniques. Fig. 2A shows the absolute profile obtained for growth on cassava, with the fitted logistic equation (Eq. /14/). Note that for fitting of the logistic curve a value of m(protein)/m(IDM) = 12.8 mg/g was subtracted from all values to take account of the high zero time value, in the manner described earlier (*5*). Af-

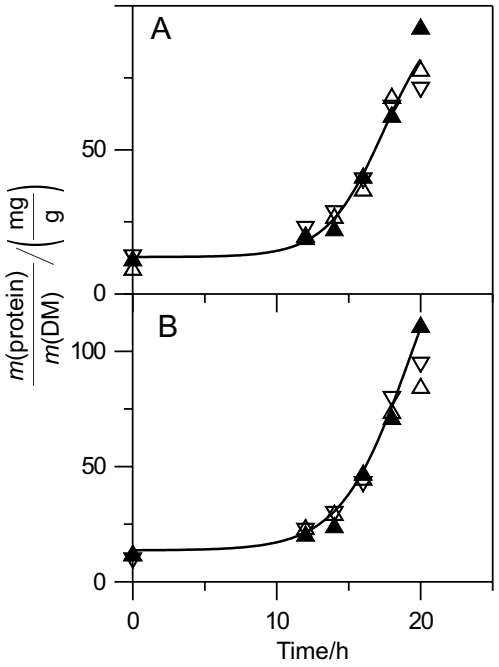

Fig. 2. Experimental results used for the estimation of Y_{XS} and m_S. (A) Fitting of the logistic equation to the experimentally determined absolute biomass profile for growth on cassava. (B) Comparison of the predicted and experimental relative mass ratio profiles of the biomass, using the absolute to relative conversion model with a value of Y_{XS} of m(protein)/m(dry substrate) = 0.2 g/g and a value m_S of 0. The various symbols represent replicate samples

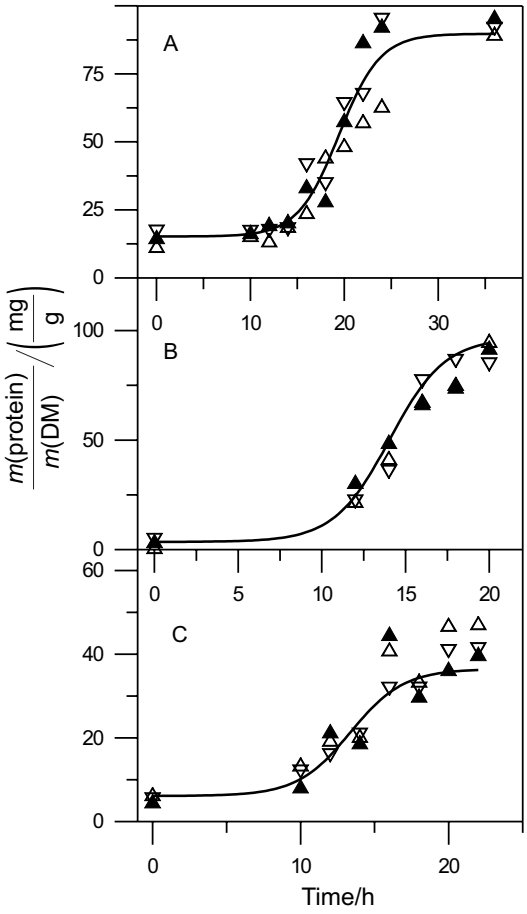

Fig. 3. Comparison of the experimental relative mass ratio profiles (symbols) of the biomass with those predicted by model A →R (curves), using the values of Y_{XS} and m_S that were used to construct Fig. 2B, namely Y_{XS} = 0.2 g/g and m_s = 0. (A) Growth on the cassava substrate; (B) Growth on the gel-based substrate with m(starch)/V = 5 g/100 mL. (C) Growth on the gel-based substrate with m(starch)/V = 25 g/100 mL. The various symbols represent replicate samples

ter this subtraction the data were analyzed by non-linear regression to give values of C_{Ao}, C_{Amax} and μ of m(protein)/m(IDM) = 0.0152 mg/g, m(protein)/m(IDM) = 88.0 mg/g and 0.497 h^{-1}, respectively. The curve plotted in Fig. 2A was obtained by adding m(protein)/m(IDM) = 12.8 mg/g to all values on the logistic curve constructed with these parameter values.

Model A→R was used to convert this growth profile, which is logistic in terms of absolute mass ratio, into the corresponding relative profile. In this case model A→R was composed of the differential form of the logistic equation, namely Eq. /18/ in Table 1, and Eqs. /7/ and /11/. Fig. 2B shows that the model predicts a relative profile that gives a good approximation of the experimental results. Note that the model was solved with the above values for C_{Ao}, C_{Amax} and μ as input, and then the m(protein)/m(DM) = 12.8 mg/g was added to all the output values of C_R. This fit was obtained with a value of Y_{XS} = m(protein)/m(dry substrate) = 0.2 g/g and a value m_S = (m(dry substrate)/m(protein))/t = 0 (g/g)/h. The value of zero for the maintenance coefficient should be interpreted as meaning that the maintenance coefficient is sufficiently small that it is not possible, within the experimental error in the data, to obtain a reliable estimate and that in this case the yield coefficient has the major effect on the shape of the curve.

In order to validate the model, these values of Y_{XS} and m_S were inserted into model A→R, which was then used to predict the relative biomass profiles in situations other than those in which the parameter values had been determined, namely growth on the gel-based substrates with the two different cassava starch mass ratios and another fermentation of cassava. Again the logistic equation (Eq. /14/) was fitted to the absolute mass ratio profiles of the biomass. Table 2 shows the parameters of the logistic equation obtained from this regression, and also the zero time value subtracted and then added back in order to construct the relative mass ratio profile of the biomass, in the same manner that was used to construct Fig. 2B.

Good agreement was obtained between the predicted and experimental relative mass ratio profiles of the biomass (Fig. 3), which not only confirms the validity of the model, but also shows that the values of Y_{XS} and m_S are of general applicability for the growth of *Rhizopus oligosporus* on starchy substrates, demonstrating the flexibility of the model.

Validation against literature growth profiles

The only experimental work already available in the literature in which the biomass was measured in terms of both the absolute mass ratio and the relative mass ra-

Table 2. Values of the parameters obtained by regression of the original experimental data, in order to obtain the kinetic equation for application in the conversion model, for the construction of various of the figures in this work

Equation and data source	Parameter values
Fig. 3. Validation of the model using experimental data of the current work	
A) Cassava substrate Logistic equation Regression with Eq. /14/ Applied in Eq. /18/	C_{Ao} = m(protein)/m(IDM) = 0.010 g/g C_{Amax} = m(protein)/m(IDM) = 57.465 mg/g μ = 0.457 h^{-1} Subtraction = m(protein)/m(IDM) = 15.173 mg/g
B) Gel-based substrate (m(starch)/V = 5/100 mL) Regression with Eq. /14/ Applied in Eq. /18/	C_{Ao} = m(protein)/m(IDM) = 0.019 g/g C_{Amax} = m(protein)/m(IDM) = 67.887 mg/g μ = 0.605 h^{-1} Subtraction = m(protein)/m(IDM) = 3.505 mg/g
C) Gel-based substrate (m(starch)/V = 5/100 mL) Regression with Eq. /14/ Applied in Eq. /18/	C_{Ao} = m(protein)/m(IDM) = 0.018 g/g C_{Amax} = m(protein)/m(IDM) = 27.063 mg/g μ = 0.558 h^{-1} Subtraction = m(protein)/m(IDM) = 6.103 mg/g
Fig. 5. Conversion of absolute profiles to the corresponding relative profiles Parameter values for the kinetic equation describing the absolute mass ratio of the biomass	
A) Logistic, data of (*9*) Regression with Eq. /14/ Applied in Eq. /18/	C_{Ao} = m(dry biomass)/m(IDM) = 0.00662 g/g C_{Amax} = m(dry biomass)/m(IDM) = 0.219 g/g μ = 0.233 h^{-1}
B) Linear, data of (*11*) Regression with Eq. /12/ Applied in Eq. /16/	C_{Ao} = m(dry biomass)/m(IDM) = 0.02 mg/g K = (m(dry biomass)/m(IDM))/t = 0.002 (mg/g)/h
C) Exponential, data of (*12*) Regression with Eq. /13/ Applied in Eq. /17/	C_{Ao} = m(dry biomass)/m(IDM) = 0.008 mg/g μ = 0.170 h^{-1}
D) Deceleration, data of (*13*) Regression with Eq. /15/ Applied in Eq. /19/	C_{Ao} = m(dry biomass)/m(IDM) = 0.01 g/g μ = 5.427 h^{-1} k = 0.053 h^{-1}
Fig. 6. Conversion of relative profiles to the corresponding absolute profiles Parameter values for the kinetic equation describing the relative mass ratio of the biomass	
A) Logistic, data of (*9*) Regression with Eq. /22/ Applied in Eq. /26/	C_{Ro} = m(dry biomass)/m(DM) = 0.0114 g/g C_{Rmax} = m(dry biomass)/m(DM) = 0.460 g/g μ = 0.192 h^{-1}
B) Linear, data of (*15*) Regression with Eq. /20/ Applied in Eq. /24/	C_{Ro} = m(protein)/m(DM) = 0.01 mg/g K = m(protein)/m(DM) = 0.0015 (mg/g)/h
C) Exponential, data of (*16*) Regression with Eq. /21/ Applied in Eq. /25/	C_{Ro} = m(dry biomass)/m(DM) = 0.00095 g/g μ = 0.099 h^{-1}
D) Deceleration, data of (*13*) Regression with Eq. /23/ Applied in Eq. /27/	C_{Ro} = m(dry biomass)/m(DM) = 0.03 g/g μ = 0.03 h^{-1} k = 0.035 h^{-1}

tio is that of Sargantanis *et al.* (*9*). In both cases the kinetics appear essentially logistic and therefore this equation was used to analyze the data.

The absolute biomass results, given in terms of grams of biomass (*9*), were converted into the corresponding values in terms of m(biomass)/m(IDM), on the basis of the stated initial dry mass of 400 g. A fit to these results gives values of C_{Ao}, C_{Amax} and μ of m(biomass)/m(IDM) = 0.00662 g/g, m(biomass)/m(IDM) = 0.219 g/g and 0.233 h^{-1}, respectively (Fig. 4). A fit to the relative mass ratio profile of the biomass gives a value of C_{Rmax} = m(biomass)/m(DM) = 0.460 g/g and a value of μ of 0.192 h^{-1}.

Model A→R was used to convert the measured absolute profile into the corresponding relative profile, using the parameter values of Sargantanis *et al.* (*9*), namely Y_{XS} = m(dry biomass)/m(dry substrate) = 0.625 g/g and m_S = (m(dry substrate)/m(dry biomass))/t = 0.02 (g/g)/h (Fig. 4). However, with these parameter values, the predicted relative biomass profile is lower than the measured relative mass ratio profile. A good agreement between the predicted and experimental relative biomass

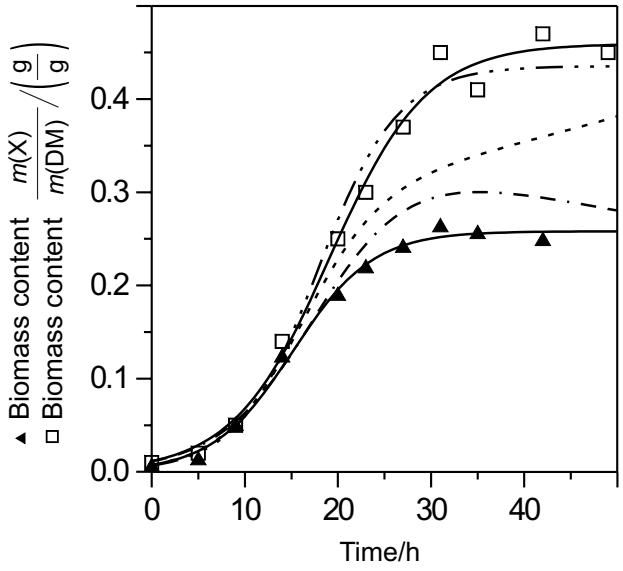

Fig. 4. Application of the model to the data of Sargantanis *et al.* (*9*). (▲) Absolute mass ratio of the biomass data; (□) Relative mass ratio of the biomass data. In each case the solid line gives the best fit of the logistic equation by non-linear regression to the respective data. (- - -) Use of model A→R with the values used by Sargantanis *et al.* (*9*), namely Y_{XS}=0.625 g/g and m_S = 0.01932 (g/g)/h; (— - - - —) Use of model A→R with Y_{XS}=0.3 g/g and m_S=0; (— - —) Use of model R→A, with the values of m_S and Y_{XS} used by Sargantanis *et al.* (*9*)

profiles is only obtained using values of Y_{XS} = 0.3 g/g and m_S = 0 in the conversion model. In fact, the data of Sargantanis *et al.* (*9*) does not give a clear indication of maintenance metabolism. They interpreted the relative profile as leveling off and the absolute profile as falling away, such as would be expected as a result of maintenance metabolism, but this is not supported by their graph since the decrease in the final stages of their absolute profile is only slight and probably within the limits of experimental error. Sargantanis *et al.* (*9*) did not explicitly show how they arrived at their estimates for Y_{XS} and m_S, so it is not possible to check their rationale.

Investigation of the effect of growth parameters on the interpretation of kinetics

The previous sections show that the appropriate version of the model can be used to convert between one measurement system and another. Therefore, the model can be used to respond to the following question: How would a curve, measured in one measurement system and giving a particular kinetic form, have looked if the biomass measurements had been expressed in the other measurement system, and what would have been concluded about the kinetics in that case? The model was used to answer this question, exploring the sensitivity of the predictions to the values of the parameters Y_{XS} and m_s. The range used for these values was based on the literature, in which Y_{XS} has been found to vary from 0.25 to 0.625 g/g and m_s has been found to vary from approximately 0 up to 0.02 (g/g)/h (*9–10*). In the case where the analysis was done on the basis of protein contents, these parameters were recalculated assuming that the dry biomass has a protein mass fraction of 50 %.

How would an absolute profile have appeared if it had been measured in relative terms?

Fig. 5 shows the effects of various combinations of Y_{XS} and m_S on the conversions of various kinetic types, obtained using absolute mass ratios, to the profiles that would have been obtained if the biomass had been measured in relative terms. Model A→R was used to perform the conversions, in this case comprised of Eqs. /7/ and /11/ and the differential form of the appropriate empirical equation (*i.e.* one of the Eqs. /16/ to /19/).

Sargantanis *et al.* (*9*) obtained logistic growth kinetics for biomass measured in absolute terms. Fig. 5A shows how the curve would have looked if it had been measured in relative terms, given various values of Y_{XS} and m_S. If the maintenance coefficient is negligible, then the profile remains logistic in shape regardless of whether the biomass measurements are made on a relative or absolute basis. Analysis of the relative profile will give almost the same μ as was determined for the original absolute profile, since the value of this parameter is most strongly affected by the early part of this curve, and during the early stages there is relatively little deviation between the absolute and relative curves. The maximum mass ratio of the biomass measured by the two methods will be different. This is to be expected since in the two different measurement systems the biomass is divided by different masses. If the maintenance coefficient is not negligible then the basic shape of the profile will change. If the biomass profile measured on the basis of an absolute mass ratio is logistic, then if the biomass had been measured on the basis of relative mass ratios, there would have been no leveling off of the biomass, rather it would have continued to increase even at the end of the fermentation, due to the consumption of dry substrate for maintenance. This curve is similar to the »rapid-acceleration slow-deceleration curve« reported by Mitchell *et al.* (*2*). Note that, in the presence of an experimental error of ±10 %, analysis of the relative profile for a Y_{XS} of 0.25 g/g and an m_S of 0.02 (g/g)/h would probably be interpreted as being linear after the first 10 h of growth.

Gumbira-Sa'id *et al.* (*11*) obtained a linear absolute biomass profile. Fig. 5B shows that the corresponding relative profile will never be linear, even in the absence of maintenance metabolism. The curvature leads to larger and larger values of the apparent growth rate (dC_R/dt) with time. However, allowing for the fact that if the relative profile were to be determined experimentally, there would be a margin of around ±10 % experimental error, for Y_{XS} = 0.625 g/g the curvature would be sufficiently small so that a straight line would give a reasonable fit to the data. At lower values of Y_{XS} the curvature would probably not be masked by the experimental error on a plot of relative biomass versus time. The degree to which the curvature appears depends on the length of the growth phase, the biomass levels reached and the amount of substrate consumed to reach those biomass levels. In other words, as the fermentation extends, the curvature becomes more and more apparent and maintenance metabolism accentuates the curvature.

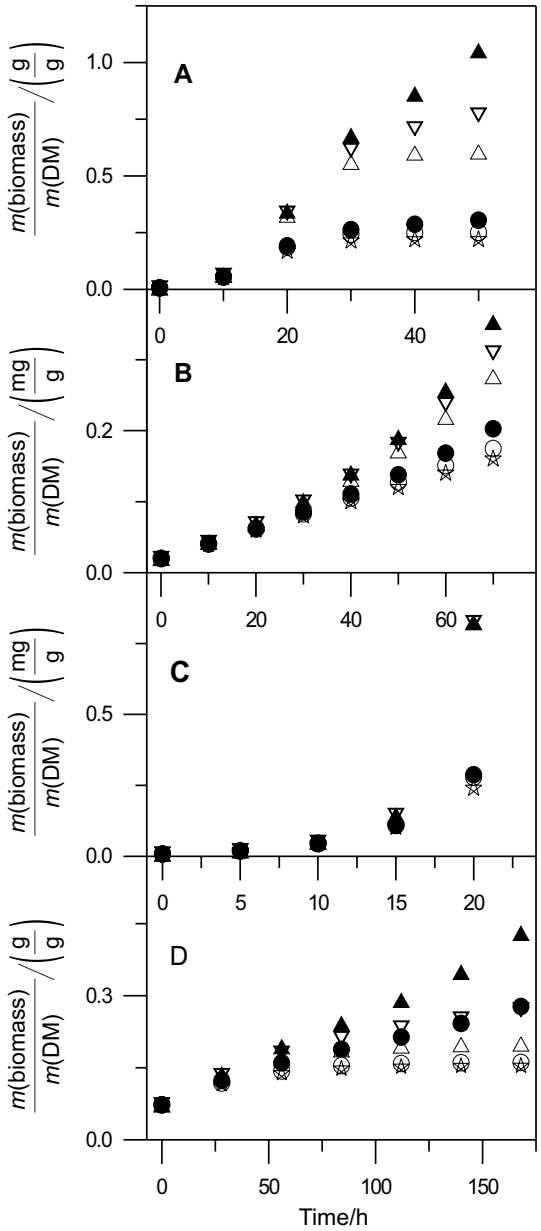

Fig. 5. Conversion from absolute profiles to relative profiles with model A→R. (A) Logistic equation applied to the data of Sargantanis *et al.* (*9*); (B) Linear equation applied to the data of Gumbira-Sa'id *et al.* (*11*); (C) Exponential equation applied to the data of Oriol *et al.* (*12*); (D) Deceleration equation applied to the data of Bravo *et al.* (*13*). Key: (☆) The absolute mass ratio profile obtained by the author, drawn using the parameter values listed in Table 2; Predicted relative mass ratio profiles with (○) Y_{XS} = 0.625 g/g and m_S=0, (●) Y_{XS} = 0.625 g/g and m_S = 0.02 (g/g)/h, (△) Y_{XS} = 0.25 g/g and m_S=0, (▽) Y_{XS} = 0.25 g/g and m_S=0.01 (g/g)/h and (▲) Y_{XS} = 0.25 g/g and m_S=0.02 (g/g)/h.

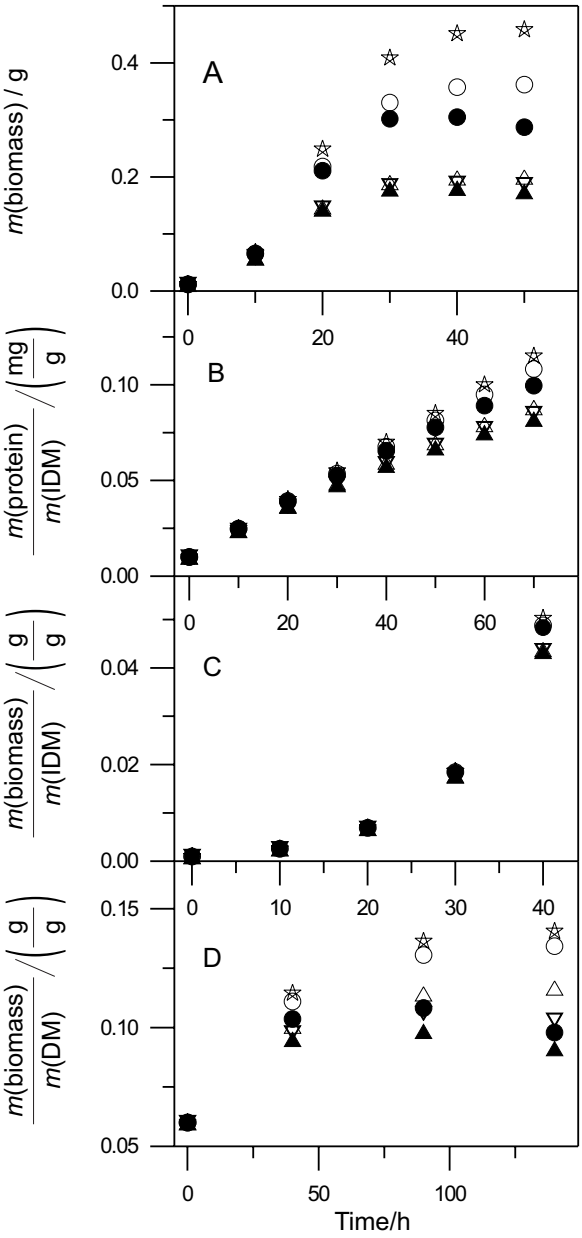

Fig. 6. Conversion from relative profiles to absolute profiles with model R→A. (A) Logistic equation applied to the data of Sargantanis *et al.* (*9*); (B) Linear equation applied to the data of Kumar and Lonsane (*15*); (C) Exponential equation applied to the data of Gutierrez-Rojas *et al.* (*16*); (D) Deceleration equation applied to the data of Bravo *et al.* (*13*). Key: (☆) The relative mass ratio profile obtained by the author, drawn using the parameter values listed in Table 2; Predicted absolute mass ratio profiles with (○) Y_{XS} = 0.625 g/g and m_S = 0, (●) Y_{XS} = 0.625 g/g and m_S = 0.02 (g/g)/h, (△) Y_{XS}=0.25 g/g and m_S=0, (▽) Y_{XS}=0.25 g/g and m_S = 0.01 (g/g)/h and (▲) Y_{XS}=0.25 g/g and m_S=0.02 (g/g)/h

Oriol *et al.* (*12*) obtained an exponential absolute biomass profile during the first 20 h of fermentation. Fig. 5C shows that the corresponding relative profile maintains its exponential appearance in the absence of maintenance metabolism. Even with maintenance metabolism the deviations from exponential shape are unlikely to be large in comparison with an experimental error of ±10 %. If the deviation between the two lines is large enough, which can happen at low values of Y_{XS} and high values of m_S, then one would obtain significantly different values of μ depending on whether the analysis was done on the basis of relative or absolute concentration profiles.

An absolute biomass profile that is well described by the deceleration model was not found in the literature. In order to explore the effects of the conversion on

the shape of the curve, the relative profile of Bravo *et al.* (*13*) was treated as though it had been obtained using absolute measurements. Fig. 5D shows that the relative profiles would have less curvature, due to the fact that substrate utilization decreases the denominator within C_R whereas the denominator within C_A stays at its original value. In fact, for the values of Y_{XS} = 0.25 g/g and m_S = 0.02 (g/g)/h, the predicted relative profile appears linear.

How would a relative profile have appeared if it had been measured in absolute terms?

Fig. 6 shows the effects of various combinations of Y_{XS} and m_S on the conversions of various kinetic types, obtained using relative concentrations, to the profiles that would have been obtained if the biomass profile had been measured in absolute terms. Model R→A was used to perform the conversion, in this case comprised of Eqs. /6/ and /11/ and the differential form of the appropriate empirical equation (*i.e.* one of the Eqs. /24/ to /27/).

The relative biomass profile of Sargantanis *et al.* (*9*) was logistic in shape. Fig. 6A shows that if the biomass profile had been plotted in absolute terms, there would be no leveling off, rather the curve would have fallen off at the end. Such profiles have indeed been reported (*14*). Such a decrease could be explained by a stoppage of growth, followed by endogenous metabolism in which part of the dry weight of the cell were metabolized to provide the maintenance energy for the remaining biomass.

The relative biomass profile of Kumar and Lonsane (*15*) was linear until 72 h. As with the conversion in the other direction, a profile that is linear with respect to relative mass ratio will never be linear with respect to absolute mass ratio (Fig. 6B), but rather a curve. In this case the growth rate (dC_A/dt) decreases constantly. However, the degree of curvature is variable and is most pronounced towards the end of the fermentation. Experimental error will hide the curvature unless a significant amount of substrate is consumed.

Gutierrez-Rojas *et al.* (*16*) obtained exponential growth kinetics in terms of relative mass ratio of the biomass. If the curve had been obtained in terms of absolute mass ratio of the biomass they would still have deduced that the kinetics were exponential (Fig. 6C). They would also have calculated a similar value for μ, since the amount of growth in their system was relatively small. If the growth had been monitored for longer, the profiles would have diverged more.

Bravo *et al.* (*13*) obtained a relative biomass profile that is described well by the deceleration equation. Fig. 6D shows that if the profile had been determined in terms of absolute biomass the profile would have had an accentuated curvature. If maintenance metabolism is significant, once the growth has decelerated sufficiently, then maintenance can be higher than the growth rate, and with consumption of the biomass through endogenous metabolism, the mass ratio of the biomass can actually fall.

Discussion

This paper provides models that can be used to convert, in either direction, between absolute and relative mass ratio profiles. These models will help systematize the analysis of kinetics in SSF, which is clearly necessary: a recent extensive review and analysis of empirical growth profiles in SSF showed that a wide range of different measurement systems has been used (*5*). It is important that the kinetics are interpreted correctly, in order to incorporate the correct empirical growth kinetic equation when an SSF bioreactor model is being developed. No attention has previously been paid to the possibility that the type of measurement method used, that is, expression of mass ratio of the biomass on a relative or absolute basis, could affect the conclusions made about the type of growth kinetics exhibited by the microorganism. The current work shows that in fact the method of measurement can affect the form of the curve and therefore the conclusion about the type of kinetics shown by the organism, although the extent of this effect depends on the length of time in which growth occurs and the values of Y_{XS} and m_S, because these affect the ratio of dry biomass to dry substrate.

In order to use the conversion models, it is necessary to determine the values of Y_{XS} and m_S. One method of doing this, as done in the current work, is to undertake experiments in which sufficient data is collected to enable the biomass to be expressed on both the relative and absolute measurement bases. This will typically involve the need to follow, in addition to the biomass or an indirect indicator of growth, the total dry weights and total moisture content as functions of time. However, it must be understood that the parameters are not necessarily constant during growth. If the variation is not large then average values for Y_{XS} and m_S would give sufficient accuracy. In the case of large variations, it would be necessary to undertake experiments to characterize how these parameters change with time and to propose equations to describe these variations. For example, Smits *et al.* (*17*) found that late in the fermentation the maintenance coefficient decreased, presumably because parts of the biomass had died and therefore did not contribute to maintenance metabolism. This dead biomass affects the measured maintenance coefficient because it is the total biomass that appears in the denominator. They proposed simple empirical equations to express the maintenance coefficient as a function of time.

As stated in the model development section and shown in the results, application of these conversion models is not limited to biomass, they can be used for any biomass component that is plotted against time and found to have a profile that can be described mathematically: the models are easily adapted for any empirical growth equation that can be expressed in both integral (X, or a component of the biomass, as a function of time) and differential form (dX/dt). Clearly the model is also of general applicability and is not limited to the experimental system used in the current work, namely the growth of *Rhizopus oligosporus* on starchy substrates.

The conversion model might itself be incorporated as part of the kinetic model within a bioreactor model.

For example, in laboratory studies the method of sacrificing individual flasks throughout the experiment is typically used, with the results being plotted as an absolute mass ratio profile of the biomass. However, since samples from a bioreactor are typically analyzed on the basis of the relative mass ratio, it is interesting for the bioreactor model to contain equations to predict the relative mass ratio of the biomass as a function of time. Eqs. /7/ and /11/ can be used in addition to the differential form of the kinetic equation that describes the absolute biomass profile.

Acknowledgements

This work was supported by the Brazilian National Council for Scientific and Technological Development (CNPq, Conselho Nacional de Desenvolvimento Científico e Tecnológico). Graciele Viccini, Nadia Krieger and David Mitchell thank CNPq for research scholarships.

Symbols

C_A absolute mass ratio of the biomass (= m(dry biomass)/m(IDM)/(g/g))

C_{Amax} maximum possible absolute mass ratio of the biomass (= m(dry biomass)/m(IDM)/(g/g))

C_{Ao} initial absolute mass ratio of the biomass (= m(biomass)/m(IDM)/(g/g))

C_R relative mass ratio of the biomass (= m(dry biomass)/m(DM)/(g/g))

C_{Rmax} maximum possible relative mass ratio of the biomass (= m(dry biomass)/m(DM)/(g/g))

C_{Ro} initial relative mass ratio of the biomass (= m(biomass)/m(DM)/(g/g))

D mass of dry material in the system (= m(DM)/g)

D_o mass of dry material in the system at zero time (= m(IDM)/g)

k first order decay constant in the deceleration model/h^{-1}

K linear growth rate constant [= m(dry biomass)/m(IDM)/t/(g/g)/h] or [= m(dry biomass)/m(DM)/t/(g/g)/h] depending on whether a absolute or relative growth profile is being described

m_S maintenance coefficient [= m(dry substrate)/m(dry biomass)/t/(g/g)/h]

S mass of total residual dry substrate in the system (= m(dry substrate)/g)

S_o mass of total residual dry substrate in the system at zero time (= m(dry substrate)/g)

t time since start of the particular phase being described/h

X total dry biomass in the system (= m(dry biomass)/g)

X_o total dry biomass in the system at zero time (= m(dry biomass)/g)

Y_{XS} true growth yield (= m(dry biomass)/m(dry substrate)/(g/g))

μ specific growth rate constant/h^{-1}

References

1. A. Pandey, C. R. Soccoll, D. A. Mitchell, *Process Biochem. 35* (2000) 1153-1169.
2. D. A. Mitchell, D. D. Do, P. F. Greenfield, H. W. Doelle, *Biotechnol. Bioeng. 38* (1991) 353-362.
3. D. A. Mitchell, D. M. Stuart, R. D. Tanner: Solid-state fermentation – microbial growth kinetics. In: *The Encyclopedia of Bioprocess Technology: Fermentation, Biocatalysis and Bioseparation, Vol. 5*, M. C. Flickinger, S. W. Drew (Eds), Wiley, New York (1999) pp. 2407-2429.
4. D. A. Mitchell, M. Berovic, N. Krieger, *Adv. Biochem. Eng./Biotechnol. 68* (2000) 61-138.
5. G. Viccini, D. A. Mitchell, S. D. Boit, J. C. Gern, A. S. da Rosa, R. M. Costa, F. D. H. Dalsenter, O. F. von Meien, N. Krieger, *Food Technol. Biotechnol. 39* (2001) 271-294.
6. L. Ikasari, D.A. Mitchell, *Biotechnol. Bioeng. 68* (2000) 619-627.
7. A. Ralston, H. S. Wilf: *Mathematical Methods for Digital Computers*, Wiley, New York (1960).
8. P. Gerhardt: *Manual of Methods for General Bacteriology*. American Society for Microbiology, Washington DC (1981)
9. J. Sargantanis, M. N. Karim, V. G. Murphy, D. Ryoo, R. P. Tengerdy, *Biotechnol. Bioeng. 42* (1993) 149-158.
10. L. P. Ooijkaas, R. M. Buitelaar, J. Tramper, A. Rinzema, *Biotechnol. Bioeng. 69* (2000) 292-300.
11. E. Gumbira-Sa'id, H. W. Doelle, P. F. Greenfield, D. A. Mitchell, *World J. Microbiol. Biotechnol. 7* (1991) 419-427.
12. E. Oriol, M. Raimbault, S. Roussos, G. Viniegra-Gonzales, *Appl. Microbiol. Biotechnol. 27* (1988) 498-503.
13. O. Bravo, A. Ferrer, C. Aieloo, A. Ledesma, M. Davila, *Biotechnol. Lett. 16* (1994) 865-870.
14. S. Tao, L. Beihui, L. Zuohu, *J. Chem. Technol. Biotechnol. 69* (1997) 429-432.
15. P. K. R. Kumar, B. K. Lonsane, *Biotechnol. Bioeng. 30* (1987) 267-271.
16. M. Gutierrez-Rojas, J. Cordova, R. Auria, S. Revah, E. Favela-Torres, *Biotechnol. Lett. 17* (1995) 219-224.
17. J. P. Smits, H. M. van Sonsbeek, J. Tramper, W. Knol, W. Geelhoed, M. Peeters, A. Rinzema, *Bioprocess Eng. 20* (1999) 391-404.

Model konverzije profila rasta tijekom fermentacije na krutoj podlozi između apsolutnih i relativnih baza mjerenja

Sažetak

Razvijen je matematički model između dviju mjernih baza koje se uobičajeno koriste pri konstrukciji profila rasta tijekom fermentacije na krutoj podlozi, tj. između apsolutnih masenih omjera (*m*(suhe tvari biomase)/*m*(početne suhe tvari)/(g/g)) i relativnih masenih omjera (*m*(suhe biomase)/*m*(suhe tvari)/(g/g)). To nisu ekvivalenti jer se fermentacijom smanjuje suha tvar izlaskom CO_2. Model se isto tako može primijeniti za bilo koju komponentu biomase, npr. proteina, prilikom indirektnog mjerenja rasta. Koristeći model konverzije profila apsolutnog masenog omjera biomase za rast *Rhizopus oligosporus*, u usporedbi s relativnom bazom, dobiveni su se profili dobro poklapali s profilima biomase dobivenim eksperimentalno. To se poklapanje dobilo pri trima različitim fermentacijama, koristeći iste vrijednosti parametara u modelu, tj. koeficijent iskorištenja *m*(proteina)/*m*(suhog supstrata) = 0,2 g/g i koeficijenta održivosti u vrijednosti 0 potvrđujući pouzdanost modela. Modelom se željelo pokazati da primijenjena baza mjerenja može utjecati na oblik krivulje pa i zaključke vezane uz tip kinetike organizma, proširujući taj učinak ovisno o vremenu rasta i vrijednosti iskorištenja te koeficijenta održivosti. U radu je istaknuto da treba paziti prilikom donošenja zaključaka o kinetici rasta tijekom fermentacije na krutoj podlozi.