Journal of
Theoretical & Computational Science

Research Article Open Access

Two Way Crabtree-Effect Model Enhancement by Maintenance Considerations Addition

Thierie J*

Free University of Brussels, Laboratory of Microbial Physiology and Ecology, Pasteur Institute of Brussels, 642 Engeland Street, B-1180 Brussels, Belgium

Abstract

This article refines tweaks and completes the mathematical model that allows accounting qualitatively and quantitatively for the Crabtree effect in eukaryotic cells cultured in the chemostat. To the respirofermentative phenomena, this work adds the representation of the often-observed maintenance phenomena. This extended two-way model accounts for the theoretical aspect of maintenance but also allows us to calculate the associated coefficient. We obtained, for *Saccharomyces cerevisiae*, a value of mGLU=0.094 h^{-1} very close with those of the literature. An unexpected relationship between ethanol produced by the yeast and its intracellular pyruvate concentration was highlighted, as well as the plausible independence of the yield coefficient from the maintenance coefficient, a relevant observation for optimization in biotechnological production processes.

Keywords: Maintenance coefficient; Crabtree effect; Two-channel model; Yeast metabolism; *Saccharomyces cerevisiae*

Introduction

We obtained the results presented in this work by adding an element to a mathematical model designed to describe the Crabtree effect and known as the "two channels" (or two ways) model [1,2]. The additional functionality is the maintenance coefficient, sometimes observed in cell cultures. The requirement to increase the possibilities of the model have emerged over time for many reasons.

The first is to complement a very powerful model that has already proven itself in both biotechnology and general microbiology [1]. This mathematical representation belongs to a series of topics that generally related to respirofermentative transitions that have discovered in mammalian cancer cells (Crabtree effect, Warburg effect). This characteristic gives it considerable potential.

However, in addition to this general already very promising aspect, the maintenance phenomenon itself has its interests. Already in 1982, S. J. Pirt wrote: "The explanation of maintenance energy requirements remains largely a physiological microbial challenge" [3]. Despite a lot of work since then, Pirt's claim remains completely relevant. Plus: its scope has expanded considerably. In the field of environment, for example, where biological wastewater treatment plants would greatly benefit from maintenance energy dissipation, exhausting pollution without the production of by-products, such as "activated sludge" [4].

In the climate change domain, some authors have drawn attention to the importance of maintenance energy on the remineralization of the organic substrate by bacterioplankton [5]. More recently, Wang and Post studied the impact of a maintenance re-evaluation for ecological soil modeling [6].

Many other examples could be provided to illustrate the interest aroused by this still misunderstood concept of maintenance, without forgetting, obviously, all the cases where this maintenance is unfavorable to the envisaged process and where one can speak of energy spilling, a term sometimes used as synonymous with "maintenance".

Let note again the example cited by Verduyn et al., who described a case where the growth kinetics do not allow to determine the maintenance coefficient in *Saccharomyces cerevisiae* cultivated in anaerobic glucose-limited chemostat cultures, a situation that our model should allow circumventing [7].

The interest of increasing the power of our two-way model is thus justified not only by a large number of situations in which this study could be applied but also by the situations in which it makes it possible to solve an otherwise insoluble problem.

Finally, let's not lose sight of the prospects of extending the model to respirofermentative transitions that could prove so important in physiology and public health.

Materials and Methods

The complete derivation of the calculations and algorithms involved is a little long and not essential to the proper understanding of this work. We refer readers who want a complete description of these two references: the most formal description of the two-ways model has been described [1]; perhaps more applied form appears [8]. As has been said, the two-way model assumes the simultaneous use of two channels for the transport of the limiting substrate in the cell; one of the channels is characterized by a high affinity for the substrate, the other by a low affinity. We are considering here the transport of glucose in yeast *Saccharomyces cerevisiae* cultured in a chemostat. This Crabtree positive yeast has the required metabolic characteristics [9].

We place ourselves in the polyphasic dispersed systems representation, which allows the explicit expression of the matter fluxes between the different phases of a system. By reducing the schematization of the bioreactor to a biphasic chemostat (a liquid - or a matrix phase - and a "solid" - or cell phase), the law of evolution of the mass balance of the limiting substrate in the cell phase, S, is given by,

$$\frac{d\tilde{C}_S^c}{dt} = -D\tilde{C}_S^c + \Phi_S^0 - q_S^c X^c + \tilde{C}_S^c \frac{d \ln N_T^c}{dt} \quad (1)$$

***Corresponding author:** Thierie J, Free University of Brussels, Laboratory of Microbial Physiology and Ecology, Pasteur Institute of Brussels, 642 Engeland Street, B-1180 Brussels, Belgium, E-mail: thieriej@gmail.com

Received October 27, 2019; **Accepted** November 07, 2019; **Published** November 14, 2019

Citation: Thierie J (2019) Two Way Crabtree-Effect Model Enhancement by Maintenance Considerations Addition. J Theor Comput Sci 5: 164. doi:10.4172/2376-130X.1000164

$\tilde{C}_S^c$ is the pseudo-homogenous concentration of the substrate associated with the cell phase;

$D=\frac{Q}{V_T}$ is the dilution rate (the ratio of the input flow to the working reactor volume); q_S^c is the net specific rate of disappearance of the substrate in the cell phase. It represents the sum of the processes that consume the substrate in the cell phase (including the phenomena of transport and metabolism). In a general manner, q_S^c can be put in the form,

$$q_S^c=\sum_i q_{S,i}^c \tag{2}$$

Where *i* represents a sub-group of given processes;

X or X^c is the total biomass.

The last term of (1) does not intervene in this reasoning relating to stationary states. We refer to Thierie [8,10]. The steady-state of (1) is then,

$$\Phi_S^0 = q_S^c X^c + D\tilde{C}_S^c \tag{3}$$

In the dispersing matric phase, the compound mass balance is

$$\frac{d\tilde{C}_S^m}{dt} = D\left(\tilde{C}_S^{m,E} - \tilde{C}_S^m\right) - \Phi_S^0 \tag{4}$$

Where $\tilde{C}_S^{m,E}$ and $\tilde{C}_S^m$ are the compound concentrations in the matric phase respectively at the inlet and in the bulk of the reactor. In the steady-state

$$\Phi_S^0 = D\left(\tilde{C}_S^{m,E} - \tilde{C}_S^m\right) \tag{5}$$

Which expresses that the flux transferred towards the cellular phase is simply the difference between the entering and the outgoing flux in the bioreactor.

The total net specific rate allowing compound metabolization into the cell is the result of several processes:

a) Compound diffusion from the matric phase towards the membrane;

b) Compound transport from outside to inside the cell;

c) Compound metabolization (complete or not) in the cell.

We will call transport/metabolization (T/M) the rate resulting from these three processes. A widely used and corroborated explicit form of the net specific form (2) consists of using a hyperbolic function to represent the specific T/M rate:

$$q_S^c=\sum_{i=1}^{n}\frac{V_S^0(i)C_S^c}{K_S(i)+C_S^c} \tag{6}$$

Where $V_S^0(i)$ is the maximum T/M rate for pathway i and $K_s(i)$ is the "affinity" for the compound corresponding to this way (affinity is the inverse of the constant). Weusthuis et al. already used similar kinetics for the description of transport phenomena making use of multiple carriers [11].

According to (6), a two way transport system can be represented by two terms

$$q_S^c = q_S^c(1) + q_S^c(2) \tag{7}$$

With

$$q_S^c(1) = \frac{V_S^0(1)C_S^c}{K_S(1)+C_S^c} \tag{8a}$$

and

$$q_S^c(2)=\frac{V_S^0(2)C_S^c}{K_S(2)+C_S^c} \tag{8b}$$

To be completely rigorous, we must use reaction concentrations (R-concentrations) rather than the extended concentrations (E-concentrations) [12]. Without justifying the demonstration, the relationship between these two types of concentrations is as follows:

$$C_S^c=\tilde{C}_S^c\frac{\delta_c}{X^c} \tag{9}$$

Where δ_c is the volumetric mass (g/L) of the cellular phase.

Relation (8a) then becomes

$$q_S^c(1) = \frac{V_S^0\tilde{C}_S^c}{K_S^*X^c+\tilde{C}_S^c} \tag{10}$$

Where $V_S^0=V_S^0(1)$ and $K_S^*=K_S(1)/\delta_c$ (note that K_S^* has no unit).

For the other pathway, we choose a system where affinity is sufficiently low so that

$$K_S(2) > C_S^c \tag{11}$$

The relation (8b) then look like a kinetics of order 1:

$$q_S^c(2)\approx k_0 C_S^c \tag{12}$$

Where, $k_0=V_S^0(2)/K_S(2)$.

Changing from R- to E-concentrations, like before, we obtain the expression of the other T/M specific rate

$$q_S^c(2) = k_0^*\frac{\tilde{C}_S^c}{X^c} \tag{13}$$

Where $k_0^*=k_0\delta_c$.

Replacing "1" with "h" (high) and "2" with "l" (low), the correct form of two-way T/M in R concentrations is expressed as two terms

$$q_S^c=q_S^c(h)+q_S^c(l)=\frac{V_S^0\tilde{C}_S^c}{K_S^*X^c+\tilde{C}_S^c}+\tilde{C}_S^c k_0^* \tag{14}$$

The mass balance in the steady state is therefore (cf. (3)):

$$\Phi_S^0=\frac{V_S^0\tilde{C}_S^c}{K_S^*X^c+\tilde{C}_S^c}X^c+\tilde{C}_S^c\left(k_0^*+D\right) \tag{15}$$

The second degree with variable coefficients associated polynomial is

$$P^2(\tilde{C}_S^c)\equiv a'_2\left(\tilde{C}_S^c\right)^2+a'_1\tilde{C}_S^c+a_0=0 \tag{16}$$

With

$$\begin{aligned} a'_2&=-K_S^*X^c\Phi_S^0 \\ a'_1&=X^c\left(V_S^0+K_S^*\left(D+k_0^*\right)\right)-\Phi_S^0 \\ a'_0&=D+k_0^* \end{aligned} \tag{17}$$

One can show that (17) only admits one not negative real solution

$$\tilde{C}_S^c=\frac{-a'_1+\sqrt{a'^2_1-4a'_2a'_0}}{2a'_2} \tag{18}$$

The above represents the core of the two-way transport model. This representation, of course, is a minimal representation of this metabolism, but we have seen how effective this model is and can account for an unexpected number of experimental phenomena.

The form (15) can be put into the implicit form using (10) and (13)

$$\Phi_S^0 - \left(q_S^c(h) + q_S^c(l)\right) X - D\tilde{C}_S^c = 0 \quad (19)$$

During the Crabtree effect, the yeast excretes ethanol, which therefore leaves the matrix phase and thus disturbs the stationary state. Imagine that the low-affinity T/M pathway is used to produce excreted ethanol: the intracellular flow of this pathway will be reduced and (19) will take the form

$$\Phi_S^0 - \left(q_S^c(h) + \beta q_S^c(l)\right) X - D\tilde{C}_S^c = 0 \quad (20)$$

With $\beta < 1$.

It is obvious that (19) and (20) cannot be satisfied simultaneously and that the system must adjust. The problem is then to determine what needs to be adjusted. We have been able to show that the pseudo-molecular formula of animal cells (its elementary composition) remains surprisingly constant, even though very diverse metabolic conditions [13]. This observation implies a comparable operative constancy in cell anabolism. As, in a chemostat, the input and output hydraulic flows are very largely imposed by the operator, the adaptation constraint can only be exerted on the cell density of the bioreactor, the only variable yet available: the biomass must adapt. It's easy to show that it will decrease and that

$$\frac{X'^c}{X^c} = \frac{q_S^c(h) + \beta q_S^c(l)}{q_S^c(h) + q_S^c(l)} \leq 1 \quad (21)$$

Previous relationships define the two-way model that accounts for the Crabtree effect. Secondary metabolites excreted or not, are calculated by the stoichiometry of the reaction once the main state variables of the model are known [1]. The following algorithm shows how to calculate model variables without maintenance (Figure 1).

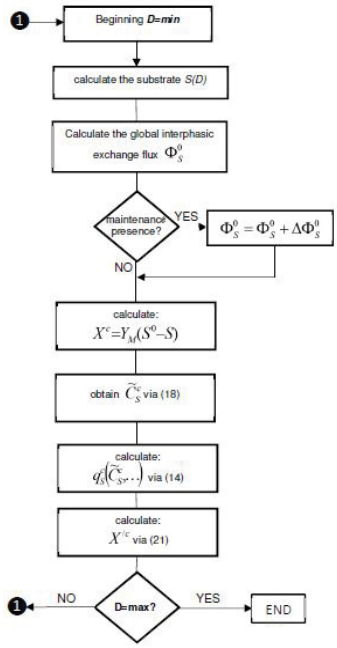

Figure 1: Flow chart showing the combined two-way model algorithm: respirofermentative transition and maintenance coefficient. The answer "YES" to the first conditional test leads to the model with maintenance and effect Crabtree. The alternative produces maintenance-free simulation. The figure shows the iteration steps from Dmin to Dmax. Conditional testing is not necessary to generate the respirofermentative transition, which is an intrinsic property of the model and not a choice of the operator. This property is one of the great strengths of the two-way model.

The flowchart above is used both for simulations and parametric estimation. In the latter case, some algorithms are used in parallel to semi-quantitatively optimize the values using a least squares method (data not shown). A simple logical test makes it possible to use this procedure in the absence or the presence of a maintenance constraint. In case of maintenance, a small supplement is added to the global interphasic exchange flow. (The consequences of such a transformation are discussed in the Discussion.)

Results

Numerical simulations

The simulation of the model above requires first the determination of the parametric coefficients by adjustments to the experimental points. Most of this determination is visual, sometimes supplemented by some algorithms based on least-squares (data not shown). The experimental data were taken and we used four state variables and eight kinetic parameters [14]. Our experience has shown that Monod's representation of the substrate as a function of D is most often questionable. We, therefore, opted for a generator model (an arbitrary representation) of glucose, given by

$$S^{-1} = a + \frac{b}{D^2} \quad (22)$$

Where S is the limiting substrate (glucose) and a = -14.372±0.664, b = 2.640±0.117 with a correlation coefficient of r^2=0.9990 (the number of significant digits is surplus, for verification purposes). The glucose concentration at the inlet of the chemostat, S^0 is constant and set at 30 g/L (3%).The kinetic constants obtained are as follows:

$K_M = 0.033$

$\mu_{max} = 0.44$

$Y_M = 0.48$

$K_S = 2.10^{-6}$

$V_S^0 = 0.55$

$k_0 = 45$

$\beta = 0.13$

The maintenance coefficient (in g/h) obtained by parametric estimation is

$$\Delta\Phi_{GLU}^0 = 0.1$$

Figure 2 shows the glucose, biomass, and ethanol profiles as a function of the dilution rate corresponding to these values.

For these three state variables, the agreement is excellent, even from a quantitative point of view (although the author didn't provide the experimental error values).

We also obtained a value of the pyruvate vs. ethanol ratio linear and quantitatively accurate on $D \in [0, D_c]$, with

$$\frac{Pyruvate}{Ethanol} = a1 + a2.D \quad (23)$$

Where $a1$=6.7831±0.0004 and $a2$=25.9875±0.0024 with a correlation coefficient of r^2=0.99999854≅1 (non-significant decimals given for verification). However, Figure 3 shows that the dependence of the two compounds cannot be reduced to a simple stoichiometric relationship of the type

$$Pyruvate \xrightarrow{\uparrow CO_2} \text{L} \longrightarrow Ethanol \quad (24)$$

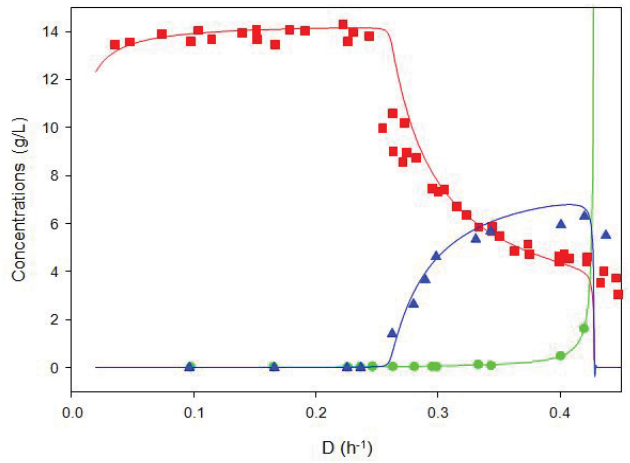

Figure 2: Steady-state profiles of main states variable as a function of D. Algorithm and kinetics parameters: see text. Green circles: Glucose (g/L); Red squares: Biomass (g DW/L); Blue triangles: Ethanol (g/L). Dc: Critical Dilution Rate. A: Convexity of X (D) indicates a nonnull maintenance coefficient. B: Steep biomass decrease due to Crabtree effect perturbation (After Von Meyenburg [14]).

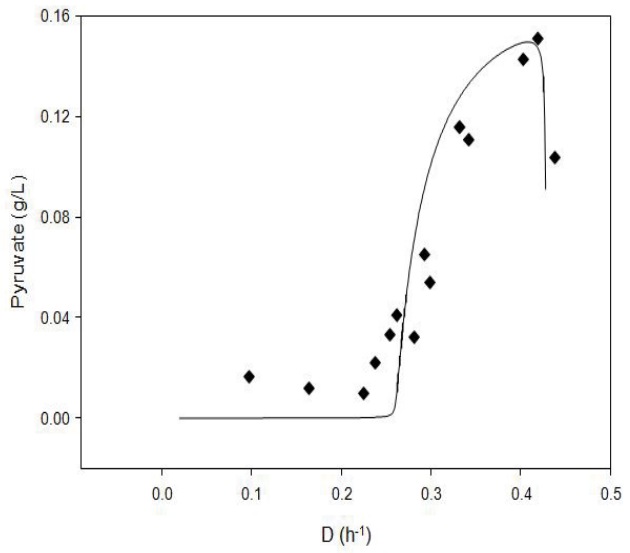

Figure 3: Comparison of experimental intracellular pyruvate and ethanol concentration at steady state. The Figure shows the concordance between ethanol (line) and intracellular pyruvate (diamonds). The graph was constructed by minimizing the residuals of pyruvate=χ.ethanol by varying χ. The general shape is roughly acceptable, but the value of χ=0.22 strongly differs from the theoretical one (around 1.9), indicating that ethanol production from pyruvate is not a simple linear process and that less than 12% pyruvate is generating EtOH (After Von Meyenburg [14]).

Which would simplify itself to elementary proportionality $EtOII \propto Pyr$.

On the other hand, the relation (23) is useful for the determination of the critical dilution rate. For a zero ordinate, we have that

$$D_c = -\frac{a2}{a1} \approx 0.26\ h^{-1} \tag{25a}$$

This value is in perfect agreement with the theoretical value of,

$$D_c = Y_M.V_S^0 = 0.48 \times 0.55 = 0.264\ h^{-1} \tag{25b}$$

Or with the intersection of the two straight-line segments of Figure 4.

$$q_S^c(D < D_c) \equiv 0.0062 + 2.0915.D \quad r^2 \cong 1$$

$$q_S^c(D > D_c) \equiv -3.4728 + 15.4374.D \quad r^2 \cong 1$$

Which is worth,

$$D_c = 0.26\ h^{-1} \tag{25c}$$

The convergence of results between theoretical or empirical evaluation methods once again demonstrates the strong coherence of the model and its suitability for the experimental.

The maintenance energy calculation

By plotting the substrate global specific interphasic exchange flux, as a function of the dilution rate D, one obtains the usual Figure 4. The ordinate at the origin of the line segment gives the conventional value of the maintenance coefficient expressed with respect to the limiting substrate, m_s, defined by (in h^{-1}) [15]:

$$q_S^c = \gamma\mu + m_S \tag{26}$$

Where g is the inverse of the yield coefficient, $\frac{1}{Y_{X,S}}$, practically constant at low dilution rates. The linear regression provides the equation (see above)

$$q_S^c = 0.0067 + 2.0868D \tag{27}$$

With a correlation coefficient $r^2 \approx 1$.

The parametric estimation of the kinetic constants of the model provided a value very close to $\Delta\Phi_S^0 \cong 0.1$ g GLU/h.

Taking into account that

$$Xq_S^c = \Phi_S^0 \tag{28}$$

and that gDW/L at small values of D, we get a maintenance coefficient of

$$Xq_S^c\big|_{D=0} = 14 \times 0.0067 = 0.0938\ h^{-1}$$

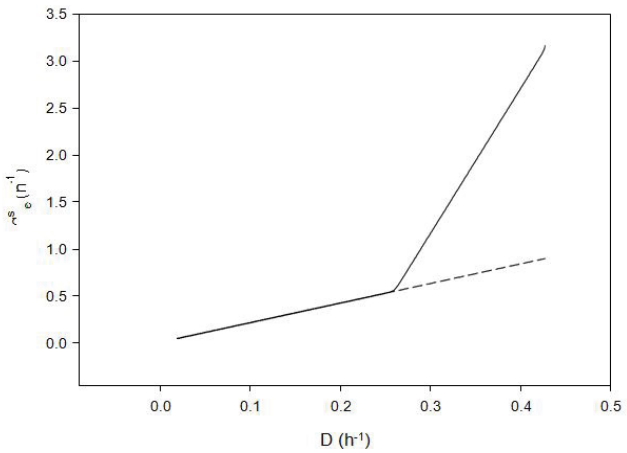

Figure 4: Specific interphasic exchange flux as a function of D. The figure shows two straight line segments before and after the critical dilution rate. The ordinate at the origin of the segment when D<Dc provided the value of the maintenance coefficient; the intersection of the two right-hand segments makes it possible to determine Dc (After Von Meyenburg [14]).

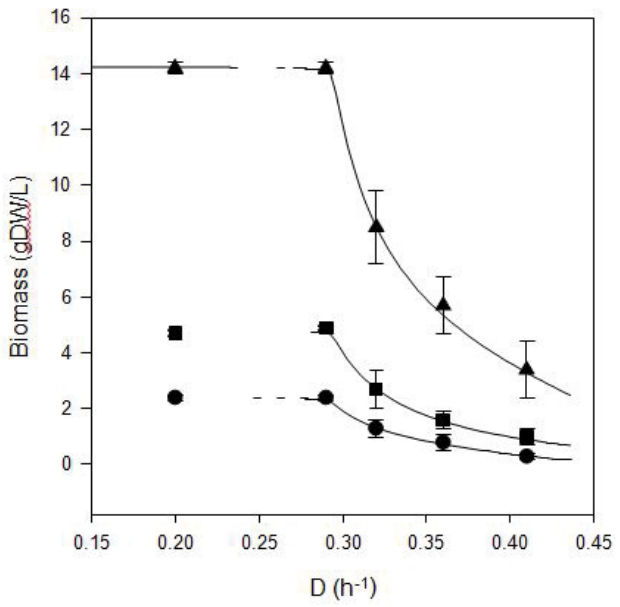

Figure 5: The curves show the sudden decrease in biomass during a Crabtree effect in Saccharomyces cerevisiae when D>Dc. Note the lack of maintenance coefficient, visible by the parallelism of the biomass curve, and the D axis for D<Dc. The critical dilution rate is $D_c \cong 0.3h^{-1}$. Glucose inlet concentration (g/L): ● 5; ■ 10; ▲ 30 (After Rieger et al., [19]).

A value very close to the parametric estimate of 0.1 h^{-1} (less than 6%, approximately).

This result is remarkable given the large number of state variables that are correctly quantitatively estimated (Figure 2) by a single set of kinetic constants (parameters). Besides, Nissen et al. gives a value of $m'_{GLU} = 0.45 \pm 0.52$ mmol.D/g [16].

The conversion to h^{-1} is $m_{GLU}(h^{-1}) = m'_{GLU} \times 180/1000\ (mmol.D/g)$ and give $m_{GLU} = 0.0864 \pm 0.0936\ h^{-1}$ a value quite compatible with the one we found. Similarly, Boender et al. gives a value of 0.5 mmol.D/g, that is $m_{GLU} = 0.09\ h^{-1}$ which is almost identical to ours (see also Vos et al.) [17,18].

As a comparison, we examined the three kinetics of Rieger et al. [19]. Figure 5 clearly shows that the biomass profiles are parallel to the x-axis and do not indicate any maintenance energy.

The linear regression of the total specific exchange flux gives, for the three curves, the same values with a coefficient of almost unitary correlation: $r^2 \cong 1$

$$q_S^c = -0.0001 + 2.1103D \quad (29)$$

Since the negative independent term is aberrant and very small, we conclude that the maintenance coefficient is zero $m_{GLU} = 0$.

It is interesting to note that the yield coefficients are very close in the two studied cases (four different kinetics, in fact):

- From (27): $Y_{X,GLU} = \frac{1}{2.0868} = 0.4792$
- From (29): $Y_{X,GLU} = \frac{1}{2.1103} = 0.4739$

The parametric estimate provided a value of $Y_{X,GLU} = 0.474$.

Discussion

We believe that concordance between experimental and theoretical results is remarkable given the metabolic complexity of the phenomenon studied: a respirofermentative transition accompanied by a non-zero maintenance coefficient. The poor stoichiometric correlation between ethanol and pyruvate should not be considered as a failure but simply as the limit of this method of modeling, sometimes promoted and recommended for its "simplicity" but which shows here that many precautions are required to use a methodology whose simplicity is only an appearance [20].

In addition to the good correlation of the results, we highlight unexpected linear relationship pyruvate to ethanol ratio. We do not seek to interpret this result here, but the precision in the data regression suggests that a precise and particular mechanism links these two variables and needs to be elucidated.

We have also demonstrated that the maintenance coefficient may vary with growth conditions without influencing the yield coefficient. This observation could be of great practical importance to optimization research processes. The confirmation by several different methods of the value of the theoretical, critical dilution ($D_c = 0.26\ h^{-1}$) reinforces the robustness of our representation.

However, we must insist on a step in our modeling that could raise a controversy. As we have long discussed in Material and Methods, the whole of our model rests on the description of the steady-state of the chemostat. The disturbance of the latter causes a decrease in biomass and the appearance of the Crabtree effect. In Figure 1, which describes the flowchart of the two-way system algorithm, it is clear that the appearance of maintenance in the model results from the addition of a small additional amount of substrate to the global interphasic exchange flow of the system: $\Phi_S^0 \Rightarrow \Phi_S^0 + \Delta\Phi_S^0$. Although this increase is very small, relatively, it could be considered that it constitutes a disturbance of the steady-state and that, consequently, the subsequent mathematical relations would no longer be guaranteed. This argument is perfectly acceptable, but we have shown that it is precisely the rebalancing of the steady-state that accounts for maintenance phenomena.

The full demonstration is too long to reproduce here. The principle of this concept is very simple: if one accepts that a quantity of additional substrate enters the cell but is quantitatively excreted, the overall mass balance will be invariant [21]. On the other hand, the energy balance can be disturbed and may cause a loss of energy at the cellular level. The following Figure 6 shows the hydraulic analogy of this concept:

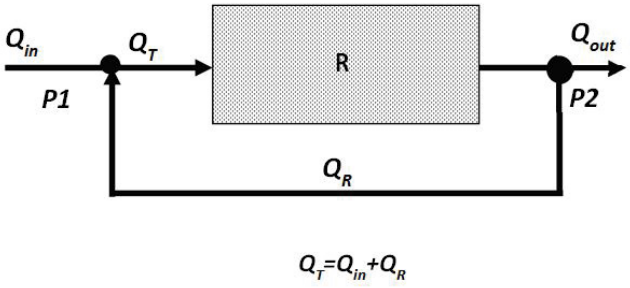

Figure 6: Figure 6 shows that internal recycling makes it possible to increase the input flow (QT) in the reservoir (R) without modifying the total mass balance: Qin=Qout. This process has an energy cost since a pump (P), or any comparable mechanism is required for recirculation.

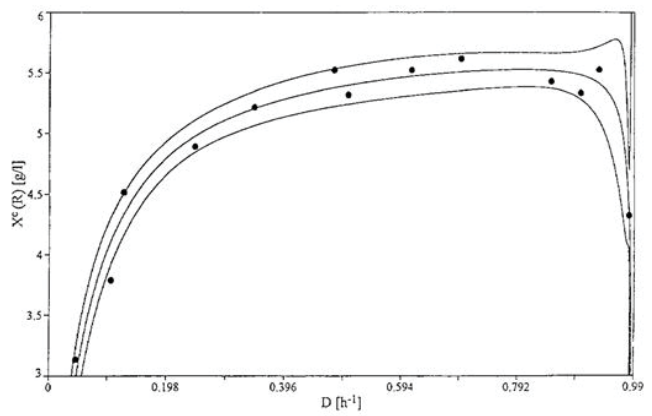

Figure 7: Biomass profile of Klebsiella pneumoniae cultivated in a chemostat showing relatively large energy spilling. Upper and lower curves show 95% confidence interval. Data: Nielsen et al. [16]; Figure after Thierie [21].

the mass balance (in g/(L.h)) around the reservoir remains invariant $Q_{in} = Q_{out}$ whatever the value of the recycled flow Q_R.

However, the use of pumps (P) required for recycling can profoundly change the energy balance of the hydraulic system in Figure 6. In fact, what is surprising in this model is that the energy balance does not seem to play a quantitative role at all.

Thus, Figure 7 was obtained using the same steady-states as for the Crabtree effect but without any secondary metabolites being excreted. There is no critical value of the dilution rate, and this time the biomass decrease is due to the recycling of the substrate. Ultimately, the biomass variation, in the case of excretion or recycling, is based on the same principle of rebalancing the global stationary state. The remarkable concordance of the experimental values of Figure 7 again shows the robustness of the representation.

Conclusion

In the original article of 2000, we assumed that the substrate was excreted without modification of chemical form; however, we can now assume that only the conservation of the recycled mass has to be taken in account, but that the chemical nature of the substrate does not necessarily have to be conserved. This consideration, of course, increases the scope of the theory but probably deserves closer examination.

The author claims that, to his knowledge, no complaint or protest about this manuscript is in progress.

Reference

1. Thierie J. Introduction to polyphasic dispersed systems theory: Application to open systems of microorganisms' culture. Springer International Publishing, Switzerland. 2015.
2. Thierie J, Penninckx MJ. Possible occurrence of a crabtree effect in the production of lactic and butyric acids by a floc-forming bacterial consortium. Curr Microbiol. 2004;48(3):224-229.
3. Pirt SJ. Maintenance energy: A general model for energy-limited and energy-sufficient growth. Arch Microbiol. 1982;133(4):300-302.
4. Low EW, Chase HA. The effect of maintenance energy requirements on biomass production during wastewater treatment. Water Res. 1999;33(3):847-853.
5. Cajal-Medrano R, Maske H. Growth efficiency, growth rate and the remineralization of organic substrate by bacterioplankton--revisiting the Pirt model. Aquat Microb Ecol. 1999;19(2):119-128.
6. Wang G, Post WM. A theoretical reassessment of microbial maintenance and implications for microbial ecology modeling. FEMS Microbiol Ecol. 2012;81(3):610-617.
7. Verduyn C, Postma E, Scheffers WA, Van Dijken JP. Physiology of saccharomyces cerevisiae in anaerobic glucose-limited chemostat culturesx. Microbiology. 1990;136(3):395-403.
8. Thierie J. Modeling threshold phenomena, metabolic pathways switches and signals in chemostat-cultivated cells: The Crabtree effect in Saccharomyces cerevisiae. J Theor Biol. 2004;226(4):483-501.
9. Maier A, Völker B, Boles E, Fuhrmann GF. Characterisation of glucose transport in Saccharomyces cerevisiae with plasma membrane vesicles (countertransport) and intact cells (initial uptake) with single Hxt1, Hxt2, Hxt3, Hxt4, Hxt6, Hxt7 or Gal2 transporters. FEMS Yeast Res. 2002;2(4):539-550.
10. Thierie J. Theoretical and conceptual derivation of threshold phenomena and metabolic switching models in a chemostat system. J Comput Biol. 2018;8(2):12-29.
11. Weusthuis RA, Pronk JT, Van Den Broek PJ, Van Dijken JP. Chemostat cultivation as a tool for studies on sugar transport in yeasts. Microbiol Rev. 1994;58(4):616-630.
12. Thierie J. Why does bacterial composition change with the chemostat dilution rate? Biotechnology Techniques. 1997;11(9):625-629.
13. Thierie J. Appraisal of the pseudo-molecular concept of biological cells using a statistical method: A trend towards universalization. Int J Biotech & Bioeng. 5(6):45.
14. Meyenburg KV. Katabolit-Repression und der Sprossungszyklus von Saccharomyces cerevisiae. ETH Zurich. 1969;114(2):113-222.
15. Pirt S J. Principles of microbe and cell cultivation. 1975, Blackwell Scientific Publications, UK.
16. Nielsen TL, Schulze U, Nielsen J, Villadsen J. Flux distributions in anaerobic, glucose-limited continuous cultures of saccharomyces cerevisiae. Microbiology. 1997;149(1):203-218.
17. Boender LG, De Hulster EA, Van Maris AJ, Daran-Lapujade PA, Pronk JT. Quantitative physiology of saccharomyces cerevisiae at near-zero specific growth rates. Appl Environ Microbiol. 2009;75(23):5607-14.
18. Vos T, Hakkaart XD, De Hulster EA, Van Maris AJ, Pronk JT, Daran-Lapujade P. Maintenance-energy requirements and robustness of saccharomyces cerevisiae at aerobic near-zero specific growth rates. Microb Cell Fact. 2016;15(1):111.
19. Rieger M, KÄPpeli O, Fiechter A. The role of limited respiration in the incomplete oxidation of glucose by saccharomyces cerevisiae. Microbiology. 1983;129(3):653-661.
20. Villadsen J, Jørgensen SB. Reflections on the aerobic fermentation stoichiometry of crabtree positive yeasts. Biotechnol Bioeng. 2014;111(3):632-637.
21. Thierie J. Cellular cycling of substrate as a possible cryptic way for energy spilling in suspended cellular continuous cultures. Biotechnol Lett. 2000;22(14):1143-1149.