Growth of *Saccharomyces cerevisiae* Is Controlled by Its Limited Respiratory Capacity: Formulation and Verification of a Hypothesis*

B. Sonnleitner† and O. Käppeli
Department for Biotechnology, Swiss Federal Institute of Technology, 8093 Zürich, Switzerland

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A novel mechanistic model for the growth of baker's yeast on glucose is presented. It is based on the fact that glucose degradation proceeds via two pathways under conditions of aerobic ethanol formation. Part is metabolized oxidatively and part reductively, with ethanol being the end product of reductive energy metabolism. The corresponding metabolic state is designated oxidoreductive. Ethanol can be used oxidatively only. Maximum rates of oxidative glucose and ethanol degradation are governed by the respiratory capacity of the cells. The model is formulated by using the stoichiometric growth equations for pure oxidative and reductive (fermentative) glucose and ethanol metabolism. Together with the experimentally determinable yield coefficients ($Y_{X/S}$) for the respective metabolic pathways, the resulting equation system is sufficiently determined. The superiority of the presented model over hitherto published ones is based on two essential novelities. (1) The model was developed on experimentally easily accessible parameters only. (2) For the modeling of aerobic ethanol formation, the substrate flow was split into two simultaneously operating (i.e., in parallel) metabolic pathways that exhibit different but constant energy-generating efficiencies (respiration and fermentation) and consequently different and constant biomass yields ($Y_{X/S}$). The model allows the prediction of experimental data without parameter adaption in a biologically dubious manner.

INTRODUCTION

Progress in biotechnology has created a need to quantify metabolic processes of microorganisms so that they can be most thoroughly and efficiently exploited. The rewards of an improved quantification are more certainly great: (1) increased yield of microbial products, (2) increased rate of product formation, (3) maintenance of microbial product quality and uniformity, and (4) attainment of process uniformity. Although these ends have been long persued, the complexity of microbial systems has presented serious obstacles to process quantification. Nevertheless, limited success has been reported in monitoring, controlling, and optimizing microbial systems using material balancing techniques and empirical process models.[1–4]

A sound approach to modeling the growth of *Saccharomyces cerevisiae* depends, in general, on the identification and quantification of the key biochemical and physiological elements that collectively determine the kinetics of this microbial system. In view of the complexity of the system, a careful determination of the conceptual parameter(s) must be the goal. In this paper we report on a model that was based on the biological data on yeast growth currently available.

In 1954 a first quantitative study of aerobic yeast growth by Lemoigne and coworkers[5] showed a diauxic growth on glucose. This growth behavior in an aerobic batch culture was confirmed by Beck and von Meyenburg[6] with a well-documented investigation.

In a first growth phase, biomass is formed and ethanol is accumulated at the expense of glucose consumption. In the subsequent growth phase, which begins after glucose is completely exhausted, the produced ethanol serves as substrate for further growth. Because major differences in the activities of enzymes of the tricarboxylic acid cycle[7] and the glyoxylate shunt[8] were measured in the presence of glucose, this growth behavior was explained on the molecular level by a repression/derepression mechanism of respiration. This repression is commonly referred to as the Crabtree effect[9] or glucose effect.

When biomass formation of baker's yeast was recorded as a function of dilution rate in a continuous culture, two metabolically different regions were distinguished.[6] In the first range of low dilution rates, the breakdown of glucose is oxidative. No other carbon-

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† To whom all correspondence should be addressed.

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containing products than biomass and carbon dioxide are formed in appreciable amounts. A linear increase in the specific oxygen uptake rate was observed in the oxidative range of catabolism.

When the dilution rate is successively increased, a particular strain-specific value is reached above which ethanol is produced by the cells. Beck and von Meyenburg[6] noticed that the specific oxygen uptake rate reached a maximum at the onset of aerobic ethanol formation and then steadily decreased with increasing dilution rates. This course of the specific oxygen uptake rate versus dilution rate was considered as the consequence of the repression of respiratory enzymes under conditions of aerobic ethanol formation.

In 1979 Barford and Hall[10] demonstrated that the oxygen uptake rate did not decrease when the maximum oxidative dilution rate was exceeded but remained constant at its maximum value. They also found that in batch cultures the maximum oxygen uptake rate was reached when the cells were properly adapted. However, these authors do not offer another concept explaining aerobic ethanol formation. The Crabtree effect was still considered as the basis for this event.

Rieger et al.[11,12] discussed the involvement of a limited respiratory capacity in the occurrence of aerobic ethanol formation of baker's yeast. It was assumed that it resulted from a saturated respiratory capacity and not from glucose repression of respiration. Following the routes of this concept, it was shown by Petrik et al.[13] that the so-called glucose effect consists of a short- and a long-term regulation. By glucose pulse experiments it was shown that ethanol formed before any repression of mitochondrial functions was noticed. It primarily represented an overflow reaction on the level of pyruvate as a consequence of a saturated oxidative capacity. A long-term adaption of mitochondrial functions to the conditions of aerobic ethanol formation was observed by dilution rate shift experiments; however, both oxidative and fermentative glucose catabolism occurred simultaneously. The corresponding physiological state was therefore referred to as oxidoreductive or respirofermentative glucose metabolism.[14,15]

The results of transient responses to changes in the growth-limiting nutrient supported the validity of the concept of a limited respiratory capacity as the basis for the occurrence of oxidoreductive glucose metabolism. A limitation of growth by anything other than glucose resulted in an imbalance between glucose flux and biosynthetic potential of the cells caused oxidoreductive glucose breakdown.[14]

The model presented here is based on the stoichiometries of the three pure metabolic routes involved in glucose breakdown as known from the batch and continuous culture experiments reviewed, i.e., oxidative and reductive (fermentative) glucose catabolism as well as ethanol utilization. Aerobic ethanol formation is represented by combining the oxidative and reductive pathways for glucose degradation, which are characterized by distinct but constant biomass yields. Their parallel operation is determined only by the respiratory capacity of the cells. Simulations with the developed model verified that the concept of a limited respiration as derived from the biological data may well exert the proposed role as a determinant of glucose metabolism in these yeasts.

MATERIALS AND METHODS

The experimental data and methods used in this report were previously published.[12] The organism studied was *Saccharomyces cerevisiae* H1022 (ATCC 32167). Simulations were calculated using FORTRAN 77 on a PDP-11/34 with a floating point processor.

THE MODEL

The model is based on the following observations and conceptual ideas:

1) Batch growth on glucose and on ethanol follows nearly ideal Monod kinetics.

2) There are no by-products of growth in significant amounts; the main products in dilute cultures are biomass, CO_2, H_2O, and, under appropriate conditions, ethanol. The experimental carbon recovery was always very close to 100%.

3) The specific oxygen uptake rate is linearly correlated with the specific glucose uptake rate under oxidative metabolism only, i.e., subcritical glucose flux or ethanol growth. Under oxidoreductive conditions of supracritical glucose flux (conditions where ethanol is produced by the cells), the specific oxygen uptake rate remains constant at its maximum value independent of the growth rate.

4) Glucose inhibits the uptake of ethanol as a substrate for growth, if present in measurable concentrations.

5) Glucose can be metabolized both aerobically and anaerobically, however, with different rates and different efficiencies.

6) Ethanol can be utilized aerobically only.

7) The elemental composition of biomass grown on glucose does not change significantly if ethanol is or is not produced. The composition of biomass grown on ethanol tends to be somewhat different from that of glucose-grown cells, but the differences are within analytical errors (Rieger, in preparation[16]).

Therefore, the formulation of the model is as follows.

1) There exist three different stoichiometric equations for the description of the growth of *S. cerevisiae*-type yeasts on glucose and/or ethanol: the purely oxidative (formulas [1] and [3]) and one purely reductive (formula [2]) metabolic pathways:

$$C_6H_{12}O_6 + a\ O_2 + b\ NX\ [NH_3] \longrightarrow b\ C_1H_{HX}O_{OX}N_{NX} + c\ CO_2 + d\ H_2O \quad [1]$$

$$C_6H_{12}O_6 + g\ NX\ [NH_3] \longrightarrow g\ C_1H_{HX}O_{OX}N_{NX} + h\ CO_2 + i\ H_2O + j\ C_2H_6O \quad [2]$$

$$C_2H_6O + k\ O_2 + l\ NX\ [NH_3] \longrightarrow l\ C_1H_{HX}O_{OX}N_{NX} + m\ CO_2 + n\ H_2O \quad [3]$$

Biomass X has the assumed molecular formula $C_1H_{HX}O_{OX}N_{NX}$, where HX, OX, NX, and the "molecular weight" are calculated from elemental analyses of biomass. Nitrogen is assumed not to change its oxidation state. Therefore, the nitrogen balance does not contribute to uniquely define the equation system (see below). However, it must not be omitted in the above equations because of its contribution to the hydrogen balance, which has to be considered.

2) From the above equations three different sets of linear algebraic equations are derived, each considering the carbon, oxygen, and hydrogen balance. So far, the equation systems are not defined and consequently cannot be solved since the number of unknowns exceeds the number of equations by one. It is, therefore, necessary to obtain empirically one coefficient for each set of equations. The most reliably measurable coefficients are the yield coefficients $Y_{\text{biomass/substrate}}$ (on a mass basis), which of course are proportional to the stoichiometric coefficients b, g, and l (on a molar basis) in [1]–[3]:

$$Y^{\text{oxidative}}_{\text{biomass/glucose}} = b\,\frac{\text{molecular weight of biomass}}{\text{molecular weight of glucose}} \quad (1)$$

$$Y^{\text{reductive}}_{\text{biomass/glucose}} = g\,\frac{\text{molecular weight of biomass}}{\text{molecular weight of glucose}} \quad (2)$$

$$Y_{\text{biomass/ethanol}} = l\,\frac{\text{molecular weight of biomass}}{\text{molecular weight of ethanol}} \quad (3)$$

3) The respiratory capacity of the cells is assumed to govern glucose or ethanol metabolism in growing cells and product formation and represents a bottleneck for oxidative substrate utilization (Fig. 1). At substrate fluxes low enough to fit accordingly into this bottleneck ("subcritical" substrate flux), pure oxidative metabolism is observed (with first priority for glucose and second priority for ethanol). If glucose flux exceeds the respiration bottleneck ("supracritical" substrate flux), the following partition of glucose flux takes place. Exactly the part of glucose saturating the respiration bottleneck is metabolized according to for-

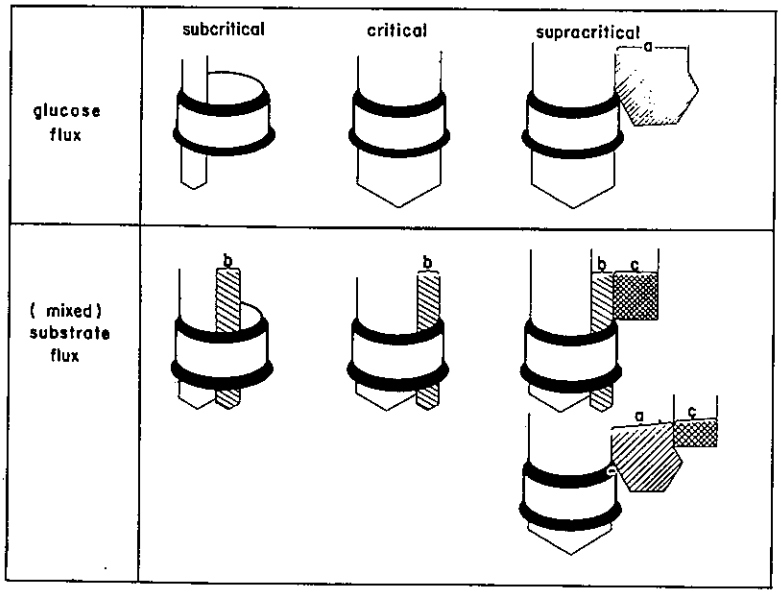

Figure 1. Limited respiratory capacity of *Saccharomyces*-type yeasts illustrated as a bottleneck. If the total amount of substrate(s) flux can pass the bottleneck, i.e., substrate(s) is metabolized purely oxidatively, substrate flux is "subcritical," or when the bottleneck is completely filled, substrate flux is critical. If substrate flux no longer completely fits into the bottleneck, i.e., "supracritical," the following situations must be considered. (1) Glucose flux is supracritical. The residual part of glucose that cannot pass the bottleneck is metabolized reductively (a) and ethanol is produced (top right). If there is additional ethanol supplied in the medium, it cannot pass the bottleneck because this is filled with glucose. Hence, this ethanol remains unused (c) because there is no reductive metabolic pathway to utilize ethanol (bottom right). (2) Glucose flux is subcritical but there is additional ethanol in the medium. Ethanol is utilized oxidatively as long as its flux fits (in addition to the fully oxidative glucose flux) into the bottleneck (marked b, middle row). The residual ethanol cannot be metabolized as is shown (c).

mula [1], and the rest is metabolized reductively according to formula [2]. Since ethanol utilization can be a purely oxidative process only, and ethanol utilization is observed to have lower priority than glucose utilization, no ethanol utilization can take place under these conditions. Consequently, growth on ethanol as the sole substrate is definitely limited by the respiratory capacity.

4) Glucose uptake follows Monod kinetics.

$$q_S = q_{S,\max} \frac{s}{s + K_s} \qquad (= q_S(s)) \tag{4}$$

5) Ethanol uptake follows Monod kinetics. The priority of glucose uptake over ethanol uptake can be formulated as inhibition by freely available glucose.

$$\mu_{\text{ethanol}} = \mu_{\max,\text{ethanol}} \frac{e}{e + K_e} \frac{K_i}{s + K_i} \tag{5}$$

However, this holds true only if there is free respiratory capacity available.

6) Respiration itself depends on the availability of dissolved oxygen.

$$q_{O_2} \leq q_{O_2,\max} \frac{o}{o + K_o} \tag{6a}$$

or

$$q^i_{O_2} \leq q^i_{O_2,\max} \frac{o}{o + K_o} \tag{6b}$$

where $q^i_{O_2}$ and $q^i_{O_2,\max}$ are the individual fluxes of oxygen for glucose or ethanol metabolism. This alternative formulation may also be considered.

7) Growth is an autocatalytic reaction.

$$r_X = \mu x \tag{7}$$

The total specific growth rate (μ_{total}) is the result of all additive substrate fluxes where the three relations are valid.

$$\mu_j = -Y_{X/S_j} q_{S_j} \tag{8}$$

$$\mu_{\text{total}} = \mu^{\text{oxidative}}_{\text{glucose}} + \mu^{\text{reductive}}_{\text{glucose}} + \mu_{\text{ethanol}} \tag{9}$$

$$\mu_{\text{total}} = -Y^{\text{oxidative}}_{\text{biomass/glucose}} q_S^{\text{oxidative}} - Y^{\text{reductive}}_{\text{biomass/glucose}} q_S^{\text{reductive}} - Y_{\text{biomass/ethanol}} q_{\text{ethanol}} \tag{10}$$

The yield coefficients are assumed to be constant for a first approximation. The quantities of the individual q values and, hence, the partial μ values, depend on the following:

7a) Glucose flux does not require maximal respiration, i.e., $q_{O_2,\text{glucose},\max} = q_S a$ is less than $q_{O_2,\max}$: Subcritical glucose flux, i.e., essentially all glucose is metabolized oxidatively, depending on the availability of dissolved oxygen.

$$q_{O_2,\text{glucose}} \leq q_{O_2,\max} \frac{o}{o + K_o} \tag{11a}$$

or, if a separate consideration of oxygen uptake for glucose and for ethanol utilization is desired:

$$q_{O_2,\text{glucose}} = q_{O_2,\text{glucose},\max} \frac{o}{o + K_o} \tag{11b}$$

with $q_{O_2(,\text{glucose}),\max}$ = minimum of $q_{O_2,\max}$ and $aq_S(s)$

$$q_S^{\text{oxidative}} = \frac{q_{O_2,\text{glucose}}}{a} \tag{12}$$

The residual respiratory capacity can be utilized for ethanol growth if ethanol is present in the culture liquid, being restricted by the oxygen availability in the same manner.

7b) Glucose flux is sufficiently high to exceed the bottleneck, $q_{O_2,\text{glucose},\max} = q_S a$ would be greater than $q_{O_2,\max}$: supracritical glucose flux, i.e., as much glucose as can be oxidatively metabolized (depending on dissolved oxygen availability) is utilized according to pathway [1], and the rest (i.e., $q_S^{\text{reductive}} = q_S^{\text{total}} - q_S^{\text{oxidative}}$) is utilized according to pathway [2]. There is

Table I. Parameters used in the model.

Parameter	Most probable value	Range of variation in Figures 3–7	Dimension
$q_{S,\max}$; maximal specific glucose uptake rate	3.5	3.0 –3.75	g g^{-1} h^{-1}
$q_{O_2,\max}$; maximal specific oxygen uptake rate	8.0	7.5 –8.25	mmol g^{-1} h^{-1}
$Y^{\text{oxidative}}_{\text{biomass/glucose}}$; yield for pathway [1]	0.49	0.47–0.50	g g^{-1}
$Y^{\text{reductive}}_{\text{biomass/glucose}}$; yield for pathway [2]	0.05	0.05–0.10	g g^{-1}
OX; oxygen content of biomass in "molecular formula"	0.57	0.54–0.63	mol mol^{-1}
$Y_{\text{biomass/ethanol}}$; yield for pathway [3]	0.72		g g^{-1}
$\mu_{\max,\text{ethanol}}$; maximal specific growth rate	0.17		h^{-1}
K_s; saturation parameter for glucose uptake	0.1–0.5		g L^{-1}
K_o; saturation parameter for oxygen uptake	0.1		mg L^{-1}
K_e; saturation parameter for growth on ethanol	0.1		g L^{-1}
K_i; inhibition parameter: free glucose inhibits ethanol uptake	0.1		g L^{-1}
CX; carbon content of biomass in "molecular formula"	1.00		mol mol^{-1}
HX; hydrogen content of biomass in "molecular formula"	1.79		mol mol^{-1}
NX; nitrogen content of biomass in "molecular formula"	0.15		mol mol^{-1}

no capacity for ethanol utilization (pathway [3]). The substrate fluxes established in this way allow the definition of the fractional μ values using the respective constant yield coefficients and, by summing them, to determine the total specific growth rate.

8) Accordingly, the fluxes of products (CO_2, H_2O, and ethanol) result from stoichiometric equations [1]–[3] and the above established substrate fluxes or fractional growth rates, respectively.

9) The mass balances for biomass, glucose, ethanol, as well as for oxygen and carbon dioxide in both liquid and gas phase, are sufficiently defined to be solved. This system of differential equations may be completed by including the mass balance for water, depending on the degree of desired accuracy. However, in the following considerations this was neglected.

RESULTS AND DISCUSSION

Steady State Solution for Growth on Glucose

The stationary behavior of the described model using parameters listed in Table I is shown in Figure 2 for considerably dilute media because the model—in the presented form—is not intended to formulate any inhibitory effects of ethanol. But, definitely, the so-called "glucose effect" can be described both qualitatively and quantitatively by the bottleneck hypothesis using fairly accurately measured experimental data as model parameters. The critical specific glucose uptake rate ($q_{s,crit} = 0.62\ h^{-1}$) and the corresponding dilution rate ($D_R = 0.3\ h^{-1}$) are obtained exactly using values of 0.49 for $Y^{oxidative}$ and 8.0 (mmol $g^{-1}\ h^{-1}$) for $q_{O_2,max}$, which are the two determinative parameters. Both can be measured very accurately and have been found to be relatively constant.[12,17]

The growth behavior characteristic for *Saccharomyces*-type yeasts was found to be a physiological phenomenon and not originating from mass transfer limitations. The oxygen transfer rate required for growth with maximum oxidative capacity is 40 mmol $L^{-1}\ h^{-1}$ at a biomass concentration of approximately 5 g L^{-1}. With the cultivation conditions applied, this value was certainly exceeded as can be derived from oxygen transfer rate measurements with similar equipment by Käppeli and Fiechter.[18] Limitations from the medium composition can also be excluded, since this medium was found to be carbon limited for several yeast strains,[19,13] which is contrary to previously used media as was clearly shown by Rieger et al.[12]

It is important to mention that growth parameters can be experimentally determined with sufficient accuracy so far at low dilution rates only, where a small change in dilution rate has only negligible influence on biomass and substrate concentrations. At dilution rates $D > D_R$, the continuous culture should be controlled using either a turbidostat technique or a chemostat

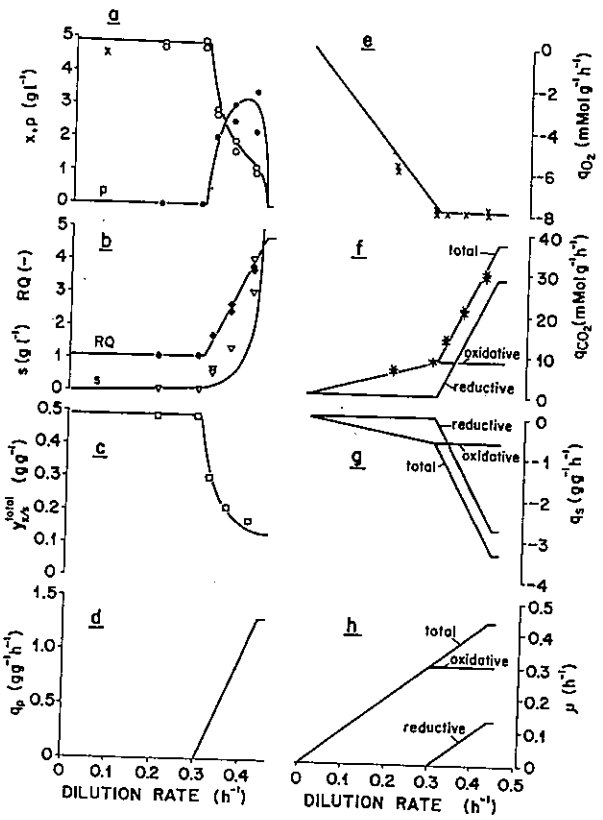

Figure 2. Simulation of the stationary behavior of the proposed model using eqs. (6a) and (11a) with a feed concentration of 10 g L^{-1} glucose in the medium. The parameters used are given in Table I ($K_s = 0.5$ g L^{-1}). Experimental data were obtained in the medium that allows definite carbon-limited growth described by Rieger et al.[12] The following dependent variables are plotted versus dilution rate: a: Biomass (x) and ethanol (p) concentration. b: Glucose concentration (s) and respiratory quotient (RQ). c: Overall biomass yield coefficient ($Y^{total}_{X/S}$). d: Specific ethanol formation rate (q_p, positive values). e: Specific oxygen uptake rate (q_{O_2}, negative values). f: Specific carbon dioxide production rate (q_{CO_2}); according to different pathways ([1], oxidative), ([2], reductive), and (total), the sum of both pathways as experimentally determined. g: Specific glucose uptake rate (q_S, negative values) analogous to f. h: Specific growth rate: upper line (total) is sum of the contributions from pathway [1] (oxidative) and pathway [2] (reductive).

equipped with a high performance control of dilution rate. So far, this was not realized properly; the time stability of so-called steady states were reported to be very poor (fluctuations of up to 80% around mean values of concentrations) and must be attributed to inappropriate equipment.[12]

The respiratory quotient (RQ) in the purely oxidative part of the x-D-diagram ($D < 0.3\ h^{-1}$) has a value of 1.07 and not, as often published earlier, of exactly 1.00. This is mainly a consequence of the assumed molecular formula for biomass. As biomass composition changes or is determined differently, the RQ value changes too, reflecting the difference in the state of oxidation of carbon in substrate and in biomass (redox balance; for comparison and detailed discussion refer to References 10, 12, and 20–23).

The dependence of respiration on oxygen availability is expressed by the Monod-type correlation between q_{O_2} and dissolved oxygen concentration (eq. 6). Therefore, two more values must be considered: the oxygen transfer coefficient of the reaction vessel ($k_L a$) influencing the dissolved oxygen concentration and the Monod saturation coefficient for oxygen (K_o). The value of 0.1 mg L^{-1} used for the simulations has been determined for *Trichosporou cutaneum* and not for *S. cerevisiae*. However, the comparable behavior of both organisms under oxygen limitation with respect to, e.g., cytochrome content indicates that K_o values must be very similar or even lower.[24, 25]

Sensitivity Toward Model Parameters

The values of the following parameters must be known for numerical solutions of the model: the maximally attainable fluxes of substrates, the yield coefficients, and the saturation/inhibition coefficients. Since some of them are very important in order to meet quantitative accordance between model and experiments, the sensitivity of the model towards these parameters is analyzed in the following. This will allow a quantitative comparison with other models that need to adapt different parameters that are, moreover, experimentally not accessible.

Maximal Specific Respiration Rate ($q_{O_2,max}$)

This parameter is the most important one in the present model because it is the only one that controls the breakdown of glucose by different pathways. However, it can be determined experimentally fairly well (8.0 mmol $g^{-1} h^{-1} \pm 3\%$). Variations of the parameters within the indicated range are displayed in Figure 3, which shows the effect of changing $q_{O_2,max}$ on the behavior of the model. Only the dilution rate for critical substrate flux (D_R) is influenced. Experimentally, this influence may be verified only when a chemostat with excellent dilution rate control is available.

Maximal Specific Glucose Uptake Rate ($q_{S,max}$)

Growth of cells is a consequence of substrate uptake and not vice versa. However, the kinetic formulation is equivalent if only one pathway for substrate utilization can be used by the cells. But the difference becomes obvious in the present case where glucose can be metabolized oxidatively and/or reductively. Therefore, the determinative kinetic equation of the present model reads $q_S = f(s)$; and $\mu = f(s)$ is derived therefrom. This concept has also been adopted earlier.[20,26–30] This requires that the value of $q_{S,max}$ instead of μ_{max} is known, but the latter can be determined more accurately. However, the values of $q_{S,max}$ derived from batch and from continuous culture experiments are very similar and in the range of 3.2–3.5 h^{-1}. Since $q_{S,max}$ does only influence the attainable μ_{max} value (see Fig. 4), provided D_R (i.e., the couple $q_{O_2,max}$ and $Y^{oxidative}$) is fixed, the more accurately and directly determinable μ_{max} values can be used to check the correct choice of $q_{S,max}$.

Biomass Yield for Purely Oxidative Glucose Metabolism ($Y^{oxidative}$)

We have chosen the stoichiometric parameter b (which is synonymous to $Y^{oxidative}$) to be empirically determined in order to make the balance equation system defined.

Experimental experience reveals that the biomass yield can be determined most accurately. In high performance reactors at dilution rates $< D_R$, $Y^{oxidative}$ was determined to be 0.48–0.50 g g^{-1} under definitely glucose-limited growth conditions (very low dilution rates are not considered here, i.e., maintenance is not modeled). It is noteworthy to state that $Y^{oxidative}$ influ-

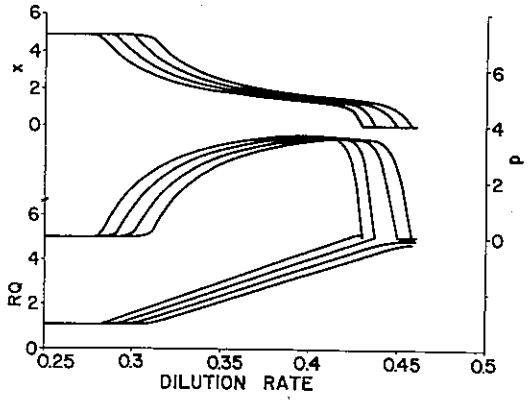

Figure 3. Sensitivity of the model towards maximal specific oxygen uptake rate. All other parameters kept constant as listed in Table I (K_s = 0.1 g L^{-1}). D_R and D_C both increase with $q_{O_2,max}$ = 7.50, 7.75, 8.00, and 8.25 mmol $g^{-1} h^{-1}$. Concentrations in g L^{-1}.

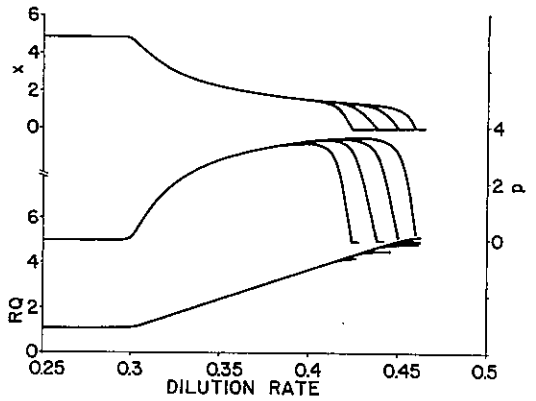

Figure 4. Sensitivity of the model towards maximal substrate uptake rate. All other parameters kept constant as listed in Table I. D_C increases with $q_{S,max}$ = 3.00, 3.25, 3.50, and 3.75 g $g^{-1} h^{-1}$.

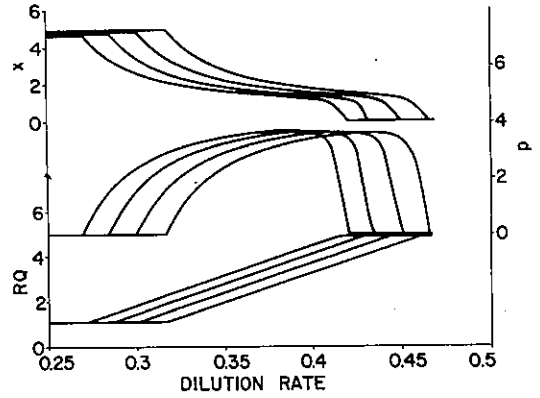

Figure 5. Sensitivity of the model towards biomass yield coefficient for pathway [1]. All other parameters kept constant as listed in Table I. D_R and D_C both increase with $Y^{oxidative}_{biomass/glucose}$ = 0.47, 0.48, 0.49, and 0.50 g g^{-1}.

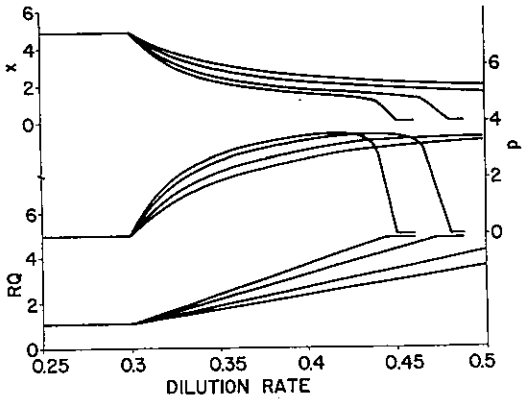

Figure 6. Sensitivity of the model towards biomass yield coefficient for pathway [2]. All other parameters kept constant as listed in Table I. D_C increases with $Y^{reductive}_{biomass/glucose}$ = 0.05, 0.06, 0.08, and 0.10 g g^{-1}.

ences not only the biomass yield, but to a much greater extent the value of D_R (Fig. 5). This latter dependence is significantly more pronounced than the dependence of D_R on $q_{O_2,max}$ based on the experimentally achievable accuracy. However, since both $q_{O_2,max}$ and $Y^{oxidative}$ influence the value of D_R, and this value can also be well determined experimentally in appropriate bioreactors, we can chose a correct couple of entirely constant values for $q_{O_2,max}$ and $Y^{oxidative}$ to mimic D_R exactly; 8.0 mmol g^{-1} h^{-1} and 0.49 g g^{-1} fulfill this requirement and, furthermore, are both equally well within the range of data so far determined for this strain when cultivated under definite carbon limitation.[12]

Biomass Yield for Purely Reductive Growth ($Y^{reductive}$)

Analogous to the parameter couple $q_{O_2,max}$ and $Y^{oxidative}$, which determine D_R, it is not $q_{S,max}$ alone but its combination with $Y^{reductive}$ that determines D_C or μ_{max}, respectively. According to the setup of the model, $Y^{reductive}$ is the yield that must be determined under anaerobic conditions. This value, however, has not been extensively studied. It is reported to be between 0.05 and 0.1 g g^{-1}. These data are presently not of great use since the value of μ_{max} is enormously influenced by the parameter $Y^{reductive}$, as shown in Figure 6. Knowing D_C (or μ_{max}, respectively) from sets of washout and batch experiments to be 0.45 h^{-1}, we can fix a value of 0.05 for $Y^{reductive}$, which, together with a value of 3.5 h^{-1} for $q_{S,max}$, allows the model to calculate μ_{max} = 0.45 h^{-1}. However, if we apply this couple of values to describe strict anaerobic growth, the predicted $\mu^{anaerobic}$ of 0.175 h^{-1} is approximately half of the value found experimentally for this strain (0.34 h^{-1}).[31] This value is obtained when the upper limit of the given range for $Y^{reductive}$ = 0.1 g g^{-1} is applied. There are at least two reasonable ways to explain this uncertainty.

1) It has been shown that the nutrient requirements are distinctly different for strictly anaerobic and for aerobic growth. Certain growth factors[31,32] are no longer essential for growth under aerobic conditions, no matter whether or not oxygen is limited. Since ethanol can be formed also under aerobic conditions, the reductive pathway must operate. There are no data that would prove a dependence of $Y^{reductive}$ on the external availability of those growth factors, but the model suggests such a dependence.

2) It has been shown that ethanol has an inhibitory effect on both growth and ethanol formation if present in higher concentrations.[33,34] One now may argue reasonably that ethanol that is formed inside the cells immediately affects the performance of metabolism negatively. But subtle experiments need to be done to identify and evaluate these effects.

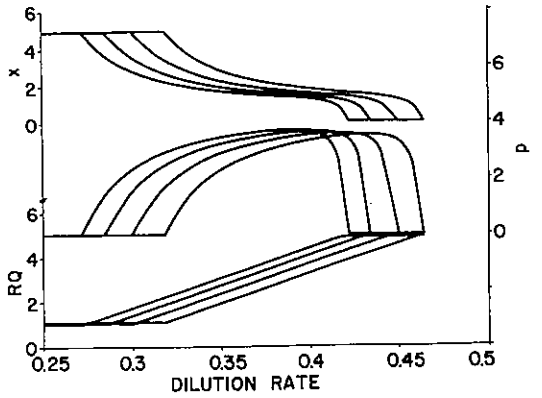

Figure 7. Sensitivity of the model towards elemental composition of biomass. All other parameters kept constant as listed in Table I. D_R and D_C both increase with decreasing oxygen content of biomass (OX) = 0.63, 0.60, 0.57, and 0.54 (mol oxygen) (mol carbon)$^{-1}$.

Composition of Biomass, e.g., Oxygen Content (OX)

So far we have considered the composition of biomass to be independent of the specific growth rate. This is reasonable because analytical data on the elemental composition of cells grown at different dilution rates do indicate this;[12,16] however, the whole range of permissive dilution rates has never been covered, and data from different laboratories vary somewhat (compare References 1, 10, 12, 16, and 23).

The sensitivity of the model on realistic changes of biomass composition is pronounced, as illustrated for changing oxygen content in Figure 7. The main effect of this variation is similar to a variation of $q_{O_2,max}$, but furthermore, the RQ-value is also influenced. However, this is only a minor effect. A substrate dependent alteration of biomass composition is suggested by experimental data rather than a growth rate dependent one. Yet for simplicity this is not implemented in the model in the present formulation.

Steady State Solution for Growth on Ethanol

There is only one possible pathway in which *S. cerevisiae* will grow on ethanol, and this is purely oxidative. It is, therefore, formally the same whether growth or substrate uptake are formulated as a primary function of available substrate concentration; both are correlated linearly by the respective yield coefficient. Using a value of 0.72 g g^{-1} for $Y_{ethanol}$ and 8.0 mmol g^{-1} h^{-1} for $q_{O_2,max}$, the bottleneck of respiration is reached at the specific growth rate of 0.165 h^{-1}, which fairly well coincides with the measured maximal specific growth rate of 0.17 h^{-1}. Using the slightly different value of 0.54 (instead of 0.57) for the ratio of oxygen to carbon in biomass (*OX*), as is suggested by data from Rieger (in preparation), the experimental value is predicted more closely. This coincidence of measured and predicted μ_{max} value for growth on a substrate that can only be metabolized oxidatively supports our hypothesis that the respiratory capacity ("bottleneck") determines the growth rate (together with the yield coefficient).

This consideration holds also true for another substrate, hexadecane, which can formally be treated similar to ethanol: μ_{max} is determined by saturation of the respiratory capacity.[35]

Growth on Mixed Substrates: Glucose and Ethanol

The concept of formulating the higher priority of glucose over ethanol uptake allows the description of mixed substrate experiments (see Fig. 8). The decrease of biomass concentration at $D < D_R$ is not a consequence of the utilization of the reductive pathway for glucose metabolism but results because less ethanol is used for growth. Part of the ethanol is not oxidized as a consequence of the saturation of the respiratory bottleneck; it is left unused in the medium because it cannot be utilized reductively. Therefore, the RQ value increases and reaches the value typical for pure glucose growth when the respiratory bottleneck is exactly saturated by glucose metabolism, i.e., at $D = D_R = 0.3$ h^{-1}. This priority of glucose over ethanol utilization can be followed better in Figure 9.

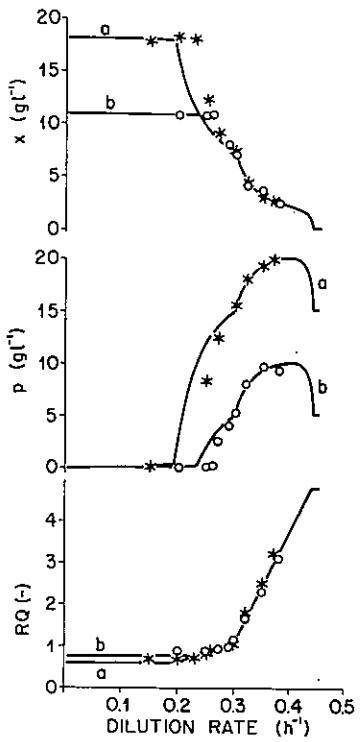

Figure 8. Simulation of the stationary behavior of the model for growth on mixed substrates: 15 g L^{-1} glucose and a, 15 g L^{-1} ethanol (*) and b, 5 g L^{-1} ethanol (○) in the inlet medium. Biomass concentration (x), ethanol concentration (p), and respiratory quotient (RQ) versus dilution rate. Model parameters as in Table I (K_s = 0.5 g L^{-1}). Experimental conditions and data (symbols) as described by Rieger et al.[12]

Qualitatively, model solutions and experiments are identical. However, the quantitative differences in the dependence of the appearance of ethanol in the supernatant on dilution rate must most likely be assigned to the use of inadequate equipment. There was no control unit available to keep dilution rate constant, which resulted in widely scattering concentration determinations (approximately ±10%).

Batch Growth

The situation of batch growth of a glucose-sensitive yeast is not fully encountered by the model since it is not designed to describe the diauxic lag phase in the present form. In Figure 10 the data of the model are

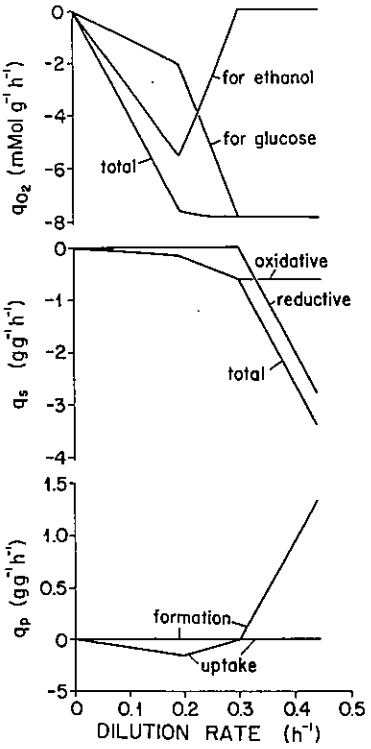

Figure 9. Simulation of the stationary behavior of the model for growth on mixed substrates: 15 g L^{-1} glucose and 15 g L^{-1} ethanol; specific oxygen uptake rate, specific glucose uptake rate, specific ethanol formation rate (positive values), and specific ethanol uptake rate (negative values) versus dilution rate. Partial contributions to total values according to the different pathways as indicated. Model parameters as in Figure 8.

represented discontinuously. The break in the time axis approximately corresponds to the actual duration of the lag phase between the two growth phases. In this way a good coincidence of experimental and model data (solid lines) was achieved.

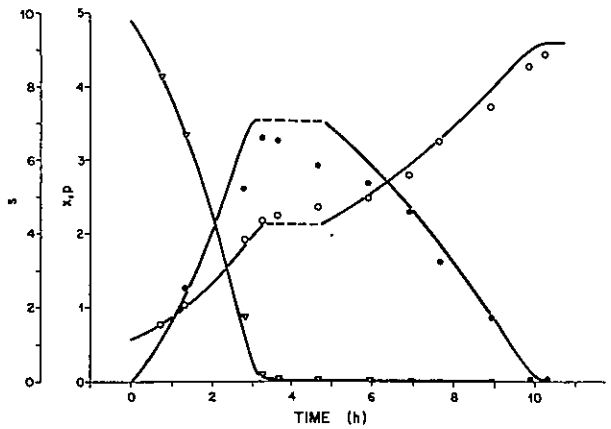

Figure 10. Batch growth on a 1% glucose medium. Symbols represent experimental data: concentrations of biomass (○), glucose (▽), and ethanol (●) scaled in g L^{-1}. Solid lines represent the model prediction if they were contiguous. The dashed lines indicate the diauxic lag phase, which is not formulated in this model.

In batch cultures, lower than maximum oxygen uptake rates and consequently high RQ values (5 to 10) have often been observed. The model predicts values for the RQ between 4 and 5. The observed differences have to be assigned to varying qualities of the inocula. However, when carefully prepared inocula (e.g., directly from a chemostat) were used, RQ values were constant and very close to the predicted ones. It is likely that high ethanol concentrations and oxygen limitation during inocula preparation in shake flasks additionally restrict the respiratory bottleneck (for comparison see also References 10, 17, and 36).

Growth Under Oxygen Limitation

When the specific respiration rate was limited by low concentrations of dissolved oxygen, the excretion of ethanol was observed at lower dilution rates than under conditions of sufficient oxygen supply.[14] We have simulated oxygen limitation by choosing fixed low k_La values. In Figure 11 the model behavior for k_La = 100 h^{-1} is compared with a k_La = 1000 h^{-1} used for all

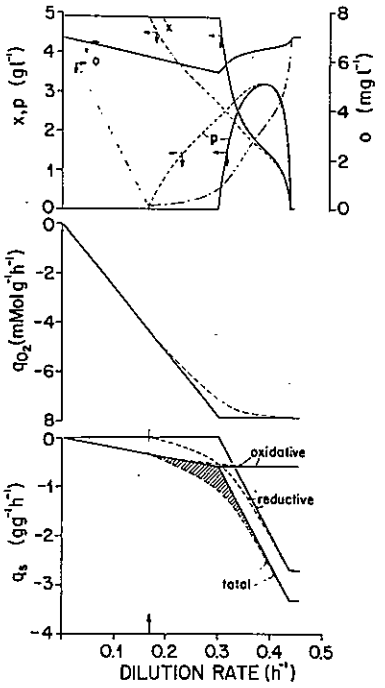

Figure 11. Simulation of yeast growth under oxygen limitation. The same model parameters as in Figure 2 were used, but here k_La = 100 h^{-1} (dotted lines) is compared with k_La = 1000 h^{-1} (as used for all other figures where dissolved oxygen concentration (o) never fell below 5 mg L^{-1}). Specific aeration rate was 2 vvm, and relative gas hold up was 0.2. Concentrations of biomass (x), ethanol (p), and dissolved oxygen, specific oxygen uptake rates (q_{O_2}), and specific substrate uptake rates (q_S) versus dilution rate. The arrow indicates the beginning of oxygen limitation, i.e., the artificial reduction of respiratory capacity by low dissolved oxygen concentration, at low k_La. The shadowed area represents the necessary increase of specific glucose uptake rate under oxygen limited growth conditions. This is generally referred to as the "Pasteur effect."

previous calculations of steady state solutions. The increase of necessary total q_S with decreasing oxygen availability (i.e., decreasing k_La) predicted by the model is generally referred to as the Pasteur effect. However, in the original definition, this effect was described the other way around as related to a lowering of q_S by the presence of oxygen.[37] The same situation may well be encountered when respiration is restricted by other medium limitations.[12,14] Our concept could easily be generalized to such situations by including additional terms that account for a restriction of the respiratory bottleneck by factors other than dissolved oxygen concentration.

Saturation Coefficients

It is not our intention to present absolutely correct values for these parameters. However, the experimental data on substrate concentrations at half-maximal substrate uptake rates under carbon-limited growth conditions were of the same order of magnitude as are the values assumed in the present simulations for K_s and K_e. A further reduction of the values of these parameters has negligible effects in the stationary solutions of the model (invisible in the presented figures). Other authors have suggested significantly higher values for K (for a compilation, see Hoppe and Hansford[33]). But this has a negligible effect on the goal of the present study, the formulation of both glucose and oxygen effect in yeast.

CONCLUSION

The model presented is characterized by important properties that distinguish it from other models.

1) Utilization of pathways [1] and [2] is under the direct control of the degree of saturation of respiration. This is contrary to the concept of Bellgardt and coworkers[28,29,38] who assumed the initial sequences of tricarboxylic acid cycle to be a similar bottleneck exerting this control function. Their concept derives most likely from the fact that the authors relied on data of enzymatic analyses reported by von Meyenburg and coworkers.[6,39] Barford and Hall,[26] however, did verbally state that the control of respiration and fermentation results from the saturation of the respiration capacity without any other effects. Actually they used a different stoichiometric concept with ethanol utilization as the only oxygen-requiring fractional growth reaction. Therefore, the maximum rate of ethanol uptake (k_2 in Ref. 26) became the crucial parameter determining the control of the oxidative fraction of growth. Hence, saturation of respiration is a necessary consequence resulting from their assumed stoichiometry rather than the cause for this type of metabolic control. In the presented concept the role of origin and effect is changed. This becomes obvious from a comparison between their and our x-D-diagrams (Figs. 11 and 12 in Ref. 26 and Figs. 2, 9, and 11 in this paper). When the observable biomass yield begins to decrease with increasing dilution rate, the respiration becomes saturated according to our model (because this is the basic design), but it is far from saturation at this point according to the Barford and Hall model.

2) We have formulated growth on glucose and/or ethanol as formally parallel reactions according to pathways [1]–[3] as did Bellgardt and coworkers.[28,29,38] On the contrary, other authors did formulate sequential reactions: glucose was assumed to be utilized by the cell via a fermentative pathway (i.e., strictly without oxygen) only, thus producing and excreting ethanol. This anaerobic concept can then be extended to aerobic conditions by adding the sequential reaction of growth on reabsorbed ethanol, which is an obligatory aerobic reaction.[21,26,27] Other sets of sequential reactions were proposed by Roels and coworkers,[16,40] but their stoichiometry and energetics were unclear if not even contradictory. Although the fractionation of an overall reaction into sequential partial reactions is a legitimate formal kinetic tool, we regarded it inferior to the concept of parallel reactions because it derives from the attempt to explain the distinct metabolic control of *S. cerevisiae*-type yeasts on the basis of the growth pattern observed phenomenologically in batch culture.

3) In order to predict biomass and/or product formation, the crucial parameters of our model can be kept constant under any aerobic growth conditions, no matter whether or not oxygen is limited or whether or not glucose or ethanol or mixed substrates are concerned. Under strictly anaerobic growth conditions, the change of one single parameter ($Y^{\text{reductive}}$) is required. This may be explained biologically by the need to add supplementary growth factors to the medium for the promotion of anaerobic growth. This comprehensive applicability of our model is the result of the conceptual additive contributions to growth by parallel reactions according to pathways [1]–[3]. These are, in fact, fully accessible to separate experimental analysis. The concept of sequential reactions applied by other authors requires the adaptation of the energetics of the overall (mixed) reaction in order to account for the changing oxidative or fermentative fraction of metabolism with varying specific growth rates. This has usually been realized by letting either Y_{ATP} or the P/O ratio float.[26,27,30,38] The 95% joint confidence regions for these parameters have been determined under different growth conditions and were shown to vary considerably.[16,40] But it was not only the above-mentioned yield coefficients, it concerned also rate parameters ("constants") that had to be adapted when different growth situations were to be predicted on the basis of otherwise proposed concepts.[26,27,38] Finally, the parameters related to the energetics of metabolism (Y_{ATP} and the

P/O ratio) are not accessible to direct experimental analysis, whereas biomass yield coefficients are.

4) The presented concept uses maximum respiration rate to explain mechanistically aerobic ethanol formation, a property typical for any strain of baker's yeast. The concept also accounts for a further "artificial" reduction of respiration by oxygen limitation. A Monod-type dependence of the oxygen uptake rate on dissolved oxygen concentration was chosen also by Péringer et al.[30] Bellgardt and coworkers[28,29] used a Blackman-type dependence. Both perform equally well. Since the actual degree of saturation of the respiratory capacity triggers the additional operation of the reductive pathway [2], the present model does also account for the so-called Pasteur effect. At any given specific growth rate the specific substrate uptake rate is minimized under conditions of excess oxygen supply and maximized with increasing oxygen limitation (i.e., with artificially reduced respiratory capacity). This is, to our knowledge, the first time that both glucose or the Crabtree effect and the Pasteur effect can be explained mechanistically by one single hypothesis.

One should, therefore, avoid the expressions "Pasteur" and "Crabtree" effects. The proposed correct alternatives are "oxidative" and "oxidoreductive." Growth is oxidative under conditions of subcritical substrate flux and pure carbon limitation. Growth is oxidoreductive under aerobic conditions of critical or supracritical glucose flux. Growth is purely reductive under anaerobic conditions only.

Glucose and ethanol can be cometabolized as long as the respiratory capacity is not exceeded (subcritical total substrate flux under given constraints of oxygen availability). Under conditions of supracritical glucose flux, ethanol is produced. In between, glucose has priority over ethanol uptake.

NOMENCLATURE

x	biomass concentration
s	substrate (= glucose) concentration
p	product (= ethanol) concentration
e	ethanol concentration, either as product or substrate
o	dissolved oxygen concentration
μ_z	(fractional) specific growth rate on substrate z
r_z	(absolute) reaction rate of reactant z
Y_{X/S_z}	yield coefficient: biomass on substrate z
q_z	specific uptake rate of substrate z (negative value) or specific production rate of product z (positive value)
a,b,c,d,g,h,i,j,k,l,m,n	(molar) stoichiometric coefficients

References

1. C. L. Cooney, H. Y. Wang, and D. I. C. Wang, *Biotechnol. Bioeng.*, **19**, 55 (1977).
2. H. Y. Wang, C. L. Cooney, and D. I. C. Wang, *Biotechnol. Bioeng.*, **19**, 69 (1977).
3. H. Y. Wang, C. L. Cooney, and D. I. C. Wang, *Biotechnol Bioeng.*, **21**, 975 (1979).
4. D. W. Zabriskie and E. Humphrey, *AIChE J.*, **24**, 138 (1978).
5. M. Lemoigne, J. P. Aubert, and J. Millet, *Ann. Inst. Pasteur*, **87**, 427 (1954).
6. C. Beck and H. K. von Meyenburg, *J. Bacteriol.*, **96**, 479 (1969).
7. E. S. Polakis, W. Bartley, and G. A. Meck, *Biochem. J.*, **97**, 298 (1965).
8. J. A. Barnett and H. L. Kronberg, *J. Gen. Microbiol.*, **23**, 65 (1960).
9. R. H. De Deken, *J. Gen. Microbiol.*, **44**, 149 (1966).
10. J. P. Barford and R. J. Hall, *J. Gen. Microbiol.*, **114**, 267 (1979).
11. M. Rieger, O. Käppeli, and A. Fiechter, in *Current Developments in Yeast Research*, G. G. Stewart and I. Russel, Eds. (Pergamon Press, Toronto, 1981), p. 369.
12. M. Rieger, O. Käppeli, and A. Fiechter, *J. Gen. Microbiol.*, **129**, 653 (1983).
13. M. Petrik, O. Käppeli, and A. Fiechter, *J. Gen. Microbiol.*, **129**, 43 (1983).
14. O. Käppeli, M. Gschwend-Petrik, and A. Fiechter, *J. Gen. Microbiol.*, **131**, 47 (1985).
15. O. Käppeli, M. Arreguin, and M. Rieger, *J. Gen. Microbiol.*, **3**, 411 (1985).
16. J. G. Dekkers, H. E. de Kok, and J. A. Roels, *Biotechnol. Bioeng.*, **23**, 1023 (1981).
17. J. P. Barford, P. M. Jeffrey, and R. J. Hall, *Adv. Biotechnol.*, **1**, 255 (1981).
18. O. Käppeli and A. Fiechter, *Biotechnol. Letters*, **3**, 541 (1981).
19. O. Käppeli and A. Fiechter, *Biotechnol. Bioeng.*, **25**, 2518 (1982).
20. C. Meyer and W. Beyeler, *Biotechnol. Bioeng.*, **26**, 816 (1984).
21. A. H. E. Bijkerk and R. J. Hall, *Biotechnol. Bioeng.*, **19**, 267 (1977).
22. H. E. de Kok and J. A. Roels, *Biotechnol. Bioeng.*, **22**, 1097 (1980).
23. J. P. Barford and R. J. Hall, *Biotechnol. Bioeng.*, **21**, 609 (1979).
24. H. Laurila, O. Käppeli, and A. Fiechter, *Arch. Microbiol.*, **140**, 257 (1984).
25. K. Furukawa, E. Heinzle, and I. J. Dunn, *Biotechnol. Bioeng.*, **25**, 2293 (1983).
26. J. P. Barford and R. J. Hall, *Biotechnol. Bioeng.*, **23**, 1735 (1981).
27. R. J. Hall and J. P. Barford, *Biotechnol. Bioeng.*, **23**, 1763 (1981).
28. K. H. Bellgardt, W. Kuhlmann, and H. D. Meyer, in *Modelling and Control of Biotechnical Processes, Proceedings in the First IFAC Workshop*, A. Halme, Ed. (Pergamon Press, Oxford, 1982), p. 67.
29. G. Auling, K. H. Bellgardt, H. Diekmann, and M. Thoma, *Appl. Microbiol. Biotechnol.*, **19**, 353 (1984).
30. P. Péringer, H. Blachère, and G. Corrieu, *Biotechnol. Bioeng.*, **16**, 431 (1974).
31. H. Schatzmann, Ph.D.-Thesis Number 5504, Swiss Federal Institute of Technology (1975).
32. A. Fiechter, G. F. Fuhrmann, and O. Käppeli, *Adv. Microb. Physiol.*, **22**, 123 (1981).
33. G. K. Hoppe and G. S. Hansford, *Biotechnol. Letters*, **4**, 39 (1982).
34. J. H. T. Luong, *Biotechnol. Bioeng.*, **27**, 280 (1985).
35. F. K. Gmünder, O. Käppeli, and A. Fiechter, *Eur. J. Appl. Microbiol. Biotechnol.*, **12**, 129 (1981).
36. N. B. Pamment and R. J. Hall, *J. Gen. Microbiol.*, **105**, 297 (1978).
37. L. Pasteur, *Bul. Soc. Chim. Paris*, Jun. **28**, 79 (1861).
38. K. H. Bellgardt, H. D. Meyer, W. Kullmann, and K. Schügerl, *Proc. 3rd Europ. Congr. Biotechnol.*, **2**, 607 (1984).
39. H. K. von Meyenburg, *Vierteljahresschrift Naturforsch. Ges. Zürich*, **114**, 113 (1969).
40. T. G. E. Geurts, H. E. de Kok, and J. A. Roels, *Biotechnol. Bioeng.*, **22**, 2031 (1980).