Multi-author Review

Developments in Biotechnology

The Editors would like to thank Dr. J. Reiser for coordinating this multi-author review.

Dedication

J. Reiser and O. Käppeli[a]

Institute for Biotechnology, Swiss Federal Institute of Technology, CH-8093 Zürich (Switzerland), and [a] Bideco AG, CH-5401 Baden (Switzerland)

This issue of EXPERIENTIA on 'Developments in Biotechnology' is dedicated to Professor Armin Fiechter who celebrated his 65th birthday in October. He is one of the most eminent promoters of biotechnology in Switzerland and he was instrumental in establishing the Institute for Biotechnology at ETH in 1982 of which he is still director. By pioneering the combination of fundamental and applied aspects he has greatly influenced the advancement of biotechnology in teaching and research in Switzerland.
Distinguished scientists from various countries were invited to contribute to this special issue of EXPERIENTIA to review aspects of yeast physiology, process optimization and control, analytics, and applications. We are well aware that the articles presented here are only a narrow selection of the current activities in biotechnology but since Professor Fiechter has devoted most of his scientific career to research on yeast physiology, process development and instrumentation it is appropriate to focus this issue on these aspects. We thank all the authors who have contributed to mark this special event.
Professor Fiechter continues to actively stimulate progress in biotechnology with a still lively awareness of future developments. May he continue to persue his objectives and, together with Mrs. Fiechter, enjoy the time to come.

Further evidence for the existence of a bottleneck in the metabolism of *Saccharomyces cerevisiae*

L. C. M. Auberson, C. V. Ramseier, I. W. Marison and U. von Stockar

Institute of Chemical Engineering, Swiss Federal Institute of Technology, CH-1015 Lausanne (Switzerland)

Summary. The growth physiology of *Saccharomyces cerevisiae* strains H1022 and Whi2+ has been studied in aerobic batch and continuous (chemostat) cultures. Results from the measurement of biomass and medium components (off-line) together with oxygen, carbon dioxide and heat measurements (on-line) have been used in an attempt to explore the existence of 'overflow' or 'bottleneck' metabolism as opposed to catabolite repression (Crabtree effect) in these strains. Chemostat experiments indicated that specific oxygen uptake rate (q_{O_2}) was linearly related to the dilution rate (D) at values below the critical dilution rate (D_{crit}), becoming constant above D_{crit}, which is in agreement with the bottleneck theory. However, batch culture experiments indicated negligible oxygen consumption during the initial glucose growth phase, the culture exhibiting purely anaerobic metabolism. The bottleneck theory would propose that q_{O_2} has a constant (maximum) value under these conditions. The results presented here suggest that while the bottleneck theory can be adequately used to describe chemostat growth of *S. cerevisiae*, some other control mechanism must be operating under conditions of high glucose concentrations, such as those initially prevailing in the batch culture experiments.
Key words. Bottleneck theory; calorimetry; chemostat; Crabtree effect; glucose effect; *S. cerevisiae*; yeast metabolism.

The Crabtree or glucose effect in *Saccharomyces cerevisiae* has important repercussions on the metabolism and the physiology of this yeast. *S. cerevisiae* growing aerobically on glucose produces ethanol, which is itself the substrate during a second growth phase. Diauxic growth is the result of the Crabtree effect in batch cul-

tures. In aerobic continuous cultures of *S. cerevisiae*, glucose is degraded oxidatively to biomass, CO_2 and H_2O at low dilution rates, and this remains the case up to the critical dilution rate, after which ethanol is detectable in the culture broth. This is the onset of the Crabtree effect in continuous cultures. Glucose or some product of its metabolism represses respiratory chain and certain tricarboxylic acid cycle enzymes, while hindering the proper development of mitochondria [8]. The mechanism of the Crabtree effect in yeast is complex and not well understood.

Barford and Hall [1] demonstrated that *S. cerevisiae* cultivated both in a glucose- and in a galactose-limited chemostat displayed a saturated respiratory capacity beyond the maximum oxidative dilution rate (critical dilution rate); that is, the specific oxygen uptake rate remained constant. Their results were 'unusual' in the sense that '... any specific glucose effect should result in a lower respiratory rate for an organism grown on glucose compared with galactose' [1]. No correlation between the saturated respiratory capacity and the aerobic ethanol formation was proposed by these authors. Rieger et al. [9] also found the same saturated behavior for their strain of *S. cerevisiae*. This observation, in addition to their results from ethanol pulse experiments with derepressed cells, led them to conclude the existence of an internal bottleneck at the enzymatic level. They further limited the bottleneck to the oxidative branch of metabolism, thus identifying the limited respiratory capacity as an inherent feature of *S. cerevisiae* [10]. Aerobic formation of ethanol would then be the consequence of overflow. This approach simplified the complexity of modelling the Crabtree effect in continuous cultures.

Continuous cultures continued to be the ideal technique for detecting the presence of a respiratory bottleneck. Other workers performed parallel analyses of cytochrome content [2, 7], biologically and physically restricted the size of the bottleneck by introducing limitations in nutrients and oxygen [4, 11], utilized co-substrates [10], and performed pulse experiments [7, 9]. The common goal of these investigations was to validate the bottleneck hypothesis for aerobic ethanol formation in *S. cerevisiae*. A predictive model for continuous cultures was formulated based on this hypothesis [11].

Whether or not the bottleneck hypothesis is applicable to all *S. cerevisiae* strains remains to be seen. It does not offer an interpretation for batch diauxic growth of *S. cerevisiae*, for example. We have cultivated *S. cerevisiae* H1022 in a glucose-limited chemostat in order to detect the presence of a respiratory bottleneck. Transient experiments with dilution rate shifts were then performed in order to assess the dynamics of the bottleneck in the oxidative region and in the region near the critical dilution rate. In addition, the heat produced by batch cultures of *S. cerevisiae* growing on glucose was monitored on-line by calorimetry, as the heat output is a direct assessment of the type of metabolism [12].

Materials and methods

Organisms

Saccharomyces cerevisiae H1022 used in these experiments was kindly supplied by the Microbiology Department of the Swiss Federal Institute of Technology, Zurich. *S. cerevisiae* Whi2+ (X4003-5B) was obtained from the Department of Genetics, University of Sheffield S1O 2TN, UK. The strains were maintained at 4 °C on glucose agar plates.

Culture medium

The synthetic medium used for *S. cerevisiae* H1022 had the following composition per liter of double-distilled water: 10.0 g glucose, 2.0 g $(NH_4)_2SO_4$, 0.65 g $(NH_4)_2HPO_4$, 0.30 g KCl, 0.15 g $MgSO_4$ 7 H_2O, 0.10 g $CaCl_2$ 2 H_2O, 0.2 ml antifoam (polypropylene glycol P2000, Fluka, Buchs, CH), 0.78 mg $CuSO_4$ 5 H_2O, 4.80 mg $FeCl_3$ 6 H_2O, 3 mg $ZnSO_4$ 7 H_2O, 3.50 mg $MnSO_4$ 2 H_2O, 10.0 µg biotin, 20 mg m-inositol, 10 mg Ca-pantothenate, 2 mg thiamin-HCl, 0.5 mg pyridoxin-HCl.

S. cerevisiae Whi2+ was grown on a YPG medium containing per liter of double-distilled water: 10 g Difco yeast extract, 20 g Difco Bacto peptone, 20 g glucose, 0.5 ml antifoam (polypropylene glycol P2000, Fluka, Buchs, CH).

All media were sterilized at 121 °C for 20 min. The vitamins and trace elements were sterilized by filtration and added to the cooled medium. Glucose for the YPG medium was sterilized separately to avoid caramelization and added to the cooled peptone-yeast extract mixture.

Experimental methods

S. cerevisiae H1022 was cultivated in a glucose limited chemostat. The fermenter (Bioengineering AG, Wald, CH) working volume was 2.5 l with a stirring speed of 1500 rpm at 30 °C. The pH was maintained at 5.0 by the addition of 4 M NaOH and 4 M H_2SO_4.

S. cerevisiae Whi2+ was cultivated in a modified Bench Scale Calorimeter (Ciba-Geigy AG, Basel, CH) described extensively elsewhere [5, 6]. The working volume was 1.35 l at 30 °C. pH regulation was as described for *S. cerevisiae* H1022.

Analytical methods

Steady state determination. The steady state for the continuous cultures was verified by cell counts and measurements of optical density at 600 nm at regular intervals for 2–3 h (Lambda 1, Perkin-Elmer, Norwalk, USA).

Biomass. Dry weight was determined by drying washed centrifuged samples at 100 °C to constant weight. The supernatant was removed and chilled for subsequent ethanol and substrate analyses.

Glucose. Residual glucose concentrations were measured using an enzyme-based glucose analyzer (Yellow Springs Instruments, Glucosimat, model 27, Ohio, USA) and by

enzymatic colorimetric assay of the appropriately diluted supernatant (Boehringer-Mannheim GmbH, GOD-PAP, No. 608 459, FRG).

Ethanol. Ethanol concentrations in the supernatant were determined enzymatically (Boehringer-Mannheim GmbH, test No. 176 290, FRG) and by gas–liquid chromatography (Shimadzu Mini-1, Kyoto, Japan).

Gas analyses. Oxygen uptake rates were measured with a paramagnetic analyzer (Servomex No. 540 A, Crowborough, GB) and carbon dioxide evolution rates were measured with an infrared analyzer (Leybold-Heraeus, Binos 1, Hanau, FRG).

Theory

The following treatment of overflow metabolism is based on the general ideas set forth by Sonnleitner and Käppeli[11]. In order to obviate the need for analyzing their model on a computer, a set of simple predictive equations will be developed below.

It is assumed that the metabolism of a yeast culture growing in continuous culture is purely aerobic (respirative) at low dilution rates. The specific growth can be related to the oxygen uptake rate as follows:

$$\mu_{ae} = D = (Y_{X/O})_{ae} \cdot q_{O_2} \tag{1}$$

$(Y_{X/O})_{ae}$ represents the biomass yield on oxygen ($g\,mole^{-1}$) during true respiratory growth. Due to the existence of a bottleneck in the aerobic metabolism, q_{O_2} cannot increase above $q_{O_2 max}$. Hence:

$$\mu_{ae} \leq (Y_{X/O})_{ae} \cdot q_{O_2 max} = D_{crit} \tag{2}$$

If the dilution rate is increased above D_{crit}, the culture will respond by maintaining the aerobic growth rate at the largest value permitted by the bottleneck as indicated by eq. (2), but it will increase the growth rate further by catabolizing the additional substrate through the anaerobic (fermentative) pathway:

$$\mu = D = (Y_{X/O})_{ae} \cdot q_{O_2 max} + \mu_{an} \tag{3}$$
$$D = D_{crit} + \mu_{an}$$

Since anaerobic growth is associated with a lower biomass yield, X will decrease but ethanol will appear in the culture broth.

The specific substrate uptake is calculated by allowing for the appropriate biomass yield for aerobic and anaerobic growth separately:

$$q_S = \frac{D_{crit}}{(Y_{X/S})_{ae}} + \frac{\mu_{an}}{(Y_{X/S})_{an}} \tag{4}$$

where $(Y_{X/S})_{ae}$ and $(Y_{X/S})_{an}$ stand for the truly aerobic and the truly anaerobic (lower) biomass yields, respectively.

Table 1. Predictive equations for the continuous culture of S. *cerevisiae*

$D < D_{crit}$	$D \geq D_{crit}$
$q_{O_2} = \frac{D}{(Y_{X/O})_{ae}}$	$q_{O_2} = \frac{D}{(Y_{X/O})_{ae}} = q_{O_{2max}}$
$q_S = \frac{D}{(Y_{X/S})_{ae}}$	$q_S = \frac{D_{crit}}{(Y_{X/S})_{ae}} + \frac{1}{(Y_{X/S})_{an}}(D - D_{crit})$
$q_P = O$	$q_P = \frac{1}{(Y_{X/P})_{an}}(D - D_{crit})$
$q_{CO_2} = \frac{D}{(Y_{X/C})_{ae}}$	$q_{CO_2} = \frac{D_{crit}}{(Y_{X/C})_{ae}} + \frac{1}{(Y_{X/C})_{an}}(D - D_{crit})$
$S \simeq O$	$S = \frac{q_S K_S}{q_{Smax} - q_S}$
$X = (Y_{X/S})_{ae} \cdot S_O$	$X = \frac{S_O - S}{\frac{D_{crit}}{D \cdot (Y_{X/S})_{ae}} + \left(1 - \frac{D_{crit}}{D}\right)\frac{1}{(Y_{X/S})_{an}}}$
$P = O$	$P = \frac{\frac{1}{(Y_{X/P})_{an}}(D - D_{crit})(S_O - S)}{\frac{D_{crit}}{(Y_{X/S})_{ae}} + \frac{1}{(Y_{X/S})_{an}}(D - D_{crit})}$

By substituting μ_{an} as given by eq. (3), one obtains, for $D > D_{crit}$:

$$q_S = \frac{D_{crit}}{(Y_{X/S})_{ae}} + \frac{1}{(Y_{X/S})_{an}} \quad (D - D_{crit}) \tag{5}$$

By re-writing eq. (4) for other q's and by dividing each term with appropriate yields applicable for either true aerobic or anaerobic growth, all specific conversion rates can be predicted as a function of $D > D_{crit}$. Below D_{crit}, only the aerobic term applies. The resulting equations are listed in table 1.

It has to be stressed that all stoichiometric coefficients and yields appearing in table 1 may be computed from only two yields: the intrinsic aerobic and anaerobic biomass yields $(Y_{X/S})_{ae}$ and $(Y_{X/S})_{an}$, which can be determined experimentally. The computations are carried out by solving the elemental balances. Convenient explicit equations for such computations are available[12].

The residual substrate concentration is predicted by solving the expression for the substrate uptake rate for S:

$$S = \frac{q_S K_S}{q_{Smax} - q_S} \tag{6}$$

X and P are predicted based on appropriate stoichiometric equations such as the following

$$X = \frac{D}{q_S}(S_O - S) \tag{7}$$

If eqs. (5) and (6) are substituted, the equations given in table 1 follows. The values of the parameters used in the model are given in table 2.

Table 2. Parameter values used in the predictive model

D_{crit} (h^{-1})	q_{O_2max} ($mM\,g^{-1}\,h^{-1}$)	q_{Smax} ($mM\,g^{-1}\,h^{-1}$)	$(X_{X/S})_{ae}$ ($g\,g^{-1}$)	$(Y^*_{X/S})_{an}$ ($g\,g^{-1}$)	K^*_S ($g\,l^{-1}$)	ash (%)
0.27	3.24	1.33	0.65	0.10	0.10	7

* Sonnleitner, B., and Käppeli, O.[11]

Results and discussion

Continuous cultures

S. cerevisiae H1022 was cultivated in a glucose-limited chemostat with a non-limiting oxygen supply (fig. 1). The dilution rate was varied from 0.11 h^{-1} to 0.49 h^{-1} with steps of 0.05 h^{-1}. This step was reduced to 0.01 h^{-1} near the critical dilution rate, that is, the dilution rate above which ethanol is detectable in the culture broth. According to the bottleneck theory, q_{O_2}, the specific oxygen uptake rate, increases linearly up to the critical dilution rate, D_{crit}, beyond which q_{O_2} remains constant. This hypothesis is in contrast to enzymatic repression whereby the specific oxygen uptake rate decreases above the critical dilution rate. In our experiments, q_{O_2} appeared constant beyond D_{crit} = 0.27 h^{-1} (fig. 2). The fact that the last point decreases is not significant, in our opinion, due to the low amount of biomass at this dilution rate.

The constructed model based on the saturated respiratory capacity predicts well both the specific uptake and production of gases as well as the residual substrate, biomass and ethanol concentrations (fig. 1).

Further proof of the applicability of the bottleneck theory can be seen in the transient responses of the culture after dilution rate shifts in the oxidative region, from 0.23 h^{-1} to 0.26 h^{-1} (fig. 3) and 'across' D_{crit}, from 0.26 h^{-1} to 0.31 h^{-1} (fig. 4), that is, from purely aerobic growth into mixed aerobic–anaerobic growth. In both cases, the oxygen uptake rate stabilized within 1–2 h to new steady-state values of 6.2 $mM\,g^{-1}\,h^{-1}$ for the first shift and 6.3 $mM\,g^{-1}\,h^{-1}$ for the second shift through D_{crit}. The q_{CO_2} also reached its new steady-state value rapidly for the shift in the oxidative region, but was still

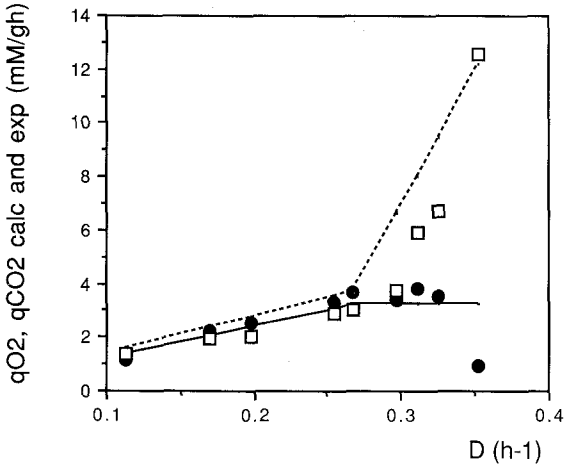

Figure 2. Specific oxygen uptake and specific carbon dioxide production rates as a function of the dilution rate. Experimental values: (●) q_{O_2}, (□) q_{CO_2}. Predicted values: (——) q_{O_2}, (- - -) q_{CO_2}.

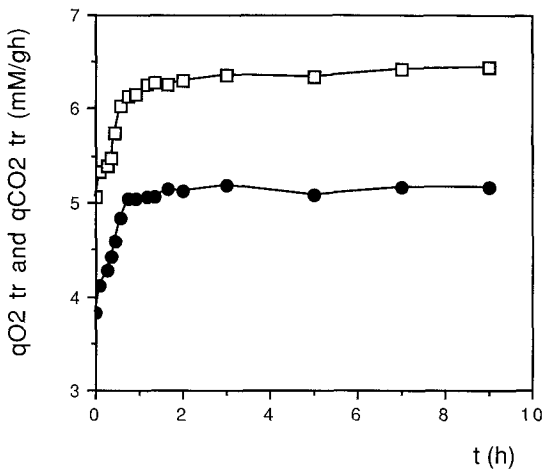

Figure 3. Transient specific oxygen uptake and specific carbon dioxide production rates as a function of time for the dilution rate shift from 0.23 to 0.26 h^{-1}. (□) q_{O_2}, (●) q_{CO_2}.

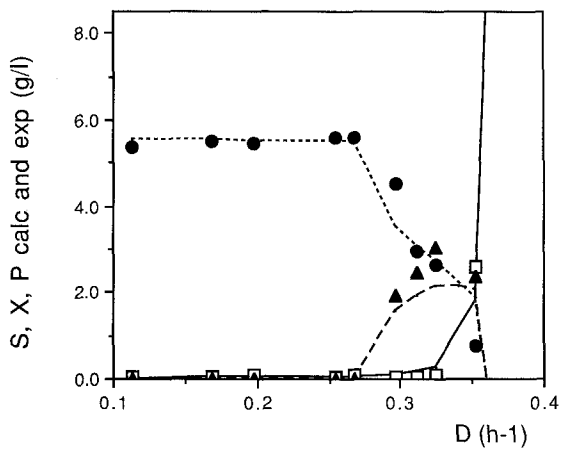

Figure 1. Substrate, biomass and product curves as a function of the dilution rate. Experimental values: (●) biomass, (▲) ethanol, (□) glucose. Predicted values: (- - -) biomass, (– –) ethanol, (——) glucose.

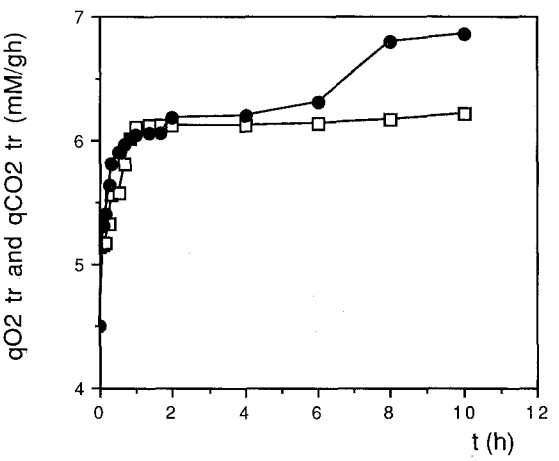

Figure 4. Transient specific oxygen uptake and specific carbon dioxide production rates as a function of time for the dilution rate shift from 0.26 to 0.31 h^{-1}. (□) q_{O_2}, (●) q_{CO_2}.

increasing after 8 h when the culture was forced beyond the critical dilution rate.

The immediate response of the culture and the rapidity with which the new steady state was attained are indicative of an overflow mechanism resulting from a bottleneck. Furthermore, the resulting $q_{O_2 max}$ was the same for both shifts.

Batch culture with S. cerevisiae Whi2 +

Due to difficulties in initiation of batch growth of *S. cerevisiae* H1022, a Whi2 + strain of *S. cerevisiae* was used for batch culture studies. The oxygen consumption is very low for the glucose growth phase in batch cultures of *S. cerevisiae*. We therefore chose to utilize a heat-flux calorimeter (BSC-81), since the extent of purely aerobic metabolism can also be measured by calorimetry [12].

S. cerevisiae Whi2 + grew diauxically on glucose as is typical for Crabtree-positive yeasts (fig. 5). The specific growth rate on glucose was 0.31 h^{-1} and 0.06 h^{-1} for growth on ethanol. A plot of the integrated heat signal as a function of the biomass concentration shows two distinct groupings of the data corresponding to the glucose and ethanol growth phases, respectively (fig. 6). The heat yields (the amount of heat released to the environment per g dry weight of cells formed) were calculated by regression analysis. The value of the yield for mixed aerobic–anaerobic growth on glucose was 4.02 kJ g^{-1} and was 22.18 kJ g^{-1} for oxidative growth on ethanol. The disparity in these two figures for the same growth experiment is indicative of the presence of two types of metabolism. The low heat yield for growth on glucose is very similar to that reported by von Stockar and Birou [12] as typical of fermentative growth. The large amount of heat produced when *S. cerevisiae* Whi2 + grew on ethanol is higher than that reported by von Stockar and Birou [12] (11–13 kJ g^{-1}) for fully aerobic growth processes. Nevertheless, the significance of the difference between the two yields is clear.

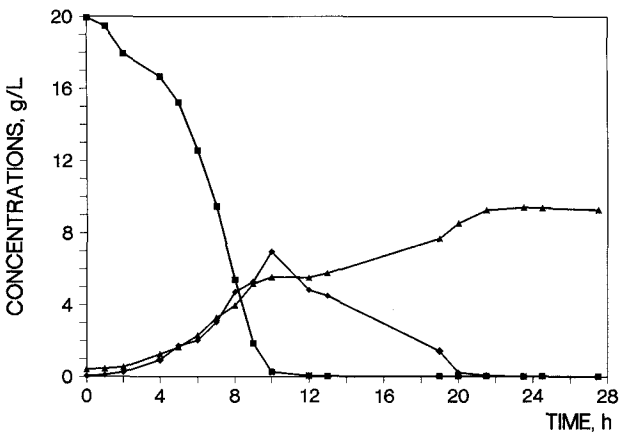

Figure 5. Diauxic growth of *S. cerevisiae* Whi2 + on YPG medium. Ethanol (◆), biomass (▲) and glucose (■) concentrations as a function of time.

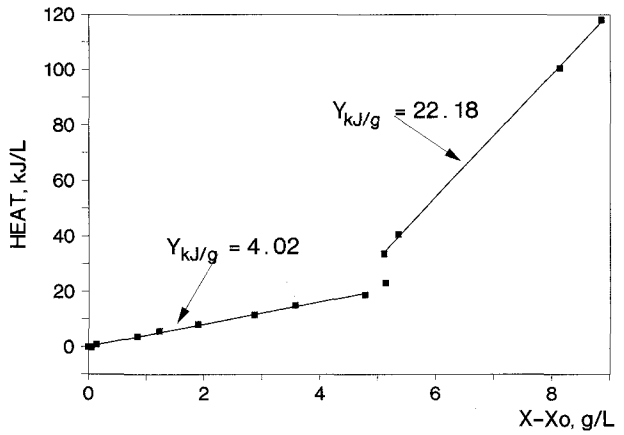

Figure 6. Calculated heat yields for the diauxic growth *S. cerevisiae* Whi2 + on YPG medium.

Conclusions

Aerobic ethanol formation by *S. cerevisiae* is the consequence of an inherently limited respiratory capacity. This hypothesis is a simplification of the Crabtree effect and allows the straightforward construction of a predictive model based on overflow metabolism. We have shown that *S. cerevisiae* H1022 displays a saturated respiratory capacity at and beyond the critical dilution rate of 0.27 h^{-1}. Furthermore, the results obtained are successfully predicted by our model. The rapidity of the culture's response and adaptation in the dilution rate shift experiments, as well as the similar values of $q_{O_2 max}$ observed at the new steady states, were further evidence for the bottleneck. However, calorimetric data for batch diauxic growth of *S. cerevisiae* Whi2 + showed distinctly that the heat output during the glucose growth phase was indicative of fermentative rather than fully aerobic metabolism. If the bottleneck is really an inherent feature of *S. cerevisiae*, then the question why the bottleneck was not observed during aerobic batch growth remains unanswered at the moment. Barford and Hall [1] have reported a saturated respiratory capacity in their strain of *S. cerevisiae* growing diauxically on glucose. They have stated that proper adaptation of the strain to aerobic conditions is mandatory if the oxygen consumption rate is to remain constant during the two phases of batch growth. This appears to be less important for continuous cultures.

Nomenclature

D	dilution rate, h^{-1}
K_S	saturation constant, $g l^{-1}$
P	product concentration, $g l^{-1}$
X	biomass concentration, $g l^{-1}$
X_O	initial biomass concentration, $g l^{-1}$
S	residual glucose concentration, $g l^{-1}$
S_O	initial glucose concentration, $g l^{-1}$
q_{O_2}	specific oxygen uptake rate, mM $g^{-1} h^{-1}$
$q_{O_2 max}$	maximum specific oxygen uptake rate, mM $g^{-1} h^{-1}$
q_{CO_2}	specific carbon dioxide production rate, mM $g^{-1} h^{-1}$
q_P	specific ethanol production rate, $g g^{-1} h^{-1}$
q_S	specific glucose uptake rate, $g g^{-1} h^{-1}$
q_{Smax}	maximum specific glucose uptake rate, $g g^{-1} h^{-1}$
$Y_{i/j}$	yield of i related to j, $g g^{-1}$ or g $mole^{-1}$
ae	aerobic growth

an anaerobic growth
calc calculated
crit critical
exp experimental
tr transient

1 Barford, J. P., and Hall, R. J., A mathematical model for the aerobic growth of *Saccharomyces cerevisiae* with a saturated respiratory capacity. Biotechnol. Bioeng. *23* (1981) 1735–1762.
2 Käppeli, O., Petrik, M., and Fiechter, A., Transient responses of *Saccharomyces cerevisiae* to a change of the growth-limiting nutrient in continuous culture. J. gen. Microbiol. *131* (1985) 47–52.
3 Käppeli, O., Arreguin, M., and Rieger, M., The respirative breakdown of glucose by *Saccharomyces cerevisiae*: an assessment of a physiological state. J. gen. Microbiol. *131* (1985) 1411–1416.
4 Käppeli, O., Regulation of carbon metabolism in *Saccharomyces cerevisiae* and related yeasts. Adv. Microbiol. Physiol. *28* (1986) 181–209.
5 Marison, I. W., and von Stockar, U., A novel bench-scale calorimeter for biological process development work. Thermochim. Acta *85* (1985) 493–496.
6 Marison, I. W., and von Stockar, U., The application of a novel heat flux calorimeter for studying growth of *Escherichia coli* W in aerobic batch culture. Biotechnol. Bioeng. *28* (1986) 1780–1793.
7 Petrik, M., Käppeli, O., and Fiechter, A., An expanded concept for the glucose effect in the yeast *Saccharomyces uvarum*: Involvement of short- and long-term regulation. J. gen. Microbiol. *129* (1983) 43–49.
8 Polakis, E. S., Bartley, W., and Meek, G. A., Changes in the activities of respiratory enzymes during the aerobic growth of yeast on different carbon sources. Biochem. J. *97* (1965) 298.
9 Rieger, M., Käppeli, O., and Fiechter, A., The respiratory capacity of *Saccharomyces cerevisiae*, in: Current Development in Yeast Research. Eds G. G. Stewart and I. Russel. Pergamon Press, Toronto 1981.
10 Rieger, M., Käppeli, O., and Fiechter, A., The role of limited respiration in the incomplete oxidation of glucose by *Saccharomyces cerevisiae*. J. gen. Microbiol. *129* (1983) 653–661.
11 Sonnleitner, B., and Käppeli, O., Growth of *Saccharomyces cerevisiae* is controlled by its limited respiratory capacity: Formulation and verification of a hypothesis. Biotechnol. Bioeng. *28* (1986) 927–937.
12 von Stockar, U., and Birou, B., The heat generated by yeast cultures with a mixed metabolism in the transition between respiration and fermentation. Biotechnol. Bioeng. *34* (1989) 86–101.

0014-4754/89/11-12/1013-06$1.50 + 0.20/0

Kinetic analysis and simulation of glucose transport in plasma membrane vesicles of glucose-repressed and derepressed *Saccharomyces cerevisiae* cells

G. F. Fuhrmann, B. Völker, S. Sander and M. Potthast

Department of Pharmacology and Toxicology, Philipps-Universität Marburg, Lahnberge, D-3550 Marburg (Federal Republic of Germany)

Summary. In this study experimental data on the kinetic parameters investigated by other authors[1–5,11] together with own data on plasma membrane vesicles, have been subjected to a computer simulation based on the equations describing facilitated diffusion. The simulation led to an ideal fit describing the above data. From this it can be concluded that glucose is transported by facilitated diffusion, and not by active transport as was postulated by Van Steveninck[14,15].

The simulation method also demonstrates that the fast sampling technique used by these authors[1–5,11] underestimates the fluxes. Thus, the parameters given do not contribute to the understanding of glucose transport under different metabolic conditions.

The K value of plasma membrane vesicles prepared from glucose-repressed cells is around 7 mM. Derepression, particularly by galactose, causes a highly significant increase in affinity as shown by a decrease in the K value to 2 mM. The highest affinity was measured in a triple kinaseless mutant grown on glycerol with a K value of 1 mM. If seems, therefore, that the kinetic parameters derived from initial uptake rates of glucose in intact cells[1–5,11] using single flux analysis, such as Eadie-Hofstee- or Lineweaver-Burk-plots, are in error.

Key words. Saccharomyces cerevisiae; growth conditions; kinaseless mutant; plasma membrane vesicles; glucose transport; kinetics and computer simulation.

Introduction

Kinetic analysis of glucose transport in *Saccharomyces cerevisiae* cells grown under different metabolic condition has so far been reported for intact cells only[1–5,11]. From these studies it has been postulated that glucose can be transported both by a transporter with a high affinity and a low apparent K_m (1–3 mM) and one of low affinity and high apparent K_m (20–100 mM). The low affinity process occurs, according to these investigators, by carrier-mediated facilitated diffusion. Examples for this process are transport of glucose under nonmetabolizing conditions (i.e., in iodo-acetate-inhibited cells) or in triple kinaseless mutants. For the high affinity process, the involvement of the internal enzymes hexokinase (PI and PII) and glucokinase has been discussed; alternatively, a vectorial phosphorylation process such as an active transport analogous to the phosphotransferase system has been proposed[14,15]. Kinase-positive cells containing one or more of the hexokinase isoenzymes (PI and PII) and glucokinase exhibited biphasic transport kinetics, with a high affinity (1–3 mM) and a low affinity (20–