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Chapter 5

Control of Specific Growth Rate in *Saccharomyces cerevisiae*

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Abstract

In this contribution we resolve the long standing dispute whether or not the Monod constant (K_S), describing the overall affinity of an organism for its growth limiting substrate, can be related to the affinity of the transporter for that substrate (K_M). We show that the two constants can be directly related to one another via the control of the transporter on the specific growth rate; they are identical if the transport step has full control. The analysis leads to the counter-intuitive result that it is to be expected that the affinity of an organism for its substrate is higher than the affinity of the enzyme that facilitates its transport. Experimentally we show this indeed to be the case for the yeast *Saccharomyces cerevisiae*, for which we determined in glucose limited chemostat cultures a K_M value for glucose more than two times higher than the K_S value. Moreover, we could calculate that at glucose concentrations of 0.03 and 0.29 mM the transport step controls the specific growth rate for respectively 78% and 49%.

Introduction

In the classic publication (Monod, 1949) in which Monod proposed his famous equation for the specific growth rate dependency on substrate concentration (eq. 1) he suggested, realizing the similarity with the Michaelis-Menten equation, that the K_S value "should be expected to bear some more or less distant relation to the apparent dissociation constant of the enzyme involved in the first step of the breakdown of a given compound".

$$\mu = \frac{\mu_{max} \cdot S}{S + K_S} \qquad\qquad (1)$$

Monod appears to be very careful in this statement and rightly so for the mechanistic interpretation of an empirical constant that is dependent on the systemic behavior of thousands of reactions is not simple and lies at the core of the relatively new research field of Systems Biology.

Whereas the Michaelis-Menten equation was derived on the basis of an enzyme kinetic mechanism and its parameters have a mechanistic interpretation, the Monod equation is purely empirical. Thus the Monod constant describes the overall affinity for an organism for its growth limiting substrate, while the Michaelis-Menten constant can be directly expressed as a ratio of the elementary rate constants acting on the enzyme-substrate complex. In a description for growth under substrate limited conditions it would seem logical to assume the substrate transporter to play an important role as this is likely to be the enzyme with which the cell senses the substrate. As such, one could expect the affinity of the cell for the growth limiting substrate (K_S) to be related to the affinity of the substrate transporter (K_M).

In addition to the Monod equation, several other functional dependencies of the specific growth rate for a single limiting substrate have been proposed. For a historical review of different models for microbial growth kinetics see Jannasch (1993) (Jannasch and Egli, 1993), for a comparison of different growth models see Button (1993) (Button, 1993). Many of these models are very similar to the Monod model, but might contain additional parameters, such as the Moser (Moser, 1958) and Contois (Contois, 1959) models, while others are fundamentally different, such as the Blackman (Blackman, 1905), Teissier (Teissier, 1936), also known as the exponential model and the logarithmic model (Westerhoff, et al., 1982). While some attempts have been made to give a mechanistic interpretation to the Monod constant, these were never really successful (Liu, 2007).

Systems Biology attempts to understand systemic behavior on the basis of the characteristics of the systems components. From such a perspective it would be extremely interesting to test whether it is possible to relate a systemic property such as the K_S of an organism to the local property of an enzyme (K_M). We have addressed this question using a theoretical and an experimental approach. In a metabolic control analysis of the problem we could relate the two constants via the growth control of the substrate transporter. In glucose-limited chemostat cultures of the yeast *Saccharomyces*

cerevisiae we experimentally determined K_S and K_M and made the intriguing observation that the overall affinity of the organism is more than a factor 2 higher than the affinity of the transporter. In addition, from the two constants we could calculate the control of the specific growth rate by the glucose transporter.

Results

Theory

The chemostat is an ideal instrument for studying the relationship between specific growth rate and the concentration of a growth-limiting substrate. In a chemostat experiment the medium is composed such that one of the substrates will be growth-rate limiting. This makes it possible to set the specific growth rate via the influx rate of the medium. At steady state the specific growth rate (μ) equals the dilution rate (D), defined as the medium flow rate/culture volume. As such it is possible to construct a plot of μ against the growth limiting substrate concentration (*S*) by varying the dilution rate and measuring the steady state residual substrate concentration. The Monod equation (eq. 1) can be fitted to such a data set, to estimate the maximal specific growth rate (μ_{max}) and Monod constant K_S.

To understand how a change in medium influx rate ultimately leads to a change in the specific growth rate, it is useful to first analyze a minimal chemostat system, consisting of four variables, *S*, S_{in}, *X* and *B* (Fig. 1).
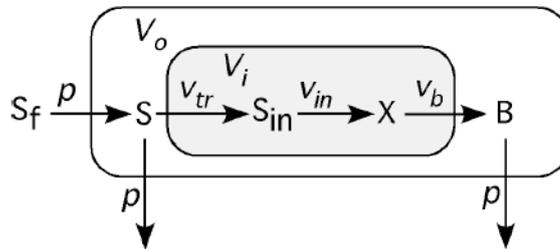

Figure 1. Scheme for the simple chemostat set-up as used in the theory and model sections. The chemostat consists of two external variables (in extracellular volume V_o): the growth limiting substrate concentration (S) and the biomass concentration (B). The biological system consists of two variables (in intracellular volume V_i), S_{in}, the internal substrate concentration and B, a precursor for biomass formation. The microorganism consists of three enzyme catalyzed reactions: v_{tr}, the substrate transporter, v_{in} an internal reaction producing X, and v_b, a biosynthetic reaction forming biomass from X. The chemostat instrument consists of pump reactions (p) leading to the influx of S and efflux of effluent from the culture (containing S and B)

For our analysis we use the metabolic control analysis framework, originally developed in Kacser and Burns (Kacser and Burns, 1973) and Heinrich and Rapoport (Heinrich and Rapoport, 1974), which has been expanded extensively since then ((Fell, 1996; Fell, 1992; Heinrich, 1996)), and has also been applied to chemostat cultures (Small, 1994;

Snoep, et al., 1994). Any effect of the pump rate on the specific growth rate must have been mediated via either of the two external variables, S or B:

$$G_p^\mu \quad = \quad G_p^S \cdot R_S^\mu + G_p^B \cdot R_B^\mu \tag{2}$$

$$= \quad G_p^S \cdot \epsilon_S^{v_{tr}} \cdot C_{v_{tr}}^\mu + G_p^B (\epsilon_B^{v_{tr}} \cdot C_{v_{tr}}^\mu + \epsilon_B^{v_b} \cdot C_{v_b}^\mu) \tag{3}$$

where G_p^μ is a global control coefficient, defined as $\dfrac{d\mu}{dp} \cdot \dfrac{p}{\mu}$, and describes the percentage change in specific growth rate μ upon a 1 percentage change in pump rate p. Since μ is proportional to the dilution rate (and thus also to p), $G_p^\mu = 1$. The change in steady state concentrations of S and B by p is quantified by G_p^S and G_p^B respectively and their effect on the local response of the microorganism is given by R_S^μ and R_B^μ respectively. These local responses are defined at the steady state concentration of S and B and can be interpreted as the change in specific growth rate of the isolated microorganism upon a change in S or B (e.g $R_S^\mu = \dfrac{d\mu}{dS} \cdot \dfrac{S}{\mu}\big|_{S*,B*}$) with * denoting the steady state concentrations of the respective variables). Assuming that S is only sensed by its transporter tr, the effect of a change in S is mediated via its effect on the transporter activity v_{tr}, quantified by its elasticity coefficient ($\varepsilon_S^{v_{tr}} = \dfrac{\partial v_{tr}}{\partial S} \cdot \dfrac{S}{v_{tr}}$). The resulting effect of the change in transport activity on steady state μ is given by the control coefficient of the transporter on the specific growth rate ($C_{v_{tr}}^\mu = \dfrac{d\mu}{dv_{tr}} \cdot \dfrac{v_{tr}}{\mu}\big|_{S*,B*}$). In a similar way eq. 3 describes the effects of p on biomass B, where both reactions v_{tr} and v_b are sensitive to changes in the biomass concentration.

The steady state biomass concentration is equal to the biomass yield on substrate Y_S^B times the substrate that is consumed by the organisms: $B = Y_S^B \cdot (S_f - S)$, with S_f the substrate concentration in the feed. Assuming that Y_S^B is relatively constant and that the growth limiting substrate concentration will generally be low and insignificant compared to S_f (at low specific growth rates), we can derive:

$$G_p^B \quad = \quad \frac{dB}{dp} \cdot \frac{p}{B} = \frac{d(Y_S^B(S_f - S))}{dp} \cdot \frac{p}{Y_S^B(S_f - S)} \tag{4}$$

$$= \quad \frac{-dS}{dp} \cdot \frac{p}{S_f - S} \tag{5}$$

$$= \quad G_p^S \cdot \frac{S}{S_f - S} \approx 0 \tag{6}$$

Thus, at low specific growth rates, changing the pump rate does not effect the biomass concentration. Then, eq. 3 reduces significantly, and substituting $G_p^\mu = 1$ leads to:

$$G_p^S = \frac{1}{\epsilon_S^{v_{tr}} \cdot C_{v_{tr}}^\mu} \equiv \frac{1}{R_S^\mu} \tag{7}$$

Eq. 7 relates the change in substrate concentration after changing the pump rate to the local elasticity and control coefficients of the substrate transporter. This equation is general, i.e. not restricted to a specific growth rate function or transport rate equation and holds if the residual substrate concentration is much lower than the medium substrate concentration, i.e. at low dilution rates.
From the Monod equation the response coefficient of μ with respect to S can be derived:

$$R_S^\mu = \frac{d\mu}{dS} \cdot \frac{S}{\mu} = \frac{K_S}{S + K_S} \tag{8}$$

If we substitute a specific rate equation for the substrate transporter we can derive a relationship between the Monod constant K_S and the Michaelis Menten constant K_M. For instance in the case of Michaelis Menten kinetics for the transporter:

$$v_{tr} = \frac{V_{MAX} \cdot S}{K_M + S} \tag{9}$$

its elasticity equals:

$$\epsilon_S^{v_{tr}} = \frac{\partial v_{tr}}{\partial S} \cdot \frac{S}{v_{tr}} = \frac{K_M}{K_M + S} \tag{10}$$

Substituting eq. 10 and eq. 8 into eq. 7 and rearranging:

$$C_{v_{tr}}^{\mu} = \frac{1 + \frac{S}{K_M}}{1 + \frac{S}{K_S}} \qquad (11)$$

When Michaelis Menten kinetics (eq. 9) are used to describe the transport step for the growth limiting substrate, this step will have full control on growth rate, i.e. $C_{v_{tr}}^{\mu} = 1$, and from eq. 11 it follows that in that case K_M equals K_S.

The Michaelis Menten equation is well known and widely used in studies on isolated enzymes but its applicability for the analysis of systems of linked reactions is very restricted, as it assumes the absence of product. However, in a system of linked reactions, product concentrations can never be zero, as they function as substrates for the next reaction. Thus, the effect of product on the enzyme activity must be taken into account. This effect can be of a kinetic (competition with the substrate for binding to the active site) or a thermodynamic type (with increasing product concentration the Gibbs free energy of a reaction becomes less negative).

If we first consider the kinetic effect of product on the enzyme activity, eq. 9 can be extended to:

$$v_{tr} = \frac{V_{MAX} \cdot \frac{S}{K_{MS}}}{1 + \frac{S}{K_{MS}} + \frac{P}{K_{MP}}} \qquad (12)$$

and its elasticity equals:

$$\epsilon_S^{v_{tr}} = \frac{K_{MS}(1 + \frac{P}{K_{MP}})}{K_{MS}(1 + \frac{P}{K_{MP}}) + S} \qquad (13)$$

Thus, if one would assay the sensitivity of the transporter for its substrate, while keeping the internal product concentration constant at the steady state concentration, eq. 13 is equivalent to eq. 10 with $K'_{MS} = K_{MS}(1 + \dfrac{P}{K_{MP}})$, and eq. 11 holds, with K'_{MS} replacing K_M.

An example of such a system could be the PTS glucose transport system in *E. coli* which is an active transport system, effectively irreversible but still product sensitive.

For a reversible reaction, e.g. assuming a symmetrical transporter for the facilitated transport of glucose in yeast, the following rate equation can be used:

$$v_{tr} = \frac{\frac{V_{MAX}}{K_M}(S - S_{in})}{1 + \frac{S}{K_M} + \frac{S_{in}}{K_M}} \tag{14}$$

and its elasticity:

$$\epsilon_S^{v_{tr}} = \frac{S}{S - S_{in}} \cdot \frac{K_M + 2 \cdot S_{in}}{K_M + S + S_{in}} \tag{15}$$

When eq. 15 (instead of eq. 10) is substituted in eq. 7, it is not possible to express K_S in terms of K_M without knowing the internal glucose concentration. If we consider the two extreme cases: $S_{in} = 0$ and $S_{in} = S$, it can be shown that in the latter case there is no relation between K_S and K_M, and the elasticity of the transporter approaches infinity, while in the first case equation 15 reduces to eq. 10 (as does eq. 13).

Importantly, when using product sensitive rate equations such as eq. 12 and 14, the transporter will not automatically have a control on growth rate of 1. More specifically, unless the transporter is completely limiting growth, its flux control coefficient will be smaller than 1. In the cases where the transporter is far away from equilibrium (as is to be expected for the uptake of the growth limiting component), it can then be derived that K_S will be smaller than K_M. This is a rather counter-intuitive result; the affinity of an organism for its growth limiting substrate is greater than the affinity of the enzyme with which the organism senses (and transports) the substrate.

Core models

To illustrate the theoretical findings core models simulating different transport kinetics were constructed. Each of the core models has the same network structure (as shown in Fig. 1), but uses a different kinetic type for the transporter. Parameter values for the different models vary, and were chosen such that the steady state behavior of the models follows Monod kinetics. The core models have four variables, S, S_{in}, X and B, described by the following ordinary differential equations:

$$\frac{dS}{dt} = D \cdot (S_f - S) - v_{tr} \tag{16}$$

$$\frac{dS_{in}}{dt} = v_{tr} - v_{in} \tag{17}$$

$$\frac{dX}{dt} = v_{in} - v_b \tag{18}$$

$$\frac{dB}{dt} = v_b - D \cdot B \tag{19}$$

The first core model uses irreversible but product sensitive kinetics for the transporter with v_{tr} as in eq. 12, (v_m = 9 mM/h, K_{MS} = K_{MP} = 0.1 mM), v_{in}, converting internal substrate to intermediate X, following reversible kinetics as in eq. 14, (v_m = 10 mM/h, K_{MS} = K_{MP} = 1 mM, K_{eq} = 100) and v_b modeled with eq. 9, (v_m = 0.52 mM/h, K_{MS} = 2 mM). Note that the internal reactions, i.e. v_{tr}, v_{in} and v_b are multiplied by B, as their rates are proportional to the biomass in the culture. In addition the internal volume was for reasons of simplicity modeled as constant (V_i/V_o = 0.0001). The medium substrate concentration S_f was chosen as 20 mM. The dilution rate (D) was scanned over a range from 0.01 to 0.45 1/h and the steady state variable values were calculated. The Monod equation gave a good description of the steady state relation between D and S, and a μ_{max} of 0.50 h^{-1} and a K_S of 0.0054 mM were determined. At a D of 0.1 h^{-1} a steady state concentration for S of 0.0013 mM was obtained and B equalled 19.998 mM, with an internal concentration of S_{in} equal to 0.0197 mM. The elasticity of the transporter with respect to S, $\varepsilon_S^{v_{tr}}$, calculated from eq. 13 was 0.989. The control of the pump on S, G_p^S, equals 1.23. From the $\varepsilon_S^{v_{tr}}$ and G_p^S using eq. 7 the control of the transporter on the specific growth rate, $C_{v_{tr}}^\mu$ can be calculated to be 0.82. The same value for $C_{v_{tr}}^\mu$ was also obtained directly when the "bacterium" in the core model was analyzed at the steady state values of S and B. Importantly, this value is close to the value estimated from the model data using eq. 11, i.e. $(1 + 0.00134/(0.1 + 0.0197))/(1 + 0.00134/0.0054)=0.81$, which indicates that in the case where the transporter kinetics can be described with eq. 12, $C_{v_{tr}}^\mu$ can be estimated from K_M, K_S and S. Note also that in the model the K_S value is much smaller than the K_M value, i.e. the organism has a higher affinity for its substrate than the transporter of the growth limiting substrate.

For the core model simulating a reversible transporter, the same parameter values were chosen, except for the transport kinetics, for which a K_{eq} = 1, and a K_M = 0.9 mM for both external and internal substrate was chosen (i.e. non-active, symmetrical transporter, eq. 14). The dilution rate was scanned from 0.01 to 0.45 h^{-1} and the Monod equation was fitted on the steady state solutions, which yielded a good description with a K_S value of 0.126 mM and a μ_{max} value of 0.51 h^{-1}. At a dilution rate of 0.1 h^{-1} an external substrate concentration of 0.030 mM and an internal concentration of 0.0197 mM was obtained, with $\varepsilon_S^{v_{tr}}$ = 2.8364, and G_p^S = 1.265, yielding (using eq. 7) a $C_{v_{tr}}^\mu$ of 0.28. The same value was obtained when the external variables (S and B) were clamped at their steady state values, and $C_{v_{tr}}^\mu$ was determined directly. The control of the transporter on the specific growth rate was also calculated from the K_M, K_S, S and S_{in} values using eqs. (15, 8 and 7), resulting in a value of 0.28, indicating that indeed a good estimation for this control can be obtained from the steady state chemostat data.

These core models only serve to illustrate the theoretical analysis section with numerical examples, the chosen parameter values are not realistic.

Experimental results

The Monod equation describes how the specific growth rate of an organism is dependent on the concentration of a single substrate. We have used chemostat cultures to achieve a single substrate limitation while having control over the specific growth rate via setting

of the dilution rate. At steady state, μ equals D and by determining the steady state residual substrate concentration at various dilution rates, the relationship between the limiting substrate concentration and the specific growth rate can be studied.

Measurement of the growth limiting substrate concentration is usually difficult because the concentrations are low and likely to change during sampling unless a method is used that rapidly stops metabolism. For these reasons we have used glucose-limited chemostat cultures of the yeast *Saccharomyces cerevisiae*, which is known to have a low affinity for this substrate and we operated the chemostat at low culture densities and stopped metabolism by rapid sampling in acid.

S. cerevisiae VIN13 was grown at a range of dilution rates between 0.05 and 0.48 h^{-1} and the specific growth rate as a function of the residual glucose concentration is plotted in Figure 2. Fitting the Monod equation on the data set yields a K_S estimate of 0.12 mM (Asymptotic SE 0.009) and a μ_{max} value of 0.50 h^{-1} (Asymptotic SE 0.011).

In the chemostat the steady state glycolytic flux (q_{glc}) can be calculated from the medium glucose concentration (Glc_f), the residual glucose concentration (Glc), the dilution rate (D) and the biomass concentration, using: ($q_{glc} = \dfrac{Glc_f - Glc}{biomass} \cdot D$). Steady state uptake rates of glucose in the chemostat were determined over the dilution range 0.05 to 0.48 h^{-1}, Figure 3. At low dilution rates, a linear relation was observed between q_{glc} and D, indicating that the $Y_{Glc}^{biomass}$ is constant.
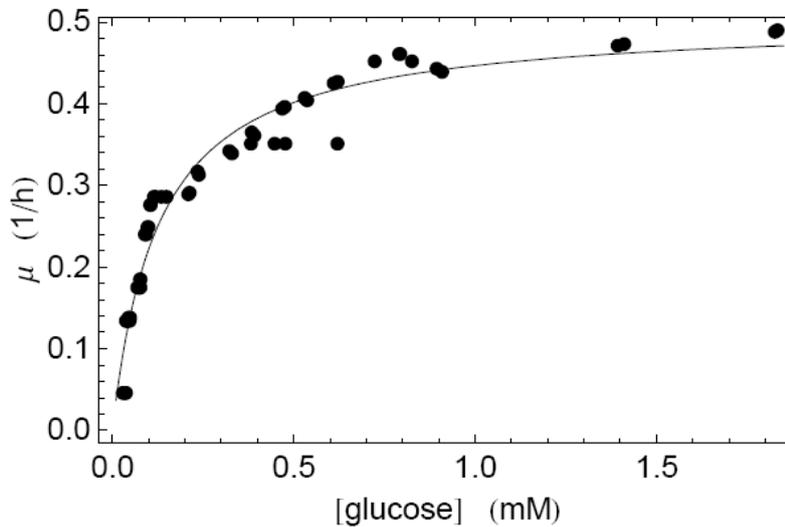

Figure 2. Specific growth rate as a function of the residual substrate concentration. *S. cerevisiae* was grown over a range of dilution rates and steady state residual glucose concentrations were determined. The Monod curve is fitted through the data points

In addition, up to a dilution rate of 0.15 h^{-1} the residual glucose concentration is < 5% of the medium glucose concentration, and no significant change in biomass concentration was observed between dilution rates 0.05 and 0.15 h^{-1} (in agreement with the assumptions on the basis of which eq. 6 was derived).

At D values higher than 0.4 h^{-1} a dramatic increase in the slope of q_{glc} against D is observed (Figure 3), indicating a decrease in $Y_{Glc}^{biomass}$. This phenomenon is well known as the Crabtree effect, and shows the shift from a purely oxidative metabolism, to a fermentative metabolism with ethanol produced.

To be used as a growth substrate, glucose first needs to be taken up, for which yeast is known to have several transporters. To investigate the relationship between the affinity of the glucose transport step (K_M) and the overall affinity of the cell for glucose (K_S) we harvested yeast cells at two dilution rates and characterized the glucose transport step.

In Figure 4 the glucose uptake kinetics for yeast grown at a dilution rate of 0.1 h^{-1} are shown in an Eadie-Hofstee plot, where the data were fitted, assuming zero trans influx kinetics, to a two component transport system, consisting of a high affinity (K_M, 0.75 (mM), v_{max}, 506 (nmol/mg prot/min)), and a low affinity transporter (K_M, 201 (mM), v_{max}, 787 (nmol/mg prot/min)). At a dilution rate of 0.35 h^{-1} similar results were obtained (K_{M1}, 0.72 (mM), v_{max1}, 632 (nmol/mg prot/min), K_{M2}, 93 (mM), v_{max2}, 569 (nmol/mg prot/min)).
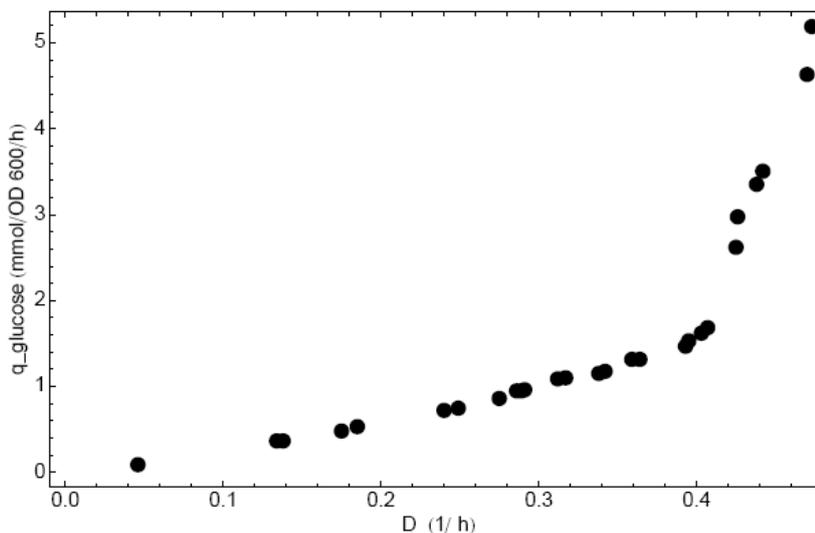

Figure 3. Steady state glycolytic flux as a function of dilution rate. *S. cerevisiae* was grown in glucose limited chemostat cultures over a range of dilution ranges and the steady state uptake rate of glucose was determined. The uptake rate is expressed per unit OD 600 nm; due to the low densities at which the cells were grown it was not possible to get an accurate dry weight measurement.

The steady state uptake rate of glucose in the chemostat equals 40 and 282 nmol Glc/min/(mg protein) at D = 0.1 and 0.35 h^{-1} respectively, (assuming 50% of the dry weight is protein). With the very low residual glucose concentration in the chemostat (0.03 and 0.29 mM at dilution rates of 0.1 and 0.35 h^{-1} respectively) the measured uptake kinetics cannot explain the measured steady state glucose uptake rates, even if the internal glucose concentration were zero. For the chemostat grown cells the internal glucose cannot be zero, otherwise there would be no glycolytic flux. An estimate for the internal glucose concentration can be made on the basis of the steady state flux and the kinetics of the glucokinase. Using the kinetic information on the glucokinase, and the steady state metabolite concentrations from the kinetic model as published by Teusink et al. (Teusink, et al., 2000) together with the expression level as determined in Daran-Lapujade et al. (Daran-Lapujade, et al., 2007) for glucose limited chemostat culture at D = 0.1 h^{-1}, an internal glucose concentration of 0.003 mM was calculated, which is close to the 1µM concentration suggested in Postma et al. (Postma, et al., 1989), (for D = 0.35 h^{-1} an internal glucose concentration of 0.025 mM was calculated, see Materials and Methods for details).
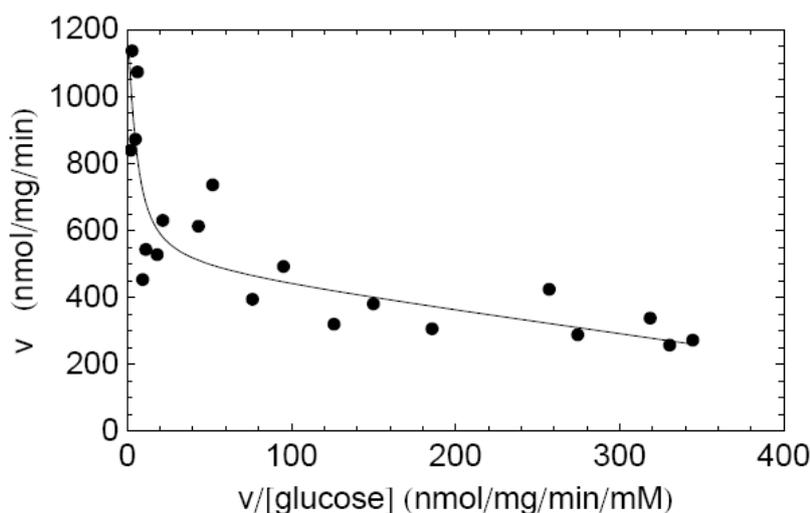

Figure 4. Eadie-Hofstee plot for glucose transport kinetics. Transport rates were determined using ^{14}C-labeled glucose in zero-trans influx assays for *S. cerevisiae*, grown in glucose limited chemostat cultures at a dilution rate of 0.1 h^{-1}. The line shows the best fit to a two component transport system.

 If this non-zero glucose concentration is taken into account for the predicted glucose uptake rate on the basis of the measured in vitro kinetics then an even greater difference between measured and predicted glucose uptake rate is observed.

The most likely explanation for this discrepancy is that the internal glucose concentration in the in vitro uptake experiments is not negligible and leads to an overestimation of the K_M for glucose. An objective function that sums the squared differences between the experimental data and the model prediction was constructed (allowing for a non-zero

internal glucose concentration in the glucose assay and adding as additional constraint the glucose uptake rate in the chemostat, see Material and Methods for details). Minimizing the objective function resulted in the following parameter values for the cells grown at D = 0.1 h^{-1}: for the high affinity transporter, K_M = 0.30 mM, and v_{max} = 494 (nmol/mg prot/min) and for the low affinity system, K_M = 187 mM and v_{max} = 786 (nmol/mg prot/min), with an internal glucose concentration in the in vitro assay of 0.15 mM. At a dilution rate of 0.35 h^{-1} an identical internal glucose concentration was obtained with the following kinetic parameters: K_{M1} = 0.27 mM, K_{M2} = 87 mM, v_{max1} = 619 (nmol/mg prot/min) and v_{max2} = 578 (nmol/mg prot/min).

Applying eqs. 8 and 15, a response coefficient of yeast for glucose of 0.8 and an elasticity of the transporter for glucose of 1.02 can be calculated at a dilution rate of 0.1 h^{-1}; at D = 0.35 h^{-1} response and elasticity coefficients of respectively 0.29 and 0.60 were obtained. From these, using eq. 7 a control of the transporter on the specific growth rate of 0.78 and 0.49 can be calculated at D = 0.1 and 0.35 h^{-1} respectively. This means that at the low external glucose concentration of 0.03 mM, the glucose transporter holds 78 % of the growth control (note that this is the control on growth rate for a bacterium that is isolated from the chemostat with the external metabolites clamped at the steady state values). Similarly a control of 49 % could be calculated at an external glucose concentration of 0.29 mM.

Discussion

Growth and reproduction are essential functions of living organisms. In growing cells, thousands of reactions are coordinated to build all cellular components necessary for the production of daughter cells. Despite its importance in many diseases (e.g. cancer), control and (dys-) regulation of growth rate is ill-understood, i.e. it is not known to what extend the different reactions in the metabolic network are limiting the specific growth rate (μ).

The growth rate dependency on external parameters is much better studied, especially for uni-cellular organisms limited in growth by the availability of a single substrate (S), where typically a hyperbolic relationship is observed between μ and S. We focused on the description of that hyperbolic relation using the Monod equation (eq. 1), which fitted our data well. Intriguingly the Monod equation, describing the coordinated response of thousands of reactions, is identical to the Michaelis-Menten equation, describing the activity of an isolated enzyme, and although Monod has stated that K_S is not equal to K_M he did suggest that there may be some relationship between the two parameters (Monod, 1949). In the chemostat the pump rate controls the specific growth rate via its effect on the growth limiting substrate concentration, G_p^S. This control can be related to the elasticity for glucose and the control on growth rate of the substrate transporter via eq. 7. This equation is general, but when a specific growth model, such as the Monod model, and a kinetic type for the transporter are inserted, it is possible to relate the K_M of the transporter to the K_S of the organism. An important constraint for the relationship is that it is dependent on whether the elasticity of the transporter can be expressed as a function of its K_M. For irreversible enzymes this is always possible (although the value may also be dependent on the internal substrate concentration), but for reversible enzymes close to equilibrium the importance of the K_M value for the elasticity coefficient becomes small.

Then it will in general not be possible to relate the K_M of the transporter to the K_S of the organism. In addition under such conditions the control of the transporter on the specific growth rate will be very small.

An important finding of our study is that from the relation between K_S and K_M it is possible to calculate the control of the transporter on the specific growth rate, if the residual substrate concentration is known (in case of reversible kinetics also the internal concentration needs to be known if it is not negligible). Thus for *S. cerevisiae* it was estimated that the transporter has 78% of the growth control, at a clamped glucose concentration of 0.03 mM. The experimental approach we followed is similar to the one in Postma et al. (Postma, et al., 1989), where a K_S of 0.11 to 0.39 mM was determined (although the data could not be described very well by Monod kinetics), with a high affinity K_M of 1 mM. In addition, a residual glucose concentration at $D = 0.1$ h^{-1} of 0.11 mM was measured and an internal glucose concentration of 0.001 mM was calculated (Postma, et al., 1989). Using our theoretical analysis, it can be calculated from these data that the glucose transporter controls the specific growth rate for 55 to 85 % (dependent on the K_S value).

The analysis we have given is general and can be applied to experimental data of other organisms as well. Good experimental data is available for another model organism, *Escherichia coli*, for which a K_S value of 0.41 µM was obtained with a residual glucose concentration of 0.20 µM at a dilution rate of 0.3 h^{-1} (Senn, et al., 1994). From these data a response coefficient of glucose for the specific growth rate of 0.67 can be calculated using eq. 8. A K_M value for glucose of the PTS of 20 µM has been reported (Stock, et al., 1982). In a first assumption to estimate the elasticity of the PTS for glucose a random order rapid equilibrium type of kinetics was used for the overall reaction catalyzed by the PTS. The elasticity for such a rate equation is equal to the one in eq. 12. From the elementary rate constants given in Rohwer et al. (Rohwer, et al., 2000) it can be calculated that the effect of the internal glucose-6-phosphate concentration is negligible due to the low affinity of the enzyme for this product. Thus an elasticity of the PTS for glucose of 0.99 can be calculated at an external glucose concentration of 0.2 µM. The elasticity of the PTS system for glucose can also be estimated directly from the detailed kinetic model (Rohwer, et al., 2000), as 0.97. These data indicate that in *E. coli* the glucose transporter controls the specific growth rate for 67 % (or 69 % if the elasticity of 0.97 is used) at the low glucose concentrations as obtained in the glucose limited chemostat cultures at $D = 0.3$ h^{-1}.

In this contribution we have shown that the Monod constant, although in itself purely empirical, can be related to the Michaelis Menten constant of the transporter for the growth limiting substrate. Key to the analysis is to use the Monod equation to express the sensitivity of the specific growth rate in terms of the Monod constant. Subsequently this sensitivity was related to the elasticity of the transporter for the substrate and the control of the transporter on the specific growth rate. Thus, K_S and K_M values can be related to each other via the control of the transporter on the specific growth rate. This leads to the interesting result that this control, which is often difficult to measure directly, can be estimated from the Monod and Michaelis Menten constants, as we have shown for *S. cerevisiae* and *E. coli*.

From a systems biology perspective these results have a wider implication then just finding the relation between a phenomenological and a mechanistic constant. For a field

that has as one of its aims to understand systemic behavior on the basis of the characteristics of its components (Snoep, 2005), it has far stretching implications that it is possible to express a systemic property, describing the overall sensitivity of an organism for its growth limiting substrate, as a function of the characteristics of the transporter for the substrate. Crucial in the analysis was to link the importance of the component, as expressed by its growth control, to its sensitivity for the growth limiting substrate. Under the specific conditions as prevailing in the glucose-limited chemostat cultures, the glucose transport step holds more than 50% of the growth control, which is quite amazing when considering that growth is a concerted action of several thousands of enzymes. The interplay between a good theoretical framework such as MCA, modeling and experiment, as used in this study, is illustrative for the type of approaches that we think will be essential for addressing systems biology problems.

Materials and Methods

Strain and cultivation

Saccharomyces cerevisiae strain VIN13, kindly provided by the Institute of Wine Biotechnology, Stellenbosch University, was used in all the experiments. Cells were grown over a range of dilution rates in glucose-limited chemostat cultures (Bioflo 110 fermentors, New Brunswick Scientific Co., Inc, New Jersey). The working volume was approximately 650 ml and pH was controlled at 5.5 ± 0.1 by automatic addition of 1M NaOH. The culture was sparged with air at a flow rate of 20 L/h and the culture was stirred at 250 r.p.m. The temperature was controlled at 30°C. The dissolved oxygen (O_2) was monitored with a DO_2 electrode Model InPro6110/160 (New Brunswick Scientific Co., Inc, New Jersey) and kept above 60% saturation. Medium composition was as described in Verduyn et al. (Verduyn, et al., 1992) and glucose concentration in the feed was either 1 or 2 mM, ensuring glucose limiting conditions and a low biomass concentration. No effect of medium glucose concentration on steady state residual glucose concentration was observed.

For determination of the residual glucose concentration, samples were extracted in an equal volume of cold (4°C) PCA (10%) utilizing under-pressure, to improve sampling rate. Samples were neutralized using 2M K_2CO_3 and left on ice for 15 minutes. To remove precipitated salts and proteins, the samples were centrifuged at 20800 g at 4°C for 10 minutes. Residual glucose concentrations were determined using the method described in Senn et al. (Senn, et al., 1994)

Cells to be used for glucose uptake experiments were grown at higher biomass concentrations in Applikon fermentors with a working volume of 1 L, using the same mineral medium but with a glucose concentration in the medium of 42 mM. The culture was stirred at 750 r.p.m. and sparged with air at a flow rate of 60 L/h, culture pH was maintained at pH 5.0 ± 0.1.

For the zero trans-influx experiments, samples were rapidly taken and washed twice in mineral medium lacking a carbon source, by centrifugation at 3000 g at 4°C (SS-34 rotor, Sorvall) for 10 minutes, and stored on ice for further use in the zero-trans influx experiments. Dry weight was determined by taking 2x 10 ml samples in preweighed tubes, centrifuged at 3000 g at 4°C (SS-34 rotor, Sorvall) for 10 minutes, washed twice with dH_2O and dried overnight at 100°C. Tubes were then weighed again and the

difference was used to calculate the dry-weight. Samples taken for metabolite analysis were immediately treated with 35% cold (4°C) perchloric acid, and stored at -20°C. Upon analysis, samples were neutralized with 7M KOH and filtered through a 0.45-µm filter. Metabolite concentrations were measured by HPLC (LKB, Bromma, Sweden) using a Rezex organic acid analysis column with an 8-mm particle size, 8% cross-linking and a hydrogen ionic form (Phenomenex, Torrance, CA) at a temperature of 45°C and with 7.2mM H_2SO_4 as an eluent. Detection was performed with an RI1530 refractive index detector (Jasco, Tokyo, Japan). Peak integration and data processing were performed with BORWIN CHROMATOGRAPHY software (Le Fontanil, France).

Transport assay

Zero trans-influx rates of sugars were determined in a 5 second assay according to Walsh et al. (Walsh, et al., 1994) at 30°C with the modification that growth medium was used instead of phosphate buffer. Cold glucose concentrations were verified by using a glucose oxidase assay, using glucose oxidase (Roche Diagnostics GmbH, Mannheim, Germany), peroxidase and ABTS in 0.5M tris-HCl, mixed with standard or sample and incubated at 37°C for 1 hour under slow agitation after which the absorbance at 415 nm was measured using a SPECTRAmax PLUS[384] Microplate Spectrophotometer (Molecular Devices Corporation, Sunnyvale, California).

Calculations

All calculations and model simulations were performed with Mathematica version 6 (Inc, 2007). For the calculation of the internal glucose concentration in the steady state chemostat cultures the following equation was used for the glucokinase reaction

$$v_{GLK} = \frac{\frac{V_M}{K_M ATP \cdot K_M GLC_i}(GLC_i \cdot ATP - \frac{G_6P \cdot ADP}{K_{eq}})}{(1 + \frac{G_6P}{K_M G_6P} + \frac{GLC_i}{K_M GLC_i}) \cdot (1 + \frac{ADP}{K_M ADP} + \frac{ATP}{K_M ATP})} \qquad (20)$$

(Teusink, et al., 2000): with:K_{eq} = 3800, $K_M GLC_i$ = 0.08 mM, $K_M G6P$ = 30 mM, $K_M ATP$ = 0.15 mM, $K_M ADP$ = 0.23 mM, $G6P$ = 2.5 mM, ATP = 2.5 mM, ADP = 1.3 mM as the parameter values and steady state metabolite concentrations taken from Teusink et al. (Teusink, et al., 2000). For the V_M of the glucokinase a value of 1779 nmol/mg prot/min was used, as determined by Daran-Lapujade et al. (Daran-Lapujade, et al., 2007) for aerobic glucose limited grown *S. cerevisiae* at a dilution rate of 0.1 h^{-1}, which is in good agreement with the 2 U/mg protein as reported in Postma et al. (Postma, et al., 1989). Using this equation the internal glucose concentration was solved at a glycolytic flux of 40 and 282 nmol/mg prot/min, as was measured in this study at D =0.1 and 0.35 h^{-1} respectively.

For fitting of the kinetic parameters of the glucose transport kinetics the following objective function was minimized:

$$\sum \left(data(n) - \frac{V_{M1}(GLC(n) - GLC_i)}{K_{M1} + GLC(n) + GLC_i} + \frac{V_{M2}(GLC(n) - GLC_i)}{K_{M2} + GLC(n) + GLC_i} \right)^2 +$$
$$w \cdot \left(J_{GLC} - \frac{V_{M1}(GLC_c - GLC_{ic})}{K_{M1} + GLC_c + GLC_{ic}} + \frac{V_{M2}(GLC_c - GLC_{ic})}{K_{M2} + GLC_c + GLC_{ic}} \right)^2$$

$$(21)$$

with *data*(*n*) the transport activity data for *n* different glucose concentrations ($GLC(n)$), GLC_i the internal glucose concentration during the transport assay, J_{GLC} the steady state glucose uptake rate, GLC_c the residual glucose concentration in the chemostat, GLC_{ic} the steady state internal glucose concentration in the chemostat, and ω a factor to give more weight to the steady state chemostat uptake rate, which is a single average value, as opposed to the large number of kinetic data points (a value of 5 was used for ω, increasing to higher values did not significantly influence the outcome of the optimization).