Growth kinetics of *Escherichia coli* with galactose and several other sugars in carbon-limited chemostat culture

Urs Lendenmann, Mario Snozzi, and Thomas Egli

Abstract: Kinetic models for microbial growth describe the specific growth rate (μ) as a function of the concentration of the growth-limiting nutrient (s) and a set of parameters. A typical example is the model proposed by Monod, where μ is related to s using substrate affinity (K_s) and the maximum specific growth rate (μ_{max}). The preferred method to determine such parameters is to grow microorganisms in continuous culture and to measure the concentration of the growth-limiting substrate as a function of the dilution rate. However, owing to the lack of analytical methods to quantify sugars in the microgram per litre range, it has not been possible to investigate the growth kinetics of *Escherichia coli* in chemostat culture. Using an HPLC method able to determine steady-state concentrations of reducing sugars, we previously have shown that the Monod model adequately describes glucose-limited growth of *E. coli* ML30. This has not been confirmed for any other sugar. Therefore, we carried out a similar study with galactose and found steady-state concentrations between 18 and 840 $\mu g \cdot L^{-1}$ for dilution rates between 0.2 and 0.8$\cdot h^{-1}$, respectively. With these data the parameters of several models giving the specific growth rate as a function of the substrate concentration were estimated by nonlinear parameter estimation, and subsequently, the models were evaluated statistically. From all equations tested, the Monod model described the data best. The parameters for galactose utilisation were μ_{max} = 0.75$\cdot h^{-1}$ and K_s = 67 $\mu g \cdot L^{-1}$. The results indicated that accurate K_s values can be estimated from a limited set of steady-state data when employing μ_{max} measured during balanced growth in batch culture. This simplified procedure was applied for maltose, ribose, and fructose. For growth of *E. coli* with these sugars, μ_{max} and K_s were for maltose 0.87$\cdot h^{-1}$, 100 $\mu g \cdot L^{-1}$; for ribose 0.57$\cdot h^{-1}$, 132 $\mu g \cdot L^{-1}$, and for fructose 0.70$\cdot h^{-1}$, 125 $\mu g \cdot L^{-1}$.

Key words: monod model, continuous culture, galactose, glucose, fructose, maltose, ribose.

Résumé : Les modèles de cinétique de la croissance bactérienne ont l'habitude de décrire le taux de croissance spécifique (μ) en fonction de la concentration de nutriments limitant la croissance (s) et d'une série de paramètres. Un exemple typique est le modèle proposé par Monod où μ est apparenté à s selon l'affinité pour le substrat (K_s) et le taux de croissance spécifique maximale (μ_{max}). La méthode couramment utilisée pour évaluer de tels paramètres est de cultiver les microorganismes en culture en mode continu et de mesurer la concentration du substrat limitant la croissance comme une fonction du facteur de dilution. Par contre, dû à l'absence de méthodes analytiques permettant de quantifier les sucres avec une précision du $\mu g \cdot L^{-1}$, il a été impossible de mesurer les cinétiques de croissance d'*Escherichia coli* lors d'une culture en chémostat. En utilisant une méthode HPLC capable de mesurer des concentrations à l'état stable des sucres réductants, nous avons précédemment démontré que le modèle de Monod décrivait adéquatement la croissance de l'*E. coli* ML30 où le glucose était le facteur limitant. Comme cela n'a jamais été vérifié avec d'autres sucres, nous avons fait une étude similaire avec du galactose et nous avons observé à l'état stable des concentrations allant de 18 à 840 $\mu g \cdot L^{-1}$ pour des taux de dilution allant de 0.2 à 0.8$\cdot h^{-1}$ respectivement. A partir de ces résultats, nous avons pu estimer les paramètres de quelques modèles utilisant le taux de croissance spécifique comme une fonction de la concentration du substrat en se servant d'une estimation non linéaire de ces paramètres et, par la suite, ces modèles ont été évalués sur le plan statistique. Par rapport à l'ensemble des équations vérifiées, c'est le modèle de Monod qui a le mieux décrit les résultats. Les paramètres de l'utilisation du galactose étaient μ_{max} = 0.75$\cdot h^{-1}$ et K_s = 67 $\mu g \cdot L^{-1}$. Les résultats ont confirmé qu'il est possible d'obtenir des valeurs K_s précises à partir d'un nombre limité de résultats obtenus à l'état stable si l'on utilise un μ_{max} mesuré durant une croissance équilibrée dans une culture en cuvée. Cette procédure simplifiée a été appliquée au maltose, au ribose et au fructose. Les valeurs μ_{max} et K_s de la croissance d'*E. coli* en présence de ces sucres ont été de 0.87$\cdot h^{-1}$, 100 $\mu g \cdot L^{-1}$ avec le maltose, 0.57$\cdot h^{-1}$, 132 $\mu g \cdot L^{-1}$ avec le ribose et de 0.70$\cdot h^{-1}$, 125 $\mu g \cdot L^{-1}$ avec le fructose.

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U. Lendenmann, M. Snozzi, and T. Egli.[1] Swiss Federal Institute for Environmental Science and Technology (EAWAG), Swiss Federal Institute of Technology, Überlandstrasse 133, CH-8600 Dübendorf, Switzerland.

[1]Author to whom all correspondence should be addressed (e-mail: egli@eawag.ch).

Mots clés: Modèle de Monod, culture en mode continu, galactose, glucose, fructose, maltose, ribose.

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Introduction

The specific growth rate of microorganisms growing in a chemically defined minimal medium is a function of the concentration of the growth-limiting medium component. Therefore, many mathematical models have been suggested to describe microbial growth as a function of concentration of the growth-limiting medium component and a set of "kinetic" parameters. Monod related the specific growth rate (μ) to the concentration of a growth-limiting substrate (s) with eq. 1 (Table 1), where μ_{max} is the maximum specific growth rate and K_s is the saturation constant (Monod 1942). Today, this model is widely used for simulation of microbial growth processes in biotechnology and microbial ecology. Nevertheless, Monod's equation has been criticized in various respects (Powell 1967) and, therefore, many alternative models have been proposed in the literature (Table 1). However, a problem inherent to most of the previous studies was the lack of good experimental data that could be used for the evaluation of the different growth models. For example, *Escherichia coli* is certainly the most extensively studied bacterium, but information available on growth kinetics is virtually limited to glucose, and surprisingly few data have been published on kinetics with other sugars (see Table 6). Furthermore, the saturation constants published for glucose and galactose vary over three orders of magnitude.

Previously, we have shown that the Monod model adequately describes glucose-limited growth in chemostat culture (Senn et al. 1994). This has not been confirmed for any other sugar, mainly because of the lack of analytical methods to quantify sugars in growth media in the kinetically relevant concentration range of a few micrograms per litre. Hence, to date, it is still unknown whether or not the Monod model can also be applied for the description of growth with carbon sources other than glucose.

Because kinetic information is crucial for understanding and modelling of microbial growth in both ecology and biotechnology, we set out to determine reliable kinetic parameters for growth of *E. coli* with galactose, maltose, ribose, and fructose in carbon-limited continuous culture. Here, we first demonstrate that the Monod model statistically most adequately describes growth with galactose. Subsequently, to determine the Monod parameter, K_s, for growth of *E. coli* in maltose-, ribose-, and fructose-limited chemostat culture, we have evaluated and applied a method that requires only a limited set of experimental data.

Growth models considered in this study

Most of the published growth models are based on the Monod equation. Herbert corrected the Monod equation for endogenous metabolism (eq. 3, Table 1) (Powell 1967), while Powell extended the model with a term for diffusion resistance in the area surrounding the bacterial cell (eq. 5) in which L is the substrate concentration required for maximum growth rate if K_s was zero. Later, Dabes et al. proposed a three-constant form of the Monod model including two slow enzymatic reactions (Dabes et al. 1973) and Koch published a model including a diffusion-limited step (substrate permeation through the outer membrane of Gram-negative bacteria) and an enzymatic step (active transport across the cytoplasmic membrane) (Koch and Wang 1982). Mathematically, the equations of Dabes and Koch are identical to that of Powell (Powell 1967). Shehata and Marr described their data with a model consisting of two Monod terms for two parallel transport systems (Shehata and Marr 1971). In this equation, the sum of μ_1 and μ_2 gives μ_{max}, whereas K_1 and K_2 are the corresponding saturation constants. For nonlinear parameter estimation, μ_2 had to be replaced with $(\mu_{max} - \mu_1)$ as indicated in eq. 7 to ensure that the sum of the estimates of μ_1 and μ_2 cannot exceed μ_{max}. The above models will be referred to as Monod-type growth models.

The oldest model used for the description of bacterial growth is the Blackman model, which was originally designed for plant growth (eq. 8) (Blackman 1905). It proposes a linear relationship between the specific growth rate and concentration of the limiting substrate until a second nutrient becomes growth limiting. Tessier published a model (Powell 1967) which is based on an exponential function (eq. 10). More recently Westerhoff and co-workers proposed a logarithmic relationship between μ and s (eq. 12) (Westerhoff et al. 1982). Because our data give only information on μ and s, models that require information on cell composition, cell volume (Kooijman et al. 1991), and culture density (Contois 1959) were not considered in this study.

Kinetic models are usually given in the form $\mu = f(s)$. In the case of the Monod model, as written in eq. 1 for batch experiments, μ is the dependent variable that is measured experimentally as a function of the independent variable, s, which is set by the operator. In contrast, in the chemostat μ is given by the dilution rate and s is measured. Thus, the appropriate form of the models for description of chemostat growth is $s = f(D)$ (Robinson 1985; Schmidt 1992). Therefore, both the original and the inverse form of most models were considered for data evaluation (Table 1).

Materials and methods

Organism

Escherichia coli ML30 (DSM 1329) was used in all experiments.

Growth medium

The mineral medium contained per litre of purified water KH_2PO_4 2.72 g, NH_4Cl 0.763 g, EDTA $Na_2{\cdot}2H_2O$ 82 mg, $MgSO_4{\cdot}7H_2O$ 59 mg, $Na_2MoO_4{\cdot}2H_2O$ 2.6 mg, $CaCO_3$ 10 mg, $FeCl_3{\cdot}6H_2O$ 2.03 mg, $MnCl_2{\cdot}4H_2O$ 4.95 mg, ZnO 1.02 mg, $CuCl_2{\cdot}2H_2O$ 0.85 mg, $CoCl_2{\cdot}6H_2O$ 1.20 mg, H_3BO_3 0.31 mg. Prior to heat sterilization, pH was adjusted to 3 with concentrated H_2SO_4. Concentrated sugar solutions were acidified to pH 3 with HCl, sterilized separately, and added as a growth-limiting carbon source to a final concentration of 100 $mg{\cdot}L^{-1}$.

Cultivation

Continuous culture with galactose was performed in a 1.8-L KLF type bioreactor (Bioengineering, Wald, Switzerland) with a working volume of 930 mL. Temperature was maintained at 37 ± 0.1°C, pH was controlled at 7.0 ± 0.05, and pO_2 was kept higher

Table 1. Growth models selected for detailed evaluation.

Author	Eq.	$\mu = f(s)$	Eq.	$s = f(\mu)$
Monod	1	$\mu = \mu_{max}\frac{s}{K_s + s}$	2	$s = \frac{\mu \cdot K_s}{\mu_{max} - \mu}$
Herbert	3	$\mu = (\mu_{max} + m)\frac{s}{K_s + s} - m$	4	$s = \frac{(\mu + m)\cdot K_s}{\mu_{max} - \mu}$
Powell	5	$\mu = \mu_{max}\frac{K_s + L + s}{2 \cdot L}\left[1 - \sqrt{\frac{4 \cdot L \cdot s}{(K_s + L + s)^2}}\right]$	6	$s = L\frac{\mu}{\mu_{max}} + K_s\frac{\mu}{\mu_{max} - \mu}$
Shehata and Marr	7	$\mu = \mu_1\frac{s}{K_1 + s} + (\mu_{max} - \mu_1)\frac{s}{K_2 + s}$		
Blackman	8	$\mu = d \cdot s$ for $s \le \frac{\mu_{max}}{d}$ $\mu = \mu_{max}$ for $s > \frac{\mu_{max}}{d}$	9	$s = \frac{\mu}{d}$ for $\mu \le \mu_{max}$ $s > \frac{\mu}{d}$ for $\mu = \mu_{max}$
Tessier	10	$\mu = \mu_{max}\left(1 - e^{\frac{-s}{T}}\right)$	11	$s = -T \cdot \ln\left(1 - \frac{\mu}{\mu_{max}}\right)$
Westerhoff et al.	12	$\mu = a + b \cdot \ln(s)$	13	$s = e^{\frac{\mu - a}{b}}$

Note: μ, specific growth rate; μ_{max}, maximum specific growth rate; K_s, Monod saturation constant; m, maintenance; L, diffusion constant in Powell's model, i.e., concentration of s which would allow the organisms to grow at μ_{max} if K_s was zero; μ_1 and ($\mu_{max} - \mu_1$), rate constants of the high- and the low-affinity transport systems in Shehata and Marr's model; d, increase of the specific growth rate per unit of substrate in Blackman's model; T, saturation constant in Tessier's model; a and b, parameters of Westerhoff's model.

than 80% of air saturation. Experiments with maltose, ribose, and fructose were carried out under the same conditions in a 2.5-L bioreactor (MBR, Wetzikon, Switzerland) with a culture volume of 1.6 L.

Maximum growth rate

The μ_{max} of the cells was determined in batch culture. Cells were precultivated aerobically at 37°C on the above medium supplied with 0.5 $g \cdot L^{-1}$ galactose, fructose, maltose, or ribose. During 4 days, the cells were repeatedly transferred into fresh medium in order to adapt the culture to maximum growth rate. After this adaptation, μ_{max} was measured in three parallel 500-mL Erlenmeyer flasks supplied with 200 mL of medium. Growth was followed spectrophotometrically at 546 nm in a cuvette of 1 cm light path.

Sugar analysis

Samples were withdrawn from the bottom of the bioreactor and immediately filtered. Analysis included desalting of samples by electrodialysis with subsequent sugar determination by HPLC. The details of the sample preparation and sugar analysis have been previously published (Lendenmann 1994, Senn et al. 1994).

Estimation of the errors of experimental data

Experimentally, the standard deviation of the dilution rate was found to be approximately 5% of the absolute value.

$$[14] \quad \sigma_D = 0.05 \cdot D$$

Between different chemostat runs, measured steady-state concentrations at identical dilution rates varied within 10–20%. Therefore, the overall standard deviation (σ_s) of steady-state substrate concentrations was assumed to be the sum of the standard deviation of the measurements (σ_{obs}) at a particular growth rate in one chemostat run plus an error of 10% of the measured steady-state concentration, but at least 5 $\mu g \cdot L^{-1}$ as shown in eqs. 15*a* and 15*b*.

$$[15a] \quad \sigma_s = \sqrt{\sigma_{obs}^2 + (0.1 \cdot s_{obs})^2} \quad \text{for } s > 50\ \mu g \cdot L^{-1}$$

Table 2. Steady-state galactose concentrations during growth of *E. coli* in carbon-limited chemostat culture at different dilution rates.

D (h^{-1})	s ($\mu g \cdot L^{-1}$)[a]	σ_D (h^{-1})[b]	σ_s ($\mu g \cdot L^{-1}$)[c]	n[d]
0.14	17	0.007	5.7	9
0.20	19	0.010	5.7	4
0.24	36	0.012	5.6	9
0.30	41	0.015	8.0	4
0.34	62	0.017	10.4	5
0.40	69	0.020	9.7	11
0.45	107	0.023	13.5	5
0.50	130	0.025	16.3	8
0.54	167	0.027	18.6	5
0.56	201	0.028	22.8	8
0.59	209	0.030	21.8	5
0.62	349	0.031	38.0	6
0.64	345	0.032	52.6	10
0.66	472	0.033	51.0	8
0.73[e]	666[e]			10
0.77[e]	749[e]			8
0.81[e]	841[e]			5

Note: Galactose concentration in the feed was 100 $mg \cdot L^{-1}$.
[a]Average steady-state galactose concentration.
[b]Standard deviation of the dilution rate (estimated with eq. 14).
[c]Standard deviation of the steady-state substrate concentration (estimated with eq. 15).
[d]Number of samples measured.
[e]Data omitted for parameter estimation because affected by wall growth.

$$[15b] \quad \sigma_s = \sqrt{\sigma_{obs}^2 + (5\mu g \cdot L^{-1})^2} \quad \text{for } s \le 50\ \mu g \cdot L^{-1}$$

Table 3. Estimated parameters, approximate standard errors, and quality of fit for fitting different models to experimental data for growth of *E. coli* with galactose in continuous culture.

Model	Eq.	Fit	μ_{max}[a], μ_1[a]	K_s[b], K_1[b], T[c]	m[d], μ_2[a], d[e], a[f]	L[g], K_2[b], b[f]	RSS(D)[h]	RSS(s)[h]	χ^2(D)[h]	Algorithm[i]
Monod	2	1	0.755±0.010	66.7±5.6			0.00375	**3′759**	24.0	Quasi
Herbert	4	2	0.753±0.014	65.4±19.4	0.009±0.124		0.00392	**3′757**	24.8	Simplex
Powell	6	3	0.752±0.023	64.4±19.4		5.2±42.8	0.003	**3′755**	24.4	Simplex
Blackman	9	4	0.66 (fix)		0.0028±0.0001		0.201	**55′641**	962	Quasi
Tessier	11	5	0.668±0.003	107±5.5			0.033	**7′748**	206	Quasi
Westerhoff	13	6			–0.142±0.053	0.132±0.009	0.026	**6′713**	271	Quasi
Monod	1	7	0.753±0.016	66.5±4.4			**0.003**	3′797	24.0	Quasi
Herbert	3	8	0.762±0.022	72.6±11.4	–0.019±0.030		**0.003**	3′917	24.8	Simplex
Powell	5	9	0.764±0.036	73.3±20.7		–9.1±26.5	**0.003**	3′884	24.2	Simplex
Shehata	7	10	0.752±0.070	66.4±10.1	0.168±0.070	$92\cdot10^3$±$6.5\cdot10^6$	**0.003**	3′793	24.0	Simplex
Blackman	8	11	0.570±0.023		0.0062±0.0007		**0.049**	22′901	167	Quasi
Tessier	10	12	0.626±0.015	73.3±5.6			**0.009**	[k]	49.9	Quasi
Westerhoff	12	13			–0.294±0.028	0.159±0.006	**0.006**	10′617	29.2	Quasi
Monod	1	14	0.753±0.030	67.1±5.8			0.003	3′982	**23.9**	Quasi
Blackman	8	15	0.553±0.035		0.0068±0.0005		0.055	12′729	**151.0**	Quasi
Tessier	10	16	0.603±0.025	63.7±5.5			0.012	[k]	**39.7**	Quasi
Westerhoff	12	17			–0.308±0.025	0.162±0.007	0.006	11′066	**27.2**	Quasi
Monod	1	18	0.920 (fix)	98.2±6.1			0.037	90′220	**70.8**	Quasi

[a] μ_{max}, μ_1, $\mu_2 = \mu_{max} - \mu_1$, specific growth rate constants (h^{-1}).
[b] K_s, K_1, K_2, saturation constants ($\mu g \cdot L^{-1}$).
[c] T, constant in Tessier's model ($\mu g \cdot L^{-1}$).
[d] m, maintenance (h^{-1}).
[e] d, constant in Blackman's model ($\mu g \cdot L^{-1} \cdot h^{-1}$).
[f] a, b, parameters of Westerhoff's model (h^{-1}).
[g] L, diffusion constant ($\mu g \cdot L^{-1}$).
[h] Quality of fits, values in bold face were minimized during parameter estimation.
[i] Fitting algorithm.
[k] RSS(s) could not be estimated because fitted μ_{max} was lower than the highest experimental D, resulting in an undefined denominator in eq. 9.

Parameter estimation and statistics

Model parameters were calculated by nonlinear parameter estimation (NPE) using SYSTAT system for statistics (Wilkinson 1990). Either the Quasi-Newton or Simplex algorithm was applied. This program provided also approximate standard errors. The quality of a fit is given by the residual sum of squares (RSS) with respect to either dilution rate (RSS(D)) or substrate concentration (RSS(s)), and models were validated applying the χ^2 goodness-of-fit test, minimized for the dilution rate (D). Competing models were compared with the F-test (Robinson 1985; Senn et al. 1994).

Results

Growth with galactose

In carbon-limited chemostat culture of *E. coli* ML30, steady-state concentrations of galactose were measured at different dilution rates between 0.14 and $0.81 \cdot h^{-1}$. The experimental data are shown in Table 2 for chemostat runs using a galactose concentration in the feed of 100 $mg \cdot L^{-1}$. At dilution rates higher than $0.7 \cdot h^{-1}$, significant wall growth affected steady-state substrate concentration. At dilution rates lower than $0.5 \cdot h^{-1}$, the possible influence of wall growth could be ruled out, because comparable steady-state concentrations were observed in a similar experiment carried out with a galactose feed concentration of 10 $mg \cdot L^{-1}$ (Lendenmann 1994). Wall growth did not allow to reliably determine steady-state concentrations of galactose at dilution rates close to μ_{max}. This was indicated by the fact that washout did not occur even at dilution rates higher than the μ_{max} of $0.92 \cdot h^{-1}$ measured experimentally in batch culture.

Parameter estimation

For parameter estimation, only data up to the dilution rate $0.66 \cdot h^{-1}$ were used which were not affected by wall growth. The estimated parameters and the quality of the fits when deviations in substrate concentrations were minimized (RSS(s)) are given in Table 3 (fits 1–6). It should be mentioned that for the Blackman model the μ_{max} had to be set to the D value of the highest data pair (fit 4) as otherwise, the RSS(s) could not be calculated.

Since the form $\mu = f(s)$ is more familiar to biologists, and, ideally, similar parameters should be obtained when RSS(D) is minimized during the fitting procedure, parameters were also estimated minimizing RSS(D). Using this procedure, parameters for the model of Shehata and Marr (eq. 7) were obtained, whereas in the RSS(s) fit neither the Quasi-Newton, nor the Simplex algorithm converged successfully. The results are listed in Table 3 (fits 7–13).

In order to validate the models statistically with a χ^2 goodness-of-fit test (Richter and Söndgerath 1990) the equations of Monod (eq. 1), Blackman (eq. 8), Tessier (eq. 10), and Westerhoff (eq. 12) were also fitted by minimizing $\chi^2(D)$ (Table 3, fits 14–17).

All above fits resulted in considerably lower estimates of μ_{max} than that observed in batch culture ($0.92 \cdot h^{-1}$). Therefore, K_s of the Monod model was also estimated using a μ_{max}

Table 4. Steady-state substrate concentrations during growth of *E. coli* in carbon-limited chemostat culture at different dilution rates.

Growth substrate[a]	D (h^{-1})	s ($\mu g \cdot L^{-1}$)	Reference
Glucose	0.30	45±5	(Senn et al. 1994)
	0.60	137±15	
Galactose	0.59	209±22	This work
	0.30	41±8	
Maltose	0.60	222±17	This work
Ribose	0.21	92±4	This work
	0.30	141±11	
	0.44	246±13	
	0.54	612±7	
Fructose	0.30	112±7	This work
	0.60	242±12	

[a]Sugar concentrations in the feed were 100 $mg \cdot L^{-1}$.

of $0.92 \cdot h^{-1}$ (fit 18). NPE for other Monod-type growth models using a fixed μ_{max} did not result in plausible parameters (results not shown).

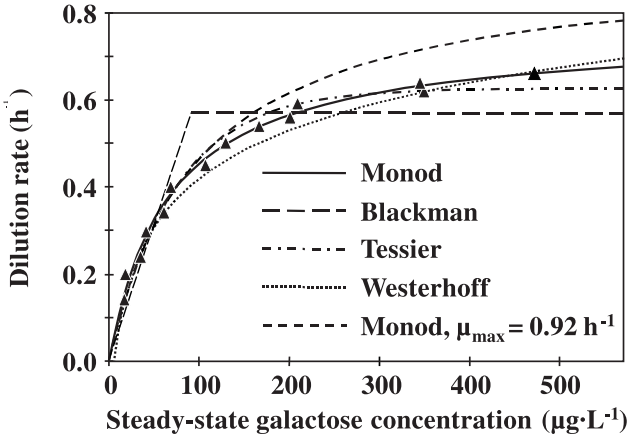
Statistical evaluation of growth models

All Monod-type growth models resulted in similar values for μ_{max} and K_s, whether RSS(s) or RSS(D) was minimized (Table 3). Hence these models adequately describe the experimental data. Considerable deviations are visible in Fig. 1 only for the models of Blackman, Tessier, and Westerhoff. This was confirmed in the χ^2 test. The models with two parameters (Monod, Blackman, Tessier, Westerhoff) have 12 degrees of freedom. Therefore, the resulting values of χ^2 should be within an interval of 4.4–23.3 for a statistical significance level α of 5% or between 3.1 and 28.3 for a significance level of 1%. For the models with three parameters (Herbert, Powell, Shehata and Marr) these intervals are 3.8–21.9 and 2.6–26.8, respectively. For the Monod-type growth models, all $\chi^2(D)$ were in the range at α = 1% if either RSS(D) or $\chi^2(D)$ was minimized. Also the Westerhoff model resulted in χ^2 values within the significance level of 1%. However, the χ^2 values in Table 6 were extremely affected by the choice of the standard deviation for D. Doubling σ_D resulted in four times smaller values of $\chi^2(D)$. Thus, if the estimates of the standard deviation were incorrect, the χ^2 values obtained must be unrealistic. This indicates the χ^2 goodness-of-fit test alone does not allow evaluation of these growth models.

Approximate standard errors of m, L, and K_2 in the models of Herbert, Powell, and Shehata and Marr, respectively, were about one order of magnitude larger than the parameters themselves (Table 3). This indicates that estimates of m (fit 2), L (fit 3), and K_2 (fit 10) have to be considered with caution. Additionally, for maintenance (m) in Herbert's equation and diffusion resistance (L) in the model of Powell, only positive values are allowed. Therefore, fits 8 and 9 must be discarded (Table 3).

With the F-test the differences in the quality of fit obtained for the models were compared (Robinson 1985). With regard to both RSS(D) and RSS(s) fits, only the Blackman model was notably poorer than the Monod model when using a significance level α of 5%. On the other hand, fits obtained for modified Monod models (eqs. 3–7) were not significantly better than the original model itself. This suggests that from the experimental data, no information can be obtained with respect to maintenance, diffusion resistance, or secondary uptake systems. Consequently, the Monod model should be preferred to the other more complex models, because always the simplest model describing data satisfactory should be selected (Schmidt 1992).

Fig. 1. Comparison of the growth models proposed by Monod (fit 7), Blackman (fit 11), Tessier (fit 12), and Westerhoff (fit 13) with galactose concentrations (▲) measured during growth of *Escherichia coli* in carbon-limited chemostat culture. With the exception of the Monod equation with $\mu_{max} = 0.92 \cdot h^{-1}$ (fit 18) where $\chi^2(D)$ was minimized, RSS(D) fits are shown.

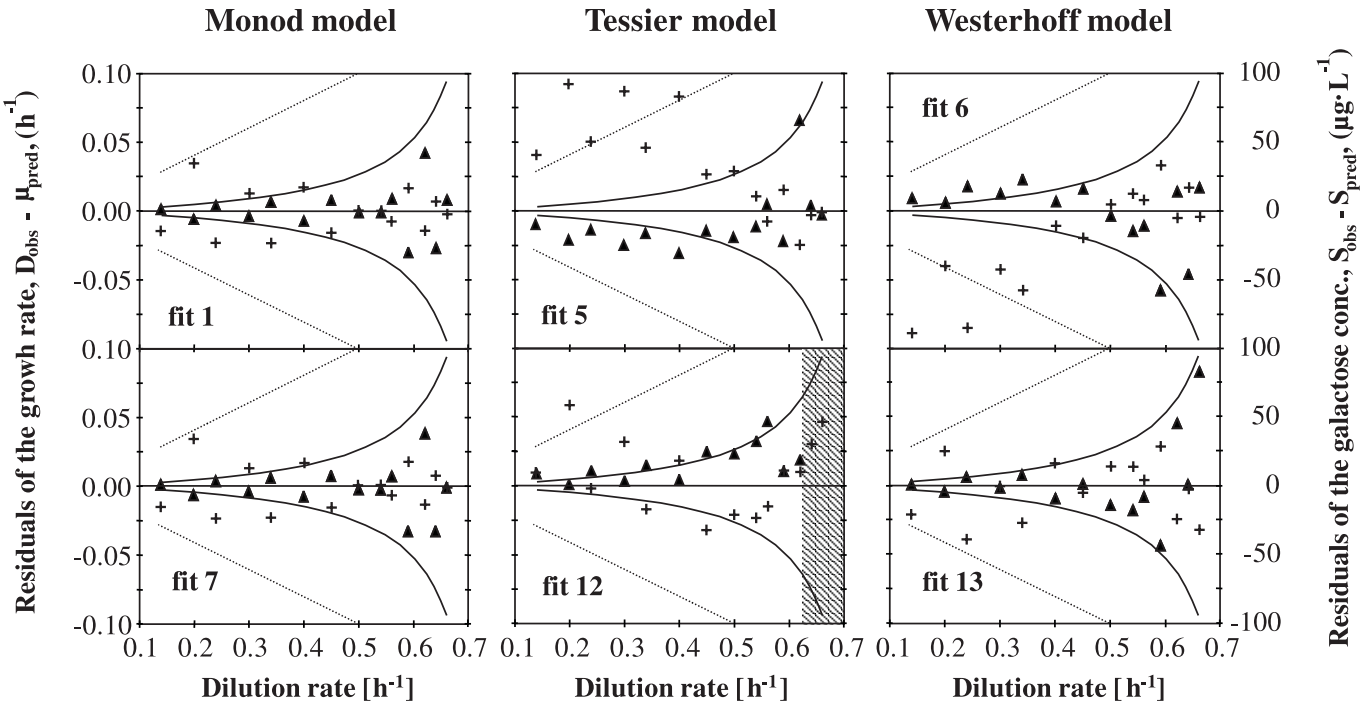


The statistical evaluation has shown that the Blackman model did not describe data satisfactorily and that the extensions of the Monod model (Herbert, Powell) were not significantly better than the Monod equation. Therefore, the remaining three models, i.e., those of Monod, Tessier, and Westerhoff, were examined further. To discriminate between these models, the residuals between the experimental data and the values predicted by the models were analysed.

The Monod model showed small random errors over the whole range of dilution rates when RSS(s) was minimized (Fig. 2, fit 1). RSS(s) fits of the Tessier and Westerhoff models described the data at the higher range of dilution rates well (Fig. 2, fits 5 and 6) but both exhibited systematic deviations, especially at low growth rates. For these reasons, the models of Tessier and Westerhoff were not able to describe the data over the whole range of dilution rates adequately. In contrast with RSS(s) fits, RSS(D) fits exhibited no systematic deviation at low dilution rates (Fig. 2, fits 7, 12, and 13). This difference can be explained by the fact that dilution rates varied only 4.7-fold, i.e., between 0.14 and $0.66 \cdot h^{-1}$, while the resulting galactose steady-state concentrations varied 28-fold, i.e., between 17 and 472 $\mu g \cdot L^{-1}$. Consequently, high (D, s) data pairs were weighted over-proportionally in RSS(s) fits, because of the larger numerical difference between experimentally measured and predicted steady-state concentrations.

This evaluation has identified the Monod model as the one describing growth of *E. coli* in galactose-limited chemostat most accurately. This is in contrast with growth of *E. coli* in glucose-limited continuous culture where the Westerhoff

Fig. 2. Residuals of D (+) and s (▲) drawn as a function of the dilution rate for the Monod, Tessier, and Westerhoff models. The upper figures represent RSS(s) fits and the lower figures represent RSS(D) fits. ·····, 20% relative deviation of the dilution rate (D); _____, 20% relative deviation of the substrate concentration (s).



model was statistically slightly better than the Monod model (Senn et al. 1994). Nevertheless, at dilution rates lower than $0.8 \cdot h^{-1}$, kinetics of growth with glucose was equally well described by both the Monod and the Westerhoff model (Senn et al. 1994). Hence, the Monod model is able to describe growth kinetics of *E. coli* with galactose and glucose adequately, and in contrast with the Westerhoff model, its parameters have a physiological meaning, i.e., maximum specific growth rate and substrate concentration at half μ_{max}.

Determination of K_s values with limited data sets

Use of the experimentally measured value of $0.92 \cdot h^{-1}$ for fitting the Monod model resulted in a poor fit (Table 3, fit 18) which can be easily noticed in Fig. 1. Nevertheless, the resulting K_s was almost equal to the substrate concentration observed at $D = 0.45 \cdot h^{-1}$, which approximately corresponds with the half of the maximum specific growth rate observed in batch culture (Table 2). A similar result was previously reported for glucose, where, when employing μ_{max} measured in batch culture ($0.92 \cdot h^{-1}$) a K_s of 73 $\mu g \cdot L^{-1}$ was obtained, which was 37% higher than when both μ_{max} and K_s were estimated from steady-state chemostat data (Senn et al. 1994). Although the difference in K_s values obtained by these two fitting procedures is significant for both galactose (this work) and glucose (Senn et al. 1994), it is negligibly small when comparing with the range of K_s values published previously for growth of *E. coli* in glucose-limited minimal media (Table 6). For a detailed listing see references (Lendenmann 1994; Senn et al. 1994). Therefore, it may be feasible to estimate realistic K_s values for chemostat growth of *E. coli* with other sugars, i.e., maltose, ribose, and fructose, with considerably smaller sets of steady-state data.

It has been argued that a least eight steady-state substrate concentrations are required for estimation of two kinetic parameters, e.g., μ_{max} and K_s (Robinson 1985). In contrast, only two data pairs are mathematically necessary to estimate the two parameters of the Monod model. However, μ_{max} and K_s can only be calculated from two data points on the assumptions that the Monod model ideally describes the dependence of growth rate on substrate concentration and that the data are free of experimental errors. Experimental systems in biology never fulfil these conditions, and therefore, more information is required for parameter estimation, for example, additional data pairs or knowledge of one of the two kinetic parameters.

The Monod model has the unique advantage that μ_{max} can be determined independently from K_s by measuring specific growth rates in batch culture. If μ_{max} is known, K_s can be derived from a single (D, s) data pair determined in the chemostat by using equation 16.

$$[16] \qquad K_s = s \cdot \frac{\mu_{max} - D}{D}$$

If more than one data pair is available, the best way to calculate K_s is a nonlinear parameter estimation using the Monod model with μ_{max} set equal to the maximum specific growth rate determined in batch culture as described above. In this study, such fits gave best results when minimizing $\chi^2(D)$. Hence, a simplified procedure can be used to obtain reasonable estimates of K_s values.

Growth with maltose, ribose, and fructose

To obtain kinetic data for growth of *E. coli* with maltose, ribose, and fructose, cells were grown at one to four differ-

ent dilution rates. The observed yield for these sugars was comparable with those obtained during growth with glucose and galactose, i.e., 0.45 ± 0.03 g(DW)·g(sugar)$^{-1}$. The steady-state data obtained are listed in Table 4.

To illustrate that estimates for K_s obtained by this method are realistic, K_s values for glucose and galactose employing only two data pairs at D = 0.3 and 0.6·h^{-1} were compared with the values previously found when all available data pairs were used for parameter estimation. K_s values of 76 and 90 μg·L^{-1} were obtained for glucose and galactose, respectively, which is in close agreement with K_s values of 73 (Senn et al. 1994) and 98 μg·L^{-1} obtained with the complete data sets (Table 3). Subsequently, kinetic data for utilization of glucose, galactose, maltose, ribose, and fructose have been determined, and the parameters obtained are given in Table 5.

K_s values of the sugars used in this study were all in the range of 50–150 μg·L^{-1}, with glucose exhibiting the lowest and ribose the highest values. However, K_s values do not directly reflect the ability of bacteria to grow at very low substrate concentrations. For example, when comparing two microorganisms of which the first has a μ_{max} of 1·h^{-1} and K_s = 100 μg·L^{-1} and the second exhibits a μ_{max} of 0.5·h^{-1} and K_s = 50 μg·L^{-1}, even at low substrate concentrations the first organism is able to grow slightly faster in spite of the higher K_s value. A better parameter to describe the ability to grow at extremely low substrate concentrations is the specific affinity (*a*) which in this study is defined as the quotient of μ_{max} and K_s. The specific affinity (*a*) corresponds with the slope of the Monod curve at zero concentration (Button 1991). The specific affinities for the five sugars tested (Table 5) varied between 0.0043 and 0.012 μg^{-1}·L·h^{-1} for ribose and glucose, respectively. Hence, among these sugars at low substrate concentrations, glucose supports the highest and ribose supports the slowest growth rate of *E. coli*.

Table 5. K_s values estimated by nonlinear parameter estimation from the steady-state data given in Table 4 using fixed μ_{max} values.

Substrate	μ_{max} (h^{-1})[a]	K_s (μg·L^{-1})	μ_{max}·K_s^{-1} (μg^{-1}·L·h^{-1})
Glucose	0.92	76 ± 2	0.012
Galactose	0.92	90 ± 13	0.010
Maltose	0.87	100[b]	0.0087
Ribose	0.57	132 ± 20	0.0043
Fructose	0.70	125 ± 48	0.0056

[a]μ_{max} measured during balanced growth in batch culture.
[b]No approximate standard error was obtained, because the degrees of freedom for estimation of K_s from a single steady-state measurement is zero.
[c]Specific affinity (μ_{max}·K_s^{-1}).

Discussion

Growth kinetics versus sugar transport kinetics

A comparison of K_s values for individual sugars obtained here (Table 5) with published Michaelis–Menten parameters for uptake of these sugars (K_m) or affinity constants for sugar-binding proteins (K_d) (Table 6) show little agreement. K_m and K_d values for maltose transport systems were approximately three times higher than K_s for growth. In contrast, K_m and K_d values for ribose were 3–5 times smaller than K_s. Only for growth with galactose a clear correlation between the measured K_s value and the high affinity K_m and K_d values for the binding-protein system (Mgl) were observed. The range of published K_m values for the low-affinity proton-driven galactose permease (GalP) is between 0.7 and 16 mg·L^{-1} for wild type strains of *E. coli* (Table 6). For the high-affinity transport system (Mgl) the corresponding value is 0.09 mg·L^{-1} (Table 6). The K_s value determined in this study for growing cells was similar to the published K_m values of the high-affinity galactose transport system, which suggests that during growth in continuous culture, up to a dilution rate of approximately 0.7·h^{-1}, the binding-protein transport system was mainly responsible for galactose uptake. Unfortunately, we are not aware of published kinetic data for the utilisation of fructose by *E. coli*.

It is interesting to note that, with the exception of galactose, kinetic data for growth do not reflect kinetic parameters obtained for sugar transport. This suggests that the correlation between growth and transport kinetics found for galactose is coincidental rather than being of a mechanistic basis. Therefore, neither biochemical characterization of sugar-uptake systems nor molecular studies on their regulation can predict the growth kinetics of bacteria in vivo.

Variability of kinetic growth parameters

Two observations indicate that the Monod growth kinetic parameters exhibit systematic variations, depending on the conditions under which the cells were grown.

First, μ_{max} values measured in batch cultures are consistently higher than those obtained from chemostat-grown cells. For example, an inoculum removed from a galactose-limited chemostat (D = 0.3·h^{-1}) reached a specific growth rate of 0.47·h^{-1} after 1 h and 0.61·h^{-1} after 2 h when exposed to excess galactose in batch culture, whereas the μ_{max} observed in batch culture was 0.92·h^{-1}. The same was reported for glucose (Harvey 1970; Lendenmann and Egli 1995). Therefore, it seems that for chemostat-grown cells, a μ_{max} is rather a theoretical value than a fixed kinetic constant. In this respect, it is not surprising that the value obtained from data fitting (μ_{max} = 0.7·h^{-1}) is somewhere between the value determined for "trained" cells in batch culture (0.92·h^{-1}) and the specific growth rate chemostat-grown cells attain when transferred into batch culture (0.61·h^{-1}) (see Table 3 for galactose; Senn et al. 1994, for glucose).

Second, enormous differences (from some 50 μg·L^{-1} to a few mg·L^{-1}) were reported for the K_s values of *E. coli* growing with glucose (Table 6) (for a detailed list, see Senn et al. 1994). Analysis of the K_s values in the literature for glucose exhibit a clear dependence on the mode of cultivation. K_s values obtained from chemostat cultures are one to two orders of magnitude lower than those measured in batch culture (Kovarova-Kovar and Egli 1998). The reason for the above differences is attributed to the ability of microorganisms to adapt to oligotrophic environments. This adaptation has been shown for *E. coli* in chemostat culture at a dilution rate of D = 0.6·h^{-1}, where the steady-state glucose concentration decreased from approximately 700 to 130 μg·L^{-1} (Senn et al. 1994). Such effects were also observed in this study for chemostat growth of *E. coli* with galactose, malt-

Table 6. Kinetic data previously published for growth with and transport of glucose, galactose, maltose, and ribose.

Substrate	Strain	Ty[a]	K_x[b] (mg·L^{-1})	μ_{max} (h^{-1})	T[c] (°C)	Reference
Glucose	H	(1)*s*	4.0	0.94	37	(Monod 1942)
		(2)*s*	8.0	0.65	20	(Jannasch 1968)
	ML30	(3)	0.068 (HA)	0.78	30	(Shehata and Marr 1971)
		(3)	12.6 (LA)	0.03	30	(Shehata and Marr 1971)
	ML308	(4)*s*	2.3	1.23	37	(Koch and Wang 1982)
		(4)*s*	0.10	0.54	37	(Koch and Wang 1982)
	ML30	(5)*s*	0.058	0.82	37	(Senn et al. 1994)
	AB257 (*met*$^-$)	(6)*m*	1.8		30	(Villarejo et al. 1978)
	AB257 (*met*$^-$)	(6)*m*	5.4		30	(Villarejo et al. 1978)
	K12 (BW2901)	(7)*m*	1.1		25	(Death et al. 1993)
Galactose	K12 (KL16)	(8)*s*	108	0.65	37	(Kornberg and Riordan 1976)
	ML32400	(9)*m*	0.72 (LA)		25	(Rotman and Radojkovic 1964)
	ML32400	(9)*m*	0.088 (HA)		25	(Rotman and Radojkovic 1964)
	K12 (D115)	(10)*m*	16 (LA)		21	(Wilson 1974)
	K12 (D115)	(10)*m*	0.09 (HA)		21	(Wilson 1974)
	K12 (W3092)	(11)*d*	0.086		4	(Zukin et al. 1977)
	B/r	(12)*d*	0.063		?	(Quiocho et al. 1979)
Maltose	RV	(13)*s*	8.64	0.76	27	(Dykhuizen and Davies 1980)
	K12 (HfrG6)	(14)*m*	0.32		21	(Szmelcman et al. 1976)
	K12 (HfrG6)	(14)*d*	0.36		21	(Szmelcman et al. 1976)
	K12 (HfrG6)	(15)*d*	0.79		21	(Schwartz et al. 1976)
Ribose	W289	(16)*m*	0.065		37	(David and Wiesmeyer 1970)
	W3092	(17)*m*	0.045		23	(Willis and Furlong 1974)
	W3092	(17)*d*	0.02		?	(Willis and Furlong 1974)

Notes: Explanations to individual reports. (1) Determined by measuring the growth rate in small intervals during retardation phase in batch culture. Actual substrate concentrations (s_t) were calculated with the equation $s_t = S_0 - S_0(x_t - x_0)/M$. S_0, initial substrate concentration; x_0, initial biomass concentration; x_t, biomass concentration at time t; M, biomass concentration reached in stationary phase. (2) Determined during chemostat growth at various dilution rates. Glucose concentrations were calculated from biomass concentration and yield data. (3) Determined by measuring growth rates at various initial glucose concentrations in batch culture. The high affinity (HA) K_s (68 μg·L^{-1}) and μ_{max} (0.775·h^{-1}) were obtained by Lineweaver–Burk linearization. The low affinity (LA) μ_{max} (0.031·h^{-1}) was obtained by subtracting the high affinity μ_{max} from the maximum specific growth rate observed in batch culture (0.806·h^{-1}). Subsequently, the low affinity K_s was obtained by data fitting using above parameters and a growth model comprising the sum of two Monod terms (Shehata and Marr 1971) (see eq. 7). (4) Determined by measuring growth continuously in a 10-cm flow-through cuvette. The first experiment was carried out with batch- and the second with chemostat-grown cells. (5) Determined according to the same method as presented above for galactose. (6) Determined by measuring of initial uptake rates of [^{14}C]glucose. Both batch and chemostat grown (D = 0.17·h^{-1}) bacteria were used. K_m of chemostat-grown cells was approximately three times lower. (7) Determined by measuring initial uptake rates of [^{14}C]glucose of cells harvested from continuous culture (D = 0.3·h^{-1}). (8) Derived from growth rate measurements at various initial galactose concentrations in batch culture. (9) Derived from [^{14}C]galactose accumulation after incubation of cells for 15 min at various external concentrations. The presence of a low affinity (LA) and high affinity (HA) uptake system was proposed on the basis of a biphasic accumulation pattern. (10) Measured as function of cellular accumulation of [^{14}C]galactose. To measure activity of GalP (low affinity proton symport transport system) Mgl (high affinity binding-protein system) was inhibited competitively with β-glycerol-galactoside. (11) Determined by equilibrium dialysis with purified binding protein. (12) Determined by equilibrium dialysis at unknown temperature. (13) Determined in chemostat culture. Steady-state substrate concentrations (s) were estimated from yield data of two identical chemostat runs with different maltose feed concentrations. (14) K_m determined by measuring initial uptake rates (40 s) of [^{3}H]maltose. K_d was determined by fluorescence titration. (15) Determined by equilibrium dialysis with osmotic shock released binding protein. (16) K_m determined by initial uptake rates (2 min) of [^{14}C]ribose. (17) K_m determined by measuring initial uptake rates (30 s) of different concentrations of [^{14}C]ribose. K_d determined by equilibrium dialysis.

[a]Type of saturation constant: *s*, K_s for growth; *m*, K_m for substrate uptake; *d*, K_d affinity constants of binding proteins.

[b]Value of saturation constant.

[c]Growth or assay temperature. A comprehensive list of previously published kinetic constants is given in (Lendenmann 1994).

ose, and fructose (data not shown) and are reported in the literature for *Klebsiella pneumoniae* (Rutgers et al. 1987, 1989) and *Cytophaga johnsonae* (Höfle 1983).

All this information suggests that the kinetic properties of microorganisms can only be described by constant Monod parameters if cells are grown under well-defined stable conditions. This is the case for microorganisms well adapted to growth in continuous culture. However, under poorly defined or fast-changing growth conditions in batch culture, K_s and μ_{max} values can vary over a large range. Competition appears to determine the direction of microbial adaptation to the growth conditions. In an oligotrophic environment, microbes with the highest affinity for the growth-limiting substrate are capable of outgrowing competitors. In contrast, under nutrient-excess conditions high substrate affinity becomes less important, because successful competition is determined by the maximum growth rate. In our experiments, the adaptation of *E. coli* to the growth conditions was manifested by the low K_s values cells exhibited for glucose and galactose after long-term continuous culture and, in contrast, μ_{max} values in batch culture that considerably exceeded that of cells grown in chemostat culture.

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