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Comparison of maximum specific growth rates and lag times estimated from absorbance and viable count data by different mathematical models

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Abstract

Maximum specific growth rate (μ_{max}) and lag time (λ) were estimated from viable count and absorbance data and compared for different microorganisms, incubation systems and growth conditions. Data from 176 growth curves and 120 absorbance detection times of serially diluted cultures were evaluated using different mathematical growth models. Accurate estimates of μ_{max} and λ were obtained from individual absorbance growth curves by using the Richard model, with values of the parameter *m* fixed to 0.5, 1.0 or 2.0 to describing different degrees of growth dampening, as well as from absorbance detection times of serially diluted cultures. It is suggested to apply the two techniques complementarily for accurate, rapid and inexpensive estimation of microbial growth parameter values from absorbance data. In contrast, considerable limitations were demonstrated for the ability of the Exponential, the Gompertz and the Logistic models to estimate μ_{max} and λ values accurately from absorbance data. Limitations of these models were revealed due the wide range of growth conditions studies.
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1. Introduction

Accurate estimation of microbial growth parameters, particularly maximum specific growth rate (μ_{max}) and lag time (λ), is essential in many areas of microbiology for example, to characterize effects of anti-microbials, optimize microbial media and to develop kinetic models for use in food and fermentation technology. Estimation of microbial growth parameters from absorbance measurements has the advantages of being rapid, non-destructive, inexpensive and relatively easy to automate as compared to many other techniques and particularly when compared to classical viable counts methods. Absorbance measuring devices typically have high detection thresholds in the range $\sim 10^6$–10^7 bacteria/ml. Consequently, growth rates determined directly from changes in absorbance can only be calculated for relatively dense microbiological cultures (Koch, 1994). Clearly, it is interesting to determine if specific growth rates (μ_t) estimated from changes in absorbance (μABS) of dense cultures corresponds to

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values determined by more sensitive measurements, particularly the classical viable count methods (μVC). This question is, e.g., very important in predictive food microbiology where growth parameters estimated from absorbance data are used increasingly for development of models. Those models are used to predict the time required for very low levels of spoilage and pathogen microorganisms to reach critical limits under various growth conditions.

Numerous techniques and mathematical growth models have been used for estimation of growth rates and lag times from absorbance data. Most early studies determined μABS values from the linear part of log-transformed absorbance growth curves (Koch, 1994). Later, absorbance data measured directly, corrected for non-linearity, or transformed into equivalent viable counts were fitted to sigmoidal growth models, e.g., the Logistic and the Gompertz equations (Corman et al., 1986; McClure et al., 1993; Dalgaard et al., 1994; Hudson and Mott, 1994; Begot et al., 1996; Chorin et al., 1997). Combinations of absorbance data and viable counts were also suggested for estimation of microbial growth parameters (Bréand et al., 1997; Stephens et al., 1997; Augustin et al., 1999). For aerobic cultures and high inoculation levels, Hudson and Mott (1994) found μABS_G values (see nomenclature in Table 1) and μVC values to be similar but the absorbance technique underestimated lag time. However, for aerobic cultures and low inoculation levels, μABS_G and μABS_E values were substantially lower than μVC (Dalgaard et al., 1994; Neumeyer et al., 1997). In studies of different microorganisms and atmospheres, μABS_L values, obtained by using different forms of

Table 1
Nomenclature

Symbol	Description and units
μ_{max}	Maximum specific growth rate (h^{-1})
$dABS_t/dt$	Volumetric growth rate
μ_t	Specific growth rate, $[dABS_t/dt]/ABS_t$ (h^{-1})
μVC	μ_{max} determined from viable count data
μABS	μ_{max} determined from absorbance measurements
μABS_L	μABS estimated by the Logistic model Eq. (2) (h^{-1})
μABS_R	μABS estimated by the Richards model Eq. (3) (h^{-1})
μABS_{RF}	μABS_R estimated by using values of m fixed as 0.5, 1.0 or 2.0 (h^{-1})
μABS_G	μABS estimated by the Gompertz model Eq. (4) (h^{-1})
μABS_E	μABS estimated by the Exponential model Eq. (5) (h^{-1})
μABS_{DT}	μ_{max} estimated from absorbance detection times of 10-fold diluted cultures by Eq. (6) (h^{-1})
μABS_{BP}	μ_{max} estimated from absorbance detection times of 10-fold diluted by the variance ratio method of Baranyi and Pin (1999) (h^{-1})
$R(\mu VC/\mu ABS)$	Ratio of μ_{max} values estimated from viable count and absorbance data
λ	Lag time (h)
λVC	λ determined from viable count data (h)
λABS	λ determined from absorbance measurements by Eq. (8) and μABS_{RF} (h)
λABS_{DT}	λABS estimated by Eq. (8) and μABS_{DT} (h)
λABS_{BP}	λABS estimated by the variance ratio method of Baranyi and Pin (1999) (h)
$D\%(\lambda VC - \lambda ABS)$	Percentage difference between λVC and λABS (se Eq. 9)
$t_{\Delta ABS}$	Time for a 0.05 unit increase in absorbance of cultures (h)
$N_{\Delta ABS}$	Cell concentration at time $t_{\Delta ABS}$ (cfu/ml)
t	Time (h)
N_t	Cell concentration at time t (cfu/ml)
ABS_t	Absorbance at time t
N_{min}, N_{max}	Parameters in Logistic model (Eq. 1) corresponding to asymptotic minimum and maximum cell concentrations (cfu/ml)
ABS_{min}, ABS_{max}	Parameters in Logistic and Richards models corresponding to asymptotic minimum and maximum absorbance
t_i	Time at inflection point in Logistic and Richards models (h)
m	Parameter in the Richards model describing growth dampening
rms	Residual mean squares = residual sum of squares/degrees of freedom

the Logistic model, were similar to μVC (Dalgaard et al., 1994; Dalgaard et al., 1997a,b; Nerbrink et al., 1999). Furthermore, absorbance detection times (DT) of serially diluted cultures allowed accurate estimation of μABS_{DT} values and a method for estimation of lag time from such data was recently suggested (Cuppers and Smelt, 1993; Baranyi and Pin, 1999). As indicated above, kinetic parameters can be estimated accurately from absorbance data but it remains unclear if the various methods and growth models used are specific, e.g., to microbiological species or are broadly applicable to different types of microorganisms and growth conditions.

The objective of the present study was to compare μ_{max} and λ values estimated from viable count and absorbance data by different mathematical growth models. Growth of five groups of bacteria, including fermentative and non-fermentative species, were studied by using different (i) incubation systems, (ii) atmospheres, (iii) temperatures and (iv) media with various levels of carbon source, electron acceptor, sodium chloride and buffer. Growth parameters were estimated from 176 viable count and absorbance growth curves as well as from 120 absorbance detection times of serially diluted cultures. The ratio of growth rates $R(\mu VC/\mu ABS)$ and the percentage difference between lag times were used to compare μ_{max} and λ values estimated from viable count and absorbance data.

2. Materials and methods

Mixtures of five to eight strains of *Brochothrix thermosphacta*, lactic acid bacteria, *Photobacterium phosphoreum*, *Pseudomonas* spp. and *Shewanella putrefaciens* were studied using incubation systems, media and temperatures as shown in Table 2. To obtain a wide range of growth yields and μ_{max} values, cultures were incubated under aerobic and anaerobic conditions with and without glucose and the electron acceptor trimethylamine-oxide (TMAO) (Table 2). Due to expected absence of growth under anaerobic conditions, *Pseudomonas* spp. was only studied with aerobic incubation. *S. putrefaciens* is

Table 2
Microorganisms, incubation systems, growth media and temperatures studied[a]

Microorganisms	Number of growth curves				
	Agitated flask[b]	Hungate tubes		Microplates	
		100% N_2[b]	100% CO_2[b]	Growth curves[b]	Detection times[c]
B. thermosphacta	6[d]	6[e]	4[e]	6[d]	4×5[d]
Lactic acid bacteria	6[d]	6[e]	6[e]	4[d]	4×5[d]
P. phosphoreum	4[f]	40[f,g,h]	42[f,g,h]	4[f]	4×5[f]
Pseudomonas spp.	6[d]	nd[i]	nd	6[d]	4×5[d]
S. putrefaciens	8[f,h]	14[f,h]	nd	8[f,h]	8×5[f,h]

[a] One hundred and seventy-six viable count and absorbance growth curves as well as 120 absorbance growth curves in microplates were generated and used for comparison of maximum specific growth rates and lag times as estimated by different mathematical models.
[b] Growth kinetics determined from individual absorbance and viable count growth curves.
[c] Growth kinetics determined from absorbance detection times of 10-fold diluted cultures.
[d] All Purpose Tween (APT) broth (Evans and Niven, 1951) with glucose (2.0 g/l) at 0, 15 or 25°C. For *B. thermosphacta,* LAB and *Pseudomonas* spp. absorbance detection times of serially diluted cultures were determined at 15 and 25°C. Furthermore, for LAB individual growth curves determined by viable counts and absorbance measurements were determined at 15 and 25°C only.
[e] APT broth with glucose (0.0 or 1.0 g/l) at 0, 15 or 25°C or Growth medium broth (GMB) (Dalgaard et al., 1994) for *P. phosphoreum* at 0 or 15°C and at 0, 15 or 25°C for *S. putrefaciens*.
[f] GMB with trimethylamine-oxide (TMAO) dihydrate (0.0 or 4.0 g/l), phosphate buffer (3.0, 6.0 or 12.0 g/l) and NaCl (0.5, 0.75 or 1.0%).
[g] Brain heart infusion (BHI) broth (Oxoid, CM225), Tryptone soya broth (TSB) (Oxoid, CM129) or Marine broth 2216 (Difco, 07891-17-4) at 0 or 15°C.
[h] GMB without TMAO was used under selected condition.
[i] Not determined.

highly CO_2 sensitive (Dalgaard, 1995b) and therefore it was not incubated with 100% CO_2.

2.1. Bacterial strains

All strains were isolated from spoiled seafood. Isolation and identification of *P. phosphoreum* (NCIMB 13476-83) and *S. putrefaciens* (S0, S15, S30, S50 and S100) from Danish seafood were previously described (Dalgaard, 1995a; Dalgaard et al., 1997b). *Brochothrix thermosphacta*, lactic acid bacteria (LAB), and *Pseudomonas* spp were isolated in Greece from Boque and Gilt head seabream (Koutsoumanis et al., 1998; Koutsoumanis and Nychas, 1999) by using streptomycin sulphate thallous acetate cycloheximide (actidione) agar (STAA, Oxoid, CM881, supplemented with SR 151), M.R.S. agar (Oxoid, CM 361) and cetrimide–fusidin–cephaloridine (CFC) agar (Mead and Adams, 1977), respectively. The following tests were used to confirm that the latter three groups of strains belonged to the expected taxonomic groups: shape, size, motility, Gram test, catalase, oxidase and glucose fermentation. Isolates from STAA and M.R.S. agar were further tested for growth on acetate agar, production of gas from glucose and gluconate, final pH in La-broth, NH_3 production and pH increase from arginine metabolism (aerobically and anaerobically with 0.1 and 2.0% glucose), and for production of polymer from sucrose. These tests were carried out as previously described (Dalgaard and Jørgensen, 2000). Isolates from CFC agar were further tested for reduction of TMAO (Gram et al., 1987) and fluorescence on King agar B (King et al., 1954).

2.2. Growth experiments

For each combination of microorganism and growth condition, duplicate growth experiments were carried out at constant temperatures. A total of 176 individual growth curves were generate by using both viable counts and absorbance measurements. In addition absorbance detection times in microplates were determined for 120 serially diluted cultures. The incubation systems and media used are shown in Table 2. Precultures were prepared as previously described (Dalgaard, 1995b) by using APT broth for *B. thermosphacta*, LAB, and *Pseudomonas* spp. and GMB for *P. phosphoreum* and *S. putrefaciens*. Precultures for the individual growth curves were diluted to provide inoculation levels of 10^2–10^4 cfu/ml for all incubation systems. Precultures to be used for determination of absorbance detection times were diluted to provide an inoculation level of 10^5–10^6 cfu/ml followed by four successive 10-fold dilutions.

Flask cultures were incubated with agitation (~100 rpm). Media in Hungate tubes were saturated with 100% N_2 or 100% CO_2 (Dalgaard et al., 1997a) and cultures were incubated without agitation. Ninety-six-well microplates (Nunc™, Roskilde, Denmark) with 250 μl of medium per well were used for incubation at 0°C whereas the automated Bioscreen C system with 100-well-microplates containing 300 μl per well (Labsystems, Helsinki, Finland) were used at 15 and 25°C.

2.3. Growth measurements

Cultures were appropriately diluted in physiological saline (0.85% NaCl) with peptone (0.1%) before enumeration by spread plating. Cultures incubated in the Bioscreen C were sampled without removing the microplates from the instrument. Holes were carefully melted in microplate lids by using a hot wire. Typically, 12–15 viable count data points were produced for each growth curve. *B. thermosphacta*, LAB, and *Pseudomonas* spp. were enumerated on APT agar plates (25°C, 3 days). Long and Hammer agar (van Spreekens, 1974) plates were used for enumeration of *P. phosphoreum* (15°C, 5 days) and *S. putrefaciens* (25°C, 3 days). Changes in absorbance of all cultures were measured at 540 nm. At each sampling time, 1.0 ml of medium from agitated flask cultures was dispensed into disposable microcuvettes (Müller ratiolab®, Dreieich, Germany) and absorbance measured. Hungate tubes were vortexed and absorbance measured directly without opening the tubes. Absorbance was measured with air as the blank with a simple spectrophotometer (Novaspec II, Pharmacia Biotech, Allerød, Denmark). Microplates were agitated for 10 s prior to measurement of absorbance by using a Multiscan RC microplate reader (Labsystems, Helsinki, Finland) or by the automated Bioscreen C system. Absorbance growth curves with at least 20 data points were

generated for all incubation systems but in most cases a higher number of data points were obtained (see, e.g., Figs. 3 and 4). Apparent growth yields were expressed as the difference between the initial and final absorbance of cultures. Non-linearity of the absorbance response was not corrected for by dilution of cultures or mathematically.

2.4. Maximum-specific growth rate (μ_{max})

$\mu_{\max}$ values were estimated from viable counts data (μVC) by the log-transformed four-parameter Logistic model (Eq. (1)). From absorbance growth curves, $\mu_{\max}$ values were estimated by the non-transformed four-parameter Logistic model (Eq. (2)), the Richards model (Eq. (3)), the modified Gompertz model (Eq. (4)), and the Exponential model (Eq. (5)) (Pruitt et al., 1979, Zwietering et al., 1990; Dalgaard et al., 1994). In addition, $\mu_{\max}$ values were estimated from absorbance detection times of 10-fold serially diluted cultures in microplates by using (i) Eq. (6) with data from a single series of detection times (μABS_{DT}) and (ii) the ANOVA method of Baranyi and Pin (1999) with data from duplicate series of detection times (μABS_{BP}).

$$\mathrm{Log}(N_t) = \mathrm{Log}\left(N_{\min} + \frac{N_{\max} - N_{\min}}{1 + \exp[-\mu \mathrm{VC}(t - t_i)]}\right) \quad (1)$$

$$ABS_t = ABS_{\min} + \frac{ABS_{\max} - ABS_{\min}}{1 + \exp[-\mu ABS_{\mathrm{L}}(t - t_i)]} \quad (2)$$

$$ABS_t = ABS_{\min} + \frac{ABS_{\max} - ABS_{\min}}{[1 + \exp(-\mu ABS_{\mathrm{R}} \cdot m \cdot (t - t_i))]^{1/m}} \quad (3)$$

$$\mathrm{Ln}\left(\frac{ABS_t}{ABS_0}\right) = A \cdot \exp\left(-\exp\left[\left(\frac{\mu ABS_{\mathrm{G}} \cdot \mathrm{e}}{A}\right) \cdot (\mathrm{lag} - t) + 1\right]\right) \quad (4)$$

$$\mathrm{Ln}(ABS_t) = k_5 + \mu ABS_{\mathrm{E}} \cdot t \quad (5)$$

$$\mathrm{Ln}(N_i) = k_6 - \mu ABS_{DT} \cdot DT_i \quad (6)$$

See Table 1 for description of parameters in growth models (Eqs. (1)–(6)). The suffix '*i*' in N_i and DT_i (Eq. (6)) indicate cell levels and absorbance detection times corresponding to different 10-fold serially diluted cultures, respectively. Fig. P (Anonymous, 1999) or Statgraphics (Anonymous, 1998) were applied for fitting of models including the non-linear regression required to estimate parameter values in several of the growth models indicated above.

The ratio $R(\mu VC/\mu ABS)$ of $\mu_{\max}$ values estimated from viable counts and absorbance data was calculated for each growth experiment and growth model. Only one model (Eq. (1)) was used for estimation of $\mu_{\max}$ values and lag time from viable count data. The reason is that the modified Gompertz model (Gibson et al., 1988; Zwietering et al., 1990) is known to overestimate $\mu_{\max}$ values of typical microbial growth cultures by approximately 10–20% (Baranyi et al., 1993; Dalgaard et al., 1994; Dalgaard, 1995b; Membré et al., 1999) and that the Baranyi model (Baranyi and Roberts, 1995), also popular in predictive microbiology, provides $\mu_{\max}$ values which are practically identical to those obtained from the less complicated Logistic model (Eq. (1)) and the exponential model (Dalgaard et al., 1994; Dalgaard, 1995b). For estimation of lag time from viable count data, very similar values have been obtained by the Baranyi model and the Logistic model whereas the modified Gompertz models may provide negative lag time estimates (Dalgaard, 1995b).

2.5. Lag time (λ)

λ values were estimated from viable count growth data (λVC) by using Eq. (7) and parameter values obtained from Eq. (1) (Dalgaard, 1995b).

$$\text{Lag time } (\lambda VC) = t_i - \frac{1}{\mu_{\max}} \cdot \ln\left(\frac{N_{\max} + N_{\max} \cdot \exp(\mu_{\max} \cdot t_i)}{N_{\max} + N_{\min} \cdot \exp(\mu_{\max} \cdot t_i)} - 1\right) \quad (7)$$

From absorbance growth curves, λ values (λABS) were estimated as shown in Fig. 1. Firstly, the time ($t_{\Delta ABS}$) and viable counts ($N_{\Delta ABS}$) corresponding to a 0.05 units increase in absorbance were determined from fitted model parameter values. Lag time (λABS) was then calculated by using Eq. (8). Values of N_0 and $N_{\Delta ABS}$ were determined from viable counts

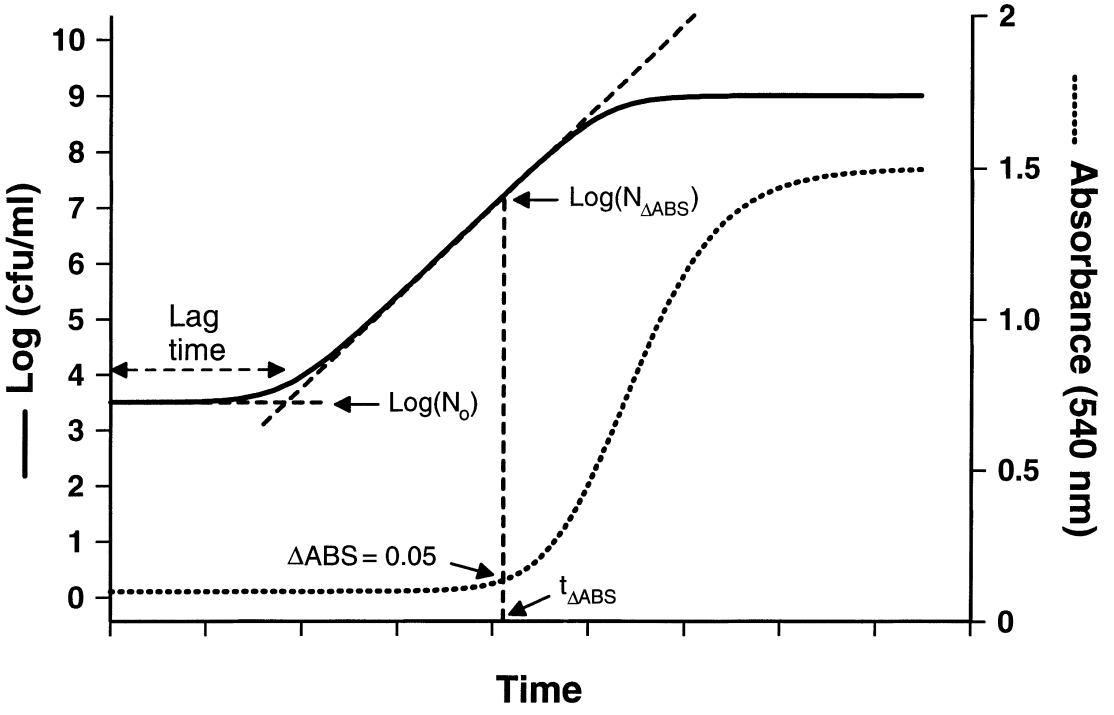

Fig. 1. Bacterial growth curve showing the general relationship between changes in viable count and absorbance of cultures. Significance of the parameters $t_{\Delta ABS}$, $N_{\Delta ABS}$, and N_0, used for estimation of lag time from absorbance growth curves, is indicated.

for each growth curve. For practical estimation of lag time from absorbance growth curves it is important to determine if $N_{\Delta ABS}$ values depends on growth condition for different microorganisms and this was evaluated. In addition, lag time (λABS_{BP}) was estimated from duplicate series of absorbance detection times by the ANOVA method of Baranyi and Pin (1999). Values of λABS_{BP} and μABS_{BP} were estimated by using a Microsoft Excel spreadsheet and Solver add-in (Baranyi and Pin, 1999).

Lag time (λABS) =

$$t_{\Delta ABS} - \left(\frac{[\log(N_{\Delta ABS}) - \log(N_0)] \cdot \ln(10)}{\mu ABS} \right) \qquad (8)$$

Lag times determined from viable count and absorbance data were compared by their difference expressed in percent of the inflection point in the Logistic model (t_i in Eq. (1)) as shown in Eq. (9) below. The inflection point t_i in the Logistic model, corresponds to the time when $N_t = N_{max}/2$, i.e., the time when log(cfu/ml) is 0.3 units below Log(N_{max}). Lag times and μ_{max} values are frequently used to predict times required for food-related microorganisms to reach high numbers, e.g., numbers corresponding to food spoilage. For this reason, the inflection point (t_i) was chosen to express discrepancy between lag times estimated from absorbance data and viable counts.

$$D\%(\lambda VC - \lambda ABS) = \frac{\lambda VC - \lambda ABS}{t_i \text{ (from Eq. (1))}} \cdot 100 \qquad (9)$$

3. Results and discussion

3.1. Maximum specific growth rate (μ_{max})

3.1.1. Effect of mathematical models and growth yield on $R(\mu VC/\mu ABS)$

Values of μ_{max} and growth yield varied from 0.009 to ~1.0 h^{-1} and from 0.1 to ~2.2 absorbance units, respectively. Within this range of growth conditions, μABS_L values were independent of growth yield but the average $R(\mu VC/\mu ABS_L)$ value of 1.28±0.74

(AVG±SD, $n=176$) indicated that some μABS_L and μVC values differed considerably as also shown in Fig. 2a. The highest $R(\mu VC/\mu ABS_L)$ values were obtained for the non-fermentative micro-organisms, *Pseudomonas* spp. and *S. putrefaciens*, growing in microplate cultures. Omitting these data, resulted in an average $R(\mu VC/\mu ABS_L)$ value of 1.14±0.43 ($n=162$). Previously, average $R(\mu VC/\mu ABS_L)$ values of 1.00–1.12 were found for various microorganisms growing under different atmospheres, temperatures, pH, NaCl, lactate and acetate levels (Dalgaard et al., 1994, 1997a; Augustin et al., 1999; Nerbrink et al., 1999). Clearly, the Logistic model has been appropriate for accurate estimation of μ_{max} values in some studies but variability of $R(\mu VC/\mu ABS_L)$ values, as found in the present study, suggests this model (Eq. (2)) as inappropriate for estimation of μ_{max} values from absorbance growth curves in general.

The Gompertz and the Exponential models (Eqs. (4) and (5)) underestimated μ_{max} values determined from absorbance growth curves resulting in average $R(\mu VC/\mu ABS)$ values of 2.9±2.2 and 3.2±2.2, respectively, (Fig. 2b,c). It is noteworthy that μABS_G and μABS_E values depended strongly on growth yield. In fact, μ_{max} values were underestimated by as much as 10–20-fold for some cultures with low growth yield (Fig. 2b,c). Clearly, these models cannot be used in general for estimation of μ_{max}

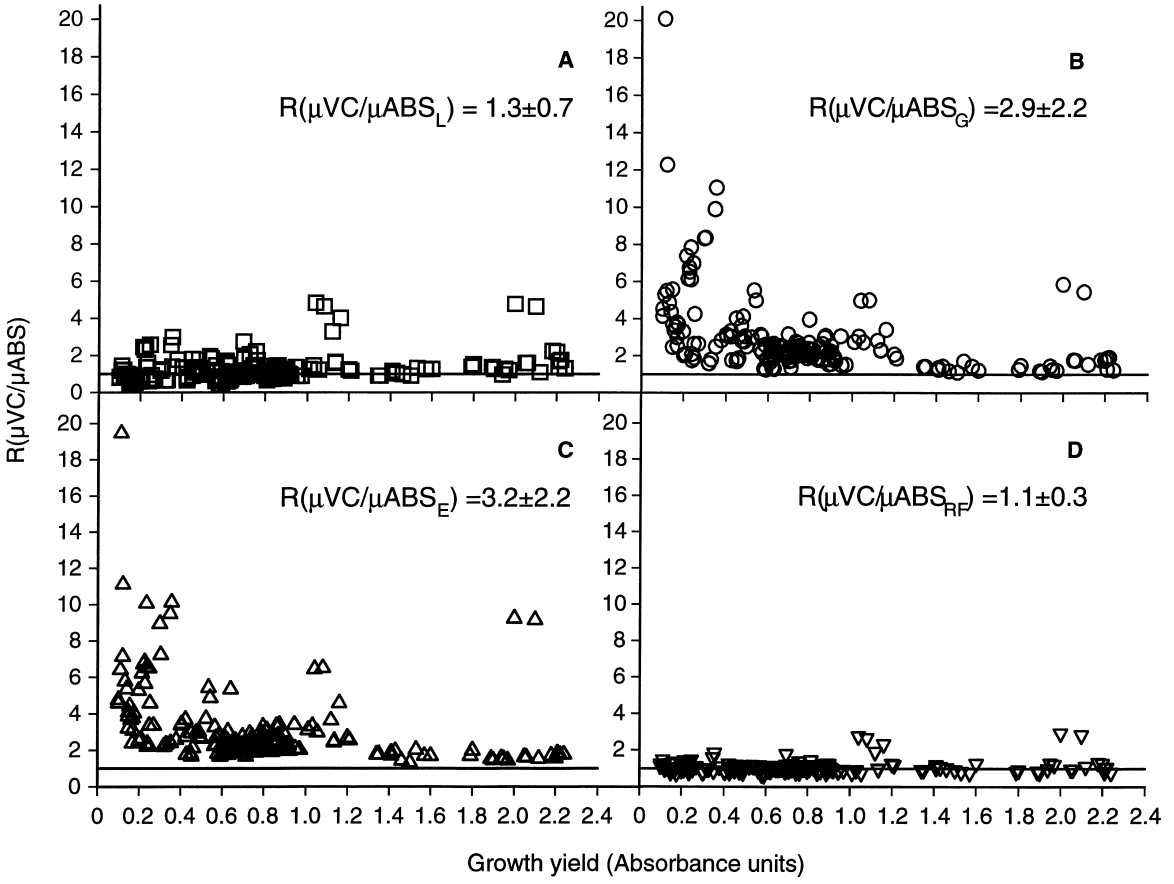

Fig. 2. Ratios, $R(\mu VC/\mu ABS)$, between maximum specific growth rates determined from viable counts by the log-transformed Logistic model (μVC) and from individual absorbance growth curves by the Logistic model (A), the Gompertz model (B), the Exponential model (C) and the Richards model (D). For the Richards model fixed values of the parameter '*m*' of 0.5, 1.0 or 2.0 was used and for each growth curve the '*m*' value that provide the lowest residual mean square (rms) value was selected. One hundred and seventy-six growth curves with different yields were evaluated by the different models.

values from absorbance growth curves. In agreement with the present study, the Gompertz and the Exponential models were found previously to under estimate μ_{max} values resulting in an average $R(\mu VC/\mu ABS)$ value of ~1.6 for aerobic microbiological cultures, with high growth yields (Dalgaard et al., 1994; Neumeyer et al., 1997). A strong effect of growth yield on $R(\mu VC/\mu ABS)$ values was not documented in those previous studies due to the limited range of environmental conditions studied. Growth yields of microbial cultures are little effected by relatively wide ranges of temperatures and water activities, at least for some microorganisms (Krist et al., 1998). However, parameters like pH, carbon substrates, electron acceptors, extreme growth conditions, incubation systems and many other factors influence microbial growth yields substantially. For studies of such growth conditions, the Gompertz and the Exponential models should be avoided because estimated μ_{max} values will not reflect actual growth rates but the combined effect of growth conditions on growth rates and growth yields. In fact, the effect of growth yield on μABS_G and μABS_E may explain the substantial differences in μ_{max} values previously estimated from absorbance of cultures in agitated flasks, microplates and fermentors at similar environmental conditions (Begot et al., 1996; Potvin et al., 1997). The present study does not support the recommendation of using the Gompertz model (Eq. (4)) for direct estimation of growth rates from absorbance growth curves (Begot et al., 1996). Furthermore, Fig. 2b shows that calibration factors of 1.5–1.6 as previously used to correct for the difference between μVC and μABS_G (Dalgaard et al., 1994; Neumeyer et al., 1997) only can be applied successfully, for a limited range of growth conditions resulting in high growth yields.

The pronounced differences in μABS values obtained by the Logistic model as compared to the Gompertz and the Exponential models arose because the two later models determine μABS values as slopes of log-transformed absorbance growth curves. In contrast, the parameter μABS_L in Eq. (2) does not correspond to any particular slope of an absorbance growth curve as is shown by the differential form of this model (Eq. (10)). The differential model shows the specific growth rate at time t (μ_t) to differ from the maximum specific growth rate (μABS_L) when absorbance of a culture (ABS_t) approaches the maximal absorbance (ABS_{max}). Consequently, the maximum specific growth rates (μABS_L) may be accurately estimated even in situation where changes in absorbance are only determined at times when growth is dampened. Of course, the Logistic model only provides accurate estimates of μ_{max} values where dampening of growth curve corresponds to the growth dampening dictated by this model (Eq. (10)).

$$\mu_t = \frac{dABS_t/dt}{ABS_t} = \mu ABS_L \cdot \left(1 - \frac{[ABS_t]}{[ABS_{max}]}\right) \quad (10)$$

$$\mu_t = \frac{dABS_t/dt}{ABS_t} = \mu ABS_R \cdot \left(1 - \frac{[ABS_t]^m}{[ABS_{max}]^m}\right) \quad (11)$$

In the present study, shapes of absorbance growth curves clearly influenced $R(\mu VC/\mu ABS_L)$ values (Fig. 3). A slow dampening of growth observed for some aerobic and microplate cultures resulted in $R(\mu VC/\mu ABS_L)$ values >1.0, whereas abrupt dampening of growth, seen, e.g., with limitation of carbon substrate, provided $R(\mu VC/\mu ABS_L)$ values <1.0. (Fig. 3). The Richards model includes a parameter (m in Eqs. (3) and (11)) that allows this model to simulate growth curves with different degrees of dampening. However, the integrated form of the Richards model (Eq. (3)) provided stable parameters estimates only for 61 out of the 176 absorbance growth curves studied (results not shown). This confirmed the Richards model to have poor statistical properties and simple reparametrization is unlikely to overcome the problem as previously reported (Ratkowsky, 1983, pp. 73–75). Nevertheless by using fixed values of 0.5, 1.0 or 2.0 for the parameter m, stable μABS_{RF} estimates were obtained and the Richards model then provided substantially more accurate estimates of μ_{max} values from absorbance growth curves than the Logistic model (Figs. 2 and 3). As seen by comparison of Eqs. (2) and (3) as well as Eqs. (10) and (11) the Logistic model is a special case of the Richards model with $m=1.0$. The average $R(\mu VC/\mu ABS_{RF})$ value was 1.07 ± 0.34 ($n=176$) as compared to 1.28 ± 0.74 for $R(\mu VC/\mu ABS_L)$ determined by the Logistic model. The most appropriate of the fixed values of 0.5, 1.0 or 2.0 for the parameter m in the Richards model can in most cases be determined simply by visual inspection of growth curves (Fig. 3). Furthermore,

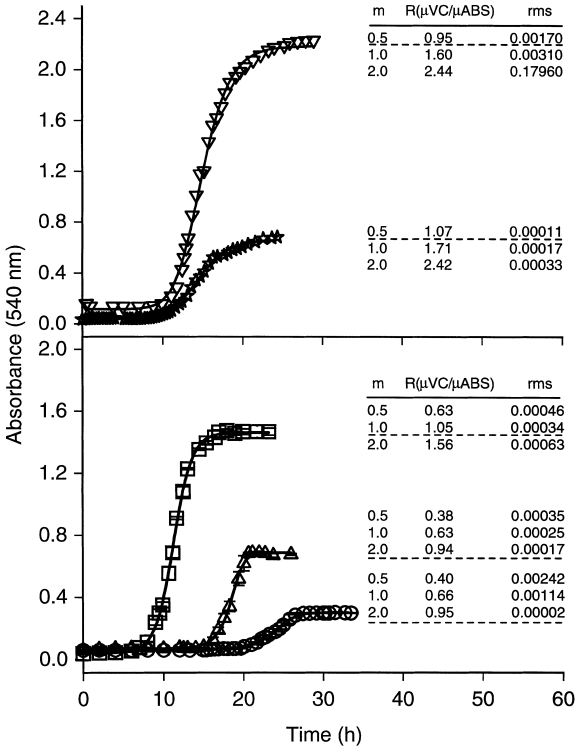

Fig. 3. Effect of growth dampening on the ratio $R(\mu VC/\mu ABS)$. μVC was estimated from viable counts by Eq. (1) and μABS from absorbance growth curves by using the Richards models (Eq. (3)) with fixed values of the parameter '*m*' of 0.5, 1.0 and 2.0. For each absorbance growth curve the solid line shows the fit of data to the Richards model with the *m* value providing the lowest residual mean squares (rms) value. (▽) *S. putrefaciens* grown aerobically at 25°C; (★) *S. putrefaciens* grown in microplate cultures at 25°C; (□) *B. thermosphacta* grown aerobically 15°C in APT broth with 2% glucose; (△) *B. thermosphacta* grown under 100% N_2 at 15°C in APT broth with 1% glucose; and (○) lactic acid bacteria grown under 100% N_2 at 15°C in APT broth without glucose.

the *m* value that provided the closest μABS_{RF} and μVC values also resulted in the lowest residual mean square (rms) value (Fig. 3). Thus appropriate fixed *m* value for estimation of μABS_{RF} values from absorbance growth curves can be determined easily.

3.1.2. Effects of incubation systems, physiology of microorganisms and media on $R(\mu VC/\mu ABS_{RF})$

The average $R(\mu VC/\mu ABS_{RF})$ value was close to 1.0 but values for microplate cultures of the non-fermentative microorganisms, *Pseudomonas* spp. and *S. putrefaciens*, differed from the remaining data (Table 3 and Fig. 2). The high $R(\mu VC/\mu ABS_{RF})$ values observed for this combination of incubation system and microbial physiology showed that μ_{max} values cannot always be accurately estimated from absorbance growth curves. Limited diffusion of oxygen into culture media in microplates most likely explain the high $R(\mu VC/\mu ABS_{RF})$ values for non-fermentative microorganisms. As an example, μVC values were similar for *Pseudomonas* spp. growing in agitated flasks and in microplates cultures but μABS_{RF} values differed for the two incubation systems (Fig. 4). Growth yields of the non-fermentative microorganisms depended strongly on oxygen availability and slow increases in absorbance of microplate cultures (Fig. 4b) most likely reflected a limited diffusion of oxygen into the culture medium rather than the growth rate potential of the non-fermentative microorganisms. Omitting data for the non-fermentative microorganisms growing in microplates, resulted in an average $R(\mu VC/\mu ABS_{RF})$ value of 1.01 ± 0.19 ($n=162$) as compared to 1.14 ± 0.43 for $R(\mu VC/\mu ABS_{L})$.

Low inoculation levels of 10^2–10^4 cfu/ml were used in the present study and μVC values therefore corresponded to μ_{max}, i.e., the growth rate potential of cultures as the conditions studied (Eq. (10)). Consequently, μABS_{RF} values, as determined from absorbance growth curves by the Richards model with fixed *m* values, were accurate estimates of μ_{max} values, except for non-fermentative organisms growing in microplates (Table 3). It is worth noting that the Richards model estimated μ_{max} values accurately despite of the fact that non-linearity of absorbance data had not been corrected for by dilution of cultures or by mathematical transformation of the absorbance data. Non-linearity of absorbance data can be very substantial but, in agreement with the present study, the effect on μABS_{L} values was previously quantified and found to be insignificant (Dalgaard et al., 1994).

The cell morphology of *B. thermosphacta* changes between coccobacilli and chains of rods depending on growth phase. Rattanasomboon et al. (1999) concluded that turbidimetry, for this reason, overestimated the specific growth rate. The present study was unable to confirm these observations. In fact, the Richards model estimated μ_{max} values and lag times

Table 3
Effect of incubation systems and atmospheres on the ratio $R(\mu VC/\mu ABS_{RF})$ of growth rates determined from viable counts by Eq. (1) and from absorbance data by using the Richards model (Eq. (3)) or absorbance detection times of serially diluted cultures (Eq. (6))

Microorganisms	$R(\mu VC/\mu ABS_{RF})$				
	Agitated flask[a]	Hungate tubes[a]		Microplates	
		100%	100%	Growth	Detection
	Aerobic	N_2	CO_2	curves[a]	times[b]
B. thermosphacta	1.05±0.17	1.00±0.08	1.11.±0.12	0.96±0.11	0.82±0.22
Lactic acid bacteria	0.92±0.04	1.00±0.06	1.00±0.14	1.00±0.25	0.97±0.06
P. phosphoreum	1.00±0.04	1.02±0.17	1.00±0.24	1.00±0.18	1.10±0.07
Pseudomonas spp.	0.95±0.22	nd[c]	nd	**2.56±0.39**	0.96±0.16
S. putrefaciens	1.08±0.14	1.07±0.23	nd	**1.19±0.28**	0.96±0.11

[a] Growth rates determined from individual growth curves.
[b] Growth rates determined by the dilution method from absorbance detection times of series of 10-fold diluted cultures.
[c] Not determined.

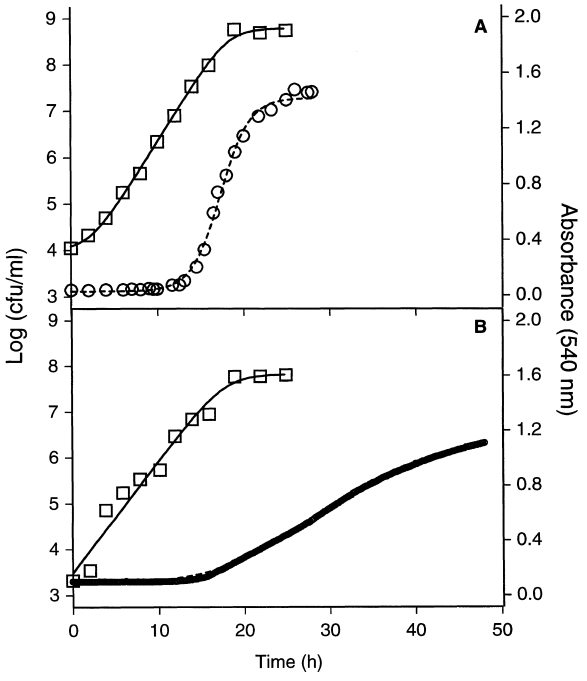

Fig. 4. Growth at 25°C of *Pseudomonas* spp. in agitated Erlenmeyer flask (A) and in Bioscreen C microplates (B). (□) Viable counts; (○) absorbance measurements. Due to a very high number of measurements in the Bioscreen experiments individual data points cannot be discriminated.

of *B. thermosphacta* accurately (Tables 3 and 4). It has also been pointed out that calibration functions were needed for estimation of μ_{max} values from absorbance growth curves when growth conditions influence the relation between absorbance and viable counts (Baranyi and Roberts, 1995; Chorin et al., 1997). However, estimation of μ_{max} values from absorbance data by the Richards model, as suggested in the present study and discussed above, was sufficiently robust to overcome effects of growth conditions on cell size and absorbance non-linearity (Table 3).

Absorbance detection times of 10-fold serially diluted cultures provided an average $R(\mu VC/\mu ABS_{DT})$ value of 0.96±0.14 (SD), when simple linear regression was applied for estimation of μABS_{DT} values (Eq. (6)). The more complicated ANOVA procedure suggested by Baranyi and Pin (1999) resulted in a similar $R(\mu VC/\mu ABS_{BP})$ value of 0.97±0.16 (S.D.). The dilution methods provided accurate estimates of μ_{max} values for all micro-organisms studied including non-fermentative micro-organisms growing in micro-plates (Table 3). This supported the hypothesis that only the upper part of absorbance growth curves for these cultures were influenced by oxygen limitation.

3.2. Lag time (λ)

3.2.1. Estimation of λ from individual absorbance growth curves

Lag times estimated from viable count growth curves varied between 0 and 175 h in the 176 growth experiments. Lag times estimated from absorbance growth curves by Eq. (9) depend on values of $t_{\Delta ABS}$, $N_{\Delta ABS}$, N_0 and μABS (see Fig. 1 and Table 1). The

Table 4
$D\%(\lambda VC - \lambda ABS)$ values and cell levels corresponding to an increase in absorbance of 0.05 units ($N_{\Delta ABS}$)

Microorganisms	Agitated flask[a]	Hungate tubes		Microplates	
		100% N_2[a]	100% CO_2[a]	Growth curves[a]	Absorbance detection times[b]
B. thermosphacta					
$D\%(\lambda VC - \lambda ABS)$[c]	3.3±8.8	−2.3±7.9	−6.5±4.4	−3.6±8.1	
$D\%(\lambda VC - \lambda ABS)$[d]	3.1±4.5	−5.6±20.9	−5.9±8.3	−5.6±18.3	−0.7±2.1
$\text{Log}(N_{\Delta ABS})$[e]	6.6±0.3	6.9±0.7	7.2±0.3	6.9±1.0	
Lactic acid bacteria					
$D\%(\lambda VC - \lambda ABS)$[c]	−5.9±3.0	−3.3±5.7	−1.6±10.0	0.2±14.0	
$D\%(\lambda VC - \lambda ABS)$[d]	−5.7±7.3	−3.3±6.3	−0.3±16.5	0.2±16.1	−7.2±10.8
$\text{Log}(N_{\Delta ABS})$[e]	7.8±0.3	7.7±0.2	7.6±0.3	6.3±0.3	
P. phosphoreum					
$D\%(\lambda VC - \lambda ABS)$[c]	0.3±2.3	0.3±9.5	−2.7±12.7	0.8±7.7	
$D\%(\lambda VC - \lambda ABS)$[d]	0.2±4.3	0.8±12.6	−4.1±15.6	0.2±5.1	9.2±7.0
$\text{Log}(N_{\Delta ABS})$[e]	6.3±0.4	6.5±0.3	6.3±0.3	6.0±0.3	
Pseudomonas spp.					
$D\%(\lambda VC - \lambda ABS)$[c]	−0.4±8.9			94.5±39.2	
$D\%(\lambda VC - \lambda ABS)$[d]	−3.3±6.9	nd[f]	nd	91.3±34.2	−11.5±10.9
$\text{Log}(N_{\Delta ABS})$[e]	6.2±0.7			6.2±0.3	
S. putrefaciens					
$D\%(\lambda VC - \lambda ABS)$[c]	2.8±5.1	3.2±14.7		9.1±14.3	
$D\%(\lambda VC - \lambda ABS)$[d]	3.7±13.8	3.2±19.1	nd	9.3±15.6	−11.6±10.3
$\text{Log}(N_{\Delta ABS})$[e]	6.4±0.7	7.4±0.5		6.8±0.3	

[a] $D\%(\lambda VC - \lambda ABS)$ values calculated from lag times determined for individual growth curve by the Richards model with values of m fixed to 0.5, 1.0 or 2.0.
[b] $D\%(\lambda VC - \lambda ABS)$ values calculated on the basis of lag times determined from duplicate series of absorbance detection times by using the ANOVA method suggested by Baranyi and Pin (1999).
[c] $D\%(\lambda VC - \lambda ABS)$ values calculated on the basis of $N_{\Delta ABS}$ values determined for each individual growth curve.
[d] $D\%(\lambda VC - \lambda ABS)$ values calculated on the basis of the average of $N_{\Delta ABS}$ value determined for each combination of microorganism and incubation system.
[e] Average $N_{\Delta ABS}$ values calculated for each combination of microorganism and incubation system.
[f] Not determined.

time until absorbance of a culture increases by 0.05 units ($t_{\Delta ABS}$) is easily determined but estimation of $N_{\Delta ABS}$ values can be more problematic as growth conditions may influence the size of microbiological cells and thereby $N_{\Delta ABS}$. With N_0 and $N_{\Delta ABS}$ determined from individual viable count growth curves, average $D\%(\lambda VC - \lambda ABS)$ values were close to zero (Table 4). Thus, lag times were accurately estimated from individual absorbance growth curves, except for micro-plate cultures of non-fermentative micro-organisms, particularly *Pseudomonas* spp., where large negative lag time estimates were obtained (Table 4). Omitting these data, that resulted from poorly estimated μ_{max} values, provided an average $D\%(\lambda VC - \lambda ABS)$ value of -0.9 ± 10.3 (SD) ($n=162$). This showed λVC and λABS to be similar but there was a very close correlation ($r=0.97$) between $R(\mu VC/\mu ABS_{RF})$ and $D\%(\lambda VC - \lambda ABS)$ values showing the small differences between λVC and λABS to be caused, almost exclusively, by differences between μVC and μABS_{RF}.

For practical estimation of lag time from absorbance growth curves, $N_{\Delta ABS}$ values obviously, cannot be determined by viable counts for each

individual growth curve. With average $N_{\Delta ABS}$ values, determined for each combination of microorganism and incubation system, $D\%(\lambda VC - \lambda ABS)$ became -0.9 ± 13.9 (SD) ($n = 162$). In this case, the correlation between $R(\mu VC/\mu ABS_{RF})$ and $D\%(\lambda VC - \lambda ABS)$ values was reduced ($r = 0.92$), indicating that both μABS and $N_{\Delta ABS}$ values influenced lag time estimation. Variation in $\text{Log}(N_{\Delta ABS})$ values, in the present study (Table 4), could not be related to systematic effects of growth conditions on cell size, possibly with the exception of *S. putrefaciens* where cell size increase with temperature of incubation (results not shown). Previously, glycerol was shown to influence cell size of *Bacillus cereus* in such a way that cultures without glycerol and with 7.74 Log(cfu/ml) had the same absorbance as cultures of 7.93 Log(cfu/ml) with 20% glycerol (Chorin et al., 1997). Growth conditions that influence the size if microbiological cells clearly reduce precision of lag times estimated by Eq. (8) from absorbance growth curves where $N_{\Delta ABS}$ in practice must be assumed to be constant. Nevertheless, if growth conditions like glycerol only change $N_{\Delta ABS}$ by 0.2 Log(cfu/ml) lag time estimates in many cases will be sufficiently accurate to be useful in practice.

As shown in the previous section, μABS values estimated by using the Logistic, the Gompertz and the Exponential models were substantially less accurate and less precise than values obtained by the Richards model. Consequently, lag times determined from μABS_L, μABS_G, and μABS_E values will often be inaccurate and of insufficient precision for use in practice. In agreement with this conclusion, lag time of micro-plate cultures of *Listeria monocytogenes* could not be determined accurately from absorbance data by using the Logistic model (Augustin et al., 1999). These authors suggested lag time could be estimated by combined use of absorbance and viable count measurements. In contrast, the present study showed that lag time could be estimated accurately and with a reasonable precision from absorbance growth curves by using the Richards model (Table 4).

Estimation of N_0 values is required for calculation of lag time by Eq. (8). However, when a single pre-culture is used for inoculation of a larger number of absorbance cultures, as in factorially designed experiments, costs and efforts required to determine N_0, e.g., by viable counting or direct microscopy are modest.

3.2.2. Estimation of λ from detection times of serially diluted cultures

Detection times for duplicated series of 10-fold diluted cultures provided an average $D\%(\lambda VC - \lambda ABS)$ value of -5.6 ± 10.9 (S.D.) when calculated by the ANOVA procedure of Baranyi and Pin (1999). Baranyi and Pin (1999) in their study evaluated a higher number of replications of serially diluted cultures but presented no experimental data to show if kinetic parameters obtained by their new method corresponded to values from classical methods like viable count. To estimate lag time from absorbance data, the present study indicates individual absorbance growth curves, in most cases, will be as accurate and more cost efficient than the ANOVA procedure requiring replicated series of diluted cultures. However, for lag time estimation of non-fermentative microorganisms in microplate cultures, dilution methods are more appropriate than individual absorbance growth curves (Table 4). Microplate systems are often convenient but can be difficult to operate at low temperatures and under modified atmosphere conditions. It seems logical to use individual growth curves and serially diluted cultures complementarily and thereby allow lag times to be estimated from absorbance data for wide ranges of microorganisms and growth conditions.

4. Conclusions

The Richards model, with values of the parameter m fixed to 0.5, 1.0 or 2.0, allowed μ_{max} values to be estimated accurately from absorbance growth curves. Accurate μ_{max} values were obtained independently of the growth yield of cultures and for wide ranges of growth conditions. In contrast, the Gompertz and the Exponential models were inappropriate for estimation of μ_{max} values under conditions that influenced growth yields. The Richards model estimated μ_{max} values more precisely than the Logistic model and this enabled lag times to be determined from individual absorbance growth curves. Accuracy and precision of μ_{max} values and lag times obtained by the Richards model, corresponded to values esti-

mated from absorbance detection times of serially diluted cultures.

The application of absorbance measurements, as suggested here, is useful in many areas of microbiology where accurate as well as rapid and inexpensive estimation of microbial growth parameters is required. Clearly, turbidimetry is limited by high detection thresholds of measuring devices and to study growth of pathogenic microorganisms, sometimes important in even very low levels, these techniques are restricted to conditions where high cell densities are reached. In contrast, spoilage bacteria are only important in foods when then growth to high levels occurs. For such microorganisms the absorbance techniques evaluated here will most often be of considerable practical importance.

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