Growth efficiency, growth rate and the remineralization of organic substrate by bacterioplankton — revisiting the Pirt model

Ramón Cajal-Medrano[1,2], Helmut Maske[1,*]

[1]Ecología, Centro de Investigación Científica y Educación Superior de Ensenada (CICESE), Apdo Postal 2732, Ensenada, CP 22880, Baja California, Mexico
[2]Facultad de Ciencias Marinas, Universidad Autónoma de Baja California, Apdo Postal 453, Ensenada CP 22880, Baja California, Mexico

ABSTRACT: The growth yield of aquatic bacteria plays a critical role in the biogeochemical cycle of organic carbon in the sea. The Pirt model (1982) proposes a relationship of specific growth rate (μ, d^{-1}), respiration rate (r, d^{-1}) and growth yield (Y, unitless) of bacteria when the growth rate is limited by the concentration of the organic substrate and carbon stoichiometry is applied. Published data from natural populations of bacterioplankton within a temperature range of 10 to 25°C follow the Pirt model, where the growth yield is approaching asymptotically a maximum value (ε) with increasing growth rate. The specific rate of the maintenance metabolism (a, d^{-1}) is the shape factor defining the curve. From the model a relationship between the specific respiration rate and the growth rate $\{r = [\mu(1/\varepsilon - 1) + a/\varepsilon]\}$ can be derived. Based on the published data these model parameters were estimated: $\varepsilon = 0.51$ and $a = 0.58$. Our model results should help explain the wide range of growth efficiencies reported for natural bacterioplankton.

KEY WORDS: Bacterioplankton · Growth efficiency · Respiration rate · Growth rate

INTRODUCTION

The efficiency of bacteria to consume dissolved organic carbon and to convert it into bacterial biomass ultimately defines their predominant role as mineralizers or biomass producers. This has been paraphrased as the 'link or sink' for organic matter in the ocean (Jumars 1993). The carbon conversion efficiency of marine bacteria greatly depends on the respiration rate, a crucial variable in the budget of the marine carbon cycle (Cole & Pace 1995, Jahnke & Craven 1995). A wide range of values has been reported in the literature, from 0.03 to 0.85. Cole & Pace (1995) showed that the values most frequently reported in the literature are between 0.30 and 0.60. There are many physiological conditions known to alter the growth efficiency of bacteria such as cellular synthesis, extracellular poly-

mer formation, excretion of low molecular weight metabolites, maintenance functions and regulatory processes (Neijssel et al. 1996). Sherr & Sherr (1996) recently suggested that seasonal differences in the growth efficiency may help to explain the temporal offset in oceanic production and respiration. However, there is no published information on the parameters that are driving the bacterial growth efficiency in the ocean ecosystem.

The carbon conversion efficiency or bacterial growth efficiency can be expressed as the growth yield, Y, the ratio of organic substrate utilized by bacteria (S, substrate carbon) that is converted into bacterial biomass (X, biomass carbon), i.e. Y is unitless. Models for bacterial utilization of carbon have been developed by various authors, ranging from the very simple models with 3 state variables (Pirt 1975) to very complex bioenergetic models with 6 state variables (Vallino et al. 1996). None of these models have been used to explain the general patterns of growth yield in the ocean.
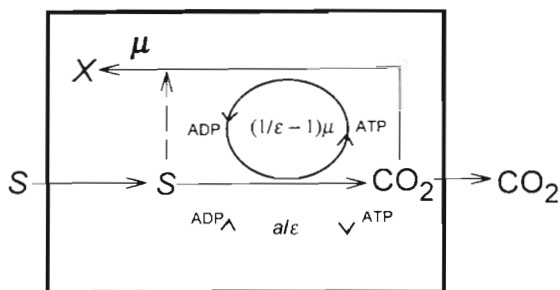
Fig 1. Organic substrate processing by the bacterial cell according to the Pirt model. The substrate, S, is incorporated into the cell, the major fraction is respired to carbon dioxide. According to the model the fraction of the substrate that is directly transformed into biomass (dashed arrow) is insignificant. Part of the respiration is supporting the cellular maintenance (a/ε), indicated by the gray circle, and the other part the biosynthesis ($1/\varepsilon - 1$) μ (black circle). μ: formation of biomass X, predominantly by de novo synthesis from CO_2

Below, the Pirt model is used to interpret published data obtained with natural bacterial populations from temperate, pelagic systems.

The most simple form of Pirt's (1975, 1982) model is considering the following state variables: organic substrate, bacterial biomass and respired carbon. Organic carbon is taken here as a metabolic energy substrate. The Pirt model was developed for conditions where bacterial growth rate is limited by organic carbon, which is probably a common condition in seawater (Morita 1997). For those waters where nitrogen or phosphorus limitation of bacterial growth rate has been documented (Horrigan et al. 1988, Kuparinen & Heinänen 1993, Zweifel et al. 1993, Rivkin & Anderson 1997) the specific form of model discussed below would not be applicable. Pirt (1975) also considered the case of growth rate limitation by inorganic nutrients, but this case will not be considered here. In the case of literature data obtained with natural seawater cultures the possibility of inorganic nutrient limitation cannot be automatically excluded. Experiments where organic substrate was added might be even more likely to lead to inorganic nutrient limitation, at least in the final stages of the logarithmic growth phase.

The Pirt model is built upon the concept that under aerobic conditions the organic substrates are taken up and mainly respired, thus providing metabolic energy (ATP) and reduction equivalents. A minor fraction of organic substrate is used directly or in modified form as biomass, as indicated in Fig. 1 by the dashed arrow, but this route is considered insignificant in the model below. The respiration products are used in principle to synthesize biomass (anabolism, solid line circle in Fig. 1), and to provide energy for the physiological activities that maintain the integrity of cellular bio-

mass, i.e. the turnover of cellular constituents, ionic equilibrium and repair processes (gray line circle in Fig. 1). The energy expended on cell movement might be subsumed under the maintenance metabolism. The model assumes a constant specific rate of respiration serving maintenance; this rate is expected not to change with the specific growth rate under conditions of growth rate limitation by organic carbon. According to the Pirt model (1975, 1982) the consumption of organic carbon (dS) equals gross bacterial production, which is the sum of anabolic bacterial growth or net growth (dX) and the remineralized carbon (dR, CO_2 liberated).

$$dS = dX + dR \qquad (1)$$

The bacterial growth efficiency (Y) is the ratio of net production or growth (dX) to gross bacterial assimilation (dS) (Pirt 1975, 1982). If X equals bacterial biomass

$$Y = \frac{dX}{dS} \qquad (2)$$

Therefore

$$Y = \frac{dX}{dX + dR} \qquad (3)$$

if

$$\mu = \frac{dX}{Xt} \qquad (4)$$

and

$$r = \frac{dR}{Xt} \qquad (5)$$

then

$$Y = \frac{\mu}{\mu + r} \qquad (6)$$

implying that growth efficiency is determined by the ratio of growth rate to remineralization rate. Above, an ideal substrate has been considered that could be converted completely to biomass, with r being independent of the growth rate and with a maximum stoichiometric efficiency of Y given by $\mu_{max}/(\mu_{max} + r)$. Pirt (1982) presented a more general model, introducing a maximum obtainable yield at maximum growth rates and a growth rate independent basal respiration rate serving the maintenance metabolism. This maximum obtainable yield, Y_{EG} (Pirt 1975) or Y_G (Pirt 1982), was conceptually related to the nutritional quality of the substrate. Here we will be using instead the unitless efficiency factor, ε, that defines the biosynthesis efficiency of the substrate into biomass or into energy equivalents for maintenance respiration because it is not the maximum growth efficiency that is defining the substrate allocated to the maintenance metabolism but the efficiency with which the substrate can be converted. According to the model, the specific rate of utilization of the energy source dS is distributed into 2 routes for energy utilization leading to 2 different but

related coefficients: a constant maintenance energy coefficient and a growth rate dependent maintenance energy coefficient. Both processes are indicated by separate cycles in Fig. 1. The substrate demand for both processes can be defined as the substrate equivalents for cellular maintenance, dA/ε, and those to support biosynthesis, i.e. the interconversion of substrate into biomass, dX/ε. If applied to Eqs. (1) and (2) we get:

$$Y = \frac{dX}{\frac{1}{\varepsilon}(dX + dA)} \tag{7}$$

To simplify the units, dA is used here as energy equivalents of carbon dioxide liberated by the oxidation of an ideal organic substrate ($\varepsilon = 1.0$). Note that dA/X is still assumed to be constant for a specific organic substrate and independent of the growth rate as long as this substrate is limiting the growth rate. Pirt (1975) and, in a similar model, Thingstad (1987) considered the efficiency factors for dX and dA in Eq. (7) as independent. Because there is no experimental information available that would allow the separate evaluation of these 2 processes, it will be assumed below that both processes have the same efficiency factors.

In Eq. (7) the consumption of organic substrate was determined by a constant energy demand for maintenance metabolism, $(1/\varepsilon)dA$ (cf. Fig. 1), and the conversion of substrate into biomass, $(1/\varepsilon)dX$ (cf. Fig. 1). The latter term includes the biomass formed, dX, and the organic substrate remineralized to provide the energy for biomass synthesis, $(1/\varepsilon)dX - dX$,

$$\frac{1}{\varepsilon}dX = dX + \left(\frac{1}{\varepsilon} - 1\right)dX \tag{8}$$

The oxidation of organic substrate or production of CO_2 (dR) depends on the maintenance energy plus energy used in biomass synthesis; therefore dR would then be

$$dR = \frac{1}{\varepsilon}dA + \left(\frac{1}{\varepsilon} - 1\right)dX \tag{9}$$

Respiration will oxidize a variable portion of assimilated dissolved organic carbon (DOC) to CO_2 and lead to the concurrent consumption of O_2. The respiration quotient (RQ) can probably vary with the efficiency factor if ε is dependent on the digestibility or the state of reduction of the organic substrate. Solving Eq. (8) for dX and substituting the right-hand part of Eq. (9) for dR in Eq. (3) yields

$$Y = \frac{dX}{\frac{1}{\varepsilon}dX + \frac{1}{\varepsilon}dA} \tag{10}$$

Eqs. (6), (7) & (10) can be recast in terms of specific growth μ and specific respiration rates. a is the specific

rate of the oxidation of organic carbon to serve the energy demand for cellular maintenance $[dA \, (X t)^{-1}]$ (Pirt 1982). The rate of a is in reference to a hypothetical substrate that could be respired with 100% efficiency.

$$Y = \frac{\mu}{\frac{1}{\varepsilon}\mu + \frac{1}{\varepsilon}a} \tag{11}$$

Eq. (9) can be expressed in terms of specific rates (d^{-1}), where $r \, [dR \, (X t)^{-1}]$ is the specific rate of cellular CO_2 production.

$$r = \frac{1}{\varepsilon}a + \left(\frac{1}{\varepsilon} - 1\right)\mu \tag{12}$$

The specific respiration rate, r, can be calculated from the measured growth yield and the growth rate by solving r from Eq. (6).

$$r = \frac{\mu}{Y} - \mu \tag{13}$$

It is clear from Eq. (13) that r will increase linearly with the growth rate.

RESULTS

Data of growth efficiency, respiration and growth rate of pelagic marine and freshwater bacteria under organic substrate limitation, and non-organic carbon limitation were taken from the published literature. The Pirt model (1975) was developed mainly from data obtained with continuous cultures, which by definition are substrate limited. The specific model applied here is only applicable to conditions of energy substrate, i.e. organic carbon limitation. Even though a good number of data of growth efficiency have been published (see Jahnke & Craven 1995, Carlson & Ducklow 1996), not many data sets include the specific growth rate. The published data chosen by us included the variables growth efficiency and specific growth rate, or these variables could be computed by us from other data provided in the publications. In an effort to include as many data sets as possible, some data obtained after the addition of organic carbon were included, but only those where inorganic nutrient limitation was deemed unlikely. Data from cultures where inorganic nutrients were added were included in the list because the addition of inorganic nutrients would not cancel the organic substrate limitation. The specific growth rate, μ (h^{-1}), is reported as the logarithm (base e) of the biomass increase over time. In Table 1 some aspects of the methodology used to obtain the data in the different publications are provided for comparison.

Goldman & Dennet (1991) prepared batch cultures with natural bacteria populations from artificial seawa-

Table 1. Comparison of the methods used to obtain the specific growth rate and the growth efficiency

Source	Specific growth rate calculation	Growth efficiency calculation
Goldman & Dennet (1991)	Logarithmic particulate carbon growth	$Y = \Delta POC/\Delta DOC$
Blum & Mills (1991)	Turnover time $\mu = 1/\text{turnover time}$ $\mu = (T^{\bullet}\, f_1)/(\text{cell } \Delta t)$	$Y = BV\, f_2/(BV\, f_2 + RQ_1\, \Delta O_2)$
Middelboe et al. (1992)	Dilution rate of continuous culture	$Y = \Delta POC/(\Delta POC + \Delta DIC)$
Middelboe & Søndergaard (1993)	$\mu = (T^{\bullet}\, f_3)/(\text{cell } \Delta t)$	$Y = \Delta POC/(\Delta POC + RQ_2\, \Delta O_2)$
Carlson & Ducklow (1996)	Logarithmic cell growth and culture cell volume growth	$Y = \Delta B\, f_4/\Delta DOC$
Kristiansen et al. (1992)	Dilution rate of continuous culture	$Y = \Delta POC/(\Delta POC + \Delta DIC)$

cell (cell ml^{-1}) = concentration of bacterial cells
BV (μm^3) = biovolume
DOC (μM) = dissolved organic carbon measured by organics added minus residual concentration (Goldman & Dennet 1991) or by HTCO (high temperature combustion) (Carlson & Ducklow 1996)
DIC (μM) = total inorganic carbon concentration measured by IRGA (infrared gas analyzer)
ΔO_2 (μM) = change in dissolved oxygen concentration
POC (μM) = particulate organic carbon measured by CHN analyzer
$RQ_1 = \Delta CO_2/\Delta O_2$; $RQ_1 = 1.0$, $RQ_2 = 0.82$
$T^{\bullet}$ (M h^{-1}) = thymidine incorporation
$f_1 = 1.3 \times 10^{18}$ cells (mol ^{3}H – TdR incorporated)$^{-1}$
$f_2 = 354$ fg C μm^{-3}
$f_3 = 2 \times 10^{18}$ cells mol^{-1} of thymidine incorporated
$f_4 = 120$ fg C μm^{-3}

ter, measuring biomass as particulate organic carbon, and they calculated the organic substrate incorporated from the organics added to the media and the residual concentration. Specific growth rate was calculated by regression analysis of the exponential portion of the curve ln POC versus time. The data on specific growth rate were taken from Goldman & Dennet's Table 1 and the corresponding growth efficiency from their Table 3. The experimental temperature was 24°C.

Blum & Mills (1991) used biodegraded seagrass as an organic substrate in their experiments with natural bacterial populations from Chesapeake Bay. Specific growth rate was measured by cell counts and thymidine incorporation, biomass by cell counts and microphotometric evaluation of biovolume using a conversion factor of 0.354 pg C μm^{-3}, and respiration by oxygen consumption with a respiration quotient (RQ) of 1.0. The growth rate was calculated as the inverse of bacterial turnover time in their Fig. 2a and bacterial growth efficiency Y is taken from their Fig. 2b. The text does not indicate the temperature, but we suggest that the temperature was within the range of temperatures given by the other authors, between 10 and 25°C.

Middelboe et al. (1992) prepared batch and continuous cultures inoculated with natural populations from an estuary. μ was calculated from the generation time (g) in their Table 2, applying $\mu = 0.693/g$. Growth efficiency (Eq. 3) was calculated from net production and

respiration data in the same table. Respiration was measured by changes in inorganic carbon and biomass production from particulate organic carbon. Bacterial carbon content was measured by cell counts and POC determination using a factor of 42 ± 7 to 56 ± 10 fg C cell^{-1}. The temperature ranged from 10 to 19°C during the measurements.

In the Middelboe & Søndergaard (1993) study, batch cultures were inoculated with a natural bacterial population from a freshwater lake to obtain bacterial biomass and bacterial production (their Fig. 2) and the corresponding bacterial growth efficiency (their Fig. 3a). The respiration was calculated from the oxygen consumption using an RQ of 0.82. The temperature during the experiments was 18°C.

Carlson & Ducklow (1996) used Sargasso Sea water for batch cultures, measuring the biovolume for biomass estimates and organic substrate incorporated was measured as DOC consumption during the log phase of batch culture growth. Their data for specific growth rate (their Table 3, column 'Growth property' and 'Abundance') and growth efficiency from each treatment were used. The temperature for their measurements varied between 19 and 25°C.

Kristiansen et al. (1992) calculated growth efficiencies in continuous freshwater cultures obtained with enriched, normal and aged lake water after removal of CO_2 and pH adjustment. Data of growth efficiency and specific growth rate were taken directly from their
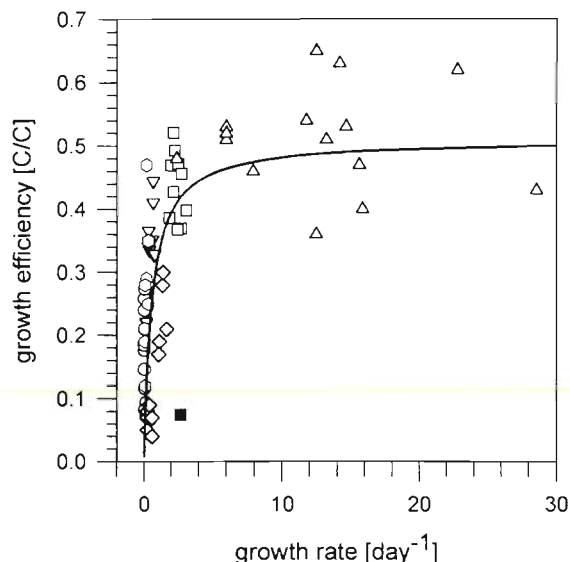
Fig. 2. Growth efficiency versus specific growth rate taken from published data. O (Blum & Mills 1991), △ (Goldman & Dennet 1991), ▽ (Middelboe et al. 1992), □ (Middelboe & Søndergaard 1993), ◇ (Carlson & Ducklow 1996), ○ (Kristiansen et al. 1992), ■ outlier (Middelboe & Søndergaard 1993). The line represents the growth efficiency modeled with $a = 0.58$ and $\varepsilon = 0.51$
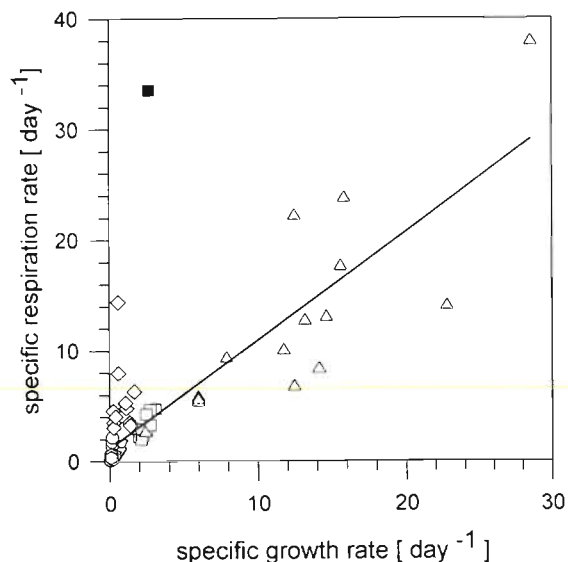
Fig. 3. Respiration versus growth rate. Respiration was calculated using Eq. (12). Linear regression yielded a coefficient of determination, $r^2 = 0.77$ ($n = 75$, $p < 0.01$) with a slope of 0.98 $\pm$ 0.06 ($p < 0.01$) and an intercept of 1.14 ± 0.4 ($p < 0.01$) from the whole data set minus the outlier (■). Symbols are the same as in Fig. 2

Table 4. Specific growth rate was calculated from their generation time (Table 4) applying $\mu = 0.693/g$. The temperature was about 10°C.

The data from the above publications are presented graphically in Fig. 2. The data show a steep increase in Y at very low growth rates and an asymptotic approach to a maximum growth efficiency of approximately 0.5 at higher growth rates. The general pattern of the data distribution suggested that the data could be interpreted by the Pirt model. Scatter of the data (Fig. 2) is expected because they were obtained in different environmental situations and with different methodologies. The different data sets were taken at temperatures ranging from 10 to 25°C; however, the data do not show any obvious co-variation with temperature.

Eq. (12) can be plotted with r on the ordinate and μ on the abscissa, with a slope of the gradient $(1/\varepsilon) - 1$ and an intercept of a/ε (Fig. 3). A linear regression of all the data presented in Fig. 2 yielded a slope (0.98 $\pm$ 0.09, $n = 76$, $p < 0.01$) and intercept (1.55 $\pm$ 0.6, $n = 76$, $p < 0.01$). One data point taken from Middelboe & Søndergaard (1993) in Fig. 2 and Fig. 3 (filled square) is an obvious outlier in comparison to the other data in the figures. This data point already deviated from the trend in the original time series (Middelboe & Søndergaard 1993, Fig. 3) and we decided to calculate the regression slope in Fig. 3 again without this data point, resulting in a slope of 0.98 $\pm$ 0.06 ($n = 75$, $p < 0.01$) and an intercept of 1.14 ± 0.4 ($n = 75$, $p < 0.01$). The reason

for excluding this single data point was not so much to increase the statistical significance of the data set but to avoid a bias in the quantitative estimate of the parameter values. All further data analysis was done with the data set where the 1 data point was excluded. From the slope ε was calculated as 0.51 and from the intercept a was estimated as 0.58. From these parameter values and Eq. (12), the respiration rate ($C_{CO_2}\,C_{biomass}^{-1}\,d^{-1}$) can be expressed as:

$$r = \mu + 1.1 \qquad (14)$$

From Eqs. (6) and (14), Y can be modeled as a function of growth rate and compared to the published values (Fig. 2). The comparison shows considerable noise at low growth yields, i.e. at low growth rates, yielding a low determination coefficient, $r^2 = 0.57$. The growth yield published and that calculated from the growth rate using Eqs. (6) & (13) are compared in Fig. 4. The intercept and slope in Fig. 4 are not significantly different from 0 and 1 respectively. In the figure the data by Carlson & Ducklow (1996) tend to lie systematically on one side of the regression line. The comparison of the respiration rate estimated from the published growth rate and yield, and the modeled respiration rate (Fig. 5) yielded a determination coefficient of 0.77 without a significant difference in the slope from 1 and in the intercept from 0. Again the data by Carlson & Ducklow (1996) appear to be separate from the other data.
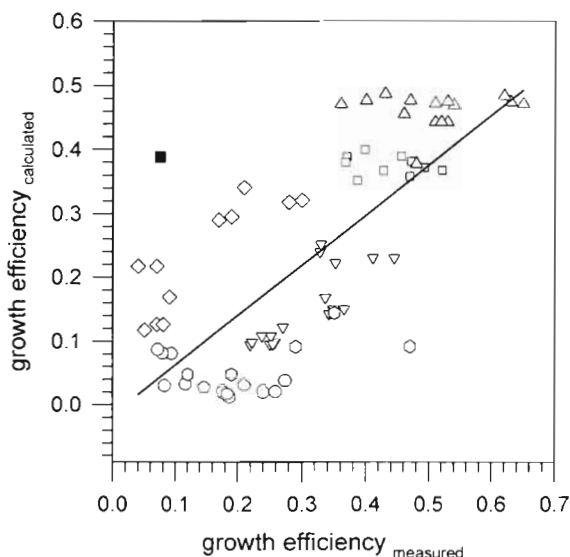
Fig. 4. Comparison of measured and calculated growth efficiency. $r^2 = 0.58$ (n = 75) and the intercept and slope are not significantly different from 0 and 1 respectively. Symbols are the same as in Fig. 2

Fig. 5. Comparison of measured and modeled respiration rate. Linear regression yielded a $r^2 = 0.77$ (n = 75, p < 0.01). The intercept and slope are not significantly different from 0 and 1 respectively. Symbols are the same as in Fig. 2

DISCUSSION

The model applied here assumes growth rate limitation by organic substrate, but the model does not calculate growth rate from ambient conditions and no mechanisms for the growth rate limitation by organic substrate are assumed. The model is inherently boundary-limited at very low growth rates, because the physiological behavior of bacterial cells in this range is little known and can vary strongly. There are reports for continued respiration of bacteria during no growth (Nyström & Kjelleberg 1989), but also of reduced physiological activity in a semi-dormant state (Novitsky & Morita 1977). The current literature discussion about the physiology of 'viable but non-culturable cells' (cf. Bloomfield et al. 1998) demonstrates the lack of knowledge about the rate of physiological activity at low growth rates. An empirical lower limit for the application of the Pirt model could be defined as 0.01 d^{-1}; this is slightly lower than the lowest published rate in the data applied here. Other boundary conditions for the model would constitute those conditions that would change the growth yield or the specific respiration rate independent of the growth rate. These conditions might include the accumulation of storage material, or the production of extra-cellular polymeric matrices. Several factors have been suggested to change the specific respiration rate, such as viral infection (Middelboe et al. 1996), the degree of substrate reduction (Vallino et al. 1996), temperature (Sherr & Sherr 1996), the C/N ratio (Goldman et al. 1987, Goldman & Dennet
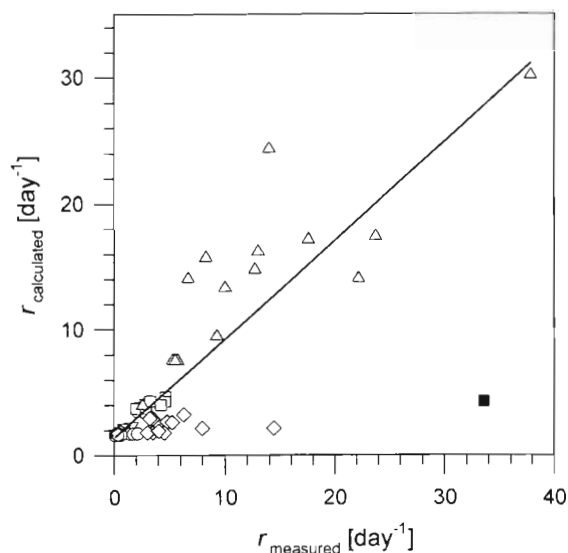
1991, Kroer 1993), addition of ammonium (Kroer 1993), inorganic nutrient limitation (Pirt 1982, Zweifel et al. 1993), oxygen partial pressure (Pirt 1975), and the excretion of organic polymers (Neijssel et al. 1996). When the Pirt model is applied to a combination of different published data sets, it has to be assumed that these data were taken under organic substrate limitation and that the above listed environmental factors did not modify the bacterial growth efficiency to a significant degree.

The data in Fig. 2 follow an asymptotic curve as expected from the Pirt model, with the growth efficiency approaching ε at high growth rates. Despite the different methods applied to obtain the data sets (Table 1), the model seems to fit the data quite well. At low growth rates the growth efficiency increases steeply. Small errors in the estimation of specific growth rate could explain much of the noise in the data in Fig. 2. The differences between data sets can be due to the type of methods applied (Table 1). These differences are difficult to evaluate quantitatively. Also, different conversion factors have been applied by the authors (Table 1), e.g. for the RQ or the conversion factors from cells to cellular carbon. One example how the results of 2 different methods based on either biovolume or cell counts to estimate growth rate can give significantly different values is given by Carlson & Ducklow (1996). When the growth rate data based on biovolume were entered into the model analysis, more data dispersion and generally higher estimates were found than with growth rate based on cell counts. Sim-
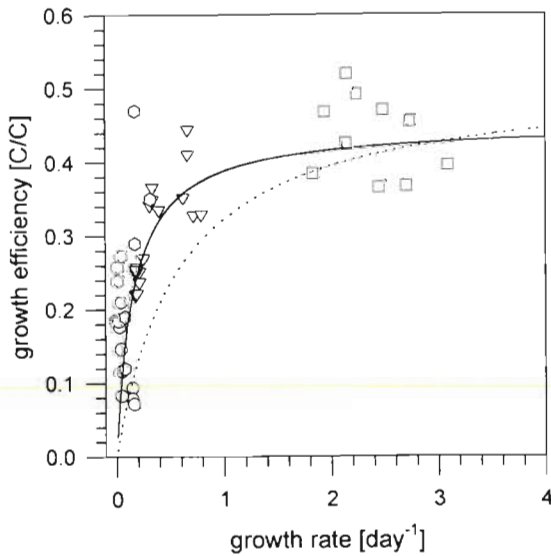
Fig. 6. Growth efficiency and specific growth rate of the data published by Blum & Mills (1991), Middelboe et al. (1992), Middelboe & Søndergaard (1993) and Kristiansen et al. (1992). (—) Modeled relationship calculated with a = 0.16 and ε = 0.4 (n = 49). ($\cdots$) Modeled relationship for all data (Fig. 2) with a = 0.58 and ε = 0.51. Symbols are the same as in Fig. 2
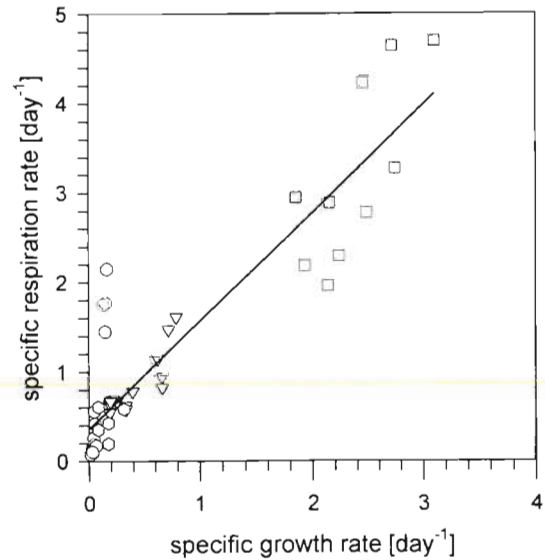
Fig. 7. Respiration versus growth rate based on the data by Blum & Mills (1991), Middelboe et al. (1992), Middelboe & Søndergaard (1993) and Kristiansen et al. (1992). The regression line yielded a = 0.16 and ε = 0.4 (r^2 = 0.85, n = 49, p < 0.01). The intercept and slope are not significantly different from 0 and 1 respectively. Symbols are the same as in Fig. 2

ilarly Troussellier et al. (1997) found that the conversion factor cell carbon per cell is more constant than the carbon per cell volume. The above discrepancies are pointed out here to demonstrate that much of the noise in Fig. 2 is probably methodological and not biological.

The most salient features of the Pirt model are the assumption of a constant maintenance respiration, a/ε, and a growth rate dependent respiration term, $(1/\varepsilon - 1)\mu$. The concept of maintenance respiration was introduced by Pirt (1965) and since then it has been supported by others (Koch 1997). There is some evidence that the value for the maintenance energy can be a large portion of the total cellular energy budget (Pirt 1982), but no typical value for maintenance energy in different physiological situations including those in the ocean ecosystem are known. Morita (1997) and others have questioned the existence of significant maintenance respiration because bacteria can survive low nutrient environments for many days without loss of biomass. As mentioned above, the Pirt model should not be applied to extremely low growth rates because of the ill-defined physiological state. In the range of measurable growth rates, it can be assumed that some energy generated from the oxidation of organic carbon must be spent on the maintenance of cell integrity and viability (e.g. turnover of macromolecules, maintenance of ion gradients, motility, etc.). Neijssel et al. (1996) suggested that a partial uncoupling of catabo-

lism from anabolism may influence the variability of a for a certain bacterial population. In Fig. 1 the direct conversion of substrate is indicated by a broken arrow. If this metabolic route would become quantitatively significant compared to the respiration of the substrate, then the biosynthesis metabolism (solid line circle, Fig. 1) would have to be modeled with an ε that would be higher than the ε for the maintenance metabolism (gray line circle, Fig. 1).

In Fig. 2 the different data sets show little overlap in growth rates. The data would represent the model better if single data sets would cover a greater range of growth rates. The asymptotic pattern of the data in Fig. 2 is largely supported by the high growth rate data from Goldman & Dennet (1991), because most of the other data lie in the region of low growth rates (<4 d^{-1}) and strongly variable growth yield. A subset of data without the Goldman & Dennet (1991) data can only be modeled with statistical significance if the data by Carlson & Ducklow (1996) and 1 outlier data point by Middelboe & Søndergaard (1993) are also excluded from the data (Fig. 6). The regression of r against μ of this reduced data set (Fig. 7) yielded a determination coefficient of 0.85 (n = 49, p < 0.01). The estimate of the ε = 0.45 and a = 0.16 parameters are somewhat different from the parameters estimated from the complete data set. The statistical significance of this reduced data set demonstrates that the general model behavior is supported by the data set without the data by Gold-

man & Dennet (1991). When the single data sets are tested by themselves, only the data from Middelboe et al. (1992) resulted in a statistically significant regression between r and μ, yielding a determination coefficient of 0.76 (n = 18, p < 0.01). The estimate of ε and a from the slope and intercept of this regression are 0.44 and 0.13 respectively. In the 3 cases calculated here, ε, the maximum attainable growth efficiency, was similar: 0.5, 0.45, 0.44. These value are close to the average value extracted from a review of literature values (Cole & Pace 1995), and they are not very different from the maximum growth yields reported for glucose or other monomeric organic substrates (Pirt 1975). In natural waters the dissolved organic substrate might be expected to yield lower growth efficiencies, because it is probably less reduced and more difficult to assimilate due to the steric configuration of the substrate molecules, but this might be compensated by the uptake of different and complementary molecules. Novitzky & Karl (1985) among others reported higher growth efficiencies for natural benthic bacterial populations. At this moment the data are insufficient to explain growth efficiencies above 0.5. There are environmental conditions known to reduce the growth efficiency in Eq. (14), e.g. inorganic nutrient limitation (Pirt 1982) and probably also higher temperature. Under inorganic nutrient limitation, r should increase at lower growth rates, i.e. under stronger inorganic nutrient limitation (Pirt 1982), which is the opposite trend to Eqs. (14) & (13). The data from Carlson & Ducklow (1996) (Fig. 2) could be best modeled with a higher maintenance respiration rate and therefore it might be argued that these data were taken under conditions of inorganic nutrient limitation. In fact Carlson & Ducklow (1996) suggest that during their experiments a secondary nutrient limitation might have occurred. The authors also discussed the relatively low growth efficiency obtained in their experiments, and the dependence of these values on the conversion factor (f_4, Table 1). Both the inorganic nutrient limitation or the conversion factor might explain the divergence of the Carlson & Ducklow (1996) data from the general trend of the other data, but given the limited data and the general lack of information on the physiology of growth efficiency, there is no sufficient basis for a comparative discussion of the data sets.

The growth efficiency function is dependent on the respiration rate. Temperature in turn has a pronounced effect on the respiration rate and therefore can be expected to affect growth efficiency. Some authors have found a linear correlation between growth efficiency and temperature that was positive (Barillier & Garnier 1993), negative (Daneri et al. 1994), or did not show any correlation at all (Kroer 1993). The temperature in the present literature data set ranged from 10 to 25°C showing no obvious co-variation with growth efficiency; however, increased respiratory activity is expected with a temperature increase (Sherr & Sherr 1996). In general it is not known if respiration and substrate uptake are affected in different ways because of technical difficulties to separate the effect of substrate and temperature.

The maintenance respiration, given by a/ε from these 3 different estimates, was 1.14, 0.36, and 0.3 respectively, for the total data set, the reduced data set and Middelboe et al. (1992). These considerable differences can partially be explained by the errors in the growth rate and the yield estimate. But it can also be expected that there are considerable physiological differences at very low growth rates, as mentioned above, and there is no consensus at this moment under what conditions the physiological activity is changing and on what scales. The Pirt model can estimate the specific respiration rate at different growth rates, even if the natural bacterial community is composed of different physiological fractions, e.g. a growing and a viable but non-growing fraction (Kogure et al. 1987, Roszak & Colwell 1987, Zweifel & Hagström 1995, Joux & LeBaron 1997). The community metabolism can then be calculated from the biomass ratios and the specific growth rates of each fraction, but only if the same ε and a would apply to the different physiological fractions.

One underlying assumption in the analysis above is a constant quality of the organic substrate and hence a constant growth efficiency. The effect of the quality of the organic substrate on bacterial utilization of organic carbon was investigated by Vallino et al. (1996). They extracted from the literature the following relationships of the state of reduction of the limiting organic substrate (φ) to the maximum growth rate (Eq. 15) and to the growth yield at maximum growth rate (Eq. 16):

$$\mu_{max} = 0.18\varphi^2 \qquad (15)$$
$$Y = 0.17\varphi - 0.038 \qquad (16)$$

If Eq. (15) is substituted into Eq. (16) a relationship of Y versus μ_{max} under unlimited substrate concentrations is found (Fig. 8). Eqs. (15) & (16) should only be valid at growth rates <6.0 d^{-1} because at higher growth rates the growth efficiency would exceed 1. Fig. 8 is fundamentally different from Fig. 2 because Fig. 8 is valid for growth conditions unlimited by the concentration of specific substrates, but limited by the quality of the substrate, whereas Fig. 2 is valid for growth conditions limited by the concentration of organic substrate. Theoretically Fig. 8 should describe an outer envelope, and the Y versus μ curves obtained for different substrate types should apply only to growth rates below the curve in Fig. 6. This is obviously not the case in Fig. 2, where the maximum Y of 0.5 is relatively low, but this Y is obtained at much higher growth rates than
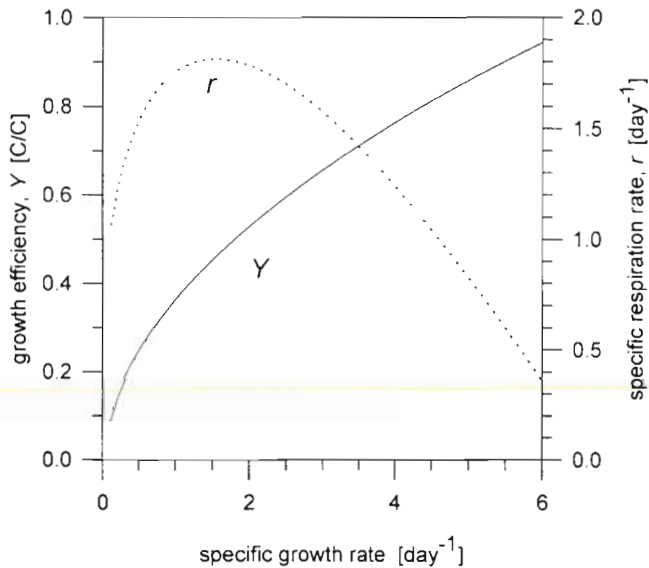
Fig. 8. Growth efficiency and specific respiration rate versus maximum specific growth rate (Vallino et al. 1996)

in Fig. 8. The difference might be explained partly by the noisy data from which Eqs. (15) & (16) were derived (Vallino et al. 1996). In addition, the bacteria in most of the data sets analyzed by us were growing on multiple substrates, whereas the data in Fig. 8 were obtained from cultures supplied with single substrates. There is an obvious need for further research to resolve this difference.

There are few data available in the literature describing the general relationship of growth efficiency, specific growth rate and CO_2 respiration of heterotrophic aquatic bacteria. The limited number of data sets analyzed here were obtained with various methods, but still a general pattern can be observed. We are proposing that, within a limited temperature range of approximately 10 to 25°C, this general pattern can be explained by the Pirt model, and Eqs. (6) & (12) can be applied to ecosystem models for regions where the bacterial growth rate is not limited by inorganic nutrients. There is an obvious need for more growth yield data obtained with natural populations and these future data will show to what extent parameters other than growth rate, as outlined above, will modify the general pattern.

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