Annu. Rev. Ecol. Syst. 1998. 29:503–41

BACTERIAL GROWTH EFFICIENCY IN NATURAL AQUATIC SYSTEMS

Paul A. del Giorgio[1] *and Jonathan J. Cole*[2]
[1]Horn Point Laboratory, University of Maryland Center for Environmental Science, P.O. Box 775, Cambridge, Maryland 21613 and [2]Institute of Ecosystem Studies, Box AB, Millbrook, New York 12545-0129

KEY WORDS: bacteria, plankton, respiration, production, organic carbon

ABSTRACT

Heterotrophic bacteria perform two major functions in the transformation of organic matter: They produce new bacterial biomass (bacterial secondary production [BP]), and they respire organic C to inorganic C (bacterial respiration [BR]). For planktonic bacteria, a great deal has been learned about BP and its regulation during the past several decades but far less has been learned about BR. Our lack of knowledge about BR limits our ability to understand the role of bacteria in the carbon cycle of aquatic ecosystems. Bacterial growth efficiency (BGE) is the amount of new bacterial biomass produced per unit of organic C substrate assimilated and is a way to relate BP and BR: BGE $=$ (BP)/(BP $+$ BR). Estimates of BGE for natural planktonic bacteria range from <0.05 to as high as 0.6, but little is known about what might regulate this enormous range. In this paper we review the physiological and ecological bases of the regulation of BGE. Further, we assemble the literature of the past 30 years for which both BP and BR were measured in natural planktonic ecosystems and explore the relationship between BGE and BP. Although the relationship is variable, BGE varies systematically with BP and the trophic richness of the ecosystem. In the most dilute, oligotrophic systems, BGE is as low as 0.01; in the most eutrophic systems, it plateaus near 0.5. Planktonic bacteria appear to maximize carbon utilization rather than BGE. A consequence of this strategy is that maintenance energy costs (and therefore maintenance respiration) seems to be highest in oligotrophic systems.

0066-4162/98/1120-0503$08.00

INTRODUCTION

Bacteria are the most abundant and most important biological component involved in the transformation and mineralization of organic matter in the biosphere (20, 109, 155). Heterotrophic bacteria contribute to the cycles of nutrients and carbon in two major ways: by the production of new bacterial biomass (secondary production) and by the remineralization of organic carbon and nutrients. Understanding this dual character of planktonic bacteria in aquatic ecosystems is a central paradigm of contemporary microbial ecology (12, 37, 109). Much of the primary production in aquatic ecosystems is ultimately processed by planktonic bacteria. Comparative studies of a wide range of natural aquatic systems show that planktonic bacterial production is correlated with and averages about 30% of net primary production (NPP) (26, 37). The real magnitude of organic carbon flow through bacterioplankton remains largely unknown, however, because measurements of bacterial production are seldom accompanied by measurements of bacterial respiration (BR) (64). The amount of organic C assimilated by bacteria (A) is the sum of bacterial secondary production (BP) and BR. In most studies of organic carbon flow in aquatic ecosystems, this respiration term is derived from measurements of BP and assumed values of bacterial growth efficiency (BGE). BGE is defined as the ratio of BP to A. Thus, $\mathrm{BGE} = \mathrm{BP}/(\mathrm{BP} + \mathrm{BR}) = \mathrm{BP}/\mathrm{A}$.

Assumed values of BGE are often based on early measurements made with simple radiolabeled organic compounds (29, 59), and these values are now widely regarded as overestimates of the real growth efficiency of natural bacterioplankton that utilize complex natural substrates (17, 24, 32, 64, 83). Relative to the large body of data that has been gathered on BP and other microbial processes in the last 20 years, surprisingly little is known about BR and BGE and their regulation in natural systems. Our lack of knowledge is due to two factors. First, BR is simply more difficult to measure accurately than is BP. Second, there has been a general belief that rates of catabolism and anabolism are tightly coupled and that maximum efficiency and economy are achieved during growth. One of the ideas that we develop here is that catabolism and anabolism are not well coupled. This uncoupling provides bacteria with the metabolic flexibility necessary to cope with the vicissitudes of a largely oligotrophic and ever-changing environment.

In this review, we attempt to synthesize the results of research on bacterioplankton growth efficiency done in the last 30 years, focusing on data from natural ecosystems. Because most research on bacterial energetics has been conducted on pure bacterial cultures growing on defined media, we also briefly review current paradigms in microbial energetics. We then assemble direct measurements of BP, BR, and BGE taken from the literature and try to synthesize

the state of knowledge of BGE and the factors that regulate it in natural aquatic ecosystems. This review focuses on aerobic, planktonic, heterotrophic bacteria. These bacteria utilize organic compounds to derive both their energy and carbon requirements and are responsible for the bulk of microbial biomass and activity in the water column of lakes and oceans.

Conceptual Framework

By definition, growth efficiency (or yield—here used interchangeably) is the quantity of biomass synthesized per unit of substrate assimilated. In the process of growth, various compounds, elements, and minerals are converted into cell material at the expense of the energy source (Figure 1). An organic substrate

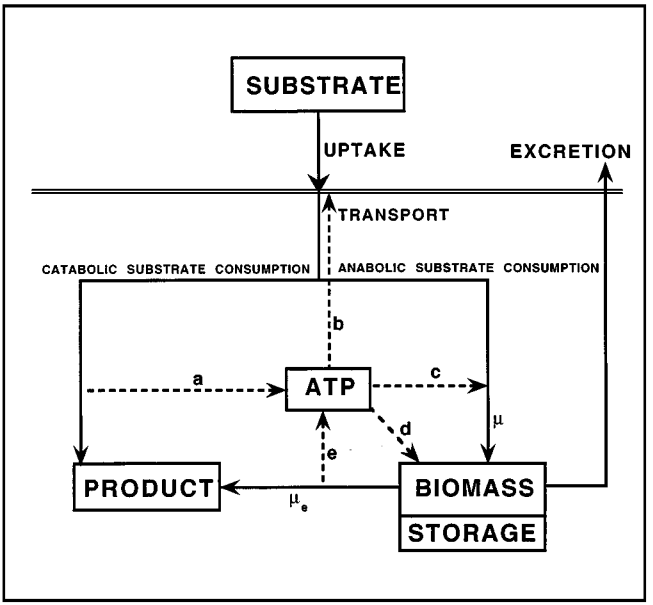


Figure 1 Simplified depiction of catabolic and anabolic pathways that influence BGE in aquatic bacteria. The oxidation of organic compounds contributes to the energy pool as ATP at a rate *a*. Active transport of substrates into the cell requires energy from this ATP pool at a rate *b*; anabolic reactions utilize ATP at a rate *c* and result in a growth rate μ. The anabolic pathways result not only in increases in biomass but also in storage products and organic compounds that may be excreted back to the medium. Maintenance expenditures consume ATP at rate *d*. In the absence of exogenous substrates, minimum maintenance energy requirements must be supported by degradation of biomass through endogenous metabolism (μe), which supplies ATP at a rate *e*. Endogenous metabolism is defined here as the state when no growth is possible, and by definition BGE is 0 under these conditions (116, 117). Adapted from Reference 6.

taken up by a bacterial cell will be partly used in catabolic reactions to generate ATP and partly used in anabolic reactions for biomass synthesis (31) (Figure 1). The purpose of this scheme is to emphasize that multiple processes determine growth efficiency. Each process may respond to a different set of controlling factors; the energy and carbon content of the organic substrate may determine whether it will be preferentially catabolized or incorporated into structural components; energy expenditures in active transport may be related to the concentration, variety, and nature of the exogenous substrates as well as to growth rate; excretion may be the result of energy-spilling reactions or may be an active process, as in the case of the production of exoenzymes; and storage may be a function of the physiological conditions of cells and also of the nature of organic substrate that is utilized. Thus, it is unlikely that growth efficiency in bacteria is regulated by a single factor.

Growth Efficiency in Bacterial Cultures with Defined Media

We cannot provide all the information on microbial bioenergetics in pure cultures, and so we refer the reader to several excellent reviews (100, 101, 117, 135, 140). In brief, there has been a continuous search for regular behavior in BGE by normalizing yield to substrate consumed (94) or to energy produced from the substrate (Y_{ATP} sensu Bauchop & Elsden [4]). The modern view, based on continuous-culture techniques, suggests that BGE is not constant, regardless of the parameter to which it is normalized (6, 116, 117, 133, 134). Continuous-culture techniques allow the growth rate to be varied over a wide range by varying the dilution rate. Whereas unconstrained growth of bacteria in batch culture often led to a rather constant yield for any given substrate, the constraining conditions of chemostat culture could provoke an enhanced rate of catabolism and variable and lower yields (134, 140). As experimental data accumulated, it become clear that Y_{ATP} was also not constant but varied at least sixfold around the presumably fixed value postulated by Bauchop & Elsden (4). Furthermore, theoretical calculations of the amount of energy (as ATP) that would be needed to synthesize bacterial biomass showed that measured bacterial yields in virtually all cases were much lower than those predicted from biochemical pathways (133). Subsequent research has confirmed that yields, whether based on substrate consumption, energy production, or thermodynamic efficiency, are usually at least 50% below expected values (57, 100–102), even in energy- and nutrient-sufficient cultures. Some of the variation and the generally low BGE can be explained by maintenance requirements.

MAINTENANCE ENERGY Metabolic energy is distributed between two kinds of demands: the demands of biosynthetic processes that produce a net increase in biomass, and the demands of processes that do not (for example, regulation of

internal pH and osmotic pressure, macromolecular turnover, membrane energization, and motility [102]). A common assumption is that the rates of energy demands of biosynthetic processes change in a continuous manner with specific growth rate while the energy demand of maintenance processes remains constant (116). Thus, maintenance energy becomes an increasingly greater fraction of the total energy flow in the cell at low growth rates, and growth efficiency declines. However, the concept of a constant maintenance energy requirement has been repeatedly challenged in recent years, because it has been experimentally shown that values vary by more than 30-fold (6, 135, 138–140). Variations in maintenance requirements have often been difficult to explain but are taken as evidence that bacteria often utilize large amounts of energy in reactions that are not directly related to growth, particularly when growth itself is constrained (117).

COUPLING BETWEEN CATABOLISM AND ANABOLISM Results from experimental studies suggest that when growth is unconstrained, as in batch cultures, there is often a high degree of coupling between catabolism and anabolism. When growth is constrained by the supply of organic substrate or inorganic nutrients, as in most chemostat studies and certainly in most natural situations, different degrees of uncoupling are invariably observed. Washed suspensions of bacteria, for example, oxidize substrates such as glucose at a high rate under conditions at which cell synthesis is severely impeded (138). This uncoupling is manifested in various ways: high rates of oxygen and organic substrate consumption, metabolite overproduction and excretion, excess heat production, and energy-spilling pathways (117). All these processes result in reduced growth efficiency. Whereas growth is dependent on efficiency (i.e. cells must consume nutrients to grow), the reverse does not follow: Cells do not have to grow to consume carbon substrate. Anomalies in BGE are often found at low growth rates when growth is limited by some substrate other than the energy source. In general, catabolism appears to proceed at the maximum rate at which the organisms are capable under the conditions, irrespective of whether the energy so produced can be used for biosynthesis (116). Under conditions of severe constraints to growth, it has been suggested that maintaining the highest possible flow of energy would be advantageous (116, 117, 140). One of the potential advantages of a high energy flux in the cell may be to maintain the energization of cell membranes and the function of active transport systems, both of which are essential conditions for resumption of growth whenever environmental conditions change (31, 96). The conclusion that it is advantageous and even necessary for bacteria to maintain a high flow of energy is supported by thermodynamic analysis of microbial energetics, which suggests that microbial growth efficiency is usually low but is optimal for maximal growth (152).

Years of experimental work on microbial energetics have shown that even under the simplest culture conditions it is often difficult to predict bacterial growth efficiency. The difficulty in predicting BGE stems from the fact that bacteria can alter the coupling between catabolism and anabolism to maximize growth according to the conditions (117, 139). These considerations are particularly relevant in our interpretation of bacterioplankton energetics, because planktonic bacteria occupy an extremely dilute environment, in which energy, carbon, and other nutrients are often limiting and growth is usually slow. Therefore, it is expected that maintenance energy requirements, as well the nature of the organic substrates utilized by bacteria and the availability of nutrients, would play a significant role in determining BGE and that bacterioplankton should generally be in a region of low BGE. It is also expected that planktonic bacteria should exhibit a relatively large degree of uncoupling between catabolism and anabolism compared to their cultured counterparts. As discussed below, the data from natural aquatic systems generally support these expectations.

BGE IN NATURAL AQUATIC SYSTEMS

Measuring BGE

Measurement of BGE continues to be a challenge to microbial ecologists. Early studies monitored the uptake, incorporation, and respiration of simple radiolabeled compounds (59). The advantage of this approach is its high sensitivity, which allows rates of uptake and respiration to be measured in short incubations even in the most unproductive aquatic systems. The main disadvantage is that during these short incubations, the intracellular carbon pools may not attain equilibrium and so BGE can be grossly overestimated (14, 73). In addition, the single model compounds may not be representative of the range of substrates utilized by bacteria in nature. The use of single radiolabeled compounds has largely been replaced by techniques that attempt to measure the BGE of bacteria utilizing the in situ pool of organic matter. Two main approaches are used for this purpose.

1. The first is simultaneous measurements of BR and BP in relatively short (usually $<$36 h) incubations (11, 19, 24, 30, 49, 81, 107). There are two difficulties here. First, although BP can be measured in an incubation of $<$1 h, obtaining a measurable change in BR can take 24 h or more depending on the system. Second, bacteria must be physically separated from other planktonic components. This is usually attempted by filtration in the 2- to 0.6-μm range. Complete separation is seldom achieved, so a variable fraction of the measured BR is due to organisms other than bacteria. In addition, filtration disrupts the structure of the bacterial assemblage. Organic C consumption is

approximated as the sum of BP and BR. BR is generally measured as O_2 consumption (11, 19, 49) or, more rarely, as CO_2 production (54). BP is generally measured from the rate of protein or DNA synthesis, using radiolabeled leucine or thymidine, although in some studies the changes in bacterial abundance and size are monitored.

2. The second approach is dilution culture, in which filter-sterilized water is reinoculated with a small amount of the native bacterial assemblage and the subsequent growth of these bacteria is monitored, generally for days or weeks (14, 17, 80, 144, 156). In this type of long-term experiment, it is possible to monitor the changes in dissolved organic carbon (DOC) and particulate organic carbon (POC), and BGE is then calculated as $\Delta DOC/\Delta POC$. The obvious difficulty is the exceedingly long incubation and possible deviation from natural conditions.

There have been no explicit comparisons of BGE estimated from short- and long-term experiments, and both approaches have problems. Whichever approach is taken, bacteria are isolated from their natural sources of DOC, and separation of bacteria from microbial grazers also uncouples pathways of nutrient regeneration which may be important in maintaining higher BGE in natural systems. In long-term experiments, there may be increasing use of refractory DOC fractions and depletion of nutrients, and therefore the resulting estimate of BGE should be generally lower than in short-term incubations, in which presumably only the most labile fraction of DOC is utilized. Growth of heterotrophic nanoflagellates is almost inevitable in long-term incubations, and the resulting grazing may heavily affect the accumulation of bacterial biomass and the apparent BGE (43, 66, 83). Also, in long-term experiments the accumulation of toxic metabolic by-products may result in lower BGE (82). The actual consumption of DOC can seldom be directly measured in short-term experiments, and the assumption that BR + BP approximates C consumption does not always hold (18). Regardless of the time of incubation, there can be considerable variation in BR and BP rates (109, 129, 142), so the length of the incubation and the integration method for these rates become critical for the calculation of BGE (129). In general, it is thought that reducing the incubation times to hours results in ecologically more relevant data, but in many natural samples this is not possible with current methods.

All the methods used in determining BGE involve assumptions and the application of conversion factors, which contribute to the large variability observed in BGE. Some critical assumptions deal with the conversion of bacterial abundance to carbon, and a wide range of factors are used. Whereas some investigators measured the bacterial cells to estimate volume (119), others assumed a fixed cell size or carbon content per cell (8, 76, 118). Likewise, there is variability

in the conversion factors used for the calculation of BP (11, 19). Respiratory quotients (RQ) assumed by authors also vary (49, 91), although it is generally assumed that RQ = 1 and it is likely that RQ is a minor source of error compared to the problems discussed above.

Patterns in BGE in Natural Aquatic Systems

UPTAKE AND INCORPORATION OF SINGLE SUBSTRATES Early research with radiolabeled single substrates demonstrated that growth efficiencies vary consistently among compounds or families of compounds. Amino acids are generally incorporated more efficiently (range, 40 to >80%) than sugars (<60%), for example; however, in general, simple substrates are incorporated with apparent efficiencies of more than 40% (29, 65). These BGE values were assumed to be representative of in situ bacterial processes, and for the next 20 years microbial ecologists applied a BGE range from 40 to 60% in ecological studies (26, 114). King & Berman (73) have shown that intracellular isotope dilution and non-steady-state conditions result in high apparent incorporation of radiolabeled compounds into biomass and therefore in an overestimation of BGE. Moreover, bacteria use many organic substrates simultaneously, and extrapolation from a single model compound may have led to significant overestimation of natural BGE (14, 49, 61, 64). The data on single compounds have been reviewed extensively by others (27, 64) and are not considered further in our review.

IN SITU MEASUREMENTS OF BGE We surveyed the literature of the past 30 years for direct measurements of in situ bacterial growth efficiency in aquatic ecosystems. Data such as temperature, bacterial growth rate, DOC concentration, and substrate C:N were also recorded whenever possible. A total of 328 estimates of BGE were extracted from 39 published articles (Table 1). We pooled these data into four categories: marine, freshwater, estuarine, and riverine systems. Although there is a wide variation in measured BGE within each type of system (Figure 2), the data suggest consistent differences among systems with BGE increasing from marine areas to estuaries. We further explored the patterns in bacterioplankton metabolism by using a subset of the data ($n = 237$) made up of simultaneous measurements of bacterial production and respiration or DOC consumption in a variety of aquatic systems. In order to assess the possible effect of method on the estimate of BGE, we grouped the data according to the method: S for short-term metabolic measurements such as leucine uptake and oxygen consumption, and L for long-term experiments in which changes in POC and DOC were usually measured. We respected the assumptions and conversions used by the different authors and assumed RQ = 1 only when converting oxygen consumption rates to carbon units.

Table 1 List of published sources of direct measurements of in situ BGE that appear in Figures 2 and 3[a]

System	Method	BGE	Reference
Oceans			
Sargasso Sea	L	0.04–0.09	54
Coastal and shelf waters	S	0.08–0.69	24
Gulf of Mexico	S	0.02–0.23	107
North Pacific	L	0.01–0.33	18
Sargasso Sea	L	0.04–0.30	17
Coastal	L	0.31–0.64	68
Weddel Sea and Scotia Shelf	L	0.38–0.40	15
North Atlantic	L	0.04–0.06	76
Coastal and enclosures	S	0.07–0.46	30
Gulf of Mexico	L	0.26–0.61	80
Mississippi River plume	S	0.10–0.32	19
Peruvian upwelling	S	0.30–0.34	131
Louisiana shelf	S	0.18–0.55	11
Coastal waters	S	0.08–0.37	96
Coastal and shelf waters	S	0.01–0.25	49
Coastal waters	S	0.1–0.3	13
Baltic and Mediterranean Seas	L	0.21–0.29	156
Baltic Sea	S	0.25	103
Coastal waters	S	0.38–0.57	118
Estuaries			
Coastal Bay and salt marsh	S	0.11–0.61	24
Danish fjord	L	0.22–0.36	90
Hudson River	S	0.18–0.61	44
Danish fjord	L	0.19–0.23	14
Coastal bay	L	0.60–0.61	80
Brackish estuary	S	0.40	82
Lakes			
Swedish lakes	L	0.12–0.36	144
Latvian lakes	S	0.26	36
Cuban lakes	S	0.14–0.30	115
German lakes	L	0.17–0.22	147
Danish lakes	S	0.15–0.37	119
Temperate reservoir	S	0.14–0.66	71
Russian lake	S	0.04–0.24	38
Danish lakes	L	0.25–0.46	79
Lake Constance	S	0.16–0.35	53
Lake Constance	S	0.09–0.80	120
Danish lakes	L	0.34–0.43	92
Rivers			
River Meuse	L	0.30	121
River	L	0.32–0.36	80
Amazon River	S	0.03–0.46	9
Ogeechee River	L	0.31	89

[a]Data are grouped by system (marine, estuaries, lakes and rivers), and by method (S for short-term and L for long-term incubations).

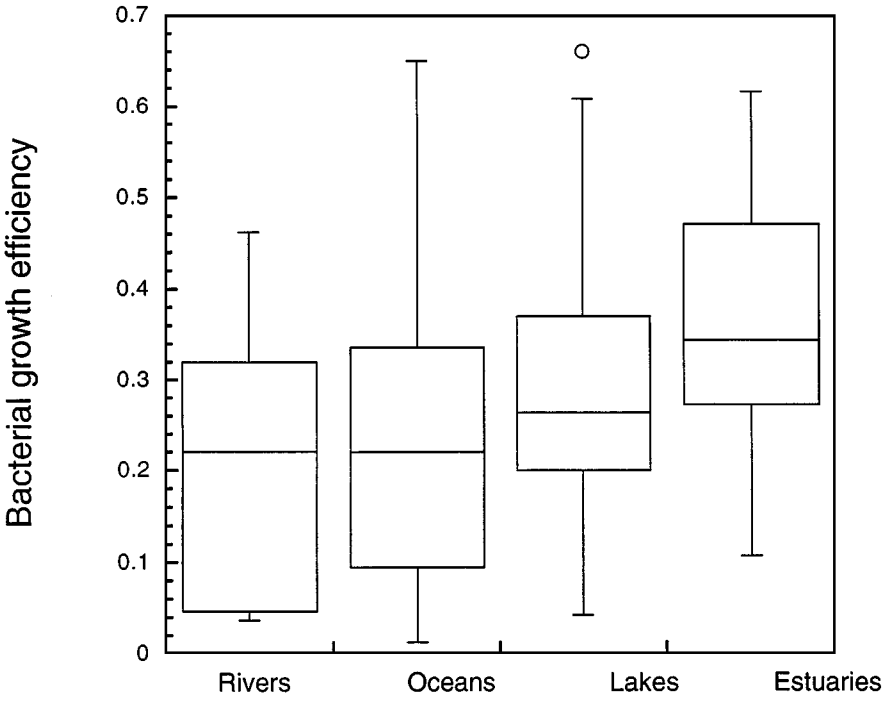


Figure 2 Summary of literature data on direct measurements of BGE in natural aquatic systems. Box-and-whisker plot shows median, and upper/lower quartiles (*box*), and range of values (*bars*). Extreme outliers are marked as *open circles*. The sources of the data are in Table 1.

There is a broad positive relationship between bacterial respiration and production (both in micrograms of C liter^{-1}h^{-1}, [Figure 3a]), with the following model I and II regression equations:

$$\mathrm{BR} = 3.70 \times \mathrm{BP}^{0.41},\ r^2 = 0.46\ (\text{model I}) \qquad 1.$$

$$\mathrm{BR} = 3.42 \times \mathrm{BP}^{0.61},\ r^2 = 0.46\ (\text{model II}) \qquad 2.$$

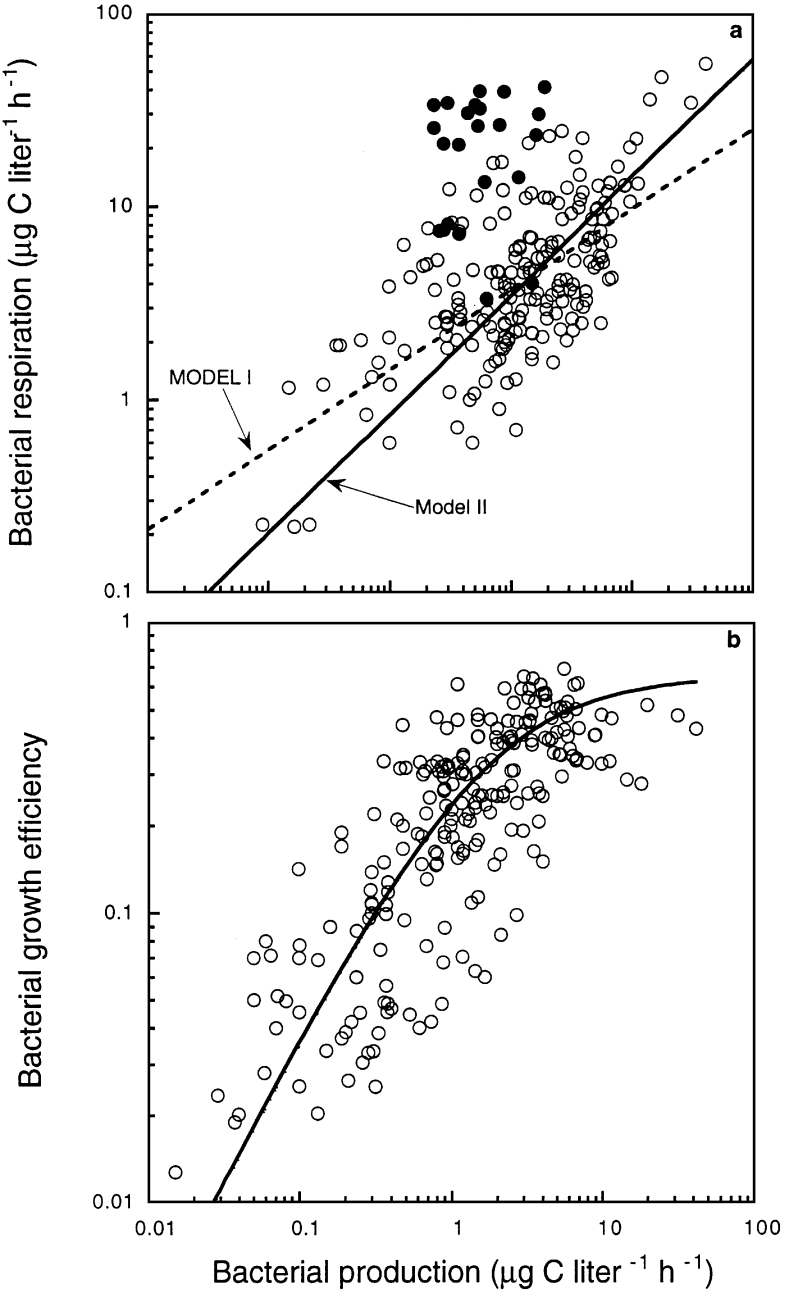
Equation 1 was obtained using ordinary least squares (OLS) regression, for comparison with previously published empirical models, and it provides the best predictive model of BR from measured BP. Because measurement error occurs in both BR and BP, the Model II regression equation, calculated following Ricker (110b), provides a better estimate of the true functional relationship between BR and BP. What these two models have in common is that the slopes of both regressions are significantly lower than 1. The ecological

relevance of this low slope is that it determines a pattern of increasing BGE along a gradient of increasing BP (Figure 3*b*). The data from Griffith et al (50) consistently had one order of magnitude higher BR relative to BP than all the other studies and were excluded from the analyses. Had we included these data, the slope of the regression between BR and BP would have been even lower. While the asymptotic form of this relationship is in part a consequence of the form of the BGE equation, the magnitudes of both the slope and the asymptote are of interest. First, BGE approaches a maximal value (near 0.5) as BP reaches 5 μg C liter^{-1} h^{-1}. The mean value of BGE at BP $>$ 5 μg C liter^{-1} h^{-1} is quite high, 0.46, a point at which BP and BR are nearly equal. Second, the lower values of BGE appear to be fixed above some minimum level. The following model best fits these data:

$$\mathrm{BGE} = \frac{0.037 + 0.65\mathrm{BP}}{1.8 + \mathrm{BP}} \qquad 3.$$

F Roland & JJ Cole (submitted) found a similar relationship between BP and BGE for data from the Hudson River by using a consistent set of methods. We explored the possible effect of method on the estimates of BGE. Although the expectation is that long-term experiments (method L) should result in lower BGE (129), this effect was not evident in our data. An analysis of covariance showed no significant effect of the type of method (S or L) on the relationship between BP and BR or that between BP and BGE. When all the data are pooled by method, the average BGE values for the two groups are very similar (0.26 for S and 0.28 for L). It is evident that the combined effect of different conversion factors and assumptions applied by various authors add significant noise to the observed variability in the relationship between BP and BR. Of greater concern, perhaps, is whether this noise drives some of the patterns in BGE that we describe in this section, but at present we have no evidence that the various sources of error introduce a systematic bias to the BGE data.

BP is positively correlated with primary production in aquatic systems (26); therefore, the pattern described here represents a tendency for increasing BGE along broad gradients of primary production in aquatic systems. The systematic differences in BGE found among systems (Figure 2) reflect differences in average primary productivity, with marine systems being generally the least productive and estuaries being the most productive. Other than differences in productivity, there are no apparent systematic differences in BGE among systems, so that in all subsequent discussions, we refer to variation in BGE along trophic gradients rather than among specific systems. Several studies had already suggested that BGE increases systematically with primary productivity (11, 24, 49), and we show here that this pattern is general and extends from ultraoligotrophic oceans to highly productive lakes and estuaries.



There is a large amount of variance in BGE for any given value of BP, which is the result of a large degree of uncoupling between bacterial production and respiration (Figure 3). Some of this variance may be simply the result of methodological artifacts. The relationship between BR and BP is also characterized by an intercept that is significantly different from zero, suggesting that at BP $= 0$ there would still be measurable rates of BR. This residual BR is not trivial, and since it corresponds to situations of virtually no bacterial growth, it must be somehow related to maintenance energy requirements of assemblages in ultraoligotrophic aquatic systems. It is clear from these patterns that maintenance energy requirements are a significant fraction of the energy flow in oligotrophic microbial assemblages.

TEMPORAL VARIATION IN BGE There have been relatively few investigations of daily and detailed seasonal variation in natural bacterioplankton BGE. Coffin et al (24) reported a marked diel cycle, with BGE ranging from 37 to 72% and increasing during the day, presumably following inputs of alga derived organic substrates. Some of the daily variation in BGE has been linked to the differential effect of light on BP and BR (99a). Seasonal variation in BGE within systems is sometimes small; for example, Schwaerter et al (119) reported a range of 28–34% in one lake throughout the summer. However, generally there are large and often rapid variations in BGE (24, 44, 120; F Roland & JJ Cole, submitted). It appears that BGE responds quickly to subtle changes in the rate of supply and the quality of substrates and to any factor that alters BP.

Relationship Between Growth Rate and Growth Efficiency

The broad trends of BGE along gradients of productivity suggest a relationship between BGE and bacterial doubling time (growth rate), which, as pointed out above, is expected from theoretical considerations. Comparative analyses have shown that growth rate or cell-specific production tends to increase with NPP and chlorophyll concentration (26, 153), so over broad productivity gradients both bacterial growth rate and BGE should covary. We further explored this pattern with a subset of our data ($n = 52$) for which we had simultaneous measurements of BGE and bacterial generation times, mostly estimates of

Figure 3 (*a*) BR a function of bacterial production in aquatic ecosystems. The data set collected from the literature is composed of 237 paired observations of BR and BP; the sources of these data appear in Table 1. *Lines* correspond to model I and II regressions fits to the data; the equations appear in the text. *Dark circles* are data from Griffith et al (50) that had significantly higher BR relative to BP and were excluded from the regression analyses. (*b*) BGE, calculated as BP/(BR + BP) by using the data in panel *a*, as a function of BP. The line is a rectilinear hyperbole with a fixed lower limit; the model is text Equation 3.

in situ rates. These data show no significant relationship between doubling time and BGE. Schweitzer & Simon (120) also found no relationship between growth rates and BGE in natural assemblages of bacterioplankton. In two studies, a positive relationship was found between growth rate and BGE in continuous cultures of natural bacterioplankton (79, 90), although Bjørnsen (14) found a negative relationship between BGE and growth rate. Søndergaard & Theil-Nielsen (129) found that the maximum BGE corresponded to the highest growth rates during batch incubations of bacterioplankton, but they found no consistent relationship between BGE and growth rates among samples. These data suggest that BGE may covary with growth rate in any given combination of temperature, organic substrates, nutrients, and other constraining factors but that the relationship between BGE and growth rate may be specific for each set of growth conditions.

One factor that uncouples growth rate from growth efficiency is the observation that bacteria may maximize growth at the expense of efficiency (116, 140, 151). This maximization is achieved with different energetic costs and different degrees of uncoupling between catabolism and anabolism. There are clear examples of this type of uncoupling for bacterioplankton. Addition of nutrients sometimes increases substrate consumption with no effect on net growth (8). Middelboe et al (93) found that viruses decreased BGE in bacterial cultures while increasing the growth rate of noninfected bacteria. They argued that lysed cells released P and N, which were used by uninfected cells for growth, at the expense of lowering BGE by the production of exoenzymes to hydrolyze polymeric P and N released by dead bacteria. Zweifel et al (156) observed a 70% increase in cell yield (number of cells) and a 20% decrease in BGE after phosphorus was added to the culture media. These authors suggested that P enhanced cell division while P-limited cells were able to store organic carbon without dividing and thus could maintain a higher carbon growth efficiency. Poindexter (104) showed that during P-limited growth in chemostats, the bacterial concentration but not the biomass was proportional to the substrate P content; conversely, during C-limited growth, the bacterial biomass but not the concentration was proportional to the substrate C content. Robinson et al (112) showed that addition of N did not increase BGE but sharply increased the rates of decomposition of detritus and the final yield of bacteria.

These examples, as well as the patterns in BGE discussed above, suggest that although the highest bacterial growth rates attained in natural aquatic systems correspond to conditions under which BGE is high, such as in estuaries and eutrophic lakes, it is not necessary to increase BGE in order to increase growth rates. This is important because total carbon consumption may be regulated by factors different from those that regulate growth or BP (75). Because most contemporary research in microbial ecology has focused on the regulation of

bacterial growth and production, we know relatively little about what may regulate total bacterial carbon consumption in aquatic ecosystems.

REGULATION OF BACTERIOPLANKTON GROWTH EFFICIENCY

Research with cultured bacteria (117), as well as models of bacterial energetics and growth in aquatic systems (2, 12, 27, 61, 83, 151), suggests that the substrate supply and complexity and mineral nutrient availability are the most important variables controlling BGE. In addition, as described above, BGE is broadly correlated with BP. Hence, it is to be expected that the factors that influence BP in experiments would also affect BGE. However, the results of experiments in various systems have been inconsistent. In this section, we explore the empirical evidence on the regulation of BGE in natural bacterioplankton assemblages in an attempt to reconcile the pattern of increasing BGE along productivity gradients with observations and experimental results on carbon and nutrient quality and supply.

Effect of Temperature, Salinity, and Pressure

The growth rate declines as temperatures move away from the optima for each type of bacteria in laboratory studies (101). However, a strong positive relationship exists between growth rates and temperature in natural bacterioplankton assemblages (110, 153). If low temperatures result in lower growth rates, a positive relationship between temperature and growth efficiency would also be expected. Newell & Lucas (97) and F Roland & JJ Cole (submitted), for example, found higher BGE in summer than in winter. However, BGE tends to decline with increasing temperature in other systems (30, 50, 62), even though growth rates tend to increase. In all these cases, however, the effect of temperature was very weak. A subset of our data ($n = 151$) for which the BP, BR, BGE, and temperature are known shows no significant relationship between BGE and temperature or any significant effect of temperature on the relationship between BP and BR. However, this data set is biased towards higher temperature (mean = 19°C), and there are relatively few measurements in the low-temperature range (<10°C), so that we can conclude only that temperature is not an overriding regulating factor of BGE in natural systems.

The changes in BGE along gradients of salinity have been the subject of only a few studies. Griffiths et al (50) found a weak negative relationship between BGE on glucose or glutamate and salinity in a large-scale study but concluded that salinity had no direct effect on BGE. A gradient of increasing salinity may have corresponded to a gradient of declining productivity from coastal to open waters and, as shown above, a pattern of declining BGE would be expected.

The only study to our knowledge that has assessed the effect of pressure on BGE is that by Turley & Lochte (150), who concluded that deep-sea bacteria were able to mineralize more organic carbon at 450 atm than at 1 atm but that BGE was lower under high pressure.

Nutrient Limitation of BGE

The dependence of BGE on the relative availability of mineral nutrients and organic carbon was originally formulated by Fenchel & Blackburn (42) and later expanded by others (2, 12, 13, 45, 58, 151). The basic idea is that bacteria regulate the catabolism of organic substrates to attain the correct intracellular stoichiometry with respect to N (and other nutrients). Because the elemental composition of bacteria is relatively constant (45), BGE should be negatively related to the C:N ratio of the substrate, at least in the range of C:N where N, and not C, is limiting. This type of model is important not only to understand the regulation of BGE but also to assess the role of bacteria in nutrient cycling in aquatic environments (12, 45, 46).

Regulation of BGE by the availability of mineral nutrients implies that increases in the supply of nutrients should result in increased BGE. Billen (12) and Goldman et al (45) have unequivocally shown that the BGE of natural assemblages of marine bacteria grown on a range of substrates is inversely related to the C:N ratio of the substrate. However, the relationship between BGE and available C:N was considerably weakened when bacteria were exposed to multiple nitrogen and carbon sources (46), and under these circumstances the source of the nitrogen (i.e. NH_4 or amino acids) became important. Bacterial assemblages growing under ambient conditions are exposed to multiple sources of nutrients and organic matter, and so it is expected that the relationship between C:N and BGE may not always hold under natural conditions. Some experimental data support the role of N in regulating BGE; for example, Kroer (80) found that BGE increased with ammonium concentration and decreased with increasing C:N ratio of the bulk dissolved substrates in coastal areas, and Benner et al (8) found that BGE was limited by N in a salt marsh and by P in a freshwater marsh. However, most experimental additions of inorganic nutrients have had little or no effect in lakes and rivers (9, 144), coastal areas (30, 68, 112, 156), and open oceans (17, 18, 75). There is thus conflicting evidence about the role of N in regulating BGE in natural aquatic systems, and there is no clear pattern along trophic gradients.

That the C:N ratio may not be the key regulator of BGE in natural bacterial assemblages is also clear in a subset of our data, for which the C:N ratio of the presumed substrate was reported. These data show a very weak negative relationship between BGE and C:N. Figure 4 contains data from natural systems as well as experimental data from laboratory cultures (45, 46) to emphasize

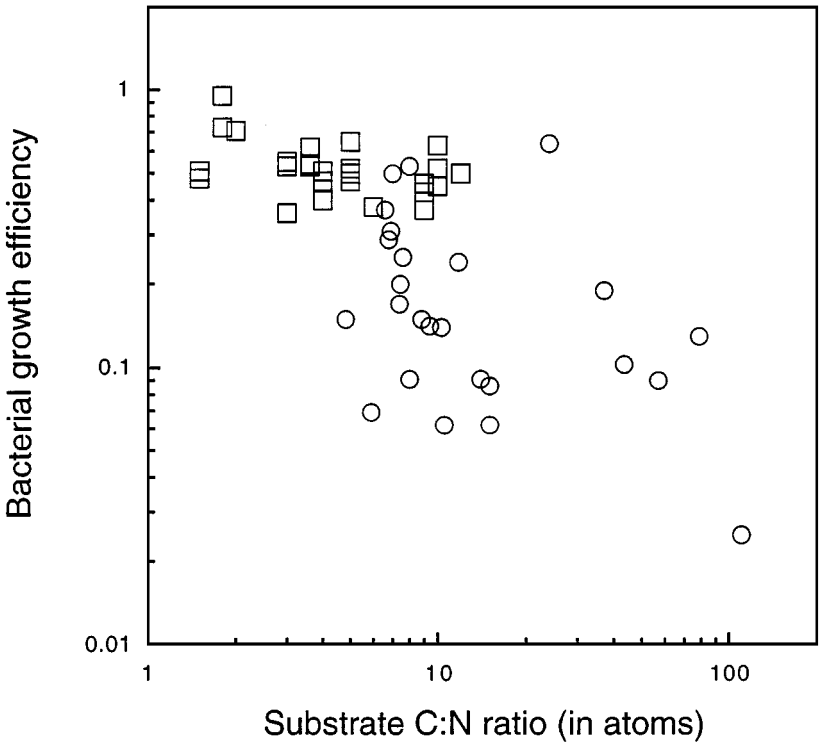


Figure 4 BGE as a function of the C:N ratio of the substrate. *Open circles* correspond to measurements of BGE on natural complex substrates. *Open squares* correspond to BGE in defined media (45, 46).

that growth in defined media creates conditions that are seldom experienced by natural bacterioplankton. From the weak relationship between substrate C:N and BGE, we draw three inferences. (*a*) Nutrients other than N may be limiting. There is increasing evidence that P may control BGE in both freshwater (9, 58) and marine (107, 156) systems and that iron may limit BGE in large areas of the oceans (143). (*b*) Bulk C:N may not be representative of the substrates actually available and taken up by bacteria (112). (*c*) Nutrients and organic carbon may colimit BGE (39, 40).

Energy and Organic Carbon Limitation of BGE

It has been repeatedly suggested that bacteria in oligotrophic systems are limited primarily by the supply of carbon and energy (18, 26, 37, 74, 75). The distinction between energy and carbon limitation is not always fully realized in microbial

studies, however. From a bioenergetic point of view, energy limitation occurs when the ATP generated during the biological oxidation of a compound is insufficient to reduce all the available carbon in the molecule to the level of bacterial cell carbon (85). Growth on relatively oxidized substrates, such as acetate, glycolate, and even glucose, is usually energy limited from this perspective, and these compounds are incorporated into biomass with low efficiency, even if inorganic nutrients are in excess. Thus, it is the ratio between biologically available energy and carbon content of the organic molecules that determines the maximum BGE (27, 84, 85, 151).

In the context of this review, we should thus distinguish between control of BGE arising from the rate of supply of organic matter and control arising from the nature of the available organic matter. Although both may result in low BGE, they are ecologically distinct. If the supply of organic matter is low, whatever its nature, a large fraction of this substrate will be catabolized and used primarily for maintenance energy requirements rather than for growth, with a resulting low growth efficiency (55, 117, 135). Conversely, there might be a large supply of organic substrates which, because of their relative energy and carbon contents, are incorporated with low efficiency even under conditions of excess mineral nutrients. Distinguishing between these two types of limitation in natural situations is difficult, especially because of confounding by possible nutrient colimitation.

Regulation of growth efficiency by the supply of organic C implies that increases in the supply rate should result in increased BGE and production. Empirical and experimental results show that this is not always the case. For example, Kirchman (74) found that growth of planktonic bacteria in the oligotrophic subarctic Pacific was not stimulated by the addition of glucose, and others have found similar patterns in other areas (79, 107). Carlson & Ducklow (17) found that addition of glucose and amino acids resulted in a higher BGE but noted that with glucose addition, cells produced storage carbon and increased in mass rather than in abundance. A similar conclusion was reached by Cherrier et al (18). Perhaps the only common result of most addition experiments is that amino acids tend to enhance both BGE and bacterial growth (17, 18, 30, 68, 74). It has been suggested that it is energetically advantageous to use preformed compounds (74), but the energetic cost of transporting amino acids across the membranes greatly offsets this advantage (101, 133). It is more likely that because amino acids have relatively high energy and carbon contents and are also a source of N, they release bacteria from multiple limitation by carbon, energy, and N. Experimental evidence suggests that the quality of the organic C, rather than the rate of supply of organic matter, may regulate BGE in most natural aquatic systems (151). The reports of DOC accumulation in oligotrophic oceanic areas during the summer (42) provide further evidence that the supply of organic matter may not be the main factor regulating BGE.

SOURCES AND QUALITY OF ORGANIC SUBSTRATES Qualitative aspects of natural DOC that are relevant to bacterial energetics are difficult to define (151). One approach has been to determine bacterial utilization of molecular size fractions of natural DOC. No clear patterns have emerged, because there are reports of highest BGE on either the low-molecular-weight (LMW) fractions (145, 148) or the high-molecular-weight (HMW) fractions (1, 89). Some of these differences can be explained by the C:N ratio of the weight fractions rather than by any qualitative characteristic of the organic carbon itself (89, 148). It is clear from these results that high bioavailability does not necessarily imply high BGE: Amon & Benner (1), for example, reported that HMW fractions were most bioreactive but LMW fractions were incorporated more efficiently into bacterial biomass. Both the absolute amount and the proportion of labile DOC, defined as DOC taken up by bacteria in batch incubations, tend to increase along trophic gradients (128), suggesting that a higher BGE in more productive systems may be the consequence of qualitative changes in the DOC pool. There is some experimental evidence for such a relationship (91).

Another approach has been to assess how different sources of organic matter affect BGE. We collected from the literature 85 direct measurements of BGE in five broad categories of organic matter depending on its source: organic matter excreted by phytoplankton (EOC), and organic detritus derived from phytoplankton, seaweeds, vascular vegetation, and animal feces (Table 2). These combined data show that the efficiency of conversion of detrital organic matter to bacterial biomass is generally low (<30%) for all categories except EOC, in which most values are above 50% (Figure 5). Algal EOC production and cycling are measured by monitoring the incorporation of ^{14}C into phytoplankton and its subsequent release and uptake by bacteria. The high values of BGE obtained for EOC most probably reflect the same type of problems that affect estimates of incorporation efficiency of single radiolabeled compounds, i.e. lack of isotopic equilibrium in the internal carbon pools of bacteria resulting in an overestimation of BGE (14, 73). Although organic carbon derived from vascular vegetation is usually considered of rather low quality and is a major component of the more refractory humic fraction of DOC, BGE measured on either vascular vegetation (Figure 5) or the humic DOC is well within the average values measured for bulk water and other organic components (3a, 8).

There are sources of organic matter in addition to the direct production of detritus from plants and animals. A potentially significant source in the open ocean is atmospheric deposition of volatile organic compounds. Heikes et al (56) estimated that formaldehyde is subject to atmospheric dry deposition rates of about 2.2 mg of C $m^{-2}\,day^{-1}$ in the Central Atlantic, and Nuncio et al (99) have shown that formaldehyde is rapidly utilized in the upper layer of the ocean. Another source of labile DOC is the photochemical oxidation of organic matter

Table 2 BGE on different types of detrital organic matter[a]

Source of detritus	System	BGE	Reference
Phytoplankton	Marine	0.13–0.22	10
Phytoplankton	Marine	0.17–0.27	5
Phytoplankton	Marine	0.07–0.13	98
Phytoplankton	Marine	0.09–0.24	83
Phytoplankton	Marine	0.50	150
Phytoplankton	Marine	0.18	130
EOC	Marine	0.71–0.81	154
EOC	Freshwater	0.57–0.75	7
EOC	Freshwater	0.50	28
EOC	Freshwater	0.31–0.56	23
EOC	Freshwater	0.77	62
Vascular plants	Marine	0.43	41
Vascular plants	Freshwater	0.09–0.11	89
Vascular plants	Freshwater	0.53	43
Vascular plants	Marine	0.025–0.10	83
Vascular plants	Freshwater/Marine	0.17–0.36	8
Vascular plants	Marine	0.11	16
Vascular plants	Freshwater	0.74–0.92	69
Vascular plants	Marine	0.04–0.17	111
Vascular plants	Freshwater	0.37–0.63	44
Vascular plants	Freshwater/Marine	0.27	95
Vascular plants	Marine	0.44	47
Vascular plants	Marine	0.19–0.64	52
Macroalgae	Marine	0.09	137
Macroalgae	Marine	0.06–0.07	86
Macroalgae	Marine	0.32	112
Macroalgae	Marine	0.25	78
Macroalgae	Marine	0.11	136
Macroalgae	Marine	0.07	97
Macroalgae	Marine	0.09–0.13	83
Tunicate feces	Marine	0.15	105
Bivalve feces	Marine	0.15	137
Bivalve feces	Marine	0.06–0.09	149

[a]Data were used in Figure 5 and are grouped according to the source of the detritus: phytoplankton detritus includes detritus from natural algal assemblages and from algal cultures; organic carbon excreted by phytoplankton (EOC), organic carbon derived from vascular vegetation, including seagrasses and other aquatic macrophytes and terrestrial vegetation; organic carbon derived from marine macroalgae; organic carbon derived from feces.

in the surface layers of lakes (110) and oceans (72, 95a). Kieber et al (72) estimated rates of pyruvate production in the Sargasso Sea of 1.6 mg m^{-2} day^{-1}, which would represent 1–4% of BR measured by Carlson & Ducklow (17). It is reasonable to expect that a wide variety of other LMW substrates, including acetate, acetaldehyde, formate, glucoxylate, and methanol, are formed together

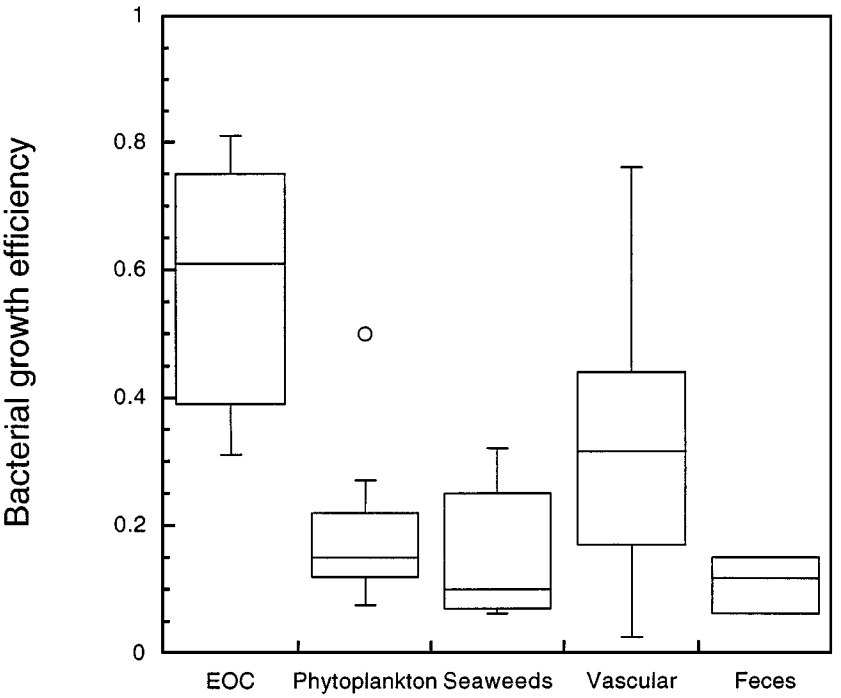


Figure 5 Summary of literature data on direct measurements of BGE for organic matter grouped according to source. Box-and-whisker plot shows median and upper/lower quartiles (*box*), and range of values (*bars*). Extreme outliers are marked as *open circles*. The sources of the data are in Table 2.

with pyruvate by the photochemical breakdown of DOC, and the summed production of these substrates may well be a significant source of C and energy for bacteria. Since many other simple organic compounds are deposited from the atmosphere, it is conceivable that these two pathways of carbon input into oceanic systems are significant to bacterial metabolism.

Atmospheric deposition and photochemical oxidation result in the production of LMW compounds that are characterized by a low heat of combustion and a high degree of oxidation relative to microbial biomass and are typically incorporated with low efficiencies (27, 85, 95a). Algal excretion is also dominated by small, low-energy organic molecules (23). In the large expanses of ultraoligotrophic ocean and even in ultraoligotrophic lakes, these compounds may form the bulk of biologically labile organic carbon. As systems become more productive, the relative importance of EOC as a bacterial substrate tends to

decline (3) and the impact of atmospheric deposition and photolysis on pelagic metabolism will decline: These qualitative changes may positively influence BGE. However, because it is difficult to differentiate energy from carbon limitation, it is unclear whether the BGE of natural bacterioplankton assemblages is limited by the supply of organic matter, the chemical nature of the organic substrates present, or both.

GROWTH EFFICIENCY, ENERGY REQUIREMENTS, AND CELL ACTIVITY IN NATURAL BACTERIAL ASSEMBLAGES

We showed in the previous section that the supply and nature of the organic substrates, as well as the availability and sources of mineral nutrients, may influence BGE, although there are no clear patterns of resource regulation among systems. Research on bacterial bioenergetics has shown that when cultures are primarily energy limited as a result of the rate of supply of organic matter, bacteria tend to maximize the efficiency of utilization of the energy source through tight coupling between catabolism and anabolism and high BGE (117). The extremely low and often variable BGE values observed in most oligotrophic systems suggest a high degree of uncoupling between catabolism and anabolism and do not support the hypothesis that the rate of supply of energy is the main limiting factor for BGE. Moreover, cell-specific respiration rates are not consistently lower in oligotrophic areas (32), suggesting that the amount of energy available on a per-cell basis may be roughly similar among systems. Rather, cell-specific maintenance requirements appear to be higher in oligotrophic areas with extremely low concentrations of organic substrates and nutrients. Thus, per unit organic carbon input, more carbon is used for maintenance in oligotrophic areas than in eutrophic areas, and it is the interaction among the rate of supply of energy, the quality of the substrate, and the energetic demands of cells that determines BGE. These high apparent maintenance respiration rates may occur (*a*) when cells must transport solutes against a large concentration gradient, (*b*) when cells must produce extracellular substances in large amounts, (*c*) when cells must maintain a wide array of active transport systems and the corresponding arrays of catabolic enzymes, and (*d*) when a large fraction of the population is in a state of starvation survival, with only minimal metabolism. We briefly discuss these possibilities below.

Transport of Nutrients

Transport of nutrients and organic C could influence maintenance energy in two ways. First, as the concentrations become lower in dilute, oligotrophic environments, the energetic cost of active transport increases (87). Second, the

appearance of nutrients and carbon sources for bacterial growth is transient, and the ability to respond to sudden increases in nutrient levels is an essential property for survival in dilute environments. It is also clear that bacteria under carbon limitation are able to simultaneously take up and catabolize a wide variety of substrates (39, 40), but there is a cost in maintaining the transport proteins, catabolic enzymes, and functional membrane systems needed to deal with both the variety and low concentrations of substrates (31, 96). The energetic cost of maintaining such a wide array of highly efficient transport systems has never been explicitly assessed in natural bacteria.

Metabolite Excretion

Most bacterial species excrete metabolites to the medium, even during aerobic growth. The causes and bioenergetic implications of excretion have been only minimally investigated in bacterioplankton, but the production of exoenzymes is perhaps the best-understood aspect of metabolite excretion in aquatic bacteria (60, 61a). A large fraction of DOC in natural aquatic systems is composed of polymeric substances that cannot be incorporated directly into bacteria. Large molecules and colloids present in the DOC pool must be acted upon by exoenzymes before they can be utilized by bacteria (61a, 125), and the hydrolysis of polymers has been suggested as the rate-limiting process to bacterial production in aquatic systems (22). The synthesis and excretion of enzymes must be coupled to active transport systems that can capture the products of extracellular hydrolysis and of enzymatic systems capable of catabolizing these substrates; it may thus represent a major energy expenditure of bacteria in natural aquatic systems. For example, Middelboe & Søndergaard (91) found an inverse relationship between lake BGE and β-glucosidase activity (Figure 6). Extracellular enzyme production increased toward the end of batch culture incubations of lake bacterioplankton, when most of the labile DOC had been consumed and the submicron and colloidal fractions were increasingly utilized (92). The need to perform extracellular hydrolysis of polymers thus makes a large energy demand on bacterial cells in natural environments. The increasing trend in BGE along productivity gradients suggests that bacteria may be deriving more of their C needs from exoenzymatic breakdown of polymeric substances in oligotrophic environments, with the consequent decline in BGE. This hypothesis remains to be empirically tested.

In addition to the excretion of enzymes, bacteria are capable of producing copious amounts of extracellular mucopolysaccharides (31a, 112), which form mucilaginous capsules around the cells (31a, 57a, 112) and also form loosely associated slimes and fibrils (82a, 112). The chemical nature of these extracellular compounds varies greatly, but uronic acids often form the bulk of these materials (71a). Metabolite excretion appears to be greater when the organic

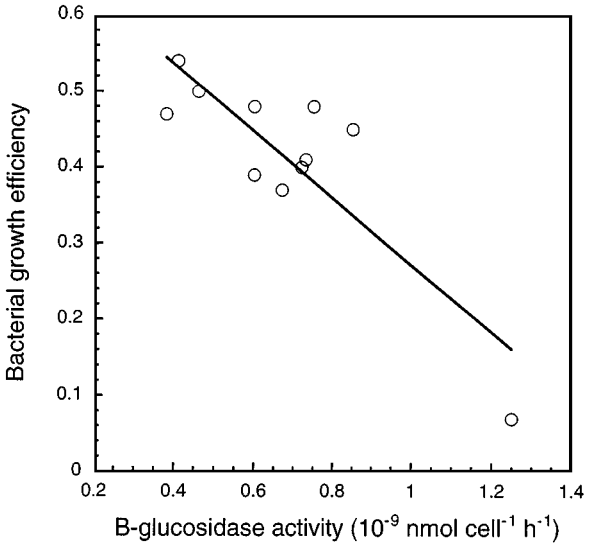


Figure 6 Relationship between BGE and β-glucosidase activity in lake bacterioplankton. Data from Middelboe & Søndergaard (91).

substrate is in excess of the growth requirement, and it also depends on the nature of the organic substrate (84, 139). It has been suggested that excretion of metabolites is a pathway of energy dissipation that may contribute to the maintenance of intracellular stoichiometry (31a, 84). However, excretion of organic metabolites, including polysaccharides, lipids, proteins, and humic-like substances, has also been found under conditions of carbon and nutrient limitation in aquatic bacteria (48, 63, 67, 146). Most excretion products are polymeric, and the biosynthesis of these substances typically exerts high energy requirements to the cell (117, 133). Not surprisingly, there is a general inverse relationship between the overproduction and excretion of metabolites and growth efficiency in bacterial cultures (84). In addition, natural bacterioplankton excrete both organic and inorganic N, even under nutrient and carbon limitation (21, 46, 141, 149). Current BP measurements, whether based on changes in bacterial biomass or on the incorporation of leucine or thymidine, are unlikely to include the production of exopolymers, and this will result in a more or less severe underestimation of BGE (31a). This underestimation of BP and BGE may not be trivial from the point of view of organic carbon flow in pelagic food webs, because there is evidence that a variety of grazers can effectively utilize bacterial exopolymers (31b,78a).

Physiologic Condition of Cells

Although often treated as a black box in ecological studies, bacterioplankton assemblages display a large internal variation in cell size and morphology, taxonomic and functional characteristics, and physiological states (77, 88, 96). In any given bacterioplankton assemblage, there are cells in the entire range of physiological states, from extremely active to slowly growing, dormant, and even dead. Distinguishing cells in these various physiological states poses major technical and conceptual difficulties (88) and is rapidly becoming a major focus of research in aquatic microbial ecology. Stevenson (132) proposed that most bacterial cells present in aquatic systems are inactive, i.e. either dormant or dead. Subsequent research has demonstrated that under conditions of low organic substrate supply, heterotrophic marine bacteria can enter a phase of long-term survival; the literature on this subject has been extensively reviewed by Morita (96). Bacteria in starvation survival mode are not completely inactive but are able to take up substrate and engage in low but measurable rates of biosynthesis (77).

Several methods are currently used to determine single-cell activity in natural bacterioplankton assemblages (88). Since these methods are used and compared in various systems, it has become apparent that different approaches yield different estimates of the proportion of bacterial cells that are alive, viable, and/or metabolically active (70, 123). However, the growing consensus among microbiologists is that bacterioplankton assemblages are composed both of highly "active" and growing bacteria, which often comprise a small fraction of the total population, and cells that are dead, dormant, or slowly growing (96, 123). Most studies in aquatic microbial ecology focus on the average growth rates of bacterioplankton assemblages, by scaling the measured production rates to the total cell abundance. However, the BGE that is commonly measured in ecological studies is the average of the BGE values of these subpopulations of bacteria, which must vary within any given assemblage at least as much as growth rates. The assumption that all bacteria are growing with the same conversion efficiency is most probably wrong.

Figure 7 (left panel) shows the traditional approach to bacterioplankton assemblages, which assumes that all cells are participating equally in the metabolic processes. The right panel shows an alternative view, i.e. that the assemblage is composed of at least two fractions characterized by very different average growth rates and by different BGEs (the nongrowing fraction will still consume organic matter to fuel basic maintenance energy requirements). Overall BGE may vary as a result of changes in the relative size of the pools of active and inactive cells without any changes in the actual growth or metabolic rates of the bacteria in each pool. Likewise, measured values of adenylate

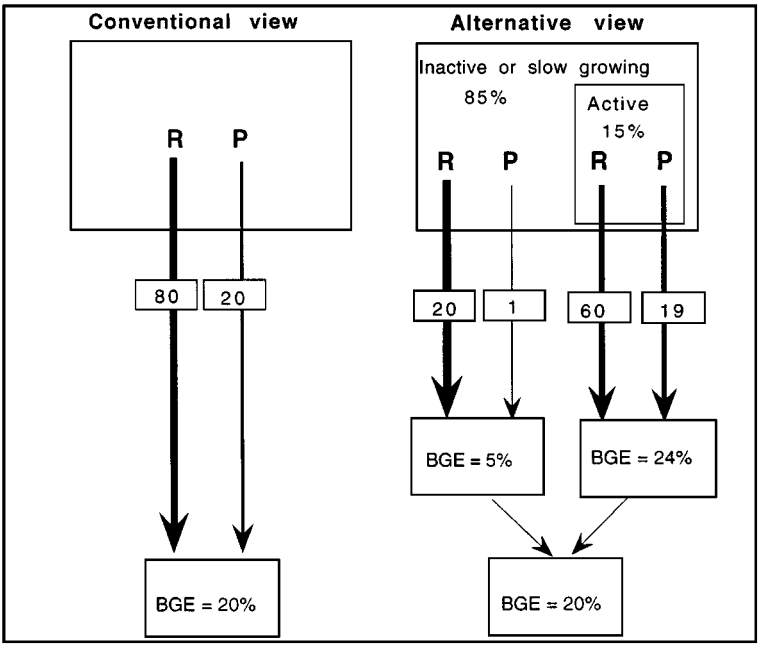


Figure 7 Two alternative depictions of the functions of bacterioplankton assemblages. The *left panel* assumes that production (P) and respiration (R) are homogeneous among all cells. BGE is related to the average growth rate. The *right panel* assumes that there are two distinct pools of cells, one highly active and the other relatively inactive. Each pool is characterized by distinct P, R, and BGE, so the resulting growth efficiency of the assemblage is dependent on the relative size of the active and the inactive pools. Numbers are for purposes of example only.

energy charge (EC_A) of marine bacterioplankton assemblages are often close to or below the range where growth and biosynthesis are theoretically possible (69a, 96a). These measurements are consistent with generally low BGE in unproductive marine waters, but EC_A, like BGE, is an average value for a mixed bacterial assemblage. A low community EC_A may indicate either a homogeneous population of bacteria severely limited by energy or the coexistence of actively growing cells (high EC_A) and dormant or inactive cells (low EC_A). We hypothesize that within bacterioplankton assemblages there is always a pool of highly active cells characterized by both high BGE and EC_A relative to the average values of the assemblage.

Previous comparative studies have found that the proportion of highly active bacteria increases along productivity gradients (34, 35), in much the same way we have shown here that BGE does. Regulation of the number and proportion

of highly active and of less active or dormant cells in natural bacterioplankton assemblages is complex; there is evidence for both resource regulation and control through trophic interactions within microbial food webs (21a, 33). Whether there is a direct link between growth efficiency and the structure of bacterioplankton assemblages must be explicitly addressed in future studies. This link is ecologically important because it implies that processes which affect the proportion of different physiological subpopulations, such as selective grazing by protozoans, may have a bearing on the in situ BGE, regardless of the supply and nature of the inorganic and organic substrates.

TAXONOMIC COMPOSITION Bacterial growth efficiency in laboratory studies is known to depend strongly on the type and supply of growth substrates, but for any given combination of growth parameters, different species of bacteria exhibit widely different patterns in growth efficiency (57). We have no information on how the taxonomic composition of the bacterioplankton assemblage may affect bacterial growth efficiency in natural aquatic systems. However, the advent of a new generation of molecular techniques is rapidly opening the genetic black box of planktonic bacteria, and soon we may be able to link broad taxonomic composition to aspects of microbial energetics and thus to explain some of the variance in BGE not accounted for by resource regulation.

ECOLOGICAL CONSEQUENCES OF PATTERNS IN BGE

Bacterial respiration is the major component of total respiration in most aquatic systems (61, 64, 126, 155), so that changes in bacterial respiration have profound effects on the overall carbon and gas balance in aquatic ecosystems (124). The magnitude and regulation of bacterial growth efficiency is of interest well beyond the realm of microbial ecology because the assumed value of BGE can greatly affect how one construes models of the C cycle in aquatic systems.

We have shown that BGE is postulated to be regulated by numerous factors. However, of all the possible factors, only BP gives a reasonable prediction of BR or BGE, and even this is associated with a high level of uncertainly. While this is perhaps intellectually not satisfying, it does allow us to estimate BGE and BR when BP is known. Since BP is much more commonly measured than is BR, this estimation is useful. We assert that it is more useful than using a generic value of BGE which is independent of measured BP. We propose a rectilinear hyperbole with a fixed lower limit as a predictive model of BGE from BP (Equation 3).

To infer the consequences of this model for BGE, we show in Figure 8 how the ratio of BR to net primary production (NPP) would vary across a gradient of NPP from ultraoligotrophic to eutrophic waters. This ratio is of interest because when it exceeds unity, the ecosystem is respiring more organic C than is fixed by photosynthesis and the system is net heterotrophic. In this exercise, we assumed that volumetric daily BP varied with NPP as specified by Cole et al (26):

$$\log(\mathrm{BP}) = -0.483 + 0.814 \log(\mathrm{NPP})$$

and that hourly BP was constant over the day. At the oligotrophic end of the spectrum, BR would exceed NPP by sevenfold; at the eutrophic end of the spectrum, NPP would exceed BR. Compared with the assumption of a constant value of BGE across the gradient, the new model produces a much larger range in the ratio of BR/NPP. In the region for which NPP is 90–300 mg C m^{-3} day^{-1}, this model is in agreement with the traditionally assumed range of BGE of 0.25–0.45 (Figure 8).

We further examined the consequences of this new model of BGE on a large oceanic data set of simultaneous measurements of BP and NPP synthesized by

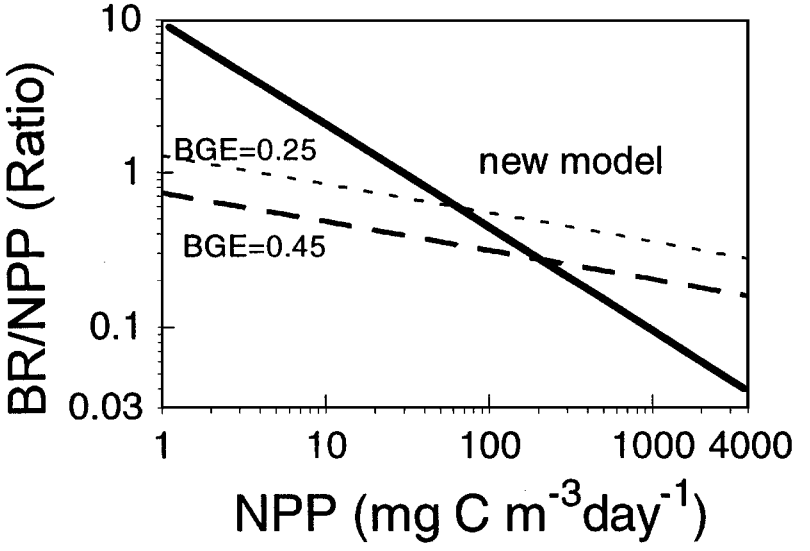


Figure 8 Model for the variation of BGE along gradients of primary productivity in aquatic ecosystems. BP was calculated along a simulated gradient of NPP by using the model in Reference 25, and BGE was then calculated from BP by using text Equation 3. The plot shows how the resulting ratio of BR to NPP would vary along a gradient of NPP and how this ratio would vary if we assumed that BGE is constant along this gradient. See text for a complete explanation.

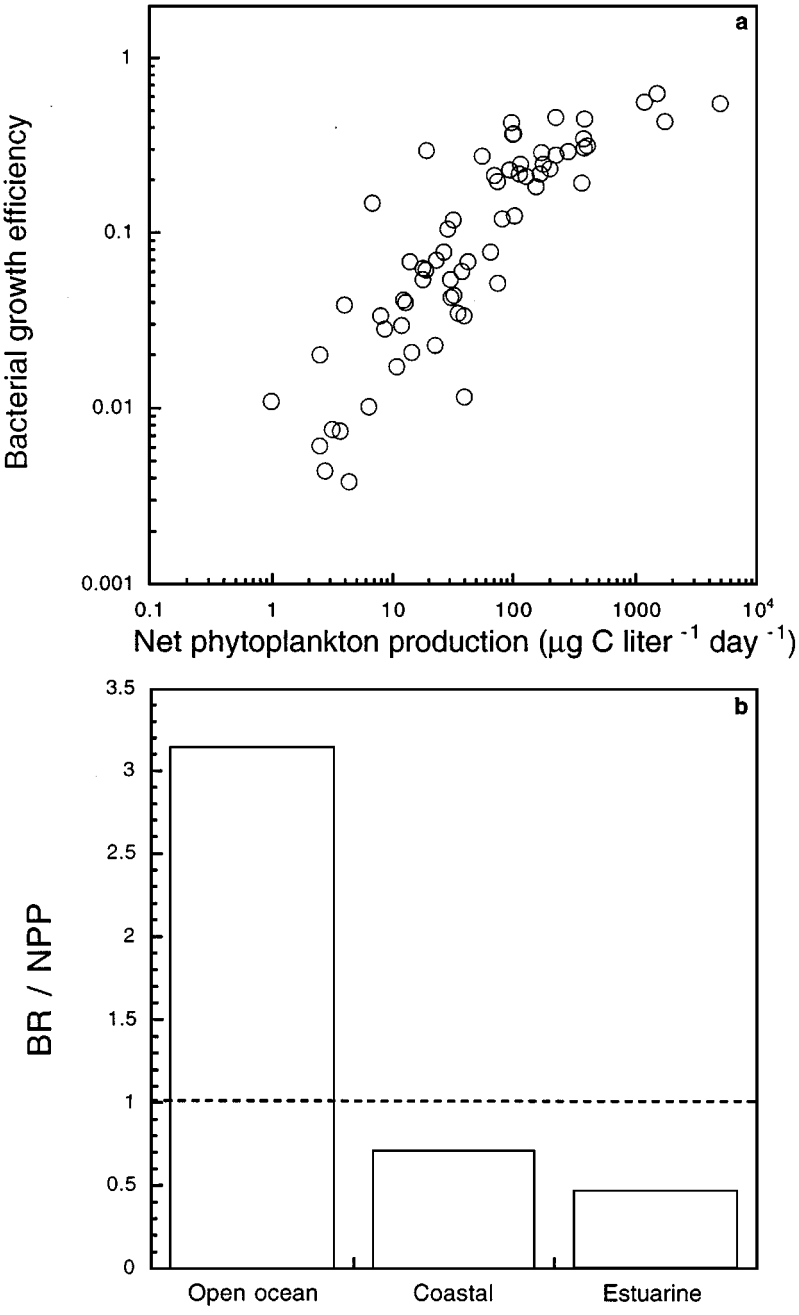


Figure 9 (*a*) BGE as a function of NPP in marine systems. The large data set on simultaneous measurements of BP and NPP in reference 37 was used, and BGE was calculated from BP by using text Equation 3. (*b*) Average ratio of BR to NPP for estuaries and coastal and open-ocean sites, calculated from the data in panel a.

Ducklow & Carlson (37). These values range in primary production from 5 to more than 5000 μg of C liter^{-1} day^{-1} and in BP from <0.01 to 11 μg of C liter^{-1} h^{-1}. There is a positive relationship between BP and NPP, not unlike previous reports (26). We used our model of BGE versus BP (Equation 3) to calculate BGE from these BP measurements, so that we could relate BGE to NPP. The estimated BGE increases with net phytoplankton production (Figure 9*a*), ranging from less than 0.1% in ultraoligotrophic ocean sites to slightly over 50% in the most productive estuarine and coastal sites. This pattern suggests virtually no bacterial net growth or production in the most unproductive aquatic ecosystems, as suggested previously (51, 126). The fact that BGE increases together with bacterial production along gradients of primary production also results in a pattern of relatively little change in bacterial respiration along this gradient. The relative invariance in microbial respiration, and even of plankton community respiration in general, has been noted before (31c, 32).

The covariation of BGE, BP, and NPP thus results in a pattern of BR being large relative to NPP in oligotrophic areas but small where NPP is high. The current paradigm generally assumes that coastal ecosystems, particularly estuaries, may be net heterotrophic (respiration exceeds primary production) because of the influence of allochthonous organic inputs (60, 127). In contrast, open-ocean systems are usually thought to be examples of autotrophic self-supporting systems because of their relative isolation from significant allochthonous sources of organic matter. However, when BGE is factored into the organic matter flow and the resulting BR is considered together with BP, the patterns in system functioning that emerge are strikingly different. Figure 9*b* shows the average balance between NPP and BR, calculated from BGE as described above by using the data of Ducklow & Carlson (37), grouped by system. There is a systematic increase in the BR:NPP ratio from estuaries, where BR:NPP is low on average, to open oceans, where the average BR:NPP ratio is well above unity (Figure 9*b*). This pattern agrees with actual measurements of bacterial metabolism relative to phytoplankton in a variety of marine systems (32, 110a).

Our model for BGE is derived from the empirical data culled from diverse ecosystems. We point out, however, that the data set is not very extensive and there is a great deal of variability in the plot of BGE versus BP. Thus, BGE may not be correctly estimated from Equation 3 in all environments. For example, in a detailed study of the organic C budget of oligotrophic Mirror Lake, Cole et al (25) concluded that BGE could not be below about 0.2 and have the C budget balance. Further, with the best estimates of all other variables in that budget, the best estimate of BGE would be near 0.4. The model derived here implies a BGE for Mirror Lake of about 0.1, which would imply a greater use of allochthonous DOC than is likely in that system. On the other hand, recent work on BGE in the Hudson River largely conforms to Equation 3: the relationship between BP

and BGE was of the same form as equation 3, with slightly different constants (F Roland & JJ Cole, submitted).

CONCLUSIONS

There is a large range of variation in BGE in aquatic systems, but we have shown a consistent increase in BGE along gradients of productivity in aquatic systems. Growth seems to be energetically more costly in dilute systems, but at present we can only speculate on the causes of this. We have argued that maintenance of active transport systems and of basic metabolic machinery, and the production of extracellular enzymes, may exert disproportionately high energy demands on bacteria inhabiting oligotrophic aquatic systems and may result in low growth efficiency. We have also suggested that the cell-specific rates of organic matter utilization is similar in oligotrophic and eutrophic conditions, so that the rate of supply of organic matter may not be a factor limiting BGE. Rather, we suggest that a combination of the quality of this organic matter, nutrient availability, and the particular energetic demands in each type of system may regulate BGE.

We propose the following scenario. Bacterioplankton in oligotrophic lakes and oceans are exposed to generally low concentrations of dissolved substrates, including organic carbon and nutrients, and it is possible that cell growth is colimited by energy, carbon, and nutrients. The generally low concentrations of nutrients impose low growth rates on bacteria and place cells in the realm in which maintenance energy expenditures are high relative to the overall energy flux. Maintenance requirements are further enhanced by high costs of active transport, the need to maintain functional transport systems even when growth is impeded by lack of suitable substrates, and the production of exoenzymes needed to supply suitable substrates for growth. We have suggested that these relatively high-maintenance and other nongrowth energy requirements are met by the catabolism of relatively oxidized, LMW compounds which provide neither enough energy nor enough carbon to sustain growth. We suggest that these labile organic molecules, which support the bulk of BR in oligotrophic aquatic systems, particularly open oceans, originate from algal excretion, photooxidation of DOC, and atmospheric inputs of DOC. Thus, the low BGE values that characterize oligotrophic areas may be the result of relatively high maintenance energy requirements, the lack of mineral nutrients, and the predominance of low-energy compounds in the labile pool of DOC. As systems become enriched in nutrients and primary production increases, both the rate of supply and the quality of DOC increase, as does the availability of nutrients, with a general increase in BGE.

There is still much uncertainty surrounding the magnitude and variation of BGE in natural aquatic systems, and the present review has revealed significant

gaps in our knowledge. We propose the following areas that should be given priority in future studies.

1. We need more and better estimates of BR of bacterioplankton in a wider variety of aquatic systems. Our current understanding of BGE is largely limited by the scarcity and uncertainty of BR measurements. We need more consistency in the methods to measure BGE.

2. We know relatively little about the regulation of BR in bacterioplankton, although this is possibly the largest single component of organic carbon flow in most aquatic systems. In particular, the concept of maintenance energy requirements has never been explicitly investigated for bacterioplankton.

3. The distinction between energy and organic carbon limitation of BGE should be further explored, at both the conceptual and experimental levels.

4. The BGE that we measure in natural assemblages is an integrated measure of the efficiency of utilization of a large number of organic compounds. Individual compounds may be incorporated with very different efficiencies, and perhaps the overall BGE that we measure is related to, and indicative of, the proportion of broad qualitative classes of organic compounds available to bacteria.

5. The BGE that we measure is the average of BGEs of different subpopulations of bacteria that coexist within the bacterioplankton assemblage. Understanding what controls the distribution of subpopulations of highly active versus dormant or slowly growing cells in bacterioplankton assemblages will no doubt advance our understanding of what controls BGE in natural aquatic systems.

6. The energetic costs of the production of exoenzymes, active solute transport, and excretion of a variety of polymers have seldom been investigated in natural bacterioplankton, but these processes may play an important role in determining differences in BGE among systems.

7. One fundamental question is whether the low BGE measured in most oceanic systems is only a reflection of external factors such as nutrient or carbon availability or whether it is genetically determined and an inherent characteristic of the dominant bacteria in these systems.

Literature Cited

1. Amon RMW, Benner R. 1996. Bacterial utilization of different size classes of dissolved organic matter. *Limnol. Oceanogr.* 41:41–51
2. Anderson TR. 1992. Modeling the influence of food C:N ratio, and respiration on growth and nitrogen excretion in marine zooplankton and bacteria. *J. Plankton Res.* 14:1645–71
3. Baines SB, Pace ML. 1991. The production of dissolved organic matter by phytoplankton and its importance to bacte-

ria: patterns across marine and freshwater systems. *Limnol. Oceanogr.* 36: 1078–90

3a. Bano N, Moran MA, Hodson RE. 1997. Bacterial utilization of dissolved humic substances from a freshwater swamp. *Aquat. Microb. Ecol.* 12:233–38

4. Bauchop T, Elsden SR. 1960. The growth of microorganisms in relation to their energy supply. *J. Gen. Microbiol.* 23:457–69

5. Bauerfeind S. 1985. Degradation of phytoplankton detritus by bacteria: estimation of bacterial consumption and respiration in an oxygen chamber. *Mar. Ecol. Prog. Ser.* 21:27–36

6. Beeftink HH, van der Heijden RTJM, Heijnen JJ. 1990. Maintenance requirements: energy supply from simultaneous endogenous respiration and substrate consumption. *FEMS Microbiol. Ecol.* 73:203–10

7. Bell WH, Sakshaug E. 1980. Bacterial utilization of algal extracellular products. 2. A kinetic study of natural populations. *Limnol. Oceanogr.* 25:1021–33

8. Benner R, Lay J, K'nees E, Hodson RE. 1998. Carbon conversion efficiency for bacterial growth on lignocellulose: Implications for detritus-based food webs. *Limnol. Oceanogr.* 33:1514–26

9. Benner R, Opsahl S, Chin-Leo G, Richey JE, Forsberg BR. 1995. Bacterial carbon metabolism in the Amazon River system. *Limnol. Oceanogr.* 40:1262–70

10. Biddanda B. 1988. Microbial aggregation and degradation of phytoplankton-derived detritus in seawater. 2. Microbial metabolism. *Mar. Ecol. Prog. Ser.* 42:89–95

11. Biddanda B, Opsahl S, Benner R. 1994. Plankton respiration and carbon flux through bacterioplankton. *Limnol. Oceanogr.* 39:1259–75

12. Billen G. 1984. Heterotrophic utilization and regeneration of nitrogen. In *Heterotrophic Activity in the Sea*, ed. JE Hobbie, PJleB Williams, pp. 313–55. New York: Plenum

13. Billen G, Fontigny A. 1987. Dynamics of a *Phaeocystis*-dominated spring bloom in Belgian coastal waters. *Mar. Ecol. Prog. Ser.* 37:249–57

14. Bjørnsen PK. 1986. Bacterioplankton growth yield in continuous seawater cultures. *Mar. Ecol. Prog. Ser.* 30:191–96

15. Bjørnsen PK, Kuparinen J. 1991. Determination of bacterioplankton biomass, net production and growth efficiency in the Southern Ocean. *Mar Ecol. Prog. Ser.* 71:185–94

16. Blum K, Mills AL. 1991. Microbial growth and activity during the initial stages of seagrass decomposition. *Mar. Ecol. Prog. Ser.* 70:73–82

17. Carlson CA, Ducklow HW. 1996. Growth of bacterioplankton and consumption of dissolved organic carbon in the Sargasso Sea. *Aquat. Microb. Ecol.* 10:69–85

18. Cherrier J, Bauer JE, Druffel ERM. 1996. Utilization and turnover of labile dissolved organic matter by bacterial heterotrophs in eastern North Pacific surface waters. *Mar. Ecol. Prog. Ser.* 139:267–79

19. Chin-Leo G, Benner R. 1992. Enhanced bacterioplankton production and respiration at intermediate salinities in the Mississippi River plume. *Mar. Ecol. Prog. Ser.* 87:87–103

20. Cho BC, Azam F. 1988. Major role of bacteria in biogeochemical fluxes in the ocean's interior. *Nature* 332:441–43

21. Cho BC, Park MG, Shim JH, Azam F. 1996. Significance of bacteria in urea dynamics in coastal surface waters. *Mar. Ecol. Prog. Ser.* 142:19–26

21a. Choi JW, Sherr BF, Sherr EB. Dead or alive? A large fraction of ETS-inactive marine bacterioplankton cells, as assessed by the reduction of CTC, can become ETS-active with incubation and substrate addition. *Aquat. Microb. Ecol.* In press

22. Chróst RH. 1990. Microbial ectoenzymes in aquatic environments. In *Aquatic Microbial Ecology: Biochemical and Molecular Approaches*, ed. J Overbeck, RJ Chróst, pp. 47–78. Berlin: Springer-Verlag

23. Chróst RH, Faust MA. 1983. Organic carbon release by phytoplankton: its composition and utilization by bacterioplankton. *J. Plankton Res.* 5:477–93

24. Coffin RB, Connolly JP, Harris PS. 1993. Availability of dissolved organic carbon to bacterioplankton examined by oxygen utilization. *Mar. Ecol. Prog. Ser.* 101:9–22

25. Cole JJ, Caraco NF, Strayer DL, Ochs C, Nolan S. 1989. A detailed carbon budget as an ecosystem-level calibration of bacterial respiration in an oligotrophic lake during midsummer. *Limnol. Oceanogr.* 34:286–96

26. Cole JJ, Findlay S, Pace ML. 1988. Bacterial production in fresh and saltwater ecosystems: a cross-system overview. *Mar. Ecol. Prog. Ser.* 43:1–10

27. Connolly JP, Coffin RB, Landeck RE. 1992. Modeling carbon utilization by

bacteria in natural water systems. In *Modeling the Metabolic and Physiologic Activities of Microorganisms*, ed. C Hurst, pp. 249–76. New York: Wiley
28. Coveney MF, Wetzel RG. 1989. Bacterial metabolism of algal extracellular carbon. *Hydrobiologia* 173:141–49
29. Crawford CC, Hobbie JE, Webb KL. 1974. Utilization of dissolved free amino acids by estuarine microorganisms. *Ecology* 55:551–63
30. Daneri G, Riemann B, Williams PJL. 1994. In situ bacterial production and growth yield measured by thymidine, leucine and fractioned dark oxygen uptake. *J. Plankton Res.* 16:105–13
31. Dawes EA. 1985. Starvation, survival and energy reserves. In *Bacteria in Their Natural Environment*, ed. M Fletcher, GD Foodgate, pp. 43–79. New York: Academic
31a. Decho AW. 1990. Microbial exopolymer secretions in oceanic environments. *Oceanogr. Mar. Biol. Annu. Rev.* 28:73–153
31b. Decho AW, Moriarty DJ. 1990. Bacterial exopolymer utilization by a harpacticoid copepod: a methodology and results. *Limnol. Oceanogr.* 35:1039–49
31c. del Giorgio PA, Cole JJ, Caraco NF. 1998. Linking planktonic biomass structure to plankton metabolism and net gas fluxes in northern temperate lakes. *Ecology.* In press
32. del Giorgio PA, Cole JJ, Cimbleris A. 1997. Respiration rates in bacteria exceed phytoplankton production in unproductive aquatic systems. *Nature* 385:148–51
33. del Giorgio PA, Gasol JM, Vaque D, Mura P, Agusti S, et al. 1996. Bacterioplankton community structure: Protists control net production and the proportion of active bacteria in a coastal marine community. *Limnol. Oceanogr.* 41: 1169–79
34. del Giorgio PA, Prairie YT, Bird DF. 1997. Coupling between rates of bacterial production and the number of metabolically active cells in lake bacterioplankton, measured using CTC reduction and flow cytometry. *Microb. Ecol.* 34:144–54
35. del Giorgio PA, Scarborough G. 1995. Increase in the proportion of metabolically active bacteria along gradients of enrichment in freshwater and marine plankton: implications for estimates of bacterial growth and production. *J. Plankton Res.* 17:1905–24
36. Drabkova VG. 1990. Bacterial production and respiration in the lakes of different types. *Arch. Hydrobiol. Ergeb. Limnol.* 34:209–14
37. Ducklow HW, Carlson CA. 1992. Oceanic bacterial production *Adv. Microb. Ecol.* 12:113–81
38. Dzyuban AN, Timakova TM. 1981. Microflora and decomposition of organic matter in the water and bottom sediments of lake Pert. *Hydrobiol. J.* 17:23–27
39. Egli T. 1991. On multiple-nutrient-limited growth of microorganisms, with special reference to dual limitation by carbon and nitrogen substrates. *Antonie Leeuwenhoek* 60:225–34
40. Egli T, Lendenmann U, Snozzi M. 1993. Kinetics of microbial growth with mixtures of carbon sources. *Antonie Leeuwenhoek* 63:289–98
41. Fallon RD, Pfaender FK. 1976. Carbon metabolism in model microbial systems from a temperate salt marsh. *Appl. Environ. Microbiol.* 31:959–68
42. Fenchel T, Blackburn TH. 1979. *Bacteria and Mineral Cycling.* New York: Academic
43. Findlay S, Carlough L, Crocker MT, Gill HK, Meyer JL, et al. 1986. Bacterial growth on macrophyte leachate and fate of bacterial production. *Limnol. Oceanogr.* 31:1335–41
44. Findlay S, Pace ML, Lints D, Howe K. 1992. Bacterial metabolism of organic carbon in the tidal freshwater Hudson Estuary. *Mar. Ecol. Prog. Ser.* 89:147–53
45. Goldman JC, Caron DA, Dennett MR. 1987. Regulation of gross growth efficiency and ammonium regeneration in bacteria by substrate C:N ratio. *Limnol. Oceanogr.* 32:1239–52
46. Goldman JC, Dennett MR. 1991. Ammonium regeneration and carbon utilization by marine bacteria grown on mixed substrates. *Mar. Biol.* 109:369–78
47. Gosselink JG, Kirby CJ. 1974. Decomposition of salt marsh grass, *Spartina alterniflora* Loisel. *Limnol. Oceanogr.* 19:825–32
48. Goutx M, Acquaviva M, Bertrand J-C. 1990. Cellular and extracellular carbohydrates and lipids from marine bacteria during growth on soluble substrates and hydrocarbons. *Mar. Ecol. Prog. Ser.* 61:291–96
49. Griffith PC, Douglas DJ, Wainright SC. 1990. Metabolic activity of size-fractioned microbial plankton in estuarine, nearshore, and continental shelf waters of Georgia. *Mar. Ecol. Prog. Ser.* 59:263–70

50. Griffiths RP, Caldwell BA, Morita RY. 1984. Observations on microbial percent respiration values in arctic and subarctic marine waters and sediments. *Microb. Ecol.* 10:151–64
51. Güde H, Jürgens K, Parth G, Walser R. 1991. Indications for low net productivity of pelagic bacterioplankton. *Kiel. Meeresforsch. Sonderh.* 8:309–16
52. Haines EB, Hanson RB. 1979. Experimental degradation of detritus made from the salt marsh plants *Spartina alterniflora* Loisel, *Salicornia virginica* L., and *Juncus roemerianus* Scheele. *J. Exp. Mar. Biol. Ecol.* 40:27–40
53. Hanisch K, Schweitzer B, Simon M. 1996. Use of dissolved carbohydrates by planktonic bacteria in a mesotrophic lake. *Microb. Ecol.* 31:41–55
54. Hansell DA, Bates NR, Gundersen K. 1995. Mineralization of dissolved organic carbon in the Sargasso Sea. *Mar. Chem.* 51:201–12
55. Harder J. 1997. Species-independent maintenance energy and natural populations sizes. *FEMS Microbiol. Ecol.* 23:39–44
56. Heikes B, McCully B, Zhou X, Lee YN, Mopper K, et al. 1996. Formaldehyde methods comparison in the remote lower trophosphere during Manua Loa Photochemistry Experiment 2. *J. Geophys. Res.* 101:15741–55
57. Heijnen JJ, van Dijken JP. 1992. In search of a thermodynamic description of biomass yields for the chemotrophic growth of microorganisms. *Biotechnol. Bioeng.* 39:833–58
57a. Heissenberger A, Leppard GG, Herndl GJ. 1996. Relationship between the intracellular integrity and the morphology of the capsular envelope in attached and free-living marine bacteria. *Appl. Environ. Microbiol.* 62:4521–29
58. Hessen DO. 1992. Dissolved organic carbon in a humic lake: effects on bacterial production and respiration. *Hydrobiologia* 229:115–23
59. Hobbie JE, Crawford CC. 1969. Respiration corrections for bacterial uptake of dissolved organic compounds in natural waters. *Limnol. Oceanogr.* 14:528–32
60. Hopkinson CS Jr, Buffam I, Hobbie J, Vallino J, Perdue M, et al. 1998. Terrestrial inputs of organic matter to coastal ecosystems: an intercomparison of chemical characteristics and bioavailability. *Biogeochemistry.* In press
61. Hopkinson CS Jr, Sherr BF, Wiebe WJ. 1989. Size fractioned metabolism of coastal microbial plankton. *Mar. Ecol. Prog. Ser.* 51:155–66
61a. Hoppe HG. 1991. Microbial extracellular enzyme activity: a new key parameter in aquatic ecology. In *Microbial Enzymes in Aquatic Environments*, ed. RJ Chróst, pp. 60–83. New York: Springer-Verlag
62. Iturriaga R, Hoppe H-G. 1977. Observations of heterotrophic activity on photoassimilated matter. *Mar. Biol.* 40:101–8
63. Iturriaga R, Zsolnay A. 1981. Transformation of some dissolved organic compounds by a natural heterotrophic population. *Mar. Biol.* 62:125–29
64. Jahnke RA, Craven DB. 1995. Quantifying the role of heterotrophic bacteria in the carbon cycle: a need for respiration rate measurements. *Limnol. Oceanogr.* 40:436–41
65. Joint IR, Morris RJ. 1982. The role of bacteria in the turnover of organic matter in the sea. *Oceanogr. Mar. Biol. Annu. Rev.* 20:65–118
66. Johnson MD, Ward AK. 1997. Influence of phagotrophic protistan bacterivory in determining the fate of dissolved organic matter in a wetland microbial food web. *Microb. Ecol.* 33:149–62
67. Jørgensen NOG, Jensen RE. 1994. Microbial fluxes of free monosaccharides and total carbohydrates in freshwater determined by PAD-HPLC. *FEMS Microbiol. Ecol.* 14:79–94
68. Jørgensen NOG, Kroer N, Coffin RB, Yang X-H, Lee C. 1993. Dissolved free amino acids, combined amino acids, and DNA as sources of carbon and nitrogen to marine bacteria. *Mar. Ecol. Prog. Ser.* 98:135–48
69. Kaplan LA, Bott TL. 1983. Microbial heterotrophic utilization of dissolved organic matter in a piedmont stream. *Freshwater Biol.* 13:363–77
69a. Karl DM. 1980. Cellular nucleotide measurements and applications in microbial ecology. *Microbiol Rev.* 44:739–96
70. Karner M, Furhman JA. 1997. Determination of active marine bacterioplankton: a comparison of universal 16s rRNA probes, autoradiography, and nucleoid staining. *Appl. Environ. Microbiol.* 63:1208–13
71. Katretskiy YA. 1978. Oxygen consumption and efficiency with which the bacterioplankton in the Tsimlyansk Reservoir utilizes the energy contained in organic matter. *Hydrobiol J.* 15:16–19

71a. Kennedy AFD, Sutherland IW. 1987. Analysis of bacterial exopolysaccharides. *Biotechnol. Appl. Biochem.* 9:12–19
72. Kieber DJ, McDaniel, Mopper K. 1989. Photochemical source of biological substrates in seawater: implications for carbon cycling. *Nature* 341:637–39
73. King GM, Berman T. 1985. Potential effects of isotopic dilution on apparent respiration in ^{14}C heterotrophy experiments. *Mar. Ecol. Prog. Ser.* 19: 175–80
74. Kirchman DL. 1990. Limitation of bacterial growth by dissolved organic matter in the subarctic Pacific. *Mar. Ecol. Prog. Ser.* 62:47–54
75. Kirchman DL, Rich JH. 1997. Regulation of bacterial growth rates by dissolved organic carbon and temperature in the equational pacific ocean. *Microb. Ecol.* 33:11–20
76. Kirchman DL, Suzuki Y, Garside C, Ducklow HW. 1991. High turnover rates of dissolved organic carbon during a spring phytoplankton bloom. *Nature* 352:612–14
77. Kjelleberg S, Flardh KBG, Nystrom T, Moriarty DJW. 1993. Growth limitation and starvation of bacteria. In *Aquatic Microbiology*, ed. TE Ford, pp. 289–320. Boston: Blackwell
78. Koop K, Newell RC, Lucas MI. 1982. Microbial regeneration of nutrients from the decomposition of macrophyte debris on the shore. *Mar. Ecol. Prog. Ser.* 9:91–96
78a. Korber DR, Lawrence JR, Lappin-Scott HM, Costerton W. 1995. Growth of microorganisms on surfaces. In *Microbial Biofilms*, ed. HM Lappin-Scott, JW Costerton, pp. 1–47. Cambridge: Cambridge Univ. Press
79. Kristiansen K, Nielsen H, Riemann B, Fuhrman JA. 1992. Growth efficiencies of freshwater bacterioplankton. *Microb. Ecol.* 24:145–60
80. Kroer N. 1993. Bacterial growth efficiency on natural dissolved organic matter. *Limnol. Oceanogr.* 38:1282–90
81. Laanbroek HJ, Verplanke JC. 1986. Tidal variation in bacterial biomass, productivity and oxygen uptake rates in a shallow channel in the Oosterschelde basin, The Netherlands. *Mar. Ecol. Prog. Ser.* 29:1–5
82. Landwell P, Holme T. 1979. Removal of inhibitors of bacterial growth by dialysis culture. *J. Gen. Microbiol.* 103:345–52
82a. Leppard GG. 1995. The characterization of algal and microbial mucilages and their aggregates in aquatic ecosystems. *Sci. Total Environ.* 165:103–31
83. Linley EAS, Newell RC. 1984. Estimates of bacterial growth yields based on plant detritus. *Bull. Mar. Sci.* 35:409–25
84. Linton JD. 1990. The relationship between metabolite production and the growth efficiency of the producing organism. *FEMS Microbiol. Rev.* 75:1–18
85. Linton JD, Stephenson RJ. 1978. A preliminary study on growth yields in relation to the carbon and energy content of various organic growth substances. *FEMS Microbiol. Lett.* 3:95–98
86. Lucas MI, Newell RC, Velimirov B. 1981. Heterotrophic utilization of kelp (*Ecklonia maxima* and *Laminaria pallida*). II. Differential utilization of dissolved organic components from kelp mucilage. *Mar. Ecol. Prog. Ser.* 4:43–55
87. Marden P, Nystrom T, Kjelleberg S. 1987. Uptake of leucine by a marine gram-negative heterotrophic bacterium during exposure to starvation conditions. *FEMS Microbiol. Ecol.* 45:233–41
88. McFeters GA, Yu FP, Pyle BH, Stewart PS. 1995. Physiological assessment of bacteria using fluorochromes. *J. Microbiol. Methods* 21:1–13
89. Meyer JL, Edwards RT, Risley R. 1987. Bacterial growth on dissolved organic carbon from a blackwater river. *Microb. Ecol.* 13:13–29
90. Middelboe M, Nielsen B, Søndergaard M. 1992. Bacterial utilization of dissolved organic carbon (DOC) in coastal waters—determination of growth yield. *Arch. Hydrobiol. Ergeb. Limnol.* 37:51–61
91. Middelboe M, Søndergaard M. 1993. Bacterioplankton growth yield: a close coupling to substrate lability and beta-glucosidase activity. *Appl. Environ. Microbiol.* 59:3916–21
92. Middelboe M, Søndergaard M. 1995. Concentration and bacterial utilization of sub-micron particles and dissolved organic carbon in lakes and a coastal area. *Arch. Hydrobiol.* 133:129–47
93. Middelboe MB, Jørgensen NOG, Kroer N. 1996. Effects of viruses on nutrient turnover and growth efficiency of noninfected marine bacterioplankton. *Appl. Environ. Microbiol.* 62:1991–97
94. Monod J. 1942. *Recherches sur la Croissance des Cultures Bactériennes.* Paris: Hermann
95. Moran MA, Hodson RE. 1989. Formation and bacterial utilization of dis-

solved organic carbon derived from detrital lignocellulose. *Limnol. Oceanogr.* 34:1034–47

95a. Moran MA, Zepp RG. 1997. Role of photoreactions in the formation of biologically labile compounds from dissolved organic matter. *Limnol. Oceanogr.* 42:1307–16

96. Morita RY. 1997. *Bacteria in Oligotrophic Environments*. New York: Chapman & Hall

96a. Nawrocki MP, Karl DM. 1989. Dissolved ATP turnover in the Bransfield Strait, Antarctica during a spring bloom. *Mar. Ecol. Prog. Ser.* 57:35–44

97. Newell RC, Lucas M. 1981. The quantitative significance of dissolved and particulate organic matter released during fragmentation of kelp in coastal waters. *Kiel. Meeresforsch.* 5:356–69

98. Newell RC, Lucas M, Linley EAS. 1981. Rate of degradation and efficiency of conversion of phytoplankton debris by marine microorganisms. *Mar. Ecol. Prog. Ser.* 6:123–36

99. Nuncio J, Seaton PJ, Kieber RJ. 1995. Biological production of formaldehyde in the marine environment. *Limnol. Oceanogr.* 40:521–27

99a. Pakulski JD, Aas P, Jeffrey W, Lyons M, Von Waasenbergen L, et al. 1998. Influence of light on bacterioplankton production and respiration in a subtropical coral reef. *Aquat. Microb. Ecol.* 14:137–48

100. Payne WJ. 1970. Energy yields and growth of heterotrophs. *Annu. Rev. Microbiol.* 24:17–52

101. Payne WJ, Wiebe WJ. 1978. Growth yield and efficiency in chemosynthetic microorganisms. *Annu. Rev. Microbiol.* 32:155–83

102. Pirt SJ. 1982. Maintenance energy: a general model for energy-limited and energy-sufficient growth. *Arch. Microbiol.* 133:300–2

103. Platpira VP, Filmanovicha RS. 1993. Respiration rate of bacterioplankton in the Baltic Sea. *Hydrobiol. J.* 29:87–94

104. Poindexter JS. 1987. Bacterial response to nutrient limitation. In *Ecology of Microbial Communities*, ed. M Fletcher, TRG Gray, JG Jones, pp. 283–317. Cambridge: Cambridge Univ. Press

105. Pomeroy LR, Hanson RB, McGillivary PA, Sherr BF, Kirchman D, et al. 1984. Microbiology and chemistry of fecal products of pelagic tunicates: rates and fates. *Bull. Mar. Sci.* 35:426–39

106. Pomeroy LR, Sheldon JE, Sheldon WM Jr. 1994. Changes in bacterial numbers and leucine assimilation during estimations of microbial respiratory rates in seawater by the precision Winkler method. *Appl. Environ. Microbiol.* 60:328–32

107. Pomeroy LR, Sheldon JE, Sheldon WM Jr, Peters F. 1995. Limits to growth and respiration of bacterioplankton in the Gulf of Mexico. *Mar. Ecol. Prog. Ser.* 117:259–68

108. Pomeroy LR, Wiebe WJ. 1993. Energy sources for microbial food webs. *Mar. Microb. Food Webs* 7:101–18

109. Pomeroy LR, Wiebe WJ, Deibel D, Thompson RJ, Rowe GT, et al. 1991. Bacterial responses to temperature and substrate concentration during the Newfoundland spring bloom. *Mar. Ecol. Prog. Ser.* 75:143–59

110. Reitner B, Herndl GJ, Herzig A. 1997. Role of ultraviolet-B radiation on photochemical and microbial oxygen consumption in a humic-rich shallow lake. *Limnol. Oceanogr.* 42:950–60

110a. Rich J, Gosselin M, Sherr E, Sherr B, Kirchman D. 1997. High bacterial production, uptake and concentrations of dissolved organic matter in the Central Arctic Ocean. *Deep-Sea Res.* 44:1645–63

110b. Ricker WE, 1973. Liner regression in fishery research. *J. Fish. Res. Bd. Can.* 30:409–34

111. Robertson ML, Mills AL, Zieman JC. 1982. Microbial synthesis of detritus-like particulates from dissolved organic carbon released by tropical seagrasses. *Mar. Ecol. Prog. Ser.* 7:279–85

112. Robinson JD, Mann KH, Novitsky JA. 1982. Conversion of the particulate fraction of seaweed detritus to bacterial biomass. *Limnol. Oceanogr.* 27:1072–79

113. Deleted in proof

114. Roman MR, Ducklow HW, Fuhrman JA, Garside C, Glibert PM, et al. 1988. Production, consumption, and nutrient recycling in a laboratory mesocosm. *Mar. Ecol. Prog. Ser.* 42:39–52

115. Romanenko VI, Perez Eiriz M, Kudryavtsev VM, Pubienes A. 1976. Microbiological processes in the cycle of organic matter in Anabanilja Reservoir, Cuba. *Hydrobiol. J.* 13:8–14

116. Russell JB. 1991. A re-assessment of bacterial growth efficiency: the heat production and membrane potential of *Streptococcus bovis* in batch and continuous culture. *Arch. Microbiol.* 155:559–65

117. Russell JB. Cook GM. 1995. Energetics of bacterial growth: balance of anabolic and catabolic reactions. *Microbiol. Rev.* 59:48–62
118. Sand-Jensen K, Jensen LM, Marcher S, Hansen M. 1990. Pelagic metabolism in eutrophic coastal waters during a late summer period. *Mar. Ecol. Prog. Ser.* 65:63–72
119. Schwaerter S, Søndergaard M, Riemann B, Jensen LM. 1988. Respiration in eutrophic lakes: the contribution of bacterioplankton and bacterial growth yield. *J. Plankton Res.* 3:515–31
120. Schweitzer B, Simon M. 1995. Growth limitation of planktonic bacteria in a large mesotrophic lake. *Microb. Ecol.* 30:89–104
121. Servais P. 1989. Bacterioplankton biomass and production in the river Meuse (Belgium). *Hydrobiologia* 174:99–110
122. Deleted in proof
123. Sherr BF, del Giorgio PA, Sherr EB. 1998. Estimating the abundance and single-cell characteristics of actively respiring bacteria via the redox dye, CTC. Aquat. Microb. Ecol. In press
124. Sherr EB, Sherr BF. 1996. Temporal offset in oceanic production and respiration process implied by seasonal changes in atmospheric oxygen: the role of heterotrophic microbes. *Aquat. Microb. Ecol.* 11:91–100
125. Sinsabaugh RL, Findlay S, Franchini P, Fischer D. 1997. Enzymatic analysis of riverine bacterioplankton production. *Limnol. Oceanogr.* 42:29–38
126. Smith REH, Harrison WG, Irwin B, Platt T. 1986. Metabolism and carbon exchange in microplankton of the Grand Banks (Newfoundland). *Mar. Ecol. Prog. Ser.* 34:171–83
127. Smith SV, Hollibaugh JT. 1993. Coastal metabolism and the oceanic organic carbon balance. *Rev. Geophys.* 31:75–89
128. Søndergaard M, Middelboe M. 1995. A cross-system analysis of labile dissolved organic carbon. *Mar. Ecol. Prog. Ser.* 118:283–94
129. Søndergaard M, Theil-Nielsen J. 1997. Bacterial growth efficiency in lakewater cultures. *Aquat. Microb. Ecol.* 12:115–22
130. Sorokin YI. 1971. On the role of bacteria in the productivity of tropical oceanic waters. *Int. Rev. Ges. Hydrobiol.* 56:1–48
131. Sorokin YI, Mameva TI. 1980. Rate and efficiency of the utilization of labile organic matter by planktonic microflora in coastal Peruvian waters. *Pol. Arch. Hydrobiol.* 27:447–56
132. Stevenson LH. 1978. A case for bacterial dormancy in aquatic systems. *Microb. Ecol.* 4:127–33
133. Stouthamer AH. 1973. A theoretical study on the amount of ATP required for synthesis of microbial cell material. *Antonie Leeuwenhoek* 39:545–65
134. Stouthamer AH. 1979. The search for correlation between theoretical and experimental growth yields. *Int. Rev. Biochem. Microb. Biochem.* 21:1–47.
135. Stouthamer AH, Bettenhaussen C. 1973. Utilization of energy for growth and maintenance in continuous and batch cultures of microorganisms. *Biochim. Biophys. Acta* 301:53–70
136. Stuart V, Lucas MI, Newell RC. 1981. Heterotrophic utilization of particulate matter from the kelp *Laminaria pallida*. *Mar. Ecol. Prog. Ser.* 4:337–48
137. Stuart V, Newell RC, Lucas MI. 1982. Conversion of kelp debris and faecal material from the mussel *Aulacomya ater* by marine micro-organisms. *Mar. Ecol. Prog. Ser.* 7:47–57
138. Tempest DW. 1978. The biochemical significance of microbial growth yields: a reassessment. *Trends Biochem. Sci.* 3:180–84
139. Tempest DW, Neijssel OM. 1992. Physiological and energetic aspects of bacterial metabolite overproduction. *FEMS Microbiol. Lett.* 100:169–76
140. Tempest DW, Neijssel OM, Texeira de Mattos MJ. 1985. Regulation of carbon substrate metabolism in bacteria growing in chemostat culture. In *Environmental Regulation of Microbial Metabolism*, ed. IS Kulaev, EA Dawes, DW Tempest, pp. 53–68. NewYork: Academic
141. Therkildsen MS, Isaksen MF, Lomstein BA. 1997. Urea production by the marine bacteria *Delaya venuts* and *Pseudomonas stutzeri* grown in a minimal medium. *Aquat. Microb. Ecol.* 13:213–17
142. Thingstad TF, Hagström Å, Rassoulzadegan F. 1997. Accumulation of degradable DOC in surface waters: Is it caused by a malfunctioning microbial loop? *Limnol. Oceanogr.* 42:398–404
143. Tortell PD, Maldonado MT, Price NM. 1996. The role of heterotrophic bacteria in iron-limited ocean ecosystems. *Nature* 383:330–32
144. Tranvik LJ. 1988. Availability of dissolved organic carbon for planktonic bacteria in oligotrophic lakes of differing humic content. *Microb. Ecol.* 16:311–22

145. Tranvik LJ. 1990. Bacterioplankton growth on fractions of dissolved organic carbon of different molecular weights from humic and clear lakes. *Appl. Environ. Microbiol.* 56:1672–77
146. Tranvik LJ. 1992. Rapid microbial production and degradation of humic-like substances in lake water. *Arch. Hydrobiol. Beih.* 37:43–50
147. Tranvik LJ, Høffle MG. 1987. Bacterial growth in mixed cultures on dissolved organic carbon from humic and clear water lakes. *Appl. Environ. Microbiol.* 53:482–28
148. Tulonen T, Salonen K, Arvola L. 1992. Effects of different molecular weight fractions of dissolved organic matter on the growth of bacteria, algae and protozoa from a highly humic lake. *Hydrobiologia* 229:239–52
149. Tupas L, Koike I. 1990. Amino acid and ammonium utilization by heterotrophic marine bacteria grown in enriched seawater. *Limnol. Oceanogr.* 35:1145–55
150. Turley CM, Lochte K. 1990. Microbial response to the input of fresh detritus to the deep-sea bed. *Paleogeogr. Paleoclimatol. Paleoecol.* 89:3–23
151. Vallino JJ, Hopkinson CS, Hobbie JE. 1996. Modeling bacterial utilization of dissolved organic matter: optimization replaces Monod growth kinetics. *Limnol. Oceanogr.* 41:1591–1609
152. Westerhoff HV, Hellingwerf KJ, Van Dam K. 1983. Thermodynamic efficiency of microbial growth is low but optimal for maximal growth rate. *Proc. Natl. Acad. Sci. USA* 80:305–9
153. White PA, Kalff J, Rasmussen JB, Gasol JM. 1991. The effect of temperature and algal biomass on bacterial production and specific growth rate in freshwater and marine habitats. *Microb. Ecol.* 21:99–118
154. Wiebe WJ, Smith DF. 1977. Direct measurement of dissolved organic carbon release by phytoplankton and incorporation by microheterotrophs. *Mar. Biol.* 42:213–33
155. Williams PJleB. 1981. Microbial contribution to overall marine plankton metabolism: direct measurements of respiration. *Oceanol. Acta* 4:359–64
156. Zweifel UL, B. Riemann B, Hagström Å. 1993. Consumption of dissolved organic carbon by marine bacteria and demand for inorganic nutrients. *Mar. Ecol. Prog. Ser.* 101:23–32

Annual Review of Ecology and Systematics
Volume 29 (1998)

CONTENTS

(*continued*)