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MINIREVIEW

Evidence for the Ubiquity of Mixotrophic Bacteria in the Upper Ocean: Implications and Consequences[▽]

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Microorganisms are usually grouped into those relying solely on harvesting light (phototrophy) or those relying solely on the assimilation of organic or inorganic compounds (chemotrophy) to meet their requirements for energy. As a carbon source for biomass production, they can use either inorganic carbon (autotrophy) or organic substances (heterotrophy) (Table 1). Most biogeochemical studies of marine environments use the dichotomy of grouping microorganisms into photo(auto)trophs (primary producers like algae and cyanobacteria) and (organo)heterotrophs (secondary producers, like most heterotrophic bacteria) (see, for example, references 2 and 74).

It is well known that metabolic modes of aquatic microorganisms are more diverse than that, and modes such as mixotrophy, chemoautotrophy, chemoheterotrophy, or photoheterotrophy do exist. For microeukaryotes, mixotrophy is a widespread phenomenon in aquatic habitats and is observed in many organisms (see, for example, references 16, 62, 82, and 83). Some *Bacteria*, like purple nonsulfur bacteria (anoxygenic photosynthetic bacteria), are also able to alter between photo-, hetero-, auto-, litho-, and organotrophy, depending on the environmental conditions (93).

Although mixotrophic bacteria, which invest in both phototrophic and heterotrophic enzymatic apparatuses and combine them with autotrophic and/or organotrophic strategies, have been isolated from various aquatic environments (see, for example, references 66, 75, and 93), their contribution to total biomass and their importance for biogeochemical processes in aquatic systems have been ignored.

However, recent genome data of two *Prochlorococcus* strains (67) and one *Synechococcus* strain (56), together with results from isotope tracer uptake experiments (45, 97, 98), suggest that at least certain strains of these ubiquitous picocyanobacteria are not pure photoautotrophs and can take up organic compounds.

Cyanobacteria are not the only bacteria that can harvest light energy. Aerobic anoxygenic photosynthetic bacteria (AAnPB) use mainly bacteriochlorophyll *a* (Bchl-*a*) for photosynthesis (for example, see references 75 and 93), whereas other bacteria can use proteorhodopsin, a light-driven proton pump (3, 13, 17, 23). Isolated representatives of these bacteria usually use organic carbon for cell synthesis and are characterized as photo(organo)heterotrophs (22–24, 63, 93).

Genetic community surveys suggest that AAnPB and proteorhodopsin-containing bacteria (PRB) are common in surface waters throughout the oceans of the world and can make up a substantial fraction of the bacterial community in oligotrophic marine environments (see, for example, references 3–5, 10, 34, 35, 69–72, 80, and 89). Furthermore, it has been shown that phylogenetic clades, including organisms with photoreceptors, can be significant in degradation processes of organic compounds (43–45). These observations suggest that mixotrophic bacteria may be major players in photo-, hetero-, auto-, and organotrophic processes in the upper ocean, which may demand a thorough revision of our understanding of how microorganisms contribute to biogeochemical cycles in the marine environment.

DEFINING A MIXOTROPHIC ORGANISM

The simple classification into phototrophs and heterotrophs cannot accurately describe the metabolic diversity regarding the role of bacteria in the carbon cycle. In order to accomplish this, bacterial metabolic modes can be defined on the basis of both the energy (photo- versus litho- versus organotroph) and carbon (auto- versus heterotroph) sources used (Table 1). The definition of phototrophy may be the most confusing, as it must be considered that not all phototrophic organisms fix inorganic carbon. Additionally, several isolated phototrophic bacteria do not produce oxygen and use compounds other than water as an electron donor. Organic compounds, hydrogen, hydrogen sulfide (H_2S), thiosulfate ($S_2O_3^{2-}$), elemental sulfur (S^0), and ferrous iron (Fe^{2+}) can all be used by purple sulfur bacteria as electron donors, each potentially producing a unique product (see, for example, references 59, 60, 91, and 94). As an example, bacteria in the genus *Ectothiorhodospira* use hydrogen sulfide as an electron donor and produce elemental sulfur as a product instead of oxygen (87).

Various definitions have been proposed for phototrophy (reviewed in references 20 and 21). In this review, the following definition of phototrophy is used (42): "Phototrophy is a series of processes in which electromagnetic energy is converted to chemical energy" (Table 1). The term mixotrophy will be used to describe a metabolic strategy in which at least three modes (photo-, organo-, auto-, and heterotrophy) are combined.

First, a broad categorization of mixotrophic strategies is

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TABLE 1. Definitions of metabolic strategies to obtain carbon and energy[a]

Metabolic strategy	Definition
Energy source	
Phototrophy	Series of processes in which electromagnetic energy is converted to chemical energy
Chemo-(organo- or litho-)trophy	Series of processes in which energy is obtained by oxidizing chemical compounds; organisms using inorganic compounds (for example, water, hydrogen, sulfide, or ammonia) for this purpose are called lithotrophs, and others that require organic compounds (e.g., sugars or organic acids) are called organotrophs
Carbon source	
Heterotrophy	Series of processes in which organic compounds are used as a carbon source for biosynthesis
Autotrophy	Series of processes (for example, Calvin cycle) in which carbon dioxide and water are synthesized to organic carbon compounds

[a] According to *Brock Biology of Microorganisms* (42).

required in order to develop a general understanding of mixotrophy as an evolutionary strategy and its influence on carbon and energy fluxes in the upper ocean. For example, if photoheterotrophy is obligate, both light as an energy source and organic substances as a carbon source are necessary for sustaining growth and maintenance; if photoautotrophy is obligate but heterotrophy is facultative, only photoautotrophy is essential for growth and maintenance, and heterotrophy can be used to supplement inorganic carbon fixation; if organoheterotrophy is obligate but phototrophy is facultative, only organic matter is necessary for sustaining growth and maintenance, but, for example, phototrophy can be used to back up ATP production; if both photo- and organotrophy to obtain energy and auto- and heterotrophy are facultative, these mixotrophs are able to grow by photo-organo-auto-heterotrophy.

To what extent marine bacteria perform the metabolic strategies mentioned above is unknown. However, as more and more naturally abundant marine *Bacteria* and *Archaea* are obtained in cultures, our knowledge about the metabolic versatility of these microorganisms in respect to obtaining energy and carbon will increase. Not only isolates of abundant microorganisms but also culture-independent studies will provide insight into how these organisms may adjust their metabolic pathways, depending on environmental conditions, and use either solar radiation or organic matter degradation to obtain energy.

However, to invest in a mixotrophic strategy has significant physiological implications for an organism. Mixotrophs have to invest in the synthesis and maintenance of both a light-harvesting apparatus and proteins for uptake and subsequent degradation of organic compounds. These energetic costs may lower their maximum growth rates. A mixotroph is therefore expected to be inferior if it competes with specialist phototrophs for light or specialist heterotrophs for dissolved organic matter. So what is the advantage of simultaneously investing in pigments and proteins to degrade organic matter? There are also inherent advantages to being mixotrophic. Mixotrophic bacteria may dominate aquatic environments due to their capability to use more resources than either photoautotrophic or organoheterotrophic bacteria. For example, if organic matter is limiting, a mixotroph can switch to using light as an energy source and use inorganic carbon as a carbon source, and as light gets limiting, it can switch back to degradation of organic substances. This has been shown, for example, for bloom-forming flagellates (68, 83) and algae (53). In a dynamic system, like a marine microbial community, this might be the factor by which an organism can survive and out-compete other organisms.

Ciliates and flagellates are not the only organisms that can be mixotrophic; mixotrophy also appears to be very common in multicellular organisms (see, for example, references 62, 82, and 83) and bacteria. For *Cyanobacteria*, mixotrophy was previously described by Rippka in 1972 (66), and some years later aerobic photo-"organo"-heterotrophic bacteria containing Bchl-*a* were isolated by Shiba and coworkers (75). Since then, many more bacteria using a mixotrophic metabolism have been identified and affiliated with various phyla, i.e., *Chloroflexi* (61), *Cyanobacteria* (see, for example, references 7, 49, 54, 66, and 95), and *Proteobacteria* (71, 76, 85). These bacteria embody a wide genetic and functional diversity, as shown in many isolates of *Cyanobacteria* (see, for example, references 7, 49, 54, 66, and 95) and AAnPB (see reviews in references 64 and 91). They can use a wide range of organic compounds and contain diverse light-harvesting pigments, like chlorophylls *a* and *b*, bacteriochlorophylls *a*, *b*, *c*, *d*, and *e*, and various carotenoids.

CHLOROPHYLL-CONTAINING BACTERIA: CYANOBACTERIA

The main light-harvesting pigment on earth is chlorophyll, and chlorophyll *a* and divinyl derivatives of chlorophyll *a* dominate in the photic zone of the ocean. Cyanobacteria that contain these pigments are considered to dominate phototrophic and autotrophic processes in marine environments (8, 28, 90). For example, the genus *Prochlorococcus* dominates the phytoplankton in the central oceanic gyres (57), while *Synechococcus* can be found almost everywhere in the upper ocean (39). Although cyanobacteria are often described as photoautotrophic organisms, the uptake of organic compounds by certain genera is well described in the literature (for example, see references 7, 9, 54, 66, 97, and 98). Accordingly, amino acid transport genes were recently described for several *Cyanobacteria* genera, like *Anabaena*, *Nostoc*, *Pseudoanabaena*, and *Planktothrix* (for examples, see references 49 and 95). Recent studies on *Prochlorococcus* and *Synechococcus* also suggest that these ubiquitous and abundant marine picocyanobacteria have the potential for partial heterotrophy. For example, *Synechococcus* contributes substantially to primary production in tropical and temperate waters (90) and can be a major consumer of organic sulfur compounds, like dimethylsulfoniopropionate and methanethiol (45). Quantification of dimethylsulfoniopropionate and methanethiol uptake by *Synechococcus* using microautoradiography-fluorescence in situ hybridization showed that this picocyanobacterium plays a major role in the cycling of these compounds. Although it has been argued that *Cyanobacteria* are unable to incorporate

some organic compounds, e.g., thymidine (19), axenic cultures of *Synechococcus* can utilize urea (9, 50) and amino acids (7, 49, 54) at a low rate and even show aminopeptidase activity (48). Experimental evidence exists that both *Synechococcus* and *Prochlorococcus* can take up amino acids in their natural environments (97, 98). Zubkov and his colleagues argue that *Prochlorococcus* can contribute up to 30% to leucine-derived bacterial production. This should result in an overestimation of secondary production based on the leucine incorporation method (32). However, considering that these picocyanobacteria are mixotrophs, they should contribute to not only primary but also secondary production. Furthermore, genome sequences of two *Prochlorococcus* strains revealed that both possess known pathways that allow for incomplete heterotrophy, although both strains lack genes for steps in the tricarboxylic acid cycle (67). Additionally, oligopeptide transporter genes were present in the two genomes (67). Thus, at least certain strains seem to back up their photoautotrophic lifestyle by using organic carbon for cell synthesis.

BACTERIOCHLOROPHYLL-CONTAINING BACTERIA: AEROBIC ANOXYGENIC PHOTOSYNTHETIC BACTERIA

As for chlorophyll, several forms of bacteriochlorophyll with various structures and stoichiometries exist. Bchl-*a*, -*b*, -*c*, -*d*, and -*e* were identified and described in the literature more than 30 years ago (59, 60). It was assumed that Bchl *a*-containing bacteria, also known as purple bacteria, require an anoxic environment for photosynthesis (59, 60). However, Shiba and coworkers (75, 76) described an obligate aerobic bacterium (*Erythrobacter longus*) containing Bchl-*a* that was isolated from seaweed and seawater. In recent decades many representatives of bacteria producing Bchl-*a* have been isolated from aerobic environments and designated AAnPB (for examples, see references 64, 65, 75–79, and 93). AAnPB have also been reviewed previously (20, 64, 78, 93), with extensive work on habitat, taxonomy, morphological diversity, development and function of the photosynthetic apparatus, carbon metabolism, and their potential for use in bioremediation.

Most representatives of the AAnBP are related to *Erythrobacter* and *Roseobacter*, but Bchl-*a* has also been detected in some physiologically distinct groups such as aerobic methylotrophic bacteria and rhizobia (for examples, see references 15, 88, and 92). Phylogenetically, AAnPB belong to the alpha, beta, and gamma subclasses of the *Proteobacteria* (for examples, see references 36–38, 64, 84, and 93) and group together with anaerobic anoxygenic photosynthetic bacteria as well as with some chemotrophic species (27, 64, 73). Most cultured planktonic AAnPB belong to only a few restricted groups within the *Alphaproteobacteria* (93) including *Roseobacter* and *Erythrobacter* spp. (for examples, see references 6 and 76–79). AAnPB have been isolated from various aquatic environments, including samples from marine algae and sediments (i.e., *Roseobacter litoralis* and *Roseobacter denitrificans* [36]), from a hypersaline Antarctic lake (*Staleya guttiformis* [37], *Roseovarius tolerans* [36], and *Roseisalinus antarcticus* [38]), from a hypersaline lake in Australia, and from freshwater environments (for a review, see reference 93; for more recent articles, see references 22, 30, 55, 65, and 84). Isolates from seawater samples are scarce, whereof *Erythrobacter* spp. are among the commonly cultured Bchl-*a*-containing bacteria (35). More marine AAnPB strains related to *Roseobacter* were recently described in the literature (1, 6, 31, 40, 41; see also a review in reference 64).

These isolates are described as photo(organo)heterotrophs, as they require organic carbon for growth. Most of these isolates are capable of oxidizing a great diversity of organic carbon sources (see reviews in references 64 and 93). They can metabolize sugars, carboxylic acids, fatty acids, and amino acids and are highly versatile metabolically in terms of their organic carbon degradation. However, none of the AAnPB isolates has yet been grown autotrophically, and the key enzyme of the Calvin cycle, ribulosebisphosphate carboxylase, has not been found in any AAnPB species (93). Nonetheless, light stimulation of CO_2 uptake was detected in several species (33, 77, 78, 85). Although the degree of CO_2 fixation seems too low for purely autotrophic growth, it could provide additional organic carbon intermediates for an otherwise purely heterotrophic metabolism. Until now, it could be suggested that most AAnPB in aquatic environments grow photo-organotrophically, using light as an additional energy source to supplement their organotrophic lifestyle.

Surprisingly, these AAnPB and their effects on carbon cycling and trophic interactions were not considered until recently, although high abundances of AAnPB in marine and freshwater environments were reported more than 15 years ago. Their wide and abundant distribution was shown by the presence of a high proportion of AAnPB to the total heterotrophic bacterial strains isolated from marine environments (10 to 30%) (79). The proportion of Bchl-*a* to chlorophyll-*a* was found to be as high as 10% in oligotrophic waters, when bulk Bchl-*a* in Pacific Ocean surface water was measured by high-pressure liquid chromatography. However, samples from off the coast of southern California yielded a significantly lower proportion of Bchl-*a* to chlorophyll *a* (0.7%) in oligotrophic waters (25).

Recent investigations using infrared fluorescence microscopy and molecular tools corroborate that AAnPB are common in the world ocean (34, 35, 72, 80). These studies revealed that the relative contribution to total bacterial abundance can vary from below 1% to up to 30% in marine environments. First indications that they are more abundant in oligotrophic environments (35) were challenged by more recent reports (72, 80). There clearly is a need to increase our knowledge about the physiology (metabolic capabilities) and ecology of aquatic AAnPB to understand what is regulating their distribution in the ocean. Since they are widely distributed and abundant in the marine environment, they may play a key role in many biogeochemical processes. However, we know almost nothing about their importance for phototrophic processes and the degradation of organic molecules. Another important question to be answered is what is regulating their metabolism, their use of phototrophy or organotrophy to obtain energy. A study on *Roseateles depolymerans*, a freshwater *Betaproteobacteria*, indicates that Bchl-*a* synthesis is induced by decreasing organic carbon concentrations, as transcription of the *Roseateles depolymerans puf* operon was controlled by changes in carbon source in addition to oxygen tension and light intensity (85). Although many AAnPB have been isolated and described, it still remains unresolved whether the retrieved isolates are truly

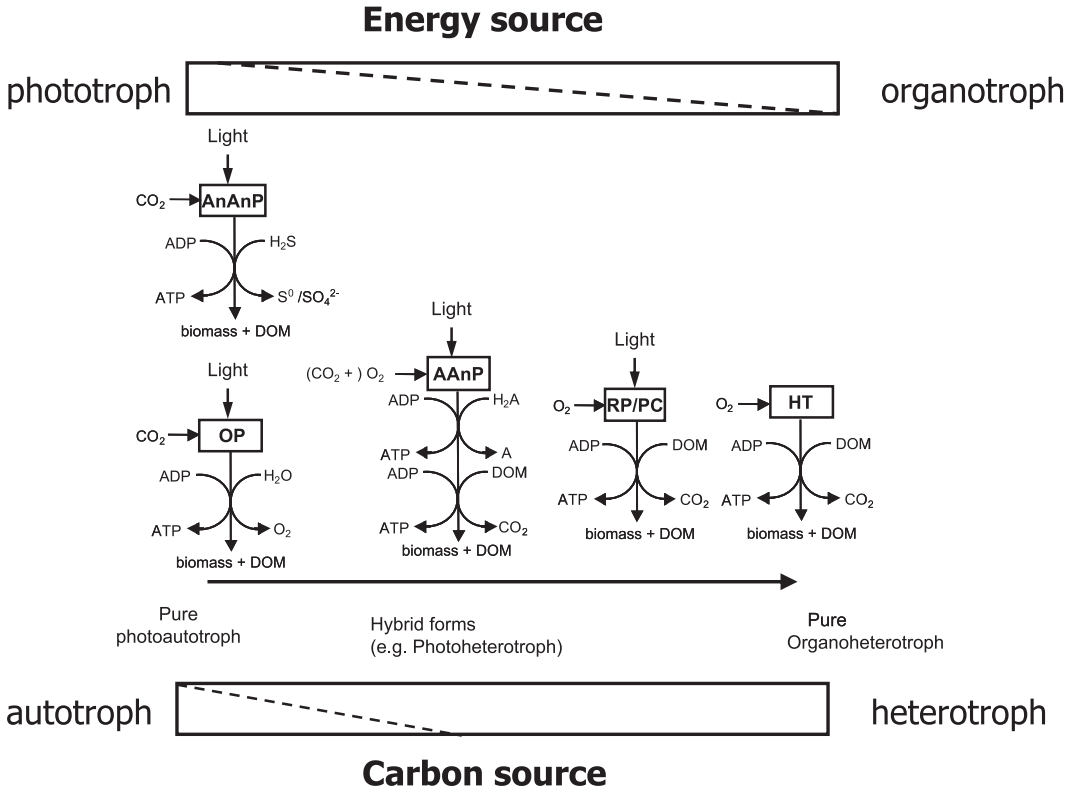

FIG. 1. Metabolic modes. AnAnP, anaerobic anoxygenic photosynthesis; OP, oxygenic photosynthesis; AAnP, aerobic anoxygenic photosynthesis; RP/PC, rhodopsin and other pigment; HT, heterotroph; DOM, dissolved organic matter. The diagram was modified from Karl (29).

representative of the populations that are abundant in the environment.

To conclude, all cultured isolates of AAnPB can be regarded as mixotrophs (photochemoheterotrophs). However, to what extent they are either photo-, litho-, or organotrophic in their natural environments is unknown.

PROTEORHODOPSIN-CONTAINING BACTERIA

Proteorhodopsin is a chromophore that can function as a light-driven proton pump and belongs to the microbial rhodopsin superfamily, type 1 rhodopsins (46). So far, only two reports provide direct evidence that proteorhodopsin actively functions as a light-harvesting pigment in seawater (4, 23).

Proteorhodopsin was first identified in the γ-proteobacterial group SAR86 using environmental genomic techniques (3). Since then, more proteorhodopsin diversity has been identified in the Red Sea (47, 69), the Mediterranean Sea (69), the Sargasso Sea (89), and the Pacific Ocean (70). Proteorhodopsin genes had only been documented in *Proteobacteria* (11); until recently, proteorhodopsin genes were detected in two *Flavobacteria* (L. Gómez-Consarnau et al., unpublished data) and in *Archaea* affiliated with the order *Thermoplasmatales* (18).

PRB appear to be ubiquitous in oceanic surface waters, since proteorhodopsin protein-encoding genes could be detected in culture-independent surveys of various coastal and open-ocean sites (4, 46, 69, 71, 89). It can be suggested that proteorhodopsin genes are very abundant in marine environments, since Venter and colleagues (89) recovered 743 proteorhodopsin-like sequences and 1,164 rRNA sequences by shotgun sequencing of a Sargasso Sea sample, which gives a ratio of 0.7 (81). A ratio of 0.13 was obtained by analyzing a bacterial artificial chromosome library from a sample of the photic zone of the Mediterranean Sea (71).

The very high abundance and diversity of bacteriorhodopsin homologues in the surface waters of the Sargasso and Mediterranean Seas suggest that these genes play an important role in harvesting light energy. PRB may also be very abundant in other parts of the ocean, since clone libraries from various marine environments revealed a high abundance of recovered 16S rRNA gene sequences of SAR86 and SAR11 (for example, see references 3, 14, 26, 51, 52, 58, 63, 86, and 96).

To date, proteorhodopsin can be assigned to a few bacterial groups: *Flavobacteria*, SAR11, and SAR86. All other proteorhodopsin sequences reported have been recovered as PCR amplicons or contigs without phylogenetic marker genes; thus, the origins (phylogeny based, for example, on the 16S rRNA gene) of these sequences remain unknown. However, fosmid libraries (11) and shotgun sequencing (89) provide evidence that proteorhodopsins are widely distributed among several different taxa of α- and γ-*Proteobacteria*. So far, SAR11 strain HTCC1062 (*Pelagibacter ubique* [23]) and two *Flavobacteria* (L. Gómez-Consarnau et al., unpublished) are the only isolated marine bacteria containing proteorhodopsin. Recently, the genome of *P. ubique* strain HTCC1062 was sequenced (24), and several more genome sequences of representatives from the

SAR11 group are on the way (S. J. Giovannoni et al., unpublished data). The published *P. ubique* strain HTCC1062 genome encodes nearly all of the basic functions for organoheterotrophy of α-proteobacterial cells. Giovannoni and colleagues showed that *P. ubique* strain HTCC1062 expressed its proteorhodopsin gene when exposed to its natural environment and that the *Pelagibacter* proteorhodopsin functions as a light-dependent proton pump (23). Hence, *P. ubique* strain HTCC1062 provides further evidence that photo-organoheterotrophic bacteria may dominate the world ocean (24), since bacteria belonging to the SAR11 clade can contribute up to 35% of the bacterial abundance (44, 51). This group of bacteria can account for more than 50% of the amino acid and glucose assimilation in oligotrophic marine water samples (44), emphasizing their important role in the degradation of organic carbon compounds.

IMPLICATIONS OF MIXOTROPHY FOR BIOGEOCHEMICAL CYCLES AND THE MICROBIAL LOOP

Classical trophodynamic models, like the microbial loop, include a photoautotrophic/organoheterotrophic dichotomy, which segregates primary and secondary producers. However, mixotrophic organisms can occupy both trophic levels at the same time. These trophic levels can be differently distributed among the described microorganisms, ranging from pure photoautotrophy to pure organoheterotrophy (Fig. 1). For example, the metabolic mode of *Cyanobacteria* may range from pure photoautotrophy to strains that can partly use organic matter for biomass synthesis. AAnPB may be the most versatile as their metabolic range may include photoautotrophy, photoheterotrophy, and organoheterotrophy, whereas PRB may back up an organoheterotrophic mode by using proteorhodopsin to obtain energy (Fig. 1).

Evidence exists that the most abundant bacteria in oligotrophic marine waters are heterotrophic bacteria (SAR11 and *Roseobacter*) that may partly be phototrophic and, conversely, photosynthetic cyanobacteria (*Prochlorococcus* and *Synechococcus*) that may partly use organic substrates heterotrophically (Fig. 1). Hence, our classical distinction between phototrophic and heterotrophic microorganisms in the ocean is now rather blurred. When we model oceanic ecosystems, we should be aware that we know little about the key players in the microbial food web. We should be cautious about conclusions, in particular, with respect to the contribution of an organism to hetero-, organo-, photo-, or autotrophic processes.

There are other implications when AAnPB and PRB are abundant. Determinations of photosynthesis based on oxygen measurements are not accurate, because AAnPB and PRB are unable to utilize water as an electron donor, since they most likely rely on organic material or other inorganic electron donors (for example, ferric iron or hydrogen sulfide) to supply reduction. Even though AAnPB and PRB may not fix inorganic carbon, in contrast to photoautotrophic bacteria (*Cyanobacteria*), the transformation of electromagnetic to chemical energy should reduce both their respiratory energy requirements in assimilating dissolved organic matter into bacterial biomass and the amount of carbon dioxide released by heterotrophic respiratory metabolism. Estimates of bacterial biomass synthesized per unit of dissolved organic matter assimilated are crucial for biogeochemical models of aquatic ecosystems. However, our estimates of heterotrophic bacterial growth efficiency are based on dark incubations (12), neglecting the bioenergetics of light-harvesting pigments when organic matter is degraded photoheterotrophically. Hence, our estimates of natural bacterial growth efficiency may be underestimated. As a consequence, our current food web models may underestimate bacterial biomass production and the importance of bacteria as a food source for higher trophic levels.

Thus, these mixotrophic microbes and their suspected diversity and ecophysiology, together with their high abundance, demand a revision of our basic concepts about carbon and energy flow in aquatic environments.

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