INSTITUT PASTEUR

Research in Microbiology 162 (2011) 858–868

www.elsevier.com/locate/resmic

Tools providing new insight into coastal anoxygenic purple bacterial mats: review and perspectives

Cédric Hubas [a,*], Bruno Jesus [b,c], Claire Passarelli [a], Christian Jeanthon [d,e]

[a] *Muséum National d'Histoire Naturelle, UMR MNHN-CNRS-IRD-UPMC 7208 BOREA, CP 53, 61 rue Buffon, 75231 Paris Cedex 5, France*
[b] *Instituto de Oceanografia, Faculdade de Ciências de Lisboa, Campo Grande, 1749-016 Lisboa, Portugal*
[c] *Centro de Biodiversidade, Genómica Integrativa e Funcional (BioFIG), Faculdade de Ciências, Universidade de Lisboa, Campo Grande, 1749-016 Lisboa, Portugal*
[d] *UPMC Univ Paris 06, UMR 7144, Adaptation et Diversité en Milieu Marin, Station Biologique de Roscoff, 29680 Roscoff, France*
[e] *CNRS, UMR 7144, Adaptation et Diversité en Milieu Marin, Station Biologique de Roscoff, 29680 Roscoff, France*

Received 15 September 2010; accepted 16 March 2011
Available online 21 April 2011

Abstract

Coastal photosynthetic microbial mats are highly structured microbial communities that populate a variety of shallow environments such as estuaries, sheltered sandy beaches, intertidal flats, salt marshes and hypersaline salterns. In soft sediments, most of these microbial mats are formed of vertically stratified, multicolored cohesive thin layers, of several functional groups of microorganisms, such as cyanobacteria, colorless sulfur bacteria, purple sulfur bacteria and sulfate-reducing bacteria, distributed along vertical microgradients of oxygen, sulfide and light. These microbial communities are highly productive and significant contributors to carbon, nitrogen and sulfur cycles and to sediment stability in shallow-water habitats. Many examples of these communities have been cited in the past, but comparatively few microbial mats have been presented for which mass developments of anoxygenic purple bacteria have been observed. Yet, application of molecular approaches has provided fresh insight into the ecology, diversity and evolution of microbial mats. In situ measurements using electrochemical and optical microprobes led to detailed characterization of their physical and chemical environment, whereas reflectance measurements revealed the spatial and temporal heterogeneity of microbial mat surfaces. We hereby report the main discoveries due to introduction of these powerful techniques and we point out the potential insight to be gained from the study of anoxygenic purple bacterial mats.

Keywords: Microbial mats; Anoxygenic phototrophs; Purple sulfur bacteria; Diversity; Photosynthesis; Spectral reflectance

1. Introduction

Microorganisms have the ability to colonize different types of habitats and interact with each other, forming more or less complex communities. Microbial mats that develop in different geographical locations are a remarkable example of these associations. They are found, for instance, in coral reefs, hypersaline ponds and lakes, salterns, thermal springs, Antarctic lakes and coastal sediments (Stal and Caumette, 1994). In the latter, they develop at the sediment–water interface in shallow environments such as estuaries, intertidal areas, sandy beaches and hypersaline salt marshes (Herbert, 1985; Stal and Caumette, 1994; van Gemerden et al., 1989a, 1989b). Coastal microbial mats are principally inhabited by bacteria (heterotrophic, autotrophic and chemotrophic) as well as eukaryotic microalgae such as benthic diatoms. In the literature, these consortia are often referred to as microbial mats, laminated microbial communities, microphytobenthos or simply biofilms (or a combination), but ultimately describe the association of different microbial cells embedded in an extracellular polymeric substance (EPS) matrix. These mats can exhibit varying morphology based on the physicochemical

* Corresponding author.
E-mail address: hubas@mnhn.fr (C. Hubas).

0923-2508/$ - see front matter © 2011 Institut Pasteur. Published by Elsevier Masson SAS. All rights reserved.
doi:10.1016/j.resmic.2011.03.010

environments they experience. Most of the time, the cells are organized according to their physiologies in vertical laminated structures consisting of successive layers.

In coastal zones, microbial mats are mostly photosynthetic and are composed of several functionally complementary groups of microorganisms whose composition can vary greatly depending on the energy and nutrient source from the top and bottom. Cyanobacteria are often the pioneer organisms and generally dominate the top layer. According to the chemical and light gradients available, they can, for instance, be followed by aerobic or facultative heterotrophic bacteria, chemolithotrophic bacteria (among them colorless sulfur bacteria), anoxygenic phototrophs (purple and green) and sulfate-reducing bacteria, forming several laminated layers distributed within the EPS matrix principally produced by cyanobacteria. Purple bacteria perform anaerobic anoxygenic (without release of oxygen) photosynthesis as, unlike cyanobacteria, they are unable to perform water photolysis due to the lack of photosystem II. As electron donors, they mostly use intermediate products of organic matter degradation from primary producers and some compounds originating from fermentation and anaerobic respiration. In microbial mats, wide diversity in purple bacteria is generally observed and the genus *Thiocapsa* is frequently represented (van Gemerden et al., 1989b).

In the last decade, community structure as well as the physical-chemical environment of microbial mats have been reviewed on several occasions (Franks and Stolz, 2009; Paerl and Pinckney, 1996; Stal and Caumette, 1994; van Gemerden, 1993). Indeed, the introduction of molecular approaches has provided new insight into the ecology of these mats by leading to characterization of community structure (Ranchou-Peyruse et al., 2006; Wieland et al., 2003). Due to the development of high resolution micro-electrodes, the physical and chemical environment of these mats was characterized at very small spatial scales (μm to mm, Revsbech and Jørgensen, 1983; Visscher et al., 1991). Pigment diversity and in situ reflectance measurements revealed the spatial and temporal heterogeneity of microbial mat surfaces (e.g. Brotas and Plante-Cuny, 2003; Paterson et al., 1998). Finally, their role in sediment biostabilization has been revealed (Paterson, 1997). This paper reports the main discoveries made through introduction of these powerful techniques, and points out gaps in current knowledge of anoxygenic phototrophic biofilms.

2. Microbial communities in coastal purple phototrophic mats

2.1. Structure of coastal purple phototrophic mats

Photosynthetic microbial mats develop in many different habitats, with salinities ranging from freshwater to hypersaline conditions (Overmann and Garcia-Pichel, 2006; van Gemerden, 1993 Some prominent marine and hypersaline habitats, where laminated microbial communities frequently develop in visible masses, are represented by coastal sediments of the Great Sippewissett Salt Marsh (USA) (Nicholson et al., 1987; Rothermich et al., 2000), coastal lagoons in southern France (Caumette, 1986; Guyoneaud et al., 1996), marine salterns in France (Caumette et al., 1994; Giani et al., 1989) and in Guerrero Negro Baja California, Mexico (Canfield and Des Marais, 1993; Ley et al., 2006), sandy flats of the Ebro Delta (Mir et al., 1991; Navarrete et al., 2000) and sheltered beaches on the Orkney Islands (van Gemerden et al., 1989a, 1989b; Wieland et al., 2003). In such ecosystems, the surface sediment layer covers a transition zone between oxic and anoxic conditions characterized by steep gradients of oxygen and sulfide. These gradients favor maturation of vertically stratified, multicolored and cohesive layers of several functional groups of microorganisms. Although the uppermost layer, brown and green in color, may contain benthic diatoms, the dense top material is typically formed by unicellular and filamentous cells of cyanobacteria that are generally a driving force, as they provide growth substrates for other organisms. For instance, newly colonized sands mainly comprised *Oscillatoria* sp. and *Spirulina* sp. (Franks and Stolz, 2009). The gliding cyanobacterium *Microcoleus chthonoplastes* often replaces these pioneer species and becomes dominant in mature intertidal mats (Stal et al., 1985; van Gemerden, 1993). Below the cyanobacteria, a distinct layer of purple sulfur bacteria is often present, sometimes overlying a layer of green sulfur bacteria. Occasionally, a white layer or patches due to sulfide-oxidizing bacteria (including *Beggiatoa* spp.) are visible at the surface of marine sediments that have a sufficiently high production of sulfide from bacterial sulfate reduction (Jorgensen, 1977). They are followed vertically by sulfate-reducing bacteria whose activity leads to the precipitation of iron sulfides visible as black mud. Aerobic heterotrophic organisms are also functionally important as their activity leads to oxygen depletion, and fermentative organisms provide growth substrates for sulfate reducers. Other numerically less important groups are nitrifying and denitrifying bacteria and methanogens. Numerous examples have been described in the literature of colored blooms and mass accumulations of phototrophic bacteria in coastal zones and lagoons (Caumette and Baleux, 1980; Imhoff, 2001, and references herein). Mass development of purple sulfur bacteria has been observed during warm summer months in intertidal zones of sandy beaches (Herbert, 1985; van Gemerden et al., 1989a, 1989b). Three different laminated microbial mats were described, distinguished by the position of the cyanobacterial layer above or beneath the purple sulfur bacterial layer, or its complete absence and therefore exclusive development of purple sulfur bacteria in the top layer. On the Orkney Islands (Herbert, 1985; Van Gemerden et al., 1989a, 1989b) and in Roscoff Aber Bay (Fig. 1; Hubas, C., Jesus B. M., Jeanthon, C., unpublished data), the latter pattern occurs seasonally when beaches are supplied with a high load of organic matter due to decomposition of macroalgae. These purple sulfur bacteria are therefore almost permanently exposed to oxygen at the sediment surface (e.g. Herbert and Welsh, 1994). Among the purple bacteria, the purple non-sulfur bacteria are also widely distributed in aquatic environments rich in organic matter (Guyoneaud et al., 1996; Hiraishi and Ueda, 1995).

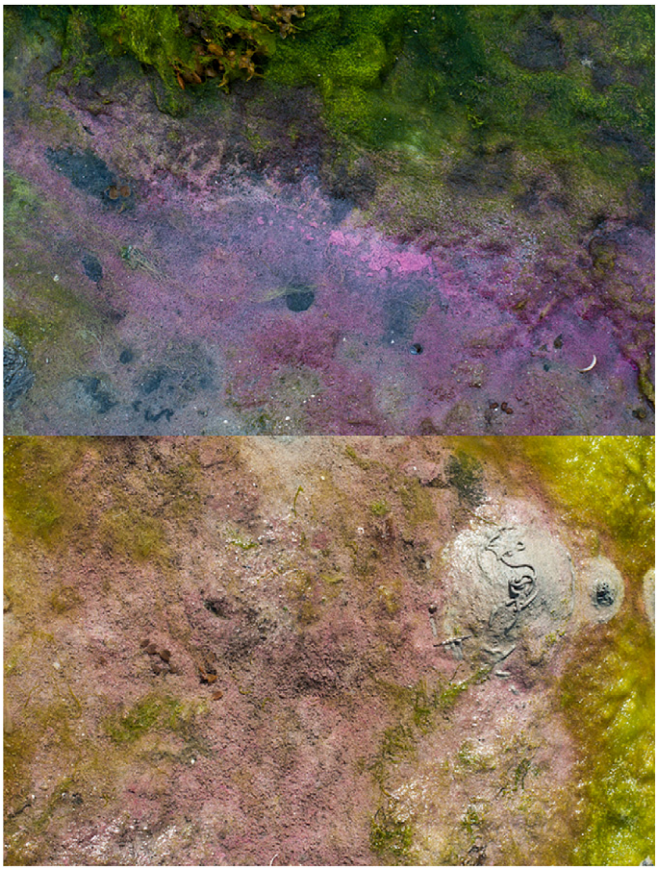

Fig. 1. Pictures of purple phototrophic mats, underwater (top panel) and emerged (bottom panel), in Roscoff Aber Bay.

2.2. Cultural and molecular diversity of purple sulfur phototrophic mats

Most ecological studies on the distribution of anoxygenic phototrophs in natural environments have been based on biochemical features such as photopigment composition (see Section 3.1) and/or on estimations of bacterial numbers, isolation and characterization of pure cultures (Guyoneaud et al., 1996; Nicholson et al., 1987; Ranchou-Peyruse et al., 2006; van Gemerden et al., 1989a). The most prominent purple sulfur bacteria, frequently observed and also isolated from marine coastal sediments, have been reviewed by van Gemerden and Mas (1995) and Imhoff (2001). They were assigned to *Thiocapsa roseopersicina*, *Thiocystis violacea* and *Allochromatium vinosum*. As an example, various organisms have been cultivated from microbial mat communities of the Ebro Delta, among the most intensively studied sites. Vacuolated bacteria such as *Thiocapsa rosea* and *Lamprobacter modestohalophilus*, as well as non-vacuolated bacteria such as *Marichromatium gracile*, *T. roseopersicina* or *Ectothiorhodospira* sp., have been isolated (Martinez-Alonso et al., 2005; Villanueva et al., 2010). *T. roseopersicina*, which is easily cultivated, is very common in marine coastal habitats and predominant in most systems, where it can reach abundances of 10^6–10^7 cells cm^{-3} (van Gemerden et al., 1989a). The adaptation to a wide range of salinities and the high metabolic versatility and flexibility of this organism (tolerance to oxygen and possible aerobic growth in the dark) are important competitive advantages that explain the success of its distribution (de Wit and van Gemerden, 1987; de Wit and van Gemerden, 1990). *Allochromatium* spp. and *Marichromatium* spp. are also often observed and may be locally dominant (Imhoff, 2001). From red layers found in mats of hypersaline environments, other members of the family Chromatiaceae such as *Halochromatium salexigens, H. glycolicum* and *Halothiocapsa halophila* have also been isolated (Caumette et al., 1988, 1997, 1991).

In the last few decades, the species composition of microbial mats has been primarily described by dissecting cores into thin horizontal layers and extracting nucleic acids or other cell components for chemical and molecular analysis (Martinez-Alonso et al., 2005; Mouné et al., 2003; Navarrete et al., 2000; Ranchou-Peyruse et al., 2006; Villanueva et al., 2010). With these techniques, a high degree of bacterial diversity was generally found. As an example, the microbial mats within hypersaline lagoons at Guerrero Negro generated more than 1500 16S rRNA sequences representing over 750 species (Ley et al., 2006). Denaturing gradient gel electrophoresis separation of 16S rRNA gene amplification products obtained using specific primer combination for Chromatiaceae, the main family of purple sulfur bacteria, showed that the diversity of members of this family in microbial mats in the Ebro Delta was high and pointed out the presence of novel species not related to any known purple sulfur bacteria (Martinez-Alonso et al., 2005).

The *pufM* gene encodes for the medium (M) subunit of the photosynthetic reaction center of anoxygenic photosynthetic bacteria of the *Alpha-*, *Beta-*, and *Gammaproteobacteria* and of the Chloroflexaceae. Molecular analyses using this functional gene have also been used to specifically study the depth distribution of anoxygenic phototrophs in mat communities (Fourçans et al., 2004; Wieland et al., 2003). Using this method, vertical diel migration of an anoxygenic phototrophic community in response to oxygen concentration and pH was detected at a microscale depth level (Fourçans et al., 2006). Only a few studies have detailed the diversity of anaerobic purple bacteria by analysis of *pufM* environmental libraries since the pioneering work of Achenbach et al. (2001) and Karr et al. (2003) on Antarctic lake waters and mats. An environmental clone library of the *pufM* gene was obtained from a thin cyanobacterial mat developed at the top of black sediment samples from the Berre lagoon (France) (Ranchou-Peyruse et al., 2006). Surprisingly, most clones were closely related to aerobic anoxygenic phototrophic bacteria related to the *Roseobacter* clade, whereas only two *Roseobacter* strains were isolated. The culture-dependent approach performed in parallel revealed the dominance of anaerobic purple sufur bacteria in these samples. The coexistence of both aerobic and anaerobic anoxygenic phototrophic bacteria has also been demonstrated in sediments from Antarctic and saline lakes (Karr et al., 2003; Thiel et al., 2010).

3. Pigment diversity and reflectance measurements

3.1. Pigment diversity of microbial mats

The microenvironment within a mat is characterized by physical-chemical gradients (e.g. light, pH, nutrients), leading to high variability in distribution of phototrophic microorganisms, both vertically within the top mm of the sediment (taxonomic stratification) and horizontally (high patchiness). Frequently, there is also significant temporal variability on biofilms that colonize intertidal areas as a result of wide physicochemical variations caused by the tide. The high variability exhibited by biofilms on such small scales causes significant sampling problems, e.g. to fully capture biofilm variability, many samples have to be taken, often more than is logistically possible. Also, until recently, most available techniques for assessing microorganism abundance or pigment diversity in biofilms were destructive (e.g. pigment extraction with organic solvents and quantification by HPLC). The destruction of the biofilm removes existing physical-chemical gradients, significantly changing the environmental conditions of the biofilm under investigation. Thus, there is growing interest in developing remote sensing techniques that allow non-destructive and non-invasive study of phototrophic microbial biofilms. Such techniques include: spectral reflectance, O_2 micro-electrodes, optodes, pulse-amplitude-modulation (PAM) fluorometry, fast repetition rate fluorometry (FRRF) and infrared CO_2 gas analyzer (IRGA) benthic chambers (e.g. Kühl, 2005; Kühl and Polerecky, 2008; Migné et al., 2002; Stephens et al., 2003; Thar et al., 2001; Vopel and Hawes, 2006; Wiggli et al., 1999). All these techniques allow repetition of measurements in the same biofilm area and some can be used to infer biofilm biomass or taxonomic composition. In this section, we focus on the use of spectral reflectance in the study of photosynthetic microbial mats.

Microbial biofilm taxonomic diversity is reflected in the presence of different pigments. Some of these pigments can be used as "signatures" of the presence of specific groups in the biofilm, e.g. diatom-dominated biofilms will show the abundant presence of fucoxanthin and chlorophyll *c*; cyanobacteria-dominated biofilms will show a variety of cyanobacterial-specific pigments (e.g. mixoxanthophyll, equinenone, etc.); and an anoxygenic bacterial biofilm will mainly show bacteriochlorophylls and carotenoids (Table 1). If, for diatom and cyanobacterial biofilms, numerous studies exist showing their pigment composition (e.g. Andréfouët et al., 2003; Brotas and Plante-Cuny, 2003; Stephens et al., 2003), only a few papers focus on anoxygenic bacterial biofilms (e.g. Massé et al., 2002). Spectral reflectance can be used to identify and quantify the presence of different pigments in the biofilms, but it is first necessary to determine the spectral signatures of these pigments. It is thus useful to have good "ground truth" studies, i.e. spectral measurements taken together with measurements of the pigments present in the

Table 1
In vivo pigment spectral "signatures" collected from available references. Numbers between brackets refer to the absorption features of each pigment. Emphasis was given to references where bacteriochlorophyll samples were found. Alo – alloxanthin, BChl – bacteriochlorophyll, β-car – β-carotene, BChl*a* – bacteriochlorophyll *a*, BChl*c* –bacteriochlorophyll *c*, BChl*d* – bacteriochlorophyll *d*, BChl*e* – bacteriochlorophyll *e*, Car – carotenoides, Chl*a* – chlorophyll *a*, Chl*b* – chlorophyll *b*, Chl*c* – chlorophyll *c*, Chl*d* – chlorophyll *d*, DD – diadinoxanthin, Fuco – fucoxanthin, Lut – lutein, Myxo – myxoxantophyll, Oke – Okenone, PC – phycocianin, PB – phycobilin pigments, PE – phycoerythrin, Per – peridinin, Spi – Spirilloxanthin, Zea – zeaxanthin.

Pigments and *in vivo* spectral signatures	Type of measurement	Type of microbial community	Reference
BChl*a* (800–810, 860–880), BChl*c* (750), Car (450–550), PC (620)	Reflectance	*Microcoleus chthonoplastes*, Chromatium sp., *Thiocapsa* sp., *Chloroflexus*	Kühl and Jørgensen (1992)
BChl*d* & BChl*e* (720), BChl*a* (835), Chl*a* (680), PE (560, 570), PC (625, 630)	Absorbance	*Chromatium*, *Thiopedia*, *Chloronema*	Steenbergen and Korthals (1982)
Bchl*a* (370, 830), Oke (520)	Absorbance	*Thiocapsa roseopersicina*	Massé et al. (2002)
Bchl*a* (805,860, 880), Spi (480,520,550)	Absorbance and reflectance	*Thiocapsa roseopersicina*	Gitelson et al. (1999)
BChl (800, 801, 804, 806, 808, 835, 837, 862, 865, 867, 868, 870, 879)	Absorbance	Review about aerobic anoxygenic phototrophic bacteria	Yurkov and Csotonyi (2008)
BChl*a* (800, 850, 890), PB (620),	Reflectance	–	Wiggli et al. (1999)
BChl*a* (790–810,865, 830–880), Chl*a* (675), Chl*c* (630–635), PC (620), PB (560–620),	Reflectance	Sediment biofilm, Cyanobacteria, diatoms and purple sulfur bacteria	Kühl et al. (1994)
Chl*a* (675), Chl*c* (623), DD (500), Fuco (550)	Reflectance	Diatom biofilms	Méléder et al. (2003)
BChl*a* (807,845), BChl*c* (745–750), Chl*a* (440, 675), PC (625)	Absorbance	Sediment cyanobacterial mat, purple and green photosynthetic bacteria	Kühl and Fenchel (2000)
Chl*a* (422, 659, 680), Car (422,448, 478), Myxo (508), PB (534, 570, 594, 628)	Reflectance	Sediment biofilm (cyanobacterial mat)	Andréfouët et al. (2003)
Alo (649), Chl*a* (412, 441, 623, 682), Chl*b* (466), Fuco (525, 540–548,) PE (574)	Reflectance	Rocky shore biofilm, diatoms with cyanobacteria	Murphy et al. (2005)
Chl*a* (422, 444, 676), Chl*b* & Chl*c* (468), Fuco & Per (672), PE (572), PC (620), Zea, lut, β-car & DD (492)	Reflectance	Sediment biofilm	Stephens et al. (2003)
Bchl*c* (732), Chl*a* (440, 680), Chl*b* (650–655), Chl*d* (710–712), PE (576), PC (626),	Hyper-spectral imaging	Bacterial mat under didemnid ascidian	Kuhl & Polerecky (2008)

biofilm. Currently, there are few studies that have attempted to establish the pigment spectral signatures of anoxygenic phototrophic biofilms. Although they are not consensual about which wavelengths should be used to detect bacteriochlorophyll, there are 3 main absorbance peaks attributed to bacteriochlorophyll *a*: around 800 nm, around 850 nm and around 870 nm. The exact wavelengths depend on the type of bacteria present (Table 1).

3.2. Spectral reflectance of microbial phototrophic mats

Spectral reflectance measurements have often been used in estimation of biofilm microalgal biomass using chlorophyll *a* as a biomass proxy (e.g. Carrère et al., 2004). Chlorophyll *a* strongly absorbs red light and reflects most infrared light. Using this information, a wide variety of chlorophyll-*a*-based reflectance studies were developed, e.g. normalized difference vegetation index (NDVI), modified soil-adjusted vegetation index (MSAVI). To our knowledge, no similar index exists to estimate anoxygenic phototrophic biofilms; although bacteriochlorophyll *a* is frequently used to infer the presence of anoxygenic phototrophic bacteria (e.g. Gitelson et al., 1999; Kühl and Jørgensen, 1992; Stal et al., 1984; Steenbergen and Korthals, 1982), it is not common to use the pigment content to quantify anoxygenic phototrophic bacterial biomass. Bacteriochlorophyll-*a*-dominated biofilms typically show absorption features in the infrared region, whereas chlorophyll-*a*-dominated biofilms do not (e.g. Stal et al., 1984). In the Roscoff Aber Bay, where anoxygenic photosynthetic biofilms seasonally developed at the sediment surface, reflectance spectra recorded from different sediment areas enabled determination of bacteriochlorophyll absorption features in the infrared region, with absorption maxima at 792 and 850 nm (Fig. 2). A spectral reflectance index is currently being developed by the authors to estimate bacteriochlorophyll content of this biofilm using bacteriochlorophyll absorption features.

Spectral reflectance has also been widely used with benthic phototrophic biofilms to follow diatom vertical migration within the sediment matrix (e.g. Serôdio et al., 2009), to follow photoregulatory vertical movements (e.g. Perkins et al., 2010) and photo-physiological mechanisms (Jesus et al., 2008), and to a lesser extent to identify the presence of different taxonomic groups, e.g. microalgae, cyanobacteria, green and purple bacteria (e.g. Bachar et al., 2008; Prášil et al., 2009; Wiggli et al., 1999). Presently, most research done with spectral reflectance on anoxygenic biofilms seems to focus on identification of the presence of different taxonomic groups in the biofilm. With the introduction of hyper-spectral (HS) imaging technology, it became possible to map sediment biofilms with high spectral and spatial resolution (e.g. Bachar et al., 2008). HS imaging is a very sensitive and minimally invasive tool that can be used in investigation of the biofilm spatial organization role in mat ecosystem functions, providing the possibility of imaging microbial identity and activity at high spatio-temporal

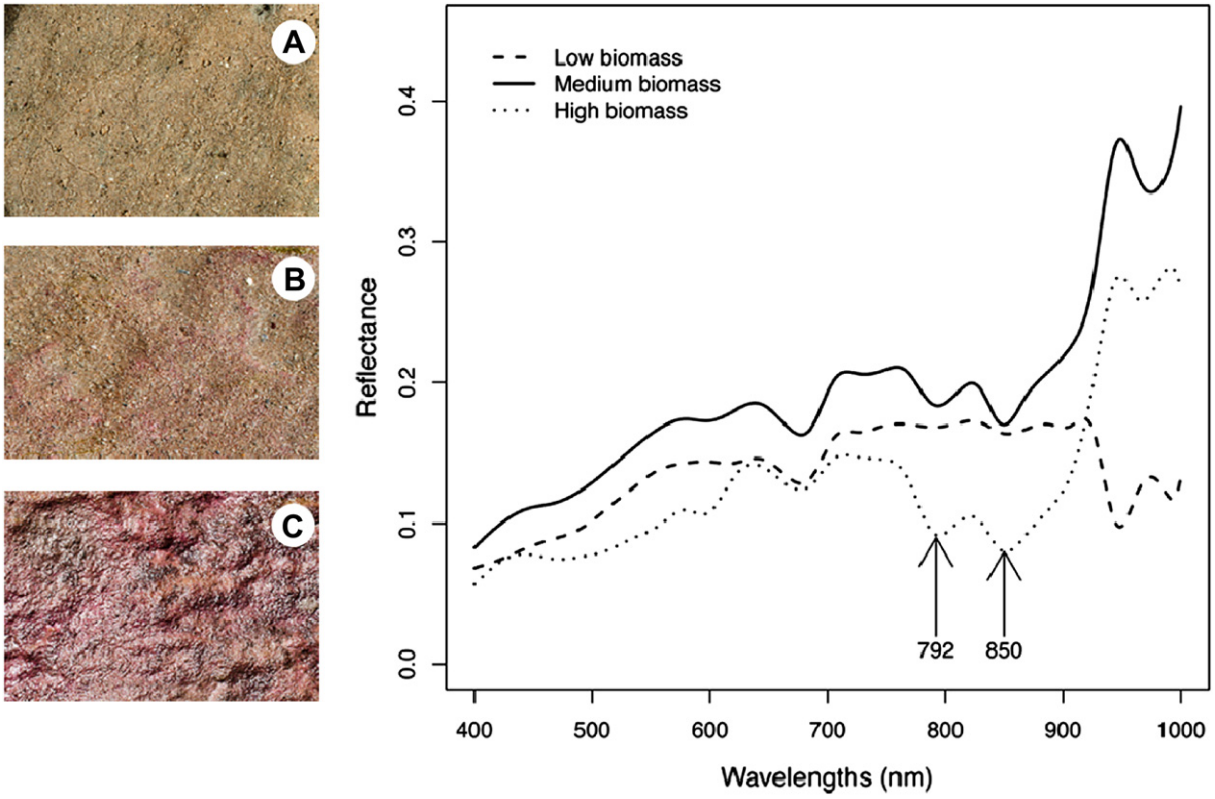

Fig. 2. Left panel: pictures of sites with low (A), medium (B) and high (C) biomass of purple sulfur bacteria, in Roscoff Aber Bay. Right panel : spectral reflectance of the sediment, in sites with low (dashed line), medium (solid line) and high (dotted line) biomass of these bacteria. The arrows point the bacteriochlorophyll *a* absorption peaks.

resolution. Presently, the majority of work involving HS imaging seems to address questions relating to spatial distribution of the different taxonomic groups that colonize the sediment, vertically and horizontally (Kühl and Polerecky, 2008; Polerecky et al., 2009). However, some research has started to emerge using combinations of imaging techniques to infer relationships between different microalgal groups and their environment. For instance, Bachar et al. (2008) used hyperspectral imaging of reflectance spectra (4th derivative of spectral images with 460–913 nm spectral resolution at 30 × 30 μm spatial resolution) and emission spectra to map distribution of different pigments (chlorophyll *a*, phycocyanin, bacteriochlorophyll *a* and bacteriochlorophyll-*c*). Both spectral methods were sensitive enough to detect biofilm stratification within the sediment, showing the spectral signatures of chlorophyll *a* and zeaxanthin closer to the sediment surface, a mid-layer 3–4 mm of bacteriochlorophyll *c* and bacteriochlorophyll *a* at deeper layers (5.5–7 mm). Using HS imagery, those authors rejected their original hypothesis that Chloroflexaceae would be closely associated with the distribution of oxygenic phototrophs and proposed an alternative hypothesis that Chloroflexaceae is maximal in locations where both photosynthate excretion and sulfate reduction occur during a light/dark cycle.

In conclusion, although considerable research on microalgae phototrophic biofilms using spectral reflectance tools already exists, there is a gap in current knowledge regarding the use of these techniques for quantification and study of anoxygenic phototrophs.

4. Role of microbial mats in the functioning of coastal ecosystems

4.1. Role of microbial mats in sediment stability

Although the cohesive strength of one sediment may depend on its physicochemical properties, such as water content, density, mineralogy, plasticity, salinity and pH (Dade et al., 1992), its stability may correlate better with biological parameters than with non-biological ones (Paterson et al., 2000). Microbial exopolymeric secretions are increasingly recognized as a major stabilizing factor (Stal, 2010). Extracellular polymeric substances (EPS) are a ubiquitous component of marine ecosystems primarily composed of carbohydrates, proteins and lesser amounts of other components. They have multiple roles in aquatic systems: attachment to substrata, flotation and locomotion, feeding, protection against desiccation/UV/pollution, development of biofilms, communication (Decho, 1990). These molecules, mostly produced by diatoms and bacteria, compose a highly hydrated matrix more or less associated with cells. Tightly-wound capsules are secreted during exponential growth phase and allegedly confer protective effects upon the cell, whereas loose slimes allow microorganisms to attach each other and to sediment (Decho, 1990). The high amounts of EPS present in the sediment glue the grains together, thus enhancing the resistance of sediment to erosion and making it more stable (Paterson et al., 2000; Stal, 2010). If resistance to erosion generally correlates well with carbohydrate and protein concentrations, variations in EPS quality also influence sediment stability (Sutherland et al., 1998; van Duyl et al., 2000). Moreover, cyanobacterial filaments trap sediment particles and reinforce cohesion (Stal, 2010). Fig. 3 summarizes the potential influence of microbial mats on sediment stability. Given the importance of sediment stability in coastal ecosystems (which are typically constrained by strong physical and geochemical gradients), microorganisms are increasingly recognized as ecosystem engineers.

In the future, more studies are required to understand how EPS composition and diversity modify sediment properties. Particularly, little is known about stabilization in mats of anoxygenic phototrophic bacteria. Yet the abundance of purple sulfur bacteria may correlate with the erosion threshold of sediment and these bacteria appear to produce far more EPS than diatoms (Grant and Gust, 1987). Thus, the erosion of sediment is lower when purple phototrophic mats are present (van Gemerden et al., 1989a). Recent measurements of sediment adhesion in Roscoff Aber Bay showed that sediment cohesion was enhanced and that sediment was stabilized by purple phototrophic bacteria, particularly with high bacterial abundance (Fig. 4). Further investigations are now required to link stabilization with the quantity or quality of the EPS produced by purple sulfur bacteria.

4.2. Production and respiration of microbial mat organic matter and its fate in the coastal food web

Microbial mats are very productive ecosystems (e.g. about 200 g Cm^{-2} y^{-1} in the Ebro Delta, Urmeneta et al., 1998). The Winkler titration method (Winkler, 1888), incorporation of ^{14}C labeled bicarbonate, rapidly responding CO_2 micro-electrodes (de Beer et al., 1997) and measurements of total DIC fluxes (e.g. Wieland et al., 2005) have been used extensively to measure primary production, but most of the estimates to date in benthic photosynthetic mats were performed with oxygen micro-electrodes (Oren, 2009), by measuring rates of oxygen depletion at different depths during light–dark shifts (Revsbech and Jørgensen, 1983). This method enables accurate estimation of gross primary production rates across the mat-water interface from profiles providing that irradiance, temperature and porosity of the substrates are known (Wieland and Kühl, 2000). Oxygen measurements can thus provide information about both gross primary production and respiration of the microbial mats at a millimeter scale. These measurements revealed that microorganisms thrive in such closeness and mutually influence each other (van Gemerden, 1993). Biological processes usually metabolically incompatible are found to occur simultaneously within the mats, implying tight coupling between them. The different members of the community are thus mutually dependent so that the entire ecosystem is often considered self-sustaining (Des Marais, 2003). The development of electrochemical and optical microprobes has attracted many microbiologists during the past decades, probably because their resolution is particularly suitable for the study of microbial environments.

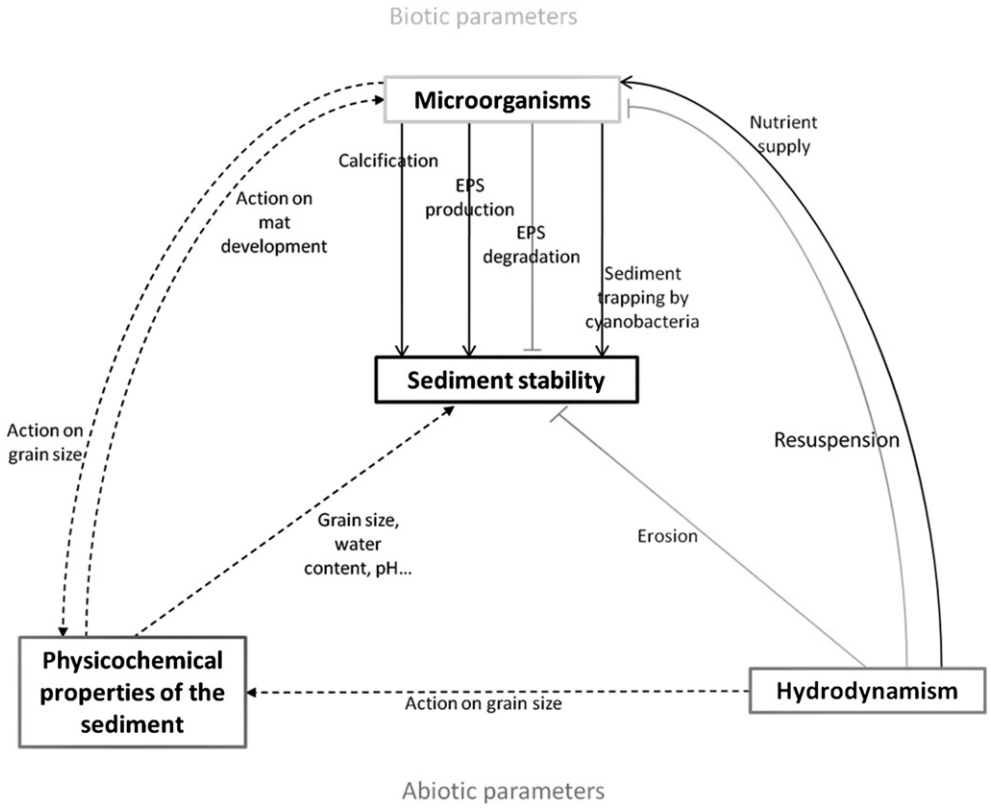

Fig. 3. Biotic and abiotic parameters influencing sediment stability. Arrow with black solid line: stimulates. Arrow with gray solid line: inhibits. Arrow with dashed line: has an influence on.

Ironically, however, whilst we now have a good understanding of the chemical and physical conditions that microorganisms experience on millimeter scales in microbial mats, we still do not know precisely which role microbes play in biogeochemical cycles on larger scales.

In addition, microbial mats can represent a significant source of fixed carbon and nitrogen to the surroundings and they may serve as an important food source for higher trophic levels (Joye and Lee, 2004). Recently, it was shown that anoxygenic microbial mats may support the diet of inhabiting mud snails (Riera, 2010). In addition, microbial mats of the intertidal area which are dominated by diatoms generally serve as a food source for many invertebrates of the macrofauna and meiofauna (e.g. Hagerthey et al., 2002; Riera and Hubas, 2003), including numerous commercial species such as penaeid shrimp postlarvae (Al-Maslamani et al., 2009) or the oyster *Crassostrea gigas* (Riera and Richard, 1996). But despite the marked role they play in the coastal food web, the fate of microbial mat organic matter has seldom been addressed. Bacterial production has proven to be a significant food source for benthic grazers and a sink of organic carbon in the food web of intertidal sediments (van Oevelen et al., 2006), but further studies are needed, particularly concerning anoxygenic microbial mats.

Indeed, mass bloom of anoxygenic phototrophic bacteria can develop at the sediment surface if the organic matter input is strong enough (Herbert and Welsh, 1994), forming purple microbial mats characterized by the absence of oxygenic photosynthesis. The accumulation of organic matter at the sediment surface stimulates respiration and, below 2–3 mm depth, sediment becomes totally anoxic and characterized by very high sulfate-reducing rates (Bolam et al., 2000; Nedergaad et al., 2002), enabling exclusive growth of anoxygenic purple bacteria. Primary production and respiration rate measurements are still scarce for these types of mats.

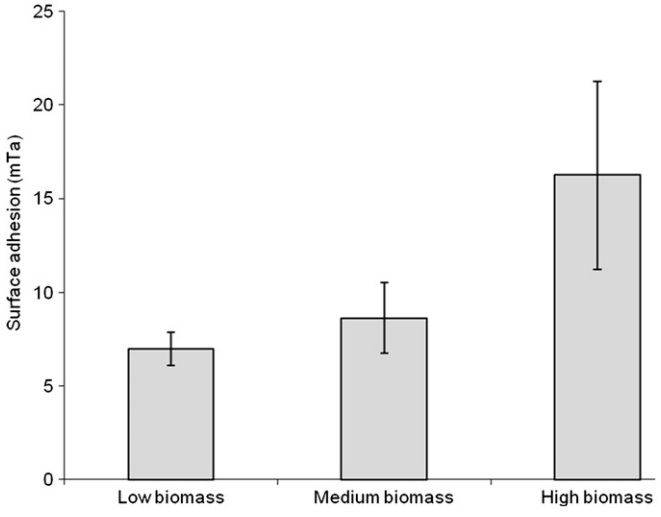

Fig. 4. Sediment cohesion (mTa; mean ± SD) in sites with low, medium and high biomass of purple sulfur bacteria, in Roscoff Aber Bay (see Fig. 2). Measurements were performed using a Magnetic Particle Inducer (MagPI), a device recently developed by Larson et al. (2009).

Recently, the strong contribution of *Chloroflexus*-like anoxygenic phototrophs (green non-sulfur bacteria) to the gross primary production and community respiration of a microbial mat was found to be strongly dependent on light availability in the near infrared region (Polerecky et al., 2007). This highlights the fact that understanding the contribution of anoxygenic phototrophs to total primary production and respiration is more complex than previously thought and that more studies on anoxygenic microbial mats are required.

Microbial mats are a remarkable example of the various forms of respiration that co-exist in aquatic habitats. Anaerobic respiration as well as aerobic respiration and re-oxidation processes have been relatively well studied in these systems. From the surface to the depth, the redox potential decreases, which influences distribution of the different respiration pathways. Along the sediment depth, chemical reactions involve different terminal electron acceptors and display apparent free energy yields which decrease with increasing depth (Hoehler, 2004). Carbon fluxes across the mat-water interface are generally deduced from oxygen measurements by applying known respiratory quotients (RQs). However, most of the RQs apply to conventional aerobic respiration and have no useful meaning if total respiration occurs mainly via anaerobic pathways (Williams and Del Giorgio, 2005). Total DIC fluxes in benthic chamber enclosures or chambers equipped with CO_2 infrared gas analyzers (Migné et al., 2002) are an efficient way of measuring C fluxes across the mat-water or mat–air interface. They have been used extensively on emersed diatom biofilms to estimate their annual carbon budgets (Hubas and Davoult, 2006; Migné et al., 2004; Spilmont et al., 2006), but rarely on other microbial mats, to our knowledge.

5. Conclusions and future directions

Current knowledge of the ecology, ecophysiology and role of anoxygenic purple microbial mats is far less well documented than for cyanobacteria and/or diatoms. Molecular approaches have provided fresh insight into the diversity of microbial mats, but the role of microbial species diversity in sustaining ecosystem processes like primary production has seldom been addressed (but see Forster et al., 2006). As revealed by spectral reflectance measurements, the distribution of phototrophic microorganisms in coastal ecosystems is highly variable. Remote sensing methods are probably an efficient way to perform a true integration of these systems on larger scales. Indeed, coastal habitats are amongst the most productive ecosystems on earth and the contribution of anoxygenic bacteria to the coastal C and N cycles and their role in sustaining local food webs are probably underestimated.

Techniques such as HS imaging combined with state-of-the art optical sensors (e.g. optodes, imaging PAM) will surely help to elucidate the distribution and trophic and geochemical roles of anoxygenic phototrophic biofilms, as well as to investigate in-depth their photobiology in intact samples. In addition, new generations of ion microprobes based on mass spectrometry of secondary ions (SIMS) are now available and are particularly appropriate to the study of microbial mats. They enable analysis of isotopic composition of a given sample surface and distribution of labeled molecules (e.g. $H^{13}CO_3^-$) on a subcellular scale. It is thus now possible to determine the rate of carbon and nitrogen fixation at the cellular level (Musat et al., 2008), which would provide valuable information on the functioning of microbial mats in the future.

Acknowledgment

We would like to thank three anonymous reviewers for their constructive remarks, David M Paterson who kindly provided the MagPI device, as well as Nicolas Spilmont and Dominique Davoult for their help with field work. This work is a contribution to the French national CNRS program EC2CO-MicroBien (Microbiologie Environnementale). Thanks are due to the École Normale Supérieure (ENS) Paris for supporting Claire Passarelli's PhD grant. Bruno Jesus was funded by an FCT postdoctoral grant (POCI/BPD/20993/2004), the project SEAPhoB (PDTC/MAR/101410/2008) and the program ASSEMBLE (n°227799).

References

Achenbach, L.A., Carey, J., Madigan, M.T., 2001. Photosynthetic and phylogenetic primers for detection of anoxygenic phototrophs in natural environments. Appl. Environ. Microbiol. 67, 2922–2926.

Al-Maslamani, I., Le Vay, L., Kennedy, H., 2009. Feeding on intertidal microbial mats by postlarval tiger shrimp, Penaeus semisulcatus De Haan. Mar. Biol. 156, 2001–2009.

Andréfouët, S., Payri, C., Hochberg, E.J., Che, L.M., Atkinson, M.J., 2003. Airborne hyperspectral detection of microbial mat pigmentation in Rangiroa atoll (French Polynesia). Limnol. Oceanogr. 48, 426–430.

Bachar, A., Polerecky, L., Fischer, J.P., Vamvakopoulos, K., de Beer, D., Jonkers, H.M., 2008. Two-dimensional mapping of photopigment distribution and activity of *Chloroflexus*-like bacteria in a hypersaline microbial mat. Fems Microbiol. Ecol. 65, 434–448.

Bolam, S.G., Fernandes, T.F., Read, P., Raffaelli, D., 2000. Effects of macroalgal mats on intertidal sandflats: an experimental study. J. Exp. Mar. Biol. Ecol. 249, 123–137.

Brotas, V., Plante-Cuny, M.R., 2003. The use of HPLC pigment analysis to study microphytobenthos communities. Acta Oecol. 24, S109–S115.

Canfield, D.E., Des Marais, D.J., 1993. Biogeochemical cycles of carbon, sulfur, and free oxygen in a microbial mat. Geochim. Cosmochim. Acta 57, 3971–3984.

Carrère, V., Spilmont, N., Davoult, D., 2004. Comparison of simple techniques for estimating chlorophyll *a* concentration in the intertidal zone using high spectral-resolution field-spectrometer data. Mar. Ecol. Prog. Ser. 274, 31–40.

Caumette, P., 1986. Phototrophic sulfur bacteria and sulfate-reducing bacteria causing red waters in a shallow brackish coastal lagoon (Prévost lagoon, France). Fems Microbiol. Ecol. 38, 113–124.

Caumette, P., Baleux, B., 1980. Étude des eaux rouges dues à la prolifération des bactéries photosynthétiques sulfo-oxydantes dans l'étang du Prévost, lagune saumâtre méditerranéenne. Mar. Biol. 56, 183–194.

Caumette, P., Baulaigue, R., Matheron, R., 1988. Characterization of Chromatium salexigens sp. nov., a halophilic Chromatiaceae isolated from Mediterranean salinas. Syst. Appl. Microbiol. 10, 284–292.

Caumette, P., Baulaigue, R., Matheron, R., 1991. Thiocapsa halophila sp. nov., a new halophilic phototrophic purple sulfur bacterium. Arch. Microbiol. 155, 170–176.

Caumette, P., Imhoff, J.F., Suling, J., Matheron, R., 1997. Chromatium glycolicum sp nov, a moderately halophilic purple sulfur bacterium that uses glycolate as substrate. Arch. Microbiol. 167, 11–18.

Caumette, P., Matheron, R., Raymond, N., Relexans, J.C., 1994. Microbial mats in the hypersaline ponds of Mediterranean salterns (Salin-de-Giraud, France). FEMS Microbiol. Ecol. 13, 273–286.

Dade, W.B., Nowell, A.R.M., Jumars, P.A., 1992. Predicting erosion resistance of muds. Mar. Geol. 105, 285–297.

de Beer, D., Glud, A., Epping, E., Kühl, M., 1997. A fast-responding CO2 microelectrode for profiling sediments, microbial mats, and biofilms. Limnol. Oceanogr. 42, 1590–1600.

de Wit, R., van Gemerden, H., 1987. Chemolithotrophic growth of the phototrophic sulfur bacterium Thiocapsa roseopersicina. FEMS Microbiol. Ecol. 45, 117–126.

de Wit, R., van Gemerden, H., 1990. Growth of the phototrophic purple sulfur bacterium Thiocapsa roseopersicina under oxic/anoxic regimens in the light. FEMS Microbiol. Ecol. 73, 69–76.

Decho, A.W., 1990. Microbial exopolymer secretions in ocean environments: their role(s) in food webs and marine processes. Oceanogr. Mar. Biol. Ann. Rev. 28, 73–153.

Des Marais, D.J., 2003. Biogeochemistry of hypersaline microbial mats illustrates the dynamics of modern microbial ecosystems and the early evolution of the biosphere. Biol. Bull. 204, 160–167.

Forster, R.M., Creach, V., Sabbe, K., Vyverman, W., Stal, L.J., 2006. Biodiversity-ecosystem function relationship in microphytobenthic diatoms of the Westerschelde estuary. Mar. Ecol. Progr. Ser. 311, 191–201.

Fourçans, A., de Oteyza, T.G., Wieland, A., Sole, A., Diestra, E., van Bleijswijk, J., Grimalt, J.O., Kuhl, M., et al., 2004. Characterization of functional bacterial groups in a hypersaline microbial mat community (Salins-de-Giraud, Camargue, France). FEMS Microbiol. Ecol. 51, 55–70.

Fourçans, A., Sole, A., Diestra, E., Ranchou-Peyruse, A., Esteve, I., Caumette, P., Duran, R., 2006. Vertical migration of phototrophic bacterial populations in a hypersaline microbial mat from Salins-de-Giraud (Camargue, France). FEMS Microbiol. Ecol. 57, 367–377.

Franks, J., Stolz, J.F., 2009. Flat laminated microbial mat communities. Earth Sci. Rev. 96, 163–172.

Giani, D., Seeler, J., Giani, L., Krumbein, W.E., 1989. Microbial mats and physicochemistry in a saltern in the Brittany (France) and in a laboratory scale saltern model. FEMS Microbiol. Ecol. 62, 151–162.

Gitelson, A., Stark, R., Dor, I., Michelson, O., Yacobi, Y.Z., 1999. Optical characteristics of the phototroph *Thiocapsa roseoparsicina* and implications for real-time monitoring of the bacteriochlorophyll concentration. Appl. Environ. Microbiol. 65, 3392–3397.

Grant, J., Gust, G., 1987. Prediction of coastal sediment stability from photopigment content of mats of purple sulfur bacteria. Nature 330, 244–246.

Guyoneaud, R., Matheron, R., Baulaigue, R., Podeur, K., Hirschler, A., Caumette, P., 1996. Anoxygenic phototrophic bacteria in eutrophic coastal lagoons of the French Mediterranean and Atlantic coasts (Prevost Lagoon, Arcachon Bay, Certes fishponds). Hydrobiologia 329, 33–43.

Hagerthey, S.E., Defew, E.C., Paterson, D.M., 2002. Influence of Corophium volutator and Hydrobia ulvae on intertidal benthic diatom assemblages under different nutrients and temperature regimes. Mar. Ecol. Progr. Ser. 245, 47–59.

Herbert, R., Welsh, D.T., 1994. Establishment of phototrophic purple sulfur bacteria in microbial mat systems. In: Stal, L.J., Caumette, P. (Eds.), Microbial mats: Structure, Development and Environmental Significance. Springer-Verlag, Heidelberg, pp. 51–60.

Herbert, R.A., 1985. Development of mass blooms of photosynthetic bacteria on sheltered beaches in Scapa Flow, Orkney Islands. P. Roy. Soc. Edinb. B. 87, 15–25.

Hiraishi, A., Ueda, Y., 1995. Isolation and characterization of Rhodovulum strictum sp. nov. and some other purple non-sulfur bacteria from colored blooms in tidal and seawater pools. Int. J. Syst. Bacteriol. 45, 882.

Hoehler, T.M., 2004. Biological energy requirements as quantitative boundary conditions for life in the subsurface. Geobiology 2, 205–215.

Hubas, C., Davoult, D., 2006. Does seasonal proliferation of Enteromorpha sp. affect the annual benthic metabolism of a small macrotidal estuary? (Roscoff Aber Bay, France). Estuar. Coast. Shelf Sci. 70, 287–296.

Imhoff, J.F., 2001. True marine and halophilic anoxygenic phototrophic bacteria. Arch. Microbiol. 176, 243–254.

Jesus, B., Mouget, J.L., Perkins, R.G., 2008. Detection of diatom xanthophyll cycle using spectral reflectance. J. Phycol. 44, 1349–1359.

Jorgensen, B.B., 1977. Distribution of colorless sulfur bacteria (Beggiatoa spp.) in a coastal marine sediment. Mar. Biol. 41, 19–28.

Joye, S.B., Lee, R.Y., 2004. Benthic microbial mats: important sources of fixed Nitrogen ans Carbon to the Twin Cays, Belize ecosystem. Atoll Res. Bull. 528, 1–24.

Karr, E.A., Sattley, W.M., Jung, D.O., Madigan, M.T., Achenbach, L.A., 2003. Remarkable diversity of phototrophic purple bacteria in a permanently frozen Antarctic lake. Appl. Environ. Microbiol. 69, 4910–4914.

Kühl, M., 2005. Optical microsensors for analysis of microbial communities. Environ. Microbiol. 166–199.

Kühl, M., Fenchel, T., 2000. Bio-optical characteristics and the vertical distribution of photosynthetic pigments and photosynthesis in an artificial cyanobacterial. Mat. Microbial. Ecol. 40, 94–103.

Kühl, M., Jørgensen, B.B., 1992. Spectral light measurements in microbenthic phototrophic communities with a fiber-optic microprobe coupled to a sensitive diode array detector Limnol. Oceanogr 37, 1813–1823.

Kühl, M., Lassen, C., Jørgensen, B.B., 1994. Optical properties of microbial mats: light measurements with fiber-optic microprobes. In: Stal, L.J., Caumette, P (Eds.), Microbial Mats: Structure Development and Environmental Significance, vol. 35. Springer-Verlag GmbH & Co, pp. 149–166.

Kühl, M., Polerecky, L., 2008. Functional and structural imaging of phototrophic microbial communities and symbioses. Aquat. Microbial. Ecol. 53, 99–118.

Larson, F., Lubarsky, H.V., Gerbersdorf, S.U., Paterson, D.M., 2009. Surface adhesion measurements in aquatic biofilms using magnetic particle induction: MagPI. Limnol. Oceanogr. Methods 7, 490–497.

Ley, R.E., Harris, J.K., Wilcox, J., Spear, J.R., Miller, S.R., Bebout, B.M., Maresca, J.A., Bryant, D.A., et al., 2006. Unexpected diversity and complexity of the Guerrero Negro hypersaline microbial mat. Appl. Environ. Microbiol. 72, 3685–3695.

Martinez-Alonso, M., Van Bleijswijk, J., Gaju, N., Muyzer, G., 2005. Diversity of anoxygenic phototrophic sulfur bacteria in the microbial mats of the Ebro Delta: a combined morphological and molecular approach. FEMS Microbiol. Ecol. 52, 339–350.

Massé, A., Pringault, O., de Wit, R., 2002. Experimental study of interactions between purple and green sulfur bacteria in sandy sediments exposed to illumination deprived of near-infrared wavelengths. Appl. Environ. Microbiol. 68, 2972–2981.

Méléder, V., Barrilé, L., Launeau, P., Carrère, V., Rincé, Y., 2003. Spectrometric constraint in analysis of benthic diatom biomass using monospecific cultures. Remote Sens. Environ. 88, 386–400.

Migné, A., Davoult, D., Spilmont, N., Menu, D., Boucher, G., Gattuso, J.P., Rybarczyk, H., 2002. A closed-chamber CO2-flux method for estimating intertidal primary production and respiration under emersed conditions. Mar. Biol. 140, 865–869.

Migné, A., Spilmont, N., Davoult, D., 2004. In situ measurements of benthic primary production during emersion: seasonal variations and annual production in the Bay of Somme (eastern English Channel, France). Conti. Shelf Res. 24, 1437–1449.

Mir, J., Martinezalonso, M., Esteve, I., Guerrero, R., 1991. Vertical stratification and microbial assemblage of a microbial mat in the Ebro Delta (Spain). FEMS Microbiol. Ecol. 86, 59–68.

Mouné, S., Caumette, P., Matheron, R., Willison, J.C., 2003. Molecular sequence analysis of prokaryotic diversity in the anoxic sediments underlying cyanobacterial mats of two hypersaline ponds in Mediterranean salterns. FEMS Microbiol. Ecol. 44, 117–130.

Murphy, R., Underwwod, A.J., Pinkerton, M., Range, P., 2005. Field spectrometry: new methods to investigate epilithic micro-algae on rocky shores. J. Exp. Mar. Biol. Ecol. 325, 111–124.

Musat, N., Halm, H., Winterholler, B., Hoppe, P., Peduzzi, S., Hillion, F., Horreard, F., Amann, R., et al., 2008. A single-cell view on the ecophysiology of anaerobic phototrophic bacteria. Proc. Nat. Acad. Sci. U.S.A. 105, 17861–17866.

Navarrete, A., Peacock, A., Macnaughton, S.J., Urmeneta, J., Mas-Castella, J., White, D.C., Guerrero, R., 2000. Physiological status and community

composition of microbial mats of the Ebro Delta, Spain, by signature lipid biomarkers. Microb. Ecol. 39, 92–99.

Nedergaad, R.I., Risgaard-Petersen, N., Finster, K., 2002. The importance of sulfate reduction associated with Ulva lactuca thalli during decomposition: a mesocosm experiment. J. Exp. Mar. Biol. Ecol. 275, 15–29.

Nicholson, J.A.M., Stolz, J.F., Pierson, B.K., 1987. Structure of a microbial mat at Great Sippewissett Marsh, Cape Cod. Massachussets. FEMS Microbiol. Ecol. 45, 343–364.

Oren, A., 2009. Saltern evaporation ponds as model systems for the study of primary production processes under hypersaline conditions. Aquat. Microb. Ecol. 56, 193–204.

Overmann, J., Garcia-Pichel, F., 2006. The phototrophic way of life. In: Dworkin, M., Falkow, S., Rosenberg, E., Schleifer, K.-H. (Eds.), The Prokaryotes, a Handbook on the Biology of Bacteria: Ecophysiology and Biochemistry. Springer, Heidelberg, pp. 32–85.

Paerl, H.W., Pinckney, J.L., 1996. A mini-review of microbial consortia: their roles in aquatic production and biogeochemical cycling. Microb. Ecol. 31, 225–247.

Paterson, D.M., 1997. Biological mediation of sediment erodibility: ecology and physical dynamics. In: Burt, N., Parker, R., Watts, J. (Eds.), Cohesive Sediments. John Wiley and Sons, pp. 215–229.

Paterson, D.M., Tolhurst, T.J., Kelly, J.A., Honeywill, C., de Deckere, E.M.G.T., Huet, V., Shayler, S.A., Black, K.S., et al., 2000. Variations in sediment properties, Skeffling mudflat, Humber Estuary, UK. Conti. Shelf Res. 20, 1373–1396.

Paterson, D.M., Wiltshire, K.H., Miles, A., Blackburn, J., Davidson, I., Yates, M.G., McGrorty, S., Eastwood, J.A., 1998. Microbiological mediation of spectral reflectance from intertidal cohesive sediments. Limnol. Oceanogr. 43, 1207–1221.

Perkins, R.G., Lavaud, J., Serôdio, J., Mouget, J.L., Cartaxana, P., Rosa, P., Barillé, L., Brotas, V., et al., 2010. Vertical cell movement is a primary response of intertidal benthic biofilms to increasing light dose. Mar. Ecol. Prog. Ser. 416, 93–103.

Polerecky, L., Bachar, A., Schoon, R., Grinstein, M., Jørgensen, B.B., de Beer, D., Jonkers, H.M., 2007. Contribution of Chloroflexus respiration to oxygen cycling in a hypersaline microbial mat from Lake Chiprana, Spain. Environ. Microbiol. 9, 2007–2024.

Polerecky, L., Bissett, A., Al-Najjar, M., Faerber, P., Osmers, H., Suci, P.A., Stoodley, P., de Beer, D., 2009. Modular spectral imaging system for discrimination of pigments in cells and microbial communities. Appl. Environ. Microbiol. 75, 758–771.

Prášil, O., Bina, D., Medová, H., Reháková, K., Zapomelová, E., Veselá, J., Oren, A., 2009. Emission spectroscopy and kinetic fluorometry studies of phototrophic microbial communities along a salinity gradient in solar saltern evaporation ponds of Eilat. Israel. Aquat. Microb. Ecol. 56, 285–296.

Ranchou-Peyruse, A., Herbert, R., Caumette, P., Guyoneaud, R., 2006. Comparison of cultivation-dependent and molecular methods for studying the diversity of anoxygenic purple phototrophs in sediments of an eutrophic brackish lagoon. Environ. Microbiol. 8, 1590–1599.

Revsbech, N.P., Jørgensen, B.B., 1983. Photosynthesis of benthic microflora measured with high spatial-resolution by the oxygen microprofile method - capabilities and limitations of the method. Limnol. Oceanogr. 28, 749–756.

Riera, P., 2010. Trophic plasticity of the gastropod Hydrobia ulvae within an intertidal bay (Roscoff, France): a stable isotope evidence. J. Sea Res. 63, 78–83.

Riera, P., Hubas, C., 2003. Trophic ecology of nematodes from various microhabitats of the Roscoff Aber Bay (France): importance of stranded macroalgae evidenced through ä13C and ä15N. Mar. Ecol. Prog. Ser. 260, 151–159.

Riera, P., Richard, P., 1996. Isotopic determination od food sources of Crassostrea gigas along a trophic gradient in the estuarine bay of Marénnes-Oléron. Estuar. Coast. Shelf Sci. 42, 347–360.

Rothermich, M.M., Guerrero, R., Lenz, R.W., Goodwin, S., 2000. Characterization, seasonal occurrence, and diel fluctuation of poly(hydroxyalkanoate) in photosynthetic microbial mats. Appl. Environ. Microbiol. 66, 4279–4291.

Serôdio, J., Cartaxana, P., Coelho, H., Vieira, S., 2009. Effects of chlorophyll fluorescence on the estimation of microphytobenthos biomass using spectral reflectance indices. Remote Sens. Environ. 113, 1760–1768.

Spilmont, N., Davoult, D., Migné, A., 2006. Benthic primary production during emersion: in situ measurements and potential primary production in the Seine Estuary (English Channel, France). Mar. Pollut. Bull. 53, 49–55.

Stal, L.J., 2010. Microphytobenthos as a biogeomorphological force in intertidal sediment stabilization. Ecol. Eng. 36, 236–245.

Stal, L.J., Caumette, P., 1994. Microbial Mats: Structure and Environmental Significance. Springer-Verlag, Heidelberg.

Stal, L.J., van Gemerden, H., Krumbein, W.E., 1985. Structure and development of a benthic marine microbial mat. FEMS Microbiol. Ecol. 31, 111–125.

Stal, L.J., Vangemerden, H., Krumbein, W.E., 1984. The simultaneous assay of chlorophyll and bacteriochlorophyll in natural microbial communities. J. Microbiol. Methods 2, 295–306.

Steenbergen, C.L.M., Korthals, H.J., 1982. Distribution of phototrophic microorganisms in the anaerobic and microaerophilic strata of Lake Vechten (The Netherlands). Pigment analysis and role in primary production. Limnol. Oceanogr. 27, 883–895.

Stephens, F.C., Louchard, E.M., Reid, R.P., Maffione, R.A., 2003. Effects of microalgal communities on reflectance spectra of carbonate sediments in subtidal optically shallow marine environments. Limnol. Oceanogr. 48, 535–546.

Sutherland, T.F., Grant, J., Amos, C.L., 1998. The effect of carbohydrate production by the diatom Nitzschia curvilineata on the erodibility of sediment. Limnol. Oceanogr. 43, 65–72.

Thar, R., Kühl, M., Holst, G., 2001. Fiber-optic fluorometer for microscale mapping of photosynthetic pigments in microbial communities. Appl. Environ. Microbiol. 67, 2823–2828.

Thiel, V., Tank, M., Neulinger, S.C., Gehrman, L., Dorador, C., Imhoff, J.F., 2010. Unique communities of anoxygenic phototrophic bacteria in saline lakes of Salar de Atacama (Chile): evidence for a new phylogenetic lineage of phototrophic *Gammaproteobacteria* from *pufLM* gene analyses. FEMS Microbiol. Ecol. 74, 510–522.

Urmeneta, J., Alcoba, O., Razquin, E., Tarroja, E., Navarrete, A., Guerrero, R., 1998. Oxygenic photosynthesis and respiratory activity in microbial mats of the Ebro Delta, Spain, by oxygen exchange method. Curr. Microbiol. 37, 151–155.

van Duyl, F.C., de Winder, B., Kop, A.J., Wollenzien, U., 2000. Consequences of diatom mat erosion for carbohydrate concentrations and heterotrophic bacterial activities in intertidal sediments of the Ems-Dollard estuary. Cont. Shelf Res. 20, 1335–1349.

van Gemerden, H., 1993. Microbial mats - a joint venture. Mar Geol 113, 3–25.

van Gemerden, H., Mas, J., 1995. Ecology of phototrophic sulfur bacteria. In: Blankenship, R.E., Madigan, M.T., Bauer, C.E. (Eds.), Anoxygenic Photosynthetic Bacteria. Advances in Photosynthesis, vol. 2. Kluwer Academic Publishers, Dordrecht, The Netherlands, pp. 49–85.

van Gemerden, H., Dewit, R., Tughan, C.S., Herbert, R.A., 1989a. Development of mass blooms of *Thiocapsa roseopersicina* on sheltered beaches on the Orkney Islands. FEMS Microbiol. Ecol. 62, 111–118.

van Gemerden, H., Tughan, C.S., Dewit, R., Herbert, R.A., 1989b. Laminated microbial ecosystems on sheltered beaches in Scapa Flow, Orkney Islands. FEMS Microbiol. Ecol. 62, 87–102.

van Oevelen, D., Middelburg, J.J., Soetaert, K., Moodley, L., 2006. The fate of bacterial carbon in an intertidal sediment: modeling an in situ isotope tracer experiment. Limnol. Oceanogr. 51, 1302–1314.

Villanueva, L., Del Campo, J., Guerrero, R., 2010. Diversity and physiology of polyhydroxyalkanoate-producing and -degrading strains in microbial mats. FEMS Microbiol. Ecol. 74, 42–54.

Visscher, P.T., Beukema, J., van Gemerden, H., 1991. In situ characterization of sediments: measurements of oxygen and sulfide profiles with a novel combined needle electrode. Limnol. Oceanogr. 36, 1476–1480.

Vopel, K., Hawes, I., 2006. Photosynthetic performance of benthic microbial mats in Lake Hoare, Antarctica. Limnol. Oceanogr. 51, 1801–1812.

Wieland, A., Kühl, M., 2000. Irradiance and temperature regulation of photosynthesis and O2 consuption in a hypersaline cyanobacterial mat (Solar Lake, Egypt). Mar. Biol. 137, 71–85.

Wieland, A., Kuhl, M., McGowan, L., Fourcans, A., Duran, R., Caumette, P., de Oteyza, T.G., Grimalt, J.O., et al., 2003. Microbial mats on the Orkney Islands revisited: microenvironment and microbial community composition. Microb. Ecol. 46, 371–390.

Wieland, A., Zopfi, J., Benthien, A., Kuhl, M., 2005. Biogeochemistry of an iron-rich hypersaline microbial mat (Camargue, France). Microb. Ecol. 49, 34–49.

Wiggli, M., Smallcombe, A., Bachofen, R., 1999. Reflectance spectroscopy and laser confocal microscopy as tools in an ecophysiological study of microbial mats in an alpine bog pond. J. Microbiol. Methods 34, 173–182.

Williams, P.J., Del Giorgio, P.A., 2005. Respiration in aquatic ecosystems: history and background. In: del Giorgio, P.A., Williams, P.J. (Eds.), Respiration in Aquatic Ecosystems. Oxford University Press, pp. 1–17.

Winkler, L.W., 1888. Die Bestimmung des im Wasser gelösten Sauerstoffen. Ber. Dtsch. Chem. Ges. 21, 2843–2855.

Yurkov, V., Csotonyi, J.T., 2008. New light on aerobic anoxygenic phototrophs. In: Hunter, C.N., Daldal, F., Thurnauer, M.C., Beatty, J.T. (Eds.), The Purple Phototrophic Bacteria. Advances in Photosynthesis and Respiration, vol. 28. Springer, Netherlands, pp. 31–55.