Optical properties of microbial mats: Light measurements with fiber-optic microprobes

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Introduction

Photosynthetic microbial mats occur as dense stratified communities in top layers of sediments, where microorganisms and sediment particles are embedded in an extracellular polymer matrix (e.g., *Farbstreifensandwatt*, Stal et al. 1985) or growing as a thin photosynthetic biofilm on solid substrata, e.g., stone or plant surfaces (Kühl 1993). In extreme environments, like hypersaline salt marshes and hot springs, regular mats several mm' to cm' thick and composed of almost pure biomass and exopolymers develop (Cohen and Rosenberg 1989). Photosynthetic microorganisms are the predominant component of these microbial mats, which often exhibit a vertical stratification of different colored layers due to the presence of photosynthetic microalgae and bacteria containing different photopigments with depth (Nicholson et al. 1987; Pierson et al. 1990). High metabolic rates due to the high density of microorganisms in mats combined with molecular diffusion acting as the major transport mechanism result in steep chemical gradients with depth as has been demonstrated by microelectrode measurements at $<$50-100 μm spatial resolution (Revsbech and Jørgensen 1986; Revsbech this volume). Production and consumption of the major electron acceptors can be calculated from measured microprofiles and different functional layers can thus be identified in microbial mats from microelectrode measurements. The typical sequence found is an upper oxygenic photosynthetic layer with concurrent oxygen respiration and a lower anoxic layer with denitrification and sulfate reduction as the predominant respiratory processes and with anoxygenic photosynthesis, provided sufficient light is penetrating from above (Jørgensen et al. 1983; Jørgensen and Des Marais 1986b; Revsbech et al. 1989; Kühl 1993). At the oxic-anoxic interface various chemoautotrophic bacteria (e.g., *Beggiatoa* or *Thiobacillus spp.*) oxidize reduced chemical species (e.g., H_2S) diffusing upwards from deeper mat layers, with oxygen or nitrate from above (Kühl and Jørgensen 1992a).

While a technique for measuring anoxygenic photosynthesis at high spatial resolution still has to be developed, oxygenic photosynthesis can be measured in microbial mats at $<$100 μm spatial resolution with the oxygen microelectrode light-dark shift technique

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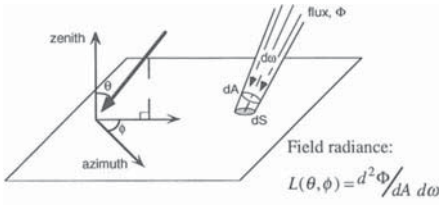
(Revsbech and Jørgensen 1983). Most studies of benthic photosynthesis have lacked information about the *in situ* spectral composition and intensity of light available for the microphytobenthos (Admiraal 1984). Thus, oxygenic photosynthesis has mostly been related to the incident quantum flux from above per unit surface area of the mat by measuring the downwelling 400-700 nm quantum irradiance with a large cosine collector. The relatively few measurements of light penetration in sediments reported in the literature, were done mostly by covering a large cosine collector with increasing amounts of sediment or by inserting miniprobes with non-ideal light collecting properties into the mat or sediment (e. g., Fenchel and Straarup 1971, Haardt and Nielsen 1980, Colijn 1982, Pierson et al. 1990).

Table 1. Basic optical parameters for light measurements in microbial mats.

Parameter	Definition	Microscale measuring technique
	The radiant flux, Φ, from a certain direction (θ, ϕ) in a spherical coordinate system per unit solid angle, $d\omega$, per unit area perpendicular to the direction of light propagation, dA	Measured by a simple, flat-cut untapered or tapered optical fiber. The radiance fiberprobe has a directional response defined by the acceptance angle of the optical fiber. Tip diameter 10-125 µm. (Jørgensen and DesMarais, 1986; Kühl and Jørgensen 1992)
 Scalar irradiance: $E_0=\int_{4\pi} L(\theta,\phi)\,d\omega$	The integral radiant flux incident from all directions about a point in the sediment. E_0 consists of a downwelling, E_{0d}, and an upwelling, E_{0u}, component corresponding to the integral flux incident from the upper or lower hemisphere, respectively	Measured by a coated and tapered optical fiber with a diffusing sphere fixed on the tip. The fiberprobe has an isotropic response for light and a tip diameter of 50-100 µm. (Lassen et al. 1992; Kühl and Jørgensen 1992)
 Downwelling irradiance $E_d=\int_{2\pi} L(\theta,\phi)\cos\theta\,d\omega$	The integral radiant flux incident from the upper (downwelling irradiance, E_d) or lower hemisphere (upwelling irradiance, E_u) per unit area of a horizontal surface element	Measured by a coated optical fiber with a diffusing disk fixed at the flat cut end of the fiber. The ir-radiance fiberprobe weights the incident radiance, $L(\theta, \phi)$, with the cosine of the incident zenith angle, θ. Tip diameter 40-125 µm. (Lassen et al. in prep.)

In order to study the optical properties and photosynthesis-light interactions in sediments and microbial mats at a spatial resolution comparable to oxygen microelectrode measurements, i.e. <100 μm, we have developed fiber-optic microprobes with defined light collecting properties for spectral measurements of the fundamental light parameters: radiance, irradiance, and scalar irradiance (see definitions in Table 1) (Jørgensen and Des Marais 1986a; Lassen et al. 1992a, Kühl and Jørgensen 1992b, Lassen et al. in prep.). The microprobes were used in several types of marine sediments and microbial mats in purely optical studies and in studies, where concurrent microscale measurements of light and photosynthesis were used to investigate the photosynthetic performance of microbenthic communities (Jørgensen and Des Marais 1988; Jørgensen et al. 1987; Lassen et al. 1992b; Kühl 1993; Kühl and Jørgensen 1993; Ploug et al. 1993). In the following, we summarize and discuss some results of these recent studies with focus on the optical properties of microbial mats. A detailed discussion of the photosynthetic performance of a marine microbial mat is presented elsewhere in this volume (Lassen et al. this volume).

Fiber-optic microprobes

Optical fibers are well-suited for building optical microprobes for several reasons: i. They have defined light collecting properties. ii. The raw fiber has a small diameter (typically 50-200 μm), and it is relatively easy to taper the fiber down to a 10-15 μm tip diameter. iii. Optical fibers are flexible and relatively sturdy when used in sediments and other dense materials.

The basic design of our optical microprobes is simple (Fig. 1B). The protective coating of a 1-1.5 m long single strand optical fiber cable is stripped of at both ends. One end of the fiber is then cut with a diamond knife to obtain a perfectly flat output end of the fiber, which is inserted into the light detection system. The other end is inserted through a hypodermic needle and fixed with a drop of epoxy resin. This light collecting end of the fiber can be modified according to the optical parameter to be measured.

For use in microbial mats and sediments it is essential to connect the fiberprobes to a sensitive detector system. Presently we are using an intensified diode array system for 3-5 nm spectral resolution light measurements of visible and infrared light (up to 900 nm) (Fig. 1A, described in Kühl and Jørgensen 1992b) and a simple battery operated photomultiplier system equipped with a heat reflecting mirror for integral measurements of 400-700 nm light (PAR).

Radiance microprobes

The most fundamental light parameter for studies of the light field is the field radiance, which is the radiant flux per unit area per unit solid angle from a certain direction (Table 1). A radiance microprobe should thus have a well-defined directional response with a narrow acceptance angle for light. The directional sensitivity is specified by the numerical aperture, $NA = n_0 \sin \Theta_a$, where n_0 is the refractive index of the surrounding medium and Θ_a, is the acceptance half angle of the optical fiber (Senior 1985). Radiance fiberprobes thus have a higher acceptance half angle in air ($n_0 = 1$) than in water ($n_0 = 1.33$).

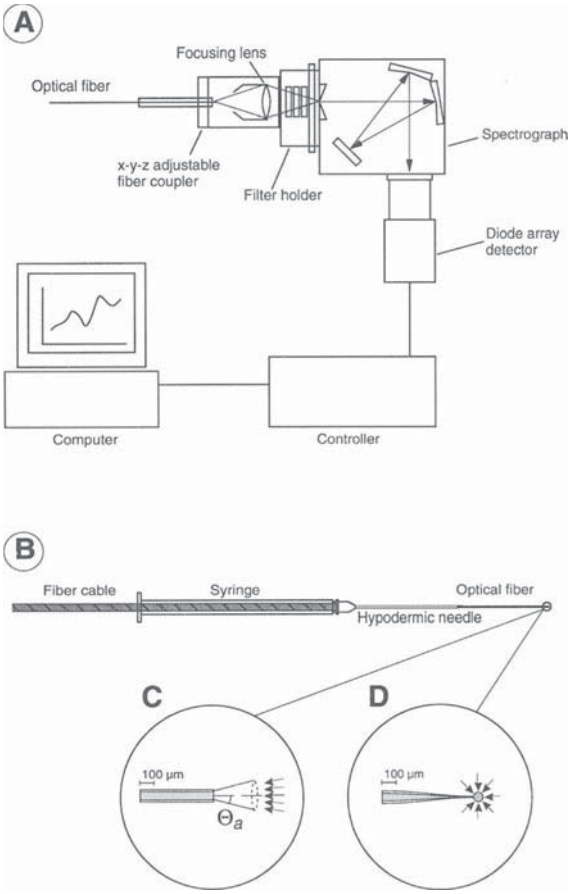


Fig.1. Diode array detector system for fiber-optic microprobes. A. Detector system. B. Fiber-optic microprobe. C. Tip of microprobe designed to measure field radiance. D. Spherical tip of microprobe designed to measure scalar irradiance (From Kühl and Jørgensen 1992b)

A simple radiance probe can be constructed by using a flat cut untapered optical fiber for collecting light (Fig. 1C). We used multimode graded-index optical fibers with an outer diameter of 125 μm and a NA of 0.2 (Newport Corp, F-MSD) giving an acceptance angle of 11.5° in air and 8.6° in water. Smaller radiance probes were made by tapering the optical fiber down to 10-50 μm in a small acetylene flame and then cutting the fiber with a diamond knife (Fig. 2A). Tapered fibers have a broader acceptance angle due to light entering through the tapered sides of the fiber tip. This problem can be solved by coating the fiber tip with an opaque enamel or a Cr-metal film (Vogelmann et al. 1991). We are able to make coated radiance probes with tip diameters down to <15 μm and acceptance angles <15-20° in air.

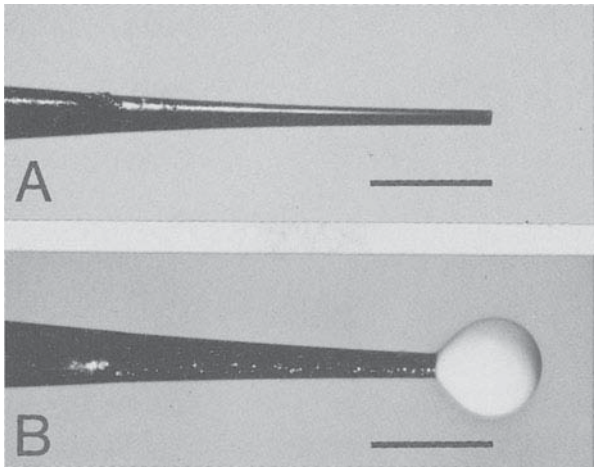


Fig. 2. Fiberoptic microprobes for field radiance (A) and scalar irradiance (B). Length of bar is 100 μm.

Scalar irradiance microprobes

Scalar irradiance is an integral measure of the total radiant flux from all directions about a point in the sediment, i. e. the spherically integrated radiance from all directions (Table 1). The scalar irradiance is the most relevant measure of total quantum flux available for the microphytobenthos which receive light from all directions around the cell in sediments and microbial mats (Jørgensen and Des Marais 1988; Kühl and Jørgensen 1992b). A scalar irradiance microprobe should thus weight incident light from all directions equally, i. e. have an isotropic angular response. This is accomplished by casting a small diffusing sphere of TiO_2-doped methacrylate onto the tip of a tapered and coated optical fiber (Fig. 1D, Fig. 2B). The construction details are described by Lassen et al. (1992a). The microprobes exhibit an isotropic ($\pm 10\%$) response in air and in water. Scalar irradiance microprobes can be made with sphere diameters from 50 to 100 μm and can thus be used for measurements of total quantum flux in microbial mats at <0.1 mm spatial resolution, i. e. the same spatial resolution as obtained by the oxygen microelectrode technique for measuring oxygenic photosynthesis.

Irradiance microprobes

Irradiance is an integral measure of the incident radiant flux per unit horizontal surface area from above (downwelling irradiance) or below (upwelling irradiance) the surface (Table 1). Light travelling at oblique angles is weighted with the cosine of the incidence angle, and irradiance thus weights scattered light less than vertically incident light. An irradiance microprobe with the described cosine response for light can be made by fixing a small droplet

of TiO_2-doped methacrylate on the end of a coated optical fiber, which is then totally covered with black opaque enamel. By careful grinding of the coated fiber end under the microscope, the white methacrylate is exposed at the tip forming a small disk surrounded by black enamel, i. e. a miniature version of the large irradiance cosine collector which is commonly used in aquatic ecology. Further details are presented elsewhere (Lassen et al. in prep.).

Radiance measurements

Simple radiance microprobes connected to a single photodiode for use in microbial mats and sediments were introduced by Jørgensen and Des Marais (1986a). Studies with this system are reviewed by Jørgensen (1989) and will not be included in the present overview. Here we present data obtained with the more sensitive (but less portable) light detection system described by Kühl and Jørgensen (1992b).

Spectral light penetration and zonation of microbial mats

Due to the directional sensitivity of radiance microprobes they are useful in studies of the dynamics and regulation of the vertical zonation of phototrophs in mats. By measuring spectral downwelling radiance by penetrating a mat sample from below or by measuring spectral upwelling radiance coming with the fiber probe from above the mat, the vertical zonation of major photopigments can be described (Jørgensen and Des Marais 1988; Kühl and Jørgensen 1992b). An example is given in Fig. 3, where downwelling spectral radiance was measured at 50 μm resolution in a laminated hypersaline microbial mat from Eilat, Israel. The radiance transmittance spectra exhibit throughs at absorption wavelengths of the major photopigments in the mat (Fig. 3A). In the upper 0.0-1.5 mm, Chl *a* (440nm and 675 nm) and carotenoids (450-550 nm) were the dominant photopigments due to the predominance of diatoms in this layer. At 1.5-2.0 mm below the mat surface, a dense cyanobacterial layer (*Microcoleus chthonoplastes* and *Phormidium spp.*) was found exhibiting strong spectral signals from Chl *a* (675 nm) and phycocyanin (620-625 nm). Below 2.5 mm, spectral signals from Bchl *a* (807 nm and 865 nm) and Bchl *c* (746 nm) were found due to the presence of green filamentous bacteria (*Chloroflexus sp.*) and purple sulfur bacteria in the bottom layer of the mat. A more detailed picture of this vertical zonation in the mat was obtained from analyzing radiance depth profiles for the absorption wavelengths mentioned above (Fig. 3B). By fitting functions (6'th or 7'th order polynomia) to the log-transformed radiance profiles it was possible to calculate depth profiles of the vertical attenuation coefficient of radiance, K_L. The attenuation coefficient is defined as the rate of change of log-transformed radiance values with depth, $K_L = -d(\ln L)/dz$ (Kirk 1983). Depth profiles of attenuation coefficients could thus be calculated by taking the first derivative with respect to depth of polynomia fitted to log transformed radiance depth profiles (Fig. 3C). In the upper 0-1 mm of the mat, the apparent high attenuation of radiance at all wavelengths was due to the scattering of photons out of the downwelling path of the

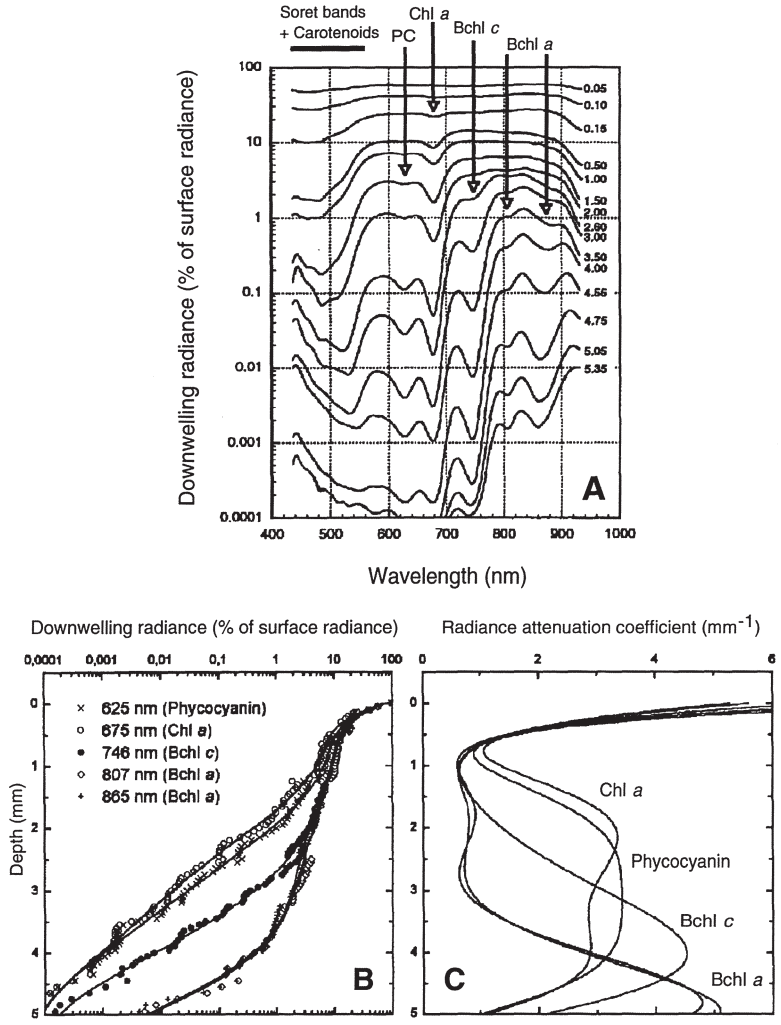


Fig.3. Measurements of downwelling radiance in a cyanobacterial mat from Eilat, Israel.
A. Radiance spectra from selected depths in the mat (indicated by numbers on graph).
B. Depth profiles of radiance at absorption wavelengths of major photopigments in the mat. Solid lines represent 6'th order polynomia fitted to the data.
C. Depth profiles of attenuation coefficients calculated from the first derivative of ln-transformed data.
(Kühl et al. unpublished data)

incident collimated light as well as light absorption by photopigments. This effect of scattering out of the light path is less important in deeper mat layers with a more diffuse light field (see

also later in this chapter). Although the results are thus unclear in the upper 0-0.5 mm of the mat, the attenuation coefficient depth profiles demonstrate the vertical zonation of the major photopigments in the microbial mat as a continuous mixture of photopigments throughout the mat, with highest Chl *a* and phycocyanin concentrations in the upper mat layers (0-3 mm) and gradually increasing amounts of Bchl *c* and Bchl *a* in the deeper mat layers (2.5-5 mm) (Fig. 3C).

Reflectance spectra and migration in microbial mats

The vertical zonation of a microbial mat is not static as many of the phototrophic microorganisms migrate in response to changes in light and in chemical gradients during a diel cycle (Jørgensen 1982). It is possible to map this migration by measuring either down- or upwelling radiance spectra with fiber-optic microprobes. Figure 4 shows the ratio between the upwelling radiance from a white $BaSO_4$ reflection standard and the reflected radiance from the

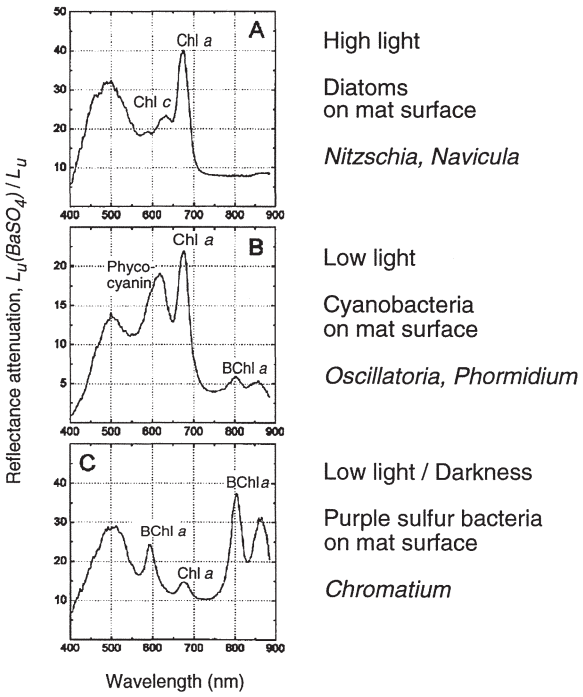


Fig. 4. Changes in attenuation of upwelling radiance due to the migration of photosynthetic microorganisms in a marine microbial mat from Limfjorden, Denmark. Data are presented as the ratio of upwelling radiance from a reflectance standard and the upwelling radiance from the mat. (Kühl et al. unpublished data)

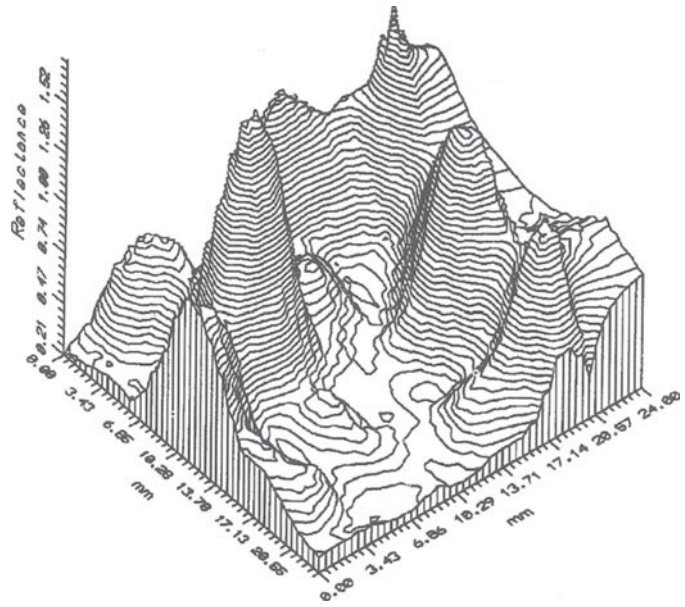


Fig. 5. Radiance reflectance of 620 nm light measured by a radiance microprobe within a 25 x 25 mm surface area of a cyanobacterial mat (Pond 5, Guerrero Negro, Baja California, Mexico). Data are expressed in % of reflected radiance from a $BaSO_4$ reflectance standard (B. B. Jørgensen and L. L. Richardson unpublished data).

surface of a marine microbial mat from Limfjorden, Denmark. The dominant migrating phototrophs were diatoms (mostly *Nitzschia spp.* and *Navicula spp.*), cyanobacteria (*Oscillatoria spp.*), and purple sulfur bacteria (*Chromatium sp.*). Under high light (> 100-200 μEinst. m^{-2} s^{-1}) the surface of the mat was covered with diatoms (Fig. 4A) exhibiting clear light absorption peaks of Chl *a* (675 nm), Chl *c* (630-35 nm), and broad absorption between 400-560 nm due to chlorophylls and carotenoids. In low light (< 50 μEinst m^{-2} s^{-1}) cyanobacteria migrated within a few hours up to the mat surface (Fig. 4B) exhibiting absorption peaks of Chl *a* (675 nm), phycocyanin (620 nm) and other phycobilins (560- 620nm). Small peaks of BChl *a* absorption (790-810 nm, and 830-880 nm) also showed up indicating that the purple sulfur bacteria had also come closer to the mat surface. After extended periods in low light or in darkness (> 6 hours) the purple sulfur bacteria migrated up to the mat surface (Fig. 4C), now exhibiting clear absorption peaks of BChl *a* at 590 nm, 805 nm and 865 nm.

By doing many point measurements of reflectance, the horizontal distribution of photopigments, i. e. the *reflectance topography* of microbial mats, can be studied at high resolution. An example is shown in Fig. 5, where the surface radiance reflectance at 620 nm (phycocyanin absorption maximum) was measured within a 25 x 25 mm area of a hypersaline

cyanobacterial mat at 1 mm spatial resolution (Pond 5, Guerrero Negro, Baja California, Mexico). The lowest reflectance values correspond to the highest concentrations of phycocyanin. The 620 nm reflectance topography of the mat is thus a mirror image of the surface distribution of cyanobacteria. In combination with spectral surface scans of larger mat areas by a CCD camera, measurements of the reflectance topography of mats with radiance microprobes could provide detailed information on the horizontal distribution of photosynthetic microorganisms and their migratory patterns (Richardson and Jørgensen, unpublished data).

In conclusion, radiance microprobes are powerful tools for investigations of the finescale vertical and horizontal distributions of photosynthetic microorganisms in microbial mats. Fiber-optic microprobes are, however, also able to resolve changes in the vertical zonation due to migration of the phototrophs in microbial mats. The method has the advantage of being non-destructive and allowing for repeated measurements in the same mat sample under different light conditions. Especially in combination with recently developed minicoring techniques for simultaneous collection of microscopy and pigment samples at high spatial resolution (Garcia-Pichel et al. 1993), fiber-optic microprobes can now be used for detailed studies of migratory behavior of microorganisms in microbial mats.

The light field in microbial mats

Angular radiance distributions

Knowledge of the light intensity in microbial mats is essential to understand the regulation of microbenthic photosynthesis. Quantification of the light intensity in a microbial mat is, however, not possible by measuring only radiance depth profiles in a single direction. This is evident from measurements of radiance depth profiles taken at different zenith angles relative to the incident collimated light (Kühl and Jørgensen 1993). Figure 6 shows such an angular radiance distribution measured at 650 nm in a marine microbial mat exhibiting a yellow-brown surface layer of sand with a few diatoms, and a dark green subsurface cyanobacterial layer on top of a pink layer of purple sulfur bacteria.

At the sediment surface the light field was highly directional and composed of the incident collimated light (0°-20° zenith angle) and diffuse backscattered light (90°-180° zenith angle) from the mat. The little forward scattered light detected at the mat surface was due to internal reflection at the water-air interface back to the sediment surface of some backscattered light from the sediment surface. Already 0.5-1.0 mm into the sand layer the angular light field changed dramatically. The collimated component of the light field decreased strongly due to intense scattering of light out of the downwelling light path, resulting in much forward scattered light. Thus, only a few tenths of a mm below the mat surface the spatial light distribution had changed from a collimated to a diffuse light field with scattered light being the major light source for photosynthetic microorganisms in the mat. In deeper mat layers the angular light distribution was affected by the lamination of the mat. At >1 mm depth the backscattered light

was attenuated more strongly due to the underlying dense cyanobacterial layer and the light distribution became more forward biased.

Thus, the laminated structure of microbial mats exhibiting varying scattering to absorption ratios with depth can interact with the general trend of the light field to become more diffuse with depth. These effects of layers with different absorption and scattering properties on the spatial light distribution are not present in non-stratified coastal sediments, where the angular light field approaches an asymptotic radiance distribution which depends on the inherent absorption and scattering properties of the sediment (Kühl and Jørgensen, 1993). The magnitude of these changes in the light field with depth also depend strongly on the ratio of scattering versus absorption at different wavelengths so that the light field is relatively more forward biased for wavelengths subject to high absorption than for wavelengths subject to low absorption. Further details on the analysis of the angular light field can be found in Kühl and Jørgensen (1993).

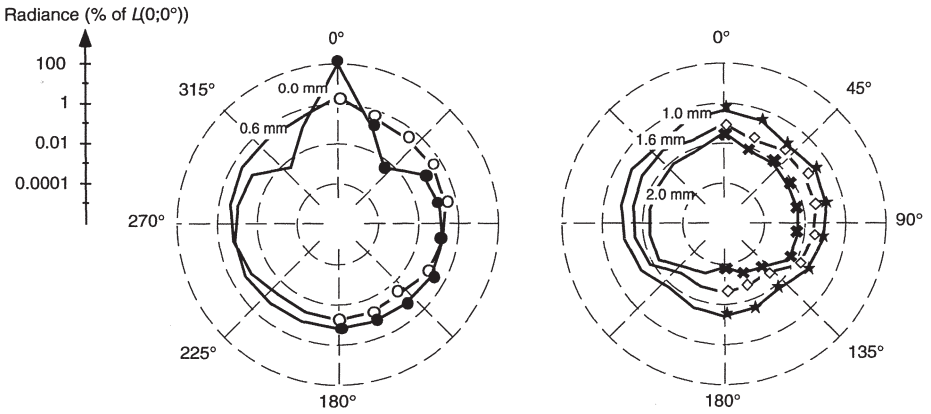


Fig. 6. Angular radiance distribution of 650 nm light in microbial mat from Kaløvig, Denmark. Numbers on curves indicate depth. Symbols indicate measured radiance values expressed on a log-scale in % of the downwelling radiance from 0° zenith angle at the mat surface (Kühl and Jørgensen unpublished data).

Irradiance and scalar irradiance

Irradiance and scalar irradiance are both common measures of light intensity. Irradiance is defined as the incident light from above (downwelling irradiance, E_d) or below (upwelling irradiance, E_u) a horizontal surface (Table 1) and weights light incident at oblique angles less than collimated light. Measurements of the angular radiance distribution (Fig. 6) show, however, that microorganisms in microbial mats live in a highly diffuse light field with primarily scattered light coming from all directions around the cell. Irradiance is therefore not

the optimal measure of total light intensity in sediments, microbial mats or other highly light scattering media. A more useful measure of light intensity is the scalar irradiance, E_0, which is an integral measure of light incident from all directions around a certain point in the mat (Table 1).

The significance of scattered light in microbial mats is illustrated in Fig. 7 and Fig. 8. In Fig. 7, spectral measurements of downwelling radiance and scalar irradiance are compared in the same marine microbial mat. The changes in spectral composition of light with depth corresponded well with the observed zonation of phototrophic microorganisms: a sandy surface layer with diatoms (Chl *a* absorption at 440 nm and 675 nm), a dense cyanobacterial layer (Chl *a* and phycocyanin absorption at 620 nm), and a bottom layer of purple sulfur bacteria (BChl *a* absorption at 805 nm and 860-880 nm). Downwelling spectral radiance was attenuated much more strongly with depth than scalar irradiance. At 0.8 mm below the mat surface, downwelling radiance was attenuated to <2-3% of the incident light throughout the spectrum (Fig. 7A), whereas scalar irradiance was only attenuated to 20-60% in the visible part of the spectrum and still exhibited 100% of the incident IR light at 0.8 mm depth (Fig. 7B). Thus, the difference between the attenuation of downwelling radiance and scalar irradiance was an order of magnitude or more.

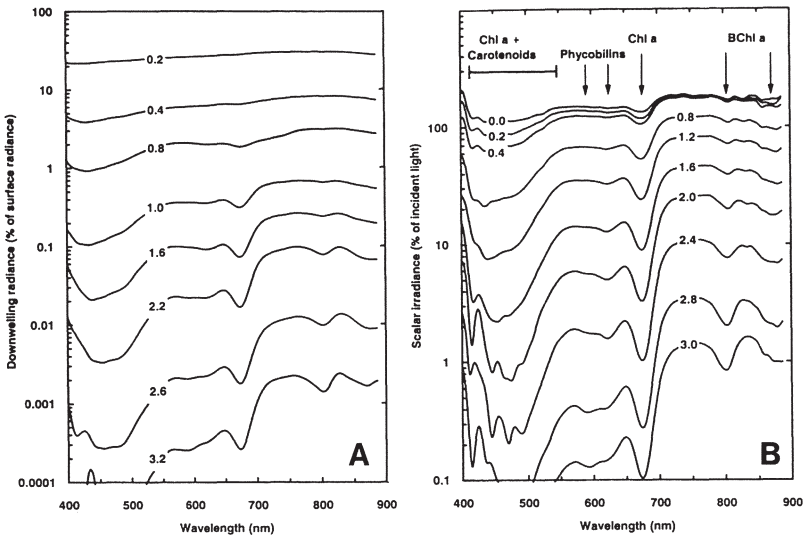


Fig. 7. Spectral light measurements in a marine microbial mat from Kaløvig, Denmark. A. Downwelling radiance. Data are normalized to downwelling radiance at the mat surface. B. Scalar irradiance. Data are normalized to incident downwelling irradiance at the sediment surface. Numbers on curves indicate depth in mm below the mat surface. Arrows indicate absorption wavelengths of major photopigments. (From Kühl and Jørgensen 1992b).

The spectral signals of photopigments were more pronounced in the scalar irradiance spectra than in the radiance spectra due to the spectral contribution of scattered light from sediment layers at some distance from the actual measuring position. The scattered light had been filtered through mat layers and was thus relatively enriched with respect to wavelengths subject to low absorption, thereby enhancing spectral differences in the light field.

A surprising result was the significant alteration of the light intensity and the spectral composition of light near the mat surface as compared to the incident light (Fig. 7B). Intense scattering resulted in a surface or subsurface maximum of scalar irradiance, i. e. of the total quantum flux to each microalgal cell, of up to 200% of the incident light intensity in the IR region of the spectrum. The spectral composition of the surface light was altered due to the spectral signal of backscattered light from deeper mat layers thereby enhancing light intensity at wavelengths with low absorption relative to absorption wavelengths of the photopigments in the mat.

The surface maximum of scalar irradiance was even more pronounced in pure quartz sand with a high scattering to absorption ratio (Fig. 8). Integrated visible (400-700 nm) light reached 200% of incident scalar irradiance at the sediment surface. The light intensity was

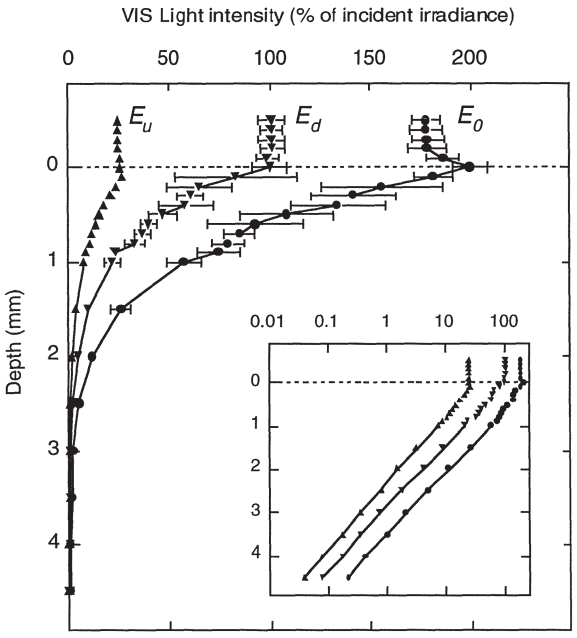


Fig. 8. Depth profiles of upwelling irradiance, E_u, downwelling irradiance, E_d, and scalar irradiance, E_0, in pure quartz sand with 125-250 μm particle size. Data are expressed in % of the downwelling irradiance at the sediment surface. Inset shows log-transformed data. (Data from Kühl et al. submitted).

attenuated exponentially with depth but remained above 100% of incident scalar irradiance throughout the upper 0.0-0.5 mm of the sediment. When comparing scalar irradiance with the irradiance, which is also shown in Fig. 8, it is evident that downwelling irradiance underestimated the total light intensity by 100% throughout the sediment. Although the measurements in pure sand represent an extreme situation with high scattering intensity and little absorption, similar scalar irradiance maxima have been calculated also from measured radiance distributions or have been measured directly in microbial mats and biofilms (e.g., Jørgensen and Des Marais 1988; Lassen et al. 1992b; Kühl 1992; Kühl and Jørgensen 1993) as well as in plant and animal tissue (e.g., Star et al. 1987; Vogelmann et al. 1991). This phenomenon of a near surface maximum of scalar irradiance thus seems to be an inherent property of compact light-scattering media in which multiple scattering is important for the radiative transfer.

Although a build-up of light intensity to a value higher than that incident onto the surface intuitively seems to be a violation of the laws of thermodynamics, the phenomenon can be explained as an effect of scattering and internal reflections at optical boundaries (e.g., Anderson et al. 1989; Seyfried 1989). Light attenuation in microbial mats is due to both absorption and multiple scattering, where scattering enhances the probability of absorption. If absorption is low, however, strong scattering retains a high flux density at a given depth in the mat (i. e., light is travelling a longer distance per vertical distance traversed). This effect of multiple scattering is especially enhanced near optical boundaries, e.g., the mat-water interface or at interfaces between different layers in a mat, where differences in refractive index could result in internal reflection at the boundaries. This results in apparent light trapping phenomena such as the local maximum of light intensity relative to the incident light from above. A more detailed discussion of the optical mechanisms behind the observed scalar irradiance maximum is beyond the scope of this overview and can be found elsewhere (e. g., Anderson et al. 1989; Seyfried 1989; Vogelmann et al. 1991; Kühl and Jørgensen 1993).

Modelling the light field in microbial mats

With the present microsensor techniques it is now possible to obtain detailed data for the optical properties of microbial mats and sediments, which can be used to model the light field in microbenthic communities. Such models must take into account the optical complexity of microbial mats with respect to multiple scattering, refractive index mismatch and the microheterogeneity in mat structure and composition. Models taking these factors into account have recently been developed for studies of light propagation in plant tissues which have an optical complexity similar to microbial mats (e.g., Fukshansky et al. 1993). These models are also to a large extent based on detailed light measurements with fiber-optic probes. A similar approach thus seems very promising for modelling the light field in microbial mats and in other compact phototrophic communities

Light and photosynthesis

The presence of a near-surface zone in microbial mats and sediments, where the light field exhibits a different spectral composition and a significantly higher light intensity has of course important experimental implications for photosynthesis studies in these systems. In studies of photosynthesis versus light intensity (P vs. I curves) or wavelength (action spectra) it is essential to use scalar irradiance as the measure of light intensity in microbial mats and sediments. By the use of scalar irradiance microprobes in combination with oxygen microelectrodes it is now possible to study the photosynthetic performance at high spatial resolution in microbial mats. A typical dataset showing depth profiles of oxygen concentration, photosynthesis, and photon scalar irradiance (400-700 nm) in a coastal microbial mat is presented in Fig. 9. The first studies in microbial mats using this type of data for measuring photosynthetic efficiency and action spectra related to the in situ measured scalar irradiance were recently published (Lassen et al. 1992b, Ploug et al. 1993). More details are presented by Lassen et al. in this volume. The technique has been applied also in other microbial habitats

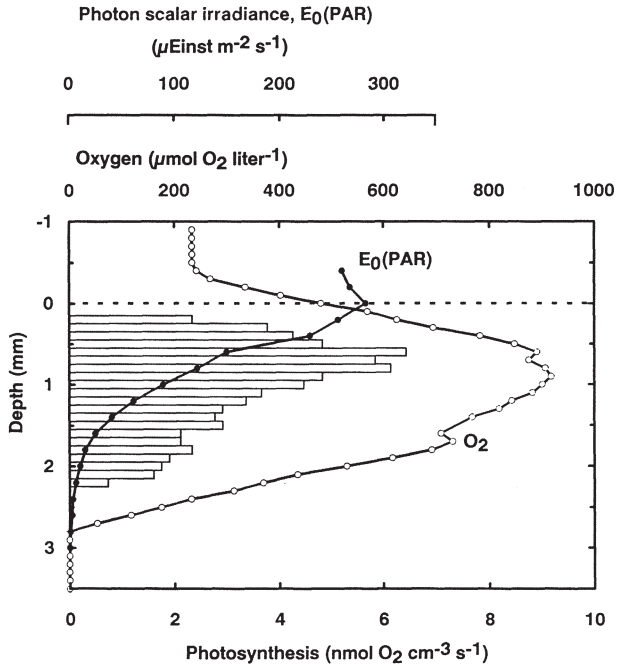


Fig. 9. Depth profiles of oxygen concentration, photosynthesis, and photon scalar irradiance in a marine microbial mat from Kaløvig, Denmark. (Data from Kühl and Jørgensen 1992b)

such as epiphytic communities of red algae and cyanobacteria on Codium macroalgae (Lassen et al. unpublished data) and in preliminary studies of symbionts in the coral tissue and of the endolithic algae in the sceleton of stony corals (Kühl et al. unpublished data).

Conclusion

The present fiber-optic microsensors used in connection with a sensitive detector system now enable studies of the light field in microbial mats at the same high spatial resolution as that with which the chemical microenvironment can be characterised by microelectrodes. From combined radiance, irradiance, and scalar irradiance measurements, the optical properties of mats and sediments can be described. Thus, sediment optics can now be analyzed at a comparable detail as in aquatic optics. The modelling of the light field in microbial mats should take into account, however, the higher optical complexity due to multiple scattering, fluorescence and microheterogeneity through which the optical properties of mats and sediments differ from those of water. Furthermore, the possibility to relate photosynthesis measured by oxygen microelectrodes to the *in situ* light intensity and spectral composition in microbial mats and sediments by concurrent measurements of scalar irradiance now makes it possible to investigate microbenthic photosynthesis at a level previously reached only in plankton research.

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