Remote Sensing of Environment 140 (2014) 196–205

Contents lists available at ScienceDirect

Remote Sensing of Environment

journal homepage: www.elsevier.com/locate/rse

# Spectral-radiometric analysis of taxonomically mixed microphytobenthic biofilms

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ARTICLE INFO

*Article history:*
Received 10 October 2012
Received in revised form 26 August 2013
Accepted 29 August 2013
Available online 23 September 2013

*Keywords:*
Chlorophyta
Cyanobacteria
Diatoms
Microphytobenthos
Pigments
Spectral reflectance signatures
Second derivative

ABSTRACT

Microphytobenthic (MPB) biofilms are taxonomically mixed microbial assemblages (e.g. cyanobacteria, chlorophyta and diatoms) responsible for important estuarine ecosystem functions (e.g. nutrient fluxes, sediment cohesion, and primary productivity). MPB biofilms exhibit high spatial–temporal variation leading to high variability in collected data and requiring high sampling replication. Thus there is an increasing interest in developing remote sensing tools to quantify and describe MPB biofilms. The objectives of the current work were to investigate the effect of MPB taxonomic diversity on biofilm spectral reflectance signatures and to investigate the possibility of using second derivative analysis to estimate MPB chlorophyll *a* (as a proxy for biomass) and pigment composition in monospecific and mixed species biofilms. Two diatoms, *Amphora coffeaeformis* (Agardh) Kützing and *Cylindrotheca closterium* (Ehrenberg) W. Smith; one green algae, *Dunaliella tertiolecta* and one cyanobacteria, *Spirulina platensis* (Gomont) Geitler were used to construct artificial MPB biofilms on glass fiber filters. Biofilms with a range of different MPB concentrations and different taxonomic groups were constructed, their spectral reflectances were measured with a ASD FieldSpec 3FR spectroradiometer and pigments were extracted and measured by HPLC. The best relationships between pigment concentration and second derivative spectra ($\delta\delta$) obtained with monospecific biofilms were: $\delta\delta_{548}$ for fucoxanthin, $\delta\delta_{590}$ for zeaxanthin, $\delta\delta_{650}$ for chlorophyll *b* and $\delta\delta_{675}$ for chlorophyll *a*. The relationship between $\delta\delta_{590}$ and zeaxanthin was probably the result of covariation with phycocyanin or phycoeritrin. $\delta\delta_{590}$ and $\delta\delta_{650}$ were strongly affected by the presence of other taxonomic groups in the mixed species biofilms and underestimated the respective pigments, while $\delta\delta_{548}$ and $\delta\delta_{675}$ could be used to estimate directly fucoxanthin and chlorophyll *a* in taxonomically mixed biofilms. The relationship between $\delta\delta_{675}$ and chlorophyll *a* was strongly affected by the presence of a second derivative double peak caused by chlorophyll fluorescence in the red region and was highly dependent on the smoothing amount applied to the original reflectance spectra. An alternative was proposed using the area of the second derivative peaks between 660 and 690 nm ($A[\delta\delta_{RED}]$). This index showed very good relationships with MPB chlorophyll *a* concentration and was almost insensitive to the amount of smoothing applied to the original spectra.



## 1. Introduction

Microphytobenthic (MPB) biofilms are important components of estuarine intertidal ecosystems (e.g. MacIntyre, Geider, & Miller, 1996), exhibiting high primary production rates (e.g. Underwood & Kromkamp, 1999), representing an import resource for grazers (e.g. Middelburg et al., 2000), being important nutrient fluxes mediators between sediment and air–water interface (e.g. Cabrita & Brotas, 2000), and playing an important role on sediment stabilization (Paterson, 1989). MPB biofilms are frequently dominated by diatoms but it is not uncommon to find taxonomically diverse assemblages, including cyanobacteria, euglenids or chlorophytes (e.g. MacIntyre et al., 1996).

Microphytobenthic biofilms exhibit high spatial–temporal variation on scales ranging from mm to km and from minutes to yearly variations (e.g. Brotas, Cabrita, Portugal, Serôdio, & Catarino, 1995; Guarini et al., 1998; Jesus, Marani, Brotas, & Paterson, 2005; Orvain et al., 2012; Spilmont, Seuront, Meziane, & Welsh, 2011) leading to high variability in collected data and requiring high sampling replication. Chlorophyll *a* (chl *a*) is a ubiquous pigment present in all MPB taxonomic groups, thus MPB biomass is often estimated using sediment chl *a* content as a proxy (e.g. Brotas et al., 1995; Jesus et al., 2009). However, this destructive sampling shows significant problems: 1) it can be costly, because large number of samples are required to capture variability; 2) it does not allow repetitive (i.e. temporal) measurements to be taken in same place; and 3) integrates chlorophyll from deeper sediment layers that

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http://dx.doi.org/10.1016/j.rse.2013.08.040

are not part of the productive active biomass. Thus, there is a growing interest in using non-destructive techniques to estimate MPB biomass such as PAM fluorescence (Honeywill, Paterson, & Hagerthey, 2002; Jesus, Mendes, Brotas, & Paterson, 2006; Serôdio, Marques da Silva, & Catarino, 1997) and spectral reflectance (Barillé, Mouget, Méléder, Rosa, & Jesus, 2011; Carrère, Spilmont, & Davoult, 2004; Combe et al., 2005; Deronde, Kempeneers, & Forster, 2006; Kromkamp et al., 2006; Méléder, Launeau, Barillé, & Rincé, 2003). Spectral reflectance is particularly useful to investigate MPB biomass at the sediment surface, e.g. normalized difference vegetation index (NDVI) and phytobenthos index (PI) (e.g. Forster & Jesus, 2006; Méléder, Barillé, Laneau, Carrère, & Rincé, 2003; Murphy, Underwood, Pinkerton, & Range, 2005), mainly because it does not require previous dark adaption or signal calibration, as in the case of PAM fluorescence (Jesus, Perkins, Mendes, Brotas, & Paterson, 2006). Additionally, it is a fast, non-destructive and relatively cheap technique.

Spectral reflectance has also been used to follow MPB vertical migration (Méléder, Launeau, Barillé, & Rincé, 2003; Perkins et al., 2010; Serôdio, Coelho, Vieira, & Cruz, 2006), to determine photo-physiological mechanisms (Jesus, Mouget, & Perkins, 2008) and, to a minor extent, to qualitatively determine MPB dominant taxonomic groups (e.g. Kazemipour, Launeau, & Méléder, 2012; Paterson et al., 1998; Stephens, Louchard, Reid, & Maffionr, 2003). Discrimination of the different taxonomic groups present in the biofilm is often carried out either by microscopical observation (e.g. Ribeiro, Brotas, Rincé, & Jesus, 2013) or by high performance liquid chromatography (HPLC), which allows the identification of MPB main classes through the quantification of their "signature" pigments, e.g. fucoxanthin for diatoms and chlorophyll *b* for chlorophytes (Brotas & Plante-Cuny, 2003). Although these two last techniques are useful and very accurate, they exhibit the same limitations as referred above for destructive biomass estimation. Thus, there is also a growing interest in developing non-destructive techniques that allow the determination of MPB taxonomic composition in mixed assemblages.

One promising technique to discriminate different taxonomic groups present in MPB biofilms is the second derivative analysis of the reflectance spectra. This technique was successfully used by Jesus et al. (2008) to follow the pigment changes that occur in diatom xanthophyll cycle, i.e. conversion of diadinoxanthin in diatoxanthin when diatoms are exposed to light. The Jesus et al. (2008) index was itself based on a reflectance index used to follow the more complex xanthophyll cycle in terrestrial plants (Peñuelas, Filella, & Gamon, 1995). In the case of the diatoms the xanthophyll cycle caused a very small change in reflectance spectra that was observed as an increase in the second derivative peak at 510 nm and a decrease in the 487 nm region. Although both changes were very small, they were successfully detected by the second derivative analysis, showing that this technique has the capacity to discriminate between small differences in reflectance spectra, resulting from changes in MPB photo-regulation mechanisms. In vivo absorption or reflectance spectra of individual pigments are difficult to characterize due to the overlapping absorbance features of biofilm pigment mixtures and of in vivo pigment maximum absorbance shifts caused by molecules attached to pigments (e.g. pigment–protein complexes)(Méléder, Barillé, Laneau, Carrère, & Rincé, 2003). This is further complicated by the presence of other substances that might affect spectral signatures (e.g. sediment type, water content, detrital organic matter).

The objectives of the current work were to investigate the effect of MPB taxonomic diversity on biofilm spectral reflectance signatures and to investigate the possibility of using second derivative analysis to estimate MPB biomass using chlorophyll *a* as a proxy and pigment composition in monospecific and mixed species biofilms.

## 2. Material and methods

### 2.1. Culture conditions

The diatom *Amphora coffeaeformis* (Agardh) Kützing, was obtained from the Canadian Phycological Culture Centre (CPCC 58); the diatom *Cylindrotheca closterium* (Ehrenberg) W. Smith, from the Nantes Culture Collection (NCC106); the green algae *Dunaliella tertiolecta* Butcher was obtained from the Provasoli-Guillard National Center for Culture of Marine Phytoplankton (CCMP364); and the cyanobacteria *Spirulina platensis* (Gomont) Geitler from Alpha Biotech Company. Two diatoms (A. *coffeaeformis* and *C. closterium*) were selected to account for some of the pigment variability that may exist between different diatom species, which are the MPB dominant group. *Cylindrotheca closterium* and A. *coffeaeformis* are common diatoms in epipelic biofilms (e.g. Archibald & Schoeman, 1984; Ribeiro et al., 2013). Algae were grown in a modified Provasoli medium (ES 1/3) as previously described (Mouget, Tremblin, Morant-Manceau, Morançais, & Robert, 1999) and the cyanobacteria were cultured in Zarrouk (1966). Cultures were grown in a semi-continuous culture mode (volume: 250 ml, temperature: 15 ± 1 °C) in Erlenmeyer flasks (500 mL) illuminated from below (14 h/10 h, light/dark photoperiod, 400 μmol photons $m^{-2}$ $s^{-1}$) by a high intensity discharge lamp (Osram HQI BT, 400 W). Irradiance was measured with a Walz US-SQS 4 waterproof light probe connected to a Li-Cor 189 logger. Temperature was measured using a custom-made digital thermometer with a thermocouple sensor probe.

### 2.2. Artificial biofilms and reflectance measurements

Two sets of artificial biofilms were constructed, one set with monospecific cultures and another set with a mixture of the different cultures with the objective of establishing a mixed species biofilm (Table 1). Monospecific biofilms were constructed by gentle filtration of different volumes (1, 2, 4, 8, 16 and 32 mL) of the monospecific cultures to GF/C Whatman filters (3.6 $cm^2$) to obtain a range of increasing pigment concentration at the filter surface, chlorophyll *a* concentration ranged from 0.6 to 79.5 mg Chl*a* $m^{-2}$. The mixed biofilms were constructed using a fixed volume (10 mL) and varying the relative amounts of the species that composed the biofilm, in order to vary pigment ratios and investigate the effect of this variation on spectral reflectance signatures. Spectral reflectance was then measured with a FieldSpec 3FR (ASD) spectroradiometer.

The ASD FieldSpec 3FR measured radiance (mW $cm^{-2}$ $nm^{-2}$ $sr^{-1}$) between 350 and 1050 nm with a spectral sampling interval of 1.4 nm. Data were collected using the ASD contact probe with its halogen light source. The optical fiber was kept at a constant distance from the target by using the probe outer rubbing distance ring, designed for this effect. Reflectance spectra were calculated by dividing the upwelling spectral

**Table 1**
Composition of the artificial biofilms used in the experiments. Monospecific biofilms were constructed with 100% of the respective species and by filtering different volumes of each culture. Mixed species biofilms were constructed by filtering a fixed culture volume (10 mL) and varying relative species composition.

| Biofilm | Species | Filtered volume (mL) | Percentage |
|---|---|---|---|
| Sp1 | *Cylindrotheca closterium* | 1, 2, 4, 8, 16 & 32 | 100 |
| Sp2 | *Amphora coffeaeformis* | 1, 2, 4, 8, 16 & 32 | 100 |
| Sp3 | *Spirulina platensis* | 1, 2, 4, 8, 16 & 32 | 100 |
| Sp4 | *Dunaliella tertiolecta* | 1, 2, 4, 8, 16 & 32 | 100 |
| Mix1 | Sp1 + Sp3 | 10 | 100 + 0; 80 + 20; 60 + 40; 40 + 60; 20 + 80; 0 + 100 |
| Mix2 | Sp1 + Sp4 | 10 | 100 + 0; 80 + 20; 60 + 40; 40 + 60; 20 + 80; 0 + 100 |
| Mix3 | Sp3 + Sp4 | 10 | 100 + 0; 80 + 20; 60 + 40; 40 + 60; 20 + 80; 0 + 100 |
| Mix4 | Sp1 + Sp3 + Sp4 | 10 | 100 + 0 + 0; 0 + 100 + 0; 0 + 0 + 100; 50 + 25 + 25; 25 + 50 + 25; 25 + 25 + 50 |