A New In Vivo Fluorimetric Technique To Measure Growth of Adhering Phototrophic Microorganisms

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We developed a noninvasive rapid fluorimetric method for the investigation of growth of adhering (benthic) phototrophic microorganisms. The technique is based on the sensitive detection of the in vivo fluorescence of chlorophylls chlorophyll *a* and bacteriochlorophyll *a* and monitors increases in signal over time as an indicator for growth. The growth fluorimeter uses modulated excitation light of blue-light-emitting diodes and a photodiode as the detector. The light-emitting diodes are mounted geometrically in an aluminum housing for efficient and uniform illumination of the bottoms of the growth containers. The fluorimeter was characterized with respect to detection limit and dynamic range. This system is capable of resolving in vivo chlorophyll *a* concentrations of $0.5 \mu\text{g liter}^{-1}$ in cyanobacteria and $0.03 \mu\text{g liter}^{-1}$ in diatoms as well as in vivo bacteriochlorophyll *a* concentrations in phototrophic bacteria of $0.3 \mu\text{g liter}^{-1}$, which points to an extremely high sensitivity compared with that of similar available techniques. Thus, the new fluorimeter allows the determination of growth at extremely low cell densities. The instrument was used successfully to measure the growth of several adhering isolates of the filamentous cyanobacterium *Microcoleus chthonoplastes* from benthic microbial mats in seawater of different salinities. The data obtained demonstrate broad growth responses for all strains, which thus can be characterized as euryhaline organisms.

While planktonic phototrophic microorganisms live permanently immersed and floating in the water column, benthic forms often live adhered to submerged or intertidal solid surfaces, attached to sediments, or as epiphytes on the macrophytobenthos. Although extensive work has been carried out on the physiology and ecology of the phytoplankton, adhering microbial-microalgal communities are difficult to study because they live in an intimate and complex relationship with their substrate and are also heterogeneously distributed in their habitat (16). Nevertheless, these microalgae and cyanobacteria are well known as important primary producers for estuarine and salt marsh food webs (18, 29). Biomass and primary productivity of some cyanobacterial communities may reach values comparable to the productivity of tropical rain forests, one of the most productive ecosystems on earth (25). These microorganisms also play an important role in nutrient cycles and in stabilizing soft sediments as primary colonizers (8, 17, 21). Adhering cyanobacteria and diatoms together with other microorganisms sometimes form complex communities, often described by the term microbial mat (26).

Until now, studies of benthic communities and microbial mats have concentrated primarily on community structure, microbial interactions, primary productivity, and nutrient cycling (26), and only little information is available on the physiological properties of the underlying species.

The growth pattern in response to environmental and/or biotic variables indicates the tolerance and adaptive potential of the organism and consequently represents a very important physiological character. For the determination of growth rates in planktonic cyanobacteria and microalgae, laboratory cultures of a single species are generally used. In these cultures, growth is normally determined from cell counts (division rate per day) or cell density (transmission) or on a cell constituent

basis (chlorophyll *a*, protein, ATP). However, these methods fail to estimate the growth of adhering forms. Only a few procedures to overcome the principal problems in acquiring some basic physiological information on these cyanobacteria and microalgae have been described. Stal and Krumbein (27) isolated and characterized cyanobacteria from marine microbial mats and determined their growth by a nonspecified optical test. Moreover, they did not provide any data on growth rate. A method to estimate growth rates of the terrestrial crust-forming cyanobacterium *Chlorogloeopsis* sp. was recently reported (4). The authors of this work determined growth by time course sacrificial sampling of filter cultures inoculated with a single suspension. Cyanobacterial dry weight was measured gravimetrically on preweighed membrane filters. This, however, is an invasive method that is unsuitable for monitoring growth over time in the same sample.

While in phytoplankton ecology in vivo fluorimetric methods are widely used for monitoring chlorophyll content as a marker for reliable measurement of algal biomass (5, 9, 14), this methodology has been employed in only a few microalgal growth studies (see reference 2 and references therein). Unfortunately, commercial chlorophyll fluorimeters either suffer from low sensitivity or have optical configurations which are not suitable for measuring the fluorescence of microorganisms that live attached to a substratum. In this study, a simple, noninvasive method for the standardized investigation of growth in large sample numbers of adhering phototrophic bacteria and microalgae is presented. With a newly developed and highly sensitive growth fluorimeter, increases in the in vivo chlorophyll fluorescence were recorded over time in cyanobacterial cultures isolated from benthic microbial mats. These measurements provide reliable growth data under specific environmental conditions.

MATERIALS AND METHODS

Cyanobacterial strains and culture. Information on the cyanobacterial strains of *Microcoleus chthonoplastes* used in the present study as well as on cultures of other phototrophic microorganisms, which were utilized for comparative pur-

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TABLE 1. Taxonomic assignments and origin of the phototrophic microorganisms used

Culture collection ^a	Strain	Assignment (in collection)	Habitat
MPI	NDN-1	<i>M. chthonoplastes</i>	Marine, adhering, intertidal; Norderney Island, North Sea, Germany
UBM	HID	<i>M. chthonoplastes</i>	Marine, adhering, brackish sediment; Hiddensee, Baltic Sea, Germany
UBM	WIS	<i>M. chthonoplastes</i>	Marine, adhering, brackish sediment; Poel Island, Baltic Sea, Germany
UBM	Bo79	<i>Synechocystis</i> sp.	Marine, planktonic; Baltic Sea, Germany
SAG	B3192	<i>M. chthonoplastes</i>	Marine, adhering, intertidal; Mellum Island, North Sea, Germany
NIVA	BAC29	<i>C. socialis</i>	Marine, planktonic
DSM	243	<i>E. shaposhnikovii</i>	Marine, adhering-planktonic, hypersaline lakes

^a MPI, Max Planck Institute for Marine Microbiology, Bremen, Germany; UBM, University of Bremen, Marine Microbiology; SAG, Sammlung von Algenkulturen, Göttingen, Germany; NIVA, Culture Collection of Algae at the Norwegian Institute for Water Research, Oslo, Norway; DSM, Deutsche Sammlung von Mikroorganismen und Zellkulturen, Braunschweig, Germany.

poses, such as the cyanobacterium *Synechocystis* sp., the diatom *Chaetoceros socialis*, and the purple bacterium *Ectothiorhodospira shaposhnikovii*, are summarized in Table 1. Isolation and cleaning procedures for *M. chthonoplastes* were done as described in previous publications (20). While strains MPI NDN-1, SAG B3192, and NIVA BAC29 were cultivated in sterile seawater and with PES/2-enriched seawater (32‰ salinity) (28), strains UBM HID, UBM WIS, and UBM Bo79 were grown under estuarine conditions at 15‰ salinity (seawater diluted with distilled water and enriched with PES/2). All strains were maintained as stock cultures in a growth incubator at 25°C, and 25 to 40 μmol of photons $\text{m}^{-2} \text{s}^{-1}$ was provided by cool-white fluorescent tubes under an 18-h light–6-h dark cycle. *E. shaposhnikovii* DSM 243 was cultivated at the Max Planck Institute in Bremen, Germany, as described in the Deutsche Sammlung für Mikroorganismen und Zellkulturen instructions.

Growth experiment. While UBM HID, UBM WIS, and MPI NDN-1 grew mainly as single filaments on the bottoms of the culture vessels, these strains sometimes also formed *Microcoleus*-typical bundles (6). SAG B3192 grew only as filaments. For the growth experiments, the cultures were shaken vigorously to suspend as many filaments as possible in the overlaying medium. From this suspension, 0.5 to 1 ml (about 10^2 to 10^3 filaments per ml) was transferred to disposable fluorimeter petri dishes with cover lids (5-cm diameter; Kleinfeld, Hannover, Germany) that were filled with different experimental media (20 ml). To check possible salt effects, SAG B3192 was incubated in medium of 5‰ salinity, which was prepared by diluting the seawater with distilled water. Growth of MPI NDN-1 was tested under hypersaline conditions at 60‰ salinity. This medium was obtained by adding artificial sea salt to normal seawater (WIMEX; Wiegand Ltd., Krefeld, Germany). While SAG B3192 and MPI NDN-1 were acclimated for at least 8 weeks before the growth experiments were conducted, both strains UBM HID and UBM WIS were investigated directly after hypo- and hypersaline shocks at 5, 32, and 60‰ salinities. All growth and treatment media were enriched with 2 mM NaHCO_3 to ensure sufficient inorganic carbon during the course of the experiment. SAG B3192 and MPI NDN-1 were utilized to compare the commercial Kleinfeld chlorophyll fluorimeter with the newly developed fluorescence method. The latter one was also used to check in more detail the growth characteristics of UBM HID and UBM WIS under salt stress. All experimental treatments were done under the standard temperature and light conditions of the incubator as mentioned above.

The first fluorescence measurement was done after 24 h, a period sufficient for the filaments to settle and attach to the surface of the bottom of the petri dish. Growth of the samples was monitored at intervals of exactly 24 h for 7 to 26 days. Before each measurement, both fluorimeters were calibrated by two-point calibrations. In the case of the Kleinfeld apparatus, a water-filled petri dish as a blank and an orange glass filter as described in the instructions of the company were used. For the new fluorimeter, the same water blank as well as 20 ml of a 10^{-6} M aqueous rhodamine B solution was used as standard calibration. The chlorophyll fluorescence signals were corrected by subtraction of the blank value. The fluorescence was measured after an illumination period of 20 s to avoid the possible influence of induction kinetics. From the fluorescence signals, the growth rate μ (day^{-1}) of exponentially growing strains was calculated by the following equation: $\mu = (\ln F_t - \ln F_o) t^{-1}$, where F_o is the initial fluorescence and F_t is the fluorescence after t days.

To verify the fluorescence data, we measured the growth of SAG B3192 by increases in dry weight. For this experiment, filaments were grown on preweighed membrane filters, and the rise in biomass over time was estimated by the method of Garcia-Pichel et al. (4).

Fluorimeter. Fluorescence measurements were performed with a self-constructed chlorophyll growth fluorimeter (Fig. 1). The basic electronic unit was taken from a Hansatech (Norfolk, United Kingdom) MFMS fluorimeter. Bright-blue-light-emitting diodes (bright blue LEDs) (Nichia, Nürnberg, Germany) with a peak wavelength of 450 nm were selected for excitation of the chlorophyll fluorescence. The LEDs were mounted in aluminum cylinders (12-mm diameter) with blue glass filters (BG 12; Schott, Mainz, Germany). The light of the LEDs was pulsed with a frequency of 870 Hz by the modulation and drive circuits of the Hansatech fluorimeter (for details, see reference 1). The detection unit of this

device, an amplified photodiode, was utilized to measure the chlorophyll fluorescence. Because of the illumination-detection setup that is based on an amplitude modulation technique, the fluorescence signal could be separated from the ambient light. A combination of a longpass glass filter (RG 665; Schott) and

FIG. 1. Schematic drawing of the LED growth fluorimeter. (A) Top view (cross section); (B) front view (cross section); cuv, cuvette, sample-filled petri dish; alh, aluminum housing block; om, optical module (LED with blue glass filter); of, optical filter (longpass filter set); det, detector unit with preamplified photodiode.

a bright-red gelatin filter (Lee, Brussels, Belgium) was used for separation of scattered excitation light from the fluorescence signals. The bacteriochlorophyll *a* fluorescence of phototrophic bacteria was separated with a different longpass glass filter (RG 830; Schott). Since longpass glass filters from Schott characterized by excellent blocking properties suffer from a strong intrinsic fluorescence, which causes a high background signal, a combination of a nonfluorescent red gelatin filter and a longpass glass filter was selected. This resulted in a maximum ratio of chlorophyll fluorescence to background signal. Nevertheless, a water blank also causes a small background signal as a result of the intrinsic fluorescence of the plastic material of the petri dish, which must be subtracted from the fluorescence signal.

A compact aluminum housing block was designed for placement of the disposable petri dishes. Four of the described LED cylinders were centrally mounted at an angle of 90° around the placement of the petri dishes (Fig. 1). This optical configuration was found to be most efficient for excitation of the *in vivo* chlorophyll fluorescence of the benthic microorganisms. The photodetector was placed at a distance of 2 cm below the petri dish bottom.

Comparative fluorescence measurements were made with a standard impulse chlorophyll fluorimeter (Kleinfeld). This instrument uses a xenon flash lamp equipped with a 435-nm interference filter for blue excitation of the sample cuvettes and a photodiode as the detector.

Chlorophyll determination. Chlorophyll *a* and bacteriochlorophyll *a* were determined by collecting cells of the appropriate species on Whatman GF/F glass microfiber filters. For chlorophyll quantification, filters were homogenized with a mortar and pestle in 100% acetone p.a. Following extraction, the homogenates were centrifuged at $10,000 \times g$ for 5 min. Supernatants were made up to 3 to 5 ml with acetone. Chlorophyll *a* was quantified spectrophotometrically by the use of previously published equations (10). For the bacteriochlorophyll determination, the filters were homogenized with 100% methanol p.a. and then centrifuged at $10,000 \times g$ for 5 min. Supernatants were made up to 4 ml with methanol, and the bacteriochlorophyll content was quantified spectrophotometrically at 770 nm with a molar extinction coefficient, E_N , of $60 \text{ (mM}^{-1} \text{ cm}^{-1})$.

RESULTS

Characterization of the measurement system. With the new growth fluorimeter, the detection limit and the dynamic range were tested for *in vivo* chlorophyll *a* determination for one unicellular planktonic cyanobacterium and a planktonic diatom; the same was done for *in vivo* bacteriochlorophyll *a* quantification for a phototrophic bacterium. Since adhering cyanobacteria and diatoms often form aggregates in culture, it was impossible to make reproducible dilution series with these forms. Therefore, planktonic organisms were chosen as representatives. The appropriate calibration curves are shown in Fig. 2. For the cyanobacterium *Synechocystis* sp., a linear relation of the calibration curve was measured for *in vivo* chlorophyll *a* concentrations of 0.5 to $108.5 \mu\text{g liter}^{-1}$. Deviations from linearity were found at chlorophyll *a* concentrations higher than $200 \mu\text{g liter}^{-1}$ because of an increase in cell density which results in higher turbidity and therefore attenuation of the excitation light.

For the diatom *C. socialis*, an extremely high sensitivity for *in vivo* chlorophyll determinations was found. The lower detection limit for chlorophyll *a* was $0.03 \mu\text{g liter}^{-1}$. The calibration curve was linear over the whole range of chlorophyll concentrations tested, i.e., 0.03 to $30 \mu\text{g liter}^{-1}$. The lower detection limit for the *in vivo* bacteriochlorophyll *a* concentration in *E. shaposhnikovii* was $0.3 \mu\text{g liter}^{-1}$, and the calibration curve was linear over the whole range of concentrations between 0.3 and $301 \mu\text{g liter}^{-1}$.

We also checked a pure chlorophyll *a* standard (Sigma). A linear relationship of the calibration curve was found for chlorophyll *a* concentrations in 100% acetone p.a. from 0.02 to $200 \mu\text{g liter}^{-1}$ (Fig. 3). For this experiment, glass petri dishes were used.

Growth rate. In the case of the tested adhering cyanobacteria, the *in vivo* chlorophyll fluorescence signals at each time point remained stable from the beginning, i.e., no chlorophyll induction kinetics were observed. The increases in fluorescence over time as a measure for growth in strain SAG B3192 incubated at 5‰ salinity and in MPI NDN-1 treated at 60‰

FIG. 2. Calibration curves for *in vivo* chlorophyll *a* in the unicellular cyanobacterium *Synechocystis* sp. and the diatom *C. socialis* as well as *in vivo* bacteriochlorophyll *a* in the phototrophic bacterium *E. shaposhnikovii* [relative fluorescence (absorbance units) versus (bacterio)chlorophyll *a* concentration (micrograms per liter)].

are shown in Fig. 4 and 6, respectively. The inset in Fig. 4 gives a higher resolution of the initial fluorescence signals. For both strains, the growth fluorimeter was compared with the commercial one. In SAG B3192, a small but significant rise in the fluorescence signal was recorded as early as day 2 with the growth fluorimeter; this small rise was followed by an exponential increase of the growth rate until the end of the experiment (day 6). Because of fast growth of this strain, the bottoms of the petri dishes were completely covered with filaments within 6 days. After this period, depletion of nutrients and self-shading occurred, and therefore the experiment

FIG. 3. Calibration curve for chlorophyll *a* dissolved in acetone (relative fluorescence [absorbance units] versus chlorophyll *a* concentration [micrograms per liter]).

FIG. 4. Increase of the in vivo chlorophyll fluorescence signals of the cyanobacterial strain SAG B3192 over time as an indicator for growth. The inset shows the data for the initial 3 days. SAG B3192 was grown at 5‰ salinity, and the data are given as mean values $\pm$ standard deviations ($n = 4$ to 5). a.u., absorbance units.

was finished. Comparative measurements with the Kleinfeld fluorimeter showed no significant growth during the first days since the signals were below the detection limit (Fig. 4). This experiment demonstrates that the new growth fluorimeter can obtain reliable data with a much smaller biomass than the Kleinfeld fluorimeter can. From the exponential part of the growth curve, a growth rate (μ) for SAG B3192 of 1.1 day^{-1} (standard deviation, less than 10%) was calculated.

The control experiment for SAG B3192 as measured by increases in dry weight gave a growth curve very similar to that obtained with the growth fluorimeter (Fig. 5). However, because of the larger amounts of biomass needed for this assay and the subsequent self-shading effects on the filters, the experiment was already finished after 5 days. The growth rate (μ) of 1.0 day^{-1} was calculated from this experiment, which confirms the fluorescence measurements to about 91% accuracy.

For MPI NDN-1 only small and continuous increases in fluorescence from days 1 to 8 could be recorded with the growth fluorimeter (Fig. 6), and consequently, it was possible to calculate growth rates. However, the data obtained from the Kleinfeld fluorimeter suggest that there was no significant growth of cyanobacteria because the biomass of these samples was too small to be measurable with this instrument. Visual observations of the petri dishes under a dissecting microscope indeed confirmed a significant increase in filament numbers. Compared with the growth rate of strain SAG B3192, the calculated growth rate (μ) of MPI NDN-1 was very low, i.e., 0.22 day^{-1} .

The influence of hypo- and hypersaline treatments on the growth rate of UBM HID and UBM WIS is given in Fig. 7 and 8. Both strains showed very similar growth response patterns, i.e., nearly no increase in biomass within the first week. However, after that treatment period at the lowest salinity for both strains, exponential growth was observed until days 12 to 14; this was followed by a stationary phase and a constant decrease in the fluorescence signal. Filaments incubated at 32‰ salinity also showed the first indication of growth after 8 days of treat-

FIG. 5. Control experiment to verify the growth rate of the cyanobacterial strain SAG B3192 obtained with the fluorimeter. Filaments were loaded on top of preweighed membrane filters, and increases in dry weight were measured over time. The data are given as mean values $\pm$ standard deviations ($n = 4$ to 5).

ment but at a significantly lower rate and a lower maximum fluorescence signal. As at 5‰ salinity, UBM HID and UBM WIS reached a biomass peak after 12 to 14 days at 32‰ salinity, which was followed by a constant decrease in the fluorescence signal (Fig. 7 and 8). In contrast to the hyposaline- and mild hypersaline-stressed filaments, cells subjected to the highest salinity treatment (60‰) exhibited a long lag phase of over 20 days before the first indication of growth could be measured. However, after this long time span, a constant increase in biomass was recorded until the end of the experiment. The effect of salinity on the appropriate specific growth rates of UBM HID and UBM WIS is summarized in

FIG. 6. In vivo chlorophyll fluorescence of the cyanobacterium *M. chthonoplastes* MPI NDN-1 over time as an indicator for growth. This isolate was grown at 60‰ salinity, and the data are given as mean values $\pm$ standard deviations ($n = 4$ to 5). a.u., absorbance units.

FIG. 7. In vivo chlorophyll fluorescence of the cyanobacterium *M. chthonoplastes* UBM HID over time as an indicator for growth. This isolate was subjected to hypo- and hypersaline treatments at 5, 32, and 60‰ salinities. The data are given as mean values $\pm$ standard deviations ($n = 4$ to 5). a.u., absorbance units.

FIG. 9. Effect of salinity on the specific growth rates ($\mu \text{ day}^{-1}$) of *M. chthonoplastes* UBM HID and UBM WIS. The data are given as mean values $\pm$ standard deviations ($n = 4$ to 5).

Fig. 9. Both isolates exhibited quite different growth responses under the range of salt treatments tested. UBM HID showed an optimum growth rate (μ) of 0.45 day^{-1} at 5‰ salinity. An increase in salinity from 5 to 32 and 60‰ was accompanied by a steady decline of the growth rate to 0.24 day^{-1} in the medium with the highest osmotic strength. In contrast to UBM HID, UBM WIS exhibited a maximum growth rate (μ) of 0.44 day^{-1} at 32‰ salinity. Both a decrease and a rise in salinity yield lower growth rates. However, the decline at 60‰ salinity to a μ of 0.18 day^{-1} was much more pronounced compared with the drop to a μ of 0.32 day^{-1} at 5‰ salinity (Fig. 9).

FIG. 8. In vivo chlorophyll fluorescence of the cyanobacterium *M. chthonoplastes* UBM WIS over time as an indicator for growth. This isolate was subjected to hypo- and hypersaline treatments at 5, 32, and 60‰ salinities. The data are given as mean values $\pm$ standard deviations ($n = 4$ to 5). a.u., absorbance units.

DISCUSSION

Fluorimeter. A special in vivo chlorophyll fluorimeter that fulfills the following requirements was developed: uses LEDs as light sources; monitors in vivo growth of adhering (benthic) phototrophic microorganisms in disposable petri dishes; is universally suitable for different groups of adhering phototrophic microorganisms (cyanobacteria, phototrophic bacteria, and diatoms); and detects in vivo chlorophyll fluorescence at extremely low biomass concentrations ($<0.5 \mu\text{g liter}^{-1}$).

The newly developed chlorophyll fluorimeter is small and compact. The LED modules and the emission filters can be exchanged quickly, and therefore the sensitivity and selectivity can be optimized for specific groups of phototrophic microorganisms and microalgae. Blue LEDs have often been preferred as light sources in optical spectroscopy and optical sensing because of their favorable properties, such as the emission of almost monochromatic light, the absence of heat production, and the ability to be modulated electronically (11). Red and yellow LEDs were also successfully used as light sources for a pulse-modulated chlorophyll fluorimeter for fluorescence quenching analysis and for solving other questions related to photosynthesis (see references 3, 12, and 23 and references therein). The growth fluorimeter described in the present report was equipped with newly developed highly intensive blue LEDs as light sources. The fluorescence of diatoms can be most efficiently excited with blue light because of the optimum absorption by chlorophyll *a* and carotenoids in this region. The fluorescence of phototrophic bacteria containing bacteriochlorophyll *a* as the main pigment together with carotenoids can also be excited with light in the wavelength range between 400 and 500 nm, but this wavelength excites only carotenoids. In contrast to the chlorophyll fluorescence of these organisms, the chlorophyll fluorescence of cyanobacteria will be most efficiently excited with yellow or orange LEDs because of the presence of the phycobilin pigments absorbing light in this range of wavelengths (24). However, our data clearly demonstrate that blue excitation of cyanobacteria is also possible but with some loss in sensitivity. Nevertheless, blue LEDs were selected as a generally useful light source for in vivo chloro-

phyll excitation of all adhering phototrophic microorganisms tested.

Although the new growth fluorimeter is equipped only with LEDs as light sources and a photodiode as the detector, it is characterized by an excellent sensitivity. The detection limit for chlorophyll *a* in diatoms of $0.03 \mu\text{g liter}^{-1}$ is lower than those of most of the *in vivo* fluorescence methods reported in the literature. Recently, a sensitive optical fiber fluorosensor for phytoplankton biomass determinations that is capable of resolving *in vivo* chlorophyll concentrations of $0.1 \mu\text{g liter}^{-1}$ has been described (15). These authors used a 250-W xenon lamp as the light source. An *in vivo* fluorimetric method using a standard spectrofluorimeter for selective determination of the biomass of cyanobacteria in mixed phytoplankton communities, with a detection limit for chlorophyll *a* of $1 \mu\text{g liter}^{-1}$, has been reported (13). Another highly sensitive chlorophyll fluorimeter that is based on a pulsed xenon lamp has been described (22). This fluorimeter has a detection limit of $0.020 \mu\text{g liter}^{-1}$ for active chlorophyll *a* which has been proven for a suspension of chloroplasts. However, a detection limit for algal suspensions was not reported (22).

There are three main reasons for the high sensitivity of the new growth fluorimeter: (i) the optical configuration enabling a very efficient illumination of the benthic microorganisms, (ii) the high light output of the blue LEDs, and (iii) the use of longpass filters instead of bandpass filters to detect the whole fluorescence.

The principle of the growth fluorimeter is based on measurements of relative increases in chlorophyll fluorescence signals over time as an indicator for growth. This methodology was found to be fully sufficient for precise determination of the growth rate. We are aware that many environmental and biotic factors, such as nutrient status, the previous light history, the physiological state, the presence of senescent cells, etc., may influence the chlorophyll concentration of the cells and consequently the fluorescence yield. However, in the present study, we controlled most environmental and biotic conditions by using a growth incubator, enriched media, and inocula with extremely low cell densities. Moreover, for SAG B3192, we determined growth by estimation of dry weight increases over time (Fig. 5). This control experiment confirmed our growth rates obtained from fluorescence measurements to approximately 91% accuracy. However, there is one problem with the growth fluorimeter. After reaching the stationary phase, strains UBM HID and UBM WIS showed a constant decrease in the fluorescence signal (Fig. 7 and 8), which, however, was not reflected by any decline in biomass (visual observation under a stereomicroscope). It seems that under these specific physiological conditions, the fluorescing chlorophyll and phycobilin pigments are preferentially degraded, and consequently, their fluorescence cannot be used as a criterion for growth.

There are only a few reports on the determination of phytoplankton reproduction rates by monitoring the increases in *in vivo* chlorophyll fluorescence (2). The authors demonstrated with control experiments that the exponential increase of *in vivo* fluorescence accurately reflects the exponential increase in cell density once the cultures have achieved acclimation under constant conditions. These data are in good agreement with our results, and we therefore conclude that the new *in vivo* growth fluorimeter represents an ideal tool to investigate growth of adhering phototrophic microorganisms on a very small scale and under undisturbed conditions for at least several days. In addition, the present method allows very time-efficient parallel measurements of large numbers of samples. In summary, all advantages of the new chlorophyll fluorimeter demonstrate a high potential for many other biological appli-

cations, for example, ecological studies of the competition between different types of phototrophic bacteria within a microbial mat.

Growth. In contrast to the growth of planktonic cyanobacteria, the growth of adhering forms is only rarely investigated. For the unicellular *Chlorogloeopsis* sp. growing as aggregates in crusts on concrete pavement, growth rates (μ) of 0.35 to 0.48 day^{-1} have been reported (4). Filamentous *Oscillatoria* species living as epiphytes on the pelagic brown alga *Sargassum* sp. exhibit very low growth rates, between 0.01 and 0.048 day^{-1} , only (19). The three strains of *M. chthonoplastes* tested in this study showed growth rates in the range of 0.22 to 0.45 day^{-1} . SAG B3192, which most probably belongs to the genus *Oscillatoria*, exhibits a much higher growth rate (μ), i.e., 1.0 to 1.1 day^{-1} . We therefore conclude that the filamentous cyanobacteria from benthic microbial communities investigated with the newly developed chlorophyll fluorimeter exhibit growth rates which are relatively high and even comparable to those of planktonic forms such as *Aphanizomenon* and *Oscillatoria* spp. ($\mu = 0.4$ to 0.6 day^{-1} [7]). However, it must be taken into account that the maximum growth rates presented in this and many other studies were obtained under optimum and controlled conditions in the laboratory, which differ considerably from those in the field. Cyanobacteria in microbial mats compete with other photosynthetic microorganisms in an environment stressed by limitations in water supply or nutrients, i.e., they encounter conditions permitting optimum growth only rarely. In summary, the new fluorimetric method and the growth data presented in this study call for further and more detailed investigations on the growth physiology of adhering diatoms, cyanobacteria, and phototrophic microorganisms.

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