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A flow-lane incubator for studying freshwater and marine phototrophic biofilms

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Abstract

Phototrophic biofilms are defined as interfacial microbial communities mainly driven by light as energy source and are studied for both ecological and technological reasons. Field investigations of biofilms usually do not offer the opportunity to study the effects of a large number of external parameters. In order to investigate the temporal development of phototrophic communities a laboratory flow-lane incubator for cultivation of freshwater and marine biofilms was developed. The incubator has four lanes which accommodate microscope slides used as substratum and for sampling. The slides can be of different material and may be employed for characterisation of phototrophic biofilms by means of gravimetry, microscopy, taxonomy, molecular biology and chemical analysis. The design allows control of irradiance, temperature and flow velocity. Furthermore, on-line control of biomass accumulation via specially adapted light sensors was proved to be a suitable indicator of temporal developmental stages (initial adhesion, active growth and mature stage). Spatial heterogeneity of the cultivated phototrophic biofilms along the flow direction within each flow-lane was low. Biofilm growth characteristics (e. g. lag time, net accrual rate, peak biomass) recorded in dependency from external conditions may be used as input data for training of artificial neural networks (ANN) and mechanistic modelling. The material and devices used in combination with low maintenance costs and ease of handling suggests the flow-lane incubator as a useful tool for studying the influence of abiotic and biotic factors on the development of freshwater and marine phototrophic biofilms.

Keywords: biofilms; flow-lane incubator; on-line biomass monitoring; photobioreactor; phototrophs

1. Introduction

Environmental phototrophic biofilms consist of complex associations of microorganisms (bacteria, fungi, cyanobacteria and algae). Fixed by a matrix of extracellular polymeric substances (EPS) secreted by the cells these microorganisms have immobilized themselves onto surfaces (substrata). By their organization in biofilms, the organisms create their own microhabitats with pronounced gradients of biological and chemical parameters, along which they can use substrates and energy effectively (Meyer-Reil, 1994). Phototrophic biofilms are studied for both ecological and technological reasons. Benthic and periphytic phototrophs, including unicellular, colonial (e.g., diatoms) or multicellular specimens (e.g., filamentous green algae) and more importantly cyanobacteria are the most successful photosynthetic organisms to exploit streams as habitats. They are ecologically important in primary production, nutrient transformation, sediment stabilization and habitat provision for other benthic organisms (Sigee, 2005; Van Dam et al., 2002). It is now well accepted that microorganisms, through their production of EPS, are at least partially responsible for the stabilization of sediments in marine and brackish near-shore environments (Wigglesworth-Cooksey and Cooksey, 2005; Meyer-Reil and Köster, 2000) as well as in lotic systems (Battin and Sengschmitt, 1999). However they can also proliferate in nutrient-enriched, stable-flowing streams, causing

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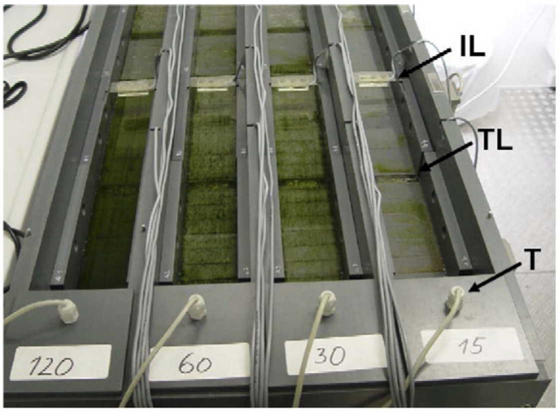

Fig. 1. Cultivation of phototrophic biofilms within four separate flow lanes of the incubator. Incident light sensors (IL) were positioned 5 cm above the substratum. Light sensors for measuring transmitted light (TL) below the substratum were inserted at 3 positions within each flow lane. Temperature sensors (T) were positioned directly within inflow device of each flow lane.

water management problems. Furthermore, streams are particularly liable to variations in water quality, because of their hydrology and the significance of terrestrial inputs. Benthic algae show a rapid response to such changes, and are potentially useful in monitoring pollution effects in lotic water systems (Biggs, 1996).

Phototrophic organisms like microalgae have an interesting and yet not fully realized potential for biotechnological exploitation (Csögör et al., 2001; Pulz and Gross, 2004). They produce natural dyes (pigments), polyunsaturated fatty acids, polysaccharides and vitamins (Cannell, 1990; Vilchez et al., 1997) as well as substances for pharmaceutical interest (Faulkner, 2000). Other applications are waste water treatment and hydrogen production (Craggs et al., 1996, Matsunaga et al., 1999; Miyake et al., 1999, Schumacher and Sekoulov, 2002). Microalgae have a high affinity for polyvalent metals due to biosorption processes. For that reason they could be used to reduce the concentration of heavy metals present in water and wastewater (Travieso et al., 2002). This is a concept for microalgae immobilization using an intensive cultivation system for an ecologically sustainable heavy metal removal process without the dangers imposed on the environment as it happens in the traditional physical–chemical purification process. Furthermore, biofilm reactors have become a focus of interest for researchers in the field of bioremediation of xenobiotic compounds and hydrocarbons (Lazarova and Manem, 2000; Wolfaardt and Korber, 2000). For example, in a study of oil-degrading biofilms, filamentous cyanobacteria function as pioneer colonizers in artificial biofilms and support hydrocarbon utilizing bacteria, namely *Acinetobacter calcoaceticus* and nocardioforms (Al-Awadhi et al., 2003).

The activities of marine biofilms were also studied from the point of view of biofouling i.e. the loss of efficiency of man-made devices and constructions in the aquatic environment because of accumulation of organisms and their products at interfaces (Characklis and Cooksey, 1983). Studies of fouling bacteria dominate the literature (Alberte et al., 1992; Holmstrom and Kjelleberg, 2000), but also algal involvement in the fouling process has been discussed recently (Callow, 2000; Cooksey and Wigglesworth-Cooksey, 2001). The major consequences of algal fouling in the marine habitat are increase of hydrodynamic drag and corrosion of metals (Callow and Edyvean, 1990). Control of algal fouling has not been completely successful, even with tributhyl tin coatings (Callow, 2000). New coatings depend on the effects of hydrodynamic shear over a ship's hull in order to remove organisms from the low-surface-energy polymers which are part of the coating systems. Successful assessment of candidate coating polymers requires laboratory-based assays that mimic conditions found in the field (Callow, 2000).

Irradiance, temperature, flow and nutritional status are environmental key factors in phototrophic biofilm development, in terms of metabolic activity, spatial organisation and growth (photosynthesis, respiration and EPS production). Apart from carbon dioxide, photosynthetically active radiation (PAR) is essential for photosynthesis in order to store energy (ATP) and reductive capacity (NADPH) for primary production. Especially the light regime under which the phototrophic cells are grown influences radically both the synthesis and the accumulation of EPS (de Brouwer et al., 2002; Perkins et al., 2001). Considering that it is almost impossible to study these phenomena in detail in

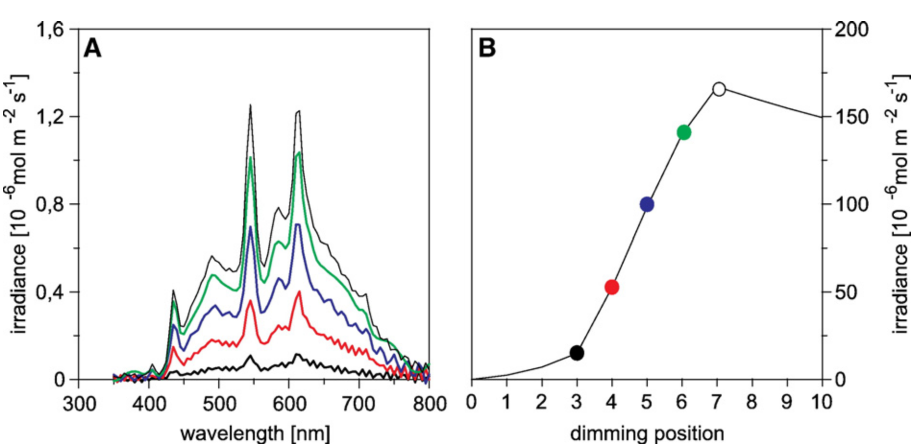

Fig. 2. Light characteristics of Truelight lamps (Auralight, Sweden). (A) spectral distribution and (B) irradiance at different dimming positions.