Available online at www.sciencedirect.com
ScienceDirect
Journal of Microbiological Methods xx (2007) xxx–xxx
Journal of Microbiological Methods
www.elsevier.com/locate/jmicmeth

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

Barbara Zippel [a,*], Jan Rijstenbil [b,1], Thomas R. Neu [a]

[a] Department of River Ecology, Helmholtz Centre for Environmental Research-UFZ, Brueckstrasse 3a, D-39114 Magdeburg, Germany
[b] Department of Marine Microbiology, NIOO-KNAW, PO Box 140, NL-4400AC Yerseke, The Netherlands

Received 23 February 2007; received in revised form 11 May 2007; accepted 12 May 2007

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

* Corresponding author. Tel.: +49 391 810 9452; fax: +49 341 235 459452.
E-mail address: barbara.zippel@ufz.de (B. Zippel).
[1] Present address: AE3 Consultancy, Fuchsialaan 8, NL-4401HV Yerseke.

0167-7012/$ - see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.mimet.2007.05.013

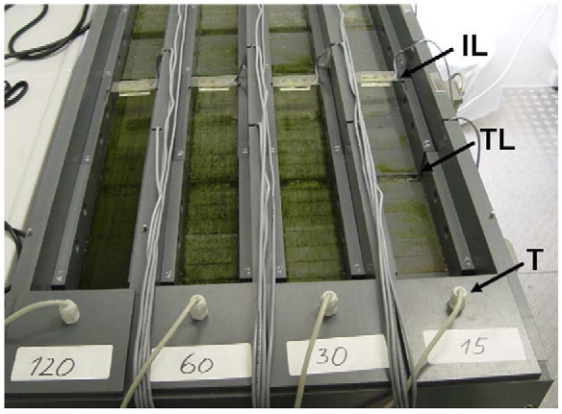

Fig. 1. Cultivation of phototrophic biofilms within four separate flow lanes of the incubator. Incident light sensors (IL) were positioned 5 cm above the substratum. Light sensors for measuring transmitted light (TL) below the substratum were inserted at 3 positions within each flow lane. Temperature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

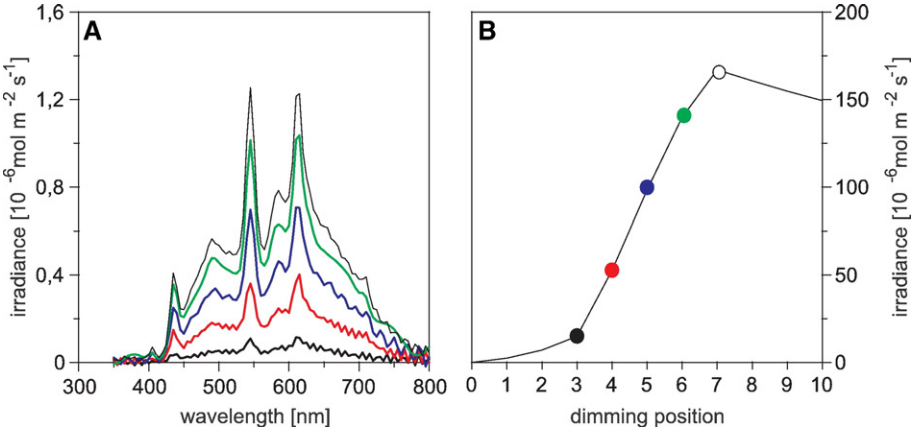

Fig. 2. Light characteristics of Truelight lamps (Auralight, Sweden). (A) spectral distribution and (B) irradiance at different dimming positions.

Please cite this article as: Zippel, B. et al. A flow-lane incubator for studying freshwater and marine phototrophic biofilms. J. Micro. Biol. Methods (2007), doi:10.1016/j.mimet.2007.05.013

the natural environment, the best way is to perform experiments in a laboratory incubator (Godillot et al., 2001). Furthermore, there is a great demand to develop a model which predicts the behaviour of the whole system (Csőgör et al., 2001).

From a mechanistic point of view it is necessary to understand how single factors of abiotic conditions influence the development of phototrophic biofilms, and which reaction can be observed if combined factors contribute to the development of biofilms. Another important issue for analysis of development and maturation of biofilms as well as for modelling purposes is the knowledge of kinetic parameters such as different developmental stages (initial adhesion, active growth phase and matured stage). In case of slow-growing phototrophic biofilms the active growth phase can last for 2–4 weeks, whereas fast-growing biofilms can reach mature stage after 2 weeks. It could be shown that for instance the ratios (EPS: Chl a) decreased with increasing age of attached consortia independent of temperature (Wolfstein and Stal, 2002).

The purpose of this study was to design a laboratory incubator for the development and investigation of environmental phototrophic biofilms from different aquatic habitats. Main focus for external cultivation conditions was on adjustment and control of irradiance, temperature as well as flow velocity of the circulating medium during whole duration of the experiments. Biomass accumulation at the illuminated substrata has to be as homogeneous as possible. Furthermore, the phototrophic biofilms have to be grown in such a way that the sampling procedure must allow in situ analysis of undisturbed biofilms (Barranguet et al., 2004a), like microsensor technique (Kühl, 2005) and laser scanning microscopy (Neu et al., 2004). Finally the differentiation of developmental stages of the growing biofilms should be possible during the whole cultivation period. The basic design used the concept of a flat reactor where the 'contact rate' between illuminated substratum (and the biofilm on it) and medium would be as intense as possible; this includes the fast recirculation of a thin liquid layer over the substratum. An additional advantage of such a setup is that there will be a negligible competition between periphyton (biofilm) and planktonic phototrophs. It was also expected that larger biofilm grazers (specialized on microalgae) will not settle easily in such an environment with a thin, fast-running liquid film.

2. Materials and methods

2.1. Flow-lane incubator

The incubator consisted of 4 separate flow lanes (length 1200 mm, width 100 mm) with integrated inlet and outlet devices at the begin and end of each lane (Figs. 1 and 3). Different substrata for biofilm attachment can be inserted into the flow lanes. In the PHOBIA (http://phobia.itqb.unl.pt) experiments 43 polycarbonate slides (76×25×1 mm) were inserted into each lane to produce a homogeneous surface for adhesion of phototrophic biofilms. Slide fixation in each lane was possible due to 12 plastic bars which were positioned along the walls of each flow lane. With this setup sampling of separate slides in different areas of the flow lane was practicable. Lamp boxes were positioned on top of each lane with a distance of 15 cm between lamp source and bottom of the flow lane. Effective maximum illuminated sampling area per lane was 840 cm^2. The incubator was used in horizontal position.

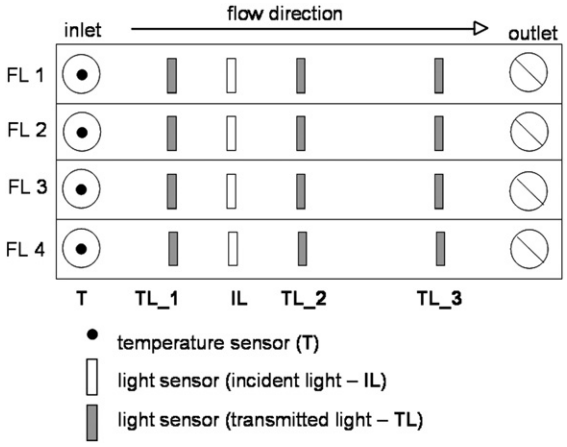

Fig. 3. Positions of process control sensors within the flow-lanes (FL) of the incubator.

2.2. Circulation system

The circulation of medium within each separate lane was maintained by a submersible aquarium pump (UP 500, aquabee, Germany) placed inside a medium beaker. The medium beakers were placed directly below the inlet devices to avoid reduction of medium flow rate due to pump characteristics. Flow meters (3–25 or 25–250 L h^{-1}, ASV Stübbe, Germany) were integrated between pump and inlet device to measure and regulate the flow rate of the circulating medium. Flow rates of 25 and 100 L h^{-1} were used in the PHOBIA experiments and produced medium flow velocities of 0.05 and 0.15 m s^{-1} above the substratum surface. Homogeneous flow conditions in the lane were created by a small turbulence reducer approximately 3 cm below fluid level in the inlet device and a little weir (height 4 mm) close to the inserted slides. The free fall outlet device at the end of each lane guaranteed circulation of the medium without backwater problems. A thermo-sensor (SMT 160-30, smartec, The Netherlands) was fixed to the turbulence reducer to measure the temperature of the circulating medium continuously during the experiments.

2.3. Illumination system

Fluorescent lamps (TrueLight 36 W, auralight, Sweden) were used as light source during experiments. Spectral light distribution in the PAR region (400–700 nm) was comparable with natural sunlight (see original spectral graphs of Auralight and Fig. 2A) but to avoid disadvantageous UV effects of the lamps, a cut-off filter (Uvilex 360Z, Schott, Germany) was used. Irradiances of 15, 30, 60 and 120×10^{-6} mol m^{-2} s^{-1} were adjusted with a potentiometer which was coupled with a digital signal switcher and a logarithmic dimming device (DSI-

Please cite this article as: Zippel, B. et al. A flow-lane incubator for studying freshwater and marine phototrophic biofilms. J. Micro. Biol. Methods (2007), doi:10.1016/j.mimet.2007.05.013

A/D, Tridonic.Atco, Austria) of the fluorescent lamps. Reflector sheets at both ends of the lamp box were integrated to guarantee a homogeneous illumination above all inserted slides per lane. Experiments were run with a 16/8 h light/dark cycle.

2.4. Light sensors — process control and biomass monitoring

Four light sensors were integrated per lane for the control of incident light (IL) during illuminating interval and for monitoring biomass accumulation during the temporal development of the biofilms (transmitted light = TL). For adjustment and control of incident light, a sensor (IL in Fig. 3) was positioned 5 cm above the slide surface and fixed at 2 opposite slide fixation bars (see Fig. 1). The other 3 light sensors were inserted at 3 positions along the flow direction of each lane (Fig. 3; 20 cm after inlet, middle of the lane and 20 cm before outlet).

Each light sensor was equipped with 3 photodiodes (BPW 3411, photoeffective area 2.7 mm^2; Siemens, Germany), which were embedded in a small polystyrene cuvette with epoxy resin. An original polycarbonate slide was fixed at the cuvette surface with small drops of transparent hot glue (Pattex, Henkel; Germany). Calibration of the light sensors was done with a planar Licor sensor (SA 12456, Licor, USA) in an adapted measuring lane with the same dimensions as in the incubator. The output of each photodiode was taken at 10 different dimming positions within the used irradiance range ($10–150 \times 10^{-6}$ mol m^{-2} s^{-1}, Fig. 2B).

At the beginning of an experiment the incident light intensity was adjusted within each flow lane via the control sensor (IL). Thereafter the readings of the inserted transmission light (TL) sensors were set to 100%. Biomass accumulation during temporal development of the growing biofilms caused a decrease of irradiance below the substratum, which was recorded with the inserted light sensors in 10-min intervals. These data were converted by a microcontroller (ATmega8, ATmel, USA) into proportional values and monitored the biomass accumulation at 3 positions along the flow direction within each lane. By this way, growth curves were obtained and allowed the definition of different developmental stages of the growing biofilms. It was differentiated between initial settlement (90% TL), active growth phase (90–10% TL) and mature stage (<10% TL) (Fig. 5).

2.5. Growth conditions and inoculum

The mineral medium used for the cultivation of freshwater phototrophic biofilms was a modification of BG11 as described by Stanier et al. (1971). Ammonium ferric citrate green was replaced by $FeCl_3$. The vitamins cyanocobalamin (40 μg L^{-1}), thiamine HCl (40 μg L^{-1}) and biotin (40 μg L^{-1}) were added. $Na_2SiO_3 \times 9H_2O$ (57 mg L^{-1}) was added to allow the growth of freshwater diatoms. Marine phototrophic biofilms were cultivated with HW Professional Sea Salt (Wiegand, Germany) at a salinity of 37 psu. Salinity was controlled by a salinity sensor after mixing of the medium. Nutrients ($NaNO_3$ — 150 mg L^{-1}, KH_2PO_4 — 16 mg L^{-1} and $Na_2SiO_3 \times 9H_2O$ — 57 mg L^{-1}) were added to the medium. The medium (4 L per lane) was exchanged twice a week in all experimental runs.

Phototrophic biofilm material was collected from an overflow weir of the sedimentation basin of the wastewater treatment plant (WWTP) at Fiumicino Airport (Rome) (Congestri et al., 2003) and used as inoculum for development of freshwater phototrophic biofilms. Marine phototrophic biofilms were inoculated with biofilm material from Oesterschelde estuary as described by Staal et al. (2007). Inocula were mechanically homogenized, frozen at −20 °C without addition of cryoprotectants to reduce abundance of protozoa and metazoa and shipped on dry ice to the experimental laboratory. This procedure had only minor effects on the species composition of phototrophic organisms (personal communication, R. Congestri) due to short time duration of storage and transport (1–2 days).

2.6. Chemical analysis

Biomass accumulation was analysed by employing gravimetric methods. Scraped off biofilm material was collected onto

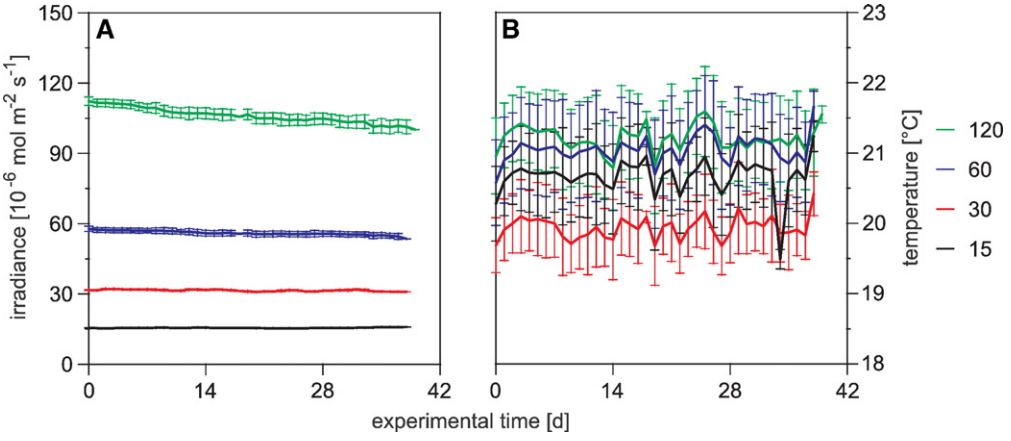

Fig. 4. External conditions during cultivation of freshwater phototrophic biofilms. (A) incident irradiance and (B) temperature was recorded every 10 minutes during experimental duration. Values in legend are in 10^{-6} mol m^{-2} s^{-1} and represent the 4 different flow lanes. Error bars show standard deviation of sensor readings during illuminated interval per day ($n = 96$).

Please cite this article as: Zippel, B. et al. A flow-lane incubator for studying freshwater and marine phototrophic biofilms. J. Micro. Biol. Methods (2007), doi:10.1016/j.mimet.2007.05.013

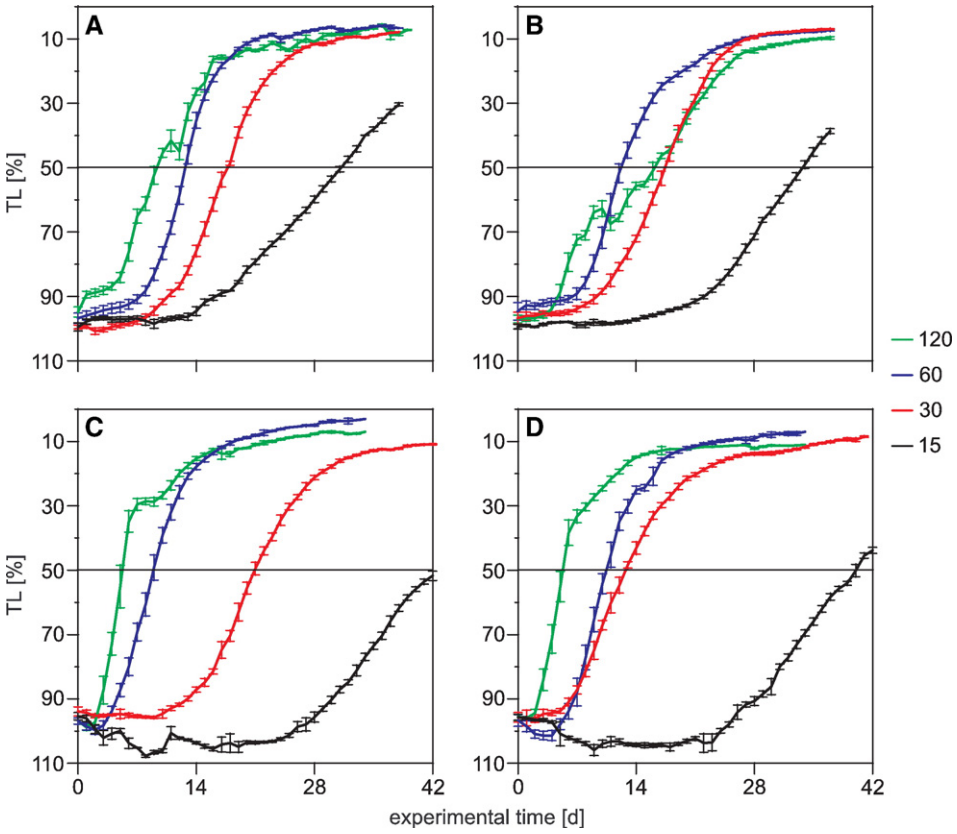

Fig. 5. Growth curves of freshwater (A, B) and marine (C, D) phototrophic biofilms at different irradiances (values in legend are in 10^{-6} mol m^{-2} s^{-1}) and flow rates of (A, C) 100 and (B, D) 25 L h^{-1}. Biomass accumulation was indicated by decrease of proportional transmitted light (TL) below the substratum. TL was measured with 3 light sensors along flow direction within each lane (see positions in Fig. 3). Error bars show standard deviation of sensor readings during illuminated interval per day (n=96).

precombusted quartz filter (QF20, Schleicher&Schuell, Germany) and dried for 24 h at 105 °C for determination of dry weight (dwt) of biomass. Biofilm thickness was calculated according to Peyton (1996).

Net accrual rate during active growth phase was calculated according to Stevenson (1996)

$$n_a = (B_t - B_{t-1})/(d_t - d_{t-1}) \qquad [g \text{ dwt. } m^{-2}d^{-1}] \qquad (1)$$

were B determines the dry weight of biomass at a sampling date. By this way, losses of biomass through erosion and sloughing were not taken into account. Time interval for gravimetric determination of biomass was in the range of 3–7 days depending on different developmental stages of the biofilms.

3. Results

3.1. Variability of external conditions (light, temperature)

The incident light intensity per lane varied only slightly (<4%, Fig. 4A) with exception of a decrescence up to 7% at the end of the experiment at the highest incident light intensity. This was maybe caused by less stable conditions in the lower logarithmic dimming range of the potentiometer. Flow-lane incubators for the cultivation of freshwater and marine phototrophic biofilms were used in climate rooms. In most cases, experiments were carried out for about 35 days. Due to different light intensities during the experiments the medium temperature varied between 20 and 21.5 °C with the highest temperature at the highest light intensity (Fig. 4B). This was caused by heat production of the fluorescent lamps depending on the adjusted light intensity.

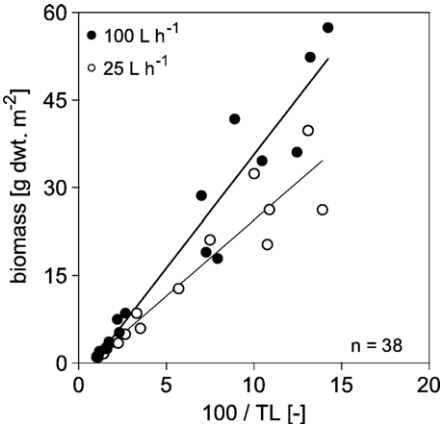

Fig. 6. Regression between proportional transmitted light (TL) and accumulated biomass during development of freshwater phototrophic biofilms grown at irradiances of 15, 30, 60 and 120×10^{-6} mol m^{-2} s^{-1}, a temperature of 20 °C and flow rates of 100 L h^{-1} (filled symbols, $f(x)=3.88\times-3{,}15$, $R^2=0{,}94$) and 25 L h^{-1} (open symbols, $f(x)=2{,}60\times-1{,}52$, $R^2=0{,}91$).

Please cite this article as: Zippel, B. et al. A flow-lane incubator for studying freshwater and marine phototrophic biofilms. J. Micro. Biol. Methods (2007), doi:10.1016/j.mimet.2007.05.013

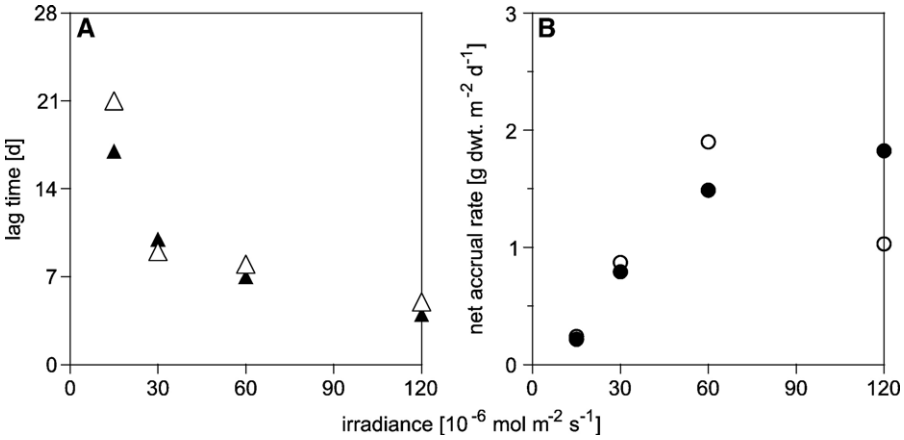

Fig. 7. Growth characteristics of freshwater phototrophic biofilms cultivated at 4 different irradiances, temperature of 20 °C and flow rates of 100 L h^{-1} (filled symbols) and 25 L h^{-1} (open symbols). (A) lag time and (B) net accrual rate were calculated from light sensor specific biomass values.

3.2. Growth curves and biomass accumulation depending on light availability

Exponential growth of phototrophic freshwater and marine biofilms at high (120×10^{-6} mol m^{-2} s^{-1}) and intermediate (60×10^{-6} mol m^{-2} s^{-1}) light intensities started 3 to 7 days after inoculation (Fig. 5). For biofilms grown at low (30×10^{-6} mol m^{-2} s^{-1}) light intensity we observed a lag time of 10 days (Fig. 5). At very low light intensity (15×10^{-6} mol m^{-2} s^{-1}) biofilm development was even more delayed and started after 16 days for freshwater and after 28 days for marine biofilms (Fig. 5). In this case, mature stage was not reached at the end of the experiment

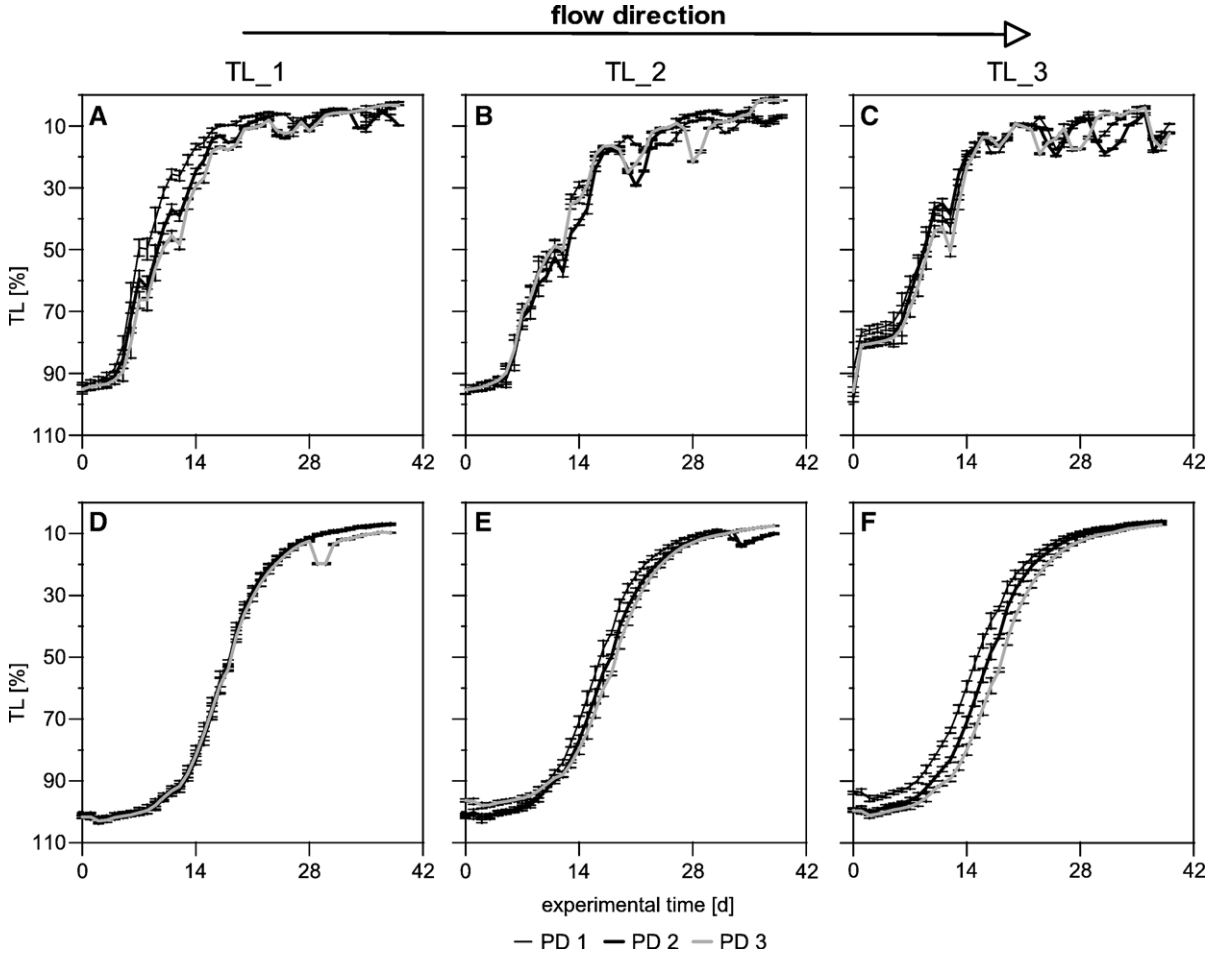

Fig. 8. Proportional transmitted light (TL) during cultivation period measured below substratum at three different positions (TL_1, TL_2, TL_3, see Fig. 3) along flow direction within the flow lanes. Each light sensor (TL) contained 3 single photodiodes (PD1–3). (A–C) 120×10^{-6} mol m^{-2} s^{-1}. (D–F) 30×10^{-6} mol m^{-2} s^{-1}. Error bars show standard deviation of sensor readings during illuminated interval per day ($n=96$).

Please cite this article as: Zippel, B. et al. A flow-lane incubator for studying freshwater and marine phototrophic biofilms. J. Micro. Biol. Methods (2007), doi:10.1016/j.mimet.2007.05.013

due to the very slow development of biofilms. Maturation of phototrophic biofilms, indicated by subsurface light values lower than 10%, was reached after approximately 21 days at high and intermediate light intensities, whereas biofilms at low light intensity reached this stage 1 week later due to their slower development. According to the law of Lambert Beer irradiance diminishes in an approximately exponential manner with depth (Kirk, 1994). In case of the described flow lanes, attenuation of light is resulted from passage through fluid depth and biofilm thickness. Assuming a constant extinction coefficient (density/pigmentation), this would imply that the ratio of incident to transmitted light must give a linear relationship with the biomass per m^2. We found such linear relationships between attached biomass and proportional transmitted light for both flow velocities investigated (Fig. 6). Interestingly, we could observe a steeper slope at high flow which means that accumulated biomass at a given proportional transmitted light is one third higher under high flow than under low flow conditions. This was may be caused by a higher amount of nutrients transferred into the biofilm due to steeper gradients within the diffusive boundary layer at higher flow velocities. Finally, we deduced from the growth curves that the lag phase became shorter as the experimental light intensity was higher (Fig. 7A). The growth rate of phototrophic biofilms during exponential phase increased proportional to the incident light intensity between 15 and 60×10^{-6} mol m^{-2} s^{-1}, but not for both flow rates at highest light intensity (Fig. 7B). In these cases, the biofilms had a less firm structure and were probably influenced by higher loss rates due to sloughing events.

3.3. Homogeneity of biomass accumulation during temporal development of biofilms

Low spatial heterogeneity of biomass accumulation on sampling devices within biofilm reactors is one of the main prerequisite for the analysis of temporal studies or determination of kinetic parameters. Uniformity of biomass accumulation during temporal development of the biofilms was analysed by growth curves obtained from each single photodiode of the inserted light sensors (Fig. 8). Growth of biofilms at high irradiances showed a more heterogeneous behaviour than at low irradiances. Especially during the second half of the active growth phase the light sensor values of the 3 photodiodes varied markedly due to sloughing processes (Fig. 8A–C). At low light intensity the growth curves showed in all cases a sigmoid pattern and variability was observed only at the end of the experiment (Fig. 8D–F).

An additional experiment was carried out to investigate spatial homogeneity of biofilm development along the flow direction within separate lanes. For this purpose, biofilms were sampled for gravimetric analysis of biomass simultaneously at three positions close to the 3 inserted light sensors (Fig. 9A–D). Biofilm thickness at sampling during active growth phase was in the range of 150 to 220 µm, whereas slightly higher values were reached at the higher light intensity. At mature stage, biofilms grown at the highest light intensity were approximately 100 µm thicker if compared to those grown at intermediate light intensity (Fig. 9B). Variation of biomass at three different positions along the flow direction was in the range between 1.2 and 22% (mean 9.1%). Higher variation (mean 12.5%) was

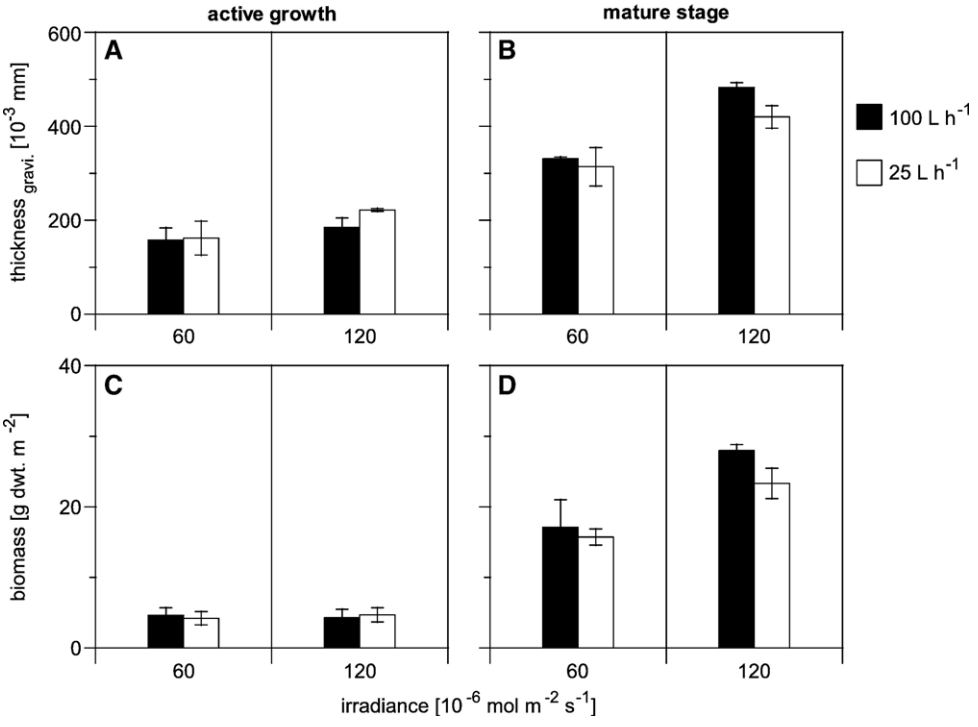

Fig. 9. Comparison of biofilm thickness (A, B) and biomass (C, D) at 3 different positions along the flow direction within the lanes. Error bars show standard deviation (n=3). Biofilms were cultivated at 60 and 120×10^{-6} mol m^{-2} s^{-1} irradiance and low or high flow rates, 25 or 100 L h^{-1} respectively.

Please cite this article as: Zippel, B. et al. A flow-lane incubator for studying freshwater and marine phototrophic biofilms. J. Micro. Biol. Methods (2007), doi:10.1016/j.mimet.2007.05.013

Table 1
Attached biomass in various natural and artificial systems

Research background	System type	Substratum	AFDM [g m^{-2}]	Chla [mg m^{-2}]	Remarks	Reference
Ecosystem function	river	ceramic tiles		5–300	influence of habitat heterogeneity	Cardinale et al. (2002)
	river	clay tiles		2–50	organic matter processing in streams	Romani and Sabater (2001)
		rocks and sand	36 – 89	2–160		
	stream	ceramic tiles		2–400	energy flow and nutrient cycling	Battin et al. (2003)
	filtration dunes	glass disk	<1.5 [a]	5–140	carbon and nutrient cycling	Barranquet et al. (2004a,b)
Biodegradation	microcosm	gravel glass	(290–420 [b]) (23–450[b])		degradation of hydrocarbons	Al-Awadhi et al. (2003)
	intertidal flat	sediment		55–186	biodegradation, carbon flow	Goto et al. (2001)
	intertidal flat	sediment		0–85	sediment stabilization	Blanchard et al. (2000)
Aquaculture	mesocosm	glass fiber tanks		0–360	water quality and food source	Thompson et al. (2002)
Ecological modelling	mesocosm	gravel PVC	20–50	10–100	interactions between turbulence and periphyton	Godillot et al. (2001)
	mesocosm	polycarbonate	0.3–52	2.2–300	temporal development of phototrophic biofilms	this study

Biomass is expressed as g ash-free dry matter (AFDM), or as mg chlorophyll *a* (Chla) per m^2 of substratum area.
[a] Converted from g carbon by assuming 48% C in AFDM.
[b] AFDM not presented, values determine fresh biomass.

observed at active growth phase and was maybe caused by sampling errors of these relatively thin biofilms. At mature stage, variation was in three out of four lanes lower than 5%.

4. Discussion

Phototrophic biofilm incubators (PBI) have been used frequently for quantitative and qualitative studies on phototrophic biofilms (Craggs et al., 1996; Schumacher and Sekoulov, 2002; Travieso et al., 2002 and others). The requirements for the described laboratory microcosms were the following, (i) adjustment and control of external conditions (light, temperature, flow velocity), (ii) easy sampling procedure for investigation of undisturbed phototrophic biofilms (e.g. LSM, microsensors), (iii) low spatial heterogeneity of attached biofilms at the sampling devices, (iv) non-destructive, real-time monitoring of biomass accumulation and (v) low purchase and maintenance costs.

None of the already described PBIs fully met the experimental requirements. Some systems used for mass cultivation of attached algae were not suitable due to destructive sampling procedure (Travieso et al., 2002). Flow lanes which were used by Gantzer et al. (1988), Godillot et al. (2001), Battin et al. (2003) had dimensions which were to large for use in normal climate laboratories. On the other hand, microscope flow chambers which were frequently used for cultivation of pure culture biofilms under controlled conditions (Christensen et al., 1999) provide an attachment area which is too small for various analytical methods. Some investigations on phototrophic biofilms from lotic and lentic systems (Lawrence et al., 2001; Neu and Lawrence, 1997) were setup in Rotating Annular Reactors (RAR). This reactor type was tested to determine a number of chemical, physical and biological parameters that influence the structure, composition and various properties of biofilms (Gjaltema et al., 1994). In most cases, adjustment of defined light conditions during cultivation of biofilms in RAR was not necessary due to origin of the inoculum (heterotrophic or chemoautotrophic bacteria from activated sludge). Beside several advantages of RAR they have two main disadvantages. Firstly, multilayer biofilms possess a high spatial heterogeneity when cultivated in RAR (Gjaltema et al., 1994; Griebe and Flemming, 2000). Secondly, on-line control of biomass accumulation during temporal development is not possible due to construction of the reactors.

Cultivation of phototrophic biofilms in the flow-lane incubator as described occurred under controlled external abiotic conditions. The once adjusted values of irradiance and temperature showed a low variability during long term experiments. This served as a basis for comparison of results obtained in parallel experiments which were run in different European laboratories using the same parameter combinations. In a comparative study, Roeselers et al. (2006) pointed out that the growth rate of phototrophic biofilms were similar in three identical flow-lane incubators. Nevertheless, the communities developed differently in terms of species richness and composition as determined by molecular techniques.

Microscope slides provided a suitable sampling unit for subsequent analysis. Biofilms attached to the inserted slides can be sampled in an easy and quick way. Due to the design of slide fixation this can be done without destruction of the biofilms sampled and the biofilms growing on the two slides next to it. In this study we used as substratum slides made from polycarbonate. But also glass slides or other transparent materials having different surface energies may be used. Whatever the specific slide material is, it can be mounted easily on the light sensor. This is of course a prerequisite for comparable measurements on light sensor and sampling slides.

To the best of our knowledge, no cultivation system for phototrophic biofilms was described in the literature which has an on-line monitoring system for biomass accumulation that is non-invasive and non-destructive. The optical approach for biomass accumulation as described by Bakke et al., 2001 was

Please cite this article as: Zippel, B. et al. A flow-lane incubator for studying freshwater and marine phototrophic biofilms. J. Micro. Biol. Methods (2007), doi:10.1016/j.mimet.2007.05.013

not suitable for our flow lanes due to dimensions of the tubes and cultivation of biofilms in a closed system. Milferstedt et al. (2006) described a non-destructively but invasively method for biomass estimation employing an adapted desktop scanner. This method is restricted to biofilm thicknesses <100 μm, and <5 g m^{-2}, which represents only young phototrophic biofilms according to Barranguet et al. (2004a). The light sensors used in the PBI were suitable for real-time monitoring of biomass accumulation during temporal development of phototrophic biofilms. They use the principle of light attenuation in dependency from biomass increase of pigmented organisms attached to the transparent substratum. In order to investigate kinetic parameters like growth rate, a high spatial homogeneity of developing biofilms is essential. This requirement could be proved by on-line monitoring of biomass accumulation at different positions along flow direction within the PBI flow lanes. Neither the photodiodes near the side walls of the flow lanes nor the photodiodes in the centre of each light sensor showed substantial differences in biomass accumulation.

Linear relation between light sensor readings and attached biomass during exponential growth and begin of matured stage was obtained. This means that the resolution of the light sensors was high enough for differentiation between accumulated biomass in the range of 1–50 g dwt. m^{-2}. Especially at the highest irradiance, matured stage, as indicated by light sensor values lower than 10%, was reached after approximately 3 weeks. This however rises the question, what about potential biomass accumulation at a later stage. In these cases, the growth rate decreased down to 25% compared to values during exponential phase. Therefore it was concluded that data presented in this study are useful to differentiate early temporal developmental stages of up to 3–5 weeks depending on the illumination conditions used. Zippel and Neu (2005) could observe different vertical stratification pattern in phototrophic biofilms developed at different irradiances at the same developmental stage. This could have implications for stability and nutrient demand of the biofilms investigated.

The last part of our requirements concerning the costs for purchase, maintenance and operation of the incubator are briefly discussed. Eight PBIs were produced by the UFZ workshop at the price of 5000 Euro per incubator. Beside the low-budget plastic material for the incubator and relatively inexpensive aquarium pumps, approximately one half of the costs were occupied for the adjustable irradiance and the automatic light sensor setup. The True-light lamps have an economic life-time in the range of 10,000 to 20,000 hours. The installation of the PBI including cleaning or changing of the single incubator parts could be done within 5 to 8 hours. Finally, we can assure from our experiences that continuous operation is possible over a period of 2–3 years.

Investigations of phototrophic biofilms from different habitats were carried out in natural and artificial systems for various reasons (sediment stability, biodegradation, modelling etc., see Table 1). Biomass and chlorophyll *a* values of the phototrophic biofilms which were cultivated in the PBI described are well within the range of previous investigations (Table 1). Nevertheless, it has to be emphasized that a laboratory microcosm like a photobioreactor can never fully reproduce the natural environment. However, the aim of this study was to investigate the effects of constant external conditions (irradiance, temperature and flow velocity) on biofilm development. For this purpose, the PBI was a reasonable conceptual approach which allowed the study of various phototrophic biofilms in the laboratory.

5. Conclusion

The flow-lane incubator designed may be used as microcosm for studying the development and behaviour of different types of environmental biofilms. The system is especially useful for controlled and reproducible cultivation of phototrophic biofilms under defined irradiance and fluid shear. The incubator with four independent lanes can be operated at low maintenance under laboratory conditions. External parameters can be easily adjusted in each lane and are controlled by robust sensors. The light sensors below the substratum allow on-line monitoring of biomass accumulation during long-term experiments. The system does prevent the formation of gradients due to homogeneous conditions along the flow direction. This results in representative biofilm samples and offers the possibility of kinetic studies of complex environmental biofilms.

Acknowledgements

This research was supported by the European Union (PHOBIA project, contract QLK3-CT-2002-01938). We are grateful to the team of the UFZ workshop Leipzig (V. Menzel) for their enormous engagement during development of the prototype. We thank L. Schulze from Betternetworks Ltd. for providing excellent light sensors and the controlling system. Technical assistance provided by H. Goretzka, U. Link, and U. Kuhlicke is highly appreciated.

References

Al-Awadhi, H., Al-Hasan, R.H., Sorkhoh, N.A., Salamah, S., Radwan, S.S., 2003. Establishing oil-degrading biofilms on gravel particles and glass plates. Int. Biodeterior. Biodegrad. 51, 181–185.

Alberte, R.S., Snyder, S., Zahuranec, B.J., Whetstone, M., 1992. Biofouling research needs for the US Navy: program history and goals. Biofouling 6, 91–95.

Bakke, R., Kommedal, R., Kalvenes, S., 2001. Quantification of biofilm accumulation by an optical approach. J. Microbiol. Methods 44, 13–26.

Barranguet, C., van Beusekom, S.A.M., Veuger, B., Neu, T.R., Manders, E.M.M., Sinke, J.J., Admiraal, W., 2004a. Studying undisturbed autotrophic biofilms: still a technical challenge. Aquat. Microb. Ecol. 34, 1–9.

Barranguet, C., Veuger, B., van Beusekom, S.A.M., Marvan, P., Sinke, J.J., Admiraal, W., 2004b. Divergent composition of algal–bacterial biofilms developing under various external factors. Eur. J. Phycol. 39, 1–8.

Battin, T.J., Kaplan, L.A., Newbold, J.D., Cheng, X., Hansen, C., 2003. Effects of current velocity on the nascent architecture of stream microbial biofilms. Appl. Environ. Microbiol. 69, 5443–5452.

Battin, T.J., Sengschmitt, D., 1999. Linking sediment biofilms, hydrodynamics, and river bed clogging: Evidence from a large river. Microb. Ecol. 37, 185–196.

Biggs, B.J.F., 1996. Patterns in benthic algae of streams. In: Stevenson, R.J., Bothwell, M., Lowe, R.L. (Eds.), Algal Ecology. Academinc Press, New York, pp. 31–56.

Please cite this article as: Zippel, B. et al. A flow-lane incubator for studying freshwater and marine phototrophic biofilms. J. Micro. Biol. Methods (2007), doi:10.1016/j.mimet.2007.05.013

Blanchard, G.F., Paterson, D.M., Stal, L.J., Richard, P., Galois, R., Huet, V., Kelly, J., Honeywill, C., de Brouwer, J., Dyer, K., Christie, M., Seguignes, M., 2000. The effect of geomorphological structures on potential biostabilisation by microphytobenthos on intertidal mudflats. Cont. Shelf Res. 20, 1243–1256.

Callow, M.E., Edyvean, R.G.J., 1990. Algal fouling and corrosion. In: Akatsuka, I. (Ed.), Introduction to Applied Phycology. SPB Academic Publishing bv, The Hague, pp. 367–387.

Callow, M.E., 2000. Algal biofilms. In: Evans, L.V. (Ed.), Biofilms: recent advances in their study and control. Harwood Academic Publishers, Amsterdam, pp. 189–209.

Cannell, R.J.P., 1990. Algal biotechnology. Appl. Biochem. Biotechnol. 21, 85–105.

Cardinale, B.J., Palmer, M.A., Swan, C.M., Brooks, S., Poff, N.L., 2002. The influence of substrate heterogeneity on biofilm metabolism in a stream ecosystem. Ecology 83, 412–422.

Characklis, W.G., Cooksey, K.E., 1983. Biofilms and microbial fouling. Adv. Appl. Microbiol. 29, 93–138.

Christensen, B.B., Sternberg, C., Andersen, J.B., Palmer, R.J., Nielsen, A.T., Givskov, M., Molin, S., 1999. Molecular tools for study of biofilm physiology. Methods Enzymol. 310, 20–42.

Congestri, R., Sangiorgi, V.C., Albertano, P., 2003. Cytomorphology and distribution of periphytiv cyanobacteria in one Italian WWTP. Arch. Hydrobiol. 109, 185–195.

Cooksey, K.E., Wigglesworth-Cooksey, B., 2001. Diatoms in biofilms. In: Flemming, H.C. (Ed.), Encyclopedia of environmental microbiology. Wiley, New York, pp. 1051–1063.

Craggs, R.J., Adey, W.H., Jessup, B.K., Oswald, W.J., 1996. A controlled stream mesocosm for tertiary treatment of sewage. Ecol. Eng. 6, 149–169.

Csőgör, Z., Herrenbauer, M., Schmidt, K., Posten, C., 2001. Light distribution in a novel photobioreactor — modelling for optimization. J. Appl. Phycol. 13, 325–333.

de Brouwer, J.F.C., Wolfstein, K., Stal, L.J., 2002. Physical characterization and diel dynamics of different fractions of extracellular polysaccharides in an axenic culture of a benthic diatom. Eur. J. Phycol. 37, 37–44.

Faulkner, D.J., 2000. Marine natural products. Nat. Prod. Rep. 17, 7–55.

Gantzer, G.J., Rittmann, B.E., Herricks, E.E., 1988. Mass transport to streambed biofilms. Water Res. 22 (6), 709–722.

Gjaltema, A., Arts, P.A.M., van Loosdrecht, M.C.M., Kuenen, J.G., Heijnen, J.J., 1994. Heterogeneity of biofilms in Rotating annular reactors: Occurrence, structure, and consequences. Biotechnol. Bioeng. 44 (2), 194–204.

Godillot, R., Caussade, B., Ameziane, T., Capblancq, J., 2001. Interplay between turbulence and periphyton in rough open-channel flow. J. Hydraul. Res. 39, 227–239.

Goto, N., Mitamura, O., Terai, H., 2001. Biodegradation of photosynthetically produced extracellular organic carbon from intertidal benthic algae. J. Exp. Mar. Biol. Ecol. 257, 73–86.

Griebe, T., Flemming, H.-C., 2000. Rotating annular reactors for controlled growth of biofilms. In: Flemming, H.-C., Griebe, T., Szewzyk, U. (Eds.), Biofilms: Investigative Methods and Applications. CRC Press, Lancaster, pp. 23–40.

Holmstrom, C., Kjelleberg, S., 2000. Bacterial interactions with marine fouling organisms. In: Evans, L.V. (Ed.), Biofilms: recent advances in their study and control. Harwood Academic Publishers, Amsterdam, pp. 101–115.

Kirk, J.T.O., 1994. Concepts of hydrologic optics. Light & Photosynthesis in Aquatic Ecosystems. Cambridge University Press, Cambridge, pp. 3–22.

Kühl, M., 2005. Optical microsensors for analysis of microbial communities. In: Leadbetter, J.R. (Ed.), Environmental Microbiology. Methods in Enzymology, vol. 397, pp. 166–199.

Lawrence, J.R., Kopf, G., Headley, J.V., Neu, T.R., 2001. Sortion and metabolism of selected herbicides in river biofilm communities. Can. J. Microbiol. 47, 634–641.

Lazarova, V., Manem, J., 2000. Innovative biofilm treatment technologies for water and waste water treatment. In: Bryers, J.D. (Ed.), Biofilms II-Process Analysis and Applications. Wiley-Liss, New York, pp. 159–206.

Matsunaga, T., Takeyama, H., Nakao, T., Yamazawa, A., 1999. Screening of marine microalgae for bioremediation of cadmium-polluted seawater. J. Biotechnol. 70, 86–92.

Meyer-Reil, L.-A., 1994. Microbial life in sedimentary biofilms — the challenge to microbial ecologists. Mar. Ecol., Prog. Ser. 112, 303–311.

Meyer-Reil, L.-A., Köster, M., 2000. Eutrophication of marine waters: Effects on benthic microbial communities. Mar. Pollut. Bull. 41, 255–263.

Milferstedt, K., Pons, M.N., Morgenroth, E., 2006. Optical method for long-term and large-scale monitoring of spatial biofilm development. Biotechnol. Bioeng. 94 (4), 773–782.

Miyake, J., Miyake, M., Asada, Y., 1999. Biotechnological hydrogen production: research for efficient light energy conversion. J. Biotechnol. 70, 89–102.

Neu, T.R., Lawrence, J.R., 1997. Development and structure of microbial biofilms in river water studied by confocal laser scanning microscopy. FEMS Microbiol. Ecol. 24 (1), 11–25.

Neu, T.R., Wölfl, S., Lawrence, J.R., 2004. Three-dimensional differentiation of photo-autotrophic biofilm constituents by multi-channel laser scanning microscopy (single-photon and two-photon excitation). J. Microbiol. Methods 56 (2), 161–172.

Perkins, R.G., Underwood, G.J.C., Brotas, V., Snow, G.C., Jesus, B., Ribeiro, L., 2001. Responses of microphythobenthos to light: primary production and carbohydrate allocation over an emersion period. Mar. Ecol., Prog. Ser. 223, 101–112.

Peyton, B.M., 1996. Effects of shear stress and substrate loading rate on *Pseudomonas aeruginosa* biofilm thickness and density. Water Res. 30, 29–36.

Pulz, O., Gross, W., 2004. Valuable products from biotechnology of microalgae. Appl. Microbiol. Biotechnol. 65, 635–648.

Roeselers, G., Zippel, B., Staal, M., van Loosdrecht, M.C.M., Muyzer, G., 2006. On the reproducibility of microcosm experiments — different community composition in parallel phototrophic biofilm microcosms. FEMS Microbiol. Ecol. 58, 169–178.

Romani, A.M., Sabater, S., 2001. Structure and activity of rock and sand biofilms in a Mediterranean stream. Ecology 82, 3232–3245.

Schumacher, G., Sekoulov, I., 2002. Polishing of secondary effluent by an algal biofilm process. Water Sci. Tech. 46, 83–90.

Sigee, D.C., 2005. Freshwater environments: the influence of physico-chemical conditions on microbial communities. Freshwater microbiology: biodiversity and dynamic interactions of microorganisms in the freshwater environment. Wiley, Chichester, pp. 47–102.

Staal, M., Thar, R., Kühl, M., van Loosdrecht, M.C.M., Wolf, G., de Brouwer, J.F.C., Rijstenbil, J.W., 2007. Carbon isotope fractionation in developing natural phototrophic biofilms. Biogeosci. Discuss 4, 69–98.

Stanier, R.Y., Kunisawa, R., Mandel, M., Cohen-Bazire, G., 1971. Purification and properties of unicellular blue-green algae (order Chroococcales). Bacteriol. Rev. 35, 171–205.

Stevenson, R.J., 1996. An introduction to algal ecology in freshwater benthic habitats. In: Stevenson, R.J., Bothwell, M.L., Lowe, R.L. (Eds.), Algal Ecology: freshwater benthic ecosystems. Academic Press, London, pp. 3–30.

Thompson, F.L., Abreu, P.C., Wasielesky, W., 2002. Importance of biofilm for water quality and nourishment in intensive shrimp culture. Aquaculture 203, 263–278.

Travieso, L., Pellón, A., Benítez, F., Sánchez, E., Borja, R., O´Farrill, N., Weiland, P., 2002. BIOALGA reactor: preliminary studies for heavy metals removal. Biochem. Eng. J. 12, 87–91.

Van Dam, A.A., Beveridge, M.C.M., Azim, M.E., Verdegam, M.C.J., 2002. The potential of fish production based on periphyton. Rev. Fish Biol. Fish. 12, 1–31.

Vilchez, C., Garbayo, I., Lobato, M.V., Vega, J.M., 1997. Microalgae-mediated chemicals production and wastes removal. Enzymol. Microbiol. Technol. 20, 562–572.

Wigglesworth-Cooksey, B., Cooksey, K.E., 2005. Use of fluorophore-conjugated lectins to study cell–cell interactions in model marine biofilms. Appl. Environ. Microbiol. 71, 428–435.

Wolfaardt, G.M., Korber, D.R., 2000. Biodegradation in biofilm communities. In: Allison, D.G., Gilbert, P., Lappin-Scott, H.M., Wilson, M. (Eds.), Community Structure and Cooperation in Biofilms. Cambridge University Press, Cambridge, pp. 279–294.

Wolfstein, K., Stal, L.J., 2002. Production of extracellular polymeric substances (EPS) by benthic diatoms: effect of irradiance and temperature. Mar. Ecol., Prog. Ser. 236, 13–22.

Zippel, B., Neu, T.R., 2005. Growth and structure of phototrophic biofilms under controlled light conditions. Water Sci. Technol. 52, 203–209.

Please cite this article as: Zippel, B. et al. A flow-lane incubator for studying freshwater and marine phototrophic biofilms. J. Micro. Biol. Methods (2007), doi:10.1016/j.mimet.2007.05.013